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Dilute and Shoot - Comprehensive LC-Q-ToF-MS analysis of beer bitter acids

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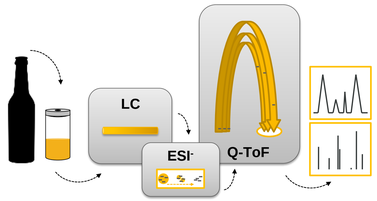
Schematic method overview



Introduction

Bitterness is a primary quality attribute of many beer styles. Depending on hopping regime, multiple bitter acids and prenylated chalconoids (e.g. xanthohumol and isoxanthohumol) are present in beer.

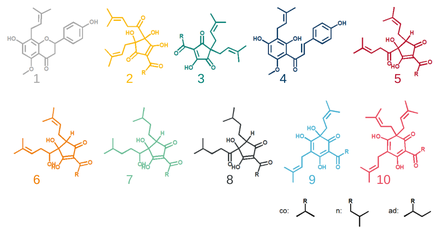


Figure 1: Beer bitter acids isoxanthohumol (1), humulinone (2), hulupone (3), xanthohumol (4), isohumulone (5), dihydro-isohumulone (6), hexahydro-isohumulone (7), tetrahydro-isohumulone (8), humulone (9) and lupulone (10).

The degradation and transformation of hop bitter acids during beer storage, effecting bitterness intensity and quality, has received considerable attention. Using high-resolution mass spectrometry (HRMS) enables substance identification in case of commercially unavailable standards. The current poster summarizes the initial steps of developing a comprehensive LC-Q-TOF-MS–based method for quantitative analysis of beer bitter acids.

Method

Chromatography: An Acquity H-Class UPLC System (Waters) with quaternary gradient pump, autosampler (10 °C) and column oven (40 °C) was used for the chromatographic separation. An Acquity UPLC-BEH C18 reversed phase column 2.1 x 100 mm (Waters) with 1.7 µm particle size was applied. Mobile phase was 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). The gradient with a total run time of 25 minutes is shown in Figure 2. The flow rate was set to 0.5 ml/min and injection volume was 1 µl.

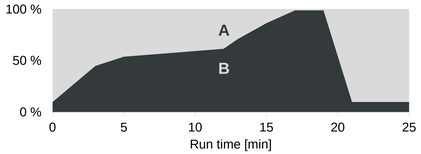


Figure 2: UPLC flow gradient

Mass Spectrometry: A Xevo G2-XS QTof mass spectrometer (Waters) with electrospray ionization (negative ion polarity, sensitivity mode) was used. The MS conditions are listed in Table 1. Mass accuracy was performed by lock mass correction with a solution of leucine and encephalin (*m/z* 554.2620). For data processing UNIFI v1.8 was used.

Table 1: MS conditions

Scan time:	0.15 s	Capillary voltage:	2.35 kV
Mass range:	*m/z* 50 to 1200	Sampling cone:	40 V
MS^E low energy:	6.0 eV	Source temp.:	120 °C
MS^E high energy:	15.0 to 45.0 eV	Desolvation temp./ gas flow:	550 °C/ 950 l/h

Standard substances, either purchased from Labor Veritas (ICS-I3, ICS-R2, ICS-T2, ICS-H1) or kindly provided by Hopsteiner* (Xanthohumol, Isoxanthohumol, DCHA-Alpha, DCHA-Beta, DCHA-Humulinone, DCHA-Hulupone), were diluted in acetonitrile with 0.1 % formic acid.

Degassed and filtered beer samples were directly injected to UPLC analysis.

Results

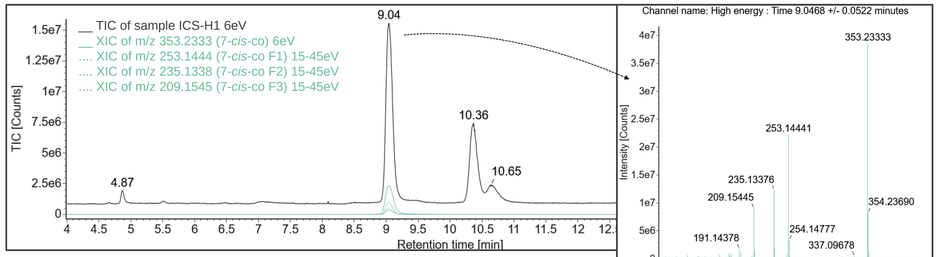


Figure 3: Chromatograms of ICS-H1 standard (left) and high energy spectrum at 9.04 min (*cis*-co-hexahydro-isohumulone) (right)

After optimization of LC-MS conditions and analysis of standard substances (Figure 3) a library of HRMS beer bitter acids data was generated (Table 2). Up to today, it includes 33 beer bitter acids (co-, n- and ad-isomers of 1-10) with their particular retention time and their 20 most intensive and characteristic high energy fragments.

The method was then applied to analyze a wide range of commercial beer samples (IPAs, lagers, light stable lagers, etc.). By checking LC-MS sample data against the library entries, all 33 beer bitter acids were confirmed to be present in the samples tested.

As an example, Figure 4 shows chromatograms (TIC) of a mixed standard solution and of a commercial beer sample (German Pilsner) in which (as expected) the isomers of isohumulones (5) and humulones (9) could be identified.

Table 2: Library excerpt with retention time (RT) in min and fragments F in *m/z*

	Item Name	Isomer	RT	F1	F2	F3	…
1	isoxanthohumol	—	4.11	119.0502	233.0819	189.0921	
		co	4.53	209.1183	249.1132	141.0557	
2	humulinone	n	5.03	263.1289	223.1340	141.0557	
		ad	5.26	263.1289	223.1340	141.0557	
		co	5.25	248.1054	180.0428	205.0506	
3	hulupone	n	5.77	262.1211	219.0663	194.0585	
		ad	6.10	262.1211	194.0585	166.0635	
4	xanthohumol	—	6.55	119.0502	233.0819	175.0037	
		trans-co	6.52	251.1289	182.0585	181.0506	
		cis-co	6.72	251.1289	182.0585	329.1758	
5	isohumulone	*trans*-n	7.39	265.1445	196.0741	195.0663	
		cis-n	7.55	265.1445	196.0741	195.0663	
		trans-ad	7.87	265.1445	196.0741	195.0663	
		cis-ad	8.20	265.1445	196.0741	195.0663	
		cis-co 1	6.74	182.0585	251.1289	233.1183	
		cis-co 2	6.96	182.0585	251.1289	233.1183	
6	dihydro-isohumulone	*cis*-n 1	7.62	196.0741	265.1445	247.1340	
		cis-n 2	7.87	265.1445	196.0741	247.1340	
		cis-ad 1	8.10	196.0741	265.1445	247.1340	
		cis-co	9.04	253.1445	235.1340	209.1547	
7	hexahydro-isohumulone	*cis*-n	10.33	267.1602	249.1496	223.1704	
		cis-ad	10.60	267.1602	249.1496	223.1704	
…							

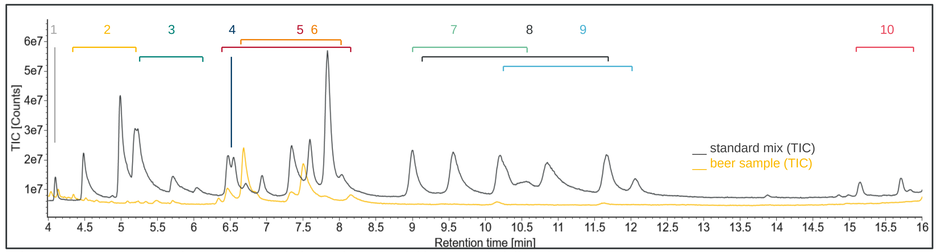


Figure 4: Chromatograms of standard mix solution and commercial beer sample (Pilsner).

Conclusion

- HRMS is a promising technique, that enables outstanding performance in substance identification.
- The presented method allows the simultaneous detection of all common beer bitter acids with simple sample preparation and short analysis run time.
- The library of accurate mass data enables unambiguous substance identification. The introduction of suitable internal standards for quantification, as well as the incorporation of bitter acid degradation products is currently under investigation.

Figure 5: © VLB Berlin

Further reading

- Hofmann et al., J. Agric. Food Chem. 2009, 57, 1172-1182
- Rodda et al., Anal Bioanal Chem 2013, 405, 9755-9767

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