CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Detection of Primary Amines (Histamine) and Polyamines in Beer

A research group raised concern among brewing chemists by reporting high concentrations of histamine, agmatine, tyramine and putrescine in a retailed beer sample (1). The data available in the literature for histamine and tyramine suggest that much lower concentrations are typical. Histamine, putrescine and cadaverine have been implicated in food spoilage. Tyramine has been linked to diet-induced migraine headaches.

The four amines listed above, in addition to cadaverine, tryptamine, and spermidine, were determined by LCEC following derivatization with o-phthalaldehyde (OPA) and tertiary-butyl-thiol (TBT). This reaction is depicted in Figure 1. The derivatives are thio-substituted isoindoles which are easily oxidized on a glassy carbon electrode. Rather than β-mercaptoethanol, which is often used for this type of reaction, TBT is used because it forms a more stable derivative with primary amines (2).

The derivatization of the samples is done before injection. A 500 µL aliquot of standard or beer is mixed with 500 µL of OPA/TBT derivatizing solution (2) and injected three minutes after mixing. The derivatives are separated on an Aquapore RP-300 column (Brownlee Labs) using an acetonitrile gradient. Chromatographic conditions are summarized below.

Figure 2 shows a chromatogram of the derivatives for a standard solution of eight amines. Figure 3 shows the chromatogram from the derivatization of a popular American beer. The chromatogram from the beer sample is complex because amino acids, containing the primary amine function, are also derivatized. The calculated concentrations of the amines of concern are listed in Table 1. The data suggest that the concentrations reported in Reference 1 may have been too high.

OPA DERIVATIZATION

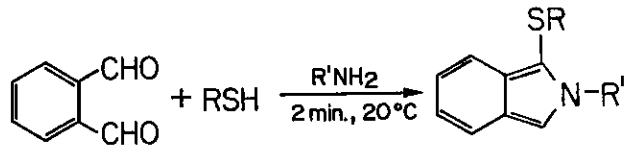

Figure 1. The reaction of o-phthalaldehyde with amines in the presence of a thiol.

Conditions

Mobile Phase: 0.05 M sodium citrate (pH 5.0), 0.01 M sodium perchlorate, gradient of acetonitrile 30 - 80%.
Column: Aquapore RP-300, 10 µm, C-18, 250 x 4.6 mm, (Brownlee Labs)
Detector: LC-4B/17A Electronic Detector, with a glassy carbon working electrode. Applied potential +0.8 V vs Ag/AgCl.
Flowrate: 1.5 mL/min.
Amount Injected: 100 µL

Since it was difficult to resolve the smaller amounts of histamine and agmatine from the amino acids, a sample clean-up procedure was used. Five mL of beer were adjusted to pH 7.3 with 6 M NaOH, and poured onto a cation exchange column (Bio-Rex cation exchange resin, 100/200 mesh). Amino acids and neutral phenolics were not retained, or were eluted with a buffer wash prior to elution of the amines of interest. The amines were eluted in 0.1 M HCl. The pH adjustment of the beer is necessary to ensure the binding of histamine to the resin.

The amines eluted from the ion exchange resin were derivatized and injected into the LCEC system. The resulting (less complicated) chromatogram is shown in Figure 4. The data indicate that histamine was present at 0.1 ppm in the beer sample.

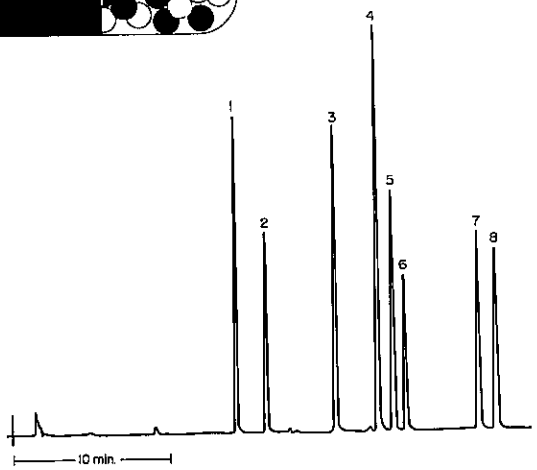

Figure 2. Chromatogram of OPA/TBT derivatives of selected amine standards. Conditions as outlined in text. Peaks represent histamine (1), agmatine (2), tyramine (3), tryptamine (4), β-phenethylamine (5), spermidine (6), putrescine (7), and cadaverine (8).

Only 7 amines were quantified in this preliminary report. However, the method could be used for the detection of other analytes with a primary amine function, in beer, as well as other food and beverage products. Other primary amines in beer that could be detected by LCEC include (but are not limited to) methylamine, ethylamine, 1,2- and 1,3-diaminopropane, isoamylamine, and ammonia.

Notes

This report outlines the separation of 8 amines, utilizing a gradient of acetonitrile and a 10 µm, C-18 reverse-phase column. By judicious choice of chromatography conditions and column parameters, selected amines can be detected using an isocratic system. An isocratic system should result in lower detection limits, since a gradient can contribute to background variations. The recommended isocratic system is the BAS 400 Electrochemical Analyzer, while the BAS 200 can provide gradient capabilities.

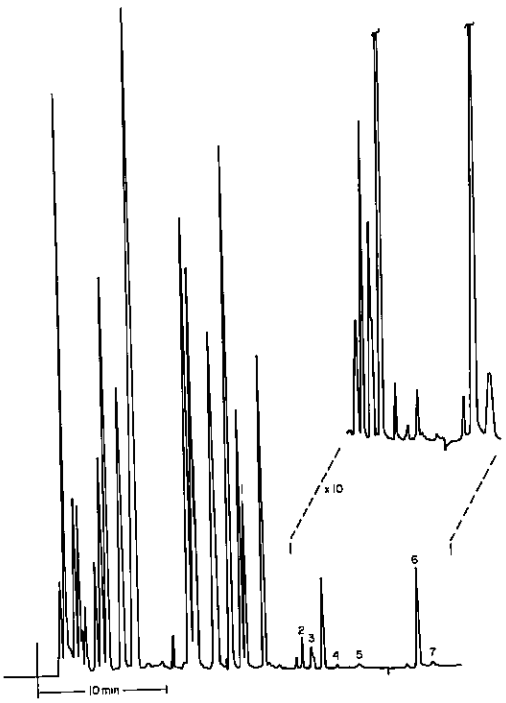

Figure 3. Chromatogram of OPA/TBT derivatives of the amines in a commercial beer sample. Conditions as outlined in text. Peaks represent agmatine (1), tyramine (2), ethylamine (3), tryptamine (4), spermidine (5), putrescine (6), and cadaverine (7). Insert: 10 x expanded scale of the indicated portion of the chromatogram.

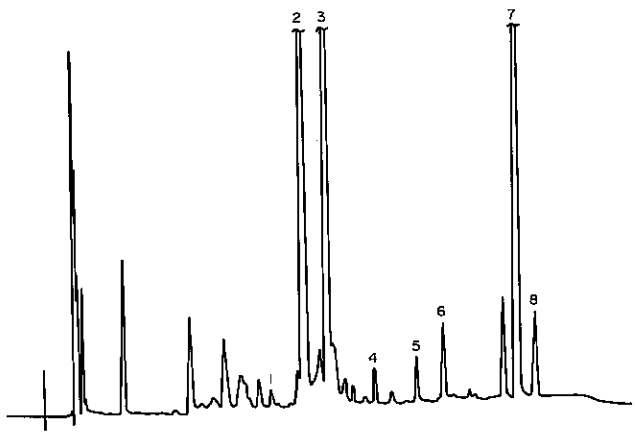

Figure 4. Chromatogram of OPA/TBT derivatives of the amines in a commercial beer sample following an initial solid phase purification procedure (see text). Conditions as described in text. Peaks represent histamine (1), agmatine (2), ammonia (3), tyramine (4), tryptamine (5), spermidine (6), putrescine (7), and cadaverine (8).

Table 1. Amines in Beer (ppm)

	Histamine	Agmatine	Tyramine	Tryptamine	Spermidine	Putrescine	Cadaverine
Ref. 1	4-8	28-35	10-15			3-8	
'BAS	0.1	15.9	1.8	0.3	0.4	3.0	0.1

References

1. J. A. Zee, R. E. Simard, and M. Desmarais, Can. Inst. Food Sci. Technol. J. 14 (1981) 119.

2. L. A. Allison, G. S. Mayer, and R. E. Shoup, Anal. Chem. 56 (1984) 1089. This paper should be consulted for details of the OPA/TBT derivatization reaction and for expected detection limits.