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Note

Determination of volatile amines in air by on-line solid-phase derivatization and high-performance liquid chromatography with ultraviolet and fluorescence detection

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Aliphatic amines and polyamines are well known as odorous substances and as precursors of N-nitrosamines, which are carcinogenic substances in the atmosphere[1]. Gas chromatography (GC)[2,3] and particularly high-performance liquid chromatography (HPLC) have been used quite extensively for the determination of volatile amines in air, due to several advantages over other analytical methods, especially high specificity and sensitivity[4–6]. Chemical derivatization techniques have been the ideal choice when GC and HPLC have been used for the above purpose. Unfortunately, all those chemical derivatizations involved homogeneous reactions, which were tedious and time consuming[7,8]. We have synthesized, characterized and evaluated a polymeric activated ester–carbonate fluorenyl (FMOC) reagent for both off-line and on-line derivatizations in HPLC[9]. These prior results showed that this polymeric reagent was extremely reactive towards nucleophiles, such as amines, due to the labelling moiety (tag) being activated by electron-withdrawing groups on the polymeric backbone. In the present study, a reaction column containing the polymeric fluorenyl reagent was slurry packed, and placed just before the separation column (on-line, pre-column mode). Trace levels of aliphatic amines and a polyamine in environmental air samples were trapped with commercially available silica gel tubes. The amines were desorbed with an acidic aqueous–organic solution and neutralized with sodium hydroxide prior to HPLC injection. Recovered amine solutions were then directly injected into the on-line, pre-column derivatization, HPLC–UV/fluorescence detection system for quantitation.

EXPERIMENTAL

Chemicals, reagents and solvents

Chemicals used were obtained from a variety of commercial sources, including Aldrich (Milwaukee, WI, U.S.A.), Burdick & Jackson Labs. (Muskegon, MI,

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U.S.A.), J. T. Baker (Philipsburg, NJ, U.S.A.) Alfa Products, Morton Thiokol (Danvers, MA, U.S.A.), and Sigma (St. Louis, MO, U.S.A.). These chemicals were all of the highest purity available and were used without further purification. HPLC solvents were obtained from EM Science (Cherry Hill, NJ, U.S.A.), as their Omnisolv HPLC brand/grade. All HPLC solvents were used after filtration through a 0.45-μm solvent filter (GVWP; Millipore, Bedford, MA, U.S.A.) and degassed under vacuum with stirring.

Apparatus

The measurements were carried out on an apparatus consisting of a Waters (Milford, MA, U.S.A.) Model 6000A pump, a Rheodyne Model 7010 injection valve with 5- and 10-μl sample loops (Rainin, Emeryville, CA, U.S.A.), a Model SE 120 dual-pen recorder (Brown, Boveri & Co., Metrawatt/Goerz Division, Vienna, Austria), an EM Science LiChrospher C_{18} reversed-phase column, 250 mm × 4.6 mm I.D., 5 μm particle size, a Waters Model 480 variable-wavelength UV–VIS detector, a Hitachi (Naka Works, Mito City, Japan) Model F1000 fluorescence spectrophotometer, and a Hitachi Model D-2000 ChromatoIntegrator.

Air sample collection

Double-sealed glass tubes (110 mm × 10 mm O.D.) containing silica gel were obtained from SKC (Eighty-Four, PA, U.S.A.). The air in the work/sampling area was sampled at a flow-rate of 400 ml/min for 4 h using a DuPont (Wilmington, DE, U.S.A.) Alpha-1 air sampler pump.

Desorption and neutralization

All silica beads and glass wool in the sampling tube were transferred to a glass vial (10 ml volume), and the sampling tubes were washed with 5 ml 1 *N* sulfuric acid–acetonitrile (1:1) into the same vial. After sonication for 1 h, the resultant solution (0.5 ml) was removed and neutralized with 0.5 ml 1.00 *N* sodium hydroxide solution to pH 10.

On-line derivatizations

The stainless-steel reaction columns (27 mm × 2.0 mm I.D.) were made in this laboratory. Using a Rheodyne Model 7060 injector as a switching valve, the reaction column was connected to the loop position on the valve. The reaction column was placed into a constant-temperature water bath (60°C). The basic sample solution (10 μl) was injected and the switching valve was switched to the bypass position at the correct time (*ca.* 6 s). The analyte was held within the reaction column for a specific time period (5 min), and the valve was then switched back to flush the derivative from the reaction column into the separation/analytical column.

Quantitation of amines

Two different sample series were performed. Amines in the sample prepared from the single blind spike experiments were quantitated via external standards. Amines in air trapped from a fish inspection laboratory of the U.S. Food and Drug Administration (FDA) were quantitated via the standard addition method. Amines spiked were in the range of 0.2–1.0 ppm. Each sample was spiked with two different concentrations. Three injections were made for each sample with or without spiked,

known concentrations of amines. Three-point calibration plots were then constructed for the quantitation of amines in the individual air sample.

RESULTS AND DISCUSSION

The structure of the polymeric reagent indicated here is simplified, the exact structure of the reagent, specific synthetic methods, and reactions will be described elsewhere, as well as methods for the characterization and loading[9]. The general solid-phase reaction to form fluorenyl (FMOC) amine derivatives is shown in Fig. 1. Authentic standards for some of the amine/polyamine derivatives were previously prepared and characterized, so that known concentrations of each could be used here for accurate quantitations.

Acetonitrile consistently provided the highest percent derivatizations for all amine substrates[9]. Using this as the solvent, the optimized temperature and times were 60°C and 5 min, the percent derivatization for propylamine was 87% with a standard deviation (S.D.) 1.5, $n = 3$, and 71% (S.D. = 1.0, $n = 3$) for diethylamine. The efficiency of sampling and the desorption procedure were investigated by using direct spiking experiments. An acidic, aqueous elution solution (1 *N* sulfuric acid) was first used to desorb the trapped amines from sampling tubes containing silica gel. Recoveries for primary and secondary amines were 85–88% and 74–82%, respectively. Recoveries increased about 5% for primary amines and about 10% for secondary amines by mixing acetonitrile with the acidic, aqueous eluting solution (1:1). This may have been caused by the increased solubility of such amines with the organic modifier present. By a comparison of the levels of amines experimentally determined *vs.* the levels spiked, percent recoveries were calculated. Recoveries greater than 90% for all amines were realized, indicating the high efficiency for this overall sampling and desorption procedure.

To validate the method further before its application to real samples, a "single blind" study was performed. The sample desorption and neutralization steps were followed, as above, by the on-line real time derivatization–HPLC separation (Fig.2). The same sample solutions were analyzed using a conventional GC–flame ionization detection method performed by another analyst in a different laboratory. The results are compared in Table I. The relative standard deviations (R.S.D.) varied from 1.1% to 4.2%. The relative errors were from −1.2% to +2.8% after calculating the amount of amines found *vs.* spiked. The final accuracies, precision and reproducibilities were acceptable and comparable to most other air sample assays reported in the literature[2–8].

The minimum amounts of amines that could be both derivatized and detected

Fig. 1. Scheme of solid-phase derivatizations of typical amines using the polymeric FMOC reagent.

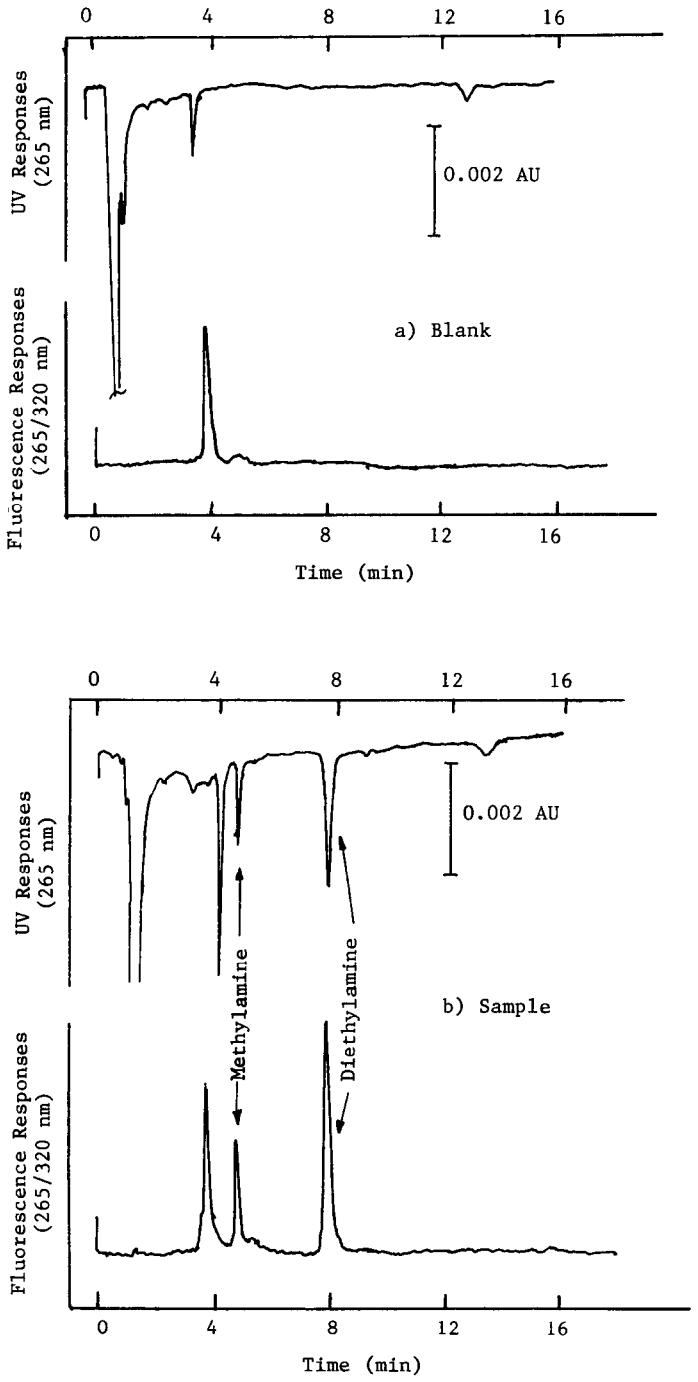

Fig. 2. Chromatogram of blind spiked experiment (a: blank; b: sample). Amines were spiked to silica adsorbent, acidicly eluted, neutralized, injected into on-line solid-phase derivatization–HPLC–UV/fluorescence detection system (10 μl), real time, room temperature, acetonitrile–water (60:40), 1.5 ml/min, LiChrospher C_{18}, 5 μm, 250 mm × 4.0 mm I.D., UV 265 nm, fluoresence 265/320 nm.

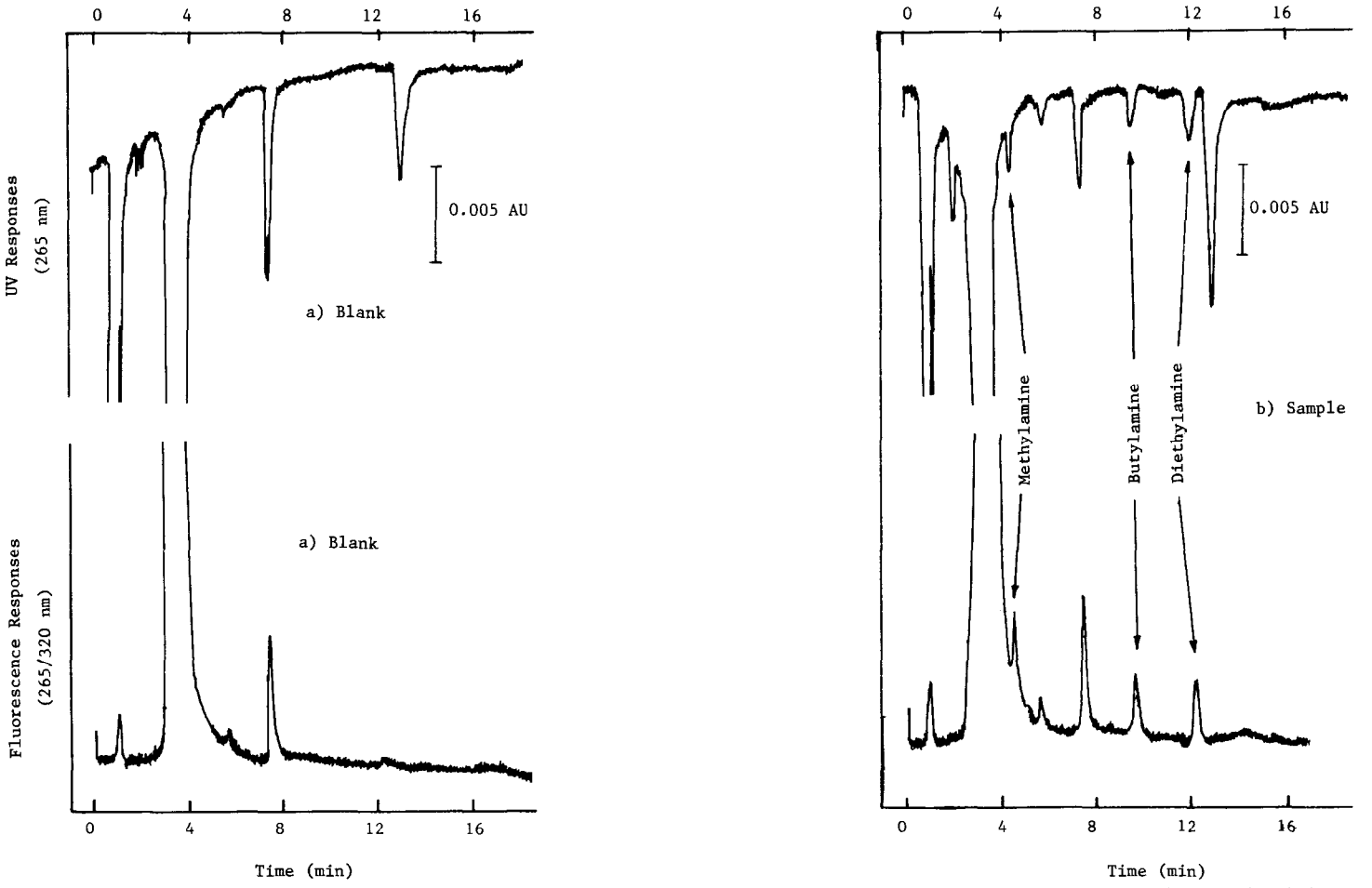

Fig. 3. On-line solid-phase derivatization–HPLC–UV/fluorescence detection for the minimum amounts of amines that could be both derivatized and chromatographically detected after air sampling procedure (a: blank; b: sample). Amines spiked to silica gel adsorbent, eluted, injected: 24 ppb for methylamine, 34 ppb for butylamine and 60 ppb for diethylamine. Specific reaction–HPLC–detection conditions: 60°C for 5 min. Other conditions as in Fig. 2.

TABLE I
SINGLE BLIND SPIKING EXPERIMENTS

See Fig. 2. Known levels of each amine, as a mixture, were spiked to the silica gel air sampling adsorbents and eluted, neutralized, and injected into the pre-column, on-line derivatization–HPLC–detection system. Comparison of levels spiked with levels experimentally determined. RE = Percent relative errors: (value found − true value)/true value × 100%.

Substrate	*Spiked (ppm)**	*Our method*			*GC–flame ionization detection****		
		*Found ± S.D. (n=3) (ppm)***	*R.S.D. (%)*	*R.E. (%)*	*Found ± S.D. (n=3) (ppm)*	*R.S.D. (%)*	*R.E. (%)*
Methylamine	14.7	15.1 ± 0.2	1.4	+2.0	14.2 ± 0.5	3.4	−3.4
Dimethylamine	31.4	32.3 ± 0.5	1.6	+2.8	32.1 ± 0.6	1.9	+2.2
Methylamine	44.1	43.2 ± 1.8	4.2	−2.0	45.4 ± 0.7	1.6	+2.7
Dimethylamine	94.4	93.3 ± 1.0	1.1	−1.2	96.2 ± 0.9	1.0	+5.1

* Sample spiked at Environmental Resource Technology, Inc.
** Amine concentration found with our method, corrected for percent recoveries.
*** NIOSH-accepted method performed at Environmental Resource Technology, Inc.

by UV/fluorescence after this sampling procedure were 24 ppb* (5.3 pmol) for methylamine, 34 ppb (5.7 pmol) for butylamine and 60 ppb (8.2 pmol) for diethylamine (Fig.3) with a signal-to-noise ratio of 3:1. Relatively higher concentrations of secondary amines were derivatized and detected due to the steric hindrance of such compounds, lowering their reactivity. The lowest concentrations of amines detected by the method were comparable with most GC and HPLC methods[2–8]. The linearities of the calibration plots were 3–4 orders of magnitude starting from the lowest concentrations of amines. The solvent front peak was the hydrolysis product of the polymeric reagent.

Amines in air were trapped from different sources, including: sewage area, fish processing company and a raw fish organoleptic (decomposition determination by odor) laboratory at the FDA. The air collections were performed according to the standard procedures issued by the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA)[10]. With the least sample preparation possible, the amine solution was directly injected into the on-line derivatization–HPLC system for quantitation. One single analysis, starting from injection, derivatization, separation, and detection of the aliphatic amines and polyamines was achieved within 30 min (Fig.4) for each sample.

Amine concentrations in a sewage area were less than the detectable levels of amines using this method. Amines at higher levels were found in the fish processing company and fish inspection/analysis laboratory, due to decomposition of the biological substances (Table II). Amine levels are known to correlate with the degree of biological decomposition. Higher levels of amines, especially of cadaverine, were found in the sample collected at the FDA fish inspection laboratory in the afternoon (P.M.) than those collected in the morning (A.M.) for the same collection time (4 h).

* Throughout this article, the American billion (10^9) is meant.

This was due to the higher degree of fish decomposition (higher temperature and less defrosting time) which occurred during the P.M. sampling period, releasing higher levels of amines. We should perhaps emphasize that the high levels of amines occurred when frozen fish was being thawed prior to organoleptic determinations. Such levels are not, we believe, routinely found within fish inspection laboratories, other than at times when all of the fish present has been thawed and is awaiting inspection.

The major limitations of the method were: (a) relatively poor derivatizations for sterically hindered compounds (secondary amines), and (b) gradual hydrolysis of the polymeric reagent when performing on-line fractions at higher temperatures for longer stop-flow times. The possible advantages in performing on-line solid-phase derivatizations in HPLC with this polymeric reagent were: (a) fast and efficient analysis, (b) sensitive for most amines in air samples, (c) accurate and precise analyses, (d) less sample work-up, (e) inexpensive, and (f) great potential for automation. It should be apparent that the application described here for volatile amines in air samples is but

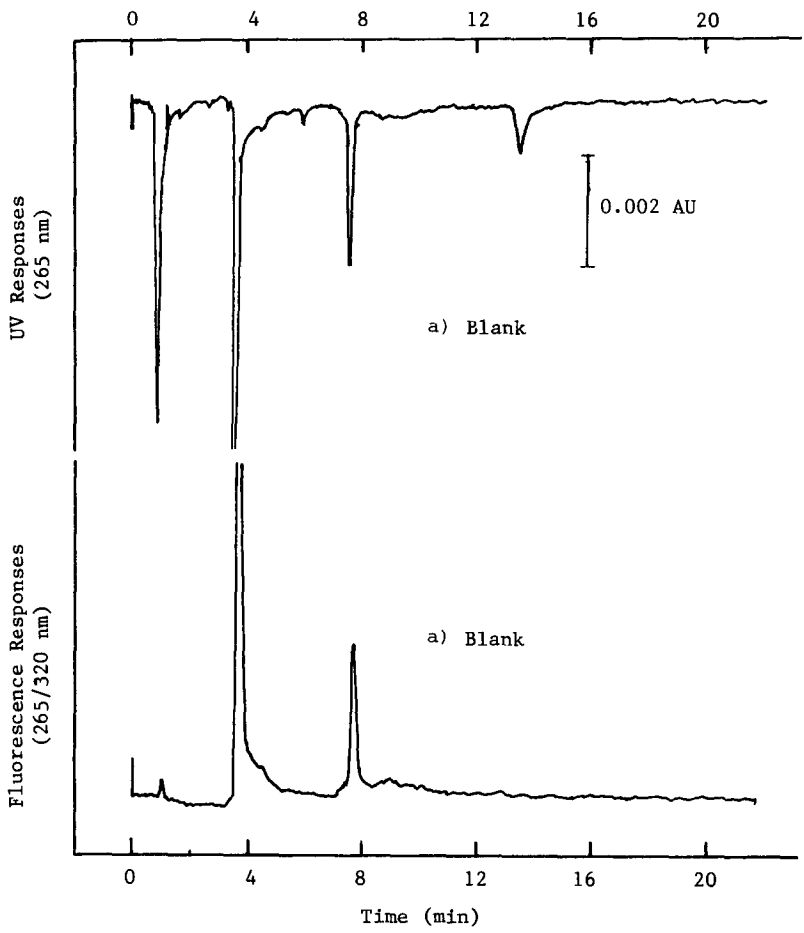

Fig. 4.

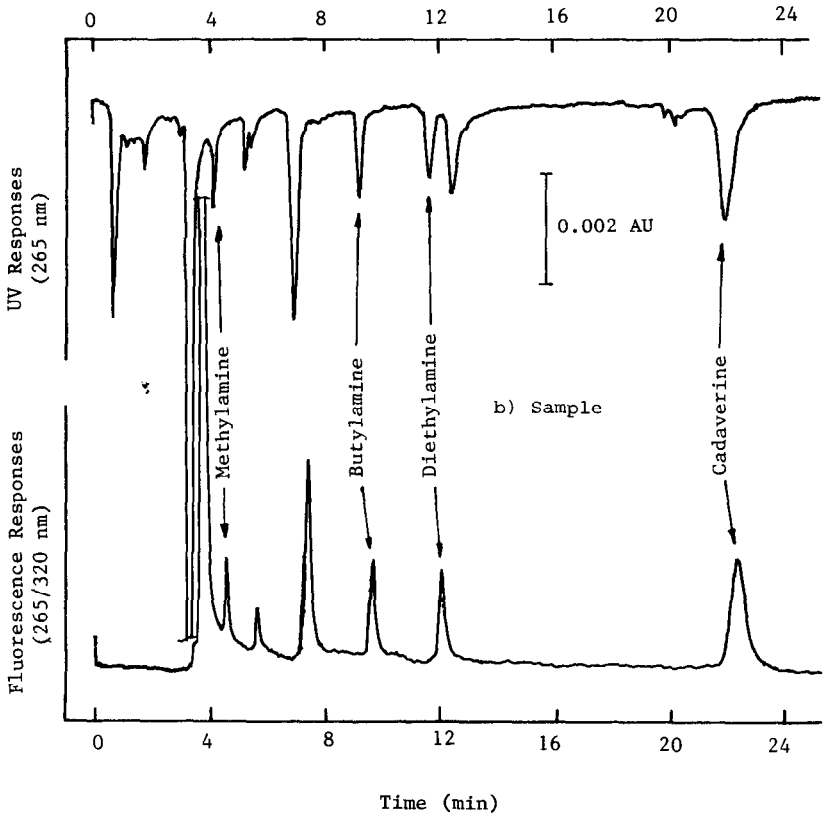

Fig. 4. HPLC–fluorescence detection of amines and polyamine determined in actual air sample taken from an FDA organoleptic laboratory (a: blank; b: sample). On-line derivatizations were at 50°C for 5 min, other conditions as in Fig. 2. See Table II for the results.

TABLE II

AMINE AND/OR POLYAMINE LEVELS IN ACTUAL AIR SAMPLES COLLECTED FROM AN FDA ORGANOLEPTIC LABORATORY

See Fig. 4.

Analyte	*Concentration*			
	Sample I (a.m.)		*Sample II (p.m.)*	
	ppm ± *S.D.* (*n*=3)	*mg/m*^{3} ± *S.D.* (*n*=3)	*ppm* ± *S.D.* (*n*=3)	*mg/m*^{3} ± *S.D.* (*n*=3)
Methylamine	0.36 ± 0.02	0.019 ± 0.001	0.50 ± 0.04	0.022 ± 0.002
Butylamine	0.40 ± 0.03	0.042 ± 0.003	0.96 ± 0.04	0.050 ± 0.005
Diethylamine	–*	–*	7.3 ± 0.30	0.380 ± 0.020
Cadaverine	0.56 ± 0.04	0.050 ± 0.002	2.2 ± 0.10	0.114 ± 0.005

* Less than the detectable levels of amines using this method.

one of many imaginable using an on-line, pre-column, solid-phase derivatization scheme in HPLC. Numerous other applications will prove possible and practical. The determination of total drug levels and/or enantiomer ratios of optically active drugs and bioorganics is one typical future application area[11].

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