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# Determination of iso-α-acids and reduced iso-α-acids (Rho, Tetra, Hexa) in hop products by HPLC using a modified version of Analytica-EBC Method 7.8

Submitted on behalf of the EBC Analysis Committee

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## 1 Introduction

After the release of four international calibration standards for HPLC analysis of isomerised α-acids (DCHA-Iso, DCHA-Rho, Tetra and DCHA-Hexa), the EBC Analysis Committee decided to test a modification of Analytica-EBC Method 7.8 for the determination of iso-α-acids and reduced iso-α-acids in hop products. The method tested is essentially the method used by the International Subcommittee for Isomerised Hop α-Acids Standards to established the declared concentration of the international calibration standards and delivered together with the standards. The method is an isocratic HPLC method with detection at 270 nm, where the extinction coefficients for all forms of iso-α-acids and reduced iso-α-acids are quite similar in the mobile phase used. In the autumn 2001 a collaborative trial was carried out using four samples of hop products each containing one particular type of non-reduced or reduced iso-α-acids. Seven members of the EBC Analysis Committee, one member of AHA (Arbeitsgruppe Hopfenanalyse), three members of BCOJ and three American hop companies participated in the collaborative trial.

## 2 Experimental

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

Four samples were circulated to 14 participants. The participating laboratories were asked to determine the non-reduced or reduced iso-α-acids contents in each sample, in duplicate, to two decimal places, according to the instructions given in the HPLC method delivered together with the samples. The participants had to aquire the international calibration standards at their own expense.

All 14 laboratories reported results on all four hop samples.

## 3 Results and discussion

The raw data returned by the participating laboratories are shown in Table 1. Sample D (hexahydroiso-α-acids) contained both *cis*- and *trans*-forms of hexahydroiso-α-acids, while the corresponding international calibration standard only contains *cis*-forms. Three participants decided only to incorporate the peaks present in the standard in the calculation of the results and thus obtained much lower results for this sample than the rest of the participants. However, they were asked to re-evaluate their results for sample D taking into consideration all hexahydroiso-α-acid peaks and these are the data reported in Table 1.

The international calibration standards available today do not contain all isomers of the different types of iso-α-acids. Commercially available hop products may therefore contain isomers not present in the standards. Applying the tested method to such samples should be done cautiously, as it is not known unambiguously whether additional peaks present in the sample chromatogram are actually isomers of the type of iso-α-acids analysed for. This is also the reason why the method tested should only be used on hop product samples containing only one particular type of iso-α-acids.

The International Subcommittee for Isomerised Hop α-Acids Standards continually modify the guidelines for using the standards to assure that the standards can be used to quantify most commercially available reduced isomerised hop products. Information regarding additional peaks in the chromatogram may therefore be found in the guidelines.

Both graphical consistency testing using Mandel's h and k statistics and numerical tests (Cochran's test and Grubbs' test) were used in the statistical evaluation of the data to identify stragglers or outliers. The results of the statistical treatment are indicated in Table 1.

Based on the results of the statistical treatment, it was decided to omit all results from laboratory number 10 and the analysis results from laboratory 12 on sample A and laboratory 5 on sample D from the calculation of the precision data. The precision data using the rest of the analytical results are summarized in Table 2. Values of $r_{95}$ and $R_{95}$ are in the range 0,52 – 0,67 % m/m and 2,13 – 2,89 % m/m, respectively, for means in the range 20,91 – 23,14 % m/m. This is comparable to the statistical data obtainable with Analytica-EBC Method 7.8, where a sample containing 21,74 % m/m iso-α-acids would give $r_{95}$ = 0,67 % m/m and $R_{95}$ = 1,98 % m/m using the formula taking into consideration all participants in the collaborative trial.

## 4 Conclusion

The EBC Analysis Committee judged both the repeatability and reproducibility values obtained in the collaborative trial as acceptable, and approved the inclusion in Analytica-EBC of the HPLC method tested for determination of one particular type of non-reduced or reduced iso-α-acids in hop products. #

**Table 1 Original data of the collaborative trial of modified EBC 7.8 (unit % m/m)**

| Laboratory | Sample | | | |
|---|---|---|---|---|
| | A | B | C | D |
| | Iso-α-acids | Rho-iso-α-acids | Tetrahydroiso-α-acids | Hexahydroiso-α-acids |
| 1 | 21,63 | 23,03 | 9,85 | 20,76 |
| | 21,47 | 22,78 | 9,74 | 20,59 |
| 2 | 19,53 | 21,26# | 9,92 | 23,12 |
| | 19,40 | 21,08# | 9,91 | 22,44 |
| 3 | 22,21 | 22,90 | 9,62 | 19,92 |
| | 22,03 | 22,79 | 9,50 | 19,85 |
| 4 | 21,05 | 22,62 | 9,07# | 21,09 |
| | 21,00 | 22,32 | 9,01# | 21,03 |
| 5 | 22,11 | 24,13 | 9,59 | 18,39## |
| | 21,78 | 24,0 | 39,57 | 17,95## |
| 6 | 22,31 | 22,97 | 9,64 | 21,29 |
| | 21,90 | 22,67 | 9,53 | 21,21 |
| 7 | 22,69 | 22,94 | 9,98 | 20,52 |
| | 22,41 | 22,66 | 9,82 | 20,28 |
| 8 | 21,68 | 24,09 | 10,10 | 20,89 |
| | 21,55 | 23,58 | 9,85 | 20,34 |
| 9 | 23,46 | 24,41 | 9,79 | 21,12 |
| | 22,97 | 24,13 | 9,59 | 20,67 |
| 10 | 22,86** | 25,40 | 10,06**++ | 23,66**+ |
| | 21,89** | 24,83 | 9,32**++ | 22,17**+ |
| 11 | 22,70 | 24,17 | 9,85 | 21,59 |
| | 22,56 | 24,11 | 9,76 | 21,37 |
| 12 | 26,12##$^{o}$ | 23,06* | 9,98 | 20,51 |
| | 25,49##$^{o}$ | 22,79* | 9,76 | 20,15 |
| 13 | 22,23 | 24,00 | 9,15# | 20,44 |
| | 22,13 | 23,87 | 9,11# | 20,40 |
| 14 | 20,61 | 22,77 | 9,66 | 21,36 |
| | 20,30 | 22,49 | 9,66 | 20,98 |

* Mandel's k statistic straggler
** Mandel's k statistic outlier
# Mandel's h statistic straggler
## Mandel's h statistic outlier
\+ Cochran's straggler
++ Cochran's outlier
$^{o}$ Grubbs' straggler
$^{oo}$ Grubbs' outlier

**Table 2 Summary of the precision data**

| Sample | Sample A | Sample B | Sample C | Sample D |
|---|---|---|---|---|
| | Iso | Rho | Tetra | Hexa |
| Number of laboratories, p | 12 | 13 | 13 | 12 |
| Grand mean, m (% m/m) | 21,74 | 23,14 | 9,65 | 20,91 |
| Repeatability standard deviation, $s_r$ (% m/m) | 0,18 | 0,18 | 0,09 | 0,24 |
| Reproducibility standard deviation, $s_R$ (% m/m) | 1,03 | 0,89 | 0,30 | 0,76 |
| Repeatability, $r_{95}$ (% m/m) | 0,52 | 0,52 | 0,26 | 0,67 |
| Reproducibility, $R_{95}$ (% m/m) | 2,89 | 2,49 | 0,83 | 2,13 |
| Coefficient of variation of repeatability, $CVS_r$ (%) | 0,8 | 0,8 | 1,0 | 1,2 |
| Coefficient of variation of reproducibility, $CVS_R$ (%) | 4,7 | 3,8 | 3,1 | 3,6 |