Use of Cold On-Column Injection for the Analysis of Putrescine and Cadaverine by Gas Chromatography

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Abstract

Putrescine and cadaverine are biogenic diamines that are produced during fermentation or spoilage of different food products. Therefore, they are used as indicators to determine food quality. Unfortunately, their chemical analysis is not an easy task. The use of "cold on-column" gas chromatographic (GC) injection together with a new base-deactivated fused silica capillary column is evaluated for the direct GC analysis of putrescine and cadaverine. Multiple injections of standard mixtures containing putrescine, cadaverine, and heptylamine are made in order to determine reproducibility. Standard mixtures at concentrations from 20 to 400 ppm are also injected to determine the linearity of response with both the column and the flame ionization detector. Excellent resolution of the three amines is achieved as well as symmetrical peaks. Multiple injections produce reproducibilities of 1% for peak areas and 0.03% for retention times. A good linear response is obtained between concentrations of 20 to 400 ppm.

Introduction

Putrescine (1,4-butanediamine) and cadaverine (1,5-pentanediamine) are produced during spoilage of fish products. Both are formed primarily by enzymatic decomposition of free amino acids present in fish tissue. Cadaverine is formed via enzymatic decarboxylation of lysine. Putrescine can be formed in a similar manner from ornithine and also from arginine via hydrolysis followed by enzymatic decarboxylation (1). Because their production increases when bacterial population increases, these amines (together with histamine) have been used as indicators of fish quality (2). Furthermore, their presence increases the toxicity of histamine, another biogenic amine produced during the spoilage of fish. Histamine is the primary causative agent in scombroid fish poisoning that may be enhanced by the presence of cadaverine and putrescine (2).

High-performance liquid chromatography (HPLC) and gas chromatography (GC) have been used for the chemical analysis of putrescine and cadaverine in food products (3–13). In both techniques, once the diamines are extracted from the fish, they are derivatized. GC requires derivatization to prevent the free amines from adsorbing irreversibly in the chromatographic system. HPLC requires derivatization to introduce chromophores to allow detection at trace levels. One of the most common HPLC derivatizing reagents is 5-dimethylamino-naphthalene-1-sulphonyl-chloride (dansyl chloride). This forms dansylated diamines that can be easily detected by ultraviolet (UV) absorption (280 nm). Usually it takes one hour to achieve complete derivatization (7). Other reagents have also been used (3). This derivatization step has made the HPLC analysis of cadaverine and putrescine a time-consuming and labor-intense procedure. These facts hinder the use of these methods on a routine basis to determine fish quality.

Earlier reports from this laboratory have shown the possibility

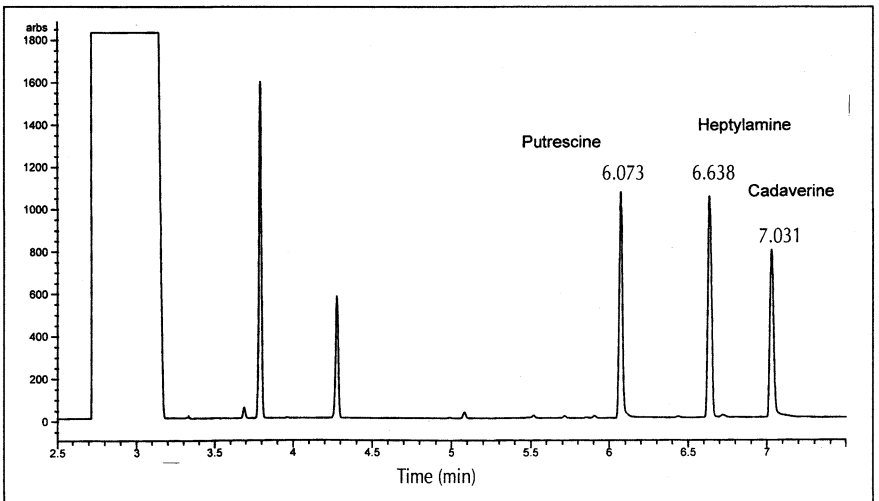

Figure 1. Chromatogram of the standard containing putrescine (200 ppm), heptylamine (154 ppm), and cadaverine (174 ppm).

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Table I. Repeatability of Direct GC Analysis of Putrescine, Cadaverine, and Heptylamine (m = 14)*

Amine	$\bar{t}_r \pm s$ (min)	%RSD (t_r)	Absolute area ($\bar{A} \pm s$)	%RSD ($\bar{A}$)	$A_{amine}/A_{hept} \pm s$	%RSD (A_{amine}/A_{hept})
Putrescine (200 ppm)	6.070 ± 0.001	0.02	1482 ± 11	0.74	0.899 ± 0.004	0.44
Heptylamine (154 ppm)	6.635 ± 0.001	0.02	1648 ± 9	0.55	na	na
Cadaverine (174 ppm)	7.028 ± 0.002	0.03	1284 ± 16	1.2	0.780 ± 0.007	0.94

*m = number of replicates; $\bar{t}_r$ = mean retention time; $\bar{A}$ = mean peak absolute areas; A_{amine}/A_{hept} = ratio of peak area from cadaverine or putrescine over peak area of heptylamine; na = not applicable.

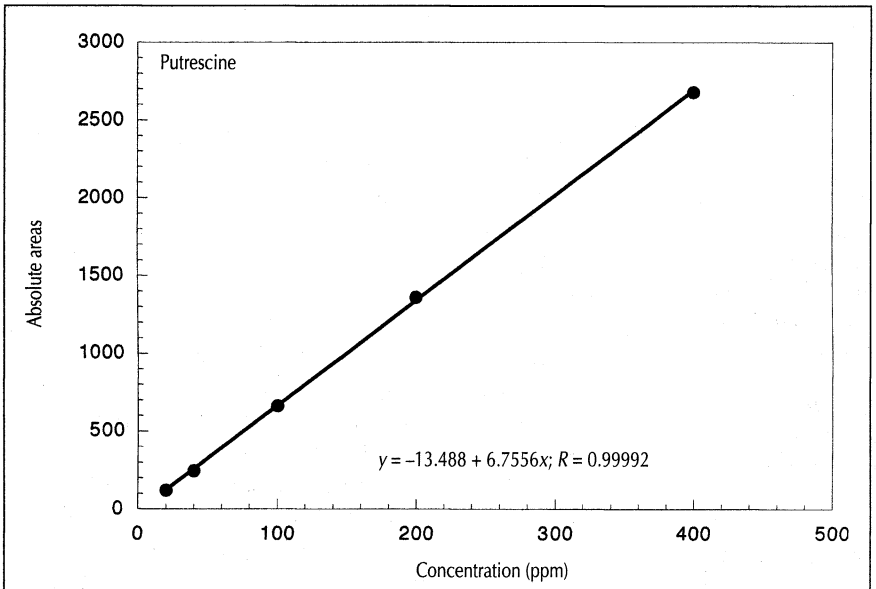

Figure 2. Calibration curve for putrescine. R is the correlation coefficient.

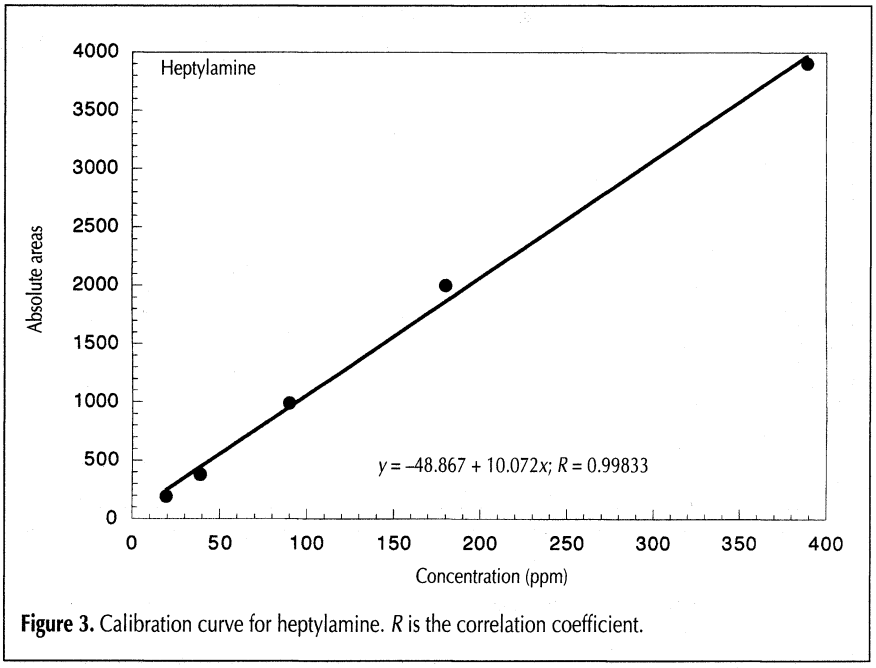

Figure 3. Calibration curve for heptylamine. R is the correlation coefficient.

of analyzing putrescine and cadaverine by GC without derivatization. In these studies, some problems with the split injection technique were encountered (primarily poor repeatability). Successive injections of a solution containing 1000 ppm each of putrescine, cadaverine, and heptylamine produced relative standard deviations (RSDs) from 6 to 9%. In addition to this, the split liner of the GC had to be treated with KOH solution, along with the glass wool in it, to obtain these RSD levels. To overcome these difficulties, the use of "cold on-column" injection was evaluated for the direct (without derivatization) analysis of putrescine and cadaverine by GC.

Experimental

Instrumentation

A Hewlett-Packard model 6890 GC (Hewlett-Packard, Wilmington, DE) equipped with a cold on-column injection port, a flame ionization detector (FID), a GC automatic liquid sampler, and a Hewlett-Packard Chem-Station was used.

Column

All the injections were made onto a Restek RT_x–5 Amine (Restek, Bellefonte, PA) fused silica capillary column. The column was 30 m × 250-µm i.d.; the film thickness was 1.0 µm.

Chemicals

Standards of 1,4-butanediamine (putrescine, 99%), 1,5-pentanediamine (cadaverine, 97%), and heptylamine (99%) were obtained from Aldrich (Milwaukee, WI). HPLC-grade dichloromethane was used as solvent and was obtained from Burdick & Jackson (Muskegon, MI).

Chromatographic separation

A standard solution containing 10.0 mg of putrescine, 8.7 mg of cadaverine, and 7.7 mg of heptylamine in 50.0 mL of CH_2Cl_2 was prepared using a plastic volumetric flask (Nalgene chemical-resistant polypropylene). The "cold on-column" technique was used to automatically inject 0.5 µL of the solution at an initial temperature of 38°C for 0.10 min, followed by a temperature increase at a rate of 50°C/min up to 250°C. The inlet was operated at a constant flow mode at a helium pressure of 13.3 psi. The oven was programmed as follows: the initial temperature was 35°C, the initial time was 0.5 min, the rate was 20°C/min, the final temperature was 200°C, and the final time was 1.0 min. The FID was used at 300°C with a helium flow of 35 mL/min, an air flow of 400 mL/min, and a nitrogen flow of 30 mL/min (makeup gas).

Statistical studies

The same standard mixture was injected 14 times onto the GC system under the same chromatographic conditions. Standard deviations (s) and relative standard deviations (RSDs) were calculated from the peak areas of each amine and from their retention times.

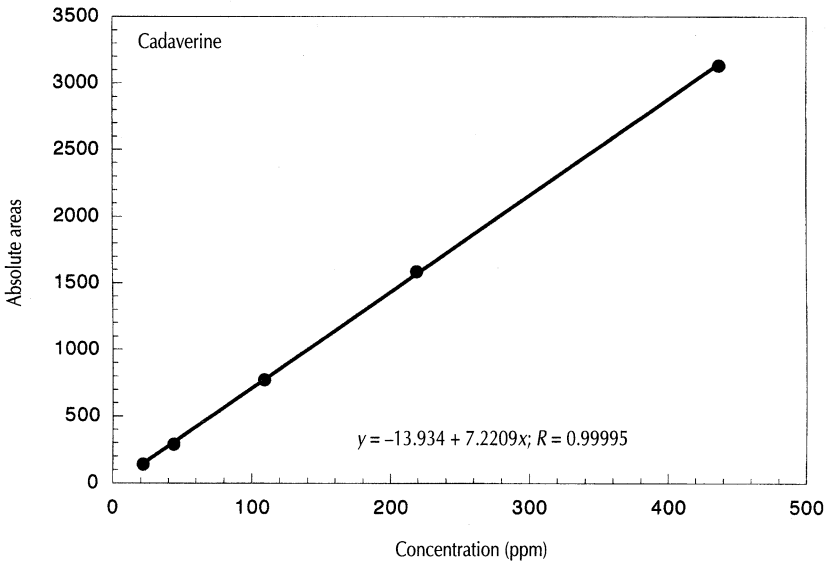

Figure 4. Calibration curve for cadaverine. R is the correlation coefficient.

Calibration curves

Five standard mixtures containing heptylamine, putrescine, and cadaverine at different concentrations were prepared using CH_2Cl_2 as the solvent. A concentration range of 20–400 ppm was used. Five injections at each concentration level were made using the conditions previously described.

Results and Discussion

The chromatographic separation of heptylamine, cadaverine, and putrescine was achieved under the conditions described here. In fact, if the sample had contained only these compounds, a faster method could have been developed. Figure 1 shows the chromatogram obtained for this separation. Putrescine eluted first at 6.073 min, followed by heptylamine at 6.638 min, and finally cadaverine at 7.031 min. Peak shapes were excellent, considering that these are underivatized free base amines.

Repeatability was evaluated by calculating s and RSD for both the peak areas and retention times. The results from these calculations appear in Table I. They show good RSDs for peak areas and excellent RSDs for retention times. These results suggest that the analysis of putrescine and cadaverine can be performed by GC without derivatization because acceptable repeatability was obtained.

Figures 2–4 show calibration curves obtained from putrescine, heptylamine, and cadaverine. Good linearities were obtained from 20 to 400 ppm, which indicates that good quantitations can be performed at these concentration levels. Repeatability at each concentration level was also evaluated. Table II shows the RSD for the peak areas of putrescine, which was representative of the other amines. The table reveals that acceptable repeatability is maintained throughout this concentration range.

Table II. Statistical Results for the Calibration Curves of Putrescine, Cadaverine, and Heptylamine

Putrescine			Cadaverine			Heptylamine		
Concentration (ppm)	$\bar{A} \pm s$	%RSD	Concentration (ppm)	$\bar{A} \pm s$	%RSD	Concentration (ppm)	$\bar{A} \pm s$	%RSD
20	120 ± 3	2.6	21.9	142 ± 4	3.1	19.5	194 ± 8	4.2
40	245.8 ± 0.8	0.34	43.7	291 ± 1	0.49	38.9	379 ± 2	0.42
100	663 ± 4	0.55	109	775 ± 4	0.48	89.8	993 ± 5	0.52
200	1359 ± 6	0.46	219	1588 ± 9	0.54	180	1999 ± 11	0.57
400	2679 ± 17	0.63	437	3132 ± 7	0.22	389	3903 ± 15	0.37

*Five injections were made at each concentration level; $\bar{A}$ = average absolute peak area.

Conclusion

It has been demonstrated in this study that the analysis of cadaverine and putrescine can be performed in GC without derivatization. Some precautions should be exercised. Plasticware should be used to handle the amine solutions to avoid their adsorption on glass surfaces. Lack of repeatability was obtained when glass vials were used in the autosampler instead of polypropylene plastic vials. The use of CH_2Cl_2 was also a key factor. Several solvents were tried, such as methanol, CH_3CN, tetrahydrofuran, and isopropanol, but CH_2Cl_2 was the best in terms of the chromatographic separations and peak shapes. Standards should be prepared using the free base amines. When solutions prepared from their hydrochloride form were injected, no peaks were detected because they are present as nonvolatile protonated species in CH_2Cl_2. The use of "cold on-column" injection was also a crucial step. Poor reproducibility was obtained when split injections were used. Previous work done in our laboratory produced RSDs from 6 to 9% for the amine peak areas at around 1000 ppm and from 0.01 to 0.05% for the retention times. The use of "cold on-column" injection produced RSDs in the peak areas from 0.74 to 1.2% at 200 ppm and from 0.02 to 0.03% for the retention times.

Future work will be done to evaluate this method for the analysis of putrescine and cadaverine in fish. The study will also be extended to other biogenic amines such as histamine, spermine, and spermidine.

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