EN

Bitterness system method

REF 984353, 984354, 984355
❶ 984353 BC System Liquid, 1 x 1 liter
❷ 984354 BC Diluent, 4 x 9 ml
❸ 984355 BC Eluent, 20 x 15 ml

INTENDED USE
Reagents for photometric determination of bittering compounds in beer and wort samples using automated Thermo Scientific™ Gallery™ Plus Beermaster analyzer.

BC System Liquid, BC Diluent and BC Eluent solutions are intended for automated Thermo Scientific Gallery Plus Beermaster analyzer for system bitterness measurement using the bitterness column (BC).

METHOD
Column extraction of bittering components from beer and wort and subsequent photometric determination. Method is performed at 37℃, using 275 nm filter.

PRINCIPLE OF THE PROCEDURE
Bittering compounds are extracted from beer or wort and measured at 275 nm.

REAGENT INFORMATION
984353 BC System Liquid, 1 x 1 liter
984354 BC Diluent, 4 x 9 ml
984355 BC Eluent, 20 x 15 ml

Reagents should be ordered separately. Typically 340-360 tests can be performed with one reagent package.

Solutions

984353 BC System Liquid: Low pH solution for conditioning
984354 BC Diluent: Sample acidification solution
984355 BC Eluent: Special sample elution solution

Preparation
The reagents are ready-to-use.
Note: After removing the cap from the 20 ml vials, check that there are no bubbles on the surface of the reagent when you insert vials into the analyzer.
Note: See the Installation manual for the instructions for how to change the BC System Liquid transportation cap to the cap with tubing installed. This cap is provided with the analyzer.

Storage and Stability
Reagents in unopened vials are stable at 15…25 ℃ until the expiry date printed on the label.

Store the reagents according to the local legal requirements.

Refer to the Application Notes of your analyzer for the on board stability of the reagents.

WARNINGS AND PRECAUTIONS
Bitterness system liquids are hazardous.
See separate sheet inside the kit for Hazardous- and Precautions-phrases:
BC System Liquid and BC Diluent: H290, H315, H319, P280, P302 + P352, P305 + P351 + P338
BC Eluent: H226, H319, H336, P210, P261, P280, P305 + P351 + P338.

Exercise the normal precautions required for handling all laboratory reagents.

SAMPLES

Sample Type
Beer, wort and process samples.

Sample bitterness concentration
All sample and method related details are in a separate application note. Typical sample concentration is 5-100 BU.

Sample preparation
- Turbid samples and samples containing precipitate must be clarified by centrifugation before analysis.
- Beer samples containing carbon dioxide and foam must be degassed by ultrasonication or magnetic stirrer. Loss of foam must be avoided and the foam must be allowed to collapse back into the beer before taking the sample to analyzer. Beer sample is recommended to pipette to the sample cup from the bottom of the flask.
- Avoid use of plastics.
- Never use a filter paper for the sample preparation step.

Note: Degassing or antifoam agents e.g. octanol must not be used. Use of degassing agents leads to fast degradation of the column performance.

TEST PROCEDURE
See the Bitter AU application in the Gallery Plus Beermaster analyzer or a separate application note. In this application AU stands for result unit, Absorbance Unit. This application contains two parts pretreatment (Pretreatment definition: Bitter AU) and measurement (Test definition: Bitter AU).

Note: The Bitter AU application <u>measurement parameters</u> must not be changed. The sample volume in the bitterness <u>pretreatment parameters can be changed to adjust the response level according to the sample level. The changes to the parameters cannot be made in the default application, but a copy of the application must be created.</u> All new applications and changes to the pretreatment parameters must be validated by the user.

Materials required but not provided
Deionized water (aseptic and free of heavy metals) and general laboratory equipment.

Consumables:
Sample Prep. block, ordering code 986366 (56 blocks for 1680 samples).
Decacell cuvettes, ordering code 986540.

CALIBRATION AND QUALITY CONTROL

Factor calibrated absolute method
Gallery Plus Bitterness system can be used as a novel bitterness method measuring at 275 nm extracted bitterness compounds as such. Results are given as Response values (AU).

For this method, application with calibration setting "none" and factor set as 1 will be used. This method is suitable for e.g laboratory internal bitterness value measurements where no correlation to the reference method is needed. This method is preinstalled in the analyzer software (Bitter AU).

Correlation to a reference method by creating a new calibrated method
If correlation to the reference method is needed and the results should be reported as units in the reference method (e.g BU, BU for Bitterness Unit), see the Appendix for the instructions. See also the Bibliography section for the list of the reference methods.

Quality Control (QC)
To verify the column condition and system reagents, a reference sample material must be run daily as a QC sample.

The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

CALCULATION OF RESULTS
Factor calibrated results are calculated automatically by the analyzer using a factor in use.

LIMITATIONS OF THE PROCEDURE

Interference
Possible matrix interference is avoided by grouping same type of samples to one application.
Due to the variability of the sample types (beer, wort, different raw materials), interference from the sample matrix should be tested by e.g sample dilutions during the method verification in the laboratory.

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MEASURING RANGE

The test has been developed to determine Bitterness concentrations within a measuring range from 5 to 100 BU.

PERFORMANCE CHARACTERISTICS

The results obtained in individual laboratories may differ from the performance data given. Picture below shows an example of method linearity.
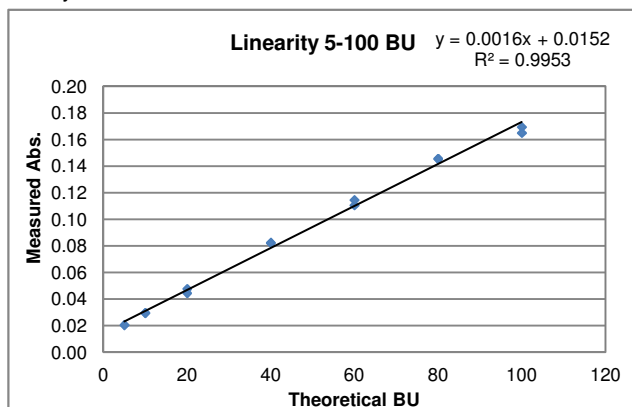
In this example, sample used was 100 BU pale lager beer and its water dilutions. In the example, 200 µl pretreated sample volume and 300 µl eluent volume was used.

Determination limit (=Test limit low)

The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 5 BU. For this determination, 300 µl sample volume and 300 µl elution volume has been used.

Repeatibility and reproducibility

Repeatibility and reproducibility study was performed with three Gallery Plus Beermaster analyzers in four days with different environmental conditions.

		Analyzer 1	Analyzer 2	Analyzer 3
18 C / 40 % rH	n	22	26	24
	Average	7.3	7.6	7.1
	SD	0.41	0.29	0.23
	CV %	5.66 %	3.81 %	3.33 %
	Max-Min	1.4	1.1	0.9
18 C / 80 % rH	n	19	24	25
	Average	7.4	7.2	7.1
	SD	0.36	0.35	0.23
	CV %	4.91 %	4.78 %	3.28 %
	Max-Min	1.3	1.8	1.0
30 C / 80 % rH	n	27	28	27
	Average	7.5	7.1	7.2
	SD	0.32	0.31	0.24
	CV %	4.24 %	4.38 %	3.25 %
	Max-Min	1.5	1.2	0.9
30 C / 40 % rH	n	28	26	25
	Average	7.4	7.7	7.6
	SD	0.31	0.42	0.27
	CV %	4.19 %	5.52 %	3.50 %
	Max-Min	1.3	1.5	1.1

OTHER REMARKS

The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

WASTE MANAGEMENT

Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.
Note: If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.

BIBLIOGRAPHY, REFERENCE METHODS

1. EBC 9.8 (Bitterness in beer)
2. EBC 8.8 (Bitter of wort)
3. ASBC Beer-23 (Beer Bitterness)
4. MEBAK 2.18.1

ADDITIONAL MATERIAL

Certificate of analysis and SDS are available at
www.e-labeling.eu/TSF

Applications for Gallery automated analyzer are available upon request from the local sales representative. Information in the Application note can change without prior notice.

MANUFACTURER

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CONTACT INFORMATION

www.thermoscientific.com
e-mail:system.support.fi@thermofisher.com

Date of revision
2015-05-22

Changes from previous version
Warnings and precautions updated.
General updates.

Appendix
Correlation to a reference method by creating a calibrated method

Calibration to a reference method
In this process, the Gallery bitterness method is calibrated to correlate to a reference method. This is achieved by using the reference method analyzed sample and its reference method assigned value as the calibrator. In this calibrated method, results are reported as BU (Bitterness unit).

Correlation of Gallery bitterness method to reference method is matrix specific. This means that different beers might need separate matrix specific calibrations. This is true at least in between dark and pale beer types.

Creating calibrated bitterness applications to the Gallery Beermaster software:
- Copy the preset Bitter AU test definition parameters to a new test definition
- Rename the new test definition as e.g Bitter BU1
- Define the calibrators and assign the reference values in the calibrator definition window
- Change the calibration type from none to linear in the new test definition. The calibrators should be analyzed as duplicates.

Additional instructions for choosing the calibrators:
- As calibrators, several different beers with different bitterness values can be used or one beer with high bitterness value and a its water dilutions.
- Calibrators should cover the entire measuring range.
- The values of the calibrators are measured with the reference method. The value of the dilution can be calculated.

Calibration interval
After setting up the methods, the daily calibration is not typically needed. To verify the calibration and column condition, calibrator material or other reference sample material must be run daily as a QC sample.

Calibration interval must be re-validated by the laboratory using the QC sample.

Quality Control (QC)
Use quality control samples at least once a day and after each calibration. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

CALCULATION OF RESULTS
The results are calculated automatically by the analyzer using a calibration curve or a factor.

Calibration Curve (example)
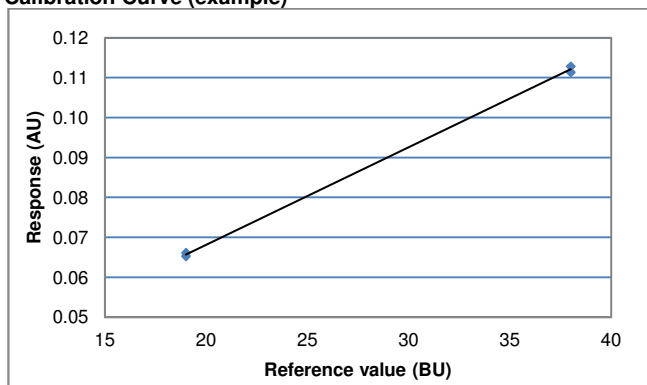
Calibrator	Response (A)	Calc. conc. (BU)
Undiluted beer	0.111	38
Undiluted beer	0.113	38
Diluted beer 1+1	0.066	19
Diluted beer 1+1	0.065	19

Calibration factor of this example is 410. The sample volume in the calibration was 100 µl.
Note that the calibration curve is calibrator material dependent.

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