

DR6000 in the Brewing Industry: Important Methods in Accordance with MEBAK and ASBC

Introduction

Compliance and consistent high quality are two of the key goals within the beverage industry. Hach® provides support for these goals through comprehensive analyses of water and beer.

The DR6000™ UV-VIS Spectrophotometer supports many of the analytical measurements necessary for monitoring throughout the entire brewing process—from raw materials to final product. The DR6000 brewing-specific software has been expanded to include the most important parameters from both MEBAK¹ and the American Society of Brewing Chemists (ASBC)². This means that the DR6000 can be used to measure beer quality around the globe.

The Key Methods in Detail

Beer Color

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 185 ff.

ASBC Beer 10-A

The EBC and ASBC units are used throughout Europe and the United States to describe the color (more specifically: the color intensity) of beer and beer wort. The value stipulated by the European Brewery Convention (EBC) or ASBC indicates how much light is absorbed by beer of a certain content of original wort. The actual color of each beer is nothing more than gradations of a brown tone, which decreases in concentration through red, copper, and amber colors, through to golden yellow and light yellow.

In addition to malt color and original wort, the color intensity of the finished beer still depends on many other factors, such as the wort preparation, the pH value and the fermentation process.

The measurement of the color may seem trivial, but it is the first impression that the customer gets before the consumption of the beer. The compliance of the beer color is therefore an important issue that can be monitored throughout the entire fermentation process.

The absorbance of the beer is measured at a wavelength of 430 nm. Historically, the beer color in EBC units is 10 x absorbance at 430 nm measured in a 1 inch (2.54 cm) cuvette. However, for MEBAK a 1 cm (10 mm) square cuvette is stipulated. Accordingly, the following calculation applies for the determination of the beer color in accordance with MEBAK:

Absorbance of the beer at 430 nm x 25 = Color in EBC units.

Historically, the beer color in ASBC units is 10 x absorbance of the beer at a wavelength of 430 nm and with the use of a ½ inch (1.27 cm) cuvette. With the use of an intermediately stipulated 1 cm (10 mm) cuvette, the following applies in accordance with ASBC method Beer-10A:

Absorbance of the beer at 430 nm * 12.7 = Color in EBC units

Additionally, the turbidity of the sample is checked in the ASBC method by means of an absorbance measurement at 700 nm. A sample is not classed as turbid if the 700 nm absorbance is $\leq 0.039 \times 430$ nm absorbance.

In the DR6000, the programs for the measurement of the beer color are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

MEBAK Beer Color	Program 2006	0–60 units
ASBC Beer Color*	Program 2020	0–60 units

The following scale of beer colors is useful for orientation:

EBC	Example	Beer color
4	Pale Lager, Witbier, Pilsener, Berliner Weisse	
6	Maibock, Blonde Ale	
8	Weißbier	
12	American Pale Ale, India Pale Ale	
16	Weißbier, Saison	
20	English Bitter, Extra Special Bitter	
26	Biere de Garde, Double IPA	
33	Dunkles Lager, Märzen, Amber Ale	
39	Brown Ale, Bock, Dunkelbier, Dunkelweizen	
47	Irish Dry Stout, Doppelbock, Porter	
57	Stout	
69	Foreign Stout, Baltic Porter	
79	Imperial Stout	

(Source: [http://de.wikipedia.org/wiki/EBC_\(Bier\)](http://de.wikipedia.org/wiki/EBC_(Bier)))

Bitterness Units

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 234 ff.
ASBC Beer-23, Wort-24

The concentration of the bitters is a key quality feature of the beer. Bitters emerge during boiling due to the isomerization of α -acids from the hops. Bitters are extracted with isoctane from the acidified sample and the absorbance is measured spectrophotometrically at a wavelength of 275 nm.

The MEBAK and ASBC methods differ only minimally in their execution. While in the MEBAK, 6 N HCl is used to acidify the samples, the ASBC uses only 3 N HCl. After extraction, the absorbance is measured in a 10 mm quartz cuvette against a blank of isoctanol of the same quality.

In accordance with the definition of MEBAK and ASBC, the results are calculated as follows:

Beer: Absorbance 275 nm * 50 = Bitterness in Bitterness Units
Wort: Absorbance 275 nm * 100 = Bitterness in Bitterness Units

The various calculations result from the dilutions of beer and/or wort samples specified in the procedure.



The standard values in accordance with MEBAK are 10–40 BU (bitterness units) for beer and 20–60 BU for wort. In accordance with ASBC, the measurement range for beer is up to 100 units (wort 200) and is reported in IBU (International Bitterness Units).

In the DR6000, the programs for the measurement of the bitter units are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

Bitterness units, beer	Program 2001	10–40 BU
Bitterness units, wort	Program 2003	20–60 BU
ASBC bitterness units, beer*	Program 2021	10–100 IBU
ASBC bitterness units wort*	Program 2011	20–200 IBU

Note:

For the analysis of bitterness units, the Hach cuvette test LCK241 (only available in Europe) can also be used. Through the introduction of the chemicals in pre-manufactured cuvettes, both time and costs for chemicals (above all, high-quality isooctane) can be saved.

Beer type	Bitterness units	mg iso-alpha acids/L beer
Wheat	15-20	15-20
Vollbier	18-24	18-24
Märzen	20-25	20-25
Export	22-26	22-26
Bock	28-36	28-36
Pils	30-38	30-38
Alt	35-50	35-50

Table 1: Bitter units of the most popular beer types (from Brauerei-Forum,

Iso- α - and β -acids

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 237 ff.

The humulones (or α -hop bitter-acids) from the hops give the beer the bitter taste. During beer production (wort boiling), the bitter iso- α -acids emerge from the hops. Therefore, the iso- α -acid content is a key factor in the taste of the beer. The β -acids also contribute to the bitter taste and are recorded with this measurement.

After the bitters (see above) have been extracted from the sample with isooctane, and after further sample washing, the iso- α - and β -acid content is determined by the measurement of the sample absorbance at 255 nm and 360 nm [1]. A 10 mm quartz cuvette is used, and both acid types are determined in a combined measurement at two wavelengths.

The standard values according to MEBAK are:

Beer:	10–40 mg/L iso- α -acids and less than 2 mg/L β -acids
Wort:	15–50 mg/L iso- α -acids and less than 1–15 mg/L β -acids

In the DR6000, the program for the measurement of the iso- α - and β -acids is available for the measurement in accordance with MEBAK.

Iso- α - and β -acids	Program 2013	0–60 mg/L iso- α -acids and 0–80 mg/L β -acids
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FAN (Free Amino Nitrogen)

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 84 ff.
ASBC Beer-31, Wort-12

The sum of the bioavailable nitrogen components in the wort is represented by the free amino nitrogen (FAN). An excessive FAN content can lead to problems, both in the taste and in the microbiological stability of the beer. Brewer's yeast and wild yeast ferment excess amino acids into long-chain alcohols (propanol, isobutanol). FAN levels are also a good indicator of when fermentation is complete. Monitoring the FAN level with the DR6000 will help to turn over tanks faster once the FAN level is low enough. The typical FAN content is 200–250 mg/L in the wort and 10–120 mg/L in the beer (MEBAK).

The methods for both MEBAK and ASBC are identical. The prepared beer or wort are mixed with a color reagent (based on ninhydrin) and the absorbance is measured at a wavelength of 570 nm in a 10 mm cuvette.

This absorbance is compared with the color produced by a 2 mg/L glycine standard as reference. For a more precise determination, the blank value, the glycine standard, and the sample are measured in triplicate and the average value is calculated. Due to the differing sample preparation of beer and wort, internal factors of 50 (for beer) or 100 (for wort) are required.

For dark beers and worts, the MEBAK method makes provision for the measurement of a sample blank value, in addition to the usual reagent blank value in order to take into account the intrinsic coloration of the sample. The measurement process and the concentration calculation for dark beers and worts are stored in the DR6000 as separate programs. In the DR6000, the programs for the measurement of free amino nitrogen are available both for the measurement in accordance with MEBAK and for the measurement in accordance with ASBC.

FAN, light beer	Program 2008	0–400 mg/L FAN
FAN, light wort	Program 2007	0–400 mg/L FAN
FAN, dark beer	Program 2016	0–400 mg/L FAN
FAN, dark wort	Program 2015	0–400 mg/L FAN
ASBC FAN, beer*	Program 2024	0–400 mg/L FAN
ASBC FAN, wort*	Program 2025	0–400 mg/L FAN

Total Polyphenols

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 223 ff.

ASBC Beer-35

Phenolic compounds from malt and hops come into the beer in differing quantities dependent on production techniques. Depending on the structure and molecule size, they have a strong influence on various beer characteristics such as color, taste, taste stability, foam and chemical–physical stability¹. Polyphenols also have an especially large impact on the final appearance of the beer. High polyphenols levels lead to a hazy beer.

The methods in accordance with MEBAK and ASBC are identical. The polyphenols in the samples react with iron(III) ions in alkaline solution forming colored iron complexes. Their absorbance is measured spectrophotometrically in a 10 mm cuvette at a wavelength of 600 nm.

The calculation is performed as follows:

$$\text{Absorbance at 600 nm} \times 820 = \text{mg/L total polyphenols}$$



Standard values in beer are 150–200 mg/L total polyphenols. The measurement range of the saved programs reaches up to 800 mg/L.

In the DR6000 the programs for the measurement of total polyphenols are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

Total polyphenols	Program 2006	0–800 mg/L phenols
ASBC total polyphenols*	Program 2020	0–800 mg/L polyphenols

Vicinal Diketones

MEBAK brew-technical analysis methods, 4th Edition, 2002, page 134 ff.

ASBC Beer-25 B

The current MEBAK¹ describes the gas chromatographic measurement of diacetyl and 2,3-pentanedione.

In the older MEBAK³ and in the ASBC two different photometric methods for the determination of the vicinal diketones are provided.

During yeast metabolism, 2-acetolactate and 2-acetohydroxybutyrate emerge in the course of fermentation. These are converted through oxidation into the vicinal diketones diacetyl and 2,3-pentanedione.

However, diacetyl can also occur as a characteristic metabolic product of certain micro-organisms¹. With too high a vicinal diketone content the beer obtains an off-flavor. This often causes a butterscotch flavor, or oily mouth feel, which is unpleasant for the consumer.

Following the MEBAK method, the two diketones diacetyl and 2,3-pentanedione react with 1,2-phenylenediamine to form a colored end product, whose absorbance is measured in a 2 cm quartz cuvette at 335 nm. This frequently used method for operational analytics is clearly faster than the gas chromatographic method, but allows no differentiation between diacetyl and 2,3-pentanedione.

Using the calibration performed by MEBAK, the content of vicinal diketones is calculated as follows:

$$\text{Absorbance at 335 nm} \times 1.2 = \text{mg/kg VDK (vicinal diketones)}$$

The target value for light beer is less than 0.15 mg/kg.

The method in accordance with ASBC is described in the method Beer-25 B under the title “Diacetyl – Broad spectrum method for VDK.” This method also does not record the diacetyl separately, but rather all present vicinal diketones.

Following ASBC Beer-25 B, diacetyl (and 2,3-pentanedione) reacts with a naphthol solution forming a color complex, which is measured at a wavelength of 530 nm. The method was calibrated by Hach with diacetyl standard solutions, and the corresponding factor stored in the programming. Periodic quality control standard measurements are recommended. As the distillation concentrates the sample by a factor of 4, un-distilled standards should be prepared at 4 times the nominal concentration. For example, a nominal 0.5 mg/kg un-distilled standard should be prepared at 2.0 mg/kg.

Using the calibration performed by Hach, the content of vicinal diketones is calculated as follows:

$$\text{Absorbance at 530 nm} \times 2.9 = \text{mg/L diacetyl (vicinal diketones)}$$



In the DR6000, the programs for the measurement of vicinal diketones are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

Vicinal diketones	Program 2014	0–1 mg/kg VDK
ASBC diacetyl*	Program 2023	0–1 mg/L diacetyl

Note:

Just as for the determination of the bitters, there is also a pre-manufactured cuvette test from Hach under the number LCK242 (only available in Europe) or TNT819 (available in U.S. in 2016) for the determination of vicinal diketones.

Reducibility

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 204 ff.

The reducibility of the beer is a key issue for taste and the biological, chemical, and physical stability of beer. Reducing compounds arising from the malt and the hops prevent and/or minimize oxidative processes in the beer. All fast-reducing compounds present in the beer are summarized as reducibility. They are measured by their reducing effect on the Tillmann’s reagent (DPI). The decoloration of this reagent in the presence of the beer sample is measured at a wavelength of 520 nm, and compared with the original coloration of the reagent. The reducibility is expressed in a dimensionless number. It indicates what percent of the reagent is reduced by the beer sample.

In the evaluation of the reducibility of beers, the following scale applies in accordance with MEBAK¹:

60	Very good
50–60	Good
45–50	Satisfactory
< 45	Poor

In the DR6000, the program for the measurement of the reducibility is available for the measurement in accordance with MEBAK.

Reducibility	Program 2004	0–100
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Thiobarbituric acid number (TAN)

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 55 ff.

The thiobarbituric acid number is a summary characteristic. It indicates the thermal load of malt and wort. Alongside 5-hydroxymethylfurfural (HMF), a large number of substances that arise from the Maillard reaction (heat promoted reaction of sugars and amino acids) react with thiobarbituric acid.

In the MEBAK test, the substances to be measured react with thiobarbituric acid and form a yellow color complex that is photometrically analyzed at a wavelength of 448 nm.

The standard values in the brewing process are (in relation to 12% original wort):

- Light kettle full wort: < 22
- Light cast wort: < 45
- Light cold wort after wort cooling: < 60



A new approach to this analysis uses a test called TBARS (thiobarbituric acid reactive substance), which essentially records malondialdehyde. Here too, the extent of the thermal load of the wort through the effect of heat is recorded by the measurement.

In the DR6000, the program for the measurement of TAN is available for measurement in accordance with MEBAK.

TAN in beer/wort Program 2011	0–100 TAN	(diluted 1/10)
TAN in congress wort	Program 2012	0–100 TAN (diluted 1/5)

Anthocyanogens

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 226 ff.

Anthocyanogens, or also leucoanthocyanidins, are a special form of anthocyanidins. Anthocyanidins are the color-giving part of anthocyanins, a group of plant colorants with a phenolic basis. The anthocyanogens (leucoanthocyanidins from the hops) are converted by hot hydrochloric acid into the red-colored anthocyanidins.

In the measurement the anthocyanogens are first adsorbed onto polyamide and then converted by hot hydrochloric acid into a red solution. The measurement is made at a wavelength of 550 nm in a 10 mm cuvette.

The standard values in accordance with MEBAK in the beer are 50–70 mg/L, dependent on production techniques. When the stabilized with PVPP the standard values are correspondingly lower.

In the DR6000, the program for the measurement of the anthocyanogens is available for the measurement in accordance with MEBAK.

Anthocyanogens	Program 2005	0–100 mg/L ATC
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Photometric Iodine Sample

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 52 ff.

After malt has been produced from grains, mostly barley, the malt is ground. The actual brewing process begins with mashing. In this process, water is heated to approximately 60 °C, then the ground malt is added and the resulting mash is heated under constant stirring to approximately 75 °C, dependent on the process. With different roasting temperatures, enzymes convert the starch from the malt into malt sugar. Alternatively, parts of the mash are boiled, which leads to a physical gelatinization of the starch. An iodine sample it is then measured to determine whether the dissolved starch is completely saccharified.

Dextrins and starch from worts or beer are precipitated, dissolved in phosphate buffer, and mixed with iodine solution. The red-to-blue coloration is measured in the spectrophotometer at a wavelength of 578 nm in a 4 cm cuvette. The standard values (in wort) per MEBAK are < 0.45.

In the DR6000, the program for the measurement of the anthocyanogens is available for the measurement in accordance with MEBAK.

Iodine sample	Program 2010	0–1 iodine value
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Iron

MEBAK, Wort, Beer, Beer-Based Beverages), 1st Edition 2012, page 423 ff. Iron can enter the beer through the raw materials as well as through filter agents and/or fining agents. It can also be picked up from apparatus, lines, or cans, or be contained in beer foam stabilizing agent. Iron negatively affects the colloidal stability, taste, foam, and gushing tendency of the beer.

Alongside AAS, iron in beer can also be determined spectrophotometrically. Trivalent iron is first reduced to bivalent iron. The bivalent iron reacts with FerroZine to form a violet-colored complex. The method stored in the DR6000 for iron determination already contains the absorbance coefficient for iron. The increase of the calibration curve is 0.037/ $\mu\text{g/L Fe}^{2+}$. Thus, the user of this program is not required to generate a proprietary iron standard series for the calibration. The reference values in beer are 0.200 mg/L.

In the DR6000, the program for the measurement of iron in accordance with MEBAK is available.

Iron Program 2017 0–1 mg/L iron

References:

- ¹ MEBAK Wort, Beer, Beer-Based Beverages, 1st Edition 2012
- ² American Society of Brewing Chemists, Methods of Analysis, 14th Edition
- ³ MEBAK, brew-technical analysis methods, volume II, 4th Edition 2002

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HACH COMPANY WORLD Headquarters: Loveland, Colorado USA

United States:	800-227-4224 tel	970-669-2932 fax	orders@hach.com
Outside United States:	970-669-3050 tel	970-461-3939 fax	int@hach.com

hach.com

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