Supelco®
Analytical Products

Manual
Analysis Methods for the Brewery Industry

Spectroquant®
Prove

Contents

* not for Spectroquant® Prove 100

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Analytical Procedures

* not for Spectroquant® Prove 100

General Information

I Safety instruction

Improper handling of reagents may result in damage to health.
The safety labels on the packaging materials and the safety instructions in the package insert must be observed in all cases. The protective measures described must be heeded exactly.

The safety data sheets for the chemicals (www.analytical-test-kits.com) contain all instructions for their safe handling, all risks that may occur, as well as preventive measures and measures to be taken in the event of an accident. Please follow these instructions for your own safety.

II Introduction

The operating steps described here are as a rule menu-driven in the photometer of the Spectroquant® Prove series. In the case of uncertainty, please refer to the corresponding sections of the functional description of the photometer.

The methods for brewery analysis are a compilation of spectrophotometric specifications of relevance in the area of brewery analysis. The working instructions are reproduced with kind permission of the "Mitteleuropäische Brautechnische Analysekommission" (MEBAK, Central European Brewery Analysis Committee) from the MEBAK method-collection volume "Rohstoffe" (Raw Materials) 1st Edition 2006 and volume II, 4th Edition 2002
and
American Society of Brewing Chemists from ASBC method collection "Methods of Analysis" 14th edition. Reproduced with permission from the American Society of Brewing Chemists for use by purchasers of specified Spectroquant® Prove Spectrophotometers. No other use or reproduction is permitted without written permission from the American Society of Brewing Chemists.

III List of abbreviations

Unless noted otherwise, all reagents used are of the GR (guaranteed reagent) grade.

H_2O	=	distilled / demineralized water
s	=	seconds
min	=	minutes
h	=	hours
mm	=	millimeter
rpm	=	revolutions per minute
nm	=	nanometer
QS	=	cells made of quartz glass
OS	=	cells made of optical glass

General Information

IV Literature

MEBAK
Brautechnische Analysemethoden

Methodensammlung der Mitteleuropäischen Brautechnischen Analysekommission (MEBAK)

Volume Rohstoffe, 1st Edition 2006
Edited by Chairman Dr Heinz-Michael Anger

Volume II, 4th Edition, revised and amended, 2002
Edited by Chairman Prof Dr H Miedaner

Published by MEBAK
D-85350 Freising - Weihenstephan, Germany

The working instructions for brewery-technical analytical methods are reproduced with kind permission of the "Mitteleuropäische Brautechnische Analysekommission" (MEBAK, Central European Commission for Brewery Analysis) from the MEBAK multiplevolume collection.

ASBC
American Society of Brewing Chemists from ASBC method collection "Methods of Analysis" 14th edition
Available under http:\\methods.asbcnet.org

American Society of Brewing Chemists
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St. Paul, MN 55121 USA
1.651.454.7250

General Information

V Zeroing

A valid zero point is required for the calculation of measurement results. Zeroing is performed as a rule by measuring the absorbance of **a cell filled with distilled water ("zero cell")** and saving the result in the photometer. In those cases in which the zero adjustment is **not** to be made using a cell filled with distilled water, this is noted in the analytical procedure of the corresponding method.

Notes

- Cells must be absolutely **clean** and **scratch-free**.
- When zeroing always use a cell of the same type as the one used for measuring the sample. Refer to the corresponding section of the functional description of the spectrophotometer for ordering information. The cells listed there are specifically designed for the Spectroquant® product range.
  Please refer to the corresponding section of the functional description of the photometer for general requirements regarding the cells. Please note that the transmission of the cell must be suited for the intended use (e. g. rectangular QS cells for the UV spectrum).
- When using rectangular cells, zeroing must be performed using the **same type of cell** (manufacturer and glass type) as the one used for the measurement. This is important since cells made by different manufacturers exhibit differing absorbance characteristics. If you exchange the cell type, please repeat the zeroing procedure with the new type.
- Clean rectangular cells prior to zeroing and fill with distilled water. **The minimum filling level is 25 mm.**
- Always insert rectangular cells into the cell compartment with the **same orientation** as the cell used for zeroing (e. g. with the cell print always on the left).

Zeroing procedure

A concentration method must be selected to start the zero adjustment. Subsequently tap the **<Settings>** button and select the **<ZERO ADJUSTMENT>** menu item. Follow the instructions shown on the display to proceed. Refer to the corresponding section of the functional description of the photometer if you are uncertain.

It is advisable to repeat the zeroing procedure in the following cases:

- When the instrument has been subjected to **mechanical stress**, e. g. to strong vibrations or after transport.
- When the ambient temperature has changed **by more than 5 °C** since the last zeroing.
- At least **once a week**.
- When using a **new type of cell** (different manufacturer, different type of glass).
- In all cases in which measurement is to yield results of the **best possible accuracy**.

VI Sample blank

The measurement and use of a sample blank can help eliminate measurement errors due to discoloration and turbidity in the sample matrix.
The sample blank is measured as per the corresponding analysis, but without the coloring reagent.
The sample blank is valid only for the subsequent measurement, and a new sample blank must be measured into the system prior to every new measurement.

Sample blank procedure

The necessary sample blanks are described in greater detail in the corresponding analytical procedures.

General Information

VII Reagent blank (blank)

The evaluation of the photometric measurement is always in relation to the reference value of a measurement solution that does not contain the analyte (reagent blank). This is to compensate the effect of the baseline absorbance of the reagents on the photometric measurement.
In the practical context, the reagent blank is measured using the same volume of deionized water in place of the sample.

Notes

- The accuracy can be enhanced by determining the **reagent blank** using reagents of **one and the same batch**, keeping the reagent blank stored until the reagents are exchanged, after which a new reagent blank must be measured.
- In order to facilitate the assignment of the data in the result documentation later on, enter the designation details (e. g. operator, date of preparation) for the respective sample (as "**Lot number**") here during the measurement of the reagent blank.
- The reagent blank can be determined either in single or in multiple measurement. In the multiple measurement mode, the reagent blank is calculated as the mean of the single measurement results.

Reagent blank procedure

After selecting a concentration method, tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>. Refer to the corresponding section of the functional description of the photometer if you are uncertain. The exact composition of the reagent blank is described in more detail in the corresponding analytical procedure.

Measurement of the reagent blank is necessary in the following cases:

- When **prompted** by the instrument.
- For each series of measurements in the case that the reagent blank changes in the course of the day of measurement.
- When **exchanging batches** of the reagents used.
- When the **saved value** is to be **overwritten**.

VIII User-defined calibration

The user must recalibrate the method in the case that calibration data (slope and reagent blank) of the method are subject to change, depending on the sample matrix or on the reagents used from one sample or, respectively, one measurement series to the next.
In the case of methods that require user-defined calibration, the procedure is described in the corresponding analytical procedures.

Procedure of user-defined calibration

After selecting a concentration method, tap the **<Settings>** button and select the **<RECALIBRATION>** menu item. Follow the instructions shown on the display to proceed. Refer to the corresponding section of the functional description of the photometer if you are uncertain.

User-defined calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the calibration is influenced by the sample matrix.

General Information

IX Method overview

Parameter	Acc.	Matrix	Measuring range	Method principle	Method No.	Photometer Prove
α Acids	MEBAK	beer	0 - 80 mg/l α acids	Inherent color	2612	100, 300, 600
α Acids	ASBC Hops-8B	hops (non-isomerized extracts)	0.0 - 100.0 % α acids	Inherent color	2637	300, 600
α and β acids	ASBC Malt 6-A	Hop / Hop pellets	0.0 - 100.0 % α acids 0.0 - 100.0 % β acids	Inherent color	2636	300, 600
Anthocyanogenes	MEBAK	beer	0 - 100 mg/l anthocyanogenes	Acidic hydrolysis (Harris and Ricketts method)	2601	100, 300, 600
Bitterness	ASBC Beer-23A	beer	1.0 - 80.0 BU	UV absorption	2603	300, 600
Bitterness	EBC, MEBAK	beer	1.0 - 80.0 BU	UV absorption	2603	300, 600
Bitterness	EBC, MEBAK, ASBC Wort-23A	wort	1.0 -120.0 BU	UV absorption	2604	300, 600
Color	ASBC Beer-10A	beer	0.0-50.0 °SRM 0.0-100.0 EBC	Inherent color	2633	100, 300, 600
Color	ASBC Wort-9A	wort	0.0-50.0 °SRM 0.0-100.0 EBC	Inherent color	2633	100, 300, 600
Color	EBC, MEBAK	wort, beer, liquid malt substitutes	0.0 - 60.0 EBC units	Inherent color	2602	100, 300, 600
Copper	EBC, MEBAK	beer (clear and light)	0.10 - 5.00 mg/l Cu	Cuprethol	2613	100, 300, 600
Diacetyl	ASBC Beer-25B	beer	0.0 - 4.0 mg/l diacetyl	a-Naphthol	2631	100, 300, 600
Flavanoids	EBC	beer	3 - 200 mg/l catechin equivalent	4-Dimethylamino-cinnamaldehyd	2626	100, 300, 600
Flocculation	ASBC Yeast-11B	yeast	-10.0 - 100.0 % flocculation	Turbidity	2635	100, 300, 600
Free Amino Nitrogen	EBC, MEBAK, ASBC Beer-31	beer, wort	0 - 400 mg/l free amino nitrogen	Ninhydrin	2606	100, 300, 600
Hop Storage Index	ASBC Hops-12	hop	0.00 - 2.00 HSI	UV absorption	2634	300, 600
Iodine Value	MEBAK	beer, wort	0.00 - 0.80 iodine value	Iodine	2615, 2616	100, 300, 600
Iron	ASBC Beer-18A	beer	0.00 - 3.00 mg/l Fe	1,10-Phenanthroline	2642	100, 300, 600
Iron	ASBC Beer-18A	beer	0.00 - 3.00 mg/l Fe	2,2'-Bipyridine	2643	100, 300, 600
Iron	ASBC Beer-18C	beer	0.00 - 0.40 mg/l Fe	Ferrozine	2644	100, 300, 600
Iron	EBC, MEBAK	beer	0.000 - 1.000 mg/l Fe	Ferrozine	2623, 2624	100, 300, 600
Iso-α acids	MEBAK	beer	0 - 60 mg/l iso-α acids	UV absorption	2611	300, 600
Nickel	EBC, MEBAK	beer	0.00 - 5.00 mg/l Ni	Dimethylglyoxime	2614	100, 300, 600
Protein	ASBC Beer-11C	beer (unstabilized)	0.0 - 100.0 % protein	UV absorption	2638	300, 600
Protein	ASBC Beer-11C	beer (stabilized)	0.0 - 100.0 % protein	UV absorption	2639	300, 600
Protein	ASBC Beer-11C	beer (dark)	0.0 - 100.0 % protein	UV absorption	2640	300, 600
Protein	ASBC Wort-17	wort (unhopped)	0.0 - 100.0 % (malt, dry basis)	UV absorption	3641	100, 300, 600
Reducing Power	MEBAK	beer	0 - 100 %	DPI	2617	100, 300, 600
Reducing Sugars	ASBC Malt-6B	malt	0.00 - 1.00 g/l dextrose	PAHBAH (Henry method)	2632	100, 300, 600
Steam-volatile Phenols	MEBAK	beer: malt:	0.00 - 0.30 mg/l steam-volatile phenols 0.00 - 3.00 mg/kg steam-volatile phenols	Aminoantipyrine by extraction	2621, 2622	100, 300, 600
Sulfur Dioxide	ASBC Malt-11	malt	0.0 - 50.0 mg/l SO_2	*p*-Rosaniline method	user-defined	100, 300, 600
Thiobarbituric Acid Number	MEBAK, ASBC Wort-21	beer, wort, malt extract	0 - 250	Thiobarbituric acid	2619	100, 300, 600
Total Carbohydrates	MEBAK	beer	0.000 - 6.000 g / 100 ml total carbohydrates	Anthrone	2625	100, 300, 600
Total Polyphenols	EBC, MEBAK, ASBC Beer-35	beer	0 - 800 mg/l total polyphenols	Iron(III)	2610	100, 300, 600
Total Sulfur Dioxide	ASBC Beer-21A	beer	0.0 - 16.0 mg/l SO_2	*p*-Rosaniline method	user-defined	100, 300, 600
Vicinale Diketones	EBC, MEBAK	beer	0.000 - 2.000 mg/kg vicinale diketones	Phenylendiamine	2620	100, 300, 600

Analytical Procedures

1 α Acids (MEBAK method)

1.1 Method

The bitter substances are extracted from the acidified sample (beer or wort) with iso-octane. Any substances that cause interference are removed by washing the extract with acidified methanol and the concentration of the α acids is determined by spectrophotometry.

1.2 Measuring range

0 - 80 mg/l α acids

1.3 Reagents and accessories

- Hydrochloric acid 6 mol/l EMPROVE®, Cat. No. 110164
- Hydrochloric acid 25 % for analysis EMSURE®, Cat. No. 100316
- Isooctane Uvasol®, Cat. No. 104718
- Sodium sulfate anhydrous for analysis EMSURE®, Cat. No. 106649
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106498
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 25-ml volumetric flask
- 100-ml volumetric flask
- 1000-ml volumetric flask
- Centrifuge glasses with solvent-proof twist-off caps, 100 - 110 ml content
- Centrifuge, 3000 rpm
- Mechanical shaker
- 25-ml mixing cylinder
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

1.4 Preparing the solutions

- **Hydrochloric acid 4 mol/l (4 N):**
  Place 521 ml resp. 583 g of hydrochloric acid 25 %
  in a volumetric flask, make up to 1000 ml with H_2O and mix
  (shelf-life 3 months)
- **Sodium hydroxide solution 6 mol/l (6N):**
  In a 100-ml volumetric flask:
  dissolve 24.0 g of sodium hydroxide pellets
  in approx. 80 ml H_2O,
  cool to room temperature,
  make up to 100 ml with H_2O and mix
- **Acidic methanol solution:**
  In a glass vessel:
  mix 64 ml of methanol and
  36 ml of hydrochloric acid 4 mol/l (4 N)
  (prepare freshly every day)
- **Alkaline methanol solution:**
  Pipette 0.2 ml sodium hydroxide solution 6.0 mol/l (6 N)
  in a volumetric flask, make up to 100 ml with methanol and mix
  (prepare freshly every day)

Analytical Procedures

1 α Acids (MEBAK method)

1.5 Preparation

- Clarify **wort** and **turbid beer** by centrifuging at 3000 rpm for **15 min** (**do not filter!**)
- Expel carbon dioxide from **sample** without losing any foam

1.6 Procedure and measurement

Reagent blank:

- Mix **5.0 ml of isooctane** and
  20.0 ml of alkaline methanol solution thoroughly

Measurement sample:

- Pipette **50.0 ml** of the sample (tempered to 20 °C) into a centrifuge glass
- Add **3.0 ml of hydrochloric acid 6 mol/l (6 N)** and **25.0 ml isooctane**
- Close centrifuge glass and **shake mechanically** at optimum mixing intensity for **30 min**
- Centrifuge at **3000 rpm** for **5 min** to separate the phases and braek the emulsion
- Draw off the **lower aqueous phase** with a pipette and **discard**
- Add sodium sulfate to the remaining **isooctane phase** until the phase **clarifies** after brief vigorous shaking
- Pipette **10.0 ml** of this phase into a 25-ml mixing cylinder
  add **10.0 ml of acidic methanol solution**
  shake for 3 min
- Transfer **5.0 ml** of the supernatant clear **isooctane phase** to a 25-ml volumetric flask
- Make up to the mark with **alkaline methanol solution** and mix thoroughly

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2612 "α Acids"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2612 it is recommended to measure a new reagent blank each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l α acids from the display.

1.7 Evaluation

Results are expressed
in mg/l

Standard values
Beer: less than 2 mg/l α acids, depending on grade, quality, type, and origin
Wort: 1 - 15 mg/l α acids, depending on degree of isomerization

1.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.18.2, page 116ff

Analytical Procedures

2 α Acids, spectrophotometric - non-isomerized hop extracts (ASBC method)

Hop extracts are concentrated liquids of hops, which can be present in isomerized and non-isomerized form. As well as hops, they contribute chemical compounds that impart bitterness and aroma to beer. α Acids are the substances that are mainly responsible for the bitterness in beer.

2.1 Method

After extraction of the hop extract with diisopropyl ether-aqueous HCl and dilution of the ether extract with methanol, a spectrophotometric measurement is carried out at multi-wavelengths.
This method applies only for non-isomerized hop extracts and requires a Prove 300 or 600 as measurements are made in the UV range.

2.2 Measuring range

0.0 - 100.0 % α acids

2.3 Reagents and accessories

- Diisopropyl ether for analysis EMSURE®, Cat. No. 100867
- Hydrochloric acid 1.0 mol/l Titripur®, Cat. No. 109057
- Sodium sulfate anhydrous for analysis EMSURE®, Cat. No. 106649
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106498
- Potassium iodide for analysis EMSURE®, Cat. No. 105043 (only necessary, if ether is tested for peroxides)
- Potassium disulfite for analysis EMSURE®, Cat. No. 105057 (only necessary, if ether is tested for peroxides)
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.0001 g
- Water bath (70 °C)
- Mechanical shaker
- 5-ml volumetric pipette
- 25-ml volumetric pipette
- 100-ml volumetric pipette
- Adjustable pipettes 0.2 - 5.0 ml
- 100-ml volumetric flask
- 250-ml extraction vessels with inert closures (e. g. conical flask with Teflon or PE closures)
- Stop watch
- Rectangular cells 10 mm, quartz, Spectroquant®, Cat. No. 100784

Analytical Procedures

2 α Acids, spectrophotometric - non-isomerized hop extracts (ASBC method)

2.4 Preparing the solutions

- **Sodium hydroxide solution 6 mol/l (6N):**
  In a 100-ml volumetric flask:
  dissolve 24.0 g of sodium hydroxide pellets
  in approx. 80 ml H_2O,
  cool to room temperature,
  make up to 100 ml with H_2O and mix
- **Alkaline methanol solution:**
  In a conical flask:
  mix 0.2 ml of sodium hydroxide solution 6.0 mol/l with
  100 ml of methanol
  (solution remains stable for 1 month in a tightly closed glass bottle in a solvent cabinet)
- **Diisopropyl ether:**
  The above mentioned isopropyl ether is stabilized and should be free of peroxides.
  Nevertheless peroxides might occur especially if the bottle is stored inappropriately or stored over a long time.
  To ensure work safety the isopropyl ether can be checked on peroxides as follows:
  Add 1 ml of potassium iodide solution 10 % (1 g potassium iodide + 9 g H_2O) to 10 ml diisopropyl ether.
  If the solution shows a definite yellow or brown color after a standing time of 1 - 10 min, peroxides should be removed from the sample. Therefor shake the solvent with 10 % potassium metabisulfite.

2.5 Preparation

Extraction of hop extract

- **Warm** hop extract for few minutes at 70 °C and homogenize it to a uniform mixture (especially important for hop extracts containing water solubles as they form separate phases)
- Weigh **2.000 g of warmed, homogenized hop extract** onto a glassine paper, fold it and place it in a 250-ml conical flask. If the hop extract contains a high amount of α acids (>30 % α acids), take only 1.000 g of the hop extract and calculate the result accordingly.
- Add **25 ml of hydrochloric acid 1.0 mol/l** and **100 ml of diisopropyl ether** by pipetting
- Stopper the flask tightly and **shake** mechanically at 20 °C and at an optimum mixing intensity for **30 min**
- Leave the suspension standing until complete separation of the phases
- **Take 30 - 35 ml of the upper ether layer** into a 50-ml conical flasks **containing 5 - 10 g sodium sulfate**
- Close the flask tightly and **shake** vigorously and quickly (attention, pressure is building up in the flask!)
- Let the solution stand until it is clear. This will take approx. 5 - 10 min

2.6 Procedure and measurement

Reagent blank:

- Dilute the diisopropyl ether-aqueous HCl solution with **methanol/alkaline methanol** in analogous manner as measurement sample

Measurement sample:

- Pipette **5 ml of the clear ether phase** into a 100-ml volumetric flask and make up to the mark with **methanol** (**dilution A**)
- **Mix dilution A** appropriately **with alkaline methanol** so that the absorption of the solution at 325 and 355 nm is within 0.1 - 0.8 A (**dilution B**). The aliquot volume of dilution A should be between 1 - 20 ml and the total volume of dilution B between 5 - 100 ml.
- Note the used volume of the aliquot of dilution A and the total volume of dilution B
- **Immediately measure** the sample to avoid decomposition

Analytical Procedures

2 α Acids, spectrophotometric - non-isomerized hop extracts (ASBC method)

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2637 "α Acids (Hop Extracts)"**.
- The aliquot of dilution A must be entered.
  An input masks pops up. Enter the aliquot of dilution A and tap **<OK>** to confirm
- The total volume of dilution B must be entered.
  An input masks pops up. Enter the total volume of dilution B and tap **<OK>** to confirm
- Press the **<START>** button to start the measurement procedure
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill reagent blank into a 10-mm rectangular quartz cell and insert cell into the cell compart ment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Reagent Blank"**.
  Confirm the message with **<OK>**.
- Fill measurement sample into a 10-mm rectangular quartz cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>**.
- Read off the result as % α acids from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

2.7 Evaluation

Results are expressed
% α acids

2.8 Literature

ASBC Methods of Analysis, online. Hops-8, Hop Extracts, B(I). Isopropyl ether spectrophotometric (I) and conductometric (II) methods [Release date 1970, revised 1977 and 2008].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Hops-8

Analytical Procedures

3 α and β Acids, spectrophotometric - hops / hop pellets (ASBC method)

Hops contribute chemical compounds that impart bitterness and aroma to beer. α acids are the substances that are mainly responsible for the bitterness in beer. The oxidation products of β acids also affect beer bitterness. The method applies to hops and hop pellets.

3.1 Method

After extraction of hops or hop pellets with an organic solvent, a spectrophotometric procedure can be used to evaluate both α and β acids.
This method requires a Prove 300 or 600 as measurements are made in the UV range.

3.2 Measuring range

0.0 – 100.0 % α acids
0.0 – 100.0 % β acids

3.3 Reagents and accessories

- Toluene for spectroscopy Uvasol®, Cat. No. 108331
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106498
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Grinder or blender jar
- Analytical balance, accurate to 0.0001 g
- Mechanical shaker or rotary shaker, 200 rpm
- Centrifuge, 2000 rpm
- Centrifuge glasses with solvent-proof twist-off caps, 100 - 110 ml content
- Polyethylene bag, suitable for covering the grinder
- 5-ml volumetric pipette
- 100-ml volumetric pipette
- Adjustable pipettes 0.2 - 5.0 ml
- 100-ml volumetric flask
- 250-ml extraction vessels with inert closures (e. g. conical flask with Teflon or PE closures)
- Stop watch
- Rectangular cells 10 mm, quartz, Spectroquant®, Cat. No. 100784

3.4 Preparing the solutions

- **Sodium hydroxide solution 6 mol/l (6N):**
  In a 100-ml volumetric flask:
  dissolve 24.0 g of sodium hydroxide pellets
  in approx. 80 ml H_2O,
  cool to room temperature,
  make up to 100 ml with H_2O and mix
- **Alkaline methanol solution:**
  In a conical flask:
  mix 0.2 ml of sodium hydroxide solution 6.0 mol/l with
  100 ml of methanol
  (solution remains stable for 1 month in a tightly closed glass bottle in a solvent cabinet)

Analytical Procedures

3 α and β Acids, spectrophotometric - hops / hop pellets (ASBC method)

3.5 Preparation

Sample preparation for pressed and unpressed hop samples

- Bring cooled samples (in a closed container) to room temperature.
- Grind sample (immediately before use). Discard the first 10 g of the ground sample. Place an appropriate polyethylene bag over the discharge of the chopper and grind the remaining sample using only small portions to avoid losses of moisture.
- Homogenize sample by inverting and rotating the bag.
- Immediately use the ground sample for analysis otherwise freeze sample.

Sample preparation for hop pellets

- Grind 75 - 125 g of the sample at high speed for 20 – 30 s using a blender jar. A heating of the sample should be avoided.
  The grind size is optimal if 95 % of the powder pass a screen with 20 opening per inch.
- Immediately use the ground sample for analysis otherwise freeze sample.

Sample Extraction

- Weigh **5.000 g of freshly ground hop sample** into a 250-ml extraction vessel.
- Add **100 ml of toluene** by pipetting.
- Stopper the extraction vessel tightly and **shake mechanically for 30 min** at room temperature and at an optimum mixing intensity or on a rotary shaker at 200 rpm.
- **Centrifuge** at 2000 rpm for 5 min or alternatively let the solution stand until the separation of the solid and liquid phases (not longer than 1 h).

3.6 Procedure and measurement

Reagent blank:

- **Toluene diluted with methanol/alkaline methanol** in analogous manner as measurement sample

Measurement sample:

- Pipette **5 ml of the clear supernatant** into a 100-ml volumetric flask and make up to the mark with **methanol** (**dilution A**)
- **Mix dilution A** appropriately **with alkaline methanol** so that the absorption of the solution at 325 and 355 nm is within 0.1 - 0.8 A (**dilution B**). The aliquot volume of dilution A should be between 1 - 20 ml and the total volume of dilution B between 5 - 100 ml.
- Note the used volume of the aliquot of dilution A and the total volume of dilution B
- **Immediately measure** the sample to avoid decomposition

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2636 "α/β Acids (Hops)"**.
- The aliquot of dilution A must be entered.
  An input masks pops up. Enter the aliquot of dilution A and tap **<OK>** to confirm
- The total volume of dilution B must be entered.
  An input masks pops up. Enter the total volume of dilution B and tap **<OK>** to confirm
- Press the **<START>** button to start the measurement procedure
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill reagent blank into a 10-mm rectangular quartz cell and insert cell into the cell compart ment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Reagent Blank"**. Confirm the message with **<OK>**.
- Fill measurement sample into a 10-mm rectangular quartz cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**. Confirm the message with **<OK>**.
- Read off the result as % α acids and % β acids from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

Analytical Procedures

3 α and β Acids, spectrophotometric - hops / hop pellets (ASBC method)

3.7 Evaluation

Results are expressed
% α acids
% β acids

3.8 Literature

ASBC Methods of Analysis, online. Hops-6, α- and β-Acids in Hops and Hop Pellets by spectrophotometry and conductometric titration, A. α- and β-Acids by Spectrophotometry [Release date 1959, revised 1976 and 2008].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Hops-6

Analytical Procedures

4 Anthocyanogenes, Harris and Ricketts method (MEBAK method)

Anthocyanogenes (leukoanthocyanidins) are phenolic compounds that are transformed into red-colored anthocyanidins by hot hydrochloric acid. The amount and the degree of condensation/polymerization of these compounds have an effect on the formation of colloidal turbidities in the beer. Stabilization measures using PVPP correlate with a reduction of the anthocyanogene content.

4.1 Method

The anthocyanogenes are adsorbed on polyamide, and the adsorbate is dissolved in butanol and hydrochloric acid and heated. This produces a red solution, the intensity of which is measured by spectrophotometry.

4.2 Measuring range

0 - 100 mg/l anthocyanogenes

4.3 Reagents and accessories

- Polyamide SC 6 (particle size 0.05 - 0.16 mm)
- 1-Butanol for analysis EMSURE®, Cat. No. 101990
- Hydrochloric acid fuming 37 % for analysis EMSURE®, Cat. No. 100317
- Iron(II) sulfate heptahydrate for analysis EMSURE®, Cat. No. 103965
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Centrifuge glasses, 100 - 110 ml content
- Centrifuge, 3000 rpm
- 50-ml mixing cylinder with ground-glass stopper
- Mechanical shaker
- Glass frit G4
- Suction flask
- Vacuum pump
- Spatula
- 30-ml test tubes with ground-glass stopper, graduation to 25 ml
- Water bath (100 °C)
- Glass rod
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

4.4 Preparing the solutions

- **Solution 1:**
  In a glass vessel:
  place 500 ml of 1-butanol with
  100 ml of hydrochloric acid 37 % and mix
  (shelf-life 4 weeks)
- **Solution 2:**
  In a glass vessel:
  dissolve 0.120 g of iron(II) sulfate in
  100 ml of solution 1
  (prepare freshly every day)

Analytical Procedures

4 Anthocyanogenes, Harris and Ricketts method (MEBAK method)

4.5 Preparation

- Centrifuge **wort and young beers** at 3000 rpm for 10 min
- Expel carbon dioxide from **sample**

4.6 Procedure and measurement

Reagent blank:

- Pipette **10 ml of H_2O** into a 50-ml mixing cylinder
- Rinse **0.5 g of polyamide powder** into the mixing cylinder with **10 ml of H_2O**
- **Shake mechanically** at optimum mixing intensity for **40 min**
- Filter suspension over a G4 frit and rinse **twice** with approx. **20 ml of H_2O**
- Suction-dry the glass frit with the polyamide powder, transfer the residue to a test tube quantitatively with the spatula, and rinse with **15 ml of solution 1**
- Add **0.5 ml of solution 2** and heat the test tube in the **boiling water bath** for **30 min** (stirring thoroughly with a glass rod during the first 5 min)
- Remove glass rod, rinse with a little of **solution 1**
- Bring the test tube to a temperature of 20 °C, and make up to 25 ml with **solution 1**

Measurement sample:

- Pipette **5.0 ml of decarbonized beer** or **wort** and **5.0 ml of H_2O** into a 50-ml mixing cylinder and mix
- Rinse **0.5 g of polyamide powder** into the mixing cylinder with **10 ml of H_2O**
- **Shake mechanically** at optimum mixing intensity for **40 min**
- Filter suspension over a G4 frit and rinse **twice** with approx. **20 ml of H_2O**
- Suction-dry the glass frit with the polyamide powder, transfer the residue to a test tube quantitatively with the spatula, and rinse with **15 ml of solution 1**
- Add **0.5 ml of solution 2** and heat the test tube in the **boiling water bath** for **30 min** (stirring thoroughly with a glass rod during the first 5 min)
- Remove glass rod, rinse with a little of **solution 1**
- Bring the test tube to a temperature of 20 °C, and make up to 25 ml with **solution 1**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2601 "Anthocyanogenes"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2601 it is recommended to measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l anthocyanogenes from the display.

Analytical Procedures

4 Anthocyanogenes, Harris and Ricketts method (MEBAK method)

4.7 Evaluation

Results are expressed
in mg/l

Standard values
50 - 70 mg/l anthocyanogens, depending on the raw materials and technical measures; correspondingly lower after stabilization with PVPP

4.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.17.2, page 109ff

Analytical Procedures

5 Bitterness - beer (ASBC method)

The most important bitter substances in wort and beer are the iso-α acids. Other α acids and δ acids may also be present, in particular in wort. In addition, wort and beer contain other derivatives of the hop bitter acids, especially oxidation products, that also contribute to the bitter taste.

5.1 Method

The bitter substances in beer and wort – in particular iso-α acids – are extracted from the acidified sample with isooctane and the concentration in the extract is measured by spectrophotometry. It can be chosen between the international method "Manual Isooctane Extraction" and the method "Reduced solvent Technique".
This method requires a Prove 300 or 600 as the measurements are made in the UV-range.

5.2 Measuring range

1.0 - 80.0 bitterness (BU)

5.3 Reagents and accessories

- Hydrochloric acid 6 mol/l EMPROVE®, Cat. No. 110164
- Isooctane Uvasol®, Cat. No. 104718
- 1-Octanol EMPLURA®, Cat. No. 100991
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Centrifuge glasses with solvent-proof twist-off caps, 35 ml content
- Centrifuge, 3000 rpm
- 1-ml volumetric pipette
- 5-ml volumetric pipette
- 10-ml volumetric pipette
- 20-ml volumetric pipette
- Pasteur pipettes, glass
- Mechanical shaker
- Stop watch
- Rectangular cells quartz, 10 mm, Spectroquant®, Cat. No. 100784

5.4 Preparing the solution

- **Hydrochloric acid 3 mol/l:**
  In a glass vessel:
  mix 5 ml of hydrochloric 6 mol/l with
  5 ml of H_2O

5.5 Preparation

- Use carbonated beer cooled to 10 °C

Analytical Procedures

5 Bitterness - beer (ASBC method)

5.6 Procedure and measurement

Acc. to method "Manual Isooctane Extraction"

Reagent blank:

- **20 ml isooctane plus one drop of 1-octanol**

Measurement sample:

- Pipette **10.0 ml** of the chilled, carbonated sample into a 50-ml centrifuge glass. Therefor use a volumetric pipette, whose tip is moistened with a small amount of 1-octanol
- Add **1.0 ml of hydrochloric acid 3 mol/l** and **20.0 ml of isooctane**
- Close centrifuge glass tightly and **shake vigorously** with a mechanical shaker **for 15 min**
- If necessary, centrifuge at **3000 rpm** for **3 min** to separate the phases and break the emulsion
- After separation of the phases, immediately transfer the clear supernatant into a 10-mm rectangular quartz cell and measure in the photometer

Acc. to method "Reduced solvent Technique"

Reagent blank

- Pipette 0.5 ml of hydrochloric acid 3 mol/l and 10.0 ml of isooctane into a 50-ml centrifuge glass
- Close centrifuge glass tightly and shake 2 - 3 times with hands and afterwards with a mechanical shaker for 15 min (at 80 % capacitity)
- Centrifuge the reagent blank in the same manner as the measurement sample

Measurement sample:

- Pipette 5.0 ml of the chilled, carbonated sample into a 50-ml centrifuge glass using a volumetric pipette
- Add 0.5 ml of hydrochloric acid 3 mol/l and 10.0 ml of isooctane
- Close centrifuge glass tightly and shake 2 - 3 times with hands and afterwards with a mechanical shaker for 15 min (at 80 % capacitity)
- Centrifuge at 400 g for 5 min. Tap the centrifuge glass and swirl to help separating the phases. Then centrifuge again for 5 min at 400 g. Repeat the tapping and swirling. If the volume is approx. 5 ml, transfer the upper phase into a cell. If not, centrifuge for another 5 min.

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2603 "Bitterness - beer"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2603 it is recommended to measure a new reagent blank each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular quartz cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in BU (= bitter units) from the display.

5.7 Evaluation

Results are expressed
in bitterness (BU)

Standard values
Beer: 10 - 40 BU, depending on grade, quality, type, and origin (source: MEBAK)

5.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.18.1, page 114ff

ASBC Methods of Analysis, online. Beer-23, Beer Bitterness, A. Bitterness Units-Manual Isooctane Extraction [Release date 1968, revised 1975].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-23

Analytical Procedures

6 Bitterness - beer (EBC / MEBAK method)

The most important bitter substances in wort and beer are the iso-α acids. Other α acids and δ acids may also be present, in particular in wort. In addition, wort and beer contain other derivatives of the hop bitter acids, especially oxidation products, that also contribute to the bitter taste.

6.1 Method

The bitter substances in beer and wort – in particular iso-α acids – are extracted from the acidified sample with isooctane and the concentration in the extract is measured by spectrophotometry.
This method requires a Prove 300 or 600 as measurements are made in the UV range.

6.2 Measuring range

1.0 - 80.0 bitterness (BU)

6.3 Reagents and accessories

- Hydrochloric acid 6 mol/l EMPROVE®, Cat. No. 110164
- Isooctane Uvasol®, Cat. No. 104718
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Centrifuge glasses with solvent-proof twist-off caps, 35 ml content
- Centrifuge, 3000 rpm
- Glass beads
- Mechanical shaker
- Rectangular cells quartz, 10 mm, Spectroquant®, Cat. No. 100784

6.4 Preparation

- Clarify **wort** by centrifuging at 3000 rpm for **15 min** (**do not filter!**)
- Expel carbon dioxide from **sample** without losing any foam

6.5 Procedure and measurement

Reagent blank:

- **Isooctane used**

Measurement sample:

- Pipette **10.0 ml** of the sample (tempered to 20 °C) into a centrifuge glass
- Add **0.5 ml of hydrochloric acid 6 mol/l**, **20.0 ml of isooctane**, and **3 glass beads**
- Close centrifuge glass and **shake mechanically** at 20°C and at optimum mixing intensity for **15 min**
- Centrifuge at **3000 rpm** for **3 min** to separate the phases and break the emulsion

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2603 "Bitterness - beer"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2603 it is recommended to measure a new reagent blank each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in BU (= bitter units) from the display.

Analytical Procedures

6 Bitterness - beer (EBC / MEBAK method)

6.6 Evaluation

Results are expressed
in bitterness (BU)

Standard values
Beer: 10 - 40 BU, depending on grade, quality, type, and origin

6.7 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.18.1, page 114ff

Analytica-EBC, Section 9 Beer, Method 9.8

Analytical Procedures

7 Bitterness - wort (EBC / MEBAK / ASBC method)

The most important bitter substances in wort and beer are the iso-α acids. Other α acids and δ acids may also be present, in particular in wort. In addition, wort and beer contain other derivatives of the hop bitter acids, especially oxidation products, that also contribute to the bitter taste.

7.1 Method

The bitter substances in beer and wort – in particular iso-α acids – are extracted from the acidified sample with isooctane and the concentration in the extract is measured by spectrophotometry.
This method requires a Prove 300 or 600 as measurements are made in the UV range.

7.2 Measuring range

1.0 - 120.0 bitterness (BU)

7.3 Reagents and accessories

- Hydrochloric acid 6 mol/l EMPROVE®, Cat. No. 110164
- Isooctane Uvasol®, Cat. No. 104718
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Centrifuge glasses with solvent-proof twist-off caps, 35 ml content
- Centrifuge, 3000 rpm
- Glass beads
- Mechanical shaker
- Rectangular cells quartz, 10 mm, Spectroquant®, Cat. No. 100784

7.4 Preparation

- Clarify **wort** by centrifuging at 3000 rpm for **15 min** (**do not filter!**)
- Expel carbon dioxide from **sample** without losing any foam

7.5 Procedure and measurement

Reagent blank:

- **Isooctane used**

Measurement sample:

- Pipette **5.0 ml** of the sample (tempered to 20 °C) and **5.0 ml H_2O** (20 °C) into a centrifuge glass
- Add **0.5 ml of hydrochloric acid 6 mol/l**, **20.0 ml of isooctane**, and **3 glass beads**
- Close centrifuge glass and **shake mechanically** at 20°C and at optimum mixing intensity for **15 min**
- Centrifuge at **3000 rpm** for **3 min** to separate the phases and break the emulsion

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2604 "Bitterness - wort"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2604 it is recommended to measure a new reagent blank each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in BU (= bitter units) from the display.

Analytical Procedures

7 Bitterness - wort (EBC / MEBAK / ASBC method)

7.6 Evaluation

Results are expressed
in bitterness (BU)

Standard values
Wort: 20 - 60 BU, depending on beer and bitter-substance utilization

7.7 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.18.1, page 114ff

Analytica-EBC, Section 8 Wort, Method 8.8

ASBC Methods of Analysis, online. Wort-23, Wort Bitterness, A. Bitterness Units by Spectrophotometry [Release date 2011].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Wort-23

Analytical Procedures

8 Color, spectrophotometric - beer (ASBC method)

This method is designed to eliminate subjective effects attributable to the human eye as well as differences in the color impression when comparing beer samples with the color comparator disk. This method is applied to beer.

8.1 Method

The absorbance is measured by spectrophotometry. The color, expressed in °SRM, is calculated by conversion with a predefined factor.

8.2 Measuring range

0.0 - 50.0 °SRM
0.0 - 100.0 EBC units

8.3 Accessories

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks)
- Membrane filters 0.45 µm or centrifuge
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

8.4 Preparation

- Expel carbon dioxide from **sample**. The beer must be free of gas bubbles as they strongly interfere the measurement
- In case of **turbidity, clarify sample** by filtration or centrifugation
- In the event of °SRM units >50.0, dilute sample with H_2O so that its color is within the measurement range and note the dilution factor

8.5 Procedure and measurement

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2633 "Color - ASBC"**.
- The dilution factor must be entered.
  An input masks pops up. Enter the dilution factor (1+x) and tap **<OK>** to confirm.
- Press the **<START>** button to start the measurement procedure.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Fill measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>**.
- Read off the result as °SRM and EBC units from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample

Analytical Procedures

8 Color, spectrophotometric - beer (ASBC method)

8.6 Evaluation

Results are expressed
in °SRM,
EBC units

The absorption values at 430 and 700 nm are also given as result.
If the absorption ratio of $Abs_{700\ nm}$: $Abs_{430\ nm}$ is higher than 0.039, the sample is turbid and needs to be clarified.

8.7 Literature

ASBC Methods of Analysis, online. Beer-10, Color, A. Spectrophotometric color method [Release date 1958, revised 1975, reviewed 2015].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-10

Analytical Procedures

9 Color, spectrophotometric celite - wort (ASBC method)

This method is designed to eliminate subjective effects attributable to the human eye as well as differences in the color impression when comparing wort samples with the color comparator disk. This method is applied to industrial wort and laboratory wort samples.

9.1 Method

The absorbance of clarified and filtered wort samples is measured by spectrophotometry. The color, expressed in °SRM is calculated by conversion with a predefined factor.

9.2 Measuring range

0.0 - 50.0 °SRM
0.0 - 100.0 EBC units

9.3 Accessories

- Kieselguhr purified and calcined GR for analysis, Cat. No. 107910
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks)
- Precision balance, accurate to 0.1 g
- Funnel with filter paper
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

9.4 Preparation

- **Immediately filter wort after sampling** through filter paper at 5 – 8 °C. Otherwise preserve wort before filtration (by storing in the refrigerator or pasteurizing in beer bottles)
- **Add 5 g Kieselguhr** to 100 ml of filtered wort, swirl and let stand for 5 min
- **Filter suspension** through filter paper. Refilter the first 30 – 40 ml of the filtrate and collect the clear filtrate in a clean flask.
- In the event of °SRM units >50.0, dilute sample with H_2O so that its color is within the measurement range and note the dilution factor

9.5 Procedure and measurement

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2633 "Color - ASBC"**.
- The dilution factor must be entered.
  An input masks pops up. Enter the dilution factor (1+x) and tap **<OK>** to confirm.
- Press the **<START>** button to start the measurement procedure.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Fill measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>**.
- Read off the result as °SRM and EBC units from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample

Analytical Procedures

9 Color, spectrophotometric celite - wort (ASBC method)

9.6 Evaluation

Results are expressed
in °SRM,
EBC units

The absorption values at 430 and 700 nm are also given as result.
If the absorption ratio of $Abs_{700\ nm}$: $Abs_{430\ nm}$ is higher than 0.039, the sample is turbid and needs to be clarified.

9.7 Literature

ASBC Methods of Analysis, online. Wort-9, Wort Color and Sample Preparation, A. Celite [Release date 1969, revised 1976, reviewed 2010].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Wort-9

ASBC Methods of Analysis, online. Beer-10, Color, A. Spectrophotometric color method [Release date 1958, revised 1975, reviewed 2015].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-10

Analytical Procedures

10 Color, spectrophotometric (EBC / MEBAK method)

This method is designed to eliminate subjective effects attributable to the human eye as well as differences in the color impression when comparing beer samples with the color comparator disk. This technical method counts as the official method of reference and can be applied to industrial worts, beers, laboratory worts (congress worts), and liquid malt substitutes of all kinds.

10.1 Method

The absorbance is measured by spectrophotometry in a 10-mm rectangular OS cell. The color, expressed in EBC units, is calculated by conversion with a predefined factor.

10.2 Measuring range

0.0 - 60.0 EBC units

10.3 Accessories

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks)
- Membrane filters 0.45 µm
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

10.4 Preparation

- Expel carbon dioxide from **sample**
- **Filter** the sample over the membrane filter; filtration can be dispensed with in the event that the turbidity of the diluted sample is **lower than 1 EBC turbidity units**
- Optionally clarify the sample by adding 0.1% kieselguhr (Kieselguhr GR for analysis, Cat. No. 107910) and filtration prior to the membrane filtration step
- In the event of EBC units > 60.0, **dilute** the sample so that its color is within the measurement range; **use** the corresponding **dilution factor** when subsequently calculating the result (**measurement result x dilution factor)**

10.5 Procedure and measurement

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2602 "Color - EBC"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill the measurement sample into a 10-mm cell and insert cell into the cell compartment. The measurement starts automatically
- Read off the result in EBC units from the display.

10.6 Evaluation

Results are expressed
in EBC units

Note
A spectrophotometric absorbance curve does not reflect the color impression perceived by the human eye, since light of identical intensity makes a different impression on the eye in different parts of the spectrum. Furthermore, the absorbance curves at 430 nm are very steep, meaning that measurement errors can easily occur. Differences also occur when comparing light beers with diluted dark beers.

Analytical Procedures

10 Color, spectrophotometric (EBC / MEBAK method)

10.7 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.13.2, page 88ff

Analytica-EBC, Section 8 Wort, Method 8.5

Analytica-EBC, Section 9 Beer, Method 9.6

Analytical Procedures

11 Copper, Cuprethol method (EBC / MEBAK method)

Copper can be imported into the beer by spray residues from the raw materials; it may also find its way into beer from apparatuses and beer pipes. Heavy-metal ions have a detrimental effect on the colloidal stability and the flavour of the beer.

11.1 Method

Copper reacts with diethanolamine and carbon disulfide to form a color complex that is measured by spectrophotometry.

Important:
This method of determination can be used only for clear and light beers.

11.2 Measuring range

0.10 - 5.00 mg/l copper

11.3 Reagents and accessories

- Diethanolamine for analysis EMSURE®, Cat. No. 116205
- Methanol for analysis EMSURE®, Cat. No. 106009
- Carbon disulfide for analysis, Cat. No. 102214
- Sodium acetate trihydrate for analysis EMSURE®, Cat. No. 106267
- Acetic acid (glacial) 100 % anhydrous for analysis EMSURE®, Cat. No. 100063
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 1000-ml volumetric flask
- 50-ml conical flask
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

11.4 Preparing the solutions

- **Solution 1:**
  In a glass vessel:
  dissolve 4 g of diethanolamine in
  200 ml of methanol
  (solution remains stable for 4 weeks when stored in tightly closed glass bottles in a solvent cabinet)
- **Solution 2:**
  In a glass vessel:
  dissolve 0.5 g of carbon disulfide in
  100 ml of methanol
  (⚠ solution remains stable for 4 weeks when stored in the dark in tightly closed glass bottles in a solvent cabinet (explosion-proof))
- **Solution 3:**
  In a glass vessel:
  mix 100 ml of methanol with
  100 ml of solution 1
  (solution remains stable for 4 weeks when stored in tightly closed glass bottles in a solvent cabinet)
- **Solution 4 (Cuprethol reagent):**
  In a glass vessel:
  mix 30 ml of solution 1 with
  10 ml of solution 2
  (prepare freshly every day)

Analytical Procedures

11 Copper, Cuprethol method (EBC / MEBAK method)

- **Buffer solution pH 4.6:**
  Dissolve 105 g of sodium acetate trihydrate in
  approx. 800 ml of H_2O,
  add 65 ml of acetic acid 100 %
  Check pH and
  make up to 1000 ml with H_2O in a volumetric flask
  (solution remains stable for 4 weeks when stored at +4°C)

11.5 Preparation

- Expel carbon dioxide from **beer**, allow froth to disintegrate
- **Buffered beer solution:**
  - Pipette **50 ml of decarbonized beer** into a 100-ml conical flask
  - Add **25 ml of buffer solution** and mix

11.6 Procedure and measurement

Sample blank:

- Pipette **20 ml of buffered beer solution** into a 50-ml conical flask
- Add **2 ml of solution 3** and mix
- Measure **within 10 min**

Measurement sample:

- Pipette **20 ml of buffered beer solution** into a 50-ml conical flask
- Add **2 ml solution 4 (Cuprethol reagent)** and mix
- Measure **within 10 min**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2613 "Copper (EBC)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill the sample blank into a 10-mm cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample Blank"**. Confirm the message with **<OK>.**
- Fill the measurement sample into a 10-mm cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**. Confirm the message with **<OK>.**
- Read off the result in mg/l copper from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

11.7 Evaluation

Results are expressed
in mg/l Cu

Specified values
Beer: <0.20 mg/l Cu

11.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.29.4, page 152ff

Analytica-EBC, Section 9 Beer, Method 9.14.2

Analytical Procedures

12 Diacetyl, Broad spectrum method - beer (ASBC method)

The metabolic processes of yeast produce 2-acetolactate and 2-acetohydroxybutyrate in the course of fermentation. 2-Acetolactate and 2-acetohydroxybutyrate are converted by oxidization to form the vicinal diketones diacetyl (2,3-butanedione) and 2,3-pentanedione. Diacetyl can also occur as metabolic product of certain microorganisms. When the threshold value is exceeded, the beer acquires an off flavor.

12.1 Method

This method is called the Broad Spectrum Method for VDK (Vicinal Diketones).
The basis of this method is the reaction of diacetyl with α-naphthol and creatine in an alkaline medium to a form a colored reaction product, which is measured by spectrophotometry.

12.2 Measuring range

0.0 - 4.0 mg/l diacetyl

12.3 Reagents and accessories

- 1-Naphthol GR for analysis, Cat. No. 106223
- 2-Propanol for analysis EMSURE®, Cat. No. 109634
- Charcoal activated for analysis, Cat. No. 102186
- Potassium hydroxide pellets for analysis EMSURE®, Cat No. 105033
- Creatine monohydrate, Cat. No. 841470
- Diacetyl analytical standard, Cat. No. 11038 (Sigma)

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks) and pipettes
- Analytical balance, accurate 0.0001 g
- Precision balance, accurate to 0.01 g
- Stirrer
- 100-ml brown glass bottle
- 100-ml polyethylene bottle
- Distillation apparatus
    - Heating mantle for boiling flasks
    - 500-ml boiling flasks, two-neck
    - Condenser
    - Tube adapter
    - 25-ml graduated measuring cylinder
- 10-ml volumetric flask
- 100-ml volumetric flask
- 1000-ml volumetric flask
- Adjustable pipettes 0.5 - 5.0 ml
- Funnel with filter
- Stop watch
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

Analytical Procedures

12 Diacetyl, Broad spectrum method - beer (ASBC method)

12.4 Preparing the solutions

- **1-Naphthol solution:**
  Dissolve 4 g of 1-naphthol
  in approx. 80 ml of 2-propanol
  in a 100-ml volumetric flask, make up to 100 ml with 2-propanol and mix.
  Transfer the solution into a conical flask,
  add ca. 0.5 g of charcoal,
  shake the mixture for 30 min and
  filter using a folded filter.

  Store the solution in a brown bottle in the dark

- **Potash solution 40 %:**
  In a glass vessel:
  dissolve 40 g of potassium hydroxide pellets in
  60 g of H_2O (⚠ exothermic reaction)

- **KOH-creatine-solution:**
  In a glass vessel:
  dissolve 0.34 g of creatine monohydrate in
  80 ml of potash solution 40 % and mix thoroughly by stirring.
  Filter using a folded filter (suitable for the filtration of alkaline solutions)

  Store in a polyethylene bottle in the refrigerator.

- **Diacetyl stock solution, 500 mg/l diacetyl:**
  Place 0.500 g of diacetyl*
  in a 1000-ml volumetric flask, make up to 1000 ml with H_2O and mix
  Immediately proceed preparing the diacetyl standard solution 5 mg/l

  * To increase accuracy adapt the amount of diacetyl on basis of the assay given in the certificate of analysis.
    Calculation: "sample weight [g] = (0.500 g · 100) / assay [%]

- **Diacetyl standard solution, 5 mg/l diacetyl:**
  Pipette 1.0 ml of diacetyl stock solution 500 mg/l
  in a 100-ml volumetric flask, make up to 100 ml with H_2O and mix
  (solution is not stable, prepare immediately before use)

12.5 Preparation

Sample:
- Expel carbon dioxide from beer

Distillation:
- Installation of the distillation apparatus:
  - Place the boiling flask in a heating mantle
  - Connect the distilling tube to the boiling flask and the condensator. The condensator should be placed so that it slopes downwards.
  - The condensator is then connected to a curved tapered tube adapter.
  - Place a 25-ml graduated cylinder filled with 5 ml H_2O below the tube adapter.
- Place **100 ml of beer** in the boiling flask.
- **Collect 15 ml distillate** in the 25-ml graduated cylinder filled with 5 ml H_2O.
- Make up to 25 ml with H_2O and mix.

12.6 Calculation of the calibration curve

A user-defined calibration is necessary.

A re-calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

Analytical Procedures

12 Diacetyl, Broad spectrum method - beer (ASBC method)

12.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solutions					
	E0 [0.00 mg/l diacetyl]	1 [0.50 mg/l diacetyl]	2 [1.00 mg/l diacetyl]	3 [1.50 mg/l diacetyl]	4 [3.00 mg/l diacetyl]	5 [4.00 mg/l diacetyl]
Diacetyl standard solution **5 mg/l diacetyl**	0.00 ml	0.50 ml	1.00 ml	1.50 ml	3.00 ml	4.00 ml
	• Pipette into separate 10-ml volumetric flasks					
H_2O	5.00 ml	4.50 ml	4.00 ml	3.50 ml	2.00 ml	1.00 ml
	• Add and mix					

- Prepare calibration solutions in the following manner:

	Calibration solutions					
	E0 [0.00 mg/l diacetyl]	1 [0.50 mg/l diacetyl]	2 [1.00 mg/l diacetyl]	3 [1.50 mg/l diacetyl]	4 [3.00 mg/l diacetyl]	5 [4.00 mg/l diacetyl]
1-Naphthol solution	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
	• Pipette into each 10-ml volumetric flask containing the standard solutions and mix					
KOH-creatine-solution	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
	• Pipette into each 10-ml volumetric flask and make up to 10 ml with H_2O • Shake vigorously for 1 min • Let the solution stand and measure between 5 - 6 min after shaking					

Reagent blank:

- Pipette **5 ml of H_2O** into a volumetric flask
- Add **1.0 ml of 1-naphthol solution** and mix by swirling
- Add **0.5 of ml KOH-creatine-solution** and make up to 10 ml with H_2O
- **Shake** vigorously for 1 min
- Let the solution stand and **measure between 5 - 6 min** after shaking

Measurement sample:

- Pipette **5 ml of the homogenized distillate-water-mixture** in a 10-ml volumetric flask
- Add **1.0 ml of 1-naphthol solution** and mix
- Add **0.5 ml of KOH-creatine-solution** and make up to 10 ml with H_2O
- **Shake** vigorously for 1 min
- Let the solution stand and **measure between 5 - 6 min** after shaking

Analytical Procedures

12 Diacetyl, Broad spectrum method - beer (ASBC method)

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2631 "Diacetyl (ASBC)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- A user-defined calibration is necessary. It is recommended to perform a re-calibration when exchanging batches of the reagents used.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.50 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **1.00 mg/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"3"** line and enter the concentration of **1.50 mg/l** for the third calibration solution.
  Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"4"** line and enter the concentration of **3.00 mg/l** for the fourth calibration solution.
  Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"5"** line and enter the concentration of **4.00 mg/l** for the fifth calibration solution.
  Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.

  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

  Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.
- For method No. 2631 it is recommended to measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l diacetyl from the display.

Analytical Procedures

12 Diacetyl, Broad spectrum method - beer (ASBC method)

12.8 Evaluation

Results are expressed
in mg/l diacetyl

12.9 Literature

ASBC Methods of Analysis, online. Beer-25, Diacetyl, B. Broad Spectrum Method for VDK [Release date 1964].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-25

Analytical Procedures

13 Flavanoids (EBC method)

Flavanoids contribute to the bitter flavour of beer. They are reputed to have antioxidative properties. They occur universally in plants.

13.1 Method

In acidified medium 4-(dimethylamino)-cinnamaldehyde reacts with flavanoids to produce a dye, which is then measured by spectrophotometry.

13.2 Measuring range

3 - 200 mg/l catechin equivalent

13.3 Reagents and accessories

- Hydrochloric acid fuming 37 % for analysis EMSURE®, Cat. No. 100317
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- 4-(Dimethylamino)-cinnamaldehyde for synthesis, Cat. No. 822034
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 100-ml volumetric flask
- 500-ml volumetric flask
- 1000-ml conical flask
- Mechanical shaker
- 250-ml measuring cylinder
- Test tubes
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

13.4 Preparing the solutions

- **Acidic methanol solution:**
  In a glass vessel:
  mix 350 ml of methanol and
  125 ml of hydrochloric acid 37 %
  (solution remains stable for 1 week when stored in dark bottles)
- **Color reagent:**
  In a volumetric flask:
  dissolve 500 mg of 4-(dimethylamino)-cinnamaldehyde in
  the entire amount of acidic methanol solution and
  make up to the 500-ml mark with methanol
  (solution remains stable for 1 week when stored in dark bottles)

13.5 Preparation

- Bring **beer** to a temperature of 20 °C
- Expel carbon dioxide from **beer** by shaking in a conical flask (gradually increase intensity) - **do not filter!**
- Dilute **beer** tenfold with H_2O (pipette 10.0 ml of decarbonized beer into a 100-ml volumetric flask, make up to the mark with H_2O, and mix: dilution 1 + 9)

Analytical Procedures

13 Flavanoids (EBC method)

13.6 Procedure and measurement

Reagent blank:

- Pipette **1.0 ml** of **H_2O** into a test tube
- Add **5.0 ml of color reagent** and mix thoroughly
- Leave to stand for **10 min**

Measurement sample:

- Pipette **1.0 ml** of the diluted **beer** into a test tube
- Add **5.0 ml of color reagent** and mix thoroughly
- Leave to stand for **10 min**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2626 "Flavanoids"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2626 it is recommended to measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l catechin equivalent from the display.

13.7 Evaluation

Results are expressed
in mg/l catechin equivalent

13.8 Literature

Analytica-EBC, Section 9 Beer, Method 9.12

Analytical Procedures

14 Flocculation, absorbance method – yeast (ASBC method)

The flocculation can give statement about consistent quality of a yeast strain if carried out routinely. If the flocculation behavior changes, changes of the yeast strain can be expected.

14.1 Method

After the breeding of the yeast a settling reaction in a calcium sulfate buffered solution is carried out. The absorbance of this suspension is measured at 600 nm.

14.2 Measuring range

-10.0 - 100.0 % flocculation

14.3 Reagents and accessories

- Calcium sulfate dihydrate for analysis EMSURE®, Cat. No. 102161
- Sodium acetate anhydrous for analysis EMSURE®, Cat. No. 106268
- Acetic acid (glacial) 100 % for analysis EMSURE®, Cat No. 100063
- Sodium hydroxide solution 1 mol/l Titripur®, Cat. No. 109137
- Ethylenediaminetetraacetic acid disodium salt dihydrate, Cat. No. 108454
- Agar extra pure EMPROVE®, Cat No. 101615
- Hopped wort
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Autoclave
- Incubator, 25 °C
- Shaker incubator, 25 °C
- Hemacytometer
- Analytical balance, accurate to 0.001 g
- pH meter
- Heating plate
- Centrifuge
- Vortex
- Stop watch
- 100-ml volumetric flask
- Petri dishes, 100 x 15 mm
- Vessels for autoclaving
- 250-ml culture flask
- 500-ml graduated measuring cylinder
- 15-ml centrifuge tubes, disposable,
- Test tubes
- Adjustable pipettes 0.1 - 10.0 ml
- Pasteur pipettes
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

Analytical Procedures

14 Flocculation, absorbance method – yeast (ASBC method)

14.4 Preparing the solutions

- **Washing solution:**
  In a glass vessel:
  dissolve 0.322 g of calcium sulfate dihydrate in
  500 ml of H_2O
- **Suspension solution pH 4.5:**
  In a glass vessel:
  dissolve 0.322 g of calcium sulfate dihydrate and
  3.4 g of sodium acetate and
  1.93 ml (or 2.03 g) of acetic acid 100 % in
  500 ml of H_2O
  Check the pH and if necessary, adjust the pH-value to 4.5 using acetic acid or sodium hydroxide 1 mol/l
- **EDTA solution 0.5 mol/l:**
  Dissolve 18.61 g of Ethylenediaminetetraacetic acid disodium salt dihydrate in
  80 ml of H_2O,
  adjust the pH value to 7.0 using sodium hydroxide 1 mol/l,
  make up to 100 ml with H_2O in a volumetric flask and mix
- **Hopped wort:**
  Collect the hopped brewery wort hot or sterilize it for 20 min at 121 °C and 15 lb/in^2
  If necessary, adjust the wort to 10 - 12 °Plato
- **Hopped wort agar:**
  In a glass vessel, suitable for autoclaving:
  add 2 g of agar to
  100 ml of hopped wort,
  warm the suspension for solving and
  sterilize for 20 min at 121 °C

14.5 Preparation

- **Grow yeast** for 4 days at 25 °C on wort agar plates
- **Inoculate** yeast from each plate at a pitching rate of 15 · 10^6 cells/ml into 100 ml hopped wort in a sterile 250-ml culture flask
- **Check the pitching rate** using a hemacytometer and adjust, if necessary, to a pitching rate of 1 - 2 · 10^7 cells/ml
- **Incubate** by shaking for 2 days at 25 °C at 150 rpm on a shaker incubator

14.6 Procedure and measurement

Measurement sample A:

- Pipette **10.0 ml of the freshly grown** yeast cultures into a 15-ml disposable centrifuge tube
- **Centrifuge** for 2.5 min at 2500 rpm
- Discard the supernatant by **decanting**
- Add **9.9 ml of H_2O** and **0.1 ml of EDTA solution 0.5 mol/l**
- **Resuspend** the pellet and vortex for 15 s
- Add **1.0 ml of the resuspended solution** to 9.0 ml H_2O in a test tube and mix
- Add **1.0 ml of the resuspended solution** to 9 ml H_2O in a test tube and mix
- Immediately measure the sample A

Analytical Procedures

14 Flocculation, absorbance method – yeast (ASBC method)

Measurement sample B:

- Pipette **10.0 ml of the freshly grown** yeast cultures into a 15-ml disposable centrifuge tube
- **Centrifuge** for 2.5 min at 2600 rpm
- Discard the supernatant by **decanting**
- Add **10.0 ml washing solution**
- **Resuspend** the pellet and vortex for 15 s
- **Centrifuge** for 2.5 min at 2500 rpm
- Discard the supernatant by **decanting**
- Add **10.0 ml of suspension solution pH 4.5**
- **Resuspend** the pellet and vortex for 15 s
- Slowly **invert** suspension (5 times in 15 s)
- Leave the suspension standing upright for exactly 6 min
- Pipette **9.0 ml of H_2O** into a separate test tube
- Add **1.0 ml of the suspension** by carefully pipetting the top 1 ml of the suspension without disturbing the remaining suspension
- **Homogenize** the content of the test tube by vortexing
- Add **1.0 ml of the resuspended solution** to 9 ml H_2O in a test tube and mix
- Immediately measure the sample B

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2635 "Flocculation (ASBC)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill measurement sample A into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample A"**. Confirm the message with **<OK>**.
- Fill measurement sample B into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample B"**. Confirm the message with **<OK>**.
- Read off the result as % flocculence from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

14.7 Evaluation

Results are expressed in % flocculence

Standard values

Flocculence >85 %:	very flocculent yeast
Flocculence 20 - 85 %:	moderately flocculent yeast
Flocculence <20 %:	nonflocculent yeast

Note

Negative flocculation values may occur for nonflocculent yeasts. This is the case when the absorption value of measurement sample A is lower than that of measurement sample B. A result below zero should be given as zero.

14.8 Literature

ASBC Methods of Analysis, online. Yeast-11, Flocculation, B. Absorbance method [Release date 1996, revised 2011].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Yeast-11

Analytical Procedures

15 Free Amino Nitrogen, Ninhydrin method - beer / wort (EBC / MEBAK / ASBC method)

Low-molecular nitrogen compounds, in particular amino acids in the wort, have an influence on the fermentation process and on the formation of fermentation byproducts. The concentration and composition of the amino acids are accordingly of relevance for the aroma profile of a beer, while the reactivity with reducing sugars (Maillard's reaction), in particular in the kilning of malt and in the boiling of mashes and worts, is a further effect. These reaction products have an effect on the redox potential, color, and aroma of the beer. In methods that are based on color reactions, the various amino acids exhibit different color intensities. Results are set in relation to a so-called "standard amino acid", in most cases glycine.
In the ninhydrin method the color yield of the individual amino acids varies between 70 % and 105 %, relative to glycine.

15.1 Method

The test sample is heated with ninhydrin at pH 6.7 and the resultant color is measured by spectrophotometry. The method measures the amino acids, ammonia, and also the terminal alpha-amino groups of peptides and proteins. Proline is also partly measured at the wavelength used. The method is not specific for alpha-amino nitrogen, since gamma-amino butyric acid, which occurs in wort, also reacts with ninhydrin to produce a color.

15.2 Measuring range

0 - 400 mg/l free amino nitrogen

15.3 Reagents and accessories

- di-Sodium hydrogen phosphate dodecahydrate for analysis EMSURE®, Cat. No. 106579
- Potassium dihydrogen phosphate for analysis EMSURE®, Cat. No. Nr. 104873
- Ninhydrin GR for analysis, Cat. No. 106762
- D(-)-Fructose, Cat. No. 105323
- Potassium iodate for analysis EMSURE®, Cat. No. 105051
- Ethanol 96 % EMSURE®, Cat. No. 159010
- Hydrochloric acid 6 mol/L EMPROVE®, Cat. No. 110164
- Sodium hydroxide solution 4 mol/l (4 N), Titripur®, Cat. No. 111584
- Glycine GR for analysis, Cat. No. 104201
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- pH meter
- 100-ml volumetric flask
- Pincer
- Test tubes with ground-glass stopper, 16 x 150 mm
- Water bath (20 °C)
- Water bath (100 °C)
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

Analytical Procedures

15 Free Amino Nitrogen, Ninhydrin method - beer / wort (EBC / MEBAK / ASBC method)

15.4 Preparing the solutions

- **Color reagent:**
  Dissolve 10.0 g di-sodium hydrogen phosphate dodecahydrate,
  6.0 g potassium dihydrogen phosphate,
  0.5 g ninhydrin, and
  0.3 g fructose
  in approx. 80 ml of H_2O,
  check pH (pH must lie between 6.6 and 6.8; adjust, if necessary, with hydrochloric acid 6 mol/l or sodium hydroxide solution 4 mol/l),
  and make up to 100 ml with H_2O in a volumetric flask
  (solution remains stable for 2 weeks when stored at +4 °C in dark bottles)
- **Dilution solution:**
  In a glass vessel:
  dissolve 2 g of potassium iodate in
  600 ml of H_2O
  add 400 ml of ethanol 96 % and mix
  (solution remains stable for 1 week when stored at +4 °C in dark bottles)
- **Glycine stock solution 200 mg/l amino nitrogen:**
  Dissolve 107.2 mg of glycine
  and make up to 100 ml with H_2O in a volumetric flask
  (solution remains stable for 1 week when stored at 0 °C in dark bottles)
- **Glycine standard solution 2 mg/l amino nitrogen:**
  1.0 ml of glycine stock solution 200 mg/l amino nitrogen
  make up to 100 ml with H_2O in a volumetric flask and mix
  (prepare freshly every day)

15.5 Preparation

- Dilute **beer** 50-fold with H_2O (dilution 1 + 49)
- Dilute **wort** 100-fold with H_2O (dilution 1 + 99)

15.6 Procedure and measurement

Important:

The amino acids occur in extremely low amounts in this analysis, meaning that contaminations must be avoided at all costs. The thoroughly cleaned glasses must be touched only on their external surfaces, and ground-glass stoppers etc. must be handled with pincers.

Analytical Procedures

15 Free Amino Nitrogen, Ninhydrin method - beer / wort (EBC / MEBAK / ASBC method)

User-defined calibration:

- Prepare calibration solutions in the following manner:

	E0 [0 mg/l FAN]	1 [2 mg/l FAN]
Water	2.0 ml	-
Glycin-Standardlösung 2 mg/l FAN	-	2.0 ml
	• pipette into separate test tubes	
Color reagent	1.0 ml	1.0 ml
	• add and mix • loosely close test tubes with glass stoppers to prevent losses by evaporation • heat in a **boiling water bath for exactly 16 min**, and subsequently allow to cool **in a water bath at 20 °C for 20 min**	
Dilution solution	5.0 ml	5.0 ml
	• add and mix • leave to stand for **3 min**; measure within 30 min	

Reagent blank:

- Pipette **2.0 ml of H_2O** into a test tube
- Add **1.0 ml of color reagent** and mix
- Loosely close test tubes with glass stoppers to prevent losses by evaporation
- Heat in a **boiling water bath for exactly 16 min**, and subsequently allow to cool **in a water bath at 20 °C for 20 min**
- Add **5.0 ml of dilution solution** and mix
- Leave to stand for **3 min**; measure within 30 min

Measurement sample:

- Pipette **2.0 ml of** the **diluted sample** into a test tube
- Add **1.0 ml of color reagent** and mix
- Loosely close test tubes with glass stoppers to prevent losses by evaporation
- Heat in a **boiling water bath for exactly 16 min**, and subsequently allow to cool **in a water bath at 20 °C for 20 min**
- Add **5.0 ml of dilution solution** and mix
- Leave to stand for **3 min**; measure within 30 min

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2606 "Free Amino Nitrogen"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The sample dilution must be entered.
  Do this by tapping the **<Settings>** button and selecting the **<DILUTION>** menu item. Activate the field for entering the dilution, enter the dilution and tap **<OK>** to confirm.
- **Measurement of the reagent blank** is necessary. Measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged.
  To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank".

Analytical Procedures

15 Free Amino Nitrogen, Ninhydrin method - beer / wort (EBC / MEBAK / ASBC method)

- **User-defined calibration** is necessary.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **2 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.
  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.
  Once all calibration solutions have been measured, save the calibration by pressing <**OK**>.

- In the case of **dark samples** (beer or wort) a sample blank must be measured.
  Do this by tapping the **<Settings>** button and selecting the **<SAMPLE BLANK>** menu item. Fill the cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the sample blank by tapping **<OK>** to confirm.

- Fill measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment.
  The measurement starts automatically.

- Read off the result in mg/l free amino nitrogen from the display.

15.7 Evaluation

Results are expressed
in mg/l FAN
The dilution is already considered in the result.

Standard values
Beer (12 %): 100 - 120 mg/l FAN

15.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.8.4.1.1, page 62ff

Analytica-EBC, Section 9 Beer, Method 9.10

ASBC Methods of Analysis, online. Beer-31, Free Amino Nitrogen [Release date 1975, revised 1976].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-31

ASBC Methods of Analysis, online. Wort-12, Free Amino Nitrogen, A. Ninhydrin Method [Release date 1975, revised 1976 and 2010].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Wort-12

Analytical Procedures

16 Hop Storage Index - hops (ASBC method)

If hop (cones and other hop products) is not stored or processed appropriately, α- and β-acids content changes reducing their potency resulting in inaccuracy of the label claim or COA. The loss is characterized by an increase in the ratio of absorbance at 275 nm to 325 nm. This can be evaluated with a UV spectrophotometer. Loss in α and β acids and increases in the hop storage index (HSI) provide a way to evaluate hops.

16.1 Method

After extraction of hops or hop pellets with an organic solvent, the absorbance ratio at 275 nm to 325 nm is determined (Hop storage index).
This method requires a Prove 300 or 600 as measurements are made in the UV range.

16.2 Measuring range

0.00 - 2.00 HSI

16.3 Reagents and accessories

- Toluene for spectroscopy Uvasol®, Cat. No. 108331
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106498
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Grinder or blender jar
- Analytical balance, accurate to 0.0001 g
- Mechanical shaker or rotary shaker, 200 rpm
- Centrifuge, 2000 rpm
- Centrifuge glasses with solvent-proof twist-off caps, 100 - 110 ml content
- Polyethylene bag, suitable for covering the grinder
- 5-ml volumetric pipette
- 100-ml volumetric pipette
- Adjustable pipettes 0.2 - 5.0 ml
- 100-ml volumetric flask
- 250-ml extraction vessels with inert closures (e. g. conical flask with Teflon or PE closures)
- Stop watch
- Rectangular cells 10 mm, quartz, Spectroquant®, Cat. No. 100784

16.4 Preparing the solutions

- **Sodium hydroxide solution 6 mol/l (6N):**
  In a 100-ml volumetric flask:
  dissolve 24.0 g of sodium hydroxide pellets
  in approx. 80 ml H_2O,
  cool to room temperature,
  make up to 100 ml with H_2O and mix
- **Alkaline methanol:**
  In a conical flask:
  mix 0.2 ml of sodium hydroxide solution 6.0 mol/l with
  100 ml of methanol
  (solution remains stable for 1 month in a tightly closed glass bottle in a solvent cabinet)

Analytical Procedures

16 Hop Storage Index - hops (ASBC method)

16.5 Preparation

Sample preparation for pressed and unpressed Hop samples

- Bring cooled samples (in a closed container) to room temperature
- Grind sample (immediately before use). Discard the first 10 g of the ground sample. Place an appropriate polyethylene bag over the discharge of the chopper and grind the remaining sample using only small portions to avoid losses of moisture
- Homogenize sample by inverting and rotating the bag
- Immediately use the ground sample for analysis otherwise freeze sample

Sample preparation for hop pellets

- Grind 75 - 125 g of the sample at high speed for 20 - 30 s using a blender jar. A heating of the sample should be avoided.
  The grind size is optimal if 95 % of the powder pass a screen with 20 opening per inch
- Immediately use the ground sample for analysis otherwise freeze sample

Extraction of hop samples

- Weigh **5.000 g of freshly ground hop sample** into a 250-ml extraction vessel
- Add **100 ml of toluene** by pipetting
- Stopper the extraction vessel tightly and **shake mechanically for 30 min** at room temperature and at an optimum mixing intensity or on a rotary shaker at 200 rpm
- **Centrifuge** at 2000 rpm for 5 min or alternatively let the solution stand until the separation of the solid and liquid phases (not longer than 1 h)

16.6 Procedure and measurement

Reagent blank:

- **Toluene diluted with methanol/alkaline methanol** in analogous manner as measurement sample

Measurement sample:

- Pipette **5 ml of the clear supernatant** into a 100-ml volumetric flask and make up to the mark with methanol (**dilution A**)
- Mix **dilution A** appropriately with **alkaline methanol** so that the absorption of the solution at 325 and 355 nm is within 0.1 - 0.8 A (**dilution B**). The aliquot volume of dilution A should be between 1 - 20 ml and the total volume of dilution B between 5 - 100 ml. Note the used volume of the aliquot of dilution A and the total volume of dilution B
- **Immediately measure** the sample to avoid decomposition

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2634 "Hop Storage Index (HSI)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Fill reagent blank into a 10-mm rectangular quartz cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Reagent Blank"**. Confirm the message with **<OK>**.
- Fill measurement sample into a 10-mm rectangular quartz cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**. Confirm the message with **<OK>**.
- Read off the result as HSI, absorbance at 275 nm and absorbance at 325 nm from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

Note
Check if the used cells are matching. The cells filled with H_2O should not differ from each other more than 0.005 Abs at 275, 325 and 355 nm. Otherwise use the same cell for reagent blank and measurement sample and rinse the cell before use with the solution to be measured.

Analytical Procedures

16 Hop Storage Index - hops (ASBC method)

16.7 Evaluation

Results are expressed
in HSI,
absorbance at 275 nm,
absorbance at 325 nm

Standard values
"Good keeper" hops (HSI range): fresh = 0.22; aged = 0.32
"Poor keeper" hops (HSI range): fresh = 0.26; aged = 0.79

16.8 Literature

ASBC Methods of Analysis, online. Hops-12, Hop Storage Index (HSI) [Release date 1979, revised 1981 and 2008].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Hops-12

Analytical Procedures

17 Iodine Value, photometric (MEBAK method)

In the practical environment, worts with iodine concentrations outside the normal range rectify only slowly and are difficult to clarify. The turbidity becomes stronger during fermentation, secondary fermentation ceases, and the beers can tend towards deviations of their flavor and odour.

17.1 Method

High-molecular dextrins and starches are precipitated by the addition of ethanol to wort or beer; they are then centrifuged off, dissolved in phosphate buffer, and iodine solution is added. Depending on the molecular weight and the degree of branching of the erythrodextrins and starch, a red to blue color emerges, the intensity of which is measured by spectrophotometry.
MEBAK specifies the use of a 40-mm rectangular cell for the photometric iodine test. It is also possible, however, to take the measurement in a 50-mm rectangular cell. Depending on the cell you are using, please select either method "**Iodine Value 40, photometric**" or "**Iodine Value 50, photometric**" on the photometer.

17.2 Measuring range

0.00 - 0.80 iodine value

17.3 Reagents and accessories

- Ethanol 96 % EMSURE®, Cat. No. 159010
- Iodine solution 0.5 mol/l (1 N), Titripur®, Cat. No. 109098
- Potassium dihydrogen phosphate for analysis EMSURE®, Cat. No. 104873
- ortho-Phosphoric acid 85 % for analysis EMSURE®, Cat. No. 100573
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 100-ml volumetric flask
- 200-ml volumetric flask
- pH meter
- Centrifuge, 2500 rpm
- Centrifuge glasses with ground-glass stopper, 50 ml content
- Mechanical shaker
- 40-mm rectangular cells OS or Rectangular cells, 50 mm, Spectroquant®, Cat. No. 114944
- Plastic spatula

17.4 Preparing the solutions

- **Iodine solution 0.01 mol/l (0.02 N):**
  Pipette 2 ml of iodine solution 0.5 mol/l (1 N)
  in a volumetric flask, make up to 100 ml with H_2O and mix
  (prepare freshly every day)
- **Potassium dihydrogen phosphate solution 0.1 mol/l:**
  Place 2.72 g of potassium dihydrogen phosphate
  in a volumetric flask, make up to 200 ml with H_2O and dissolve
  (shelf-life 4 weeks)
- **Phosphoric acid 0.1 mol/l:**
  Place 2.3 g resp. 1.35 ml ortho-phosphoric acid 85 %
  in a volumetric flask, make up to 200 ml with H_2O and mix
  (shelf-life 3 months)

Analytical Procedures

17 Iodine Value, photometric (MEBAK method)

- **Buffer solution pH 3.5 (phosphate buffer 0.1 mol/l):**
  In a glass vessel:
  potassium dihydrogen phosphate solution 0.1 mol/l with
  phosphoric acid 0.1 mol/l
  adjust pH to 3.5 on the pH meter
  (shelf-life 4 weeks)

17.5 Preparation

- Centrifuge **wort**
- Expel carbon dioxide from **beer**

17.6 Procedure and measurement

Buffer blank:

- Use **10 ml of buffer solution**

Reagent blank:

- To **10 ml of buffer solution** add
  0.5 ml of iodine solution 0.01 mol/l (0.02 N) and mix

Sample blank:

- Pipette **10.0 ml of centrifuged wort** or decarbonized **beer** into a centrifuge glass
- Add **40.0 ml of ethanol**, close centrifuge glass, and **shake mechanically** at optimum mixing intensity for **10 min**
- Centrifuge at **2500 rpm** for **5 min**
- Decant the supernatant as completely as possible and discard
- Add **20 ml of buffer solution** to the **residue** and
  form a suspension by **shaking mechanically** at optimum mixing intensity for **10 min**
- Centrifuge suspension at **2500 rpm** for **5 min**
- Pipette **2 ml of supernatant directly** into the rectangular cell (see 17.3)
- Add **8.0 ml of buffer solution** and mix

Measurement sample:

- After measuring the sample blank, add **0.5 ml of iodine solution 0.01 mol/l (0.02 N) to the sample blank** and mix **immediately** with a plastic spatula
- Leave to stand for **30 s**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2615 "Iodine Value 40, photometric"** or **method No. 2616 "Iodine Value 50, photometric"**.
- A zero adjustment must be made for each separate measurement series.
  The photometer automatically prompts the zero adjustment for the measurement series.
  For this fill buffer solution into a 40-mm or, respectively, a 50-mm rectangular cell.
  After prompting, insert the filled rectangular cell into the cell compartment; the zero adjustment starts automatically.
  Confirm the conduct of the zero adjustment by tapping **<OK>**.
- Fill reagent blank into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Reagent Blank"**.
  Comfirm the message with <**OK**>.
- Fill Sample blank into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample Blank"**.
  Comfirm the message with <**OK**>.

Analytical Procedures

17 Iodine Value, photometric (MEBAK method)

- Fill measurement sample into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Comfirm the message with <**OK**>.
- Read off the result as iodine value from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.
  The system does **not prompt a repeat of the zero adjustment**.

17.7 Evaluation

Results are expressed
as iodine value

Standard values
Wort: <0.30 iodine value

17.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.3.2, page 34ff

Analytical Procedures

18 Iron, spectrophotometric, Phenanthroline method - beer (ASBC method)

Iron may be imported into beer via the raw materials and also via filtering aids/clarifying agents, as well as from apparatuses, pipes, or cans, and it may also be contained in beer-froth stabilizing agents. Iron has a detrimental effect on the colloidal stability, flavour, and the gushing tendency of the beer.

18.1 Method

All iron ions are reduced to iron(II) ions by ascorbic acid. In a buffered medium iron ions react with 1,10-phenanthroline to form a red complex that is determined photometrically.

18.2 Measuring range

0.00 - 3.00 mg/l iron

18.3 Reagents and accessories

- 1,10-Phenanthroline (anhydrous), Cat. No. 841491
- L(+)-Ascorbic acid for analysis EMSURE®, Cat. No. 100468
- Ammonium iron(II) sulfate hexahydrate for analysis EMSURE®, Cat. No. 103792
- Hydrochloric acid fuming 37% for analysis EMSURE®, Cat. No. 100317
- Nitric Acid 65 % EMPLURA®, Cat. No. 100443

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.001 g
- Water bath (60 °C)
  or
- Thermoreactor Spectroquant®, Cat. No. 171200 or 171201 or 171202
  with
- Empty cells 16 mm with screw caps Spectroquant®, Cat. No. 114724
- Adjustable pipettes 1 - 5-ml
- 10-ml volumetric pipette
- 20-ml volumetric pipette
- 25-ml volumetric pipette
- 30-ml volumetric pipette
- 100-ml volumetric pipette
- 100-ml volumetric flask
- 50-ml conical flask
- 100-ml graduated measuring cylinder
- Funnel with folded filter (iron free)
- Stop watch
- 10-mm rectangular cells, Spectroquant®, Cat. No. 114946

18.4 Preparing the solutions

- **Nitric acid 40 %:**
  In a glass vessel:
  mix 40 ml of H_2O
  with 60 ml of nitric acid 65 % (⚠ exothermic reaction)
  Use the nitric acid 40 % to acid-wash all glassware used for analysis by rinsing with small amounts of acid. Wash the glass ware with H_2O until it is acid-free.

Analytical Procedures

18 Iron, spectrophotometric, Phenanthroline method - beer (ASBC method)

- **Iron stock solution 1000 mg/l Fe^{2+}:**
  Dissolve 3.511 g of ammonium iron(II) sulfate hexahydrate in
  approx. 400 ml H_2O,
  add 0.1 ml of hydrochloric acid 37 % and
  make up to 500 ml with H_2O in a volumetric flask and mix

- **Iron standard solution 10.0 mg/l Fe^{2+}:**
  Pipette 1.00 ml of iron standard solution 1000 mg/l Fe^{2+}
  into a 100-ml volumetric flask,
  make up to 100 ml with H_2O and mix
  (prepare freshly every day)

- **Phenanthroline reagent:**
  In a glass vessel:
  dissolve 0.300 g of 1,10-phenanthroline in
  100 ml of H_2O, heated to 70 °C

18.5 Preparation

- Expel carbon dioxide from beer and allow froth to disintegrate (avoid filtration, if possible). For beer samples with high iron content, degas by shaking only.
- Filter turbid samples over an iron-free folded filter.

18.6 Calculation of the calibration curve

A calibration for this method is preprogrammed in the photometer. It is recommended to check the calibration before the first use of the method as the calibration curve may, however, be influenced by the batch of reagents.
In general, to enhance the accuracy of the measurement, it is advisable to perform a user-defined calibration when exchanging batches of the reagents used. After making this user-defined calibration it is also necessary to measure a sample blank.

User-defined calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

18.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution					
	E0 [0.00 mg/l Fe^{2+}]	1 [0.20 mg/l Fe^{2+}]	2 [0.50 mg/l Fe^{2+}]	3 [1.00 mg/l Fe^{2+}]	4 [2.00 mg/l Fe^{2+}]	5 [3.00 mg/l Fe^{2+}]
Iron standard solution **10.0 mg/l Fe^{2+}**	0.0 ml	2.0 ml	5.0 ml	10.0 ml	20.0 ml	30.0 ml
	• Pipette into separate 100-ml volumetric flasks and make up to 100 ml with H_2O					

Analytical Procedures

18 Iron, spectrophotometric, Phenanthroline method - beer (ASBC method)

- Prepare calibration solutions in the following manner:

	Calibration solution					
	E0 [0.00 mg/l Fe^{2+}]	1 [0.20 mg/l Fe^{2+}]	2 [0.50 mg/l Fe^{2+}]	3 [1.00 mg/l Fe^{2+}]	4 [2.00 mg/l Fe^{2+}]	5 [3.00 mg/l Fe^{2+}]
Each standard solution (E0 - 5)	25.0 ml	25.0 ml	25.0 ml	25.0 ml	25.0 ml	25.0 ml
	• Pipette into separate 50-ml conical flasks					
Phenanthroline reagent	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	• Add to each flask and mix by swirling					
Ascorbic acid	25.0 mg	25.0 mg	25.0 mg	25.0 mg	25.0 mg	25.0 mg
	• Add to each flask, stopper the flasks and mix until the ascorbic acid is completely dissolved • Heat to 60 °C for 15 min* • Cool to room temperature					

Sample blank:

- Pipette **25.0 ml of sample** into a 50-ml conical flask
- Add **2.0 ml of H_2O** and mix by swirling
- Add **25 mg of ascorbic acid****,
  stopper the flasks and mix until it is completely dissolved
- Heat the solution for 15 min at 60 °C*
- Cool to room temperature

Measurement sample:

- Pipette **25.0 ml of sample** into a 50-ml conical flask
- Add **2.0 ml of phenanthroline reagent** and mix by swirling
- Add **25 mg of ascorbic acid****,
  stopper the flasks and mix until it is completely dissolved
- Heat the solution for 15 min at 60 °C*
- Cool to room temperature

* Alternatively a thermoreactor can be used. Preheat the thermoreactor to 60 °C. Transfer 10 ml of the solution into a 16-mm round cell and place the cell for 15 min in the thermoreactor. After 15 min remove the cell from the thermoreactor and cool it to room temperature.

** According to ASBC the addition of ascorbic acid is only required for beer samples with high iron content or highly oxidized samples. Nevertheless, it is recommended to perform this step for all beer samples to ensure that Fe^{3+} ions are also determined.

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2642 "Iron (ASBC) Phen"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- It is advisable to perform a user-defined calibration when exchanging batches of the reagents used.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.20 mg/l** for the first calibration solution.

Analytical Procedures

18 Iron, spectrophotometric, Phenanthroline method - beer (ASBC method)

Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"2"** line and enter the concentration of **0.50 mg/l** for the second calibration solution.
Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"3"** line and enter the concentration of **1.00 mg/l** for the third calibration solution.
Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"4"** line and enter the concentration of **2.00 mg/l** for the fourth calibration solution.
Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"5"** line and enter the concentration of **3.00 mg/l** for the fifth calibration solution.
Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Activate the **<U-CAL on>** and **<linear>** fields.

Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.

- **Measurement of the reagent blank** is necessary. Measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged.
  To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank".
- Measure a sample blank.
  Do this by tapping the **<Settings>** button and selecting the **<SAMPLE BLANK>** menu item. Fill the cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the sample blank by tapping **<OK>** to confirm.
- Fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l iron from the display.

18.8 Evaluation

Results are expressed
in mg/l Fe

Specified values
<0.200 mg/l iron (acc. to MEBAK)

18.9 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.29.3, page 149ff

ASBC Methods of Analysis, online. Beer-18, Iron, A. Analysis by Colorimetry [Release date 1958, revised 1975 and reviewed 2015].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-18

Analytical Procedures

19 Iron, spectrophotometric, 2,2'-Bipyridine method – beer (ASBC method)

Iron may be imported into beer via the raw materials and also via filtering aids/clarifying agents, as well as from apparatuses, pipes, or cans, and it may also be contained in beer-froth stabilizing agents. Iron has a detrimental effect on the colloidal stability, flavour, and the gushing tendency of the beer.

19.1 Method

All iron ions are reduced to iron(II) ions by ascorbic acid. In a buffered medium iron ions react with 2,2'-bipyridine to form a red complex that is determined photometrically.

19.2 Measuring range

0.00 - 3.00 mg/l iron

19.3 Reagents and accessories

- Acetic acid (glacial) 100 % anhydrous for analysis EMSURE®, Cat. No. 100063
- 2,2'-Bipyridine GR for analysis, Cat. No. 103098
- L(+)-Ascorbic acid for analysis EMSURE®, Cat. No. 100468
- Ammonium iron(II) sulfate hexahydrate for analysis EMSURE®, Cat. No. 103792
- Hydrochloric acid fuming 37% for analysis EMSURE®, Cat. No. 100317
- Nitric Acid 65 % EMPLURA®, Cat. No. 100443
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.001 g
- Water bath (60 °C)
  or
- Thermoreactor Spectroquant®, Cat. No. 171200 or 171201 or 171202
  with
- Empty cells 16 mm with screw caps Spectroquant®, Cat. No. 114724
- Adjustable pipettes 1 - 5-ml
- 10-ml volumetric pipette
- 20-ml volumetric pipette
- 25-ml volumetric pipette
- 30-ml volumetric pipette
- 100-ml volumetric pipette
- 100-ml volumetric flask
- 50-ml conical flask
- 100-ml graduated measuring cylinder
- Funnel with folded filter (iron free)
- Stop watch
- 10-mm rectangular cells, Spectroquant®, Cat. No. 114946

19.4 Preparing the solutions

- **Nitric acid 40 %:**
  In a glass vessel:
  mix 40 ml of H_2O
  with 60 ml of nitric acid 65 % (⚠ exothermic reaction)

  Use the nitric acid 40 % to acid-wash all glassware used for analysis by rinsing with small amounts of acid. Wash the glass ware with H_2O until it is acid-free.

Analytical Procedures

19 Iron, spectrophotometric, 2,2'-Bipyridine method – beer (ASBC method)

- **Iron stock solution 1000 mg/l Fe^{2+}:**
  Dissolve 3.511 g of ammonium iron(II) sulfate hexahydrate in
  approx. 400 ml H_2O,
  add 0.1 ml of hydrochloric acid 37 % and
  make up to 500 ml with H_2O in a volumetric flask and mix

- **Iron standard solution 10.0 mg/l Fe^{2+}:**
  Pipette 1.00 ml of iron standard solution 1000 mg/l Fe^{2+}
  into a 100-ml volumetric flask,
  make up to 100 ml with H_2O and mix
  (prepare freshly every day)

- **Acetic acid solution:**
  In a glass vessel:
  mix 20 ml of H_2O and
  10 ml of acetic acid 100 %

- **2,2'-Bipyridine reagent:**
  Dissolve 0.200 g of 2,2'-bipyridine in
  4 ml of acetic acid solution and, in a 100-ml volumetric flask,
  make up to 100 ml with H_2O and mix

19.5 Preparation

- Expel carbon dioxide from beer and allow froth to disintegrate (avoid filtration, if possible). For beer samples with high iron content, degas by shaking only.
- Filter turbid samples over an iron-free folded filter.

19.6 Calculation of the calibration curve

A calibration for this method is preprogrammed in the photometer. It is recommended to check the calibration before the first use of the method as the calibration curve may, however, be influenced by the batch of reagents.
In general, to enhance the accuracy of the measurement, it is advisable to perform a "User-defined Calibration" when exchanging batches of the reagents used. After making this user-defined calibration it is also necessary to measure a sample blank.

User-defined calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

Analytical Procedures

19 Iron, spectrophotometric, 2,2'-Bipyridine method – beer (ASBC method)

19.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution					
	E0 [0.00 mg/l Fe^{2+}]	1 [0.20 mg/l Fe^{2+}]	2 [0.50 mg/l Fe^{2+}]	3 [1.00 mg/l Fe^{2+}]	4 [2.00 mg/l Fe^{2+}]	5 [3.00 mg/l Fe^{2+}]
Iron standard solution **10.0 mg/l Fe^{2+}**	0.0 ml	2.0 ml	5.0 ml	10.0 ml	20.0 ml	30.0 ml
	• Pipette into separate 100-ml volumetric flasks and make up to 100 ml with H_2O					

- Prepare calibration solutions in the following manner:

	Calibration solution					
	E0 [0.00 mg/l Fe^{2+}]	1 [0.20 mg/l Fe^{2+}]	2 [0.50 mg/l Fe^{2+}]	3 [1.00 mg/l Fe^{2+}]	4 [2.00 mg/l Fe^{2+}]	5 [3.00 mg/l Fe^{2+}]
Each standard solution (E0 - 5)	25.0 ml	25.0 ml	25.0 ml	25.0 ml	25.0 ml	25.0 ml
	• Pipette into separate 50-ml conical flasks					
2,2'-Bipyridine reagent	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	• Add to each flask and mix by swirling					
Ascorbic acid	25.0 mg	25.0 mg	25.0 mg	25.0 mg	25.0 mg	25.0 mg
	• Add to each flask, stopper the flasks and mix until the ascorbic acid is completely dissolved • Heat to 60 °C for 15 min* • Cool to room temperature					

Sample blank:

- Pipette **25.0 ml of sample** into a 50-ml conical flask
- Add **2.0 ml of H_2O** and mix by swirling
- Add **25 mg of ascorbic acid****, stopper the flasks and mix until it is completely dissolved
- Heat the solution for 15 min at 60 °C*
- Cool to room temperature

Measurement sample:

- Pipette **25.0 ml of sample** into a 50-ml conical flask
- Add **2.0 ml of 2,2'-bipyridine reagent** and mix by swirling
- Add **25 mg of ascorbic acid****, stopper the flasks and mix until it is completely dissolved
- Heat the solution for 15 min at 60 °C*
- Cool to room temperature

* Alternatively a thermoreactor can be used. Preheat the thermoreactor to 60 °C. Transfer 10 ml of the solution into a 16-mm round cell and place the cell for 15 min in the thermoreactor. After 15 min remove the cell from the thermoreactor and cool it to room temperature.

** According to ASBC the addition of ascorbic acid is only required for beer samples with high iron content or highly oxidized samples. Nevertheless, it is recommended to perform this step for all beer samples to ensure that Fe^{3+} ions are also determined.

Analytical Procedures

19 Iron, spectrophotometric, 2,2'-Bipyridine method – beer (ASBC method)

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2643 "Iron (ASBC) Bipyridine"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- It is advisable to perform a user-defined calibration when exchanging batches of the reagents used. Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.20 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **0.50 mg/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"3"** line and enter the concentration of **1.00 mg/l** for the third calibration solution.
  Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"4"** line and enter the concentration of **2.00 mg/l** for the fourth calibration solution.
  Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"5"** line and enter the concentration of **3.00 mg/l** for the fifth calibration solution.
  Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.

  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

  Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.
- **Measurement of the reagent blank** is necessary. Measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged.
  To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank".
- Measure a sample blank.
  Do this by tapping the **<Settings>** button and selecting the **<SAMPLE BLANK>** menu item. Fill the cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the sample blank by tapping **<OK>** to confirm.
- Fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l iron from the display.

Analytical Procedures

19 Iron, spectrophotometric, 2,2'-Bipyridine method – beer (ASBC method)

19.8 Evaluation

Results are expressed
in mg/l Fe

Specified values
<0.200 mg/l Fe (acc. to MEBAK)

19.9 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.29.3, page 149ff

ASBC Methods of Analysis, online. Beer-18, Iron, A. Analysis by Colorimetry [Release date 1958, revised 1975 and reviewed 2015].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-18

Analytical Procedures

20 Iron, spectrophotometric, Ferrozine method – beer (ASBC method)

Iron may be imported into beer via the raw materials and also via filtering aids/clarifying agents, as well as from apparatuses, pipes, or cans, and it may also be contained in beer-froth stabilizing agents. Iron has a detrimental effect on the colloidal stability, flavour, and the gushing tendency of the beer.

20.1 Method

Bivalent iron reacts with the sodium salt of 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid hydrate (FerroZine™) to form a violet-colored complex with very high molar absorbance coefficient. Trivalent iron must be reduced to bivalent iron with ascorbic acid prior to the determination. The color intensity is measured by spectrophotometry.

20.2 Measuring range

0.00 - 0.40 mg/l iron

20.3 Reagents and accessories

- Ammonium acetate for analysis EMSURE®, Cat. No. 101116
- Acetic acid (glacial) 100 % anhydrous for analysis EMSURE®, Cat. No. 100063
- FerroZine™ Iron Reagent, 97.0% (Sigma-Aldrich) Cat. No. 160601
- L(+)-Ascorbic acid for analysis EMSURE®, Cat. No. 100468
- Ammonia solution 25 % for analysis EMSURE®, Cat. No. 105432
- Ammonium iron(II) sulfate hexahydrate for analysis EMSURE®, Cat. No. 103792
- Hydrochloric acid fuming 37% for analysis EMSURE®, Cat. No. 100317
- Nitric Acid 65 % EMPLURA®, Cat. No. 100443
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.001 g
- pH meter
- Adjustable pipettes 0.5 - 5-ml
- 10-ml volumetric pipette
- 30-ml volumetric pipette
- 50-ml volumetric flask
- 100-ml volumetric flask
- 200-ml volumetric flask
- Pasteur pipettes
- Test tube
- Funnel with folded filter (iron free)
- Stop watch
- 10-mm rectangular cells, Spectroquant®, Cat. No. 114946

20.4 Preparing the solutions

- **Iron stock solution 1000 mg/l Fe^{2+}:**
  Dissolve 0.7022 g of ammonium iron(II) sulfate hexahydrate in approx. 80 ml H_2O,
  add 1 ml of hydrochloric acid 37 % and
  make up to 100 ml with H_2O in a volumetric flask and mix

Analytical Procedures

20 Iron, spectrophotometric, Ferrozine method – beer (ASBC method)

- **Nitric acid 40 %:**
  In a glass vessel:
  mix 40 ml of H_2O
  with 60 ml of nitric acid 65 % (⚠ exothermic reaction)

  Use the nitric acid 40 % to acid-wash all glassware used for analysis by rinsing with small amounts of acid. Wash the glass ware with H_2O until it is acid-free.

- **Iron standard solution 5.0 mg/l Fe^{2+}:**
  Pipette 0.50 ml of iron standard solution 1000 mg/l Fe^{2+}
  into a 100-ml volumetric flask,
  make up to 100 ml with H_2O and mix
  (prepare freshly every day)

- **Buffer solution pH 4.3:**
  Dissolve 15 g of ammonium acetate and
  30 ml of acetic acid 100 %
  in approx. 100 ml of H_2O,
  check pH (if necessary, adjust the pH-value to 4.3 by adding dropwise acetic acid or ammonia solution 25 %),
  make up to 200 ml with H_2O in a volumetric flask and mix
  (solution remains stable for 4 weeks when stored at +4 °C)

- **FerroZine™ reagent:**
  Dissolve 0.263 g of FerroZine™ in
  approx. 80 ml of buffer solution pH 4.3
  in a 100-ml volumetric flask,
  make up to 100 ml with buffer solution pH 4.3 and mix
  (solution remains stable for 1 month)

- **Ascorbic acid solution 0.63 %:**
  Dissolve 0.63 g of ascorbic acid in
  approx. 80 ml of H_2O
  in a 100-ml volumetric flask,
  make up to 100 ml with H_2O and mix
  (prepare freshly every day)

20.5 Preparation

- Expel carbon dioxide from beer and allow froth to disintegrate (avoid filtration, if possible)
- Clarify turbid beers be centrifugation. Centrifugation is preferred to filtration. If filtration is performed, make sure to use an iron-free folded filter.
- If the iron content is >0.40 mg/l Fe, dilute the sample so that the iron content lies within the measuring range. Use the corresponding dilution factor when subsequently calculating the result (measurement result x dilution factor) or insert the dilution factor under <Settings>.

20.6 Calculation of the calibration curve

A calibration for this method is preprogrammed in the photometer with a factor given in the ASBC method. It is recommended to check the calibration before the first use of the method as the calibration curve may, however, be influenced by the batch of reagents and the sample matrix. It has been observed that a substantially lower gradient is found when performing a calibration for dark beers.
In general, to enhance the accuracy of the measurement, it is advisable to make a "User-defined Calibration" of the method in the sample matrix and when exchanging batches of the reagents used. After making this user-defined calibration it is also necessary to measure a sample blank.

User-defined calibration is necessary in the following cases:

- When the calibration is influenced by the **sample matrix**.
- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

Analytical Procedures

20 Iron, spectrophotometric, Ferrozine method – beer (ASBC method)

20.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution					
	E0 [0.00 mg/l Fe^{2+}]	1 [0.05 mg/l Fe^{2+}]	2 [0.10 mg/l Fe^{2+}]	3 [0.20 mg/l Fe^{2+}]	4 [0.30 mg/l Fe^{2+}]	5 [0.40 mg/l Fe^{2+}]
Iron standard solution **5.0 mg/l Fe^{2+}**	0.0 ml	0.5 ml	1.0 ml	2.0 ml	3.0 ml	4.0 ml
	• Pipette into separate 50-ml volumetric flasks					
H_2O	4.0 ml	3.5 ml	3.0 ml	2.0 ml	1.0 ml	0.0 ml
	• Add, make up to 50 ml with degassed beer (known to be low in iron) and mix					

- Prepare calibration solutions in the following manner:

	Calibration solution					
	E0 [0.00 mg/l Fe^{2+}]	1 [0.05 mg/l Fe^{2+}]	2 [0.10 mg/l Fe^{2+}]	3 [0.20 mg/l Fe^{2+}]	4 [0.30 mg/l Fe^{2+}]	5 [0.40 mg/l Fe^{2+}]
Each standard solution (E0 - 5)	10.0 ml	10.0 ml	10.0 ml	10.0 ml	10.0 ml	10.0 ml
	• Pipette into separate test tubes					
Ascorbic acid solution 0.63 %	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
	• Add and mix • Leave to stand for 5 min					
FerroZine™ reagent	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
	• Add and mix • Leave to stand for 1 min					

Sample blank:

- Pipette **10.0 ml of sample** into a test tube
- Add **1.0 ml of ascorbic solution 0.63 %** and mix
- Leave to stand for 5 min
- Add **1.0 ml of H_2O** and mix
- Leave to stand for 1 min

Measurement sample:

- Pipette **10.0 ml of sample** into a test tube
- Add **1.0 ml of ascorbic solution 0.63 %** and mix
- Leave to stand for 5 min
- Add **1.0 ml of FerroZine™ reagent** and mix
- Leave to stand for 1 min

Analytical Procedures

20 Iron, spectrophotometric, Ferrozine method – beer (ASBC method)

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2644 "Iron (ASBC) Ferrozine"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- It is advisable to perform a user-defined calibration when exchanging batches of the reagents used.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.05 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **0.10 mg/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"3"** line and enter the concentration of **0.20 mg/l** for the third calibration solution.
  Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"4"** line and enter the concentration of **0.30 mg/l** for the fourth calibration solution.
  Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"5"** line and enter the concentration of **0.40 mg/l** for the fifth calibration solution.
  Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.

  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

  Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.
- After recalibration <Recalibration> menu must not be opened again as the calibration function is then changed due to the measurement of the reagent blank. Open the <Recalibration> menu only when a new recalibration should be performed.
- After making a user-defined calibration, or in the case of an exchange of the batch of the reagents used, it is recommended to measure a new reagent blank **prior** to measuring the sample blank.
  To do this, fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank".
- Measure a sample blank.
  Do this by tapping the **<Settings>** button and selecting the **<SAMPLE BLANK>** menu item. Fill the cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the sample blank by tapping **<OK>** to confirm.
- Fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l iron from the display.

Analytical Procedures

20 Iron, spectrophotometric, Ferrozine method – beer (ASBC method)

20.8 Evaluation

Results are expressed
in mg/l Fe

Specified values
<0.200 mg/l Fe (acc. to MEBAK)

20.9 Literature

MEBAK Brautechnische Analysenmethoden 4th Edition 2002
Volume II, Method 2.29.3, page 149ff

ASBC Methods of Analysis, online. Beer-18, Iron, C. Analysis by Ferrozine [Release date 1992, reviewed and revised 2015].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-18

Analytical Procedures

21 Iron, spectrophotometric (EBC / MEBAK method)

Iron may be imported into beer via the raw materials and also via filtering aids/clarifying agents, as well as from apparatuses, pipes, or cans, and it may also be contained in beer-froth stabilizing agents. Iron has a detrimental effect on the colloidal stability, flavour, and the gushing tendency of the beer.

21.1 Method

Bivalent iron reacts with the sodium salt of 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid (Ferrozine) to form a violet-colored complex with very high molar absorbance coefficient. Trivalent iron must be reduced to bivalent iron with ascorbic acid prior to the determination. The color intensity is measured by spectrophotometry.
The MEBAK method specifies the use of a 40-mm rectangular cell for the determination of iron. It is, however, also possible to perform the measurement in a 50-mm rectangular cell. Depending on the cell you are using, please select either method "**Iron (EBC) 40**" or "**Iron (EBC) 50**" on the photometer.

21.2 Measuring range

40-mm rectangular cell: 0.000 - 1.000 mg/l iron

50-mm rectangular cell: 0.000 - 0.800 mg/l iron

21.3 Reagents and accessories

- Ammonium acetate for analysis EMSURE®, Cat. No. 101116
- Acetic acid (glacial) 100 % anhydrous for analysis EMSURE®, Cat. No. 100063
- Ferrozine for spectrophotometric det. of Fe, ≥97.0% (Sigma-Aldrich) Cat. No. 82950
- Ascorbic acid for analysis
- Iron standard solution Certipur®, 1000 mg/l Fe, Cat. No. 119781
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- pH meter
- 50-ml volumetric flask
- 100-ml volumetric flask
- 1000-ml volumetric flask
- Funnel with folded filter
- 40-mm rectangular cells OS or Rectangular cells, 50 mm, Spectroquant®, Cat. No. 114944

21.4 Preparing the solutions

- **Iron standard solution 10.0 mg/l iron:**
  1.00 ml of iron standard solution 1000 mg/l Fe^{3+}
  make up to 100 ml with H_2O in a volumetric flask and mix
  (shelf-life 1 week)
- **Buffer solution pH 4.3:**
  Dissolve 75 g of ammonium acetate and
  150 g of acetic acid 100 %
  in approx. 800 ml of H_2O,
  check pH, and
  make up to 1000 ml with H_2O in a volumetric flask
  (solution remains stable for 4 weeks when stored at +4 °C)
- **Ferrozine reagent:**
  In a glass vessel:
  dissolve 0.257 g of Ferrozine in
  50 ml of buffer solution pH 4.3
  (shelf-life 2 weeks)

Analytical Procedures

21 Iron, spectrophotometric (EBC / MEBAK method)

- **Ascorbic acid solution 2.5 %:**
  In a glass vessel:
  dissolve 2.5 g of ascorbic acid in
  97.5 g of H_2O
  (prepare freshly every day)

21.5 Preparation

- Expel carbon dioxide from **beer**, allow froth to disintegrate, and subsequently filter over a folded filter.

21.6 Calculation of the calibration curve

A calibration for this method is preprogrammed in the photometer. The calibration curve may, however, be influenced by the sample matrix. It has been observed that a substantially lower gradient is found when performing a calibration for dark beers.
To enhance the accuracy of the measurement, it is advisable to make a user-defined calibration of the method in the sample matrix. After making this user-defined calibration it is also necessary to measure a reagent blank.

User-defined calibration is necessary in the following cases:

- When the **stored calibration** is to be overwritten.
- When **exchanging batches** of the reagents used.
- When the calibration is influenced by the **sample matrix**.

21.7 Procedure and measurement

User-defined calibration:

- Prepare calibration solutions in the following manner:

	Calibration solution*				
	E0 [0.000 mg/l Fe^{3+}]	1 [0.050 mg/l Fe^{3+}]	2 [0.100 mg/l Fe^{3+}]	3 [0.200 mg/l Fe^{3+}]	4 [0.400 mg/l Fe^{3+}]
Sample	40.0 ml	40.0 ml	40.0 ml	40.0 ml	40.0 ml
Iron standard solution 10.0 mg/l Fe^{3+}	-	0.2 ml	0.4 ml	0.8 ml	1.6 ml
Ferrozine reagent	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Ascorbic acid solution 2.5 %	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
make up with H_2O in the volumetric flask **ad**	50 ml	50 ml	50 ml	50 ml	50 ml
* The respective concentrations in mg/l Fe^{3+} are relative to the volumes of the sample!					

Reagent blank:

- Pipette **2.0 ml of Ferrozine reagent** and
  1 ml of ascorbic acid solution
  into a 50-ml volumetric flask
- Make up to the mark with H_2O and mix

Sample blank:

- Pipette **40.0 ml of sample** and
  1 ml of ascorbic acid solution
  into a 50-ml volumetric flask
- Make up to the mark with H_2O and mix

Analytical Procedures

21 Iron, spectrophotometric (EBC / MEBAK method)

Measurement sample:

- Pipette **40.0 ml of sample**,
  2.0 ml of Ferrozine reagent, and
  1 ml of ascorbic acid solution
  into a 50-ml volumetric flask
- Make up to the mark with H_2O and mix

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2623 "Iron (EBC) 40"** or **method No. 2624 "Iron (EBC) 50"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- It is advisable to perform a user-defined calibration for each sample matrix.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.050 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **0.100 mg/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"3"** line and enter the concentration of **0.200 mg/l** for the third calibration solution.
  Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"4"** line and enter the concentration of **0.400 mg/l** for the fourth calibration solution.
  Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.

  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

  Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.
- After recalibration <Recalibration> menu must not be opened again as the calibration function is then changed due to the measurement of the reagent blank. Open the <Recalibration> menu only when a new recalibration should be performed.
- After making a user-defined calibration, or in the case of an exchange of the batch of the reagents used, it is recommended to measure a new reagent blank **prior** to measuring the sample blank.
  To do this, fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank".
- Subsequently measure the sample blank.
  Do this by tapping the **<Settings>** button and selecting the **<SAMPLE BLANK>** menu item. Fill the cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the sample blank by tapping **<OK>** to confirm.

Analytical Procedures

21 Iron, spectrophotometric (EBC / MEBAK method)

- Fill measurement sample into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l iron from the display.

21.8 Evaluation

Results are expressed in mg/l Fe

Specified values
<0.200 mg/l Fe

21.9 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.29.3, page 149ff

Analytica-EBC, Section 9 Beer, Method 9.13.2

Analytical Procedures

22 Iso-α Acids (MEBAK method)

22.1 Method

The bitter substances are extracted from the acidified sample (beer or wort) with iso-octane. Any substances that cause interference are removed by washing the extract with acidified methanol and the concentration of the α-acids is determined by spectrophotometry.
This method requires a Prove 300 or 600 as measurements are made in the UV range.

22.2 Measuring range

0 - 60 mg/l iso-α acids

22.3 Reagents and accessories

- Hydrochloric acid 6 mol/L EMPROVE®, Cat. No. 110164
- Hydrochloric acid 25 % for analysis EMSURE®, Cat. No. 100316
- Isooctane Uvasol®, Cat. No. 104718
- Sodium sulfate anhydrous for analysis EMSURE®, Cat. No. 106649
- Methanol for spectroscopy Uvasol®, Cat. No. 106002
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106498
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 25-ml volumetric flask
- 100-ml volumetric flask
- 1000-ml volumetric flask
- Centrifuge glasses with solvent-proof twist-off caps, 100 – 110 ml content
- Centrifuge, 3000 rpm
- Mechanical shaker
- 25-ml mixing cylinder
- Rectangular cells quartz, 10 mm, Spectroquant®, Cat. No. 100784

22.4 Preparing the solutions

- **Hydrochloric acid 4 mol/l (4 N):**
  Place 521 ml resp. 583 g of hydrochloric acid 25 %
  in a volumetric flask, make up to 1000 ml with H_2O and mix
  (shelf-life 3 months)
- **Acidic methanol solution:**
  In a glass vessel:
  mix 64 ml of methanol and
  36 ml of hydrochloric acid 4 mol/l (4 N)
  (prepare freshly every day)
- **Sodium hydroxide solution 6 mol/l (6N):**
  In a 100-ml volumetric flask:
  dissolve 24.0 g of sodium hydroxide pellets
  in approx. 80 ml H_2O,
  cool to room temperature,
  make up to 100 ml with H_2O and mix
- **Alkaline methanol solution:**
  Pipette 0.2 ml Sodium hydroxide solution 6.0 mol/l (6 N)
  in a volumetric flask, make up to 100 ml with methanol and mix
  (prepare freshly every day)

Analytical Procedures

22 Iso-α Acids (MEBAK method)

22.5 Preparation

- Clarify **wort** and **turbid beer** by centrifuging at 3,000 rpm for **15 min** (**do not filter!**).
- Expel carbon dioxide from **sample** without losing any foam.

22.6 Procedure and measurement

Measurement sample:

- Pipette **50.0 ml** of the sample (heated to 20 °C) into a centrifuge glass
- Add **3.0 ml of hydrochloric acid 6 mol/l (6 N)** and **25.0 ml isooctane**
- Close centrifuge glass and **shake mechanically** at optimum mixing intensity for **30 min**
- Centrifuge at **3000 rpm** for **5 min** to separate the phases and braek the emulsion
- Draw off the **lower aqueous phase** with a pipette and **discard**
- Add sodium sulfate to the remaining **isooctane phase** until the phase **clarifies** after brief vigorous shaking
- Pipette **10.0 ml** of this phase into a 25-ml mixing cylinder
  add **10.0 ml of acidic methanol solution**
  shake for 3 min
- Transfer **5.0 ml** of the supernatant clear **isooctane phase** to a 25-ml volumetric flask
- Make up to the mark with **alkaline methanol solution** and mix thoroughly

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2611 "Iso-α Acids"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill the sample blank into a 10-mm cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>.**
- Read off the result in mg/l iso-α acids from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

Note
The MEBAK method of analysis for the determination of iso-α acids recommends measurement against a mixture consisting of 5.0 ml of isooctane and 20.0 ml of alkaline methanol solution. This can be dispensed with when the recommended reagents are used, since these exhibit the same absorbance as distilled water at the measurement wavelengths.
When using reagents of different quality grades or of other origin, it is recommended to zero the system as described in section V "Zeroing", using a mixture consisting of 5.0 ml of isooctane and 20.0 ml of alkaline methanol solution instead of H_2O.

22.7 Evaluation

Results are expressed
in mg/l

Standard values
Beer: 10 - 40 mg/l iso-α acids, depending on grade, quality, type, and origin
Wort: 15 - 50 mg/l iso-α acids, depending on the beer and bitter-substance utilization

22.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.18.2, page 116ff

Analytical Procedures

23 Nickel (EBC / MEBAK method)

Nickel can be imported into the beer by contact with stainless steel; it may also be present in beer-froth stabilizers. Like all heavy metals, nickel exerts an effect on the froth stability and the colloidal stability of the beer.

23.1 Method

After the sample has been digested, a complex is formed by reaction with dimethylglyoxime that is then measured by spectrophotometry.

23.2 Measuring range

0.00 - 5.00 mg/l nickel

23.3 Reagents and accessories

- Nitric acid 65 % for analysis EMSURE®, Cat. No. 100456
- Perchloric acid 70 % for analysis EMSURE®, Cat. No. 100514
- Hydroxylammonium chloride GR for analysis, Cat. No. 104616
- tri-Sodium citrate dihydrate for analysis EMSURE®, Cat. No. 106448
- Ammonia solution 25 % for analysis EMSURE®, Cat. No. 105432
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106498
- Dimethylglyoxime GR for analysis, Cat. No. 103062
- Ethanol 96 % EMSURE®, Cat. No. 159010
- Chloroform for analysis EMSURE®, Cat. No. 102445
- Hydrochloric acid 0.5 mol/l (0.5 N) Titripur®, Cat. No. 109058
- Sodium tartrate dihydrate apura®, Cat. No. 106664
- Potassium peroxodisulfate for analysis EMSURE®, Cat. No. 105091
- Phenolphthalein solution 1 % in ethanol, Cat. No. 107227
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 100-ml volumetric flask
- 50-ml-conical flask
- Kjeldahl flask
- 125-ml separating funnel
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

23.4 Preparing the solutions

- **Hydroxylammonium chloride solution 10 %:**
  In a glass vessel:
  dissolve 10 g of hydroxylammonium chloride in
  90 ml of H_2O
  (shelf-life 3 months)
- **Sodium citrate solution:**
  In a glass vessel:
  dissolve 20 g of tri-sodium citrate dihydrate
  with H_2O up to 100 ml
  (shelf-life 3 months)
- **Dilute ammonia solution:**
  Mix 1 ml of ammonia solution 25 % with
  49 ml of H_2O in a glass vessel
  (shelf-life 1 week)

Analytical Procedures

23 Nickel (EBC / MEBAK method)

- **Sodium hydroxide solution 5 mol/l (5 N):**
  In a glass vessel:
  dissolve 200 g of sodium hydroxide pellets in
  1000 ml H_2O
  (shelf-life 12 months)
- **Alcoholic dimethylglyoxime solution 1 %:**
  In a glass vessel:
  dissolve 1 g of dimethylglyoxime in
  99 g of ethanol 96 %
  (solution remains stable for 3 months when stored in dark bottles)
- **Alkaline dimethylglyoxime solution:**
  Dissolve 1 g of dimethylglyoxim in
  5 ml of sodium hydroxide solution 5 mol/l (5 N)
  in a volumetric flask, make up to 100 ml with H_2O
  (solution remains stable for 3 months when stored in dark bottles)
- **Hydrochloric acid 0.33 mol/l (0.33 N):**
  Place 66.0 ml resp. 66.7 g of hydrochloric acid 0.5 mol/l
  in a volumetric flask, make up to 100 ml with H_2O and mix
  (shelf-life 3 months)
- **Sodium tartrate solution:**
  In a glass vessel:
  dissolve 20 g of sodium tartrate dihydrate
  with H_2O ad 100 ml
  (shelf-life 4 weeks)
- **Potassium peroxodisulfate solution 4 %:**
  In a glass vessel:
  dissolve 4 g of potassium peroxodisulfate in
  96 ml of H_2O
  (shelf-life 1 week)

23.5 Preparation

- Reduce **100 ml of beer** almost to dry in a Kjeldahl flask
- Evaporate **three times** with separate **5-ml portions of nitric acid 65 %**
- Evaporate again **twice** with separate **5-ml portions of perchloric acid 70 %** to destroy any organic substances
- Rinse the residue into a 125-ml separating funnel with approx 30 ml of H_2O

23.6 Procedure and measurement

Reagent blank:

- In a 50-ml volumetric flask
  Place **15 ml of hydrochloric acid 0.33 mol/l**,
  2 ml of sodium tartrate solution,
  10 ml of potassium peroxodisulfate solution,
  0.6 ml of alkaline dimethylglyoxime solution, and
  2.5 ml of sodium hydroxide solution 5 mol/l (5 N) and mix
- Make up to the mark with H_2O

Analytical Procedures

23 Nickel (EBC / MEBAK method)

Measurement sample:

- To the residue in the separating funnel add
  2 ml of hydroxylammonium chloride solution,
  5 ml of sodium citrate solution, and
  3 drops of phenolphthalein solution and mix
- Add **ammonia solution 25 %** drop by drop until a **pink color** occurs
- Add a further **4 drops of ammonia solution 25 %** and
  2 ml of alcoholic dimethylglyoxime solution and mix
- Add 20 ml of H_2O to make a total volume of approx. 60 ml
- Shake out **three times**, for 30 s each time, with separate **5-ml portions of chloroform**
  Collect the chloroform phases

(• any interfering copper compounds that may be present can be eliminated from the chloroform extract by shaking out with 5 ml of diluted ammonia solution for 1 min)

- Shake out the **chloroform phase once** with **10 ml of hydrochloric acid 0.33 mol/l** for 60 s and then once again with
  5 ml of hydrochloric acid 0.33 mol/l for a further 60 s
  (nickel passes over into the hydrochloric acid solution; take care that no residual chloroform remains in the combined hydrochloric acid extracts)
- Collect the **hydrochloric acid extracts** in a 50-ml volumetric flask
- Add **2 ml of sodium tartrate solution**,
  10 ml of potassium peroxodisulfate solution,
  0.6 ml of alkaline dimethylglyoxime solution, and
  2.5 ml of sodium hydroxide solution 5 mol/l and mix
- Make up to the mark with H_2O
- Leave to stand for at least **30 min to** at most **120 min**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2614 "Nickel (EBC)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2614 it is recommended to measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- After the reagent blank has been measured or, respectively, the stored reagent blank has been selected, fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l nickel from the display.

23.7 Evaluation

Results are expressed
in mg/l Ni

Specified values
Beer: <0.05 mg/l nickel

23.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.26.6, page 156ff

Analytica-EBC, Section 9 Beer, Method 9.15

Analytical Procedures

24 Protein, spectrophotometric - beer (ASBC method)

Proteins are high molecular macro molecules consisting of amino acids.
The amount of proteins has an effect on the full-body and taste characteristics as well as on the colloidal and foam stability. A too high amount of proteins can lead to haziness and a too low protein amount impairs the foam stability.

24.1 Method

In this method a diluted beer sample is measured spectrophotometrically to determine the protein content (%, w/w) in finished beer. Absorbance of the sample is measured at 215 and 225 nm. From these values (plus the total polyphenol content for stabilized beer samples), the protein content can be determined. The method can be used for beer stabilized with polyvinyl polypyrrolidone (PVPP), beer not treated with PVPP, and dark beer with ASBC color in the range of 15 - 50.
This method requires a Prove 300 or 600 as measurements are made in the UV range.

24.2 Measuring range

0.0 - 100.0 % protein

24.3 Accessories

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 6-ml volumetric pipette
- Precision balance, accurate to 0.01 g
- 1000-ml-conical flask
- 125-ml separating funnel
- Rectangular cells quartz, 10 mm, Spectroquant®, Cat. No. 100784

24.4 Preparing the solutions

No preparing necessary

24.5 Preparation

- Expel carbon dioxide from beer

24.6 Procedure and measurement

Measurement sample:

- Pipette **6 ml of degassed beer** into a tared 1000-ml conical flask and record the weight (= sample weight)
- Add **1000 ml of H_2O** into the conical flask and note the weight (=total weight)
- **Mix** the solution

Analytical Procedures

24 Protein, spectrophotometric - beer (ASBC method)

Measurement:

- Open the method list (**<Methods>**) and select depending on the beer sample
  method No. 2638 "Protein Beer, unstabilized" for unstabilized beer
  method No. 2639 "Protein Beer, stabilized" for stabilized beer
  method No. 2640 "Protein Beer, dark" for dark beer.
- The sample weight must be entered.
  An input masks pops up. Enter sample weight and tap **<OK>** to confirm
- The total weight must be entered.
  An input masks pops up. Enter total weight and tap **<OK>** to confirm
- If **method 2639 "Protein Beer, stabilized"** is selected, the amount of total polyphenols in mg/l must be entered additionally.
  An input masks pops up. Enter the amount of total polyphenols in mg/l and tap **<OK>** to confirm
- Press the **<START>** button to start the measurement procedure
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Fill measurement sample into a 10-mm rectangular quartz cell and insert cell into the cell compartment.
  The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>**.
- Read off the result as % (wt/wt) from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

24.7 Evaluation

Results are expressed
in % (wt/wt)

The result can also be expressed in mg/l by calculating as follows:

Protein [mg/l] = Protein [%,wt/wt] · ρ [g/ml] · 10

Where "ρ" = density of the beer sample

24.8 Literature

ASBC Methods of Analysis, online, Beer-11, Protein, C. By Spectrophotometer [Release date 2005, revised 2013, reviewed 2015].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-11.

Analytical Procedures

25 Protein, spectrophotometric - unhopped wort (ASBC method)

Proteins are high molecular macro molecules consisting of amino acids. One of the most important protein suppliers for beer is malt. The amount of proteins in beer has an effect on the full-body and taste characteristics as well as on the colloidal and foam stability. A too high amount of proteins can lead to haziness and a too low protein amount impairs the foam stability.

25.1 Method

With this method the soluble protein content in wort, prepared from malt, can be measured spectrophotometrically. The method is based on the different absorption of proteins at 215 and 225 nm.
For quantification a user-defined calibration must be performed. The standards are laboratory worts prepared of malt samples with different protein content. Of those worts the protein content is determined in advance according to ASBC Beer 11-A (Kjeldahl) or ASBC Beer 11-B (Combustion).
This method requires a Prove 300 or 600 as measurements are made in the UV range.

25.2 Measuring range

0.0 - 100.0 % protein (malt, dry basis)

25.3 Reagents and accessories

- Sodium chloride for analysis EMSURE®, Cat. No. 106404
- Different malts, used for calibration
  The protein content of the malts, used for calibration, should cover a slightly broader range as expected in the routine analysis
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.001 g
- Mill, fine-grind (laboratory mill of Buhler type), standardized acc. to ASBC Malt-4
- Mill, coarse-grind (laboratory mill of Buhler type), standardized acc. to ASBC Malt-4
  For details of standardizing see ASBC Malt-4
- Density meter
- Oven, 103 - 104 °C (for the determination of the moisture)
- Desiccator (for the determination of the moisture)
- Mashing apparatus, 70 °C (suitable for raising temperature by 1 °C/min)
- Mash beaker (brass, pure nickel or stainless steel)
- Stirrer (brass, pure nickel or stainless steel)
- Heating plate
- Thermometer
- 1-ml volumetric pipette
- 100-ml volumetric flasks
- 100-ml graduated measuring cylinder
- 200-ml graduated measuring cylinder
- 500-ml conical flask
- Watch glasses, Ø 20 cm
- Evaporating dish
- Fluted filter paper, Ø 32 cm
- Funnel, Ø 20 cm
- Glass rod
- Stop watch
- Rectangular cells quartz, 10 mm, Spectroquant®, Cat. No. 100784

Analytical Procedures

25 Protein, spectrophotometric - unhopped wort (ASBC method)

Only necessary when user-defined calibration is carried out

- 6-ml volumetric pipette
- Precision balance, accurate to 0.01 g
- 1000-ml conical flask 1000

25.4 Preparing the solution

- **Sodium chloride solution 0.5 %:**
  Dissolve 0.5 g of sodium chloride in
  approx. 80 ml of H_2O
  in a 100-ml volumetric flask, make up to 100 ml with H_2O and mix

25.5 Preparation

Prepare a laboratory wort from malt samples, for which the moisture has been determined, and determine the % extract (dry basis) of the wort.
Therefor follow the procedure described below:

Grinding procedure
Finely ground samples
- Grind **55 g of sample using a fine-grind mill**. The sample should be tempered to room temperature.
- Collect ground malt in a tared mash-beaker tightly connected to the mill and transfer malt remaining in the mill into the mash-beaker by carefully brushing
- Rapidly homogenize the sample
- Immediately after homogenizing, place the mash beaker on the balance and adjust the weight to 50 g. Therefor remove the surplus of malt into a tared evaporating dish. Determine the moisture using the surplus malt (according to Malt-3).

Coarsely ground samples (only necessary if user-defined calibration is performed)
- Grind **50.5 g of sample using a coarse-grind mill**
- Collect ground malt in a tared mash-beaker tightly connected to the mill
- Rapidly homogenize the sample
- Place the mash beaker on the balance and adjust the weight to 50 g. Therefor discard the surplus of malt

Mashing procedure
For the protein determination in malt samples the mashing is only necessary for the finely ground samples. If a user-defined calibration is performed the mashing has to be performed for both, the finely and the coarsely ground samples.

- Mash the ground malt with 200 ml H_2O, warmed to 46 - 48 °C. The temperature of the mixture should be 45 °C.
- Stir using a glass rod to avoid lumps
- Rinse the malt remaining on the glass rod and the beaker walls with a small amount of H_2O into the mash beaker. The temperature of the water should be 45 - 48 °C
- Immediately place the mash beaker in a mashing apparatus containing water heated to a temperature so that the temperature of the mixture is 45 °C.
- Start the stop watch and stir the mixture for 30 min at 45 °C
- After stirring begins, place a thermometer in the beaker and cover the mash beaker with a watch glass
- After 30 min at 45 °C, raise the temperature of the mash up to 70 °C by 1 °C/min
- Add 100 ml H_2O, heated to 70 - 71 °C
- Start the stop watch and keep the temperature at 70 °C for 60 min
- After 60 min, cool mash to room temperature by adding cooled water to the mashing apparatus. This should take between 10 - 15 min.

Note
The temperature of the mash may not deviate more than $\pm$0.5 °C of the given values.

Analytical Procedures

25 Protein, spectrophotometric - unhopped wort (ASBC method)

Filtration procedure

- After the mash is cooled to room temperature, remove the mash beaker from the mashing apparatus.
- Rinse the thermometer and the stirrer with a small amount of H_2O removing all mash adhering to the surface.
- Rapidly dry the outside of the mash beaker, so that the beaker is completely dry.
- Immediately place the beaker on the balance and adjust the weight of the mixture to 450.0 g by adding H_2O
- Homogenize the mixture using a glass rod
- Without delay start filtering by filling the complete mixture into a funnel containing a fluted filter. Place a 500-ml conical flask below the funnel.
- Take the first 100 ml filtrate (wort) to quantitatively transfer all particles remaining in the mash beaker into the funnel
- Collect 200 ml wort prepared from the coarse ground malt and determine the specific gravity at 20 °C using a density meter. (Only necessary, if user-defined calibration is performed)
  On basis of the specific gravity and the moisture of the malt the % extract can be read of from a table given by the ASBC (Tables for Extract Determination in Malt and Cereals).
  Note the % extract.
- Take the wort of the finely ground malt for the determination of the protein content in unhopped beer.

25.6 Calculation of the calibration curve

A user-defined calibration is necessary. The calibration is performed with malt of which the protein content has been determined according to ASBC Beer 11-A (Kjeldahl) or ASBC Beer 11-B (Combustion).

User-defined calibration is necessary in the following cases:

- When method is used for the first time
- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

25.7 Procedure and measurement

User-defined calibration:

Take a series of malts as calibration standards. The protein content of the malt, used for calibration, should cover a slightly broader range as expected in the routine analysis.

Analysis of protein content:

- Produce laboratory worts from malts samples for which the moisture has been determined acc. to ASBC-Malt-3 and determine the % extract content (dry basis) of the wort. Therefor follow the procedure described in chapter 25.5 “Preparation”.
  For the determination of the moisture finely ground malt is used, for the determination of the % extract use the laboratory wort prepared from the coarsly ground malt.

- Determine the protein content of the laboratory wort prepared from finely ground malt according to the method ASBC Beer 11-A (by Kjeldahl) or ASBC Beer 11-B (by combustion) and calculate the wort protein in % of malt, dry basis.

Absorption measurement:

- Pipette 1 ml of the laboratory wort prepared from finely ground malt into a 100-ml volumetric flask and make up to the mark with sodium chloride solution 0.5 %
- Measure the absorbance of the diluted wort sample in a rectangular quartz cell at 215 and 225 nm in the Ad hoc menu on the Prove spectrophotometer against H_2O
- Calculate the absorbance, adjusted to dry basis, as follows:

$$Abs,db = \frac{(Abs_{215\ nm} - Abs_{225\ nm}) \cdot 100}{100\ g\ Malt - moisture}$$

Analytical Procedures

25 Protein, spectrophotometric - unhopped wort (ASBC method)

- Perform a linear calibration (e.g. using a spreadsheet software) by plotting the absorbance, adjusted to dry basis, on the x-axis and the wort protein content in % of malt, dry basis, on the y-axis. Form a linear regression line to get the regression equation with the intercept a and the slope b:

$$\text{Wort protein [\% of malt,db]} = a + b \cdot \text{Abs,db}$$

- Note the factors a and b

Measurement sample:

- Pipette 1 ml of the laboratory wort into a 100-ml volumetric flask and make up to the mark with sodium chloride solution 0.5 %

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2641 "Protein wort"**.
- The moisture must be entered.
  An input masks pops up. Enter the moisture and tap **<OK>** to confirm
- Press the **<START>** button to start the measurement procedure
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- In case that a calibration has been performed, the factors from the linear regression equation of the user-defined calibration must be entered.
  Do this by tapping the **<Settings>** button and selecting the **<FACTORS>** menu item. An input mask pops up.
  Select the **"a"** field and enter factor a (intercept of the equation).
  Select the **"b"** field and enter factor b (slope of the equation).
  Confirm with **<OK>**.
- Fill measurement sample into a 10-mm rectangular quartz cell and insert cell into the cell compartment.
  The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>**.
- Read off the result as % malt, db from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

Note
The ASBC method of analysis for the determination of protein in unhopped beer recommends measurement against a sodium chloride solution 0.5 %. This can be dispensed with when the recommended reagents are used, since these exhibit the same absorbance as distilled water at the measurement wavelengths.
When using reagents of different quality grades or of other origin, it is recommended to zero the system as described in section V "Zeroing", using the sodium chloride solution 0.5 % instead of H_2O.

25.8 Evaluation

Results are expressed
in % (malt, dry basis)

25.9 Literature

ASBC Methods of Analysis, online, Wort-17, Protein in unhopped wort by spectrophotometry, [Release date 1990, revised 2010].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Wort-17.

ASBC Methods of Analysis, online, Malt-4, Extract [Release date 1958, revised 1976, 1991 and 2011].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Malt-4.

ASBC Methods of Analysis, online, Malt-3, Moisture [Release date 1958, revised 1976 and 2011].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Malt-3.

Analytical Procedures

26 Reducing Power, spectrophotometric (MEBAK method)

The reducing power is a measure of the rapidly reducing substances present in beer. Reductones are found in relatively small amounts in beer, but are of considerable significance for the chemicophysical and biological stability of beer, as well as the long-term constancy of its flavour.

26.1 Method

Reductones reduce a specific amount of Tillmann's reagent (2,6-dichlorophenol indophenol, DPI) within a given period of time. The decoloration of the reagent is measured with a spectrophotometer and calculated.

26.2 Measuring range

0 - 100 %

26.3 Reagents and accessories

- 2,6-Dichlorphenol-indophenol sodium salt dihydrate (DPI) GR for analysis, Cat. No. 103028
- Potassium iodide for analysis EMSURE®, Cat. No. 105043
- Sulfuric acid 25 % for analysis EMSURE®, Cat. No. 100716
- Sodium thiosulfat solution for 0.01 mol/l (0.01 N), Titrisol®, Cat. No. 109909
- Zinc iodide starch solution for analysis, Cat. No. 105445

- Standard laboratory pipettes
- 100-ml glass beaker
- 50-ml volumetric flask
- White-band filter
- 150-ml conical flask
- 25-ml burette
- Vacuum pump
- 30-ml test tubes with ground-glass stopper
- Stopwatch
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

26.4 Preparing the solutions

- **2,6-dichlorphenol indophenol solution (DPI solution):**
  Weigh approx. **113 mg of DPI** into a glass beaker,
  add approx. **25 ml of H_2O**, and
  dissolve by heating at approx. 60 °C

  Allow to cool and rinse into a 50-ml volumetric flask with H_2O,

  make up to the 50-ml mark with H_2O, and
  filter over a white-band filter.

  - Assay:
    In an 150-ml conical flask:
    place **10 ml of DPI filtrate**,
    1 g of potassium iodide,
    1 ml of sulfuric acid 25 %, and
    1 ml of H_2O and mix

    titrate with **sodium thiosulfate solution 0.01 mol/l (0.01 N)**
    against **zinc iodide starch solution** until there is a change in color

    Consumption of sodium thiosulfate solution [ml] x 145 = DPI [mg/l]

  - Dilute remaining filtrate to **1450 mg/l DPI**
    (solution remains stable for approx. 1 week when stored full to the brim in dark bottles at +4 °C)

Analytical Procedures

26 Reducing Power, spectrophotometric (MEBAK method)

26.5 Preparation

- Bring **beer** to a temperature of 20 °C and **expel carbon dioxide** under vacuum

26.6 Procedure and measurement

Sample blank:

- Use decarbonized beer

Measurement sample:

- Pipette **10 ml** of the decarbonized **beer** at 20 °C into a test tube with a ground-glass stopper
- Carefully run **0.25 ml of DPI solution** down the side of the **inclined test tube** into the sample
- **Immediately close** and mix by inverting the test tube **twice**; **after turning the tube the first time** start the stopwatch: **reaction time 60 s**
- **Immediately after mixing** fill into a 10-mm rectangular cell and measure immediately **after the reaction time has elapsed**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2617 "Reducing Power"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill the sample blank into a 10-mm cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample Blank"**. Confirm the message with **<OK>.**
- Fill the measurement sample into a 10-mm cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**. Confirm the message with **<OK>**.
- Read off the result in % reducing power from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

26.7 Evaluation

The dimensionless figure that is shown expresses the percentage of the DPI content reduced by 10 ml of beer within 60 s.

Assessment

>60 %	excellent
50 - 60 %	good
45 - 50 %	satisfactory
<45 %	poor

Remarks

Light beers that exhibit extremely high values (over 80 %) possibly contain added antoxidizing agents (e. g. L-ascorbic acid).
Dark beers can reach value in the region of 90 % even without the addition of reducing agents. Any addition (improbable in the case of dark beers) would accordingly yield a reducing-capacity result of 100 %. The measurement should be taken immediately after filling.

26.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.16.1, page 104ff

Analytical Procedures

27 Reducing Sugars, Henry method - malt (ASBC method)

Malt contains diastatic enzymes which catalyze the breakdown of starch into smaller, fermentable sugar molecules. The amount of diastatic enzymes is important for the fermentation process as the fermentable sugar is converted into alcohol.

27.1 Method

This method provides a more rapid but less precise method of determining diastatic power of malt. The method uses a spectrophotometer measuring absorbance at 415 nm.
The Ferricyanide procedure, Malt 6A which is a titration method, or automated flow analysis procedure, Malt 6C should be used if greater precision is required.

27.2 Measuring range

0.00 - 1.00 g/l dextrose

27.3 Reagents and accessories

- D(+)-Glucose anhydrous for biochemistry, Cat. No. 108337, (Dextrose)
- Sodium acetate anhydrous for analysis EMSURE®, Cat. No. 106268
- Acetic acid 1 mol/l Titripur®, Cat No. 160305
- Sodium hydroxide solution 0.5 mol/l Titripur®, Cat. No. 109138
- Special starch
  Starch manufactured specifically for diastatic power determinations is available from the American Society of Brewing Chemists, 3340 Pilot Knob Road, St. Paul, MN 55121. It is designated "soluble starch special for diastatic power determination." When purchasing new batches of starch, test them in parallel with the lot in use. Variations of more than ±2% diastatic power in averages of a series of parallel tests indicate an unsuitable batch of starch.
- Tri-Sodium citrate anhydrous for analysis EMSURE®, Cat. No. 106448
- Calcium chloride dihydrate for analysis EMSURE®, Cat. No 102382
- Sodium hydroxide pellets for analysis EMSURE®, Cat. No. 106495
- 4-Hydroxybenzhydrazide, Cat. No. 841410
- Ammonia solution 25 % for analysis EMSURE®, Cat No. 105432
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.0001 g
- Mash-beaker, brass, nickel, or stainless steal
- Mill, fine-grind
- Water bath (20 °C)
- Heating stirrer
- Drying oven, 103 °C
- 5-ml volumetric pipette
- 10-ml volumetric pipette
- 20-ml volumetric pipette
- 25-ml measuring cylinder
- 250-ml measuring cylinder
- Adjustable pipettes 0.2- 5.0 ml
- 100-ml volumetric flask
- 250-ml volumetric flask
- 500-ml volumetric flask
- 50-ml conical flasks

Analytical Procedures

27 Reducing Sugars, Henry method - malt (ASBC method)

- 500-ml infusion flasks (as alternative conical flasks or glass-stoppered bottle)
- Test tubes, 25 x 150 mm
- Filter paper, fluted, Ø 32 cm
- Funnel, Ø 20 cm
- Stop watch
- Vortex
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

27.4 Preparing the solutions

- **Acetate buffer solution:**
  Dissolve 6.8 g of sodium acetate in
  50 ml of acetic acid 1 mol/l
  in a 100-ml volumetric flask, make up to 100 ml with H_2O and mix
- **Alkaline diluent solution:**
  In a 250-ml beaker:
  dissolve 7.35 g of tri-sodium citrate in
  approx. 150 ml of H_2O

  In a 250-ml beaker:
  dissolve 0.97 g of calcium chloride dihydrate in
  approx. 150 ml of H_2O

  In a 500-ml volumetric flask:
  Transfer both solutions into a 500-ml volumetric flask,
  add 10 g of sodium hydroxide pellets and mix
  Make up to 500 ml with H_2O
- **4-Hydroxybenzhydrazide solution:**
  Dissolve 0.500 g of 4-hydroxybenzhydrazide in
  approx. 50 ml of alkaline diluent solution
  in a 100-ml volumetric flask, make up to 100 ml with alkaline diluent solution and mix
  (solution remains stable overnight in the dark at 4 °C. If solution becomes yellow, discard it.)
- **Ammonia solution 1 mol/l:**
  Place approx. 30 ml of H_2O in a 100-ml volumetric flask,
  add 7.54 ml (=6.312 g) of ammonia solution 28 - 30 % using a pipette and make up to 100 ml with H_2O
- **Ammonia solution 6 mmol/l:**
  Place approx. 400 ml of H_2O in a 500-ml volumetric flask,
  add 3 ml of the ammonia stock solution 1 mol/l using a pipette and make up to 500 ml with H_2O

27.5 Preparation

Special starch solution, 2 %

- Place 12.5 ml of cold and freshly distilled H_2O in a mash beaker
- Add 5 g of special starch (100 %, dry mass). If the assay is not 100 %, adapt the amount according to the assay
- Macerate the suspension into a smooth paste
- Add the paste slowly to 190 ml boiling H_2O. Adapt the addition speed so that the boiling does not cease.
- Quickly transfer the starch paste remaining on the beaker with a small amount of hot water
- After addition, boil for 2 min (a boiling time over 4 min must be avoided)
- Quantitatively transfer the starch solution into a 250-ml volumetric flask using about 25 ml cold H_2O and mix by inverting
- Wash down the starch remaining on the glass with a small amount of H_2O
- Cool the solution to 20 °C using a water bath
- Add 5 ml acetate buffer solution and make up the mark with H_2O
- Stopper the flask tightly and keep it at 20 °C until use

Analytical Procedures

27 Reducing Sugars, Henry method - malt (ASBC method)

Malt infusion
- Grind 10.5 g of malt (according to ASBC Malt-4) using a mill (fine-grind) and homogenize it
- Quickly weigh 10 g of the finely ground malt into a mash beaker and transfer into an infusion flask
- Add 200 ml of ammonia solution 6 mmol/l, stopper the flask and swirl
- Leave the solution to stand for exactly 10 min in a water bath at 20 °C and carefully swirl by rotating in 2-min intervals. A splashing of the flask contents should be avoided.
- Filter the suspension over a 32-cm fluted filter using a 20-cm funnel. Refilter the filtrate after a filtration time of 5 min
- Collect filtrate for 15 min

Diastasis sample solution
- Pipette 0.2 ml of filtered malt infusion solution into a 50-ml conical flask
- Temper to 20 °C using a water bath
- Add 20 ml of special starch solution at 20 °C using a fast-flowing pipette while rotating the flask and immediately start the stop watch from the moment of the addition
- Leave the solution to stand for exactly 10 min in a water bath 20 °C
- After 10 min, rapidly add 1.2 ml of sodium hydroxide 0.5 mol/l and mix by swirling

Diastasis sample blank solution
- Pipette 1.2 ml of sodium hydroxide 0.5 mol/l in a 50-ml conical flask
- Add 0.2 ml of filtered malt infusion solution by pipetting
- Temper to 20 °C using a water bath
- Add 20 ml of special starch solution at 20 °C using a fast-flowing pipette while rotating the flask and immediately start the stop watch from the moment of the addition
- Leave the solution to stand for exactly 10 min in a water bath 20 °C

27.6 Calculation of the calibration curve

A calibration for this method is preprogrammed in the photometer with a factor given in the ASBC method. It is recommended to check the calibration before the first use of the method as the calibration curve may, however, be influenced by batches of reagents.
In general, to enhance the accuracy of the measurement, it is advisable to perform a "User-defined Calibration" whenever batches of reagents are exchanged. After making the user-defined calibration it is also necessary to measure a sample blank.

User-defined calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

27.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution					
	E0 [0.00 g/l dextrose]	1 [0.20 g/l dextrose]	2 [0.40 g/l dextrose]	3 [0.60 g/l dextrose]	4 [0.80 g/l dextrose]	5 [1.00 g/l dextrose]
Dextrose (Oven dried at 103 °C for 4 h)	-	0.2000 g	0.4000 g	0.6000 g	0.8000 g	1.0000 g
	• Quantitatively transfer the amount in a 1000-ml volumetric flask and make up to the mark with H_2O					

Analytical Procedures

27 Reducing Sugars, Henry method - malt (ASBC method)

- Prepare calibration solutions in the following manner:

	Calibration solution					
	E0 [0.00 g/l dextrose]	1 [0.20 g/l dextrose]	2 [0.40 g/l dextrose]	3 [0.60 g/l dextrose]	4 [0.80 g/l dextrose]	5 [1.00 g/l dextrose]
4-Hydroxybenzhydrazide solution	5.0 ml	5.0 ml	5.0 ml	5.0 ml	5.0 ml	5.0 ml
	• Pipette into separate test tubes					
Prepared dextrose standard solution (E0-5)	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
	• Pipette into each test tube and mix • Place the test tube in a boiling water bath for 4 min • Cool in a 20 °C water bath for 5 - 10 min					
H_2O	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
	• Pipette into each test tube and mix well by vortexing					

Sample blank:

- Place **5.0 ml of 4-hydroxybenzhydrazide solution** in a test tube
- Add **0.2 ml of diastasis sample blank solution** and mix
- Place the test tube in a **boiling water bath** for 4 min
- Cool in a 20 °C water bath for 5 - 10 min
- Add **10 ml of H_2O** and mix well by vortexing

Measurement sample:

- Place **5.0 ml of 4-hydroxybenzhydrazide solution** in a test tube
- Add **0.2 ml of diastasis sample solution** and mix
- Place the test tube in a **boiling water bath** for 4 min
- Cool in a 20 °C water bath for 5 - 10 min
- Add **10 ml of H_2O** and mix well by vortexing

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2632 "Reducing Sugars (ASBC)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- A user-defined calibration is necessary.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.20 g/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **0.40 g/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"3"** line and enter the concentration of **0.60 g/l** for the third calibration solution.
  Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Analytical Procedures

27 Reducing Sugars, Henry method - malt (ASBC method)

Select the **"Conc."** field in the **"4"** line and enter the concentration of **0.80 g/l** for the fourth calibration solution.
Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"5"** line and enter the concentration of **1.00 g/l** for the fifth calibration solution.
Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Activate the **<U-CAL on>** and **<linear>** fields.

Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.

- After making a user-defined calibration measure a sample blank.
  Do this by tapping the **<Settings>** button and selecting the **<SAMPLE BLANK>** menu item. Fill the cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the sample blank by tapping **<OK>** to confirm.
- Fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in g/l dextrose from the display.

27.8 Evaluation

Results are expressed
in g/l dextrose

27.9 Literature

ASBC Methods of Analysis, online. Malt 6, Diastatic Power, B. Diastatic Power (Rapid Method/Henry Method) [Release date 1991, revised 2010].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Malt-6

Analytical Procedures

28 Steam-volatile Phenols (MEBAK method)

The degree of fumigation of whisky malts is determined by analyzing steam-volatile phenols. In the beer industry, small amounts of smoke-dried malts are used to produce "Rauchbiere" (smoked beers), a specialty of Franconia (Germany). Technical problems during kilning can, however, impart a smoky taste to malts that are intended for the production of normal beers. This taste is carried through into the finished product, resulting in complaints from consumers.
Besides organoleptic checks, spectrophotometric determination of the steam-volatile phenols has proved to be the best method of identifying malt batches that will impart the undesirable smoky taste, and of determining the extent to which tank beer and beer that has gone through the filling stage is affected.

28.1 Method

The phenol fraction obtained with steam reacts in an alkaline environment with 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (4-aminophenazone) and the oxidizing agent potassium hexacyanoferrate(III) to form a coloring substance; after extraction with chloroform this substance can be measured with a spectrophotometer.
MEBAK specifies the use of a 40-mm rectangular cell for the determination of steam-volatile phenols. It is also possible, however, to take the measurement in a 50-mm rectangular cell. Depending on the cell you are using, please select either method "**Phenols, steam-volatile 40**" or "**Phenols, steam-volatile 50**" on the photometer.

28.2 Measuring range

Malt: 0.00 - 3.00 mg/kg steam-volatile phenols
Beer: 0.00 - 0.30 mg/l steam-volatile phenols

Note
Beers with a phenol concentration >0.3 mg/l can also be analyzed. To achieve this, dilute the chloroform extract accordingly. Consider the dilution in the evaluation.

28.3 Reagents and accessories

- Chloroform for analysis EMSURE®, Cat. No. 102445
- Silicon anti-foaming agent, Cat. No. 107743
- ortho-Phosphoric acid 85 % for analysis EMSURE®, Cat. No. 100573
- Copper(II) sulfate pentahydrate for analysis EMSURE®, Cat. No. 102790
- Ammonia solution 25 % for analysis EMSURE®, Cat. No. 105432
- Ammonium chloride for analysis EMSURE®, Cat. No. 101145
- 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one GR for analysis, Cat. No. 107293
- Potassium hexacyanoferrate(III) for analysis EMSURE®, Cat. No. 104973
- Phenol GR for analysis, Cat. No. 100206

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- pH meter
- 25-ml volumetric flask
- 500-ml volumetric flask
- 1000-ml volumetric flask
- Paper filter
- Funnel
- Steam distillation apparatus
- DLFU mill for milling malt, mill aperture 1 mm
- 1000-ml separating funnel
- 40-mm rectangular cells OS or Rectangular cells, 50 mm, Spectroquant®, Cat. No. 114944

Analytical Procedures

28 Steam-volatile Phenols (MEBAK method)

28.4 Preparing the solutions

- **Copper sulfate solution 10 %:**
  In a glass vessel:
  dissolve 10 g of copper(II) sulfate pentahydrate in
  90 g of H_2O
  (shelf-life 3 months)
- **Dilute ammonia solution:**
  In a glass vessel:
  mix 10 ml of ammonia solution 25 % with
  40 ml of H_2O
  (shelf-life 4 weeks)
- **Ammonium chloride solution 5 %:**
  In a glass vessel:
  dissolve 5 g of ammonium chloride in
  95 g of H_2O
  (shelf-life 4 weeks)
- **Aminoantipyrine solution 2 %:**
  In a glass vessel:
  dissolve 2 g of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one in
  98 g of H_2O
  (prepare freshly every day)
- **Potassium hexacyanoferrate(III) solution 8 %:**
  In a glass vessel:
  dissolve 8 g of potassium hexacyanoferrate(III) in
  92 g of H_2O
  (prepare freshly every day)
- **Phenol stock solution 1000 mg/l phenol:**
  Dissolve 1.000 g phenol in
  approx. 800 ml H_2O
  in a volumetric flask and make up to 1000 ml with H_2O
  (solution remains stable for 1 week when stored at +4 °C)
- **Phenol standard solution 10 mg/l phenol:**
  Pipette 5.0 ml of phenol stock solution 1000 mg/l phenol into a 500-ml volumetric flask, make up to the mark with H_2O, and mix

28.5 Preparation

Steam distillation for **malt**:

- Place **50 g of coarse malt** in the distillation flask together with **500 ml of H_2O**
- Add **3 ml of copper sulfate solution**
- Add **phosphoric acid 85 %** to achieve a **pH <4**
- Add **1 drop of silicon anti-foaming agent**
- Carry out steam distillation until a volume of **300 ml of distillate** has been obtained

Steam distillation for **beer**:

- Place **300 ml of beer** in the distillation flask
- Add **3 ml of copper sulfate solution**
- Add **phosphoric acid 85 %** to achieve a **pH <4**
- Add **1 drop of silicon anti-foaming agent**
- Carry out steam distillation until a volume of **300 ml of distillate** has been obtained

Analytical Procedures

28 Steam-volatile Phenols (MEBAK method)

28.6 Calculation of the calibration curve

As a measure to compensate any fluctuations due to the reagents used, a user-defined calibration curve must be recorded.

The calibration for the analysis of malt and beer samples is identical. The selection of the type of sample prior to the measurement (see "Measurement") automatically prompts the calculation of the content in mg/kg for malt and, respectively, in mg/l for beer, taking the different sample-preparation procedures required for the two types of sample into due account.

User-defined calibration is necessary in the following cases:

- When the **stored calibration** is to be overwritten.
- When **exchanging batches** of the reagents used.

28.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution		
	E0 [0.0 mg/l phenol]	1 [0.05 mg/l phenol]	2 [0.10 mg/l phenol]
Phenol standard solution **10 mg/l phenol**	0 ml	2.5 ml	5.0 ml
	• Pipette into separate 500-ml volumetric flasks, make up each flask to the mark with H_2O, and mix		

- Prepare calibration solutions in the following manner:

	Calibration solution		
	E0 [0.0 mg/l phenol]	1 [0.05 mg/l phenol]	2 [0.10 mg/l phenol]
Each standard solution (E0 - 2)	300 ml	300 ml	300 ml
	• Carry out the **steam-distillation procedure** for each solution. Use only fresh solutions!		
Distillate	300 ml	300 ml	300 ml
Ammonium chloride solution	10 ml	10 ml	10 ml
	• Add and mix • Adjust **pH to 10.1 - 10.3 with ammonia solution** • Transfer to the separating funnel		
Aminoantipyrine solution	3 ml	3 ml	3 ml
	• Add		
Potassium hexacyanoferrate(III) solution	3 ml	3 ml	3 ml
• Add and mix • **Leave to stand for 3 min** • Shake out **three times**, for **1 min each time**, with separate portions of **10 ml of chloroform**, and leave to settle for approx. 10 min • **Collect chloroform phases** • Filter combined chloroform phases over a paper filter into a 25-ml volumetric flask • Rinse filter with chloroform • Fill volumetric flask to the mark with chloroform and mix			

Analytical Procedures

28 Steam-volatile Phenols (MEBAK method)

Reagent blank:

- **To 300 ml of H_2O**
  add **10 ml of ammonium chloride solution** and mix
- Adjust **pH to 10.1 - 10.3 with ammonia solution**
- Transfer to the separating funnel
- Add **3 ml of aminoantipyrine solution**
- Add **3 ml of potassium hexacyanoferrate(III) solution** and mix
- **Leave to stand for 3 min**
- Shake out **three times**, for **1 min each time**, with separate portions of **10 ml of chloroform**, and leave to settle for approx. 10 min
- **Collect chloroform phases**
- Filter combined chloroform phases over a paper filter into a 25-ml volumetric flask
- Rinse filter with chloroform
- Fill volumetric flask to the mark with chloroform and mix

Measurement sample:

- **To the distillate** (for smoke-dried malt or whisky malts less than 300 ml)
  add **10 ml of ammonium chloride solution** and mix
- Adjust **pH to 10.1 - 10.3 with ammonia solution**
- Transfer to the separating funnel
- Add **3 ml of aminoantipyrine solution**
- Add **3 ml of potassium hexacyanoferrate(III) solution** and mix
- **Leave to stand for 3 min**
- Shake out **three times**, for **1 min each time**, with separate portions of **10 ml of chloroform**, and leave to settle for approx. 10 min
- **Collect chloroform phases**
- Filter combined chloroform phases over a paper filter into a 25-ml volumetric flask
- Rinse filter with chloroform
- Fill volumetric flask to the mark with chloroform and mix

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2621 "Phenols, steam-volatile 40"** or **method No. 2622 "Phenols, steam-volatile 50"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- For method No. 2621 and 2622 it is recommended to measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank".
- **User-defined calibration** is necessary.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.05 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **0.10 mg/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.

  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

  Once all calibration solutions have been measured, save the calibration by pressing <**OK**>.

Analytical Procedures

28 Steam-volatile Phenols (MEBAK method)

- Fill measurement sample into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Select the **"Beer"** or **"Malt"** sample type by tapping the **<Citation Form>** display field.
- Read off the result in mg/kg steam-volatile phenols (**malt**) or mg/l steam-volatile phenols (**beer**) from the display.

28.8 Evaluation

Results are expressed
malt: in mg/kg steam-volatile phenols
beer: in mg/l steam-volatile phenols

Assessment
Malts: <0.20 mg/kg: no smoky taste is to be anticipated
Beers: <0.03 mg/l: no smoky taste is to be anticipated

Remark
The impact of the smoky taste is to a certain degree dependent on the composition of the beer, which is why the stated lower limit applies only with restrictions.

Wheat beers cannot be analyzed by this method, because the activity of the top-fermenting yeast results in the presence of a considerable amount of steam-volatile phenols, which, however, do not impart a smoky taste.

28.9 Literature

MEBAK Brautechnische Analysemethoden, Volume Rohstoffe,
1st Edition 2006, Method 3.1.4.13, page 219ff

Analytical Procedures

29 Sulfur Dioxide, *p*-Rosaniline method - malt (ASBC method)

29.1 Method

Sulfur dioxide reacts with para-rosaniline and formaldehyde to form a coloring substance, which can be measured spectrophotometrically.
The method is **not preprogrammed**. A "User-defined Concentration Method" incl. calibration must be created.

29.2 Measuring range

0.0 - 50.0 mg/kg SO_2

29.3 Reagents and accessories

- Pararosaniline (chloride) Certistain®, Cat. No. 107509
- Hydrochloric acid fuming 37% for analysis EMSURE®, Cat. No. 100317
- Formaldehyde solution about 37% for analysis, Cat. No. 104003
- Mercury(II) chloride for analysis EMSURE®, Cat. No. 104419
- Sodium chloride for analysis EMSURE®, Cat. No. 106404
- Glycerol for analysis EMSURE®, Cat. No. 104092
- Sodium azide ReagentPlus®, Cat. No. S2002 (Sigma-Aldrich)
- Starch soluble GR for analysis ISO, Cat. No. 101252
- Sodium disulfite (sodium metabisulfite) for analysis EMSURE®, Cat. No. 106528
- Iodine solution 0.05 mol/l Titripur®, Cat. No. 109099
- Sodium thiosulfate solution c($Na_2S_2O_3$ x 5 H_2O) = 0.1 mol/l Titripur®, Cat. No. 109147
- Unsulfured malt

- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.001 g
- Water bath 70 °C
- Water bath 25 °C
- 50-ml buret
- 50-ml brown glass bottle, glass stoppered
- 500-ml brown glass bottle, glass stoppered
- 50-ml volumetric flask, glass-stoppered
- 100-ml volumetric flask, glass-stoppered
- 500-ml volumetric flask, glass-stoppered
- 1000-ml volumetric flask, glass-stoppered
- 250-ml conical flask
- Adjustable pipettes 1.0 - 5.0 ml
- 10-ml volumetric pipette
- 30-ml volumetric pipette
- 50-ml volumetric pipette
- 100-ml volumetric pipette
- Test tubes
- Funnel with paper filter, Whatman No. 1
- Stop watch
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

Analytical Procedures

29 Sulfur Dioxide, *p*-Rosaniline method - malt (ASBC method)

29.4 Preparing the solutions

- **Glycerole solution 5 %:**
  In a glass vessel:
  mix 25 ml of glycerol with
  475 ml of H_2O
- **Sodium azide solution:**
  Dissolve 0.3 g sodium azide in
  approx. 80 ml glyercole solution 5%
  in a 100-ml volumetric flask, make up to 100 ml with glycerol solution 5% and mix
- **Stock extracting solution:**
  Dissolve 6.8 g of mercury chloride,
  2.925 g of sodium chloride and
  2.5 ml of sodium azide solution in
  approx. 80 ml glycerol solution 5%
  in a 250-ml volumetric flask, make up to 250 ml with glycerol solution 5% and mix
- **Diluted extracting solution:**
  In a glass vessel:
  mix 60 ml of stock extracting solution with
  900 ml of H_2O
  (if no calibration is performed, amount can be reduced)
- **Formaldehyde solution:**
  Place 0.54 ml of formaldehyde solution 37 % in
  a 100-ml volumetric flask, make up to 100 ml with H_2O and mix.
  (prepare freshly every day)
- ***p*-Rosaniline solution:**
  In a 500-ml volumetric flask:
  dissolve 200 mg of pararosaniline in
  approx. 400 ml of H_2O by heating to 70 °C in a water bath for 20 min (swirl from time to time)
  cool to 20 °C,
  add 30 ml of hydrochloric acid 37 % and mix
  make up to 500 ml with H_2O.
  After preparation, transfer the solution into a brown, glass-stoppered bottle and before use, let it stand for 15 min at room temperature.
  (solution is stable for one month at 5 °C in a brown bottle)
- **Color reagent:**
  Mix 25 ml of *p*-rosaniline solution with
  25 ml of formaldehyde solution
  Store in a brown, glass stoppered bottle
  (prepare freshly every day)
- **Starch solution 1 %:**
  In a glass vessel:
  dissolve 1 g starch in
  99 g of H_2O
- **Sulfur dioxide stock solution 500 mg/l SO_2:**
  Accurately weigh the calculated amount of sodium disulfite based on the assay determined acc. to chapter 29.5 into a 1000-ml volumetric flask, dissolve in water, make up to 1000 ml with H_2O and mix.
  Immediately proceed with preparing the standard solution 25 mg/l SO_2.

  The amount of sodium disulfite needed for preparing the stock solution is calculated as follows:

$$\text{Sample weight [g]} = \frac{0.742\ \text{g} \cdot 100}{\text{assay [\%]}}$$

  with assay assay of sodium disulfite determined in section 29.5

Analytical Procedures

29 Sulfur Dioxide, *p*-Rosaniline method - malt (ASBC method)

- **Sulfur dioxide standard solution 25 mg/l SO_2:**
  Pipette 2.5 ml of sulfur dioxide stock solution 500 mg/l SO_2
  in a 50-ml volumetric flask, make up to the mark with stock extracting solution and mix
- **Sulfur dioxide standard solution 10 mg/l SO_2:**
  Pipette 20 ml of sulfur dioxide stock solution 25 mg/l SO_2
  in a 50-ml volumetric flask, make up to the mark with diluted extracting solution and mix

29.5 Precise assay of the sodium disulfite

To ensure an accurate standard solution the assay of the sodium disulfite must be determined in advance by titration with sodium thiosulfate solution

Procedure:
- Weigh 118.8 mg of sodium disulfite into a 250-ml conical flask and note the amount
- Add **50 ml of iodine solution 0.05 mol/l** and immediately stopper the flask
- Swirl to dissolve sodium disulfite and let the solution stand in a **dark place for 5 min** at room temperature
- Add **1 ml of hydrochloric acid 37 %**
- After mixing the contents of the flask, subsequently **titrate with sodium thiosulfate solution 0.1 mol/l** until the yellow iodine color has disappeared
- Add **1 ml of starch solution 1%**, and continue to titrate from blue to colorless
- Note the volume of sodium thiosulfate solution 0.1 mol/l
- Calculate the assay of sodium disulfite as follows:

$$\text{assay [\%]} = \frac{0.05\ \text{mol/l} \cdot (50\ \text{ml} - V_{Na_2S_2O_3}\ \text{[ml]}) \cdot 0.5 \cdot 190.107\ \text{g/mol} \cdot 100}{m_{\text{sample weight}}\ \text{[mg]}}$$

$$\text{assay [\%]} = \frac{475 \cdot (50\ \text{ml} - V_{Na_2S_2O_3}\ \text{[ml]})}{m_{\text{sample weight}}\ \text{[mg]}}$$

with $V_{Na_2S_2O_3}$ Volume of sodium thiosulfate solution 0.1 mol/l in ml
$m_{\text{sample weight}}$ weight of sample in mg

29.6 Preparation

- Remove rootlets from the malt
- Grind 25 g of malt using a mill (fine grind) (acc. to ASBC Malt-4). The sample should be tempered to room temperature
- Homogenize the finely ground malt

29.7 Calculation of the calibration curve

The method is **not preprogrammed** in the photometer. A "User-defined Concentration Method" has to be created and a user-defined calibration must be performed.
It is advisable to recalibrate the method when exchanging batches of the reagents used. After making this user-defined calibration it is also necessary to measure a reagent blank.

A re-calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.

Analytical Procedures

29 Sulfur Dioxide, *p*-Rosaniline method - malt (ASBC method)

29.8 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution					
	E0 [0.0 mg/kg SO_2]	1 [10.0 mg/kg SO_2]	2 [20.0 mg/kg SO_2]	3 [30.0 mg/kg SO_2]	4 [40.0 mg/kg SO_2]	5 [50.0 mg/kg SO_2]
Sulfur dioxide standard solution 10 mg/l SO_2	0.0 ml	2.0 ml	4.0 ml	6.0 ml	8.0 ml	10.0 ml
	• Pipette into separate 100-ml volumetric flasks, make up to 100-ml with diluted extracting solution and mix					

- Prepare calibration solutions in the following manner:

	Calibration solution					
	E0 [0.0 mg/kg SO_2]	1 [10.0 mg/kg SO_2]	2 [20.0 mg/kg SO_2]	3 [30.0 mg/kg SO_2]	4 [40.0 mg/kg SO_2]	5 [50.0 mg/kg SO_2]
Unsulfured, finely ground malt	2.0 g	2.0 g	2.0 g	2.0 g	2.0 g	2.0 g
	• Weigh in separate 250-ml conical flasks (glass stoppered)					
Each standard solution (E0-5)	• Transfer whole content of the 100-ml volumetric flask of each standard solutioninto the separate 250-ml conical flasks					
	• Stir or shake contents for 30 min or blend for 5 min • Immediately after shaking filter through a filter paper • Refilter the first few ml of the filtrate					
Filtrate of each standard solution	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
	• Transfer into separate test tubes					
Color reagent	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
	• Pipette into each test tube and mix by inverting twice • Leave the solution to stand for 35 min at 25 ±2 °C					

Reagent blank:

- Weigh **2.0 g of finely ground, unsulfured malt** into a 250-ml conical flask (glass stoppered)
- Add **100 ml of diluted extracting solution**
- **Stir or shake** contents for 30 min or blend for 5 min
- **Immediately** after shaking **filter** through a filter paper
- **Refilter** the first few ml of the filtrate
- Transfer **10 ml of the filtrate** into a test tube
- Add **2 ml of color reagent** and mix by inverting twice
- Leave the solution to stand for **35 min at 25 ±2 °C**

Measurement sample:

- Weigh **2.0 g of finely ground, malt sample** into a 250-ml conical flask (glass stoppered)
- Add **100 ml of diluted extracting solution**
- **Stir or shake** contents for 30 min or blend for 5 min
- **Immediately** after shaking **filter** through a filter paper
- **Refilter** the first few ml of the filtrate
- Transfer **10 ml of the filtrate** into a test tube
- Add **2 ml of color reagent** and mix by inverting twice
- Leave the solution to stand for **35 min at 25 ±2 °C**

Analytical Procedures

29 Sulfur Dioxide, *p*-Rosaniline method - malt (ASBC method)

Note
The extraction conditions of the measurement sample and the blank must be exactly the same as those of the prepared calibration standards.

Creating a user-defined method:
The method is **not preprogrammed** in the photometer. A "User-defined Concentration Method" has to be created. Do this as follows:

- Open the method list (<Methods>) and tap on the field **<Add New Method>**
- Select the method type "Concentration"
- Fill out the input field:

Input field	Input
Name	Sulfur dioxide in malt
Wavelength	560 nm
Cell	10 mm*
Citation form	SO_2
Unit	mg/kg
Resolution	0.1
Lower and upper limit of the measuring range	0.0-50.0 mg/kg
User-defined range	user-defined

* There is no cell specified in the ASBC method. All cell sizes appropriate for the analysis may be used. In those cases ensure to perform all measurements (calibration, sample blank, measurement sample) with cells of the same size.

- Tap on the calibration field.
- Tap on the field **<value pairs>**.
- An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **10.0 mg/kg** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **20.0 mg/kg** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"3"** line and enter the concentration of **30.0 mg/kg** for the third calibration solution.

  Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"4"** line and enter the concentration of **40.0 mg/kg** for the fourth calibration solution.
  Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Analytical Procedures

29 Sulfur Dioxide, *p*-Rosaniline method - malt (ASBC method)

Select the **"Conc."** field in the **"5"** line and enter the concentration of **50.0 mg/kg** for the fifth calibration solution.
Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
Activate the **<linear>** field.

Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.

Measurement:

- Open the method list (**<Methods>**) and select **method "Sulfur dioxide in malt"**.
- Tap the **<START>** button to start the measurement procedure for the next sample.
- Measure a reagent blank.
  Do this by tapping the **<Settings>** button and selecting the **<REAGENT BLANK>** menu item. Fill the cell with the reagent blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the reagent blank by tapping **<OK>** to confirm.
  Activate the reagent blank by activating the **<USER RB>** field and accept with **<OK>**.
  It is recommended to measure a new reagent blank each new working day and each time the batch of reagents used is changed. In those cases proceed as described in section VII "Reagent blank"
- Fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/kg sulfur dioxide from the display.

29.9 Evaluation

Results are expressed
in mg/kg sulfur dioxide

29.10 Literature

ASBC Methods of Analysis, online. Malt-11, Sulfur Dioxide, [Release date 1982, revised 2009].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Malt-11

ASBC Methods of Analysis, online. Beer-21, Total sulfur dioxide, A. p-Rosaniline method [Release date 1960, revised 1975].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-21

Analytical Procedures

30 Thiobarbituric Acid Number (TAN) - beer / wort (MEBAK / ASBC method)

The thiobarbituric acid number counts as a sum parameter for the thermal effects on malt and wort. It is a parameter that measures not only 5-hydroxymethylfurfural (HMF) but also a multitude of products of the Maillard reaction and other organic compounds.

30.1 Method

A reaction is started in the test sample (wort, beer, congress wort/malt extract) with acetic thiobarbituric acid solution, and the resultant yellow color is measured by spectrophotometry.

30.2 Measuring range

Thiobarbituric acid number: 0 - 250

30.3 Reagents and accessories

- Acetic acid (glacial) 100 % anhydrous for analysis EMSURE®, Cat. No. 100063
- 2-Thiobarbituric acid, Cat. No. 108180
- if clarification is necessary (acc. to MEBAK): Kieselguhr GR for analysis, Cat. No. 107910
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 100-ml volumetric flask
- Water bath (70 °C)
- Brown test tubes with ground-glass stopper, 20 - 25 ml content
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946
- if clarification is necessary (acc. to ASBC): Glass fiber filter paper

30.4 Preparing the solutions

- **Acetic acid 90 %:**
  In a glass vessel:
  mix 225 g of acetic acid 100 % with
  25 g of H_2O
  (shelf-life 3 months)
- **Thiobarbituric acid solution 0.02 mol/l:**
  With heating dissolve 0.288 g of 2-thiobarbituric acid in
  approx. 90 ml of acetic acid 90 % in a 100-ml volumetric flask
  Cool to 20 °C and make up to the mark with acetic acid 90 %
  (prepare freshly every day)

30.5 Preparation

- Clarify **turbid samples**:
  - acc. to MEBAK: Clarify over Kieselguhr
  - acc. to ASBC: Clarify using glass fiber filter paper
- **Dilute the sample** in such a way that the absorbance of the measurement sample is at least 0.1 A higher than that of the sample blank. As a rule:
  - **congress worts:** dilute 1 + 4 with H_2O (dilution factor: 5)
  - **worts and beer:** dilute 1 + 9 with H_2O (dilution factor: 10)
  - **dark strong beer:** dilute 1 + 99 with H_2O (dilution factor: 100)

  If this is not the case, the message **"Condition not met"** appears in the display at the end of the measurement procedure. The analysis should be repeated with a lower dilution.

Analytical Procedures

30 Thiobarbituric Acid Number (TAN) - beer / wort (MEBAK / ASBC method)

30.6 Procedure and measurement

Sample blank:

- Pipette **10 ml of the diluted sample** into a test tube with a ground-glass stopper
- Add **5 ml of acetic acid 90 %** and mix
- **Close** test tube and heat in a water bath at **70 °C** for **70 min (acc. to MEBAK)** or at **70 °C** for **60 min (acc. to ASBC)**
  (avoid direct sunlight and use amber tubes or wrap tubes with aluminium foil. Ensure that the temperature of the bath drops only briefly by 1 - 2 °C when the test tubes are introduced)
- **After the temperature time has elapsed swiftly cool** the test tubes **to 20 °C** (cooling bath or under a strong flow of tapwater)

Measurement sample:

- Pipette **10 ml of the diluted sample** into a test tube with a ground-glass stopper
- Add **5 ml of thiobarbituric acid solution** and mix
- **Close** test tube and heat in a water bath at **70 °C** for **70 min (acc. to MEBAK)** or at **70 °C** for **60 min (acc. to ASBC)**
  (avoid direct sunlight and use amber tubes or wrap tubes with aluminium foil. Ensure that the temperature of the bath drops only briefly by 1 - 2 °C when the test tubes are introduced)
- **After the temperature time has elapsed swiftly cool** the test tubes **to 20 °C** (cooling bath or under a strong flow of tapwater)
- **Immediately** fill into a 10-mm rectangular cell and **measure immediately**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2619 "Thiobarbituric Acid No."**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The sample dilution must be entered.
  An input mask pops up. Enter the dilution and tap **<OK>** to confirm.
- Tap the **<START>** button to start the measurement procedure.
- Subsequently fill sample blank into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample Blank"**. Confirm the message with <**OK**>.
- Fill measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**. Confirm the message with <**OK**>.
- Read off the result as thiobarbituric acid number from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.
- If the message "Condition not met" appears, proceed as described in section 30.5.

Analytical Procedures

30 Thiobarbituric Acid Number (TAN) - beer / wort (MEBAK / ASBC method)

30.7 Evaluation

Results are expressed
as thiobarbituric acid number (TAN)

Standard values

Light wort (before cooking):	<22 (for 12 % original wort)
Light finished wort:	<60 (for 12 % original wort)
Light cold wort (after wort cooling):	<60 (for 12 % original wort)

30.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.4, page 35ff

ASBC Methods of Analysis, online. Wort-21, Thiobarbituric acid index [Release date 2009, revised 2010].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Wort-21

Analytical Procedures

31 Total Carbohydrates (EBC / MEBAK method)

31.1 Method

5-Hydroxymethylfurfural formed by hydrolysis with sulfuric acid and the dehydration of carbohydrates reacts with anthrone to produce a blue-green color, which is measured by spectrophotometry.

31.2 Measuring range

0.000 - 6.000 g/100 ml total carbohydrates

31.3 Reagents and accessories

- Sulfuric acid 95 - 97 % for analysis EMSURE®, Cat. No. 100731
- Anthrone GR for analysis, Cat. No. 101468
- D-Glucose anhydrous
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 100-ml volumetric flask
- 500-ml volumetric flask
- 1000-ml volumetric flask
- Water bath (2 - 4 °C)
- Water bath (95 ± 5 °C)
- test tubes with ground-glass stopper, 20 x 150 mm
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

31.4 Preparing the solutions

- **Sulfuric acid 85 % (V/V):**
  To 150 ml of H_2O carefully and with cooling
  add 850 ml of H_2SO_4 95 - 97 %, allow to cool completely to room temperature, mix, and make up to 1000 ml with H_2O in a volumetric flask
  (shelf-life 3 months)
- **Anthrone reagent:**
  Dissolve 1 g of anthrone
  in approx 800 ml of H_2SO_4 85 %,
  after complete dissolution make up to 1000 ml with H_2SO_4 85 % in a volumetric flask
  (prepare fresh, cool to 2 - 4 °C prior to use)
- **D-glucose standard:**
  Dry anhydrous D-glucose for 4 h at 100 °C and 100 mbar in a vacuum drying cabinet
- **D-glucose stock solution 400 mg/l glucose:**
  Dissolve 0.4 g of D-glucose anhydrous
  in approx. 800 ml H_2O,
  after complete dissolution make up to 1000 ml with H_2O in a volumetric flask
  (solution remains stable for 1 week when stored at +4 °C)
- **D-glucose standard solution 40 mg/l glucose:**
  Place 10 ml of D-glucose stock solution 400 mg/l glucose
  in a 100-ml volumetric flask, make up to 100 ml with H_2O and mix
  (prepare fresh)

Analytical Procedures

31 Total Carbohydrates (EBC / MEBAK method)

31.5 Preparation

- Expel carbon dioxide from **beer**, allow froth to disintegrate.
- In a volumetric flask dilute 2 ml of beer with H_2O to make 500 ml (dilution 1 + 249).

31.6 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	E0 [0.000 g/100 ml total carbohydrates]	1 [0.004 g/100 ml total carbohydrates]
Water	3.0 ml	-
Glucose standard solution 40 mg/l	-	3.0 ml
	• Pipette into separate test tubes	
Anthrone reagent (cooled to 2 - 4 °C)	10 ml	10 ml
	• Add and mix thoroughly with cooling • Loosely close test tubes with glass stoppers to prevent losses by evaporation • Heat in a **water bath at 95 ± 0.5 °C** for **exactly 20 min** • After the **temperature time has elapsed swiftly cool test tubes to 20 °C** (cooling bath or under a strong flow of tapwater	

Reagent blank:

- Pipette **3.0 ml of H_2O** into a test tube, cool to 2 - 4 °C
- Add **10 ml of anthrone reagent** (cooled to 2 - 4 °C)
  mix thoroughly with cooling
- Loosely close test tubes with glass stoppers to prevent losses by evaporation
- Heat in a **water bath at 95 ± 0.5 °C** for **exactly 20 min**
- After the **temperature time has elapsed swiftly cool test tubes to 20 °C** (cooling bath or under a strong flow of tapwater)

Measurement sample:

- Pipette **3.0 ml of diluted beer** into a test tube, cool to 2 - 4 °C
- Add **10 ml of anthrone reagent** (cooled to 2 - 4 °C)
  mix thoroughly with cooling
- Loosely close test tubes with glass stoppers to prevent losses by evaporation
- Heat in a **water bath at 95 ± 0.5 °C** for **exactly 20 min**
- After the **temperature time has elapsed swiftly cool test tubes to 20 °C** (cooling bath or under a strong flow of tapwater)

Analytical Procedures

31 Total Carbohydrates (EBC / MEBAK method)

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2625 "Total Carbohydrates"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The sample dilution must be entered.
  Do this by tapping the **<Settings>** button and selecting the **<DILUTION>** menu item. Activate the field for entering the dilution, enter the dilution and tap **<OK>** to confirm.
- **Measurement of the reagent blank** is necessary. Measure a new reagent blank each new working day and each time the batch of the reagents used is exchanged.
  To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank".
- **User-defined calibration** is necessary.
  Do this by tapping the **<Settings>** button and selecting the **<RECALIBRATION>** menu item. An input mask pops up.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **0.004 g/100 ml** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Activate the **<U-CAL on>** and **<linear>** fields.
  Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.
  Once all calibration solutions have been measured, save the calibration by pressing <**OK**>.
- Fill measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in g/100 ml total carbohydrates from the display.

31.7 Evaluation

Results are expressed
in g/100 ml

31.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.11, page 85ff

Analytica-EBC, Section 9 Beer, Method 9.26

Analytical Procedures

32 Total Polyphenols (EBC / MEBAK / ASBC method)

Depending on the technological measures being used, phenolic compounds are imported from malt and hops into beer in varying amounts. Depending on their structure and molecular size they exert a strong influence on various beer characteristics such as color, flavour, flavour stability, froth, and chemicophysical stability. Unfavorable conditions, e. g. a high content of polymerizable and/or condensable compounds and atmospheric oxygen, result in protein-precipitating byproducts with undesirable effects on the flavour.

32.1 Method

In alkaline solution polyphenols react with iron(III) ions to form colored iron complexes; the resultant brownish color is measured by spectrophotometry.

32.2 Measuring range

0 - 800 mg/l total polyphenols

32.3 Reagents and accessories

- Carboxymethylcellulose, Sodium Salt, Low Viscosity, Calbiochem®, Cat. No. 217277
- Ethylenedinitrilotetraacetic acid disodium salt dihydrate, Cat. No. 108454 or Titriplex® III, Cat. No. 108418
- Ammonium iron(III) citrate, Cat. No. 103762
- Ammonia solution 25 % for analysis EMSURE®, Cat. No. 105432
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 25-ml volumetric flask
- 100-ml volumetric flask
- 1000-ml volumetric flask
- Mechanical shaker
- for turbid samples: centrifuge and centrifuge glasses
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

32.4 Preparing the solutions

- **Carboxymethylcellulose-ethylenediaminotetraacetic acid solution (CMC-EDTA solution):**
  With stirring slowly dissolve 10 g of CMC and
  2 g of EDTA-Na_2
  in approx. 500 ml of H_2O,
  after complete dissolution make up to 1000 ml with H_2O in a volumetric flask,
  if necessary clarify by centrifuging
  (shelf-life 1 month)
- **Iron(III) solution:**
  In a glass vessel:
  dissolve 3.5 g of ammonium iron(III) citrate in
  approx. 80 ml of H_2O
  in a 100-ml volumetric flask, make up to 100 ml with H_2O and mix
  (solution must be completely clear and remains stable for approx. 1 week when stored in dark bottles)

Analytical Procedures

32 Total Polyphenols (EBC / MEBAK / ASBC method)

- **Ammonia solution:**
  Prepare the ammonia solution depending on the chosen standard method as follows:

	MEBAK/EBC	ASBC
Ammonia solution 25 %	5 ml	5.8 ml
	• Place in a glass vessel	
H_2O	10 ml	9.2 ml
	• Add and mix	

32.5 Preparation

- Expel carbon dioxide from **beer** by shaking.
- Clarify turbid worts or beers by centrifuging.

32.6 Procedure and measurement

Important:

The accuracy is influenced to a major degree by the clarity of the test solution. At the same time, the elimination of any turbidity may result in the stripping of polyphenols, which may in turn result in a false-low finding.

Sample blank:

- Pipette **10 ml of decarbonized beer** or **wort** and **8 ml of CMC-EDTA solution** into a 25-ml volumetric flask and mix thoroughly
- Add **0.5 ml of ammonia solution** and mix thoroughly
- Make up to the mark with H_2O and mix thoroughly
- Leave to stand for **10 min**

Measurement sample:

- Pipette **10 ml of decarbonized beer** or **wort** and **8 ml of CMC-EDTA solution** into a 25-ml volumetric flask and mix thoroughly
- Add **0.5 ml of iron(III) solution** and mix thoroughly
- Add **0.5 ml of ammonia solution** and mix thoroughly
- Make up to the mark with H_2O and mix thoroughly
- Leave to stand for **10 min**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2610 "Total Polyphenols"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill sample blank into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample Blank"**. Confirm the message with **<OK>**.
- Fill measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**. Confirm the message with **<OK>**.
- Read off the result in mg/l total polyphenols from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

Analytical Procedures

32 Total Polyphenols (EBC / MEBAK / ASBC method)

32.7 Evaluation

Results are expressed
in mg/l total polyphenols

Standard values
Beer: 150 - 200 mg/l total polyphenols

32.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.17.1, page 107ff

Analytica-EBC, Section 8 Wort, Method 8.12

Analytica-EBC, Section 9 Beer, Method 9.11

ASBC Methods of Analysis, online. Beer-35, Total Polyphenols [Release date 1978, reviewed and revised 2015].
American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-35

Analytical Procedures

33 Total Sulfur Dioxide, *p*-Rosaniline method - beer (ASBC method)

Sulfur dioxide is produced by the yeast during the fermentation process. It has an anti-oxidative function and avoids the production of carbonyl compounds which lead to a stale taste of the beer.
A too high amount of sulfur dioxide on the other side leads to a bad taste of the beer.

33.1 Method

Sulfur dioxide reacts with para-rosaniline and formaldehyde to form a coloring substance, which can be measured spectrophotometrically.
The method is **not preprogrammed**. A "User-defined Concentration Method" incl. calibration must be created.

33.2 Measuring range

0.0 - 16.0 mg/l SO_2

33.3 Reagents and accessories

- Pararosaniline (chloride) Certistain®, Cat. No. 107509
- Hydrochloric acid fuming 37% for analysis EMSURE®, Cat. No. 100317
- Formaldehyde solution about 37% for analysis, Cat. No. 104003
- Mercury(II) chloride for analysis EMSURE®, Cat. No. 104419
- Sodium chloride for analysis EMSURE®, Cat. No. 106404
- Sodium hydroxide solution 0.1 mol/l Titripur®, Cat. No. 109141
- Sulfuric acid 0.05 mol/l Titripur®, Cat. No. 109074
- Starch soluble GR for analysis ISO, Cat. No. 101252
- 1-Hexanol for synthesis, Cat. No. 804393
- Sodium disulfite (sodium metabisulfite) for analysis EMSURE®, Cat. No. 106528
- Iodine solution 0.05 mol/l Titripur®, Cat. No. 109910
- Sodium thiosulfate solution c($Na_2S_2O_3$ x 5 H_2O) = 0.1 mol/l Titripur®, Cat. No. 109147
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- Analytical balance, accurate to 0.001 g
- Water bath (25 °C)
- 50-ml buret
- 250-ml brown glass bottle, glass stoppered
- 1000-ml brown glass bottle, glass stoppered
- 50-ml volumetric flask, glass-stoppered
- 100-ml volumetric flask, glass-stoppered
- 250-ml volumetric flask, glass-stoppered
- 500-ml volumetric flask, glass-stoppered
- 250-ml conical flask
- 10-ml graduated flask
- Adjustable pipettes 1.0 - 5.0 ml
- 6-ml volumetric pipette
- 8-ml volumetric pipette
- 10-ml volumetric pipette
- 15-ml volumetric pipette
- 20-ml volumetric pipette
- 25-ml volumetric pipette
- 50-ml volumetric pipette

Analytical Procedures

33 Total Sulfur Dioxide, *p*-Rosaniline method - beer (ASBC method)

- Pasteur pipettes
- Stop watch
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

33.4 Preparing the solutions

- **Color reagent:**
  Dissolve 100 mg of pararosaniline in
  approx. 200 ml of H_2O in a 250-ml volumetric flask,
  add 20 ml of hydrochloric acid 37 % and mix
  Make up to 250 ml with H_2O
  After preparation, transfer the solution into a brown, glass-stoppered bottle
  and before use, leave to stand for 15 min at room temperature
  Store at 0 - 4 °C

- **Formaldehyde solution:**
  Place 5.0 ml of formaldehyde solution 37 % in
  a 1000-ml volumetric flask, make up to 1000 ml with H_2O and mix.
  Store in a brown, glass-stoppered bottle in the refrigerator.

- **Stabilizing solution:**
  Dissolve 2.72 g of mercury chloride and
  1.17 g of sodium chloride in
  approx. 80 ml of H_2O
  in a 100-ml volumetric flask, make up to 100 ml with H_2O and mix

- **Starch solution 1 %:**
  In a glass vessel:
  issolve 1 g starch in
  99 g of H_2O

- **Sulfur dioxide stock solution 500 mg/l SO_2:**
  Accurately weigh the calculated amount of sodium disulfite based on the assay determined acc. to 33.5
  into a 1000-ml volumetric flask, dissolve in water, make up to 1000 ml with H_2O and mix.
  Immediately proceed with preparing the standard solution 20 mg/l SO_2.

  The amount of sodium disulfite needed for preparing the stock solution is calculated as follows:

$$\text{Sample weight [g]} = \frac{0.742\ \text{g} \cdot 100}{\text{assay [\%]}}$$

  with assay assay of sodium disulfite determined in section 29.5

- **Sulfur dioxide standard solution 20 mg/l SO_2:**
  Pipette 20 ml of stabilizing solution and
  4 ml of sulfur dioxide stock solution 500 mg/l SO_2
  in a 100-ml volumetric flask, make up to the mark with H_2O and mix
  (solution is not stable and must be used immediately)

Analytical Procedures

33 Total Sulfur Dioxide, *p*-Rosaniline method - beer (ASBC method)

33.5 Precise assay of the sodium disulfite

To ensure an accurate standard solution the assay of the sodium disulfite must be determined in advance by titration with sodium thiosulfate solution

Procedure:

- Weigh 118.8 mg of sodium disulfite into a 250-ml conical flask and note the amount
- Add **50 ml of iodine solution 0.05 mol/l** and immediately stopper the flask
- Swirl to dissolve sodium disulfite and let the solution stand in a **dark place for 5 min** at room temperature
- Add **1 ml of hydrochloric acid 37 %**
- After mixing the contents of the flask, subsequently **titrate with sodium thiosulfate solution 0.1 mol/l** until the yellow iodine color has disappeared
- Add **1 ml of starch solution 1%**, and continue to titrate from blue to colorless
- Note the volume of sodium thiosulfate solution 0.1 mol/l
- Calculate the assay of sodium disulfite as follows:

$$\text{assay [\%]} = \frac{0.05\ \text{mol/l} \cdot (50\ \text{ml} - V_{Na_2S_2O_3}\ \text{[ml]}) \cdot 0.5 \cdot 190.107\ \text{g/mol} \cdot 100}{m_{\text{sample weight}}\ \text{[mg]}}$$

$$\text{assay [\%]} = \frac{475 \cdot (50\ \text{ml} - V_{Na_2S_2O_3}\ \text{[ml]})}{m_{\text{sample weight}}\ \text{[mg]}}$$

with $V_{Na_2S_2O_3}$ Volume of sodium thiosulfate solution 0.1 mol/l in ml

$m_{\text{sample weight}}$ weight of sample in mg

33.6 Preparation

- **Cool** sample before use
- Do **not degas** beer

33.7 Calculation of the calibration curve

The method is **not preprogrammed** in the photometer. A "User-defined Concentration Method" has to be created and a user-defined calibration must be performed. Due to matrix effects the calibration is performed with standard solutions prepared with the beer sample.
It is advisable to recalibrate the method when exchanging batches of the reagents used or the beer matrix changes. After making this user-defined calibration it is also necessary to measure a reagent blank.

A re-calibration is necessary in the following cases:

- When **exchanging batches** of the reagents used.
- When the **stored calibration** is to be overwritten.
- When the calibration is influenced by the **sample matrix**.

Analytical Procedures

33 Total Sulfur Dioxide, *p*-Rosaniline method - beer (ASBC method)

33.8 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

	Standard solution							
	E0 [0.0 mg/l SO_2]	1 [2.0 mg/l SO_2]	2 [4.0 mg/l SO_2]	3 [6.0 mg/l SO_2]	4 [8.0 mg/l SO_2]	5 [10.0 mg/l SO_2]	6 [12.0 mg/l SO_2]	7 [16.0 mg/l SO_2]
1-Hexanol	• Add 1 drop of 1-hexanol in a 10-ml graduated measuring cylinder							
Cold, undegassed beer	• Measure 10 ml of cold, undegassed beer into the measuring cylinder							
	• Transfer the content of each measuring cylinder into 8 separate 100-ml volumetric flasks							
Sulfur dioxide standard solution 20 mg/l SO_2	0.0 ml	1.0 ml	2.0 ml	3.0 ml	4.0 ml	5.0 ml	6.0 ml	8.0 ml
	• Pipette into each flask, make up to 100-ml with H_2O and mix							

- Prepare calibration solutions in the following manner:

	Calibration solution							
	E0 [0.0 mg/l SO_2]	1 [2.0 mg/l SO_2]	2 [4.0 mg/l SO_2]	3 [6.0 mg/l SO_2]	4 [8.0 mg/l SO_2]	5 [10.0 mg/l SO_2]	6 [12.0 mg/l SO_2]	7 [16.0 mg/l SO_2]
Each standard solution (E0-7)	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml
	• Pipette into separate 50-ml volumetric flasks							
Color reagent	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
	• Add and swirl							
Formaldehyd solution	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
	• Add and swirl							
	• Make up to 50-ml with H_2O and mix • Place the flask in a water bath, 25 °C and let it stand for 30 min							

Reagent blank:

- Place **1 drop of 1-hexanol** in a 10-ml graduated measuring cylinder
- Measure exactly **10 ml of undegassed, cold beer** into the measuring cylinder
- **Transfer the beer** from the graduated measuring cylinder completely as possible into a 100-ml volumetric flask and swirl
- Add **0.5 ml of starch solution 1 %**
- **Dropwise add iodine solution 0.025 mol/l** (iodine solution 0.05 mol/l diluted 1+1 with H_2O) until the solution is of permanent blue color.
- Add one drop more of iodine solution 0.025 mol/l, make up to 100-ml with H_2O and mix
- When blue color has faded, pipette **25 ml of the solution** into a 50-ml volumetric flask
- Add **5 ml of color reagent** and swirl
- Add **5 ml of formaldehyde solution** and swirl
- Make up to 50-ml with H_2O and mix
- Place the flask in a water bath, 25 °C and leave to stand for **30 min**

Analytical Procedures

33 Total Sulfur Dioxide, *p*-Rosaniline method - beer (ASBC method)

Measurement sample:

- Place **1 drop of 1-hexanol** in a 10-ml graduated measuring cylinder
- Measure exactly **10 ml of undegassed, cold beer** into the measuring cylinder
- Pipette **2 ml of stabilizing solution** and **5 ml of sulfuric acid 0.05 mol/l** into a 100-ml volumetric flask
- **Transfer the beer** from the graduated measuring cylinder completely as possible into a 100-ml volumetric flask and swirl
- Pipette **15 ml of sodium hydroxide solution 0.1 mol/l** into the flask and swirl
- Wait for 15 s
- Pipette **10 ml of sulfuric acid 0.05 mol/l** into the flask, make up to 100-ml with H_2O and mix
- Pipette **25 ml of the solution** into a 50-ml volumetric flask
- Add **5 ml of color reagent** and swirl
- Add **5 ml of formaldehyde solution** and swirl
- Make up to 50-ml with H_2O and mix
- Place the flask in a water bath, 25 °C and leave to stand for **30 min**

Creating a user-defined method:

The method is **not preprogrammed** in the photometer. A "User-defined Concentration Method" has to be created. Do this as follows:

- Open the method list (<Methods>) and tap on the field **<Add New Method>**
- Select the method type "Concentration"
- Fill out the input field:

Input field	Input
Name	Sulfur dioxide in beer
Wavelength	550 nm
Cell	10 mm*
Citation form	SO_2
Unit	mg/l
Resolution	0.1
Lower and upper limit of the measuring range	0.0-16.0 mg/l
User-defined range	user-defined

* There is no cell specified in the ASBC method. All cell sizes appropriate for the analysis may be used. In those cases ensure to perform all measurements (calibration, reagent blank, measurement sample) with cells of the same size.

- Tap on the calibration field.
- Tap on the field **<value pairs>**.
- An input mask pops up.
  Tap on **<+>** in the numerical keyboard to create an additional input line.

  Select the **"Absorbance"** field in the **"E0"** line (selected fields are shown in a blue frame).
  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"1"** line and enter the concentration of **2.0 mg/l** for the first calibration solution.
  Select the **"Absorbance"** field in the **"1"** line. Fill calibration solution 1 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

  Select the **"Conc."** field in the **"2"** line and enter the concentration of **4.0 mg/l** for the second calibration solution.
  Select the **"Absorbance"** field in the **"2"** line. Fill calibration solution 2 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Analytical Procedures

33 Total Sulfur Dioxide, *p*-Rosaniline method - beer (ASBC method)

Select the **"Conc."** field in the **"3"** line and enter the concentration of **6.0 mg/l** for the third calibration solution.

Select the **"Absorbance"** field in the **"3"** line. Fill calibration solution 3 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"4"** line and enter the concentration of **8.0 mg/l** for the fourth calibration solution.
Select the **"Absorbance"** field in the **"4"** line. Fill calibration solution 4 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"5"** line and enter the concentration of **10.0 mg/l** for the fifth calibration solution.
Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"6"** line and enter the concentration of **12.0 mg/l** for the sixth calibration solution.
Select the **"Absorbance"** field in the **"6"** line. Fill calibration solution 6 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Select the **"Conc."** field in the **"7"** line and enter the concentration of **16.0 mg/l** for the seventh calibration solution.
Select the **"Absorbance"** field in the **"7"** line. Fill calibration solution 7 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

Activate the **<linear>** field.

Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.

Measurement:

- Open the method list (**<Methods>**) and select **method "Sulfur dioxide in beer"**.
- Tap the **<START>** button to start the measurement procedure for the next sample.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Measure a reagent blank.
  Do this by tapping the **<Settings>** button and selecting the **<REAGENT BLANK>** menu item. Fill the cell with the reagent blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the reagent blank by tapping **<OK>** to confirm.
  After measuring the reagent blank the <Recalibration> menu must not be opened again as the calibration function is then changed due to the measurement of the reagent blank. Open the <Recalibration> menu only when a new recalibration should be performed.
- Fill the measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/l sulfur dioxide from the display.

33.9 Evaluation

Results are expressed
in mg/l sulfur dioxide

33.10 Literature

ASBC Methods of Analysis, online. Beer-21, Total sulfur dioxide, A. p-Rosaniline method [Release date 1960, revised 1975].

American society of brewing Chemists, St. Paul, Mn, U.S.A. doi: 10.1094/ASBCMOA-Beer-21

Analytical Procedures

34 Vicinal Diketones (Diacetyl, 2,3-Pentandione), spectrophotometric (EBC method)

The metabolic processes of yeast produce 2-acetolactate and 2-acetohydroxybutyrate in the course of fermentation. These are converted by oxidization to form the vicinal diketones diacetyl (2,3-butanedione) and 2,3-pentanedione. Diacetyl can, however, also occur as a characteristic metabolic product of certain microorganisms. When the threshold value is exceeded, the beer acquires an off-flavour.

34.1 Method

The basis of the method is the reaction of diacetyl or 2,3-pentanedione with 1,2-phenylenediamine to form 2,3-dimethylquinoxaline, which is measured by spectrophotometry.

34.2 Measuring range

0.000 - 2.000 mg/kg vicinal diketones

34.3 Reagents and accessories

- Hydrochloric acid 25 % for analysis EMSURE®, Cat. No. 100316
- 1,2-Phenylenediamine GR for analysis, Cat. No. 107246
- Silicon anti-foaming agent, Cat. No. 107743
- Standard laboratory glass equipment (e. g. glass beakers, conical flasks, measuring cylinders) and pipettes
- 25-ml volumetric flask
- 1000-ml volumetric flask
- 50-ml-conical flask
- Steam distillation apparatus
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946, or rectangular cells, 20 mm, Spectroquant®, Cat. No. 114947

34.4 Preparing the solutions

- **Hydrochloric acid 4 mol/l (4 N):**
  Place 521 ml resp. 583 g of hydrochloric acid 25 %
  in a volumetric flask, make up to 1000 ml with H_2O and mix
  (shelf-life 3 months)
- **Phenylenediamine solution 1 %:**
  In a glass vessel:
  dissolve 1 g of 1,2-phenylenediamine in
  99 g of hydrochloric acid 4 mol/l and store in a dark place
  (prepare freshly every day)

34.5 Preparation

Steam distillation:

- Place **100 g of beer**, **not decarbonized**, in the preheated distillation apparatus
- Add **1 drop of silicon anti-foaming agent**
- Regulate the steam supply so that **approx. 25 ml of distillate** is obtained in **2 min**
- Collect the distillate in a 25-ml volumetric flask
- Make up to the 25-ml mark with H_2O

Analytical Procedures

34 Vicinal Diketones (Diacetyl, 2,3-Pentandione), spectrophotometric (EBC method)

34.6 Procedure and measurement

Sample blank:

- Pipette **10 ml of the** thoroughly mixed **distillate** in a 50-ml conical flask
- Add **2.5 ml of hydrochloric acid 4 mol/l (4 N)** and mix

Measurement sample:

- Pipette **10 ml of the** thoroughly mixed **distillate** in a 50-ml conical flask
- Add **0.5 ml of phenylenediamine solution** and mix
- Allow to stand **in a dark place for 30 min** (reaction time)
- Add **2 ml of hydrochloric acid 4 mol/l (4 N)** and mix
- Measure within **20 min**

Measurement:

- Open the method list (**<Methods>**) and select **method No. 2620 "Vicinal Diketones"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill sample blank into the corresponding rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample Blank"**.
  Confirm the message with **<OK>**.
- Fill measurement sample into the corresponding rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The **"✓"** symbol appears in the line **"Insert Sample"**.
  Confirm the message with **<OK>**.
- Read off the result in mg/kg vicinal diketones from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

34.7 Evaluation

Results are expressed
in mg/kg vicinal diketones

Specified values
Light "Vollbier" (beer with a high original gravity): <0.15 mg/kg vicinal diketones

34.8 Literature

MEBAK Brautechnische Analysemethoden 4th Edition 2002
Volume II, Method 2.23, page 134ff

Analytica-EBC, Section 9 Beer, Method 9.24.1

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