Analysis of Carbohydrates in Beer Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

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ABSTRACT

Purpose: This work presents a simple technique for quantification of carbohydrates in beer and beer-like beverages by triple quadrupole mass spectrometry coupled to HILIC chromatography.

Methods: A Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer in negative electrospray mode with a Thermo Scientific™ Vanquish™ HPLC system and Thermo Scientific™ Accucore™ Amide HILIC column were utilized for all measurements. Selective reaction monitoring (SRM) transitions for each analyte were designed and source condition optimization was performed. 10 µL of each beer sample were consumed for the analysis.

Results: Using available pure substances, SRM transitions were determined and operation of the TSQ Quantis triple quadrupole mass spectrometer optimized. The TSQ Quantis mass spectrometer provided sensitive quantitation results while being sufficiently robust during exposure to very difficult sample matrices with high carbohydrate content. A sample set of ten beers was analyzed using the developed method. Different beers showed variations in carbohydrate profile, as expected based on their different recipes. The findings were in accordance with general expectations: radlers showed high concentration of fructose, peanut butter stout of lactose and lagers of maltose, maltotriose and maltotetraose. The developed method is suited for rapid analysis of mono, di, tri and tetra saccharides in beverages for screening and quantitative purposes.

INTRODUCTION

Analysis of carbohydrate profile can be used for quality and stability control, estimation of calories count, and in some cases for identification of counterfeiting. Beer was one of the oldest beverages humans have produced and nowadays beer and beer-like drinks represent the most consumed alcoholic beverage in the world. Many different types of beer are produced for local and global markets and manufactures must ensure consistency and shelf-life stability in compliance with multiple regulations. Liquid chromatography triple quadrupole mass spectrometry is a well-suited technique for identification and quantification of carbohydrates and can be used for carbohydrate profile determination and beer quality monitoring.

MATERIALS AND METHODS

Sample Preparation

1. 1mL of each beer sample was degassed by sonication
2. 10uL of degassed beer was mixed with 990uL of 50%ACN and centrifuged
3. The supernatant was pipetted into an autosampler vial and analyzed

1. Corona
2. La Fin
3. Smoked Porter
4. Peanut Butter Stout
5. Framboise
6. Paulaner Salvator
7. Warsteiner
8. Imperial Stout
9. London Porter
10. Stiegl Radler

LC-MS

TSQ Quantis triple quadrupole mass spectrometer (negative HESI mode)

Vanquish Flex HPLC system

Accucore 150 Amide HILIC; 100x2.1mm; 2.6um column

Mobile Phases

Mobile phase A: 80/20 ACN/H_2O with 0.1% NH_4OH
Mobile phase B: 30/70 ACN/H_2O with 0.1% NH_4OH
Reference [1]

Figure 1. LC Gradient

No	Time	Flow [ml/min]	%B	Curve
1	0.000		Run	
2	0.000	0.150	0.0	5
3	0.500	0.150	0.0	5
4	15.500	0.150	70.0	5
5	17.000	0.150	70.0	5
6	18.000	0.150	0.0	5
7	28.000	0.150	0.0	5
8	New Row			
9	28.000		Stop Run	

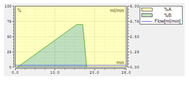


Figure 2. HESI Source and CID Parameters

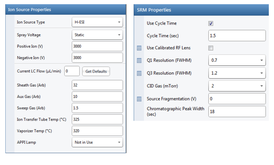


Table 1 List of SRM Transitions

Compound	Mode	Precursor	Product	CE	RF lens
Dextrose	Negative	179	59	15.1	66
Dextrose	Negative	179	89	10.2	66
Dextrose	Negative	179	119	10.2	66
Fructose	Negative	179	59	14.1	56
Fructose	Negative	179	71	12.9	56
Fructose	Negative	179	89	10.2	56
Sucrose	Negative	341	89	18.7	141
Sucrose	Negative	341	119	16.6	141
Sucrose	Negative	341	179	12.6	141
Lactose	Negative	341	100	13.3	73
Lactose	Negative	341	161	10.2	73
Lactose	Negative	341	179	10.2	73
Maltose	Negative	341	101	13.4	84
Maltose	Negative	341	161	10.2	84
Maltose	Negative	341	179	10.2	84
Maltotriase	Negative	503	161	10.2	106
Maltotriase	Negative	503	179	10.2	106
Maltotriase	Negative	503	341	10.2	106
Maltotetraose	Negative	665	341	13.3	185
Maltotetraose	Negative	665	383	27.0	185
Maltotetraose	Negative	665	503	10.2	185
Maltotetraose	Negative	665	545	20.6	185

Data Analysis

Data were analyzed using Thermo Scientific™ TraceFinder™ 4.1 SP2 and FreeStyle™ 1.3 SP1 software

RESULTS

Figure 3. SRM chromatograms of the blank

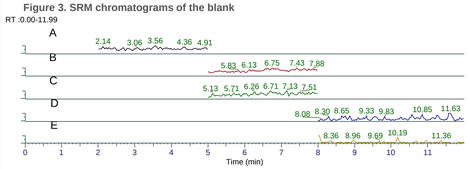


Figure 4. SRM chromatograms close to LOD level

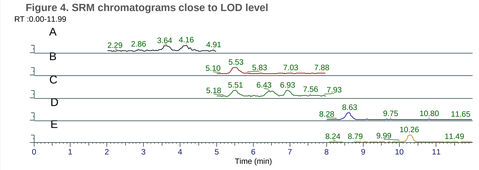
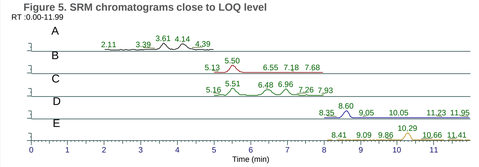


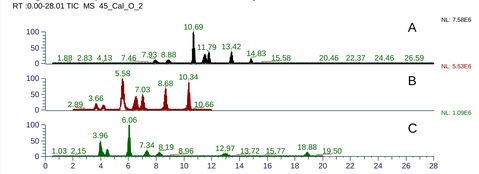
Figure 5. SRM chromatograms close to LOQ level



A: fructose (1) dextrose (2)
B: sucrose
C: sucrose (1) maltose (2) lactose (3)

D: maltotriase
E: maltotatraose

Figure 6. Comparison of selected commercially available off-the-shelf columns



A: Vendor X, comparable column 1.7um
B: Accucore Amide HILIC; 100x2.1mm; 2.6um
C: Vendor Y, comparable column 3um

Figure 7. Separation of carbohydrates by number of saccharide units

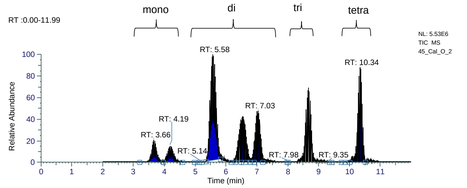


Figure 8. Low concentration calibration curves < 0.5 ppm

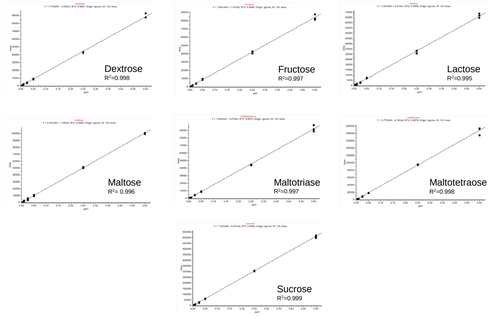


Figure 9. Calibration curves for dynamic range 0.005 - 50 ppm

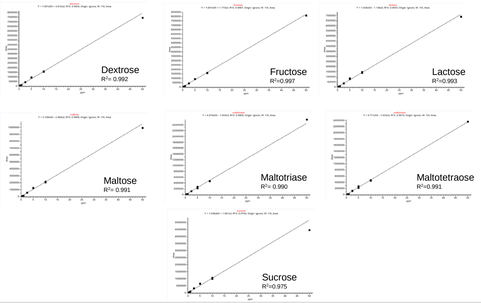


Table 2 Analysis of carbohydrates in beer samples

Beer samples: 1-Corona 2-La Fin 3-Smoked Porter 4-Peanut Butter Stout 5-Framboise 6-Paulaner Salvator 7-Warsteine 8-Imperial Stout 9-London Porter 10-Stiegl Radler;

N/D = not detected; N/I = not possible to integrate

	Fructose g/L	Dextrose g/L	Sucrose g/L	Maltose g/L	Lactose g/L	Maltotriose g/L	Maltotetraos g/L
Beer 1	0.12	0.031	0.039	0.40	0.19	1.09	4.41
Beer 2	0.33	0.047	0.040	0.31	0.26	3.61	3.50
Beer 3	0.13	0.089	0.35	0.15	N/D	1.51	9.81
Beer 4	N/I	N/I	N/D	0.12	15.4	0.21	6.47
Beer 5	36.1	13.8	0.062	1.05	0.099	0.49	0.70
Beer 6	0.11	0.029	0.039	0.56	0.37	1.18	7.30
Beer 7	0.31	0.030	0.041	0.19	0.33	0.44	2.17
Beer 8	0.63	0.49	0.041	0.16	0.088	2.23	5.99
Beer 9	0.61	0.14	0.036	1.24	N/D	1.48	7.29
Beer 10	26.1	1.3	0.24	0.38	0.16	0.66	1.18

CONCLUSIONS

- Analysis of carbohydrates in beer was demonstrated using LC-MS/MS on the TSQ Quantis mass spectrometer with Vanquish Flex UHPLC system.
- LoD was estimated to be 5-10ppb, LoQ was determined at 25ppb level and dynamic range was demonstrated up to 50 000 ppb.
- The Accucore 150 Amide HILIC column achieved separation of mono-, di-, tri- and tetra-saccharides better or comparable with other HILIC columns available for carbohydrates applications.

REFERENCES

1. Fountain K., et al. UPLC-MS Analysis of Carbohydrates, Waters Application Note, October 2009.

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