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Rapid gas chromatographic profiling and screening of biologically active amines[1]

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Abstract

An efficient method is described for the simultaneous determination of 57 amines including volatile aliphatic amines, nonvolatile polyamines and catecholamines present in aqueous samples. The method is based on two-phase isobutyloxycarbonylation (isoBOC) with a pH shift. In 1.0 M phosphate buffer at pH 7.5, phenolic hydroxyl groups were allowed to react with isobutyl chloroformate in the dichloromethane phase, and subsequently pH of the aqueous phase was increased to 12.0 for the reaction of basic amino functions. The resulting *N(O)*-isoBOC amines were recovered by solid-phase extraction using Chromosorb P in normal phase partition mode, with subsequent *tert.*-butyldimethylsilylation of the remaining hydroxyl groups for gas chromatographic analysis. Using this combined procedure, linear responses were obtained in the concentration range of 0.2–12 ppm, with correlation coefficients varying from 0.945 to 0.999 for most of the amines studied except for 5-methoxytryptamine (0.864). Temperature-programmed retention index (I) sets as measured on DB-5 and DB-17 dual-capillary columns of different polarity were characteristic of each amine and thus, useful in the screening for amines by computer I matching. When applied to saliva samples, the present method allowed rapid screening for each spiked amine and unspiked polyamines such as 1,3-diaminopropane, putrescine, cadaverine and spermidine. © 1997 Elsevier Science B.V.

Keywords: Biogenic amines; Two-phase *N(O)*-isobutyloxycarbonylation; *tert.*-butyldimethylsilylation; Solid-phase extraction; Retention index matching

1. Introduction

The amines, a group of widely occurring compounds in living organisms, are important indicators of a wide variety of biochemical, clinical, toxicological and fermentation processes. Biogenic amines generally include aliphatic mono-,

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di- and polyamines, catecholamines, indolyl and imidazolyl amines. The simultaneous detection and identification of all these amines in a single analysis become utmost important for clinical and toxicological monitoring, as well as for controlling food quality [1,2].

In recent years, multicomponent profiling analysis by high resolution capillary gas chromatography (GC) and GC-mass spectrometry (MS) is widely used in systematic screening works to detect new and unexpected compounds and also to determine changes in the ratios of different compounds [3,4]. In the literature, a number of GC methods have been developed to separate small groups of target amines such as catecholamines [5–9], polyamines [10–14], and amines of aliphatic and aromatic nature [15–21], but few have been applied to amine profiling analysis [15,16].

GC analysis of the amines requires one or more appropriate derivatization procedures to block active protons in amino and other polar groups, and mainly acylation [1,8,14,18], silylation [7], benzoylation [9], sulfonylation [19], phosphorylation [21] and alkyloxycarbonylation (AOC) have been used [5–7,10,12,13,16,20]. Extensive works have been done on the direct AOC in alkaline aqueous solution with methyl-, ethyl- and isobutyl chloroformate as reagents to make polar amines extractable into organic solvents [5,6,10–13,16,17]. The direct AOC procedure, however, requires large amounts of reagents because of their instability in alkaline solution [13,17]. With two-phase AOC procedure (mainly toluene-phosphate buffer) [13,17,20], the derivatization takes place in organic phase and thus, the reaction, requiring smaller amount of reagents, is more efficient and rapid. The major advantage of the two-phase AOC method is the simultaneous solvent extraction and derivatization of amines present in aqueous solution.

The reaction rate and derivative yield of AOC procedures strongly depend on the pH of aqueous media. The appropriate pH is generally chosen depending on the basicities of amines of interest [17]. Most of AOC reactions have been performed under strongly alkaline conditions (pH 10 or 12). However, this high pH condition is not suitable for labile catecholamines which are prone to oxidative degradation. As a different approach [7] in the literature, the optimal pH conditions were provided by reacting catechol functions first with methyl chloroformate as reagent under mild alkaline condition (pH 7.2), followed by a pH shift to 9.2 to react basic amino functions under alkaline condition.

In the course of our amino acid profiling and screening studies for pattern recognition [22–24], single-phase isobutyloxycarbonyl (isoBOC) reaction in the alkaline aqueous solution (pH 11), with subsequent solid-phase extraction (SPE) using Chromosorb P, was found to be efficient for the recovery of zwitterionic amino acids. The resulting *N*(*O*,*S*)-isoBOC amino acids were converted into the corresponding stable *tert*-butyldimethylsilyl (TBDMS) derivatives in a single step for direct analysis by dual-capillary column GC. The accurate measurements of retention index (I) and area ratio (AR) values on dual columns of different polarities, allowed positive peak identification by library matching based on the two I sets and AR comparison. However, the single-phase isoBOC procedure at a fixed pH used for amino acids was not suitable for the simultaneous derivatization of structually diverse amines.

As the first step toward amine profiling and screening works for pattern recognition, the present study was undertaken to examine the optimum conditions of isoBOC reaction of low-mass volatile aliphatic amines as well as non-volatile polyamines, catecholamines, imidazoyl and indolyl amines. This method was based on two-phase reaction with a pH shift. The resulting *N*(*O*)-isoBOC amines were subjected to our SPE and TBDMS derivatization with subsequent dual-column GC profiling analysis [22,23].

2. Experimental

2.1. Materials

The 57 amine standards examined in this study were obtained mostly as hydrochloride salts, sulfates, or phosphates from Sigma (St. Louis, MO, USA) and other vendors. *N*-methyl-*N*-(*tert.*-

Table 1
Effect of organic solvent phase on the recoveries of amines

Amine	Recovery (peak area ratio ± S.D.)[a]		
	No solvent[b]	Diethyl ether	Dichloromethane
Ethylmethylamine	3.9 ± 0.1	3.0 ± 0.4	4.4 ± 0.2
sec-Butylamine	3.3 ± 0.1	2.5 ± 0.1	4.5 ± 0.1
n-Butylamine	5.8 ± 0.4	4.5 ± 0.2	8.1 ± 0.2
Piperidine	4.3 ± 0.3	3.3 ± 0.2	6.3 ± 0.1
Dibutylamine	4.9 ± 0.4	4.4 ± 0.1	7.2 ± 0.2
n-Heptylamine	5.3 ± 0.4	4.9 ± 0.1	7.3 ± 0.4
m-Toluidine	5.0 ± 0.1	5.8 ± 0.2	8.6 ± 0.2
3,4-Dimethoxyphenethylamine	1.2 ± 0.2	2.8 ± 0.2	3.8 ± 0.2
Cadaverine	0.7 ± 0.1	4.1 ± 0.3	5.1 ± 0.4
Tyramine	0.7 ± 0.1	2.8 ± 0.3	5.7 ± 0.4

A 1.0 ml volume of phosphate buffer (pH 7.5) cotainining each amine at the concentration of 2 ppm and *n*-eicosane as I.S. at 1 ppm was subjected to isoBOC reaction followed by SPE as described in the text.
[a]Peak area ratio relative to *n*-eicosane as I.S. and standard deviation for triplicate runs.
[b]Direct isoBOC reaction in the aqueous phase without solvent.

butyldimethylsilyl)trifluoroacetamide (MTB-STFA) of silylation grade was obtained from Pierce (Rockford, IL, USA), 3,3′,-thiodipropionic acid (TDPA), isobutyl chloroformate (isoBCF) from Sigma, and *n*-hydrocarbon standards (C_{10}–C_{36}, even numbers only) from Polyscience (Niles, IL, USA). Isooctane and dichloromethane of spectroanalyzed grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA), diethyl ether from Oriental Chemical Industry (Seoul, South Korea). All other chemicals were of analytical grade and used as received. Chromosorb P (80–100 mesh) was obtained from Supelco (Bellefonte, PA, USA). A luer-tipped glass tube (5 mm I.D.) packed with Chromosorb P (2.0 g) was washed successively with 1.0 M sulfuric acid, distilled water, 1.0 M sodium hydroxide, methanol, acetone, dichloromethane and diethyl ether, followed by activation under vacuum (150°C for 3 h) prior to being used as a solid-phase extraction (SPE) tube.

2.2. Amine and internal standard solutions

Each stock solution of the amines was made up at 1.0 μg μl^{-1} in 0.1 M HCl containing 0.2% (w/v) TDPA, except for diphenylamine, dibenzylamine, β-hydroxyphenethylamine, 2,4-dichlorobenzylamine and 3,4-dichlorobenzylamine which were made in 0.1 M HCl containing 50% acetonitrile and stored frozen. Working solutions were made by combining aliquots of each stock solution and diluting with 0.1 M HCl containing 0.2% (w/v) TDPA and stored in a refrigerator. Two separate internal standard solutions were prepared by dissolving 3,4-dichlorobenzylamine at 0.2 μg μl^{-1} in 0.1 M HCl and *n*-eicosane at 1 μg μl^{-1} in isooctane.

2.3. N(O)-isobutyloxycarbonylation

Aliquots of the amine working solutions were diluted to the desired concentrations (0.2–12 ppm) with 1.0 M sodium phosphate buffer (pH 7.5) and 3,4-dichlorobenzylamine used as I.S. was added at 1.0 ppm. Two-phase isoBOC reaction in two-steps was performed by vortex mixing 1.0 ml of the diluted amine solutions with 1.0 ml of dichloromethane containing 5 μl of isoBCF. After the first mixing for 10 min, the pH of the mixture was increased to 12.0 with 5 M sodium hydroxide. Following the second mixing for 10 min the whole mixture was subjected to the solid-phase extraction with subsequent silylation as described next. Amines which were not resolved on Ultra-2 column were separately processed for the linearity

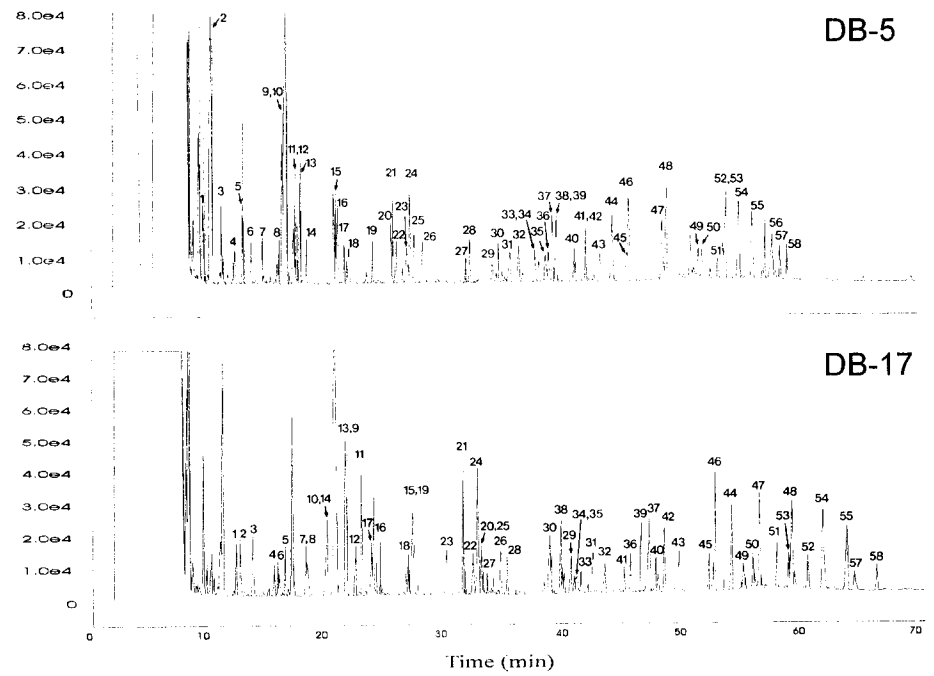

Fig. 1. Dual chromatograms of amines as *N(O)*-isoBOC, *O*-TBDMS derivatives separated on DB-5 and DB-17 (both 3.0 m × 0.25 mm I.D., 0.11 μm film thickness) dual-capillary column system. GC conditions are described in the text. Peaks: 1 = ethylmethylamine; 2 = *tert*-butylamine; 3 = diethylamine; 4 = *sec*.-butylamine; 5 = isobutylamine; 6 = diisopropylamine; 7 = *n*-butylamine; 8 = dipropylamine; 9 = pyrrolidine; 10 = isoamylamine; 11 = morpholine; 12 = piperidine; 13 = *n*-amylamine; 14 = diisobutylamine; 15 = thiazolidine; 16 = *n*-hexylamine; 17 = dibutylamine; 18 = cyclohexylamine; 19 = *n*-heptylamine; 20 = diphenylamine; 21 = *o*-toluidine; 22 = benzylamine; 23 = *n*-octylamine; 24 = m-toluidine; 25 = *p*-toluidine; 26 = *β*-phenethylamine; 27 = dihexylamine; 28 = *n*-decylamine; 29 = 2,4-dichlorobenzylamine; 30 = dicyclohexylamine; 31 = 1,3-diaminopropane; 32 = 3,4-dichlorobenzylamine; 33 = *β*-hydroxyphenethylamine; 34 = norephedrine; 35 = ephedrine; 36 = putrescine; 37 = 3,4-dimethoxyphenethylamine; 38 = diethanolamine-1; 39 = dibenzylamine; 40 = cadaverine; 41 = diethanolamine-2; 42 = histamine; 43 = 1,6-diaminohexane; 44 = tryptamine; 45 = 1,7-diaminoheptane; 46 = tyramine; 47 = 3-methoxytyramine; 48 = 5-methoxytryptamine; 49 = synephrine; 50 = octopamine; 51 = metanephrine; 52 = 3,4-dihydroxybenzylamine; 53 = normetanephrine; 54 = dopamine; 55 = spermidine; 56 = serotonin; 57 = epinephrine; 58 = norepinephrine (peak numbers correspond to Table 2).

test. To test the effect of dichloromethane as the organic solvent phase on the amine recoveries, isoBOC reaction was carried out in the same manner without any organic solvent phase and also using diethyl ether as the organic solvent phase. Sodium phosphate buffer (pH 7.5) spiked with 10 amines at 2 ppm each and *n*-eicosane as I.S. at 1 ppm was used for this test.

2.4. Solid-phase extraction and silylation

After saturation with sodium chloride, the whole mixture including the solvent phase was loaded onto a preactivated Chromosorb P tube, using a solid-phase extractor (Supelco, USA). The aqueous phase was allowed to advance homogeneously until 80% of the tube was moist. Next, the *N(O)*-isoBOC amines were eluted with diethyl ether (4 ml) and the eluate was collected in isooctane (50 μl). Most of ether and dichloromethane were removed by evaporation (N_2 stream, 45°C) followed by reaction with 20 μl of MTBSTFA (60°C, 2 h) in order to silylate remaining hydoxyl groups to TBDMS ethers. All the samples were individually prepared in triplicate and directly examined by GC and GC-MS.

2.5. Sample preparation

Aliquots of 1 ml of saliva samples without spiking or after spiking with amines were mixed with I.S. at 1.0 ppm. Each solution was made acidic (pH $\leq$ 1) with hydrochloric acid, followed by washing with *n*-hexane (3 × 0.5 ml). The aqueous layer was adjusted to pH 7.5 with solid dibasic sodium phosphate and then subjected to isoBOC reaction with subsequent SPE as described above.

2.6. Gas chromatography and gas chromatography-mass spectrometry

GC analyses were performed with a Hewlett-Packard HP model 5890A series II gas chromatograph (Hewlett Packard, Avondale, PA, USA) equipped with a split/splitless capillary inlet system and two flame ionization detectors (FID). Samples (ca. 1.0 μl) were injected in the splitless mode with a purge delay time of 0.8 min. The oven temperature was held at 60°C for 2 min, then programmed to 280°C at a rate of 4°C min^{-1}. The injector and detector temperatures were 260 and 280°C, respectively. The inlet pressure of helium was set to 100 Kpa. For the retention index measurements, a dual-capillary column system made of DB-5 (SE-54 bonded phase) and DB-17 (OV-17 bonded phase) fused-silica capillary columns (J and W Scientific, Rancho Corodova, CA, USA; dimensions 30 m × 0.25 mm I.D., 0.11 μm film thickness) was used. The two FID signals were processed simultaneously in dual-channel mode by the HP 3365 Chem software program. A standard solution of *n*-hydrocarbons (C_{10}–C_{36} even numbers only) in isooctane was injected as the external references for I measurements. Temperature-programmed I values were computed via built-in retention index program by linear interpolation between the retention times of adjacent hydrocarbon standards. For peak identification by computer I matching, a database of reference I library using the I sets of 57 amines measured on the dual columns was built in the GC computer system. For optimization of the procedure and precision and linearity tests, an Ultra-2 (SE-54 bonded phase) capillary column (25 m × 0.20 mm I.D., 0.11 μm film thickness) was used under the identical operating conditions.

To obtain mass spectra, an HP 5890A series II gas chromatograph with an Ultra-2 (SE-54 bonded phase) capillary column (25 m × 0.20 mm I.D., 0.11 μm film thickness), interfaced to an HP 5970B mass selective detector (70 eV, electron impact mode) and on-line to an HP 59940A MS Chemstation was used. Samples were introduced in the split injection mode (10:1) and the oven temperature was initially 60°C for 2 min and then raised to 280°C at 4°C min^{-1}. The injector and interface temperatures were 260 and 280°C, respectively. The mass range scanned was 50–650 U at a rate of 0.99 scan s^{-1}.

2.7. Calculations

All the quantitative calculations for the recoveries and linearity tests were based on the peak area ratios relative to the internal standard (I.S.). Least-squares regression analysis was performed on the measured peak area ratios against increasing weight ratios of amines to I.S. in order to test linearity of the whole procedure and to plot calibration curves for the quantitative measurements of amines.

3. Results and discussion

It was not an easy task to design a single analytical procedure for the complete profiling of structurally diverse biogenic amines. In this study, the two-phase isoBOC reaction method [14,17,20] was combined with a pH shift method [7] for the simultaneous derivatization of 57 amines which differ in their basicities and volatilities. The subsequent SPE procedure requires the use of low boiling organic solvents [22] and a number of preliminary experiments were thus, perfomed for the choice of a suitable solvent. Dichloromethane was chosen as the optimal solvent phase to which 5 μl volume of isoBCF reagent was added. The pH of the 1.0 M phosphate buffer as the aqueous phase was initially adjusted to 7.5 for the optimal reaction of phenolic hydoxyl functions and then

Table 2
Retention indices of amines as N(O)-isoBOC, *O*-TBDMS derivatives

Number	Amine	GCI[a] data set		Number	Amine	GCI[a] data set	
		DB-5	DB-17			DB-5	DB-17
1	Ethylmethylamine	1101.0	1254.0	30	Dicyclohexylamine	1972.6	2202.9
2	*tert*-Butylamine	1120.9	1261.4	31	1,3-diaminopropane	2004.5	2358.4
3	Diethylamine	1161.8	1301.0	32	3,4-Dichlorobenzylamine	2036.7	2403.9
4	*sec.*-Butylamine	1206.8	1360.5	33	*β*-Hydroxyphenethylamine	2095.4	2313.7
5	Isobutylamine	1233.6	1390.3	34	Norephedrine	2096.7	2250.2
6	Diisopropylamine	1251.6	1367.6	35	Ephedrine	2111.7	2299.0
7	*n*-Butylamine	1282.2	1450.0	36	Putrescine	2154.0	2519.3
8	Dipropylamine	1323.1	1450.8	37	3,4-Dimethoxyphenethylamine	2161.8	2587.5
9	Pyrrolidine	1336.3	1553.8	38	Diethanolamine-1	2168.4	2235.6
10	Isoamylamine	1345.5	1500.1	39	Dibenzylamine	2176.0	2558.9
11	Morpholine	1370.5	1592.9	40	Cadaverine	2253.9	2628.5
12	Piperidine	1378.5	1578.0	41	Diethanolamine-2	2283.9	2486.4
13	*n*-Amylamine	1380.1	1551.5	42	Histamine	2287.5	2655.7
14	Diisobutylamine	1399.3	1501.1	43	1,6-Diaminohexane	2341.7	2720.2
15	Thiazolidine	1476.1	1741.0	44	Tryptamine	2387.2	2966.3
16	*n*-Hexylamine	1486.4	1648.9	45	1,7-Diaminoheptane	2446.6	2859.2
17	Dibutylamine	1500.1	1620.5	46	Tyramine	2461.1	2888.6
18	Cyclohexylamine	1514.6	1729.4	47	3-Methoxytyramine	2605.7	3107.6
19	*n*-Heptylamine	1580.3	1747.7	48	5-Methoxytryptamine	2616.4	3263.6
20	Diphenylamine[b]	1621.6	1966.8	49	Synephrine	2770.7	3034.0
21	*o*-Toluidine	1628.9	1900.8	50	Octopamine	2789.2	3085.4
22	Benzylamine	1649.2	1938.6	51	Metanephrine	2866.5	3196.6
23	*n*-Octylamine	1681.7	1849.1	52	3,4-Dihydroxybenzylamine	2889.6	3377.5
24	*m*-Toluidine	1687.5	1949.7	53	Normetanephrine	2901.4	3249.0
25	*p*-Toluidine	1700.5	1965.7	54	Dopamine	2964.8	3401.6
26	*β*-Phenethylamine	1731.3	2028.8	55	Spermidine	3035.8	3477.7
27	Dihexylamine	1863.4	1980.9	56	Serotonin	3093.1	n.d.[c]
28	*n*-Decylamine	1881.3	2050.3	57	Epinephrine	3172.1	3510.0
29	2,4-Dichlorobenzylamine	1957.0	2284.3	58	Norepinephrine	3205.2	3571.8

[a]Mean retention index (I) values measured on DB-5 and DB-17 (both 30 m × 0.25 mm I.D., 0.11 μm film thickness) dual-capillary columns programmed from 60°C (held for 2 min) to 280°C at 4°C/min. Relative standard deviations ranged from 0.01 to 0.3% for three measurements.
[b]Underivatized.
[c]Not detected.

raised to 12.0 for the reaction of basic amino functions. The reaction time was set to 10 min at each pH condition. Unknown peaks derived from isoBCF interfered with GC resolution of some amines due to overlapping. Therefore, use of excess amount of isoBCF was avoided and 5 μl was chosen as the optimal amount for the two-phase isoBOC reaction of amines in the concentration range of 0.2–12 ppm.

The effect of organic solvent phase on the derivative yields for ten amines was evaluated. As shown in Table 1, without solvent phase, the detector responses (expressed as peak area ratio) of *m*-toluidine, 3,4-dimethoxyphenethylamine, cadaverine and tryptamine were significantly reduced upon GC analysis. This is probably due to the instability of isoBCF when added directly into the alkaline aqueous phase [13]. Dichloromethane gave much higher responses for all the amines evaluated with good overall precision compared to diethyl ether. Dichloromethane being denser than water forms the lower phase and thus a pH shift of the upper aqueous phase with 5 M sodium hydroxide could be made without disturbing the

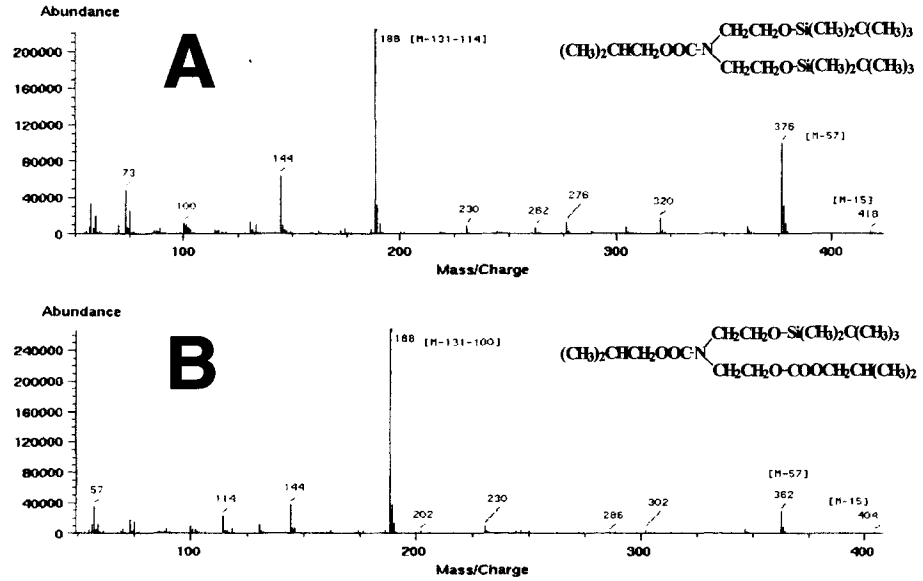

Fig. 2. Electron-impact mass spectra of (A) peak 38 and (B) peak 42 derived from diethanolamine after *N(O)*-isoBOC and *O*-TBDMS reactions.

dichloromethane phase. This appears to minimize the degradation of isoBCF and also the losses of amine derivatives during the sample work-up.

Under the present isoBOC reaction conditions, all amino and phenolic hydoxyl groups of the amines studied except for the secondary amino group in diphenylamine were converted to their isoBOC derivatives. Unlike the previous reports in the literature [7,16], one of the two hydoxyl groups in diethanolamine was partially (about 60%) converted to *O*-isoBOC group.

Following the isoBOC reaction, *N(O)*-isoBOC amines were recovered from the aqueous and solvent phases by SPE in normal phase partition mode using hydrophilic Chromosorb P as the adsorbent and diethyl ether as the eluent. Compared with the laborious solvent extraction [5–7,10,13,17,20], the SPE method permitted an efficient and rapid extraction of the *N(O)*-isoBOC amines in a few steps.

Upon reaction with MTBSTFA at 60°C in isooctane for better GC separation, all remaining *β*-hydroxyl and primary hydroxyl groups of 10 amines, including norepinephrine and epinephrine, were silylated to *O*-TBDMS groups within 2 h, yielding a single derivative for each amine. Unlike *O*-methyloxycarbonyl derivatives of phenolic hydroxyl groups reported in the literature [7], substitution of *O*-isoBOC by an *O*-TBDMS group was not observed under the present TBDMS derivatization condition. Similar to the fragmentation patterns of general TBDMS derivatives [22,23], each *O*-TBDMS derivative showed characteristic pair of $[M-57]^+$ and $[M-15]^+$ ions, thus, enabling rapid confirmation of amines possessing alcoholic hydroxyl groups. However, silylation step reduced the yields of the inherently labile amines such as thiazolidine, histamine and 5-methoxytryptamine which do not possess additional hydroxyl groups. Moreover, unknown peaks formed from the reagents, which were confirmed by sample blank runs, disturbed the GC separation of several amines. Therefore, the quantitative analysis of those amines was carried out before silylation.

The separation of 57 amines on dual-capillary columns is shown in Fig. 1. Each amine displayed a single peak, except for diethanolamine which exhibited two peaks (peak 38 and 41), labeled as diethanolamine-1 and diethanolamine-2. Under-

Table 3
Linear regression analysis of relative responses against relative weights of amines as their *N(O)*-isoBOC, *O*-TBDMS derivatives

Amine	Regression line[a]		Correlation coefficient,	Amine	Regression line[a]		Correlation coefficient
	m	b	r		m	b	r
Ethylmethylamine	1.639	0.028	0.999	Dicyclohexylamine	1.143	−0.023	0.981
tert-Butylamine	1.604	−0.036	0.996	1,3-Diaminopropane	1.527	0.099	0.999
Diethylamine	1.852	0.058	0.997	*β*-Hydroxyphenethylamine	0.549	0.258	0.998
sec-Butylamine	1.510	−0.107	0.981	Norephedrine	2.168	−0.043	0.998
Isobutylamine	2.013	−0.117	0.985	Ephedrine	1.413	−0.053	0.994
Diisopropylamine	1.024	−0.037	0.994	Putrescine	1.473	0.023	0.995
n-Butylamine	2.217	−0.136	0.988	3,4-Dimethoxyphenethylamine	0.735	−0.030	0.999
Dipropylamine	1.200	0.015	0.995	Diethanolamine-1	1.737	0.063	0.994
Pyrrolidine	2.235	−0.407	0.996	Dibenzylamine	0.651	0.117	0.999
Isoamylamine	1.363	0.275	0.998	Cadaverine	1.346	0.058	0.999
Morpholine	1.979	−0.122	0.982	Histamine	0.142	−0.194	0.997
Piperidine	1.755	0.316	0.996	1,6-Diaminohexane	1.029	0.114	0.998
Diisobutylamine	1.784	−0.177	0.984	Tryptamine	0.444	−0.968	0.982
Thiazolidine	0.792	−0.024	0.988	1,7-Diaminoheptane	0.832	0.082	0.999
n-Hexylamine	3.242	−0.130	0.998	Tyramine	1.045	0.079	0.998
Dibutylamine	2.433	−0.023	0.981	3-Methoxytyramine	0.702	−0.304	0.984
Cyclohexylamine	2.411	−0.271	0.978	5-Methoxytryptamine	0.038	0.063	0.864
n-Heptylamine	2.322	−0.155	0.984	Synephrine	0.746	−0.005	0.999
Diphenylamine	0.869	−0.168	0.964	Octopamine	0.676	−0.054	0.999
o-Toluidine	1.838	0.733	0.991	Metanephrine	0.715	−0.246	0.998
Benzylamine	2.100	−0.157	0.985	3,4-Dihydroxybenzylamine	0.035	0.073	0.945
n-Octylamine	2.320	−0.217	0.985	Normetanephrine	0.422	0.067	0.999
m-Toluidine	1.696	0.639	0.993	Dopamine	0.265	−0.340	0.985
p-Toluidine	2.178	−0.190	0.989	Serotonin	0.211	−0.397	0.980
β-Phenethylamine	2.266	−0.185	0.987	Epinephrine	0.173	−0.045	0.978
Dihexylamine	1.054	−0.097	0.993	Norepinephrine	0.041	0.171	0.995
n-Decylamine	1.862	−0.178	0.996				

[a]m, Slope = relative mass response = mean peak area ratio of amine × mass of I.S./mass of amine; b = y-intercept.

ivatized diphenylamine was eluted as a symmetrical peak (peak 20) from both columns. Serotonin was not eluted from DB-17 column of medium polarity within the analysis time. Several pairs of peaks unresolved on DB-5 column of low polarity were well separated on DB-17 column, and vice versa. Therefore, DB-5 and DB-17 dual-capillary column system, together provided a complete separation. Moreover, the elution orders of most amines on the two columns were very different. The temperature-programmed I sets of amines measured on dual-capillary columns were thus, characteristic (Table 2) and useful as a cross-check for each amine.

The structures of diethanolamine derivatives were confirmed by mass spectra as shown in Fig. 2. The characteristic pair of $[M-57]^+$ ion at m/z 376 and $[M-15]^+$ ion at m/z 418 (Fig. 2A) indicates that the compound of peak 38 is bis *O*-TBDMS derivative of *N*-isoBOC diethanolamine. In

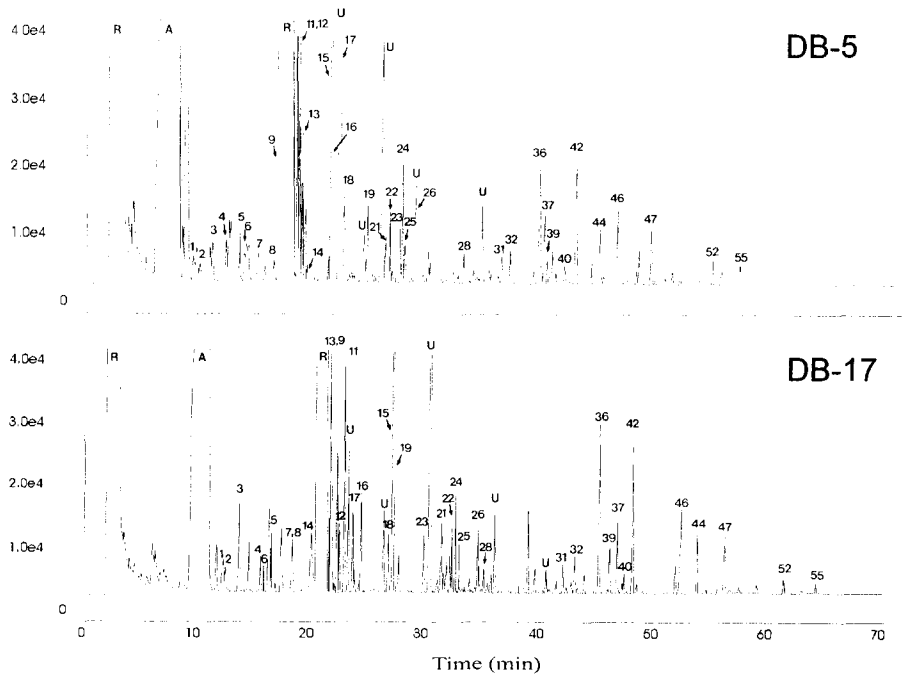

Fig. 3. Dual chromatographic profiles of saliva spiked with 33 amines. GC conditions and peak numbers are the same as those in Fig. 1. Peaks: R = peak derived from reagents; A = ammonia; U = unidentified; 31, 36, 40, 55 are unspiked polyamines.

the same manner, ions at m/z 362 and 404 of peak 41 correspond to the characteristic $[M-57]^+$ and $[M-15]^+$ ions of $N(O)$-isoBOC, mono O-TBDMS diethanolamine.

The combined method of isoBOC reaction and SPE with or without subsequent TBDMS derivatization of amines, under the selected conditions, was examined to test the linear relation between detector responses and amounts of amines. As listed in Table 3, linear responses were obtained for most amines in the range of 0.2–12 ppm with correlation coefficients varying from 0.945 to 0.999, except for 5-methoxytryptamine (0.864). The relative standard deviations ranged from 0.1 to 10.0% with the exception of thiazolidine, histamine, tryptamine, 5-methoxytryptamine, epinephrine and norepinephrine. The overall reproducibility and linearity of the three combined steps for GC separation of most amines appear to be satisfactory for their quantitative measurements in unknown samples.

When applied to saliva samples, the present method demonstrated the usefulness for amine profiling analysis as exemplified in Fig. 3. Simultaneous screening and accurate confirmation of each spiked amine and unspiked endogenous polyamines such as 1,3-diaminopropane, putrescine, cadaverine and spermidine could be achieved by computer comparison of I sets with the reference values in the home-built I library as described elsewhere [23,24]. Each amine was confirmed by GC-MS. Four peaks which appear to be endogenous to saliva remained unidentified due to the limitation of the database in the I library. The I library will continue to be expanded to include other biogenic amines for the identification of unknown amines in screening work.

4. Conclusions

A significant advantage of the present isoBOC derivatization is that labile phenolic hydroxyl group and basic amino group are sequentially reacted with minimal amount of isoBCF in the dichloromethane-phosphate buffer under each op-

timal pH condition at 7.5 and 12.0, respectively. The subsequent SPE method using Chromosorb P in partition mode allows efficient and rapid extraction of the resulting *N(O)*-isoBOC amines from the reaction mixture in a few steps. The remaining hydroxyl functions were silylated to TBDMS derivatives which generate diagnostically useful $[M-57]^+$ and $[M-15]^+$ ion pairs in their mass spectra. Dual columns with differing polarity, together provide complete separation of 57 amines with characteristic I sets that can be used for routine amine screening work. An extension of the present method for the rapid profiling and screening of fermented food products and clinical samples for biogenic amines and their quantitative measurements is in progress.

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