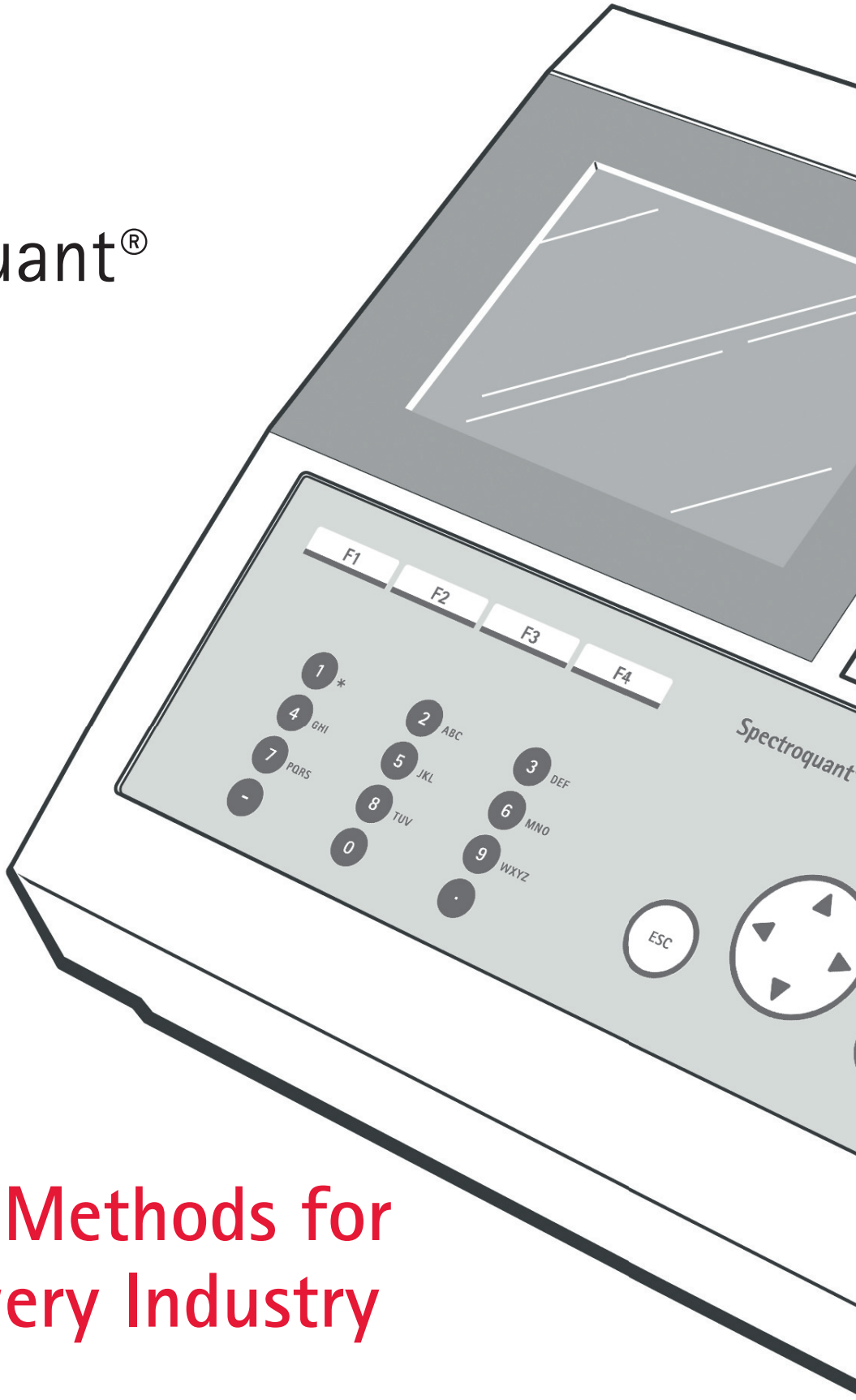
Spectroquant®

Pharo



Manual

Analysis Methods for the Brewery Industry

Release 03/2011

Contents

* only for Spectroquant® Pharo 300

Contents

Contents

* only for Spectroquant® Pharo 300

Contents

Contents

General informations

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.merck-chemicals.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. In the case of uncertainty, please refer to the corresponding section 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.

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

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 multiple-volume collection.

V Zeroing

A valid zero point is required for the calculation of measurement results. Zeroing is performed by measuring the absorption of **a cell filled with distilled water ("blank cell")** and saving the result in the photometer.
Zeroing must be performed separately for each type of cell and for each method used. The photometer will instruct you to make a zero measurement whenever one is due. Start the corresponding menu by entering <BLANK ZERO>.

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 section 8 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 section 7 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 absorption 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

The procedure is menu-driven in the photometer. Via <BLANK ZERO> open the selection list "Adjust", select "Zero adjustment", and confirm. Follow the instructions shown on the display to proceed. Refer to section 4.4 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**.

The time of the most recent zeroing of the method is shown in the top right corner of the display when the method is called up,
e.g. [ZERO 09.12.2010 12:16]

VI Sample blank value

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

Sample blank value procedure

The procedure is menu-driven in the photometer.
The necessary sample blank values are described in greater detail in the corresponding analytical procedures.

VII Reagent blank value (blank value)

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 value). This is to compensate the effect of the baseline absorption of the reagents on the photometric measurement.
In the practical context, the reagent blank value 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 value** using reagents of **one and the same batch**, keeping the reagent blank value stored until the reagents are exchanged, after which a new reagent blank value 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 value.

- The reagent blank value can be determined either in single or in multiple measurement. In the multiple measurement mode, the reagent blank value is calculated as the mean of the single measurement results.

Reagent blank value procedure

The procedure is menu-driven in the photometer.
Via <BLANK ZERO> open the selection list "Adjust", select "Reagent blank", and confirm. Follow the instructions shown on the display to proceed. Refer to section 4.5.9 of the functional description of the photometer if you are uncertain. The exact composition of the reagent blank value is described in more detail in the corresponding analytical procedure.

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

- When **prompted** by the instrument.
- For each series of measurements in the case that the reagent blank value 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 value) 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

The procedure is menu-driven in the photometer.
Via <BLANK ZERO> open the selection list "Adjust", select "Calibrate the method", and confirm. Follow the instructions shown on the display to proceed.

User-defined calibration is necessary in the following cases:

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

Analytical Procedures

1 α Acids

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 Uvasol®, Cat. No. 106002
- Sodium hydroxide solution 6 mol/l (6 N), TitriPUR®, Cat. No. 199062

- Standard laboratory glass equipment (e.g. glass beakers, conical flasks, measurement 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)
- **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 mol/l (6 N)
  in a volumetric flask, make up to 100 ml with methanol and mix
  (prepare freshly every day)

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 value:

- 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5012 "α Acids"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the reagent blank value and of the measurement sample is menu-driven.
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5012 it is recommended to measure a new reagent blank value each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank value".
- After the reagent blank value has been measured or, respectively, the stored reagent blank value 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

2 Anthocyanogenes, Harris and Ricketts 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.

2.1 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.

2.2 Measuring range

0 - 100 mg/l anthocyanogenes

2.3 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 Uvasol®, Cat. No. 106002

- Standard laboratory glass equipment (e.g. glass beakers, conical flasks, measurement 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

2.4 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)

2.5 Preparation

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

2.6 Procedure and measurement

Reagent blank value:

- 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5001 "Anthocyanogenes"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the reagent blank value and of the measurement sample is menu-driven.
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5001 it is recommended to measure a new reagent blank value 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 value".
- After the reagent blank value has been measured or, respectively, the stored reagent blank value 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.

2.7 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

2.8 Literature

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

3 Bitterness - beer (EBC 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.

3.1 Method

The bitter substances in beer and wort – in particular iso-α-acids – are extracted from the acidified sample with iso-octane and the concentration in the extract is measured by spectrophotometry.

3.2 Measuring range

10 - 80 bitterness (BU)

3.3 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, measurement 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

3.4 Preparation

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

3.5 Procedure and measurement

Reagent blank value:

- **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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5003 "Bitterness Beer"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the reagent blank value and of the measurement sample is menu-driven.
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5003 it is recommended to measure a new reagent blank value each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank value".

- After the reagent blank value has been measured or, respectively, the stored reagent blank value 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.

3.6 Evaluation

Results are expressed
in bitterness (BU)

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

3.7 Literature

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

Analytica-EBC, Section 9 Beer, Method 9.8

4 Bitterness - wort (EBC 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.

4.1 Method

The bitter substances in beer and wort – in particular iso-α-acids – are extracted from the acidified sample with iso-octane and the concentration in the extract is measured by spectrophotometry.

4.2 Measuring range

1 - 120 bitterness (BU)

4.3 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, measurement 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

4.4 Preparation

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

4.5 Procedure and measurement

Reagent blank value:

- **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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5004 "Bitterness Wort"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the reagent blank value and of the measurement sample is menu-driven.
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5004 it is recommended to measure a new reagent blank value each time the batch of the reagents used is exchanged. In this case proceed as described in section VII "Reagent blank value".

- After the reagent blank value has been measured or, respectively, the stored reagent blank value 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.

4.6 Evaluation

Results are expressed
in bitterness (BU)

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

4.7 Literature

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

Analytica-EBC, Section 8 Wort, Method 8.8

5 Colour, spectrophotometric (EBC method)

This method is designed to eliminate subjective effects attributable to the human eye as well as differences in the colour 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.

5.1 Method

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

5.2 Measuring range

0.0 - 60.0 EBC units

5.3 Accessories

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

5.4 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 colour is within the measurement range; **use** the corresponding **dilution factor** when subsequently calculating the result (**measurement result x dilution factor)**

5.5 Procedure and measurement

Measurement:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5002 "Colour (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.

5.6 Evaluation

Results are expressed
in EBC Units

Note
A spectrophotometric absorption curve does not reflect the colour 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 absorption 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.

5.7 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

6 Copper, Cuprethol method (EBC 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.

6.1 Method

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

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

6.2 Measuring range

0.10 - 5.00 mg/l copper

6.3 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, measurement cylinders) and pipettes
- 1000-ml volumetric flask
- 100-ml conical flask
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

6.4 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)

- **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)

6.5 Preparation

- Expel carbon dioxide from **beer**

6.6 Procedure and measurement

Sample blank value:

- Pipette **50 ml of decarbonized beer** into a 100-ml conical flask
- Add **25 ml of buffer solution** and mix
- Add **2 ml of solution 3** and mix

Measurement sample:

- Pipette **50 ml of decarbonized beer** into a 100-ml conical flask
- Add **25 ml of buffer solution** and mix
- Add **2 ml Solution 4 (Cuprethol reagent)** and mix
- Measure **within 10 min**

Measurement:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5013 "Copper (EBC Cuprethol)"**.
- 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 following message appears in the display

  Next step: Sample
  Proceed with <START ENTER>

- Comfirm the message with <START ENTER>.
- 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 mg/l copper from the display.

6.7 Evaluation

Results are expressed
in mg/l Cu

Specified values
Beer: <0,20 mg/l Cu

6.8 Literature

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

Analytica-EBC, Section 9 Beer, Method 9.14.2

7 Flavanoids (EBC method)

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

7.1 Method

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

7.2 Measuring range

3.0 - 200.0 mg/l catechin equivalent

7.3 Reagents and accessories

- Hydrochloric acid fuming 37 % for analysis EMSURE®, Cat. No. 100317
- Methanol Uvasol®, Cat. No. 106002
- 4-(Dimethylamino)-cinnamaldehyde for synthesis, Cat. No. 822034
- Standard laboratory glass equipment (e.g. glass beakers, conical flasks, measurement 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

7.4 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)
- **Colour 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)

7.5 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)

7.6 Procedure and measurement

Reagent blank value:

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

Measurement:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5026 "Flavanoids"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the reagent blank value and of the measurement sample is menu-driven.
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5026 it is recommended to measure a new reagent blank value 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 value".
- After the reagent blank value has been measured or, respectively, the stored reagent blank value 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.

7.7 Evaluation

Results are expressed
in mg/l catechin equivalent

7.8 Literature

Analytica-EBC, Section 9 Beer, Method 9.12

8 Free Amino Nitrogen, Ninhydrin method - light beer (EBC 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, colour, 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 colour yield of the individual amino acids varies between 70 % and 105 %, relative to glycine.

8.1 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 colour.

8.2 Measuring range

0 - 400 mg/l free amino nitrogen

8.3 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, measurement cylinders) and pipettes
- pH meter
- 100-ml volumetric flask
- Pincer
- Test tubes with ground-glass stopper, 16 x 150 mm
- Water bath (100 °C and 20 °C)
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

8.4 Preparing the solutions

- **Colour 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)

8.5 Preparation

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

8.6 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.

User-defined calibration:

- Prepare calibration solutions in the following manner:

	E0 [0 mg/l FAN]	1 [2 mg/l FAN]
Water	2.0 ml	-
Glycine standard solution 2 mg/l FAN	-	2.0 ml
	• pipette into separate test tubes	
Colour 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 value:

- Pipette **2.0 ml of H_2O** into a test tube
- Add **1.0 ml of colour 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 colour 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5008 "FAN light beer"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- **Measurement of the reagent blank value is necessary.** To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank value".
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5008 it is recommended to measure a new reagent blank value 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 value".
- **User-defined calibration is necessary.**
  In the case that a calibration is already present, a message will appear in the display asking whether this should be used.
  For method No. 5008 it is recommended to perform recalibration each time the batch of the reagents used is exchanged.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.
  A table with the necessary calibration solutions appears.
  Press <START ENTER>. The following message appears in the display

  To start measurement, insert cell…

  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is "Applied", the table with the calibration solutions reappears and the corresponding absorption field shows the value.
  Follow the procedure for solution E0 for calibration solution 1.
  Once all calibration solutions have been measured, save the calibration by pressing <F4>.
- The following message appears in the display

  To start measurement, insert cell…

- 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.

8.7 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

8.8 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

9 Free Amino Nitrogen, Ninhydrin method - light wort (EBC 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, colour, 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 colour yield of the individual amino acids varies between 70 % and 105 %, relative to glycine.

9.1 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 colour.

9.2 Measuring range

0 - 400 mg/l free amino nitrogen

9.3 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, measurement cylinders) and pipettes
- pH meter
- 100-ml volumetric flask
- Pincer
- Test tubes with ground-glass stopper, 16 x 150 mm
- Water bath (100 °C and 20 °C)
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

9.4 Preparing the solutions

- **Colour 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)

9.5 Preparation

- Dilute **wort** 100-fold with H_2O (dilution 1 + 9)

9.6 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.

User-defined calibration:

- Prepare calibration solutions in the following manner:

	E0 [0 mg/l FAN]	1 [2 mg/l FAN]
Water	2.0 ml	-
Glycine standard solution 2 mg/l FAN	-	2.0 ml
	• pipette into separate test tubes	
Colour 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 value:

- Pipette **2.0 ml of H_2O** into a test tube
- Add **1.0 ml of colour 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 colour 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5009 "FAN light wort"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- **Measurement of the reagent blank value is necessary.** To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank value".
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5009 it is recommended to measure a new reagent blank value 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 value".
- **User-defined calibration is necessary.**
  In the case that a calibration is already present, a message will appear in the display asking whether this should be used.
  For method No. 5009 it is recommended to perform recalibration each time the batch of the reagents used is exchanged.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.
  A table with the necessary calibration solutions appears.
  Press <START ENTER>. The following message appears in the display

To start measurement, insert cell…

  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is "Applied", the table with the calibration solutions reappears and the corresponding absorption field shows the value.
  Follow the procedure for solution E0 for calibration solution 1.
  Once all calibration solutions have been measured, save the calibration by pressing <F4>.
- The following message appears in the display

To start measurement, insert cell…

- 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.

9.7 Evaluation

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

Standard values
Finished wort (12 %): 200 - 250 mg/l FAN

9.8 Literature

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

Analytica-EBC, Section 8 Wort, Method 8.10

10 Free Amino Nitrogen, Ninhydrin method - dark beer (EBC 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, colour, 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 colour yield of the individual amino acids varies between 70 % and 105 %, relative to glycine.

10.1 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 colour.

10.2 Measuring range

0 - 400 mg/l free amino nitrogen

10.3 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, measurement cylinders) and pipettes
- pH meter
- 100-ml volumetric flask
- Pincer
- Test tubes with ground-glass stopper, 16 x 150 mm
- Water bath (100 °C and 20 °C)
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

10.4 Preparing the solutions

- **Colour 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)

10.5 Preparation

- Dilute **beer** 50-fold with H_2O (dilution 1 + 49)
- Determine the EBC. For beers with less than 100 EBC units, use method 8 (for light beer).

10.6 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.

User-defined calibration:

- Prepare calibration solutions in the following manner:

	E0 [0 mg/l FAN]	1 [2 mg/l FAN]
Water	2.0 ml	-
Glycine standard solution 2 mg/l FAN	-	2.0 ml
	• pipette into separate test tubes	
Colour 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	

Sample blank value:

- Pipette **2.0 ml of** the **diluted sample** into a test tube
- Add **1.0 ml of H_2O** 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

Reagent blank value:

- Pipette **2.0 ml of H_2O** into a test tube
- Add **1.0 ml of colour 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 colour 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5006 "FAN dark beer"**.
- **Measurement of the reagent blank value is necessary.** To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank value".
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5006 it is recommended to measure a new reagent blank value 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 value".
- **User-defined calibration is necessary.**
  In the case that a calibration is already present, a message will appear in the display asking whether this should be used.
  For method No. 5006 it is recommended to perform recalibration each time the batch of the reagents used is exchanged.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.
  A table with the necessary calibration solutions appears.
  Press <START ENTER>. The following message appears in the display

  To start measurement, insert cell…

  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is "Applied", the table with the calibration solutions reappears and the corresponding absorption field shows the value.
  Follow the procedure for solution E0 for calibration solution 1.
  Once all calibration solutions have been measured, save the calibration by pressing <F4>.

- The following message appears in the display

  Sample blank
  To start measurement, insert cell…

- Subsequently fill sample blank value into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

  Proceed with <START ENTER>

- Press <START ENTER>. 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.

10.7 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

10.8 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

11 Free Amino Nitrogen, Ninhydrin method - dark wort (EBC 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, colour, 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 colour yield of the individual amino acids varies between 70 % and 105 %, relative to glycine.

11.1 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 colour.

11.2 Measuring range

0 - 400 mg/l free amino nitrogen

11.3 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, measurement cylinders) and pipettes
- pH meter
- 100-ml volumetric flask
- Pincer
- Test tubes with ground-glass stopper, 16 x 150 mm
- Water bath (100 °C and 20 °C)
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

11.4 Preparing the solutions

- **Colour 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)

11.5 Preparation

- Dilute **wort** 100-fold with H_2O (dilution 1 + 99)
- Determine the EBC. For worts with less than 100 EBC units, use method 9 (for light wort).

11.6 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.

User-defined calibration:

- Prepare calibration solutions in the following manner:

	E0 [0 mg/l FAN]	1 [2 mg/l FAN]
Water	2.0 ml	-
Glycine standard solution 2 mg/l FAN	-	2.0 ml
	• pipette into separate test tubes	
Colour 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	

Sample blank value:

- Pipette **2.0 ml of** the **diluted sample** into a test tube
- Add **1.0 ml of H_2O** 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

Reagent blank value:

- Pipette **2.0 ml of H_2O** into a test tube
- Add **1.0 ml of colour 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 colour 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5007 "FAN dark wort"**.
- **Measurement of the reagent blank value is necessary.** To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank value".
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5007 it is recommended to measure a new reagent blank value 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 value".
- **User-defined calibration is necessary.**
  In the case that a calibration is already present, a message will appear in the display asking whether this should be used.
  For method No. 5007 it is recommended to perform recalibration each time the batch of the reagents used is exchanged.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.
  A table with the necessary calibration solutions appears.
  Press <START ENTER>. The following message appears in the display

  To start measurement, insert cell…

  Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is "Applied", the table with the calibration solutions reappears and the corresponding absorption field shows the value.
  Follow the procedure for solution E0 for calibration solution 1.
  Once all calibration solutions have been measured, save the calibration by pressing <F4>.

- The following message appears in the display

  Sample blank
  To start measurement, insert cell…

- Subsequently fill sample blank value into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

  Proceed with <START ENTER>

- Press <START ENTER>. 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.

11.7 Evaluation

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

Standard values
Finished wort (12 %): 200 - 250 mg/l FAN

11.8 Literature

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

Analytica-EBC, Section 8 Wort, Method 8.10

12 Iron, spectrophotometric (EBC 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.

12.1 Method

Bivalent iron reacts with the disodium salt of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine-4,4-disulfonic acid (Ferrospectral®) 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 colour 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.

12.2 Measuring range

40-mm rectangular cell: 0.000 - 1.000 mg/l iron
50-mm rectangular cell: 0.000 - 0.800 mg/l iron

12.3 Reagents and accessories

- Ammonium acetate for analysis EMSURE®, Cat. No. 101116
- Acetic acid (glacial) 100 % anhydrous for analysis EMSURE®, Cat. No. 100063
- Ferrospectral® GR for analysis, Cat. No. 111613
- 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, measurement 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

12.4 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)
- **Ferrospectral® reagent:**
  In a glass vessel:
  dissolve 0.257 g of Ferrospectral® in
  50 ml of buffer solution pH 4.3
  (shelf-life 2 weeks)

- **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)

12.5 Preparation

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

12.6 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 value.

User-defined calibration is necessary in the following cases:

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

12.7 Procedure and measurement

User-defined calibration:

- Prepare calibration solutions in the following manner:

	Standard 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
Ferrospectral® 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 value:

- Pipette **2.0 ml of Ferrospectral® 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 value:

- 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

Measurement sample:

- Pipette **40.0 ml of sample**,
  2.0 ml of Ferrospectral® 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5023 "Iron (EBC) 40"** or **method No. 5024 "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.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.
  A table with the necessary calibration solutions appears.
  Press <START ENTER>. The following message appears in the display

  To start measurement, insert cell…

  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 absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is "applied", the table with the calibration solutions reappears and the corresponding absorption field shows the value.
  Follow the procedure for solution E0 for calibration solutions 1 - 4.
  Once all calibration solutions have been measured, save the calibration by pressing <F4>.
- The following message appears in the display

  Sample blank
  To start measurement, insert cell…

- 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 value **prior** to measuring the sample blank value.
  In this case please proceed as described in section VII "Reagent blank value". After the reagent blank value has been measured the following message reappears in the display

  Sample blank
  To start measurement, insert cell…

- Subsequently fill sample blank value into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

  Proceed with <START ENTER>

- Press <START ENTER>. 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.

12.8 Evaluation

Results are expressed
in mg/l Fe

Specified values
<0.200 mg/l iron

12.9 Literature

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

Analytica-EBC, Section 9 Beer, Method 9.13.2

13 Iso-α Acids

13.1 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.

13.2 Measuring range

0 - 60 mg/l iso-α acids

13.3 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 Uvasol®, Cat. No. 106002
- Sodium hydroxide solution 6 mol/l (6 N), TitriPUR®, Cat. No. 199062

- Standard laboratory glass equipment (e.g. glass beakers, conical flasks, measurement 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

13.4 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)
- **<u>Acidic</u> 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)
- **<u>Alkaline</u> methanol solution:**
  Pipette 0.2 ml sodium hydroxide solution 6 mol/l (6 N)
  in a volumetric flask, make up to 100 ml with methanol and mix
  (prepare freshly every day)

13.5 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.

13.6 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5011 "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.
- Read off the result in mg/l iso-α acids from the display.

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 absorption 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.

13.7 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

13.8 Literature

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

14 Nickel (EBC 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.

14.1 Method

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

14.2 Measuring range

0.00 - 5.00 mg/l nickel

14.3 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, measurement 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

14.4 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:**
  In a glass vessel:
  mix 1 ml of ammonia solution 25 % with
  49 ml of H_2O
  (shelf-life 1 week)

- **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)
- **<u>alcoholic</u> 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)
- **<u>alkaline</u> dimethylglyoxime solution:**
  Dissolve 1 g of dimethylglyoxim in
  5 ml of sodium hydroxide solution 5 mol/l (5 N) and
  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 mixn
  (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)

14.5 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

14.6 Procedure and measurement

Reagent blank value:

- 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

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 colour** 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5014 "Nickel (EBC)"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the reagent blank value and of the measurement sample is menu-driven.
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5014 it is recommended to measure a new reagent blank value 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 value".
- After the reagent blank value has been measured or, respectively, the stored reagent blank value 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.

14.7 Evaluation

Results are expressed
in mg/l Ni

Specified values
Beer: <0.05 mg/l nickel

14.8 Literature

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

Analytica-EBC, Section 9 Beer, Method 9.15

15 Photometric Iodine Test

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.

15.1 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 colour 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 value40**" or "**Iodine value50**" on the photometer.

15.2 Measuring range

0.00 - 0.80 iodine value

15.3 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, measurement cylinders) and pipettes
- 100-ml volumetric flask
- 200-ml volumetric flask
- pH meter
- Centrifuge, 2500 rpm
- Centrifuge glasses with ground-glass stopper, 100 - 110 ml Inhalt
- Mechanical shaker
- 40-mm rectangular cells OS or Rectangular cells, 50 mm, Spectroquant®, Cat. No. 114944
- Plastic spatula

15.4 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)

- **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)

15.5 Preparation

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

15.6 Procedure and measurement

Buffer blank value:

- Use **10 ml of buffer solution**

Reagent blank value:

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

Sample blank value:

- 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 p 51)
- Add **8.5 ml of buffer solution** and mix

Measurement sample:

- After measuring the sample blank value, 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5015 "Iodine value40"** or **method No. 5016 "Iodine value50"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The measurement of the buffer blank value, reagent blank value, sample blank value, and measurement sample is menu-driven.
- Fill buffer blank into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

> Next step: Reagent blank
> Proceed with <START ENTER>

- Comfirm the message with <START ENTER>.
- Fill reagent blank value into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.

The following message appears in the display

Next step: Sample blank
Proceed with <START ENTER>

- Comfirm the message with <START ENTER>.
- Fill sample blank value into a 40-mm or, respectively, a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

Next step: Sample
Proceed with <START ENTER>

- Comfirm the message with <START ENTER>.
- 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 as iodine value from the display.

15.7 Evaluation

Results are expressed
as iodine value

Standard values
Wort: <0.30 iodine value

15.8 Literature

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

16 Reducing Power, spectrophotometric

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.

16.1 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.

16.2 Measuring range

0 - 100 %

16.3 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

16.4 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 colour

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

  - Dilute remaining filtrate to **1450 mg/l DPI**

  (The solution remains stable for approx. 1 week when stored full to the brim in dark bottles at +4 °C)

16.5 Preparation

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

16.6 Procedure and measurement

Sample blank value:

- 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5017 "Reducing Power"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill sample blank value into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

  Next step: Sample
  Proceed with <START ENTER>

- Comfirm the message with <START ENTER>.
- 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 % reducing power from the display.

16.7 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.

16.8 Literature

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

17 Steam-volatile Phenols

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.

17.1 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 colouring 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 “**Phenol volat40**” or “**Phenol volat50**” on the photometer.

17.2 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.

17.3 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, measurement 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

17.4 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)

17.5 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

17.6 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 photometer either demands user-defined calibration or else suggests the use of any already existing calibration. 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 **prompted** by the instrument.
- When the **stored calibration** is to be overwritten.
- When **exchanging batches** of the reagents used.

17.7 Procedure and measurement

User-defined calibration:

- Prepare standard solutions in the following manner:

Phenol standard solution to be prepared	10 mg/l phenol		
Phenol standard solution **1000 mg/l phenol**	5.0 ml		
	• Pipette into a 500-ml volumetric flask, make up to the mark with H_2O, and mix		
Phenol standard solutions to be prepared	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 solutions	E0	1	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		

Reagent blank value:

- **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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5021 "Phenol volat40"** or **method No. 5022 "Phenol volat50"**.
- **Measurement of the reagent blank value is necessary.** To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank value".
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5021 and 5022 it is recommended to measure a new reagent blank value 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 value".
- **User-defined calibration is necessary.**
  In the case that a calibration is already present, a message will appear in the display asking whether this should be used.
  For method No. 5021 and 5022 it is recommended to perform recalibration each new working day and each time the batch of the reagents used is exchanged.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.
  A table with the necessary calibration solutions appears.
  Press <START ENTER>. The following message appears in the display

To start measurement, insert cell…

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 absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is "applied", the table with the calibration solutions reappears and the corresponding absorption field shows the value. Follow the procedure for solution E0 for calibration solutions 1and 2.
Once all calibration solutions have been measured, save the calibration by pressing <F4>.

- The following message appears in the display

  To start measurement, insert cell…

- Via <F3> selection menu "Citation form" select **malt** or **beer**.
- 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/kg steam-volatile phenols (**malt**) or mg/l steam-volatile phenols (**beer**) from the display.

17.8 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.

17.9 Literature

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

18 Thiobarbituric Acid Number (TAN)

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.

18.1 Method

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

18.2 Measuring range

Thiobarbituric acid number: 0 - 100

18.3 Reagents and accessories

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

18.4 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 0.282 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)

18.5 Preparation

- Clarify **turbid samples** over kieselguhr

As a rule samples are diluted as follows:

- **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)

Select the dilution in such a way that the absorption of the measurement sample is at least 0.1 A higher than that of the sample blank.
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.

18.6 Procedure and measurement

Sample blank value:

- 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**
  (avoid direct sunlight and take care to 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**
  (avoid direct sunlight and take care to 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5019 "TAN"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- The dilution factor is asked for. Press <START ENTER> to enter the value.
- Enter the dilution factor via the alphanumeric keys and confirm by pressing <START ENTER>.
  The following message appears in the display

  Next step: Sample blank
  Proceed with <START ENTER>

- Confirm the message with <START ENTER>.
- Subsequently fill sample blank value into a 10-mm rectangular cell and insert cell into the cell compartment.
  The measurement starts automatically.
  The following message appears in the display

  Next step: Sample
  Proceed with <START ENTER>

- Confirm the message with <START ENTER>.
- Measurement sample into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result as thiobarbituric acid number from the display.
- If the message "Condition not met" appears, proceed as described in section 18.5.

18.7 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)

18.8 Literature

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

19 Total Carbohydrates (EBC method)

19.1 Method

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

19.2 Measuring range

0.000 - 6.000 g/100 ml total carbohydrates

19.3 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, measurement cylinders) and pipettes
- 100-ml volumetric flask
- 500-ml volumetric flask
- 1000-ml volumetric flask
- Water bath (2 - 4 °C)
- Water bath (95 $\pm$ 5 °C)
- test tubes with ground-glass stopper, 20 x 150 mm
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

19.4 Preparing the solutions

- **Sulfuric acid 85 %:**
  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 100 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 volumetric flask, make up to 100 ml with H_2O and mix
  make up to 100 ml with H_2O in a volumetric flask
  (prepare fresh)

19.5 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.

19.6 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 ± 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 value:

- 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 ± 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 ± 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5025 "Σ Carbohydrates"**.
- **Measurement of the reagent blank value is necessary.** To do this fill the reagent blank into a 10-mm rectangular cell and proceed as described in section VII "Reagent blank value".
  In the case that a reagent blank value is already present, a message will appear in the display asking whether this should be used.
  For method No. 5025 it is recommended to measure a new reagent blank value 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 value".
- **User-defined calibration is necessary.**
  In the case that a calibration is already present, a message will appear in the display asking whether this should be used.
  For method No. 5025 it is recommended to perform recalibration each new working day and each time the batch of the reagents used is exchanged.
  To do this, go to <BLANK ZERO> to open the selection list "Adjust", select "Calibrate the method", and confirm.

A table with the necessary calibration solutions appears.
Press <START ENTER>. The following message appears in the display

To start measurement, insert cell…

Fill calibration solution E0 into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorption is shown in the display. You now have the opportunity to run further measurements of the calibration solutions (<F1>), to reject the measurement (<F2>), or to apply the measurement result in the memory (<F4>). After the result is “Applied”, the table with the calibration solutions reappears and the corresponding absorption field shows the value.
Follow the procedure for solution E0 for calibration solution 1.
Once all calibration solutions have been measured, save the calibration by pressing <F4>.

- The following message appears in the display

To start measurement, insert cell…

- 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.

19.7 Evaluation

Results are expressed
in g/100 ml

19.8 Literature

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

Analytica-EBC, Section 9 Beer, Method 9.26

20 Total polyphenols (EBC 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 colour, 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.

20.1 Method

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

20.2 Measuring range

0 - 800 mg/l total polyphenols

20.3 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, measurement cylinders) and pipettes
- 25-ml volumetric flask
- 1000-ml volumetric flask
- Mechanical shaker
- for turbid samples: centrifuge and centrifuge glasses
- Rectangular cells, 10 mm, Spectroquant®, Cat. No. 114946

20.4 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 H2O in a volumetric flask,
  if necessary clarify by centrifuging
  (shelf-life 1 month)
- **Iron(III) Solution:**
  In a glass vessel:
  dissolve 3.1 g of ammonium iron(III) citrate in
  96.9 g of H_2O
  (Solution must be completely clear and remains stable for approx. 1 week when stored in dark bottles.)
- **Ammonia solution:**
  In a glass vessel:
  mix 25 ml of ammonia solution 25 % with
  50 ml of H_2O
  (shelf-life 4 weeks)

20.5 Preparation

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

20.6 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 value:

- 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5010 "Total Polyphenols"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill sample blank value into a 10-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

> Next step: Sample
> Proceed with <START ENTER>

- Confirm the message with <START ENTER>.
- 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 total polyphenols from the display.

20.7 Evaluation

Results are expressed
in mg/l total polyphenols

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

20.8 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

21 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.

21.1 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.

21.2 Measuring range

0.00 - 1.00 mg/kg vicinal diketones

21.3 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, measurement cylinders) and pipettes
- 25-ml volumetric flask
- 1000-ml volumetric flask
- 50-ml-conical flask
- Steam distillation apparatus
- Rectangular cells, 20 mm, Spectroquant®, Cat. No. 114947

21.4 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)

21.5 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

21.6 Procedure and measurement

Sample blank value:

- Pipette **10 ml of the** thoroughly mixed **distillate** in a 50-ml conical flask
- Add **2 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:

- Select [Concentration] in the home menu. Via [Method list] (F2) you can now select and start **method No. 5020 "Vicinal Diketones"**.
- It is recommended to zero the method each new working day. Proceed as described in section V "Zeroing".
- Subsequently fill sample blank value into a 20-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
  The following message appears in the display

  Next step: Sample
  Proceed with <START ENTER>

- Confirm the message with <START ENTER>.
- Fill measurement sample into a 20-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/kg vicinal diketones from the display.

21.7 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

21.8 Literature

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

Analytica-EBC, Section 9 Beer, Method 9.24.1

Merck KGaA, 64271 Darmstadt, Germany
Tel. +49(0)6151 72-2440, Fax +49(0)6151 72-7780
environmental.analysis@merck.de **www.merck-chemicals.com**