JAP&T TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2013, 19(2), 253-258

Journal of Agroalimentary Processes and Technologies

The internal validation of HPLC and conductometric method for the determination of hop bitter acids

Liana-Claudia Salanţă*, Maria Tofană, Sonia Socaci, Elena Mudura, Anca Fărcaş

University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăştur street, 400372 Cluj-Napoca, Romania

Received: 02 May 2013; Accepted: 14 June 2013

Abstract

Conductometry and high-performance liquid chromatography (HPLC) methods are validated in the Food Safety and Quality Testing Laboratory (FQSL) of USAMV Cluj-Napoca. The determination of alpha and beta acids, from Magnum and Huller Bitterer variety, was carried out according to Analytica EBC, method 7.4 and 7.7. The aim of internal validation study was to assure quality results. For the HPLC method, the following quality parameters were determined: specificity, linearity, precision and accuracy. The obtained conductometric values for alpha bitter acids are similar with those obtained by HPLC analysis. Standard deviations were obtained in conditions of repeatability. The recovery coefficient determined for conductometric method was 92 % and for HPLC method was 95%.

Keywords: hop pellets, bitter acids, HPLC, conductometric method

1. Introduction

The bitterness and aroma of the beer is provided by the cones, the female flower of the hop plant [1]. The chemical source of hops' bitterness are the α- and β-acids. The α-acids have three major analogous (cohumulone, humulone and adhumulone) as well as β-acids (colupulone, lupulone and adlupulone) with a six-membered ring structure.

The β-acids differ structurally from the α-acids by having one moreprenyl group. In addition, there are several homologues and analogues including posthumulone / postlupulone, prehumulone / prelupulone and adprehumulone [2].

Considering the significant impact that α- and β-acids have on the flavor of beer, it is important for brewers to be able to accurately measure their concentration in order to maintain standards for a known brand or to create a new brew with desired characteristics. The average α- and β-acid weight percent varies among the varieties of hops but is typically between 3 and 15% with β-acid concentration between 2 and 8% [3].

The methods for determination of content of bitter acids are presented in Analytica EBC. These methods are validated through interlaboratory studies and are recommended to be used in the analysis of hop, hop products and beer.

In 2008 and 2009, our group of researchers in the field of hops [4,5], performed HPLC and conductometric method validation. However, in order to assess the laboratory capacity and to assure quality results, periodically method validations are required . The purpose of our study was to determine the hop bitter acids content using the above mentioned methods in conditions of repeatability, as well as standard deviations and recovery coefficient.

2. Materials and Methods

The study was carried out for pellets from two varieties of hop: Magnum (MG) and Huller Bitterer (HB), cultivated in pedo-climatic areas from Transilvania.

The used conductometric and HPLC methods are: EBC- Method 7.4.[7] and EBC- Method 7.7. [8].

Corresponding author: *e-mail:* lili_sml@yahoo.com

The conductometric analyses for alpha bitter acids were performed with automatic titrator and conductometer system (Schott, tip TA 20 PLUS) and the following reagents were used: methanol [chem-Lab NV], lead acetate solution, (20g/l), diethyl ether (peroxides free) [lab scan analytical science], hydrochloric acid [Nordic invest] (0.1M).

The quantitative determination of α- and β-acids in the extracts was accomplished using an HPLC equipment from Shimadzu, with a UV detector. The column was a Grom Bier Bitter, 7µm, 125x 4 mm, from Alltech. The mobile phase used was methanol [Merck], water, ortho-phosphoric acid [Nordic invest] (775: 210: 9, v/v/v). The individual hop extract standard, namely, calibration standard ICE 2 (alpha and beta bitter acids in known concentration), was obtained from Labor Veritas- distribution for European Brewery Convention.

3. Results and discussion

Specificity . No interferences with the retention times of cohumulone, N/adhumulone, colupulone and N/adlupulone were observed. The method is selective for: Cohumulone and Colupulone, but isn't selective for the separation of normal-humulone from ad-humulone, which appear together as a single peak. The same thing goes for the normal-lupulone and ad-lupulone peak. In figure 1 is presented the chromatogram of a blank sample overlaid with a chromatogram of standard.

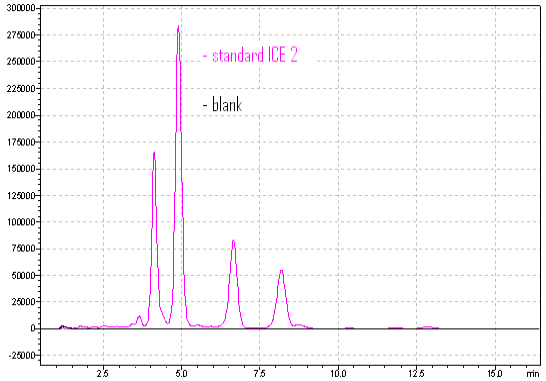

Figure 1. The chromatogram of a blank sample overlaid with a chromatogram of standard sample

Linearity. The calibration curves were linear –with adequate precision and accuracy –in the concentration interval presented in table 1. The regression coefficient for all the investigated compounds is higher than 0.99, the calibration curve for Cohumulone being presented in fig.2.

*Precision.*All the performed analyses were obtained in conditions of repeatability. The quality parameters of the HPLC method of the bitter acids are presented in table 2 -3. The results obtained were around the same value leading to a small SD (0.002- 0.058).

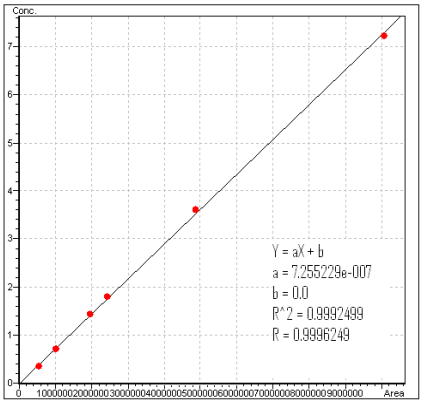

Figure 2. Calibration curve for Cohumulone

The chromatograms of HB sample, HB sample with standard ICE2 and standard ICE2 are presented in figure 3. In table 4 are presented the values of alpha bitter acids used for the calculation of recovery coefficient.

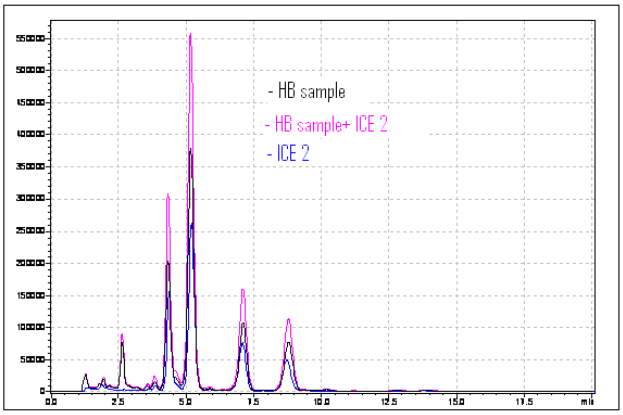

Figure 3. The overlapping HPLC chromatograms of HB sample, sample with standard ICE2 and standard ICE2

R % = {[(Sample + Std.) – Sample] / Std.} x 100 =95% The value for the recovery coefficient is 95%, indicating a good analysis.

The results obtained by conductometric method are presented in the tables 5-6. The results obtained in the two days by the two different analysts were around the same value leading to a small SD (0.068- HB sample and 0.032- MG sample).

Table 5 shows the results obtained in repeatability conditions, for bitter acids determination from Huller Bitterer variety hop pellets.

Table 1. Concentrations and mean areas for Cohumulone standard solution

Level	Conc.	Mean Area	SD	%RSD
1	0.3612500	551064	18229.81	3.308113
2	0.7225000	1028037	7089.226	0.6895885
3	1.445000	1963037	27050.16	1.377975
4	1.806250	2433267	14995.78	0.6162816
5	3.612500	4865443	48343.52	0.9936099
6	7.225000	10071656	75799.05	0.7525977

Table 2. The quality parameters of the HPLC method of the bitter acids from HB hop pellets

Analysis HB	Concentration%					
	Co-humulone	N/Ad-humulone	Total α	Co-lupulone	N/Ad-lupulone	Total β
Repeatability conditions						
1	1.91	4.56	6.47	2.09	1.85	3.94
2	1.91	4.56	6.47	2.09	1.85	3.94
3	1.78	4.47	6.27	2.06	1.83	3.89
4	1.77	4.46	6.23	2.06	1.83	3.89
5	1.79	4.51	6.29	2.09	1.85	3.94
6	1.81	4.54	6.36	2.10	1.87	3.97
7	1.77	4.41	6.18	1.77	1.81	3.85
8	1.79	4.43	6.23	1.79	1.82	3.87
9	1.79	4.42	6.21	1.79	1.81	3.85
10	1.81	4.44	6.24	1.81	1.81	3.86
The quality parameters of the method						
Min. Conc. %	1.77	4.42	6.18	1.77	1.81	3.85
Max. Conc. %	1.91	4.56	6.47	2.10	1.87	3.97
Mean% Conc.	1.81	4.48	6.29	2.07	1.83	3.90
SD %	**0.004**	**0.006**	**0.02**	**0.002**	**0.002**	**0.006**

Table 3. The quality parameters of the HPLC method of the bitter acids from MG hop pellets

Analysis MG	Concentration%					
	Co-humulone	N/Ad-humulone	Total α	Co-lupulone	N/Ad-lupulone	Total β
Repeatability conditions						
1	1.97	8.7	10.66	2.13	3.47	5.6
2	1.96	8.69	10.65	2.12	3.47	5.59
3	1.93	8.52	10.45	2.08	3.39	5.47
4	1.95	8.61	10.56	2.09	3.43	5.52
5	1.87	8.37	10.25	2.05	3.34	5.39
6	1.94	8.66	10.6	2.12	3.46	5.58
7	1.91	8.52	10.43	2.07	3.38	5.46
8	1.95	8.65	10.59	2.10	3.43	5.53
9	2.00	8.85	10.84	2.14	3.51	5.65
10	2.02	8.90	10.92	2.15	3.52	5.67
The quality parameters of the method						
Min. Conc. %	1.87	8.37	10.25	2.05	3.34	5.39
Max. Conc. %	2.02	8.90	10.92	2.15	3.52	5.67
Mean% Conc.	1.95	8.65	10.60	2.11	3.44	5.62
SD %	**0.012**	**0.062**	**0.058**	**0.016**	**0.02**	**0.036**

Table 4. HPLC values of alpha bitter acids used for the calculation of recovery coefficient

No.	Alpha acids in sample % as is	Alpha acids in standard % as is	Alpha acids (sample + standard) %as is
1	10.66	2.52	13.04
2	10.66	2.52	13.04

Table 5. Alpha bitter acids content for Huller Bitterer variety hop pellets

Analysis HB	Date	$PbAc_2$ Titre	Alpha acids % as is
1	06.02.2013	1.9974	7.17
2	06.02.2013	1.9974	7.17
3	06.02.2013	1.9974	7.35
4	06.02.2013	1.9974	7.17
5	06.02.2013	1.9974	6.98
6	07.02.2013	1.9974	7.17
7	07.02.2013	1.9974	6.98
8	07.02.2013	1.9974	6.79
9	07.02.2013	1.9974	7.17
10	07.02.2013	1.9974	6.98
Mean	**7.10**		
Standard deviation -SD	**0.068**		

Table 6. Alpha bitter acids content for Magnum variety hop pellets

Analysis	Date	$PbAc_2$ Titre	Alpha acids % as is
1	06.02.2013	1.9974	11.41
2	06.02.2013	1.9974	11.41
3	06.02.2013	1.9974	11.41
4	06.02.2013	1.9974	11.24
5	06.02.2013	1.9974	11.24
6	07.02.2013	1.9974	11.24
7	07.02.2013	1.9974	11.24
8	07.02.2013	1.9974	11.24
9	07.02.2013	1.9974	11.08
10	07.02.2013	1.9974	11.24
Mean	**11.28**		
Standard deviation -SD	**0.032**		

Table 7. Conductometric values of alpha bitter acids used for the calculation of recovery coefficient

No.	$PbAc_2$ Titre	Alpha acids in sample % as as	Alpha acids in standard % as is	Alpha acids (sample + standard) %as is
1	1.9974	7.17	2.65	9.62
2	1.9974	7.17	2.65	9.62

Table 6 shows the results obtained in repeatability conditions, for alpha bitter acids determination from Magnum variety, standardized hop pellets.
The value for the recovery coefficient (table 7) is R % = {[(Sample + Std.) – Sample] / Std.} x 100 =92%.

4. Conclusion

- The amounts of α- and β-acids in two varieties of hops were determined using liquid chromatography and conductometric methods
- The obtained conductometric values for alpha bitter acids are similar with those obtained by HPLC analysis
- The effectiveness of the methods to give reproducible results is evident in the fact that, allowing for extraction efficiency, the data achieved resemble with the literature values of α-acids in each variety of hops
- Standard deviations were obtained in conditions of repeatability
- The recovery coefficient determined for conductometric method was 92% and for HPLC method was 95%.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

References

1. Patzak, J., Nesvadba,V., Alena Henychova,Karel Krofta, Assessment of the genetic diversity of wild hops (*Humulus lupulus* L.) in Europe using chemical and molecular analyses, *Biochemical Systematics and Ecology*, **2010**, *38*, 136-145
2. Negri, Giuseppina, Santi D., , Tabach, R., Bitter acids from hydroethanolic extracts of *Humulus lupulus* L.,Cannabaceae, used as anxiolytic, Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy, **2010**, *20*(6), 850-859
3. Sahlstrom, A., Rostad, S., HPLC determination of α- and β-acids in hops, *Concordia College Journal of Analytical Chemistry*, *2*, **2011**, 78-83

4. Tofană, Maria, Sonia A. Socaci, Sevastiţa Muste, Elena Mudura, Single laboratory validation of conductometric method for the determination of hop pellets bitter acids, Journal of Agroalimentary Processes and Technologies, **2008**, *14*, 271-276
5. Tofană, Maria, Elena Mudura, Sevastiţa Muste, Sonia Ancuţa Socaci, Crina Mureşan, The validation of the HPLC Hop Bitter acids method, Journal of Agroalimentary Processes and Technologies, **2009**, *15* (1), 50-54
6. EBC- Method 7.4./*Analytica-EBC*, Section 7 Hops: Lead conductance Value of Hops, Powders and Pellets
7. EBC- Method 7.7./*Analytica-EBC*, Section 7 Hops: Alfa- and beta-Acids in Hops and Hop Products by HPLC