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Nota de aplicación

Determination of Amino Acids in Beers Using the UPLC Amino Acid Analysis Solution

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Abstract

This application note we demonstrate the efficacy of the Waters UPLC Amino Acid Analysis Solution to resolve 27 amino acids and an internal standard in less than 10 minutes and apply this capability to amino acid analysis of several beer and ale samples.

Introduction

Beer is a complex matrix consisting of over 100 components. Water, ethanol and carbohydrates are the major constituents of beers and ales. However, there are many minor compounds, some of which are critical for proper taste and quality. One class of compounds, amino acids, is metabolized by yeast during fermentation, leading to the formation of critical flavor components. Therefore, the monitoring of amino acids is essential to demonstrate product consistency and ensure customer satisfaction.

Current HPLC methods for amino acids require run times that exceed 30 minutes, with poor resolution between many amino acids. Here we shall demonstrate the efficacy of the Waters UPLC Amino Acid Analysis Solution to resolve 27 amino acids and an internal standard in less than 10 minutes and apply this capability to amino acid analysis of several beer and ale samples.

Experimental

Conditions

System:	ACQUITY UPLC with Tunable UV Detector
Method:	Cell culture
Column:	AccQ•Tag Ultra, 2.1 x 100 mm
Temperature:	60 °C
Injection volume:	1.0 µL
Detection:	UV @ 260 nm
Data:	Empower software

Standard Preparation

A stock 1000 pmol/µL stock mixed amino acid standard was prepared per the cell culture method.[1,2,3] An intermediate 100 pmol/µL mixture was prepared by mixing 100 µL of stock with 900 µL water. The working derivatized standard was prepared by adding 10 µL of the 100 pmol/µL mixture to 70 µL borate buffer followed by 20 µL AQC derivatization reagent in a total recovery vial and mixing well. The mixture was heated for 10 minutes at 55 °C, cooled to room temperature then injected. The concentration is 10 pmol/µL for the analytes of interest except Cystein (Cys) which is 5 pmol/µL.

Sample Preparation

14 samples of beer and ale were purchased commercially. These included domestic regular, light, non-alcoholic, dark beers, and an imported Belgian ale. Approximately 100 mL of each beer was sonnicated to remove carbonation. If the sample appeared excessively cloudy or turbid, it was filtered through a 0.45 micron hydrophilic filter. 200 µL of each beer and ale was mixed thoroughly with 160 µL water and 40 µL of a 1000 pmol/µL Norvaline (Nva - internal standard) solution. The preparation of the internal standard is described in the

Cell Culture Method.[1,2,3] This resulted in a 1:2 dilution (400 µL total volume) of the beer made 100 pmol /µL in internal standard.

10 µL of this mixture was then mixed with 70 µL of borate buffer and 20 µL of AQC derivitazation reagent and heated as described in the standard preparation section. This working sample mixture, now a 20 fold dilution of the beer, made 10 pmol /µL in internal standard (similar to the working standard) was injected.

Results and Discussion

Figure 1 is a chromatogram of the cell culture standard. Table 1 is reproducibility data (RSD) for retention time and area for 5 injections of this standard. An overlay of the chromatograms of several of the beers analyzed is found in Figure 2. Table 2 lists the quantitated amounts for the amino acids in the 14 beer samples tested.

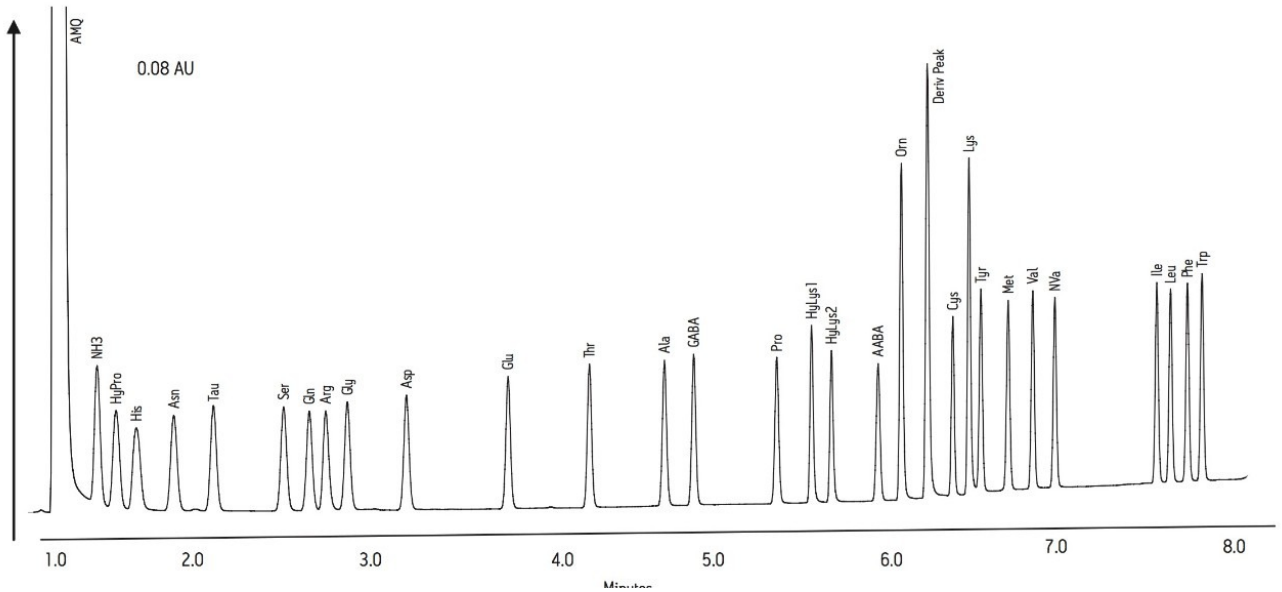

Figure 1. Chromatogram of 10 pmol/µL amino acid standard.

Analyte	RT	Area	Analyte	RT	Area
Hypro	0.522	0.736	HyLys1	0.013	0.874
His	0.581	0.743	HyLys2	0.014	0.86
Asn	0.417	0.853	AABA	0.017	0.856
Tau	0.329	0.798	Orn	0.017	0.846
Ser	0.236	0.708	Cys	0.014	0.885
Gln	0.219	0.725	Lys	0.014	0.841
Arg	0.217	0.762	Tyr	0.013	0.846
Gly	0.167	0.754	Met	0.012	0.809
Asp	0.126	0.817	Val	0.012	0.822
Glu	0.074	0.413	Nva	0.012	0.413
Thr	0.05	1.029	Ile	0.01	0.964
Ala	0.035	0.903	Leu	0.01	0.983
GABA	0.03	0.816	Phe	0.01	0.857
Pro	0.019	0.845	Trp	0.01	0.9

Table 1. Reproducibility data for amino acid standard, RT, and Area (RSD), 5 injections.

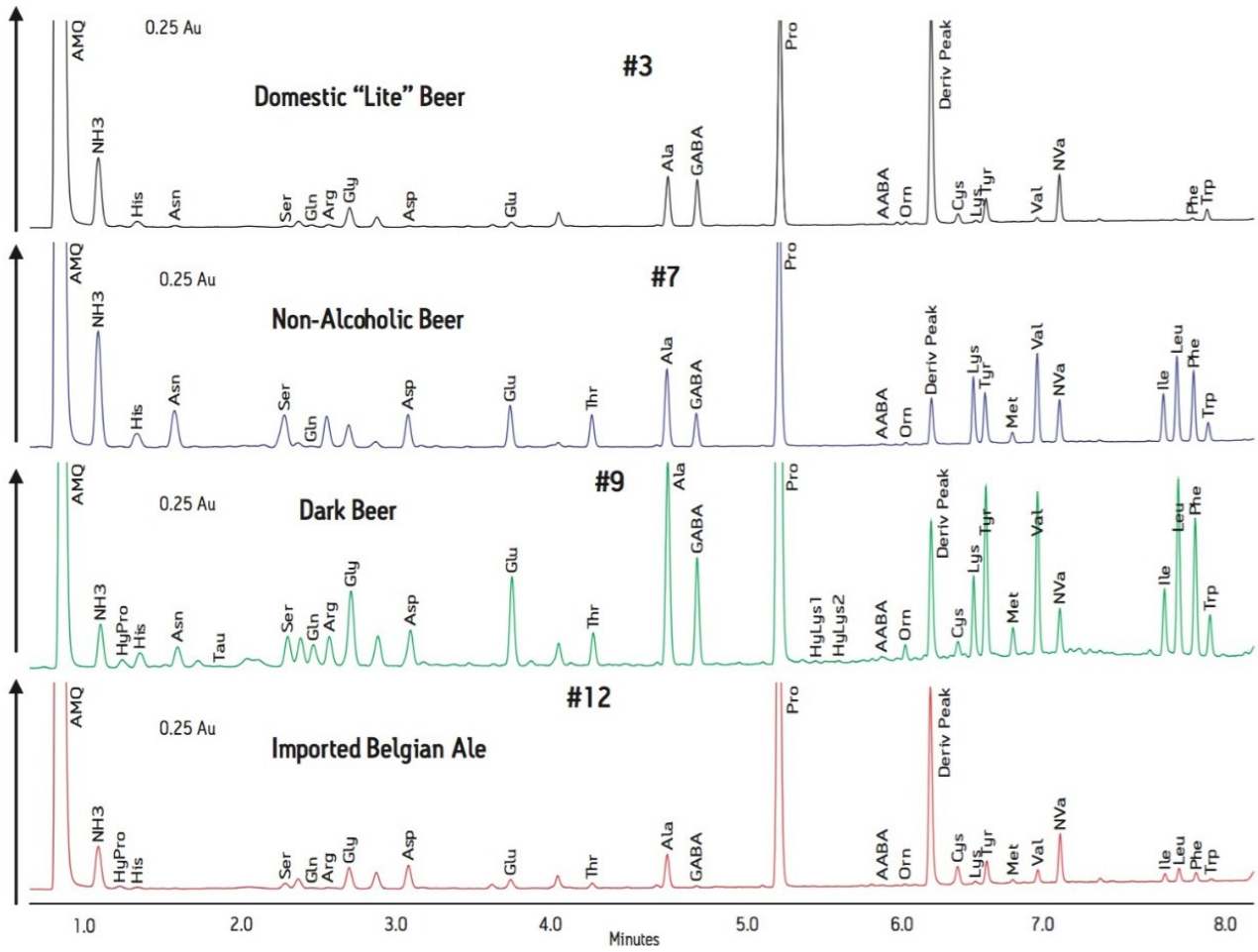

Figure 2. Chromatographic profiles of amino acid content for various beer types.

Sample#	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Type	**American Beer**	**American Beer**	**Lite Beer**	**Lager Beer**	**Mexican Beer**	**Dutch Beer**	**Non-Alcoholic**	**NY Micro Brew**	**Dark American Beer**	**Chinese Beer**	**Canadian Stout**	**Belgian Ale**	**California Micro Brew**	**American Brown Ale**
HyPro	17.4	15.1	ND	27	13.9	23.3	ND	39.8	66.6	22.9	24.4	17.7	28	29.6
His	152.3	96.9	53.8	120.8	78.4	156.3	153.7	117	136.1	76.8	180	12.4	122	140.8
Asn	272.3	69.7	12.6	87.6	14.1	16.2	346.4	55.3	172.3	ND	25.7	ND	55.7	19.5
Tau	ND	ND	ND	ND	ND	ND	ND	ND	4	ND	ND	ND	19.8	ND
Ser	113.2	25.4	9.3	40.5	19.3	17.3	333.4	52.1	240.3	13.4	29.9	37.5	64.7	24.7
Gln	129.2	51.4	19.5	24	29	36.5	9.4	22.4	196.9	11.9	97.9	3.3	24.1	14.5
Arg	137.6	29.9	21.8	96.5	175.2	186.6	ND	40.6	249.4	156.6	193.8	4.1	ND	50.4
Gly	326.3	258.8	147.7	256.3	249.7	263	ND	342.3	605.6	238.7	429.6	147.9	380.6	362.1
Asp	231.7	113.8	6.5	67.5	14.8	19.4	252.3	40.4	287.7	11.8	70.3	153.7	23.7	20.6
Glu	243.3	132.6	32.3	117.1	65.4	105.7	278.8	100.9	564.4	64.9	166	56.4	98.2	63.9
Thr	66.5	10.5		24.9	9.2	4.3	197.8	23.9	192.9	3.5	7.8	27.4	27.3	7
Ala	928.5	567.5	268.2	582	520.4	614	463.9	1219.6	1162.9	383.6	1114.3	168.2	807.5	531.8
GABA	431.9	368.1	244.8	700.1	522	457.7	190.8	911.8	586.1	494.5	484.2	7.8	930.9	903.5
Pro	2007.9	1733.4	1359.6	4473.7	2067.1	3421.5	1867.5	5797.5	6238.7	2858.7	3940.8	2685.2	4271.7	2806.2
HyLys1	ND	ND	ND	ND	ND	ND	ND	5.3	12.1	ND	ND	ND	ND	ND
HyLys2	ND	ND	ND	ND	ND	ND	ND	2.8	15.6	ND	ND	ND	ND	ND
AABA	12.2	ND	4.2	15.7	8.5	7.7	6.5	25.1	42.2	ND	13.9	5	20.5	11.1
Orn	12.3	9.1	5.4	7.6	11.2	7.5	6.7	53.9	34.6	7.3	15	3.4	1.1	27.2
Cys	26.4	33.3	26.9	38.6	27.6	26.4	ND	66.1	38	36.4	29.9	49	35.9	25.4
Lys	95.1	26.5	5.2	36.2	13.5	9.8	172.5	2.8	199.7	8.2	35.7	6.1	9.2	27
Tyr	379.4	251.3	99.5	491.8	320.5	264.5	235.3	527.9	686.7	336.9	616.8	86.7	533.5	587.1
Met	26.7	8.5	ND	17.4	12.6	7.3	49.9	14.9	114.2	ND	21	12.6	23.8	7.7
Val	406.6	181.2	14.9	378.8	294.4	244	388.6	885.9	670.9	179.3	666.8	48.7	446.7	430.5
Ile	134.9	38.3	ND	108.4	59.7	41.2	203.9	95.3	293.5	19.1	191.5	29.3	114.4	30.6
Leu	267.3	69.6	ND	184	98.5	54.8	375.2	115.5	765.5	29.1	416.7	51.6	189.9	158.9
Phe	311.3	140	8.2	260.6	236.6	174.2	302.8	333.2	586.5	148.5	487.2	33.2	241.7	339.2
Trp	99.8	65.6	40.8	119.8	85.5	149.1	76.1	157	166.4	107.6	123.5	7.6	125.3	151.9

Table 2. Amino acid content of beers sampled, units are pmol/µL, ND = not detected.

The differences in amino acid content, both qualitative and quantitative, for the samples tested are quite evident. Proline (Pro) was found in all samples tested and at a high level, not surprising given the fact that beer yeast cannot ferment proline. On the other hand, Taurine (Tau) and Hydroxy-L-lysine (HyLys) were absent or at a very low level. In general the darker beers had higher amino acid content than light beers.

Also note that in Figure 2 there are many unidentified peaks, possibly amino acids not included in the standard

mixture or other compounds that contain an amino group that would react with the AccQ▪Fluor reagent. Since the methodology is fully compatible with mass spectrometry detection, it is possible to positively identify these additional compounds, which may also be of critical importance to product consistency.

Conclusion

We have successfully demonstrated application of the Waters UPLC amino acid analysis solution (AAA) to determine the amino acids found in several different beers and ales. This method demonstrated excellent resolution of all sample components with a 10 minute cycle time. Simple sample preparation and analysis times that are approximately three times faster than traditional HPLC methods make the UPLC AAA solution ideal for demonstrating consistency of beer production.

References

1. Wheat, E, Grumbach, E and Mazzeo *et al*. A new Amino Acid Analysis Application Solution. Waters Corporation, 2006: 720001683EN < https://www.waters.com/waters/library.htm?cid=511436&lid=1512453&lcid=1512454> .
2. UPLC Amino Acid Analysis Application Solution System Guide Sections 4, 6, Waters Corporation, 2006: 720001565EN.
3. Paula Hong, Private Communication.

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