

Direct quantitative analysis of α - and β -acids by electrospray ionization mass spectrometry

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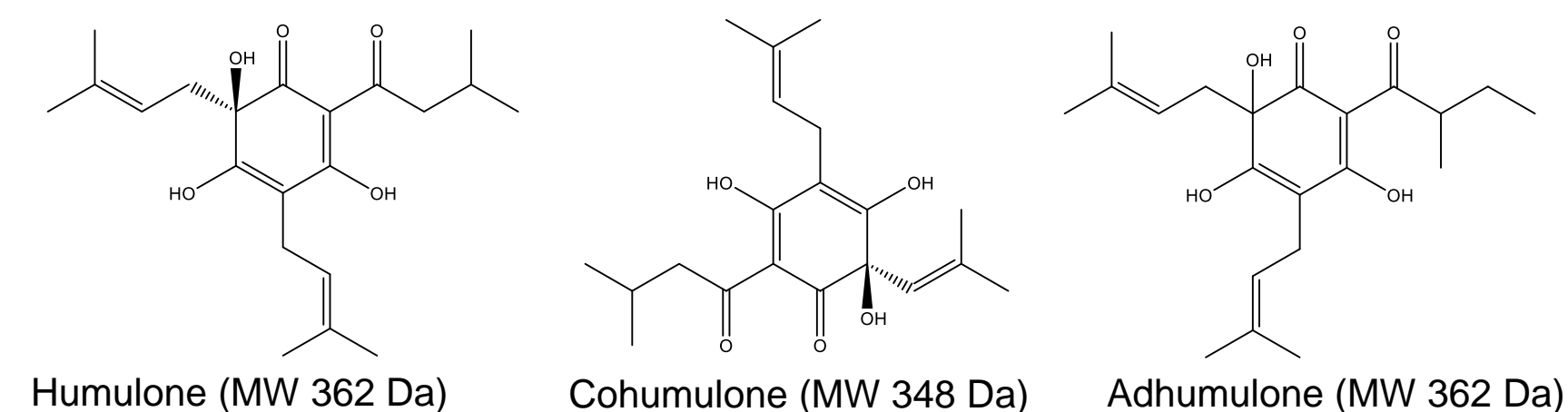
Overview

- The use of direct electrospray ionization mass spectrometry is investigated as an alternative method for quantitative determination of α - and β -acids of hops in a timely, reproducible manner.

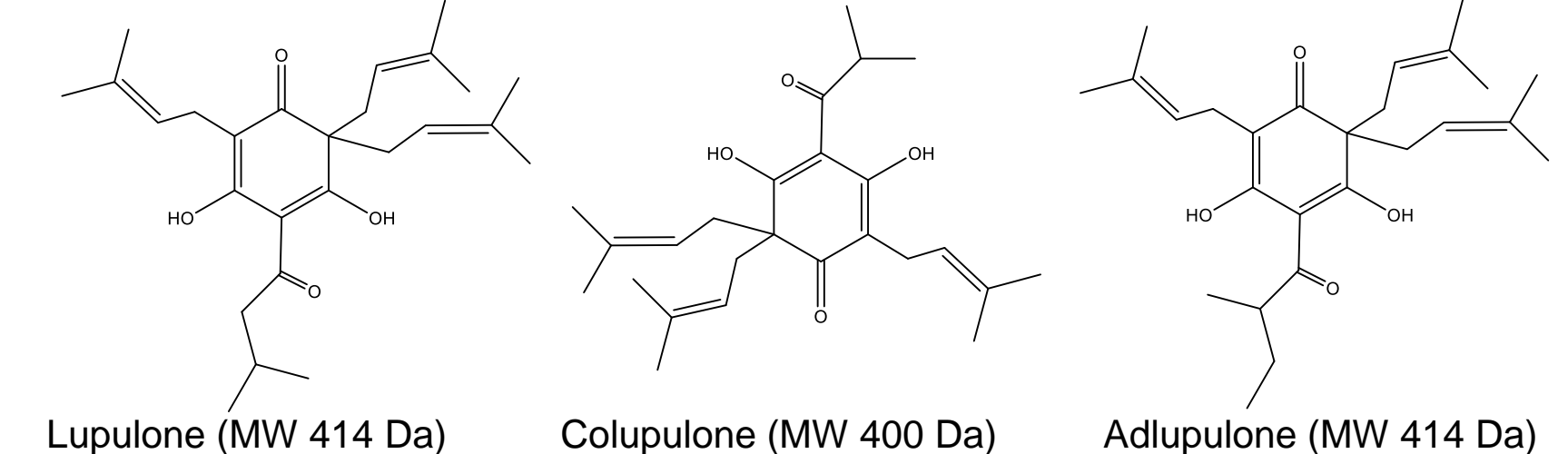
Introduction

- Electrospray ionization mass spectrometry (ESI-MS) was used in a direct analysis without chromatographic separation.
- Hops 6a (UV-Vis) uses large volumes of toxic solvents and is labor intensive and only acquires total α - acid and total β - acid content, but analysis time is only 6 minutes per sample.²
- Hops 14 (HPLC) uses large volumes of solvents but provides relative concentrations of the different congeners of both α - and β - classes of hop acids. However, analysis time can be as long as 56 minutes per sample, in addition to the ICE-3 calibration time.¹

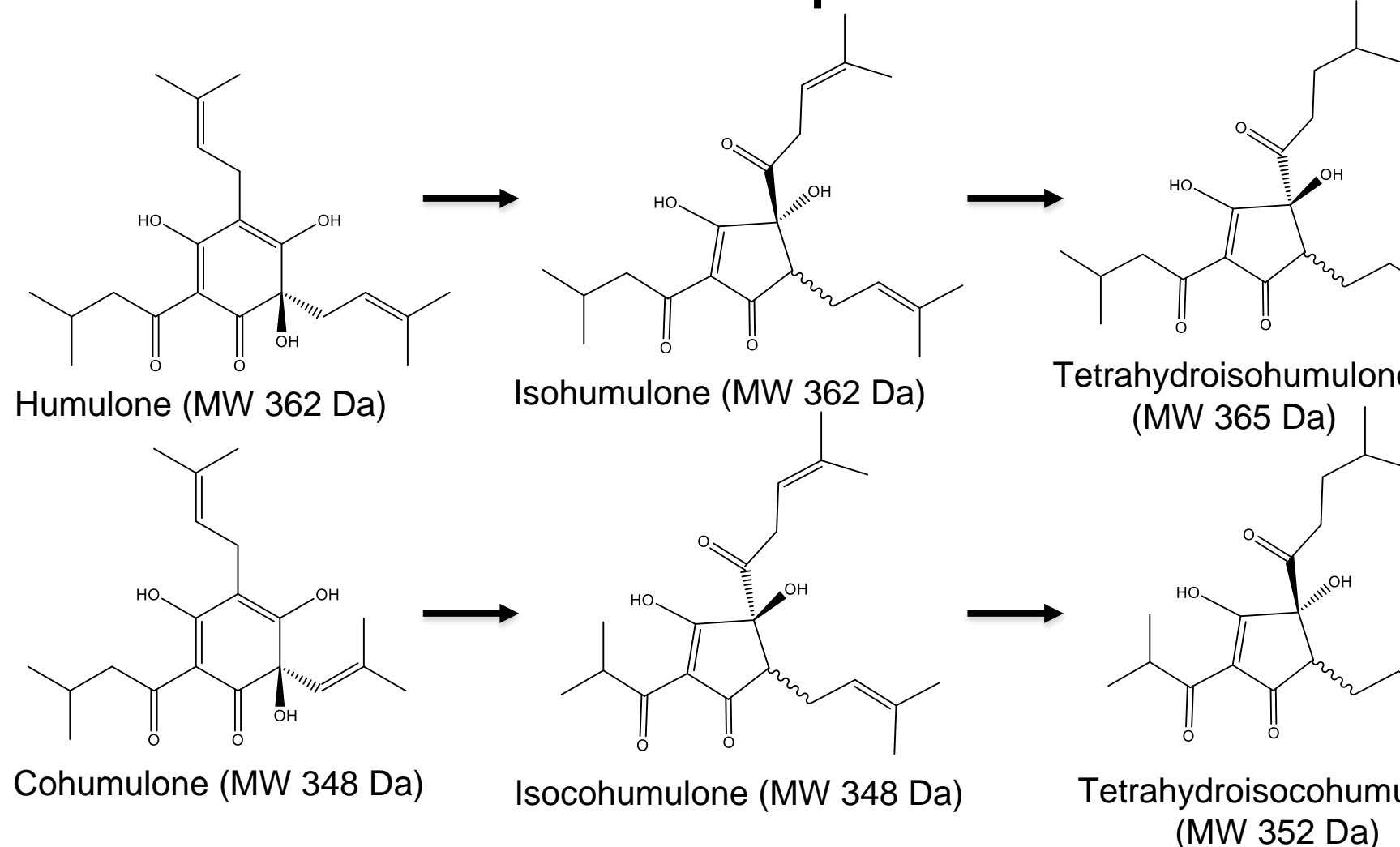
α -Acids



β -Acids



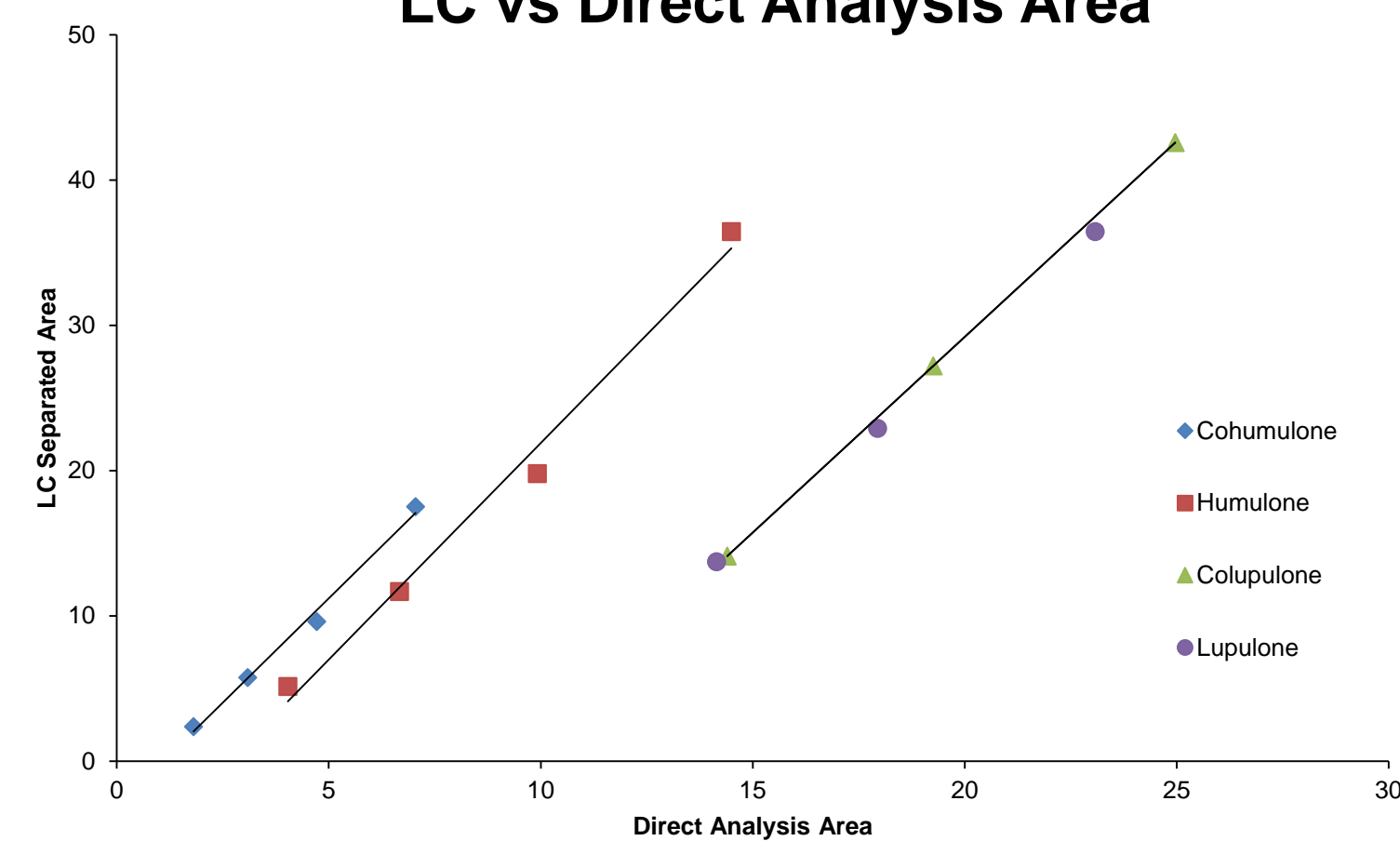
Isomerized Hop Acids



Ion Suppression

- Above a certain concentration, charge competition begins to affect ionization of analyte.⁵
- β -acids are more hydrophobic than the α -acids and therefore could suppress the signal of the α -acids due to an increase in surface activity.
- Varying concentrations of ICE-3 were analyzed via LC and direct analysis and the absolute area percentage of the hops acids were monitored.
- The intensities for the acids deviated minimally and the intensities were determined to be statistically equal for the 0.5-4 μ M concentration range.

LC vs Direct Analysis Area



HPLC Conditions

- Column: Phenomenex Kinetex XB-C18
- 2.6 μ m, 2.1 mm x 100 mm, 100 Å
- Mobile phase: 80:20:0.1 MeOH:H₂O:Formic acid
- 0.25 mL/min solvent flow rate
- UV detection: 314 nm

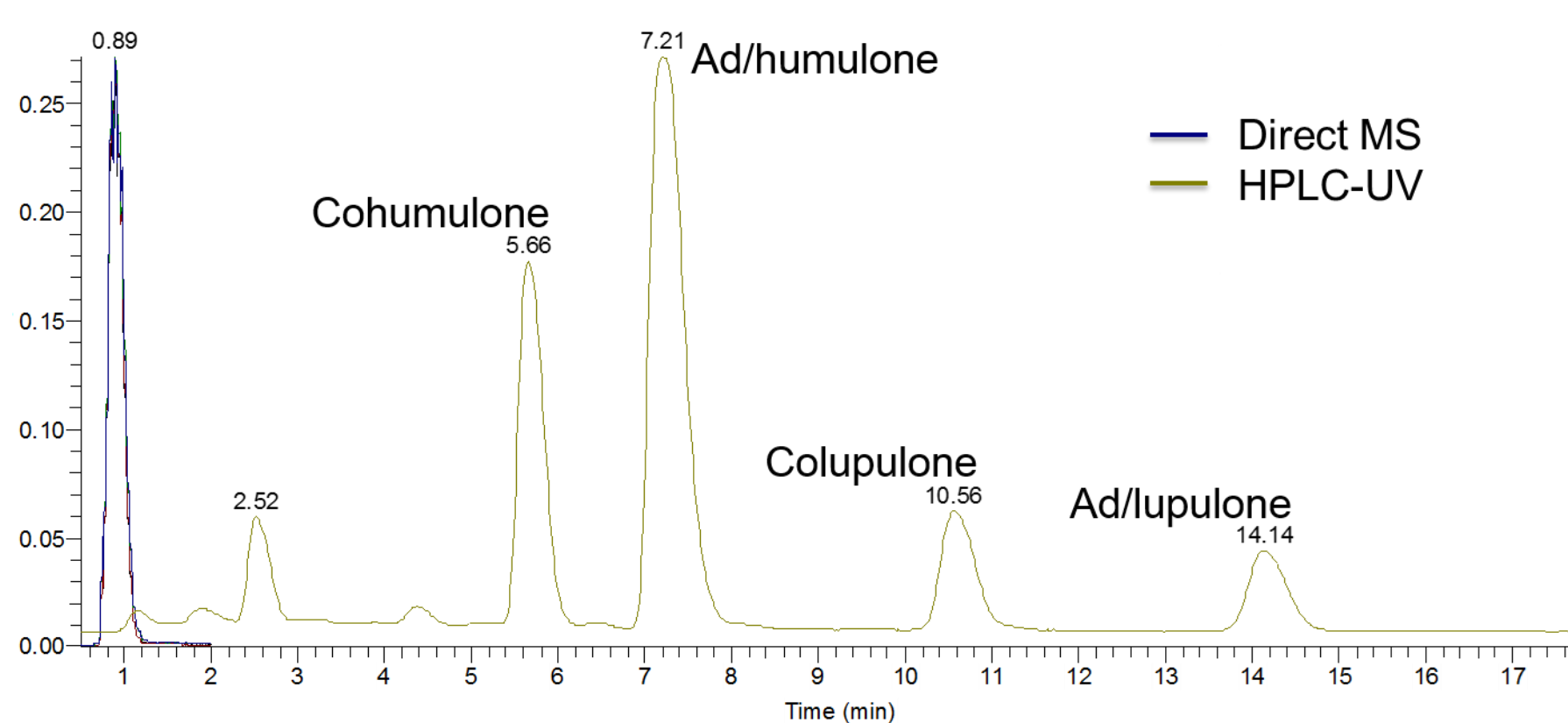
Thermo Scientific LTQ MS Conditions

- Sample prep method: Hops-14 extraction
- Spray voltage: -3.5 kV
- Capillary voltage: -20 V
- Tube lens voltage: -60 V
- Source temperature: 250° C
- 90% methanol spray solvent
- 10 μ L/min solvent flow rate
- Internal standard: ICS-T2 (Tetrahydroiso- α -acids)
- Hops standard: ICE-3

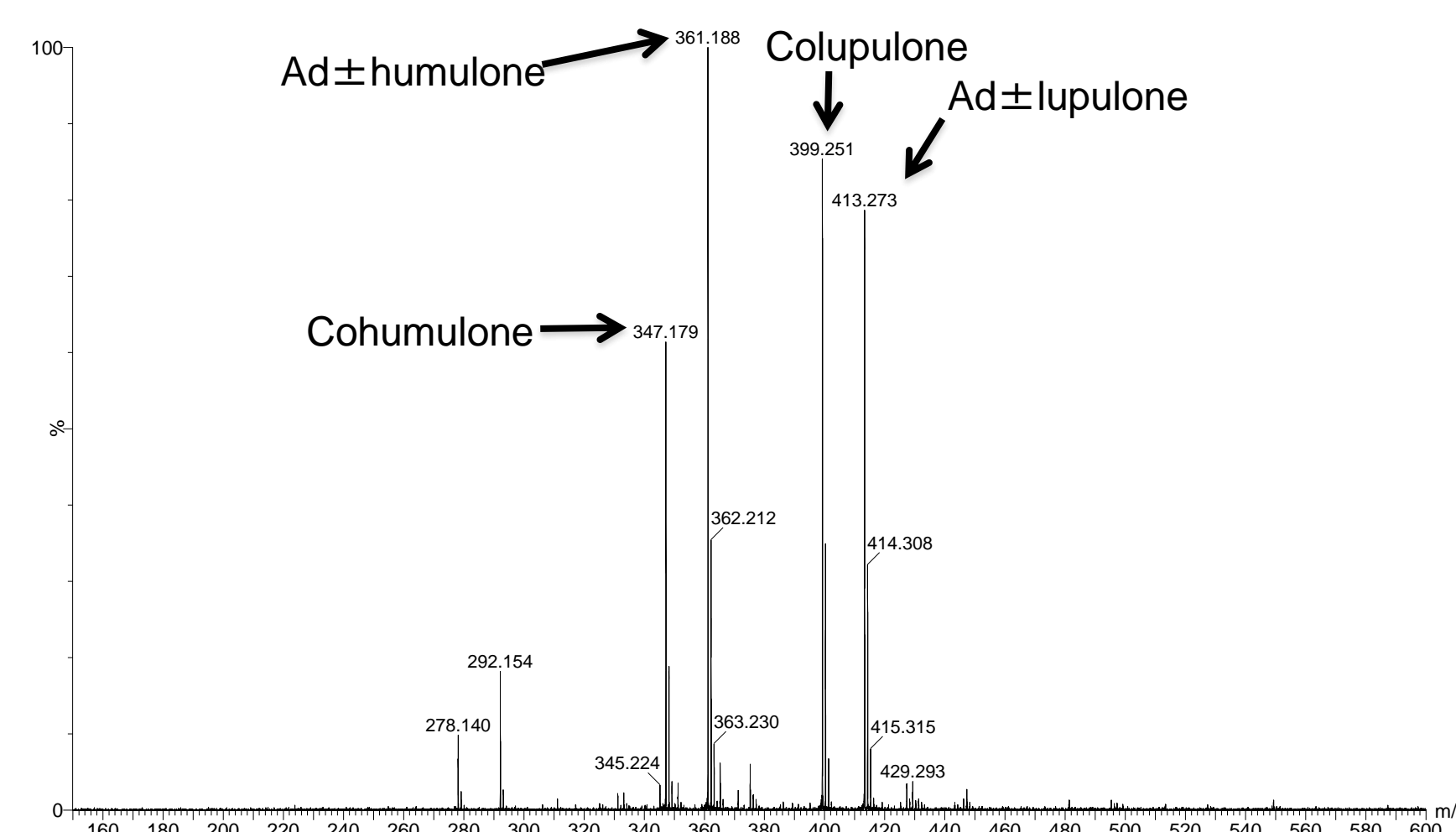
Waters Synapt MS Conditions

- Spray voltage: -3.0 kV
- Sampling cone voltage set point: 26 V
- Extraction cone voltage set point: 5.0 V
- Source temperature: 135° C
- Desolvation gas temperature: 300°
- 90% methanol spray solvent
- 0.500 mL/min solvent flow rate
- Internal standard: ICS-T2
- Hops standard: ICE-3

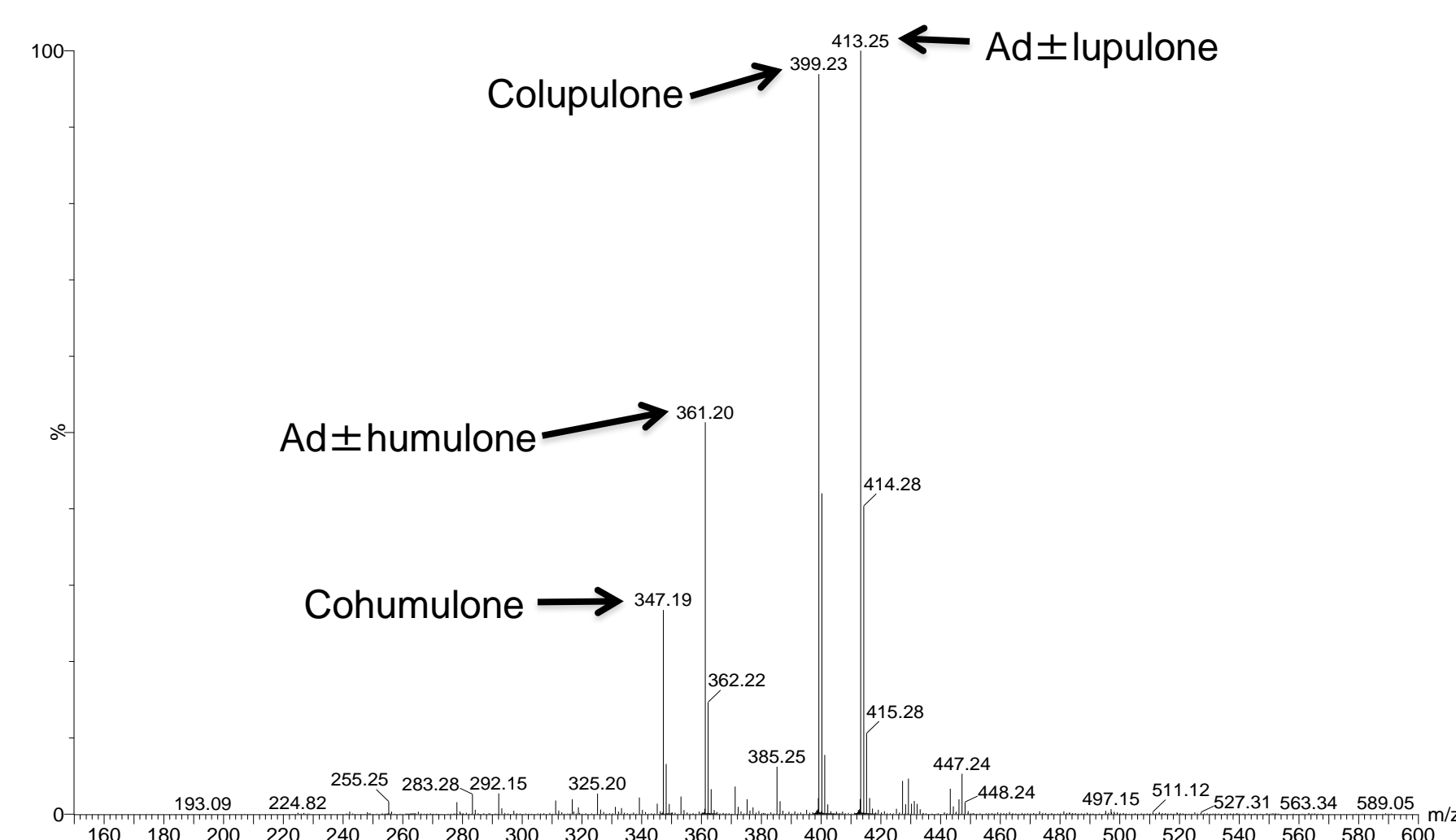
Extracted Ion Chromatograms for HPLC and Direct Analysis



Mass Spectrum for ICE-3



Mass Spectrum for Willamette Hops



ASBC Check Sample Results

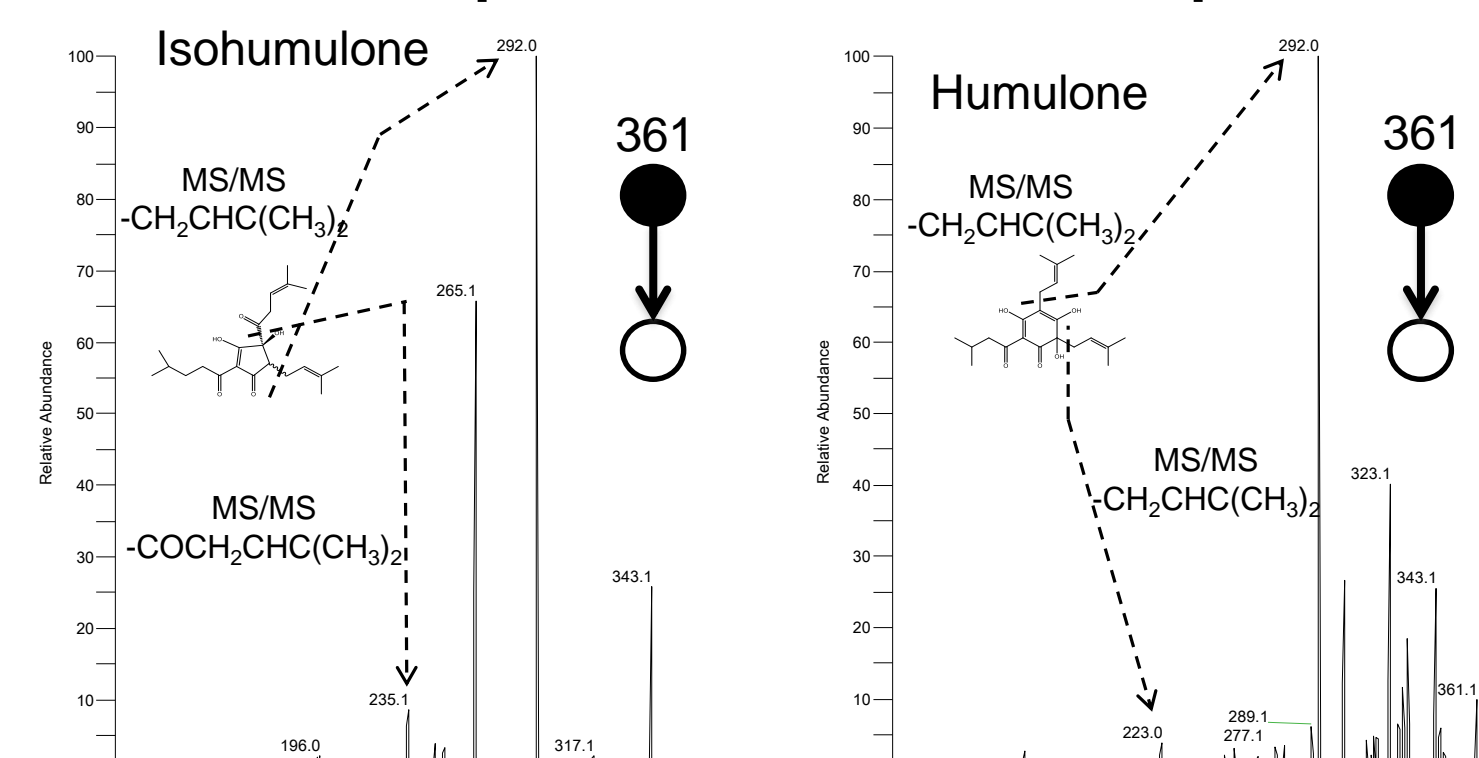
HPW-1	HPLC Nat Average	UV-Vis Nat Average	Synapt Direct Analysis MS	LTQ Direct Analysis MS
α_1	3.33%	-	3.42%	3.18%
α_2	7.65%	-	8.26%	7.03%
α_{Tot}	10.98%	11.36%	11.67%	10.21%
β_1	2.67%	-	2.31%	2.76%
β_2	2.37%	-	2.22%	2.22%
β_{Tot}	5.04%	5.52%	4.53%	4.98%

HPL-2	HPLC Nat Average	UV-Vis Nat Average	Synapt Direct Analysis MS	LTQ Direct Analysis MS
α_1	4.74%	-	5.03%	5.02%
α_2	11.07%	-	11.32%	10.92%
α_{Tot}	15.81%	17.54%	16.35%	15.94%
β_1	2.86%	-	2.72%	3.82%
β_2	2.86%	-	2.78%	3.33%
β_{Tot}	5.72%	5.78%	5.50%	7.15%

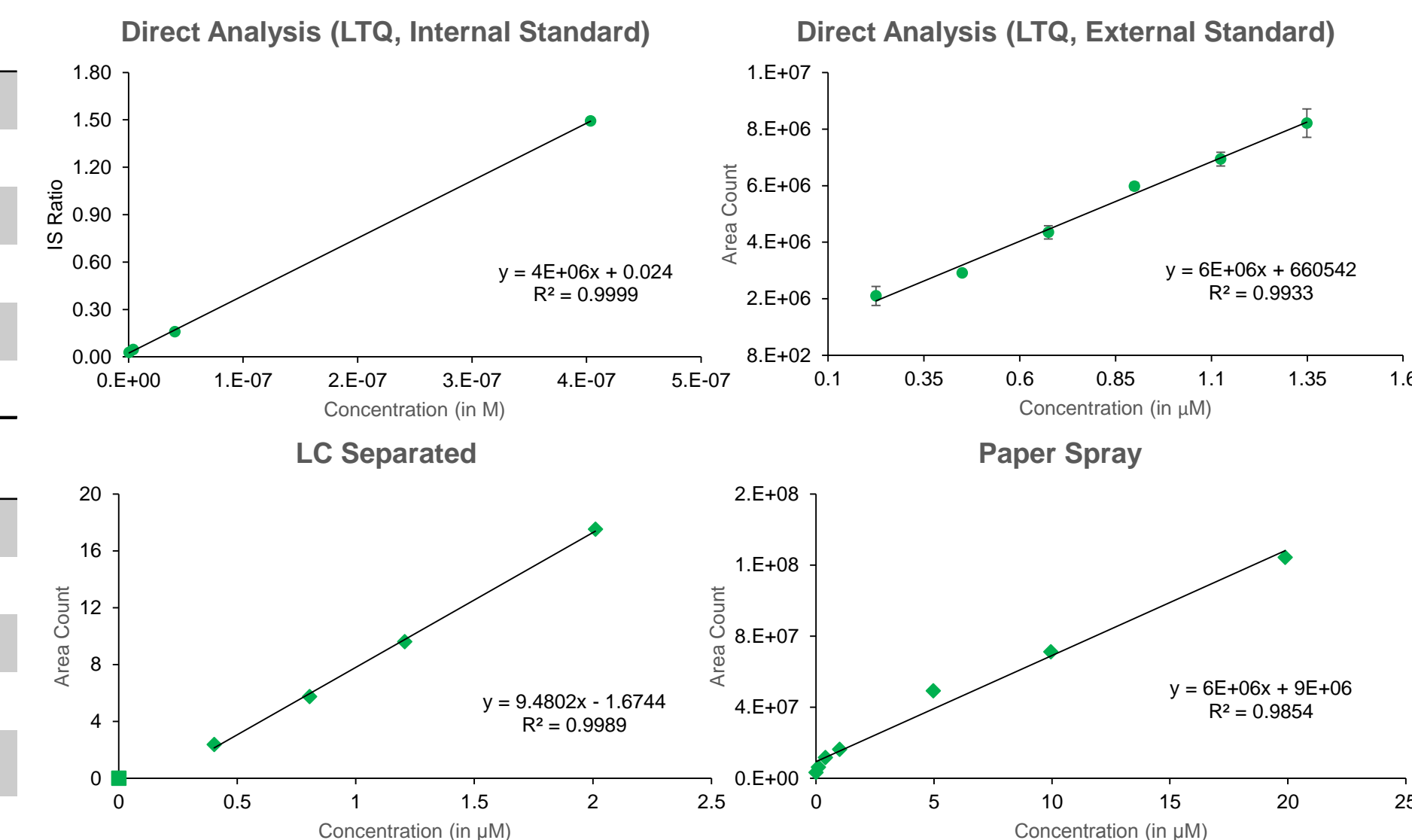
HPE-3	HPLC Nat Average	UV-Vis Nat Average	Synapt Direct Analysis MS	LTQ Direct Analysis MS
α_1	16.74%	-	17.38%	17.00%
α_2	39.52%	-	39.66%	39.82%
α_{Tot}	56.26%	60.38%	57.04%	56.83%
β_1	7.94%	-	5.88%	8.28%
β_2	6.67%	-	6.09%	7.25%
β_{Tot}	14.61%	14.94%	11.97%	15.53%

HPW-4	HPLC Nat Average	UV-Vis Nat Average	Synapt Direct Analysis MS	LTQ Direct Analysis MS
α_1	4.13%	-	3.89%	4.35%
α_2	9.60%	-	8.89%	10.09%
α_{Tot}	13.73%	14.09%	12.78%	14.44%
β_1	2.43%	-	2.94%	3.09%
β_2	1.88%	-	2.20%	2.39%
β_{Tot}	4.31%	4.85%	5.14%	5.48%

Differentiation of isohumulone and humulone for quantitation in beer samples



Cohumulone Calibration Curves



Electrospray Ionization Advantages

- Electrospray ionization mass spectrometry yields the detailed results of the Hops-14 method while only requiring less than one minute per sample.²
- The resulting mass spectrum of a hop extract gives information about other constituents, such as the oxidized hop products.
- Direct analysis by mass spectrometry only consumes 1 mL of spray solvent, whereas HPLC analysis consumes significantly more solvent for the mobile phase.²
- Direct analysis by mass spectrometry only requires 12 μ L of solvent per test while UV/VIS requires up to 9 mL per test.³

Conclusions

- Electrospray ionization mass spectrometry can determine the α - and β -acid content in hops with a turnaround of about one minute per sample.
- Tetrahydroiso- α -acids are suitable internal standards for electrospray ionization mass spectrometry when quantitating the hop acids.
- Future work include
 - Improving the sample preparation procedure for speed and reduced solvent consumption.
 - Implementing the internal standard in paper spray ionization mass spectrometry.
 - Using multivariate analysis to identify hop strains.
- A sample of beer can be directly analyzed via Paper Spray Mass Spectrometry without additional sample preparation.
- Completeness of the isomerization of the acids from the brewing process can be quantified through tandem mass spectrometry by collision induced dissociation.

Acknowledgements

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- MillerCoors and Hopsteiner for hop acids standards

References

- α -acids and β -acids in hops and hop extracts by HPLC (ASBC Hops 14)
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