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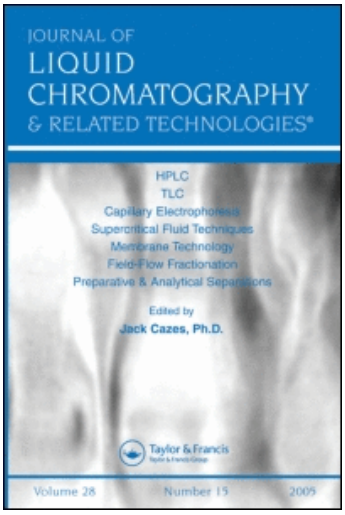

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HPLC Laser Fluorometric Determination of Amines in Beer

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HPLC LASER FLUOROMETRIC DETERMINATION OF AMINES IN BEER

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ABSTRACT

A method has been developed for the rapid and accurate determination of the predominant aliphatic amines in beer. Chromatography was performed on a reverse phase C_{18} column using an acetonitrile-water solvent gradient. Chromatographic detection was facilitated by pre-column derivatization with 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) which fluoresces under visible light (in this case an argon ion laser operating at 488 nm) after reaction with an amine. Response was linear over four decades of concentration with detection limits at approximately five picograms injected.

INTRODUCTION

The determination of amines in foodstuffs and beverages has become increasingly important with investigations showing the in-vivo production of carcinogenic nitrosamines from amines (1). Amines have also been considered of some importance by the brewing industry for their effect on beer flavor and palatability (2). Previous methods for the

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determination of these amines have required laborious and time consuming methodologies along with a large quantity of sample (2,3,4). Also as trace quantities of these amines may lead to adverse effects, highly sensitive methods are needed for their determination.

A method has been developed to detect trace quantities of aliphatic primary and secondary amines in beer, without extensive wet chemical procedures, that is quick and accurate as compared to procedures currently or previously in use. The advantages and utility of laser fluorimetry as a method of detection for liquid chromatography have been well described (5). In order to utilize its selectivity and sensitivity for this analysis the amines undergo a pre-column derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3 diazole (NBD-Cl), which is not fluorescent of itself but yields a highly fluorescent derivative when reacted with an amine (6,7). Although many aromatic amines will also undergo this reaction, the spectroscopic characteristics of most of these amines are such that they are not readily observed at the wavelengths utilized herein. The sample is introduced to a reverse phase C_{18} column for separation of its amine constituents and detection is accomplished utilizing excitation provided by an argon ion laser. The fluorescence emission is collected by an optical fiber using a previously described flow cell (8), isolated with a monochromator, and monitored with a photomultiplier tube and quantum photometer.

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METHODS AND MATERIALS

Apparatus

Separations were performed using an Alltech Associates, Deerfield, Illinois, C_{18} reverse phase column (25 cm

x 4.6 cm I.D., 10 μm) in conjunction with an Altex Scientific Inc., Berkeley, California, Model 312-12 MP Programmable Liquid Chromatography System. Excitation was provided by an argon ion laser, Spectra Phyics, Mountain View, California, Model 171 operating at 488 nm at a power of one watt. Detection was performed using a previously described flow cell and an Instruments S.A., Metuchere, New Jersey, Model 1200-vis-H-20 monochromator attached to a RCA 1P28 photomultiplier tube which was operated at 900 volts. Photocurrents were monitored with a Pacific Precision Instruments, Concord, California, Model 126 quantum photometer. The recorder used was a Kipp and Zonen, Delft, Holland, Model BD 40.

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Chemicals

NBD chloride (7-chloro-4-nitrobenzo-2-oxa-1,3 diazole) was purchased from Plaltz and Bauer, Stanford, Connecticut as were the isobutyl and n-propyl amines. Methylamine, ethylamine, sodium hydroxide, sodium acetate, and pyrollidine were purchased from Fisher Scientific, Fair Lawn, New Jersey. Dimethylamine was purchased from Eastman Kodak, Rochester, New York, and isoamylamine from Polyscience Corporation, Evanston, Illinois. All were reagent grade. The eluent was composed of HPLC grade acetonitrile from Fisher Scientific Company and distilled, deionized water. Light and dark domestic beers were acquired locally.

Procedure

Five mL of beer with its pH adjusted to 9.0 by a 6.0 M sodium hydroxide solution are added to a mixture of each of the following; 5 mL of methanol saturated with sodium acetate and 5 mL of a solution of 10 mg/mL of NBD-Cl in

methanol. The resultant mixture is heated in a closed container to 60°C for a period of one hour. This solution is filtered and a one mL alinquot is pipetted into a 50 mL volumetric flask and diluted with methanol to volume. Twenty µL of the diluted mixture are injected into the column and eluted using a gradient that proceeds from 40% acetonitrile in H_2O to 100% acetonitrile over a period of 40 minutes. Quantitative measurements based are on peak heights.

RESULTS AND DISCUSSION

Identification of Amines in Beer

Identification of the aliphatic amines in beer samples was based on their selective reaction with NBD-Cl and comparison of retention times with those of authentic standards. The amines identified in the beers tested (see Table 1) correlate well with those generally reported to be present in beer (2). Ammonia and the amino acid proline, whose presence in beer is well established, were also identified. Typical chromatograms for a dilute amine

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TABLE 1

Data for Amines in Beer Determination

Amine	t_R	LOD (pg injected)	Reproducibility (% RSD)	ppm in Beer Light	Dark
methyl	8.90	25	6.1	2.5	4.3
dimethyl	12.25	5	5.7	1.5	1.4
ethyl	13.00	4	---	---	---
pyrollidine	22.00	4	19.1	0.2	8.2
n-propyl	22.90	6	---	---	---
iso-butyl	33.45	5	11.6	0.4	3.0
iso-amyl	39.05	4	6.8	0.7	2.0

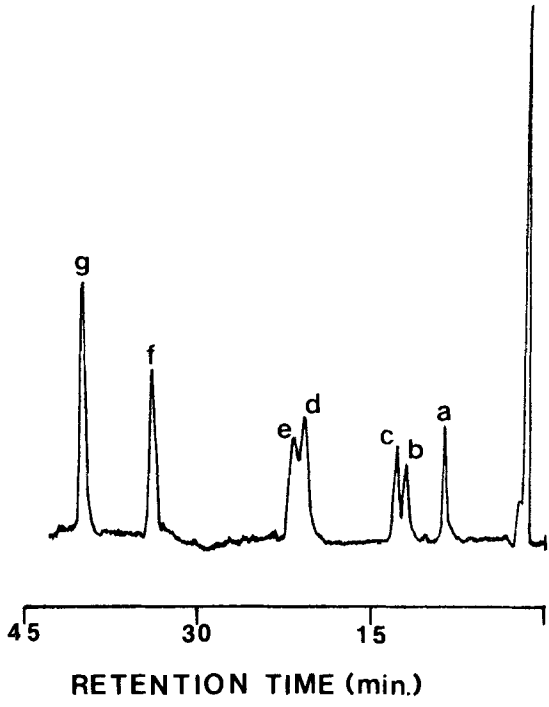

FIGURE 1. Chromatogram of a derivatized standard amine mixture; (a) methyl amine, (b) dimethyl amine, (c) ethyl amine (d) pyrollidine,. (e) n-propyl amine, (f) iso-butyl amine, (g) iso-amyl amine. Approximately 0.1 ng of each amine was injected.

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standard mixture and a beer sample appear in Figures 1 and 2, respectively. Although the efficiency for the separation is good, it was not possible to baseline resolve all the amines in our standard mixture. In fact a small shoulder on the dimethyl amine peak of one of the beer samples indicated a relatively low level of ethylamine. A column with 5 μm particles might provide the efficiency needed to baseline resolve all of the amines tested.

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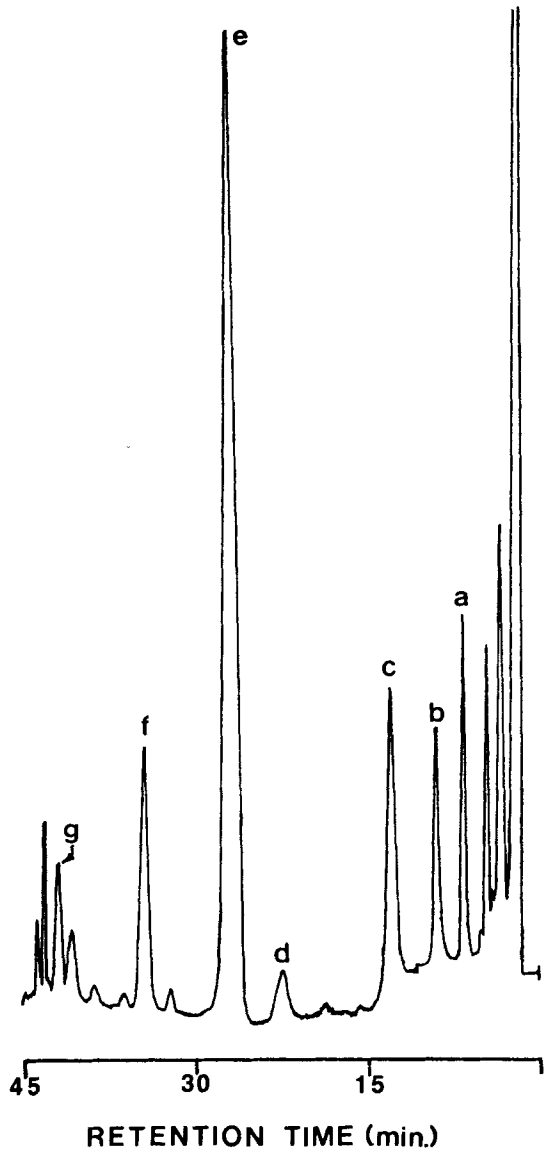

FIGURE 2. Chromatogram of a derivatized beer sample; (a) ammonia, (b) methyl amine, (c) dimethyl amine, (d) pyrollidine, (e) proline, (f) iso-butyl amine (g) iso-amyl amine.

Quantitation of Amines in Beer

An external standard method of quantification, involving the work-up of standards of the amines in distilled water, yielded poor recoveries. Since the derivatization reaction is pH dependent and liberates acid, the poor recoveries were presumed to be a result of the better buffering capacity of the pH adjusted beer relative to the pH adjusted distilled water. When the distilled water was buffered, the recoveries were greater than 100%. In order to avoid problems with maintaining the same pH for standards and samples, a standard addition method was employed. This method also served to minimize the chances of incorrectly identifying peaks in the relatively complicated sample chromatograms.

A preliminary study using graduated standards was used to estimate the spike necessary for the standard addition. The large proline peak was used as an internal standard for this work. The results of our amine determinations appear in Table 1. As expected the dark beer showed higher levels of most of the amines analyzed. This is assumed to be due to the brewing process wherein the malt is subjected to more heating during roasting and also to the greater number of ingredients used in the brew.

The reproducibility of the method was evaluated by performing six determinations on one of the beer samples. The relative standard deviations (RSD) obtained are given in Table 1. The limits of detection (LOD) and linear dynamic range of the laser fluorometric HPLC detection were also evaluated using amine standards. The LOD shown in Table 1 were more than adequate for the determination of

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amines in beer. A calibration plot for n-propyl amine standards yielded a linear regression constant of 0.9999 over the range 9.7 pg ot 97 ng injected.

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