HACH LANGE

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DOC022.52.00730.SEP05

Supplementary Software LZV 570 Brewery Analysis

DR 5000

User Manual

09/2005 edition1

ck 09/05 edition 1

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Section 1 General Information

1.1 Safety Information

Before you install the software, you should read this manual thoroughly. Take note of all information labelled "Danger" or "Note".

Besides the instructions in this manual, users must comply with the national general safety and accident prevention regulations of the country in which the instrument is used.

1.1.1 Use of Hazard Information

DANGER
Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

Important Note: *Information that the user needs to take into account when handling the instrument.*

Note: *Additional operating information for the user.*

1.2 Installation

1. Touch **Instrument Update** in the "System Check" menu.
2. Plug the USB memory stick into the USB socket (type A) of the DR 5000.
3. Confirm by touching **OK**.
4. The connection is established automatically and the software is updated.

Touch **OK** to return to the "System Check" menu.

1.3 Important information about the manual

Copyright
The copyright to this User Manual remains with the manufacturer.

The manual contains instructions and notes that may not be fully or partially

- duplicated
- disseminated
- used without authorization for competitive purposes or

communicated in any other way.

1.3.1 Chemical and Biological Safety

DANGER
Handling chemical samples, standards and reagents can be dangerous. Users of this product are advised to familiarize themselves with safety procedures and the correct use of chemicals, and to carefully read all relevant Material Safety Data Sheets.

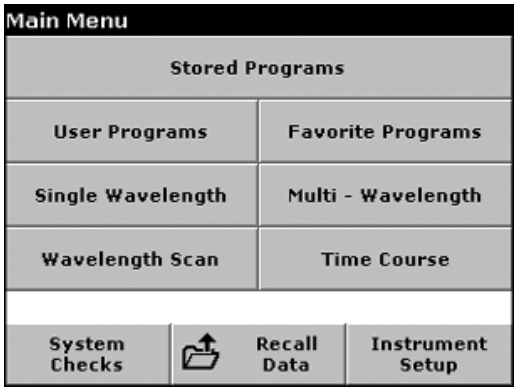
During the analysis of the sample it may be necessary to use toxic, readily flammable or corrosive chemicals.

- The user must observe all cautionary information printed on the original solution containers and safety data sheet prior to their use.
- All waste solutions must be disposed in accordance with local and national law.

1.4 Introduction

The LZV 570 Supplementary Software for Brewery Analysis is a collection of all spectrophotometric applications that are of relevance for brewery analysis. The working instructions are taken from the MEBAK manuals. Most of the procedures are from the 4th edition, 2002. For many analyses, the sipper module can be used to carry out the tests more conveniently.

1.5 Selecting a stored test



1. Select **Stored Programs** in the "Main Menu". An alphabetically sorted list of all available tests is displayed.
2. Select a test by touching the corresponding line.

   Note: *Use the scroll bar to run quickly through the list.*

   Note: *If you already know the number of the desired test, touch* ***Select by Number****. Use the alphanumeric keypad to enter the test number and confirm your input by touching* ***OK****.*
3. Touch **Start** to start the test program.

1.6 List of abbreviations

General information

Unless otherwise indicated, reagents should be analytical grade.
Unless otherwise indicated, solutions are aqueous.

dist. H_2O	distilled or demineralised water
sec	seconds
min	minutes
h	hours
SD	standard deviation
r	reproducibility
R	comparability
V_c	variation coefficient

1.7 Literature

MEBAK
Brautechnische Analysenmethoden (Analysis methods for the brewing industry)

Collected methods of the Mitteleuropäischen Brautechnischen Analysenkommision (Central European commission for brewery analysis) (MEBAK)

Published by the Chairman, Dr. Heinrich Pfenninger

Publishing house of the MEBAK

D-85350 Freising-Weihenstephan

4th Edition, 2002

Section 2 Working procedures

2.1 Bitter units (EBC method)

Principle
The bitter substances, mainly iso-a-acids, are extracted from the acidified sample with iso-octane and the concentration in the extract is determined with a spectrophotometer.

Fields of application
Beer, worts

Measuring range
20–60 BU

Accessories

- Centrifuge tubes with solvent-tight stoppers (35 ml)
- Glass beads
- Shaker
- Centrifuge (3000 rpm)
- Spectrophotometer (275 nm)
- 10 mm rectangular cuvette (QS grade)

Reagents

- Hydrochloric acid, 6N
- Iso-octane (2,2,4-trimethylpentane), spectroscopically pure (absorbance measured in 10 mm rectangular cuvette (QS grade) at **275 nm** against H_2O < 0.010) (for example Uvasol)

Sample preparation

1. Clarify wort and cloudy beer by centrifuging at **3000 rpm** for **15 min** (do not filter sample).
2. Expel carbon dioxide from sample without losing any foam.
3. Bring the sample to **20°C** and pipette **10 ml** (5 ml wort + 5 ml dist. H_2O) into a centrifuge tube.
4. Add **0.5 ml** 6N hydrochloric acid, **20 ml** iso-octane and **3** glass beads.
5. Close centrifuge tube and shake mechanically for **15 min** at **20°C**.
6. Centrifuge for **3 min** at **3000 rpm**.
7. Measure the absorbance of the iso-octane extract in a 10 mm rectangular cuvette at **275 nm** against iso-octane of the same quality (blank value).

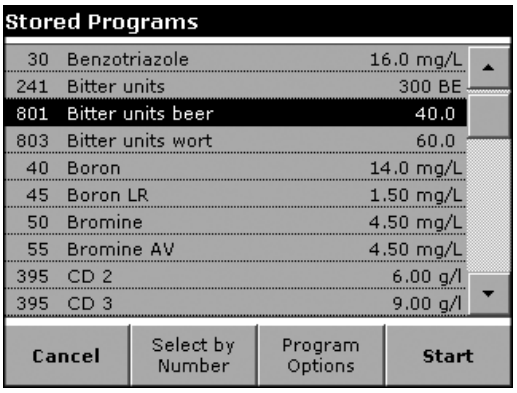
Results
Bitter units (BU) without any decimal places

Accuracy
V_{cr} = ± 2.4%
V_{cR} = ± 6.5%

Standard values
Beer: 10–40 BU, depending on type and origin
Wort: 20–60 BU, depending on beer and utilisation of bitter substances

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002, Volume II, pp 114ff

2.1.1 Procedure for measuring bitter units in beer



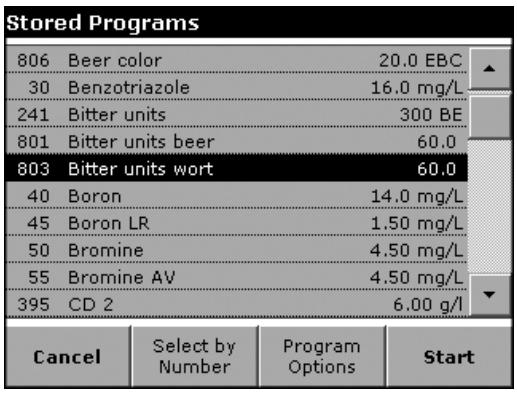
1. Prepare samples as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **801**.
3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert the sample cuvette containing the prepared iso-octane into the cell compartment and close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*

2.1.1.1 Executing the test with the sipper module

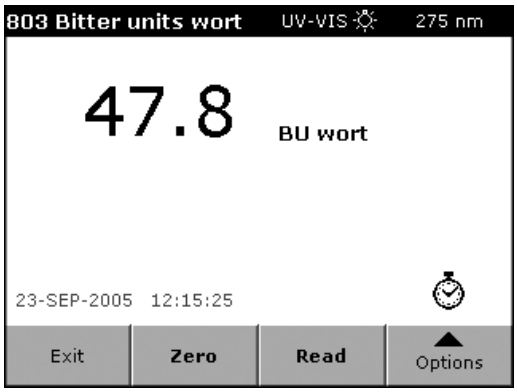
Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.1.2 Procedure for measuring bitter units in wort



1. Prepare samples as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **803**.
3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert the sample cuvette containing the prepared iso-octane into the cell compartment and close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*



2.1.2.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.2 Total polyphenols (EBC method)

Principle
Polyphenols react with iron(III) ions in an alkaline solution to form coloured iron complexes; the resulting brown colour is measured with a spectrophotometer.

Fields of application
Beer, worts

Measuring range
0–800 mg/l

Accessories

- Centrifuge
- Spectrophotometer (600 nm)
- 10 mm rectangular cuvette (OS grade)

Reagents

- Carboxymethylcellulose-ethylenediaminetetracetic acid solution (CMC-EDTA-Na):

  a. Weigh out **10 g** CMC (low viscosity) and **2 g** EDTA-Na.

  b. Dissolve these substances in about **500 ml** H_2O while stirring. When they are completely dissolved, fill up to **1 l** with H_2O. If necessary, clarify by centrifuging.

- Ammonium iron(III) citrate (3.5%):

  a. Dissolve **3.5 g** ammonium iron(III) citrate, green (16% Fe), in H_2O and make up to **100 ml**. The solution must be completely clear. It remains stable for about 1 week.

  b. Ammonia, dilute:
  Dilute **1 part** concentrated ammonia (d = 0.91) with **2 parts** H_2O.

Sample preparation

1. Shake beer to expel carbon dioxide.

2. Clarify cloudy wort or beers by centrifuging.

3. Mix **10 ml** test solution and **8 ml** CMC-EDTA solution thoroughly in a 25 ml measuring flask.

4. Add **0.5 ml** iron(III) solution and mix **thoroughly**.

5. Add **0.5 ml** dilute ammonia solution and mix **thoroughly**.

6. Make up to **25 ml** with H_2O and mix.

7. Wait **10 min**, then measure the absorbance in a 10 mm rectangular cuvette at **600 nm** against a blank sample.

8. **Blank value**

   a. Introduce **10 ml** sample (expel carbon dioxide from beer by shaking; clarify turbid wort or beer by centrifuging) into a 25 ml measuring flask.

   b. Add **8 ml** CMC-EDTA solution and mix **thoroughly**.

   c. Add **0.5 ml** dilute ammonia solution and mix **thoroughly**.

   d. Make up to **25 ml** with H_2O and mix again.

Note: *Mix thoroughly after adding each individual solution*

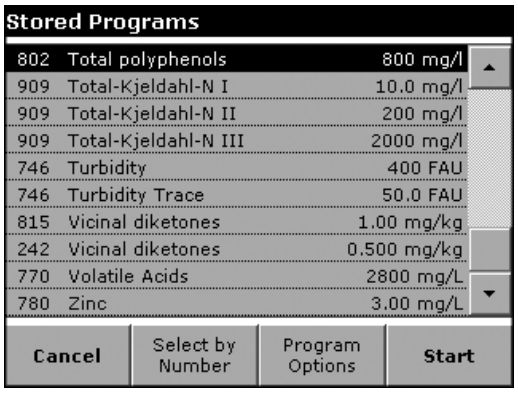
Result
The result is expressed in mg/l without any decimal places.

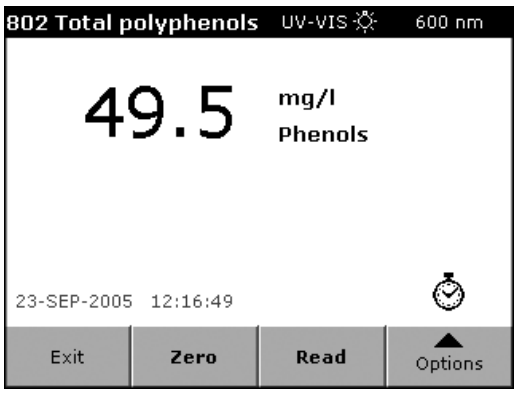
Accuracy
SD = ± 9

Standard values
Beer: 150–200 mg/l

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002, Volume II, pp 107ff

2.2.1 Procedure for measuring total polyphenols





1. Prepare samples and blank value solutions as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **802**.

3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.

4. Insert the sample cuvette containing the prepared sample into the cell compartment and close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 3. In other words, each sample has to have its own specific blank value solution.*

2.2.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.3 Reducing power (spectrophotometric method)

Reducing power is a measure of the rapidly reducible 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 taste.

Principle
Reductones reduce a certain amount of Tillmann's reagent (2,6-dichlorphenol-indophenol sodium, DPI) within a given period of time. The decolouration of the reagent is measured with a spectrophotometer and calculated.

Measuring range
0–100

Accessories

- Spectrophotometer (520 nm)
- 10 mm rectangular cuvette (OS grade)
- Stopwatch
- Water-jet pump

Reagents

- 2,6-Dichlorphenol-indophenol (0.005M) (DPI solution, molecular weight of the sodium salt 290.08):

  a. Weigh approx. **100 mg** DPI into a beaker, add approx. **25 ml** H_2O, and dissolve by heating to about **60°C**.

  b. Allow to cool, then rinse into a 50 ml measuring flask, make up to **50 ml** and pass through a tinstrip filter.

  c. Introduce **10 ml** filtrate, **1 g** KI and 2 ml H_2SO_4 (1+6) into a 150 ml Erlenmeyer flask, titrate with 0.01N sodium thiosulphate solution until a colour change occurs against starch paste.

  d. Added volume (ml) x 14.5 = mg indicator in 100 ml.

  e. Dilute remaining filtrate so that 100 ml contain 145 mg.

  f. The solution remains stable for about 1 week if kept at **+4°C** in brown bottles filled to the brim.

- Phosphate-citrate buffer (pH 4.35):
- Dissolve **31.60 g** disodium hydrogen phosphate (Na_2HPO_4 x 12 H_2O) and **11.75 g** citric acid ($C_6H_8O_7$ x H_2O) in H_2O and dilute to **1 l**.

Sample preparation

1. Heat the beer to **20°C** and expel carbon dioxide under a vacuum (water-jet pump).

2. After the carbon dioxide has been expelled, pipette **10 ml** beer into a test tube with a glass stopper, then **tilt** the tube **slightly** and add **0.25 ml** 0.005M DPI solution.

3. Close the test tube **immediately** and invert it **twice** to mix the contents, starting the stopwatch after the first inversion.

4. Immediately fill a 10 mm rectangular cuvette with the mixture. **60 sec** after adding the reagent, measure the absorbance at **520 nm** against a blank value solution (decarbonated beer without added reagent).

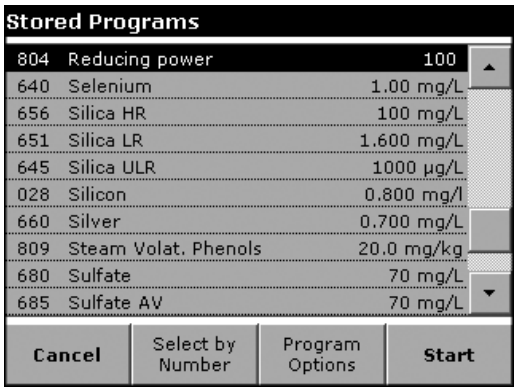
Results
The results are expressed as the proportion of the sample (in %) that was reduced by 10 ml beer in 60 sec.

Accuracy
V_{cr} = ± 1%

Standard values
> 60 very good
50–60 good
45–50 satisfactory
< 45 poor

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 104ff

2.3.1 Procedure for measuring reducing power

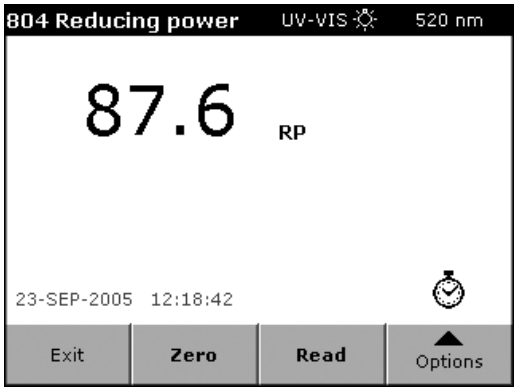


1. Prepare samples as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **804**.

3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.

4. Insert the sample cuvette containing the prepared sample into the cell compartment and close the cell compartment. After 60 sec touch **Read**. The result is displayed.



Note: *Analysis of additional samples: Repeat working procedure from point 3. In other words, each sample has to have its own specific blank value solution.*

2.3.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.4 Anthocyanogens (Harris and Ricketts method)

Anthocyanogens (leucoanthocyanidins) are phenolic compounds, which are transformed into red anthocyanidins by hot hydrochloric acid. The amount and degree of condensation or polymerisation of these compounds influence the formation of colloidal turbidities in beer. Stabilisation measures with PVPP correlate with a reduction in the anthocyanogen content.

Principle
The anthocyanogens are adsorbed on polyamide. The adsorbate is dissolved in butanol and hydrochloric acid and heated. A red solution is formed, whose intensity is measured with a photometer.

Fields of application
Beer, worts

Measuring range
0–100 mg/l

Accessories

- Shaker
- Centrifuge
- Mixing cylinder with ground-glass stopper (50 ml)
- Frit (1 G4)
- Suction flask
- Test tubes with ground-glass stoppers (30 ml, graduations to 25 ml)
- Vacuum pump
- Spectrophotometer (550 nm)
- 10 mm rectangular cuvette (OS grade)

Reagents

- MN polyamide SC 6
- **Solution 1**: n-butanol/37% hydrochloric acid 5+1 (V/V).
- **Solution 2**: Dissolve **120 mg** iron(II) sulphate ($FeSO_4$ x 7 H_2O) in 100 ml solution 1.

Sample preparation

1. Centrifuge worts and young beers for **10 min** at **3000 rpm**.
2. Pipette **5 ml** beer or wort and **5 ml** dist. H_2O into a 50 ml mixing cylinder.
3. Pipette **10 ml** distilled water (blank value) into a 50 ml mixing cylinder
4. Use **10 ml** dist. water to rinse **0.5 g** polyamide powder into each mixing cylinder.
5. Shake the two mixing cylinders mechanically for **40 min**.
6. Filter each suspension through a 1 G4 frit, rinsing twice with about **20 ml** H_2O.
7. Suction-dry the frits and polyamide powder. Use a spatula to transfer each residue to a test tube, rinsing the final traces into each test tube with **15 ml** of solution 1.
8. Add **0.5 ml** of solution 2 and heat both test tubes for **30 min** in a bath of boiling water (stir well with a glass rod for the first 5 min).
9. Remove the glass rod, rinse with a little of solution 1, bring the test tubes to 20°C and make each one up to 25 ml with solution 1.
10. Measure the absorbance of the solution in a 10 mm rectangular cuvette at **550 nm** against a similarly treated blank value solution (10 ml dist. water instead of beer).

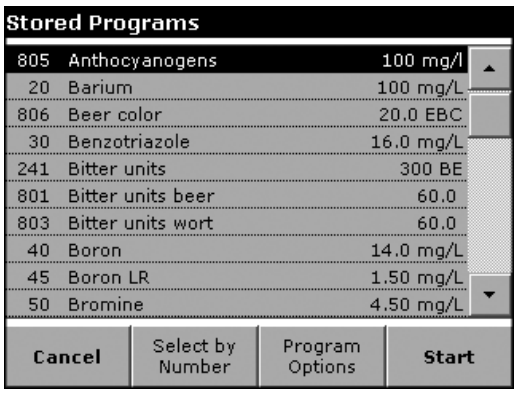
Results
The result is expressed in mg/l, without decimal places.

Accuracy
r = 9

Standard values
50–70 mg/l depending on the raw materials and technical measures; correspondingly lower after stabilisation with PVPP.

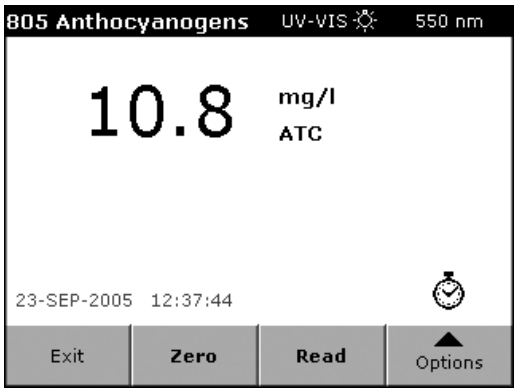
Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 109ff

2.4.1 Procedure for measuring anthocyanogens



1. Prepare samples as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **805**.
3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert the sample cuvette containing the prepared sample into the cell compartment and close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*



2.4.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.5 Beer colour (spectrophotometric EBC method)

Principle
This method is designed to eliminate subjective effects attributable to the human eye as well as differences in the colour impression when the beer samples are compared with the colour card. This method is an official reference method.

The absorbance is measured in a 10 mm rectangular cuvette at a wavelength of exactly 430 nm. The colour in EBC units is obtained by converting with a suitable factor.

Fields of application
Plant wort, beer, liquid malt substitutes of all kinds.

Measuring range
0–20 units

Accessories

- Spectrophotometer (430 nm ± 0.5 nm)
- 10 mm rectangular cuvettes (OS grade)

Sample preparation

1. Dilute the sample so that the absorbance is within the linearity range of the spectrophotometer.

2. Filter the sample through a membrane filter. Filtration is not necessary if the turbidity of the diluted sample is less than 1 EBC turbidity unit.

3. If necessary, clarify the sample by adding 0.1% kieselguhr and filtering before the membrane filtration is carried out.

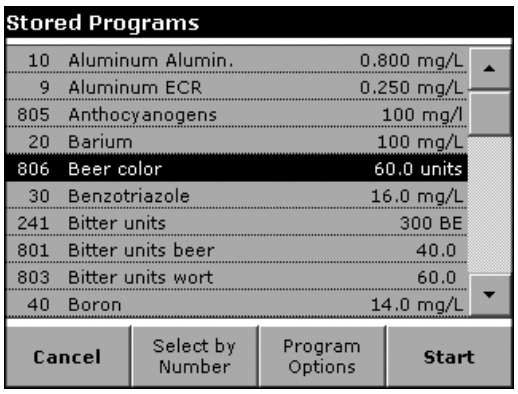
4. Measure the absorbance (A) at **430 nm** against dist. water (blank value).

Results
The results are expressed in EBC units to 2 significant decimal places.

Interferences
A spectrometric absorbance curve does not reflect the colour impression of the human eye, because light of the same intensity does not have a uniform effect on the eye in different parts of the spectrum. In addition the absorbance curves at 430 nm are very steep, so slight measurement errors may occur. Moreover, there are differences when light beers are compared with diluted dark beers.

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 88ff

2.5.1 Procedure for measuring beer colour



1. Prepare samples as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **806**.
3. Insert blank value cuvette (distilled water) into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert the sample cuvette containing the prepared sample into the cell compartment and close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*

2.5.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.6 Free amino nitrogen (ninhydrin method based on EBC method)

Principle
The test solution is heated with ninhydrin at pH 6.7 and the resulting colour is measured at 570 nm. The method covers amino acids, ammonia and also the terminal alpha-amino groups of peptides and proteins. Proline is partially detected at the wavelength used. The method is not specific for alpha-amino-nitrogen, because gamma-amino butyric acid, which occurs in worts, also reacts with ninhydrin to produce a colour.

Fields of application
Beer, worts

Measuring range
0–400 mg/l

Accessories

- Test tubes with ground-glass stoppers (16 x 150 mm)
- Variable pipette (1.0–5.0 ml) (BBP 065)
- Pipette tips for pipette (BBP 068)
- Water bath suitable for boiling water
- Water bath (20°C)
- Spectrophotometer (570 nm)
- 10 mm rectangular cuvette (OS grade)

Reagents

- **Colour reagent**: Dissolve **10.0 g** disodium hydrogen phosphate (Na_2HPO_4 x 12 H_2O), **6.0 g** potassium dihydrogen phosphate (KH_2PO_4), **0.5 g** ninhydrin and **0.3 g** fructose in H_2O and make up to 100 ml. This solution remains stable for 2 weeks in a dark bottle. The pH must be 6.6–6.8.
- **Dilution solution**: Dissolve **2 g** potassium iodate in **600 ml** H_2O and add **400 ml** 96% ethanol
- **Stock solution**: Dissolve **107.2 mg** glycine in 100 ml H_2O. Keep this stock solution at 0°C.
- **Standard solution**: Make up **1 ml** stock solution to **100 ml** with H_2O. This standard solution contains 2 mg/l amino-nitrogen.

Sample preparation

1. Dilute wort 100-fold, beer 50-fold (1–3 mg/l amino-nitrogen)
2. Analyse sample, standard solution and blank value solution three times.
3. Pipette **2 ml** of the diluted sample or the standard solution or H_2O into a test tube.
4. Add **1 ml** colour reagent and mix.

5. Loosely close test tube with glass stopper to prevent evaporation losses.

6. Heat for exactly **16 min** in boiling water in a water bath, then cool for **20 min** in a water bath at 20°C.

7. Add **5 ml** dilution solution.

8. Measure the absorbance within 30 min in a 10 mm rectangular cuvette at **570 nm** against a blank value solution treated in the same way (H_2O + colour reagent).

9. **Correction for dark worts and beers (perform three times).**

   a. Introduce **2 ml** of the diluted sample into a test tube.

   b. Add **1 ml** H_2O instead of the colour reagent, then proceed as described above.

   c. Measure against H_2O after adding **5 ml** dilution solution

Results
The results are expressed in mg/l without decimal places.

Accuracy
r = 17
R = 28

Standard values
Finished wort (12%): 200–250 mg/l
Beer (12%): 100–120 mg/l

About 220–250 mg/l free amino-nitrogen should be present in the original wort to ensure satisfactory primary and secondary fermentation.

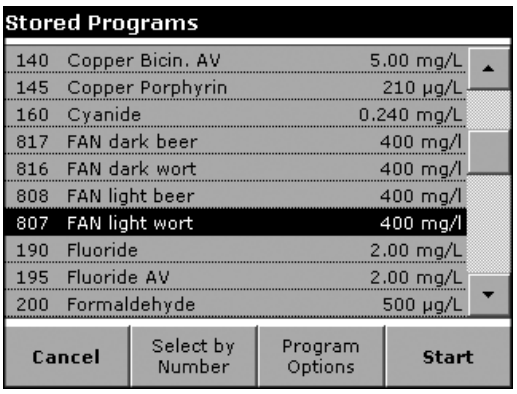
Interferences
The amino acids are present in very small amounts, so contamination must be avoided at all costs. The carefully cleaned test tubes should only be touched on the outside. Ground-glass stoppers, etc., should be picked up with forceps.

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 62ff

Remark
The working procedure described below specifies that the blank value solutions, standard solution and sample should be measured three times without correction when light beer and wort are analysed.

In the case of dark beers, the working procedure specifies that the blank value solution, standard solution, correction and sample should be measured three times.

2.6.1 Procedure for measuring free amino nitrogen (FAN) in light worts



1. Prepare three blank value solutions, three standard solutions and three samples as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **807**.
3. Insert zero value solution (distilled water) into the cell compartment, close the cover and touch **Zero**.
4. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.

   Note: *Repeat the procedure with blank value cuvettes 2 and 3. Display:* ***E2*** *and* ***E3****.*
5. Insert standard cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E4**.

   Note: *Repeat the procedure with standard cuvettes 2 and 3. Display:* ***E5*** *and* ***E6****.*
6. Insert the sample cuvette with the first prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E7**.

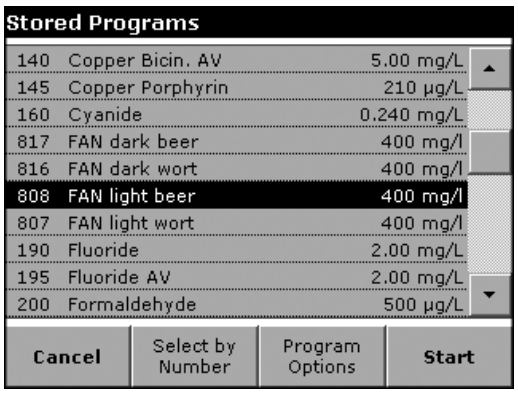
   Note: *Repeat the procedure with sample cuvettes 2 and 3. Display:* ***E8*** *and then, after the final measurement, the result.*
7. The FAN result is displayed in mg/l.

Note: *Analysis of additional samples: Repeat working procedure from point 6.*

2.6.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.6.2 Procedure for measuring free amino nitrogen (FAN) in light beer



1. Prepare three blank value solutions, three standard solutions and three samples as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **808**.
3. Insert zero value solution (distilled water) into the cell compartment, close the cover and touch **Zero**.
4. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.

   Note: *Repeat the procedure with blank value cuvettes 2 and 3. Display:* ***E2*** *and* ***E3****.*
5. Insert standard cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E4**.

   Note: *Repeat the procedure with standard cuvettes 2 and 3. Display:* ***E5*** *and* ***E6****.*
6. Insert the sample cuvette with the first prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E7**.

   Note: *Repeat the procedure with sample cuvettes 2 and 3. Display:* ***E8*** *and then, after the final measurement, the result.*

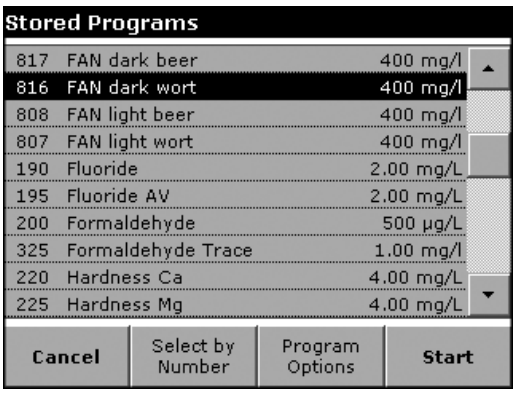
7. The FAN result is displayed in mg/l.

Note: *Analysis of additional samples: Repeat working procedure from point 6.*

2.6.2.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.6.3 Procedure for measuring free amino nitrogen (FAN) in dark worts



1. Prepare three blank value solutions, three standard solutions and three samples as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **816**.

3. Insert zero value solution (distilled water) into the cell compartment, close the cover and touch **Zero**.

4. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.

   Note: *Repeat the procedure with blank value cuvettes 2 and 3. Display:* ***E2*** *and* ***E3****.*

5. Insert standard cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E4**.

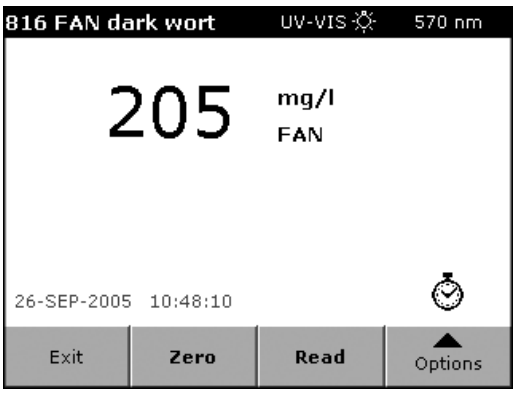
   Note: *Repeat the procedure with standard cuvettes 2 and 3. Display:* ***E5*** *and* ***E6****.*

6. Insert correction cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E7**.

   Note: *Repeat the procedure with correction cuvettes 2 and 3. Display:* ***E8*** *and* ***E9****.*

7. Insert the sample cuvette with the first prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E10**.

   Note: *Repeat the procedure with sample cuvettes 2 and 3. Display:* ***E11*** *and then, after the final measurement, the result.*



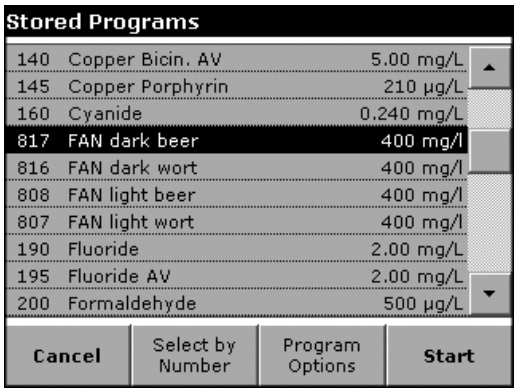
8. The FAN result is displayed in mg/l.

Note: *Analysis of additional samples: Repeat working procedure from point 7.*

2.6.3.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.6.4 Procedure for measuring free amino nitrogen (FAN) in dark beers



1. Prepare three blank value solutions, three standard solutions and three samples as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **817**.

3. Insert zero value solution (distilled water) into the cell compartment, close the cover and touch **Zero**.

4. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.

   Note: *Repeat the procedure with blank value cuvettes 2 and 3. Display:* ***E2*** *and* ***E3****.*

5. Insert standard cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E4**.

   Note: *Repeat the procedure with standard cuvettes 2 and 3. Display:* ***E5*** *and* ***E6****.*

6. Insert correction cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E7**.

   Note: *Repeat the procedure with correction cuvettes 2 and 3. Display:* ***E8*** *and* ***E9****.*

7. Insert the sample cuvette with the first prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E10**.

   Note: *Repeat the procedure with sample cuvettes 2 and 3. Display:* ***E11*** *and then, after the final measurement, the result.*

8. The FAN result is displayed in mg/l.

Note: *Analysis of additional samples: Repeat working procedure from point 7.*

2.6.4.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.7 Steam-volatile phenols

The degree of fumigation of whisky malts is determined by analysing steam-volatile phenols. In the beer industry, small amounts of smoke-dried malts are used to produce "Rauchbiere" (smoked beers), a speciality of Franconia. Technical problems during kilning can 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.

Principle
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 oxidising agent potassium hexacyanoferrate(III) to form a colour substance, which can be measured with a spectrophotometer after being extracted with chloroform.

Fields of application
Malt, beer

Measuring range
0–20 mg/kg

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

Accessories

- DLFU mill (aperture 1 mm)
- Steam distillation unit
- Separating funnels (1 l)
- Spectrophotometer (460 nm)
- 40 mm rectangular cuvette (OS grade)

Reagents

- Chloroform, ultrapure
- Silicone antifoam emulsion
- Phosphoric acid, conc. (d = 1.71)
- Copper sulphate, $CuSO_4$ x 5 H_2O (10%)
- Ammonium chloride (5%)
- 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (2%): prepare freshly each day

- Potassium hexacyanoferrate(III), $K_3[Fe(CN)_6]$, 8%: prepare freshly each day
- Phenol standard solution:
  Dissolve 1,000 g phenol in H_2O giving 1000 ml (1ml = 1mg). The solution must be clear and colourless. Use this solution to prepare dilutions with which to obtain the calibration curve between 0.02 and 0.1 mg/l when needed.
- Ammonia, dilute (1+4):
  Dilute 1 part conc. ammonia (d = 0.91) with 4 parts H_2O.

Sample preparation

1. Steam distillation

a. Introduce **50 g** coarse malt and **500 ml** H_2O (for beer analyses 300 ml) into a distillation flask.

b. Add **3 ml** copper sulphate solution.

c. Add phosphoric acid until the pH is less than 4.

d. Add silicone antifoam emulsion.

e. Carry out steam distillation until 300 ml have been obtained.

2. Colour reaction

a. Add **10 ml** ammonium chloride solution to all of the distillate (or correspondingly less in the case of genuine smoke-dried malts or whisky malts, for example 100 ml). To prepare a blank value solution, use 300 ml H_2O instead of the distillate and add **10 ml** ammonium chloride solution.

b. Shake.

c. Adjust the pH of the distillate and blank value solution to 10.2 ± 0.1 by adding ammonia.

d. Transfer to 1 l separating funnels.

e. Add **3 ml** 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one and **3 ml** potassium hexacyanoferrate(III) to each funnel.

f. Shake.

g. Leave to stand for **3 min**.

h. Extract by adding **10 ml** chloroform to each funnel and shaking each funnel **3 times** (1 min).

i. Wait **10 min** for phase separation to occur.

j. Filter the chloroform extracts through a paper filter into 25 ml measuring flasks.

k. Rinse the filters with a little chloroform.

l. Fill each flask up to the mark with chloroform.

m. Measure the chloroform extract (prepared distillate) in a 40 mm rectangular cuvette at **460 nm** against a blank value solution obtained by following the above procedure but using 300 ml H_2O instead of the distillate.

3. Calibration values

a. Carry out steam distillation on phenol standard solutions with concentrations between 0.02 and 0.1 mg/l (use 300 ml), then proceed as described above.

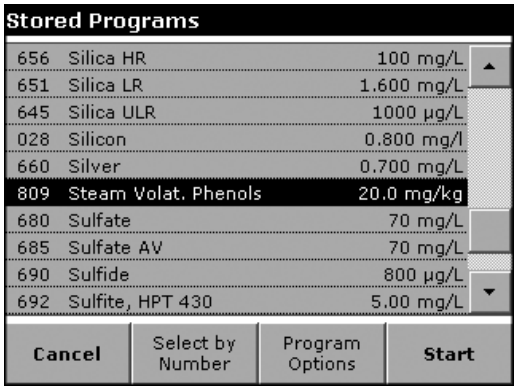
Results
The results are expressed in mg/kg to two decimal places (or in mg/l in the case of beer)

Accuracy
Vc = ± 5% (repeat error)

Required values
Malts: < 0.2 mg/kg: no smoky taste to be expected.
Beers: < 0.03 mg/l: negligible effect in most cases.
The intensity of the smoky taste is partly dependent on the composition of the beer. The specified lower limit therefore only applies with reservations.

Literature
MEBAK Brautechnische Analysenmethoden 3rd Edition, Volume I

2.7.1 Procedure for measuring steam-volatile phenols



1. Prepare the samples and blank value solution as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **809**.

3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.

4. Insert the sample cuvette containing the prepared sample into the cell compartment and close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*

2.7.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.8 Photometric iodine sample

Photometric iodine sample by new method (MEBAK from 1993).

Principle
High-molecular dextrins and starches are precipitated by adding ethanol to wort and beer, separated by centrifuging, and dissolved in a phosphate buffer, to which iodine solution is then added. Depending on the molecular weight and the degree of branching of the erythrodextrins and starch, a red to blue colour appears, whose intensity is measured with the help of a photometer.

Fields of application
Wort, beer (samples whose iodine value is > 0.8 must be diluted.)

Measuring range
0–1 iodine value

Accessories

- Centrifuge
- Centrifuge tubes with ground-glass stoppers (100–110 ml content)
- Shaker
- Pipettes (0.5 ml, 2 ml, 10 ml, 20 ml, 40 ml)
- Spectrophotometer (578 nm)
- 40 mm rectangular cuvette (OS grade)
- Plastic spatula

Reagents

- Ethanol, 95%
- Iodine solution, 1N (stock solution)

- Iodine solution, 0.02N (prepare freshly each day from the stock solution)
- Phosphate buffer solution, 0.1M, pH 3.5: adjust the pH of a 0.1M KH_2PO_4 solution to 3.5 with 0.1M phosphoric acid

Sample preparation

1. Pipette **10.0 ml** centrifuged wort, or carbon-dioxide-free beer, into a centrifuge tube.
2. Add **40.0 ml** ethanol and shake mechanically for **10 min**.
3. Centrifuge for **5 min** at **2500 rpm**.
4. Carefully decant as much of the clear phase as possible.
5. Dissolve residue in **20.0 ml** phosphate buffer solution by shaking mechanically for **10 min**.
6. Centrifuge the solution for **5 min** at **2500 rpm**.
7. Pipette **2 ml** of the supernatant liquid and **8 ml** phosphate buffer solution into a 40 mm rectangular cuvette and measure at **578 nm** against phosphate buffer solution.
8. Add **0.5 ml** 0.02N iodine solution, mix the contents immediately with the plastic spatula, then measure after **30 sec**.
9. **Iodine blank solution**
   a. Pipette **10 ml** phosphate buffer solution and **0.5 ml** 0.02N iodine solution into a 40 mm rectangular cuvette and mix.
   b. Measure absorbance at **578 nm** against phosphate buffer solution.

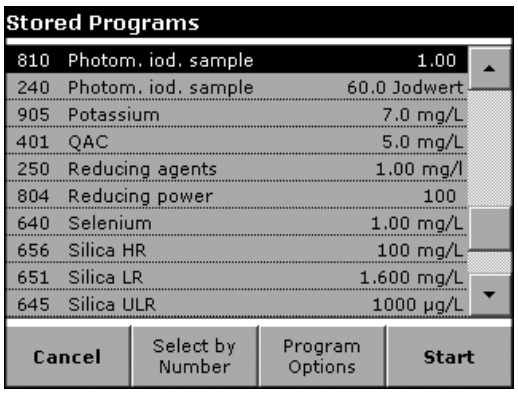
Results
The results are expressed as absorbance to 2 decimal places.

Accuracy
Vcr = ± 3%

Standard values
< 0.3 (wort)

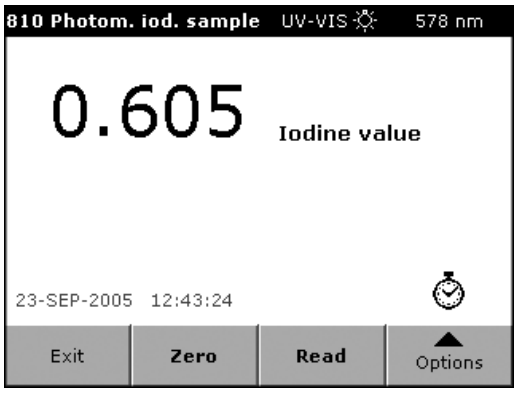
Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 34ff

2.8.1 Procedure for measuring photometric iodine sample



1. Prepare samples and iodine blank solution as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **810**.
3. Insert cuvette containing phosphate buffer into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert blank value cuvette containing the prepared iodine blank solution (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.
5. Insert the sample cuvette containing the prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E2**.
6. Introduce **0.5 ml** 0.02N iodine solution into sample cuvette. Mix the contents **immediately** with plastic spatula, and after 30 sec insert the cuvette into the cell compartment, close the cover and touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4. The prepared iodine blank solution can be used for the complete series of measurements.*



2.8.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.9 Thiobarbituric acid number (TAN)

The thiobarbituric acid number is a sum parameter for the thermal effects on malt and wort. It is a parameter that, apart from 5-hydroxymethylfurfural (HMF), covers a large number of products of the Maillard reaction and other organic compounds.

Principle
The test sample reacts with a solution of thiobarbituric acid and acetic acid and the resulting yellow colour is measured with the help of a spectrophotometer.

Fields of application
Beer, wort, congress wort or malt extract

Measuring range
0–100

Accessories

- Water bath (70°C)
- Brown test tubes with ground-glass stoppers (20 ml or 25 ml)
- Spectrophotometer (448 nm)
- 10 mm rectangular cuvettes (OS grade)

Reagents

- Acetic acid (90%):
  Dilute **225 g** 100% acetic acid (glacial acetic acid) with H_2O to 250 g.
- Thiobarbituric acid (0.02 mol/l):
  Dissolve **0.288 g** 2-thiobarbituric acid (M = 144.15 g/mol) in a 100 ml measuring flask with 90% acetic acid by heating in a water bath. Cool to 20°C then make up to the mark with 90% acetic acid. Prepare freshly each day.
- Kieselguhr

Sample preparation

Note: *The analysis procedure is empirical and should therefore be adhered to exactly.*

1. Clarify turbid test solutions by filtration over kieselguhr.

2. Dilution

- Dilute worts and beers 10-fold with H_2O
- Dilute congress worts 5-fold with H_2O

3. Blank value

a. Add **5 ml** 90% acetic acid to **10 ml** diluted sample, shake and proceed as for the main value.

4. **Main value**

   a. Add **5 ml** thiobarbituric acid to **10 ml** diluted sample and shake.

   b. Place in a 70°C water bath for **70 minutes** (avoid direct sunlight and ensure that, at most, the temperature in the bath decreases only briefly by 1–2°C when the test tubes are introduced).

   c. When the reaction time has elapsed, cool the test tubes quickly to 20°C (strongly flowing cold water or cooling bath).

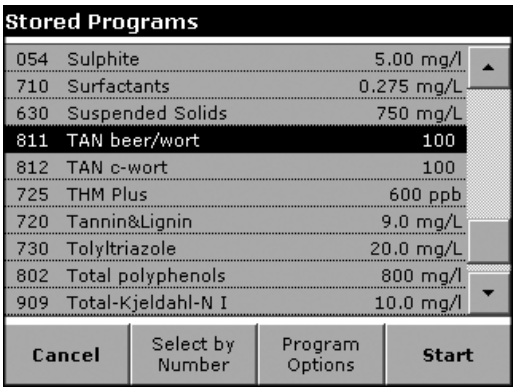
   d. Measure the yellow colour **immediately** in 10 mm rectangular cuvettes at **448 nm** against H_2O.

Results
Thiobarbituric acid number (TBN); dimensionless number

Standard values
Light finished wort < 45
Light cold wort (after wort cooling) < 60

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 35ff

2.9.1 Procedure for measuring thiobarbituric acid number in beer and wort



1. Prepare samples and blank value solution as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **811**.

3. Insert zero value cuvette containing distilled water into the cell compartment. Close the cell compartment. Touch **Zero**.

4. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.

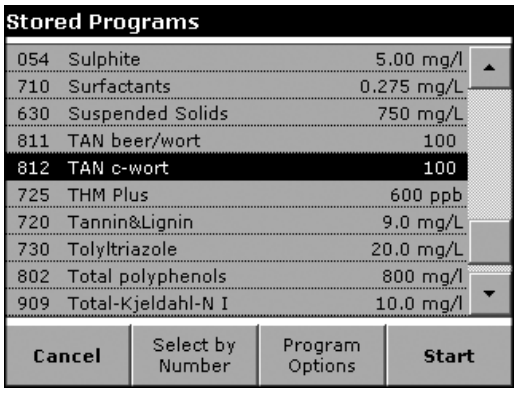
5. Insert the sample cuvette containing the prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*

2.9.1.1 Executing the test with the sipper modulel

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.9.2 Procedure for measuring thiobarbituric acid number in congress wort



1. Prepare samples and blank value solution as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **812**.
3. Insert zero value cuvette containing distilled water into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Read**. Display: **E1**.
5. Insert the sample cuvette containing the prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4.*

2.9.2.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.10 Iso-α-acids and α-acids

Principle
The bitter substances are extracted from the acidified sample with iso-octane. Certain substances that cause interference are removed by washing the extract with acidified methanol, and the concentrations of iso-α-acids and α-acids are determined by measuring the absorbance in alkaline methanol at 255 nm and 360 nm.

Fields of application
Beer, wort

Measuring range
0–60 mg/l

Accessories

- Centrifuge tubes with solvent-tight screw tops (100–110 ml content)
- Shaker
- Centrifuge (3000 rpm)
- Spectrophotometer (255 nm and 360 nm)
- 10 mm rectangular cuvettes (QS grade)

Reagents

- Hydrochloric acid, 6N
- Iso-octane (2,2,4-trimethylpentane), of spectroscopic purity
- Sodium sulphate, anhydrous
- Methanol
- Hydrochloric acid (4N)
- Sodium hydroxide (6N, carbonate-free)
- Acidic methanol solution: Mix 64 ml methanol and 36 ml 4N hydrochloric acid (prepare freshly each day).
- Alkaline methanol solution: Take 0.2 ml 6N sodium hydroxide and make up to 100 ml with methanol (prepare freshly each day).

Sample preparation

1. Clarify wort or cloudy beer by centrifuging at **3000 rpm** for **15 min** (do not filter).
2. Expel carbon dioxide from beer without losing any foam.
3. Bring the sample to 20°C, then pipette **50 ml** into a centrifuge tube.
4. Add **3 ml** 6N hydrochloric acid and **25 ml** iso-octane.
5. Close the centrifuge tube and shake mechanically for **30 min** at optimal mixing intensity.

6. Separate the phases and break the emulsion by centrifuging for **5 min** at **3000 rpm**.

7. Use a pipette to draw off and discard the bottom aqueous phase. Add sodium sulphate to the iso-octane phase until the phase clarifies after being shaken vigorously for a short time.

8. Pipette **10 ml** of the iso-octane phase into a 25 ml mixing cylinder.

9. Add **10 ml** acidic methanol solution, and mix the contents of the cylinder by inverting it 100 times

10. Pipette **5 ml** of the supernatant clear iso-octane phase into a 25 ml measuring flask.

11. Make up to the mark with alkaline methanol solution and mix.

12. Measure the absorbance of the iso-octane solution at **255 nm** and **360 nm** against a blank value solution.

13. **Preparation of the blank value solution**

    a. Pipette **5 ml** iso-octane into a 25 ml measuring flask.

    b. Fill up to the mark with alkaline methanol solution and mix.

Results
The result is expressed in mg/l without any decimal places

Accuracy
Vcr = ± 5%

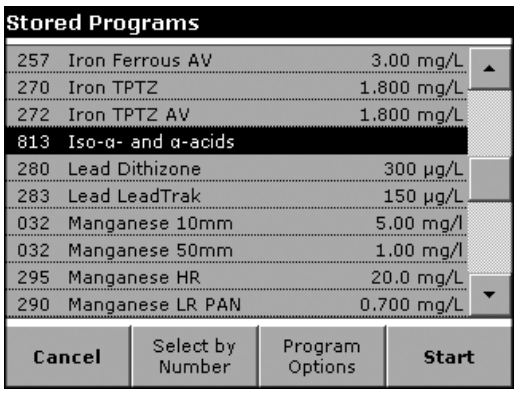
Standard values
Beer: 10–40 mg/l iso-α-acids, depending on type and origin
(< 2mg/l a-acids)

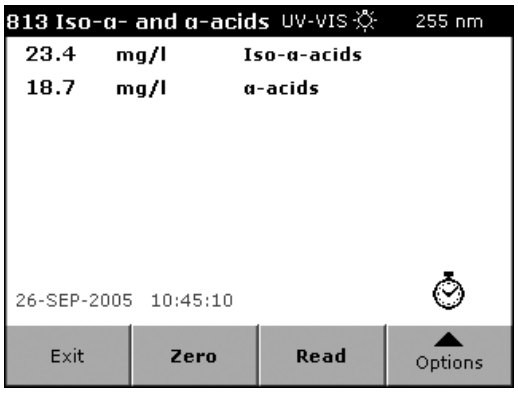
Wort: 15–50 mg/l iso-α-acids, depending on the beer and the level of bitter substance utilisation

1–15 mg/l a-acid depending on degree of isomerisation

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 116ff

2.10.1 Procedure for measuring iso-α-acids and α-acids





1. Prepare samples and blank value solution as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **813**.
3. Insert blank value cuvette (see sample preparation) into the cell compartmentcell compartment. Close the cell compartment. Touch **Zero**.
4. Insert the sample cuvette containing the prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 4. The prepared blank value solution can be used for the complete series of measurements.*

2.10.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.11 Vicinal diketones (diacetyl, 2,3-pentanedione)

The metabolic processes of yeast produce 2-acetolactate and 2-acetohydroxibutyrate during fermentation. These are oxidised to 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.

The photometric determination method is often used in preference to the gas chromatographic method in operational checks, because it can be carried out quickly and without the need for expensive apparatus. Unfortunately it is not capable of differentiating between diacetyl and pentanedione.

Principle
The basis of the method is the reaction of diacetyl or 2,3-pentanedione with 1,2-phenylenediamine to form 2,3-dimethylquinoxaline, which exhibits specific absorbance at 335 nm.

Fields of application
Beer

Measuring range
0–1 mg/kg

Accessories

- Macro version of apparatus for nitrogen determination, with heating jacket (for example from Schott). The accompanying cooler may need to be replaced by a larger one if the distillate is not cooled sufficiently. Other, similar units (for example from Büchi) are equally suitable.
- Spectrophotometer (335 nm)
- 20 mm rectangular cuvettes (QS grade)

Reagents

- Hydrochloric acid (4N)
- 1,2-Phenylenediamine (1% in 4N hydrochloric acid). Prepare the solution freshly on the day when it is needed, and keep it is a dark place. 1,2-Phenylenediamine is toxic and allergenic; handle it carefully, and wear gloves while working.
- Antifoam emulsion (free of diketones

Sample preparation

1. Introduce **100 g** beer, from which the carbon dioxide has not been removed, into a preheated distillation apparatus.
2. Add one drop of antifoam emulsion.
3. Regulate the steam supply so that about 25 ml distillate are obtained in 2 min.
4. Collect the distillate in 25 ml measuring flasks.

5. Pipette **10 ml** of the mixed distillate into each of two 50 ml Erlenmeyer flasks (main value solution, blank value solution).

6. **Blank value solution**

   - Add **2.5 ml** 4N hydrochloric acid.

7. **Main value solution**

   - Add **0.5 ml** 1,2-phenylenediamine solution, mix and allow to stand in a dark place for **30 min**.
   - Then add **2 ml** 4N hydrochloric acid.

8. Within **20 min**, measure the absorbance of the main value solution against the blank value solution at **335 nm** in 20 mm rectangular cuvettes.

Results
The result is expressed in mg/kg to two decimal places.

Accuracy
SD = ± 0.01

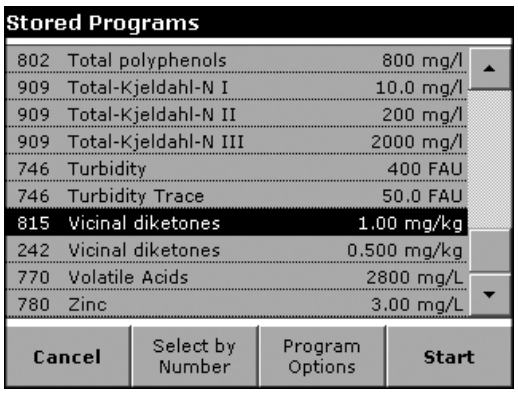
Required value
For light "Vollbier" (beer with a high original gravity) < 0.15 mg/kg.

Remarks
If series of analyses are carried out, the apparatus need not be cleaned or rinsed between the individual determinations but can be refilled immediately with beer after it has automatically emptied. After a series of distillations the adhering residues should be removed with sodium hydroxide solution or some other suitable cleaning agent.

Any acetohydroxy acids that are present in beer after the filling stage are oxidised to diketones in the presence of O_2. Before the actual analysis the beer sample can be thermostated at 70°C for 1.5 hours for the purpose of analysing the total diketone content.

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 134ff

2.11.1 Procedure for measuring vicinal diketones



1. Prepare samples and blank value solution as described in the working procedure.
2. Select **Stored Programs** in the "Main Menu". Select test number **815**.
3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.
4. Insert the sample cuvette containing the prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 3.*

2.11.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

2.12 Iron

Iron in beer may originate from raw materials, filter aids, apparatus, pipes or cans, or beer foam stabilising agents. Iron has a disadvantageous effect on colloidal stability, taste and the gushing tendency of the beer.

Principle
Divalent iron reacts with the disodium salt of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine-4,4-disulphonic acids (Ferrozin) to form a violet-coloured complex with a very high molar absorbance coefficient. Trivalent iron must be reduced to the divalent form before the determination is carried out. The colour intensity is measured with a spectrophotometer.

Measuring range
0–1 mg/l

Accessories

- Analytical balance readable to 0.1 mg
- Pipettes (0.1 ml, 2 ml, 5 ml)
- Spectrophotometer (560 nm)
- 40 mm rectangular cuvette (OS grade)

Reagents
Prepare all solutions with iron-free H_2O.

- Buffer solution (pH 4.3):
  Dissolve **75 g** ammonium acetate and **150 g** conc. acetic acid in about **800 ml** H_2O, check the pH and make up to 1 l.
- Ferrozin reagent:
  Dissolve **0.257 g** Ferrozin or Ferrospectral in **50 ml** buffer (the solution remains stable for 2 weeks).
- Ascorbic acid (2.5%) Prepare freshly each day.
- Hydrochloric acid, conc.
- Iron(III) standard solution for obtaining the calibration curves:
  Dissolve **863.4 mg** ammonium iron(III) sulphate $[NH_4Fe(SO_4)_2 \times 12\ H_2O]$ in H_2O in a 1 l measuring flask. Add **0.1 ml** conc. hydrochloric acid and make up to the mark with H_2O. Dilute 50 ml of this solution with H_2O to 1 l to obtain a standard solution containing 5 mg/ml Fe^{3+}.

Sample preparation

1. Expel carbon dioxide from beer and allow the foam to completely collapse.
2. Pipette **40 ml** beer, **2 ml** Ferrozin reagent and **1 ml** ascorbic acid solution into a 50 ml measuring flask.
3. Make up to the mark with H_2O.

4. Prepare a blank value solution in exactly the same way, but without adding the Ferrozin reagent. Prepare a blank value solution for each beer.

5. Measure the absorbance of the solution in a 40 mm rectangular cuvette at **560 nm** against the corresponding blank value solution.

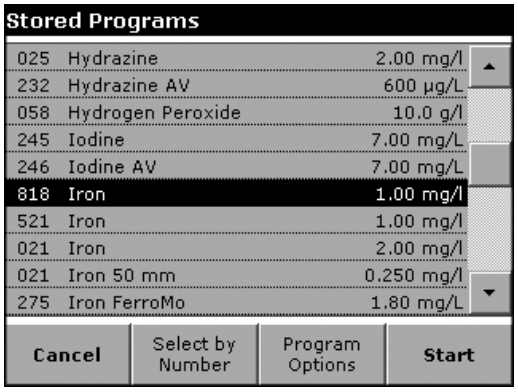
Results
The results are expressed in mg/l to three significant places

Accuracy
r = 0.008

Required value
< 0.200 mg/l

Literature
MEBAK Brautechnische Analysenmethoden
4th Edition 2002 Volume II, pp 149ff

2.12.1 Procedure for measuring iron



1. Prepare samples and blank value solution as described in the working procedure.

2. Select **Stored Programs** in the "Main Menu". Select test number **818**.

3. Insert blank value cuvette (see sample preparation) into the cell compartment. Close the cell compartment. Touch **Zero**.

4. Insert the sample cuvette containing the prepared sample into the cell compartment. Close the cell compartment. Touch **Read**. The result is displayed.

Note: *Analysis of additional samples: Repeat working procedure from point 3.*

2.12.1.1 Executing the test with the sipper module

Information about the installation, module configurations and sample introduction of the sipper module can be found in the user manual of the DR 5000 Spectrophotometer (15.3.3. Installation Sipper Module, page 133).

Obtaining the calibration curve
The factor 1 = 0.037 is an empirical variable and has to be individually determined from a calibration line. The factor is the gradient of the calibration line.

- Pipette **40 ml** beer into each of four 50 ml measuring flasks.
- Pipette respectively **0.40 ml**, **0.80 ml**, **1.60 ml** and **3.20 ml** iron standard solution (5 mg Fe^{3+}/ml) into the measuring flasks.
- Add **2 ml** Ferrozin reagent and **1 ml** ascorbic acid solution to each measuring flask.
- Make up to the mark with H_2O.
- Measure the absorbance of the solution in a 40 mm rectangular cuvette at 560 nm against the corresponding blank value solution.
- Blindwert messen.

Deduct the absorbance of the sample from the absorbance values of the standard solutions.

Section 3 Replacement Parts

Description	Cat. No.
Macro-cuvette (OG grade; path length = 20 mm)	LZP331
Cuvette set (path length = 1 cm; matched pair)	2095100
Cuvette (QS grade; path length =10 mm)	2624410
Pour-through cuvette (QS grade; path length = 10 mm; fill height = 10 mm; total height = 40 mm)	LZV510

Section 4 How To Order

Orders/Repair service

Please contact your representative:

HACH LANGE GMBH
Willstätterstraße 11
D-40549 Düsseldorf
Tel.: +49 (0)2 11 5288-0
Fax: +49 (0)2 11 5288-143
info@hach-lange.de
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HACH LANGE LTD
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Manchester, M50 1DL
Tel. +44 (0)161 8 72 14 87
Fax +44 (0)161 8 487324
info@hach-lange.co.uk
www.hach-lange.co.uk

Information Required

- Hach account number (if available)
- Billing address
- Your name and phone number
- Shipping address
- Purchase order number
- Catalog number
- Brief description or model number or series-production number
- Quantity