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Modification of the method Analytica-EBC 7.7 (Alpha- and Beta-acids in Hops and Hop Products by HPLC)

Submitted on behalf of the EBC Analysis Committee

Descriptor: Analytica-EBC, method development, alpha- and beta-acids

1 Introduction

During early 2004 a collaborative trial was carried out to test two modifications of the method Analytica-EBC 7.7. Four samples of hop pellets and four samples of hop extracts were sent to members of the EBC Analysis Committee, ASBC (American Society of Brewing Chemists), BCOJ (Brewery Convention of Japan) and AHA (Arbeitsgruppe Hopfenanalyse).

2 Experimental

The organisation of the collaborative trial and the statistical treatment of the data were performed according to the international standard ISO 5725.

The eight samples were circulated to 14 participants. The participating laboratories were asked to determine the alpha- and beta-acids contents in each sample, in duplicate, and to report the results to two decimal places, according to the instructions given in the modified EBC method 7.7. In the first modification methanol, diethyl ether and hydrochloric acid were added to the hop sample at the same time and the whole mixture was then shaken for 40 minutes. The second modification concerned the HPLC chromatography. As an alternative to the column "Nucleosil" (currently recommended in the method EBC 7.7) a new column (Nucleodur 5-100 C18 ec, 125 x 4 mm) recently developed by Macherey-Nagel was stipulated. The use of this new column required some changes to the HPLC parameters. The mobile phase methanol/water/phosphoric

acid was prepared in a ratio of 775/210/9 (v/v/v). The eluent flow was set at 1,0 ml/minute and the column temperature was held at 40 °C. All participants carried out HPLC analysis with the "Nucleodur" column using these modified parameters. The international calibration standard ICE 2 was used for external calibration. 13 laboratories reported results on all eight samples. One laboratory only reported results on the four samples of hop extract.

3 Results

The results returned by the participating laboratories are shown in Table 1. Numerical consistency testing using Cochran's test and Grubb's test was used in the statistical evaluation of the data to identify stragglers or outliers. The results of the statistical treatment are also indicated in Table 1. Based on the results of the statistical treatment, it was decided to omit the outliers from the calculation of the precision data.

The precision data using the remaining analytical results are summarized in tables 2-5. In the case of alpha-acids the repeatability values r_{95} depend on the mean for both pellets and extract, but the reproducibility R_{95} only depends on the mean for pellets. Reproducibility R_{95} of alpha-acids in extracts and all precision figures for beta-acids do not depend on the mean.

4 Conclusion

The EBC Analysis Committee judged both the repeatability and reproducibility values obtained in the collaborative trial to be acceptable, and approved the modifications of the method 7.7 for inclusion in Analytica-EBC.

Appendix

Table 1 Laboratory mean values of total alpha- and beta-acids (% m/m)

Alpha-acids								
Laboratory	Pellet 1	Pellet 2	Pellet 3	Pellet 4	Extract 1	Extract 2	Extract 3	Extract 4
1	3,06	6,07	11,18	15,42	34,75	38,72	46,94	54,59
2	2,83	6,18	11,12	14,67	34,48	36,64	45,57	54,84
3	2,75	5,93	10,53	14,88	33,85**	37,94**	47,59	52,37
4	2,78	6,17	10,72	14,44	34,45	38,36	46,25	54,52
5	2,84	6,15	10,80	14,51	34,57	38,63	46,65	54,34
6	2,95	6,63	11,29	15,19	34,69	39,45	47,01	56,17
7	2,98	6,40	11,11	14,79	35,32	35,00°	46,49	55,16
8	2,89	6,33	11,03	14,95	35,90	40,35	48,40	57,08
9	2,92	6,30	10,85	14,75	34,42	38,69	46,51	54,98
10	2,90	6,32	11,17	14,99	34,17	38,42	46,32	54,38
11	-	-	-	-	34,79	39,23	46,74	55,28
12	2,63	6,00	10,53	14,60	55,54°°	38,91	46,94	55,56
13	2,92	6,21	9,25°°	11,54°°	35,39	39,11	45,92	52,09
14	2,96	6,69	10,99	13,74	34,75	38,75	44,32	54,77

Beta-acids								
Laboratory	Pellet 1	Pellet 2	Pellet 3	Pellet 4	Extract 1	Extract 2	Extract 3	Extract 4
1	5,38	5,65	4,08	6,43	28,85	26,56	26,79	17,62
2	5,16	6,25	4,40	6,21	30,45	26,30	27,14	19,39
3	5,10	5,85	4,14	6,15	28,21**	25,93**	27,08	16,51
4	5,18	6,05	4,11	6,11	28,88	26,88	26,86	17,82
5	5,08	5,69	3,97	5,98	28,40	26,30	26,37	17,24
6	5,40	6,28	4,24	6,41	28,86	26,53	26,49	17,40
7	5,41	5,71	3,95*	5,95	29,52	24,38°°	27,01	18,09
8	5,05	5,83	3,96	6,10	29,42	27,44	27,22	18,14
9	5,06	5,76	3,84	6,04	28,16	26,48	26,25	17,14
10	5,53**	5,92	4,12	6,32	29,68	26,79	27,10	18,33
11	-	-	-	-	28,59	26,32	26,28	17,42
12	5,33	5,93	4,01	6,38	18,33°°	27,49	27,83	18,42
13	5,16	6,09	4,20	5,84	29,21	26,80	26,92	17,43
14	5,35	6,19	4,07	5,65	28,93	26,71	25,44	17,44

* Cochran's statistic straggler

** Cochran's statistic outlier

° Grubb's statistic straggler

°° Grubb's statistic outlier

Table 2 Summary of the precision data for total alpha-acids (% m/m) in pellets

	P 1	P 2	P 3	P 4
n	13	13	12	12
m	2,88	6,26	10,94	14,74
s _r	0,050	0,068	0,202	0,224
s _R	0,117	0,227	0,293	0,452
s _L	0,106	0,216	0,212	0,392
r	0,14	0,19	0,57	0,64
R	0,33	0,64	0,83	1,28
r ₉₅		0,045 m		
R ₉₅		0,122 + 0,075 m		

Table 3 Summary of the precision data for total beta-acids (% m/m) in pellets

	P 1	P 2	P 3	P 4
n	12	13	13	13
m	5,22	5,94	4,08	6,12
s _r	0,092	0,075	0,073	0,114
s _R	0,155	0,223	0,155	0,246
s _L	0,125	0,210	0,137	0,218
r	0,26	0,21	0,21	0,32
R	0,44	0,63	0,44	0,70
r ₉₅		0,25		
R ₉₅		0,55		

Table 4 Summary of the precision data for total alpha-acids (% m/m) in extracts

	E 1	E 2	E 3	E 4
n	12	13	14	14
m	34,81	38,48	46,54	54,72
s _r	0,322	0,298	0,378	0,460
s _R	0,543	1,349	0,979	1,334
s _L	0,437	1,316	0,903	1,253
r	0,91	0,84	1,07	1,30
R	1,54	3,82	2,77	3,78
r ₉₅	0,079 + 0,022 m			
R ₉₅	2,98			

Table 5 Summary of the precision data for total beta-acids (% m/m) in extracts

	E 1	E 2	E 3	E 4
n	12	12	14	14
m	29,08	26,71	26,77	17,61
s _r	0,320	0,238	0,226	0,281
s _R	0,662	0,436	0,596	0,572
s _L	0,580	0,366	0,552	0,498
r	0,90	0,67	0,64	0,80
R	1,87	1,23	1,69	1,62
r ₉₅	0,75			
R ₉₅	1,6			