HPLC determination of biogenic amines in beer by AQC derivatization

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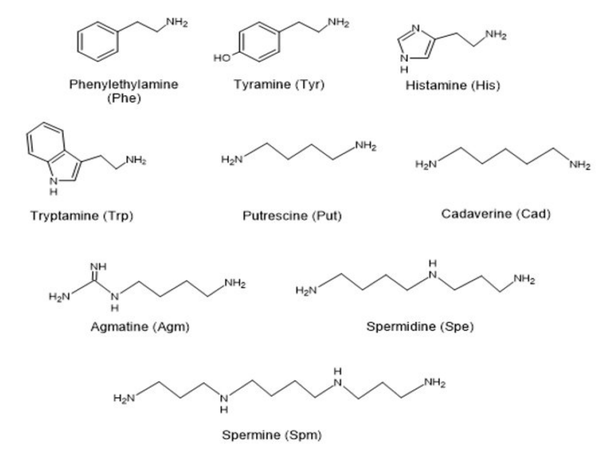
Abstract

Purpose: To develop a reversed-phase method for the analysis of biogenic amines in beer, based on sample derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC).

Methods: Biogenic amines in beer and standard were labeled with AQC. A Thermo Scientific™ Accucore™ aQ C18 column (2.1x100 mm, 2.6 µm) was run on a Thermo Scientific™ Vanquish™ Core HPLC System with UV and fluorescence detection. Biogenic amines in beer and standard were labeled with AQC.

Results: Sample preparation was straightforward. All amines were detected by fluorescence, except for tryptamine, which was detected by UV. Biogenic amines content was determined for ten beers.

Figure 1. Chemical structure of biogenic amines found in beer



Introduction

Biogenic amines in beer originate from the raw materials or during fermentation. High amines content in beer is an indicator of bacteria contamination, for instance caused by poor hygienic conditions.

Analysis of biogenic amines can be performed by HPLC. Most of biogenic amines lack chromophores and cannot be detected by UV absorption. To overcome this limitation, a typical approach is to chemically label the amines with fluorescent molecules. AQC is a powerful reagent for pre-column derivatization because of the stability of AQC-derivatized component and the reproducibility of the derivatization reaction with both primary and secondary amines.

In this work, a mixture of nine biogenic amines labeled with AQC are separated with a Accucore aQ C18 polar end-capped column operated by a Vanquish Core HPLC System. Biogenic amines content was afterward determined in ten beers by UV and fluorescence detection (FLD).

Materials and methods

Sample preparation

1 M borate buffer was diluted to 0.2 M by adding 200 µL buffer to 800 µL water. 4 mg of AQC was weighed into a vial and diluted to 1 mL with anhydrous ACN.

70 µL borate buffer diluted solution were mixed with a 20 µL AQC solution and 10 µL of sample or standard. The mixture was placed in a heating block and heated at 55° C for 10 minutes. It was then removed and cooled to room temperature. Finally, the solution was diluted 1:5 with water and stored at room temperature.

Beer samples were filtered and diluted 1:1 before labeling.

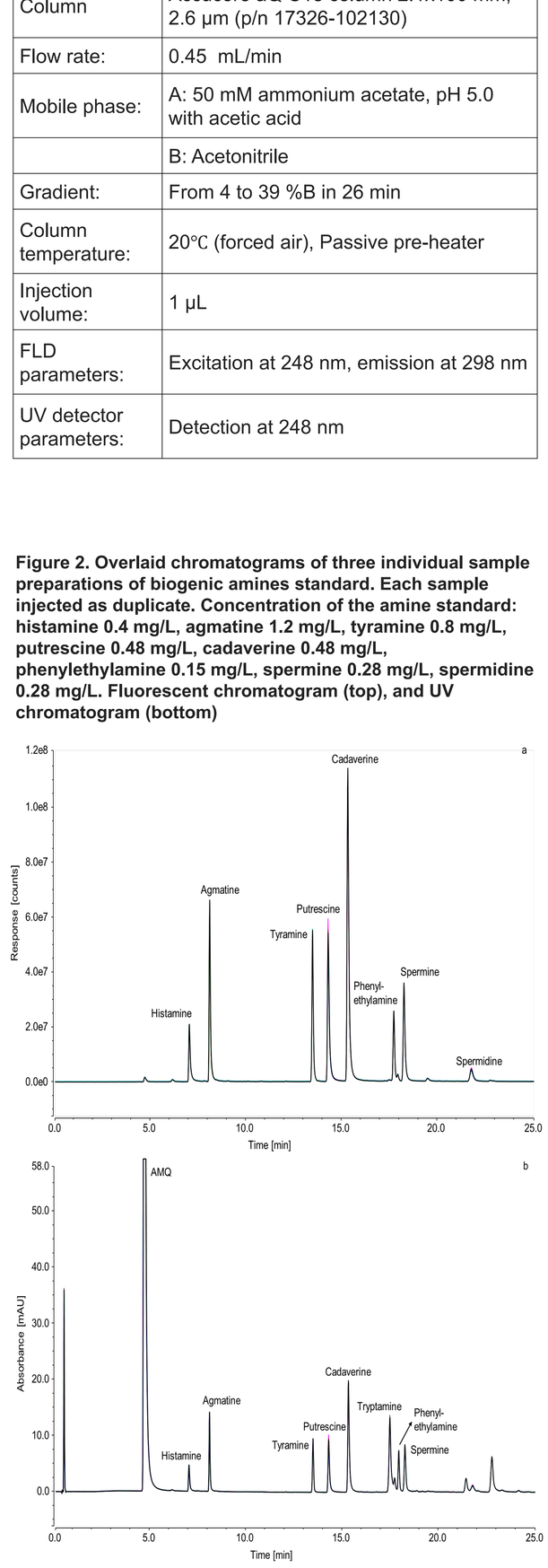
Instrumentation

Vanquish Core Quaternary HPLC System with Thermo Scientific™ Vanquish™ Diode Array Detector CG and Thermo Scientific™ Vanquish™ Fluorescence Detector F. Data collection and evaluation was performed using Thermo Scientific™ Chromeleon™ Chromatography Data Software version 7.3.

Table 1. Chromatographic conditions

Column	Accucore aQ C18 column 2.1x100 mm, 2.6 µm (p/n 17326-102130)
Flow rate:	0.45 mL/min
Mobile phase:	A: 50 mM ammonium acetate, pH 5.0 with acetic acid
	B: Acetonitrile
Gradient:	From 4 to 39 %B in 26 min
Column temperature:	20°C (forced air), Passive pre-heater
Injection volume:	1 µL
FLD parameters:	Excitation at 248 nm, emission at 298 nm
UV detector parameters:	Detection at 248 nm

Figure 2. Overlaid chromatograms of three individual sample preparations of biogenic amines standard. Each sample injected as duplicate. Concentration of the amine standard: histamine 0.4 mg/L, agmatine 1.2 mg/L, tyramine 0.8 mg/L, putrescine 0.48 mg/L, cadaverine 0.48 mg/L, phenylethylamine 0.15 mg/L, spermine 0.28 mg/L, spermidine 0.28 mg/L. Fluorescent chromatogram (top), and UV chromatogram (bottom)



Results

Biogenic amines standard

The HPLC method was successfully applied to the separation of biogenic amines pooled standard. Fluorescence detection provided the best signal-to-noise ratio for all amines except tryptamine. Signal-to-noise ratio of tryptamine was higher with UV detection. The low sensitivity found for this molecule with fluorescence detection was ascribed to a fluorescence-quenching effect.

Pooled standards were used to prepare the external calibration curve.

Biogenic amines in beer

All amines of Figure 1 could be detected except tryptamine and spermidine. Histamine eluted in the tail of an interference peak found in all beers, as confirmed in the experiment with histamine spiked sample of Figure 4. Histamine could not be observed in non-spiked samples, indicating that the component is not present or present at very low level (Figure 5). The histamine content is reported in Table 1 as gross overestimation based on the interference peak area. Spermidine and tryptamine were not detected in any sample.

Figure 4. Example of Lager beer spiked with 0.13 mg/L histamine (blue trace) and unspiked beer (black trace). Histamine peak elutes in the tail of a strong matrix interference

Figure 5. Detailed view of overlaid chromatograms of derivatized beer samples. Histamine is either absent or at very low level

Figure 3. Examples of chromatograms of a lager (top) and a wheat beer (bottom) with fluorescence detection.

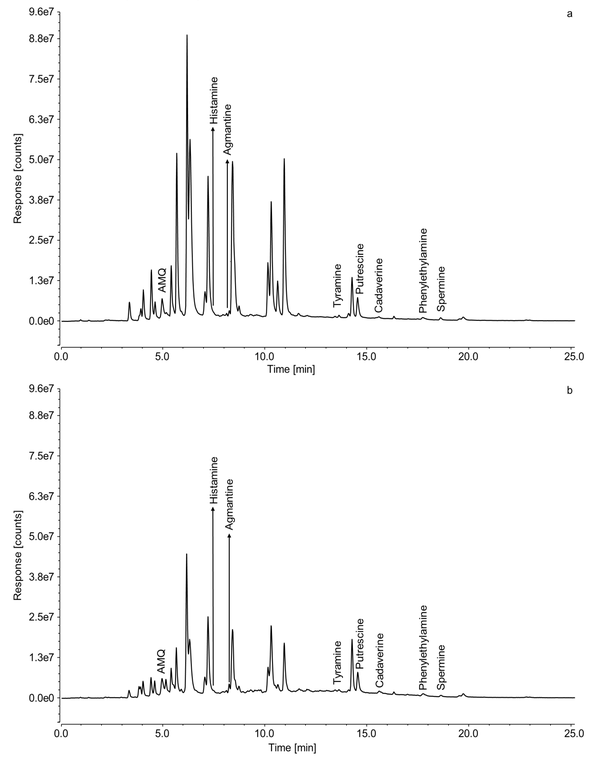


Table 3. Concentration of biogenic amines in beer ± standard deviation in mg/L (n=3). Concentration corrected by recovery. Lager beers are denoted with 'H', wheat beers are denoted with 'W'. Amount of histamine reported as overestimation except for W3. Tryptamine and spermidine were not detected in any of the samples.

Amine	H1	H2	H3	H4	H5
His	<36.1	<3.9	<98.7	<60.2	<73.4
Agm	9.07 ±0.07	3.17 ±0.02	2.77 ±1.30	3.97 ±0.01	2.77 ±0.03
Tyr	< LOQ (0.5)	0.69 ±0.08	1.01 ±0.06	0.95 ±0.06	1.33 ±0.03
Put	3.03 ±0.03	3.52 ±0.12	4.94 ±0.17	5.09 ±0.09	4.62 ±0.06
Cad	< LOQ (2.0)	< LOQ (2.0)	4.93 ±0.06	< LOQ (2.0)	< LOQ (2.0)
Phe	< LOQ (0.38)	< LOQ (0.38)	< LOQ (0.38)	< LOQ (0.38)	< LOQ (0.38)
Spm	< LOQ (0.97)	0.84 ±0.02	1.19 ±0.03	0.94 ±0.06	1.21 ±0.10

Amine	W1	W2	W3	W4	W5
His	<54.4	<55.0	< LOQ (1.0)	<85.9	<55.6
Agm	4.54 ±0.04	4.91 ±0.08	6.49 ±0.06	5.73 ±0.00	4.09 ±0.01
Tyr	0.83 ±0.05	0.62 ±0.09	0.72 ±0.03	1.03 ±0.06	1.40 ±0.08
Put	4.35 ±0.05	4.31 ±0.17	4.31 ±0.07	4.55 ±0.09	6.76 ±0.33
Cad	< LOQ (2.0)	< LOQ (2.0)	< LOQ (2.0)	< LOQ (2.0)	< LOQ (2.0)
Phe	< LOQ (0.38)	< LOQ (0.38)	< LOQ (0.38)	< LOQ (0.38)	< LOQ (0.38)
Spm	0.81 ±0.01	1.79 ±0.07	< LOQ (0.97)	1.15 ±0.04	< LOQ (0.97)

Conclusions

We developed a HPLC method for detection and quantitation of biogenic amines in beer.

- Amines are labeled with AQC with an easy and rapid procedure
- Signal to noise ratio of AQC-tryptamine is higher with UV than fluorescence detection due to fluorescence quenching
- Method is robust and sensitivity is exceeding the requirements for analysis of beer or other fermented beverages

We tested ten beer samples purchased from local stores and quantified the amines content by external calibration.

- Tryptamine and spermidine were not detected in any beer
- An unknown interference in the sample hindered accurate quantitation of histamine. However, it can be stated that histamine is either absent or at very low level in all samples analyzed in the study
- All detected amines were below or in agreement with previous reports from literature (*Izquierdo-Pulido M. et al. Biogenic Amines in European Beers, 1996, J. Agric. Food Chem., 44 (10), 3159–3163*)

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