

Evaluation of a Gallery Total Polyphenol Method Performance in Beer (and Wort) using the EBC / MEBAK protocol

Liisa Otama and Annu Suoniemi-Kähärä

Thermo Fisher Scientific Oy, P.O.Box 100, 01621 Vantaa, Finland

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Abstract

The purpose of this study was to automate the EBC protocol to measure Total Polyphenols in beer and wort samples using automated Thermo Scientific Gallery analyzer. Results of the Gallery were compared to the results of commercial BABS reference samples.

As a result of this study, for the 7 beer samples analyzed, the BABS assigned values showed good correlation to Gallery Total Polyphenol application performed according to the EBC 9.11 protocol. Recovery rates varied within 94 - 106 % and total precision (n=50) was 2.4 % or less for all samples. No significant run-to-run variation was noticed. Method linearity was determined in range of 79 - 199 mg/l but it can be extended with automated dilutions. No sample pretreatment was needed for the beer samples.

Advantages of automation include easier and faster measurement. It also enables small sample sizes with less waste. For light sensitive reagents, the analyzer enables dispensing without reagent being exposed to excess light. In addition, with Gallery it is possible to perform simultaneously several other analytes for the same sample. For Total Polyphenol measurement the analysis turnaround time is approximately 10 minutes for one request and 55 minutes for 100 requests.

Introduction

Total polyphenols are becoming interesting group of compounds because of their beneficial, antioxidant effects in food. There are currently several analytical methods for the Total Polyphenol measurement. Folin-C based method and measurement at 280 nm are widely used methods in the wine industry but due to the unspecificity of these methods the result levels differs from the EBC method used for beer and wort samples.

In the brewing industry, polyphenols are considered as beer flavonoids such as the flavan-3-ols and their products, the proanthocyanidins, represent a class of readily oxidizable compounds capable of hindering or preventing the oxidation of other molecules present in beer (3).

Total Polyphenols are analyzed due to haze formation, primarily resulting from protein-polyphenol interactions within the product. Polyphenols are also one of the two chemical entities which control the colloidal stability of beer, the other being proteins.

In this study, the EBC / MEBAK Total Polyphenol method (1,2) was adapted to Thermo Scientific Gallery discrete analyzer and automated method was optimized to 37 °C reaction temperature, which is the basic operation temperature in the Gallery analyzer. Results are reported in mg/l to the nearest whole number as Gallic acid equivalent. Seven BAPS reference beers from LGC Standards were used as samples in method comparison.

Materials and Methods

Instruments

Discrete photometric Thermo Scientific Gallery analyzer (manufactured by Thermo Fisher Scientific) is a fully automated bench-top system.

Samples

Seven BAPS reference beers from LGC Standards were used as samples in method comparison. Reference samples included both lager and bitter beers. Assigned values for each sample were determined as statistical median of results reported by participants.



Reagents

Reagents were prepared according to EBC protocol 9.11 with slight modification in Ammonia dilution ratio to avoid evaporation. However, the original reagent ratio was kept. Carboxymethyl cellulose/ ethylenediamine tetraacetic acid (CMC/EDTA) was prepared by weighting 1.0 g sodium CMC and 0.2 g disodium EDTA and diluted to 100 ml with H₂O. If necessary, the reagent can be clarified by centrifugation. Reagent is stable one month at 2-8 °C.

Ferric reagent was prepared by weighting 3.1 g ammonium iron citrate with iron concentrate of 16 % and diluting it to 100 ml with H₂O. Reagent is stable for one week at 2-8 °C if not exposed to light. It is recommended to store the reagent in a dark place in brown glass container or covered with foil.

Ammonia reagent was prepared by diluting concentrated ammonia (d=0.92 g/ml) to deionized water with dilution ratio 1:39.

All chemicals were purchased from Sigma-Aldrich.

Application

80 µl of CMC/EDTA reagent, 100 µl of sample and 65 µl of ammonia reagent were dispensed to the cuvette and blank was measured at 600 nm. 5 µl of ferric reagent was added and incubated in 420 s. The absorbance was measured at 600 nm. The Gallery analyzer computes sample concentration automatically using the formula:

$$P = 820 \times (A - \text{BIAS})$$

where P is the polyphenol content (mg/l) and A is the absorbance at 600 nm. The BIAS correction is done by measuring the response of deionized water.

Method Calibration

The value of the calibration type is set to "Bias". The Bias calibration is measured with deionized water as calibrator by using one measurement point. The factor is set to 820 before measuring the bias of the calibration line.

Sample Pretreatment

No additional sample pretreatment was needed for beer samples. Turbid wort samples were centrifuged before the analysis.

Precision

Precision was determined by measuring seven reference beer samples as ten replicates in five batches, total number of results being 50. Precision within run, between run and in total was determined for each sample.

Linearity

Method linearity was determined with reference beer samples in range of 79 - 199 mg/l. All samples were measured as three replicates.

Method Comparison

Seven BAPS reference samples from LGC Standards were analyzed with Gallery and results were compared to assigned value of inter laboratory results.

Results

Precision

The total precision of the measurement for all samples were very good, 2.4 % or better (see Table 1). No significant variation was noticed between runs.

Linearity

Samples analyzed were in range of 79 - 199 mg/l (see Figure 1).

Method comparison

Method comparison between Total Polyphenol performed by Gallery and reference method performed by EBC Ferric method gave good correlation. Recovery rates varied within 94 - 106 % (see Table 2).

Figure 1. Linearity

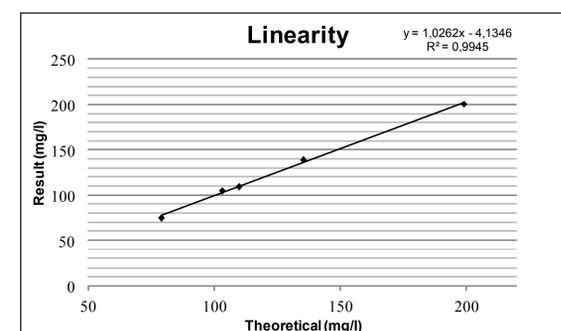


Table 1. Precision (n=50)

	Mean 198 mg/l		Mean 207 mg/l		Mean 74 mg/l		Mean 108 mg/l	
	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Within Run	1,4	1,1	1,3	0,9	1,3	1,2	0,5	0,4
Between Run	2,9	2,2	2,0	1,5	2,0	2,0	0,5	0,4
Total	3,2	2,4	2,3	1,7	2,3	2,3	0,7	0,6

	Mean 133 mg/l		Mean 136 mg/l		Mean 101 mg/l	
	SD	CV%	SD	CV%	SD	CV%
Within Run	1,4	1,1	1,3	0,9	1,3	1,2
Between Run	2,9	2,2	2,0	1,5	2,0	2,0
Total	3,2	2,4	2,3	1,7	2,3	2,3

Table 2. Method comparison

Sample	Assigned value (mg/l)	Measured value (mg/l)	Recovery %
Lager 1	199	198	99
Lager 2	75	74	94
Lager 3	110	108	98
Lager 4	135	136	101
Lager 5	103	101	98
Bitter 1	195	207	106
Bitter 2	142	133	94

Conclusion

The photometric method used in Thermo Scientific Gallery analyzer for analyzing Total Polyphenol in beer and wort correlates well with the EBC reference method. The advantage of using automated analyzer is the speed of analysis and the possibility to measure several analytes from the same sample in addition of Total Polyphenol measurement. This automated method can perform more than 100 Total Polyphenol measurements per hour with very little hands-on time.

Reference

1. Analytica-EBC Method 8.12 Total Polyphenols in Wort by Spectrophotometry and Analytica-EBC Method 9.11 Total Polyphenols in Wort by Spectrophotometry
2. Brautechnische Analysenmethoden Band II, Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK). 2.17.1 Gesamtpolyphenole (EBC Methode).
3. A Discussion of Polyphenols in Beer Physical and Flavour Stability. P. Aron and T. Shellhammer. J. Inst. Brew. 116(4), 369-380, 2010.