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DETERMINATION OF MONO-, DI- AND POLYAMINES IN FOODS USING A SINGLE-COLUMN AMINO ACID AUTO-ANALYZER

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SUMMARY

A fully automated, rapid and sensitive method was developed to analyze fourteen different biogenic amines in food. Using a Technicon C_4 ion-exchange resin column (20 m × 0.5 cm), adapted to an automatic Technicon TSM amino acid analyzer, the following amines were separated and quantified: adrenaline, noradrenaline, 1,3-diaminopropane, putrescine, cadaverine, histamine, spermidine, dopamine, spermine, agmatine, tyramine, serotonin, phenethylamine and tryptamine. Five buffers were required to elute the amines using a gradient of pH from 5.6 to 12.7; the column temperature was maintained at 65 C. The method was also assayed on ground beef, cheese and wine samples. Amines from cheese and ground beef samples were extracted with 0.6 *M* perchloric acid. No extraction of wine samples was necessary.

INTRODUCTION

Considerable interest has been generated in recent years regarding biogenic amines in foodstuffs[1–4]. The amine content of fish and other foods has recently been reviewed by Smith[5] and Ienistea[6]. Microbial spoilage in foods leads to the production of decarboxylases which under appropriate conditions can convert amino acids into their corresponding amines. Thus, the diamines cadaverine and putrescine, which are formed from the amino acids lysine and ornithine, are products of bacterial decomposition of animal tissue, and histamine is formed from the amino acid histidine in some foods by a similar mechanism. In this respect, biogenic amines might be a useful indicator of spoilage when their levels in foods are measured. Some of these amines have been found to be vasoactive or psychoactive agents[6–8].

The separation of these nitrogenous compounds is generally accomplished by gas and liquid chromatography or by high-performance liquid chromatography (HPLC) but the sample preparation steps are tedious[7–9]. Fluorimetry is a sensitive method but it is limited in its specificity to a few amines. Ion-exchange column

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chromatography (IEC) with automated instruments is the most favored method for routine analysis of mono-, di- and polyamines in biological material. However, most of the ion exchange methods only involve separations of putrescine, cadaverine, spermidine, spermine, histamine and a few other related compounds such as tyramine and tryptamine[10–12]. Few of these techniques permit separation of more than seven amines by ion exchange on a single column[13,14]. Moreover, no method has been developed to allow separation of sixteen amines in a relatively short time of elution (150 min).

The purpose of the present work was to develop an accurate and reproducible chromatographic method using single-column ion exchange to separate and quantify a large number of amino compounds in a shorter time and which would be applicable to a range of untreated materials, such as wine, dairy and meat products.

EXPERIMENTAL

Apparatus

A single-column Technicon TSM amino acid analyzer (Technicon, Tarrytown, NY, U.S.A.) with a 20 × 0.5 cm I.D. resin bed packed with Chromobead C_4 was used; the sample cartridge also contained Chromobead C_4 [0.1 ml, 50% (w/w) slurry]. The column temperature was maintained at 65°C, the buffer flow-rate was 0.50 ml/min and the transmittance of the ninhydrin-treated eluent was followed at 570 nm. The bath temperature for ninhydrin was maintained at 85°C. The chromatograms were integrated with an Autolab system A.A. computing integrator (Technical Marketing Assoc., Toronto, Canada). Direct sample injection onto the sample cartridge system was used for liquid samples.

Solid samples were prepared for analysis using Gehrke's extraction method[15] with 0.6 *M* perchloric acid. The homogenate was obtained with a Polytron Pt 45 (Kinewatica, Luzern, Switzerland), followed by centrifugation with a Sorvall RC2 centrifuge (DuPont Instruments, Newtown, CT, U.S.A.).

Chemicals

A solution of 40 g of ninhydrin (Pierce, Chicago, IL, U.S.A.) in 2 l of methyl cellosolve (Fisher Scientific, Montréal, Canada) was added to 1 l of 4 *M* sodium

TABLE I

COMPOSITION AND NATURE OF ELUTING BUFFERS

Reagent	*Buffer*				
	A	*B*	*C*	*D*	*E*
Sodium citrate dihydrate (g/l)	19.6	19.6	19.6	19.6	19.6
Sodium chloride (g/l)	17.5	134.4	149.0	146.1	146.1
Sodium tetraborate (g/l)	0	0	0	8.64	8.64
Isopropanol (ml/l)	55	60	105	105	105
Methyl cellosolve (ml/l)	0	0	0	50	100
Brij 35 (ml/l)	10	10	10	15	15
pH ± 0.03	5.55	5.65	6.10	9.60	12.75
Time (min)	36	38	24	21	31

TABLE II

STOCK SOLUTIONS AND STANDARDS USED

Amine	*Abbreviation*	*Stock solution (g/100 ml)*	*Standard (μg/ml)*
Adrenaline	A	0.1000	100–600
Noradrenaline	NA	0.0250	25.0–130.0
1,3-Diaminopropane	DAP	0.0009	0.85–5.10
Putrescine	PUT	0.0017	1.7–10.2
Histamine	HA	0.0045	4.5–28.0
Cadaverine	Cd	0.0019	1.9–9.4
Spermidine	Sd	0.0030	3.0–18.0
Dopamine	DA	0.1500	150–700
Hexamethylenediamine	HDA	0.0040	4.0
Agmatine	Agm	0.0090	9.0–54.0
Spermine	Spm	0.0023	2.3–13.8
Tyramine	TA	0.0088	8.78–52.68
β-Phenethylamine	PA	0.0145	14.5–87.0
Serotonin	5-HT	0.1540	154.0–214.0
Tryptamine	T	0.0048	4.8–28.8

acetate buffered at pH 5.5 (BDH, Toronto, Canada). The volume was adjusted to 4 l with distilled water.

A solution of 1.049 g of hydrazine sulfate in 350 ml of distilled water was added to 20 ml of Brij wetting agent (Technicon). The volume was adjusted to 4 l with distilled water, and four drops of concentrated sulfuric acid were added.

Buffer preparation

The compositions and nature of the eluting buffers (A–E) are shown in Table I. To prevent microbial growth, four drops of pentachlorophenol (5 mg/ml in 95% ethanol) were added per liter of buffer using a pH gradient from 5.55 to 12.75 to elute sixteen amines.

TABLE III

SPLIT-PLOT DESIGN USED FOR THE ANALYSIS OF VARIANCE

Source of variance	*Degree of freedom*
Replicates, R	3
Concentration, c	5
Error a: $R \cdot c$	15
Sample, S	1
Sample · concentration	5
Error b: $R \cdot S$, $S \cdot R \cdot c$	18

TABLE IV

REPRODUCIBILITY FOR BIOGENIC AMINES IN STANDARD SOLUTIONS

$\bar{X} \pm$ S.E. = Mean $\pm$ standard error; C.V. = coefficient of variation.

ml of stock solution diluted in 10 ml of 0.01 M $HClO_4$		*Logarithm of relative peak area*													
		A	*NA*	*PUT*	*DAP*	*HA*	*Cd*	*Sd*	*DA*	*Agm*	*Spm*	*TA*	*PA*	*5-HT*	*T*
1	$\bar{X}$*	0.89	1.25	1.33	1.20	1.30	1.22	1.47	1.18	1.38	1.42	1.66	1.73	1.57	1.88
	S.E.	0.09	0.20	0.12	0.09	0.12	0.20	0.12	0.19	0.16	0.25	0.09	0.09	0.08	0.20
	C.V	11.1	16.2	10.02	7.69	9.8	17.61	8.09	16.7	11.86	16.98	5.55	5.60	11.64	10.75
2	$\bar{X}$	1.29	1.59	1.68	1.62	1.76	1.60	1.87	1.62	1.76	1.75	2.02	2.07	1.88	2.28
	S.E.	0.12	0.22	0.16	0.16	0.15	0.13	0.14	0.15	0.07	0.19	0.13	0.16	0.09	0.17
	C.V.	10.3	12.99	10.44	10.28	8.78	8.24	7.94	9.50	4.28	11.26	6.87	7.71	4.86	7.69
3	$\bar{X}$	1.44	1.74	1.83	1.65	1.80	1.65	1.96	1.80	1.93	1.96	2.15	2.27	2.11	2.46
	S.E.	0.09	0.17	0.16	0.14	0.28	0.28	0.09	0.14	0.09	0.15	0.15	0.15	0.14	0.15
	C.V.	7.28	10.19	8.84	3.88	8.31	16.80	5.07	7.80	4.84	7.76	6.87	6.45	3.48	6.27
4	$\bar{X}$	1.60	1.80	2.04	1.84	2.00	1.89	2.16	2.02	2.11	2.11	2.39	2.50	2.32	2.60
	S.E	0.01	0.18	0.15	0.06	0.13	0.10	0.11	0.11	0.06	0.18	0.12	0.12	0.13	0.18
	C.V.	8.75	10.83	7.55	3.83	6.65	6.64	5.68	5.68	3.08	8.73	5.04	4.92	3.6	6.98
5	$\bar{X}$	1.73	2.03	2.08	1.96	2.11	1.96	2.24	2.02	2.21	2.23	2.37	2.49	2.47	2.77
	S.E.	0.09	0.24	0.21	0.08	0.20	0.20	0.08	0.14	0.91	0.17	0.11	0.13	0.20	0.18
	C.V.	6.50	12.15	9.98	4.08	9.53	10.28	3.56	6.98	4.12	7.74	4.62	5.19	7.92	6.40
6	$\bar{X}$	1.83	2.09	2.22	2.05	2.22	2.09	2.46	2.01	2.30	2.25	2.49	2.59	2.61	2.82
	S.E.	0.16	0.13	0.12	0.13	0.18	0.15	0.13	0.26	0.10	0.11	0.12	0.45	0.19	0.16
	C.V.	9.20	6.4	8.49	6.37	8.13	6.97	5.27	12.69	4.30	4.89	5.16	6.75	7.20	5.87

* n = 8 replicates for each $\bar{X}$ value.

Calibration standards

The following biogenic amines were obtained from Sigma (St. Louis, MO, U.S.A.): 1,3-diaminopropane (DAP); histamine dihydrochloride (HA); cadaverine (Cd); spermidine trihydrochloride (Sd); agmatine sulfate (Agm); dopamine (DA); noradrenaline D-L hydrochloride (NA); adrenaline (A); β-phenethylamine hydrochloride (PA); sperminc tetrahydrochloride (Spm); tyraminc (TA); tryptamine hydrochloride (T) and 5-hydroxytryptamine (5-HT) as a creatinine sulfate derivative. Hexamethylenediamine (HDA) used as internal standard and putrescine (PUT) were purchased from ICN Pharmaceuticals (New York, NY, U.S.A.).

The concentrations of the stock solution for each amine and the working solutions are shown in Table II. Prior to injection, the sample cartridge was washed with 0.2 *M* NaOH and regenerated with sodium maleate buffer containing 19.2 g of sodium maleate and 10.5 g of sodium chloride in 1 l of water adjusted to pH 2.0. Digital integration of each peak was carried out by the integrator. Each amine response was expressed by the ratio of the individual amine peak surface to the internal standard peak surface. The relative peak area of each amine was calculated from the individual ninhydrin response.

Statistical analysis

To determine the reproducibility of the method and the best-fit relation of colorimetric response for each amine concentration, a split-plot design (Table III) was used for the statistical analysis[16]. Six dilutions of the stock solution were prepared: 1, 2, 3, 4, 5 and 6 ml of the stock solution were made up to 10 ml of 0.1 *M* $HClO_4$ to give six different concentrations for each amine. Four replicates of the stock solution were prepared and each diluted as described above. Each diluted solution was split into samples and injected twice for IEC. The significance of each source of variation was determined by analysis of the variance of each source such as replicates, concentration and sample effect. The Bartlett test was used to verify the

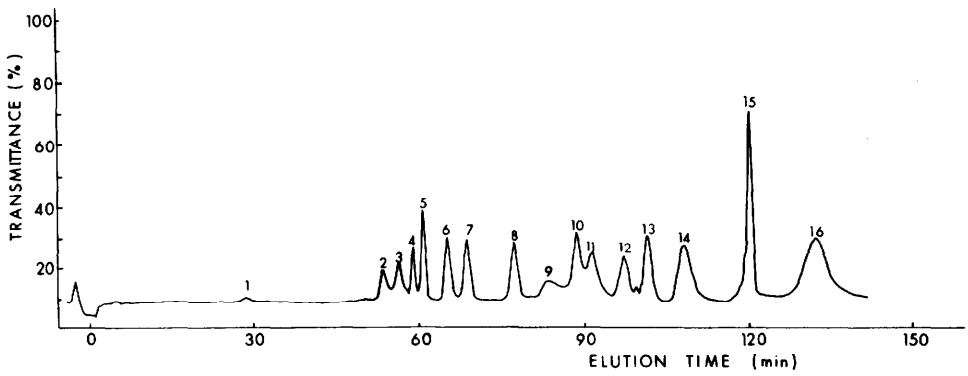

Fig. 1. IEC chromatogram of a mixture of mono-, di- and polyamines. Conditions: injection, 1.0 ml; detection, 570 nm; column, 20 × 0.5 cm I.D., packed with Technicon C_4 resin; temperature 65°C; flow-rate 30 ml/h; reaction-coil volume, 302.4 cm^3; reaction time, 20 min; reaction temperature, 85°C. Sample and amounts (μg/ml): 1 = NH_3; 2 = A, 200; 3 = Na, 50; 4 = DAP, 1.70; 5 = PUT, 3.4; 6 = HA, 9.0; 7 = Cd, 3.8; 8 = Sd, 6.0; 9 = DA, 300.0; 10 = HDA, 4.0; 11 = Agm, 18.0; 12 = Spm, 4.6; 13 = TA, 17.56; 14 = PA, 29.0; 15 = 5-HT, 308.0; 16 = T, 9.6.

homogeneity of the standard deviation with the mean of each concentration before the analysis of variance. The ninhydrin color response at different concentrations was submitted to linear and curvilinear regression analysis. The regression coefficients were calculated to provide the highest coefficient of determination (R^2) for standard curve.

RESULTS AND DISCUSSION

The chromatogram presented in Fig. 1 shows the separation, resolution and order of elution of various mono-, di- and polyamines at the μg/ml level. The total elution time was 145 min and no amine appears during the first 30 min. The first buffer elutes amino acids from the samples, then ammonia is eluted followed by the fifteen amines. The elution time is shortened considerably by the use of a borate–citrate buffer at a high pH gradient (5.55–12.75) compared with the Villanueva method[14]; the saving in time is 30 min per sample. Moreover, noradrenaline was separated from adrenaline and other amines for the first time.

A closer examination of the chromatogram of different concentrations of these biogenic amines indicates that the standard error increased with concentration, giving a positive result for the Bartlett test of homogeneity of variance. Logarithmic transformation of the relative peak area was necessary to overcome this inhomogeneity, and the coefficients of variation calculated after this transformation for each amine at six different concentrations are shown in Table IV. For NA, Cd, DA and Spm the coefficients of variation were relatively high when the concentration was low (1 ml per 10 ml). The analysis of variance indicated that for all amines no significant difference

TABLE V

PRECISION AND ACCURACY OF THE ANALYSIS OF BIOGENIC AMINES

Amine	*Abbreviation*	*Retention time* (min)*	*Regression coefficient in* $y = A + Bx$**		*Coefficient of determination,* R^2	*Standard error, S.e.*
			A	*B*		
Adrenaline	A	61.70 ± 1.20	−0.29	1.19	0.99	0.02
Noradrenaline	NA	64.60 ± 1.20	0.82	1.06	0.99	0.05
1,3-Diaminopropane	DAP	68.20 ± 1.45	−0.79	1.04	0.97	0.06
Putrescine	PUT	69.90 ± 1.40	1.07	1.12	0.99	0.03
Histamine	HA	74.80 ± 1.96	0.80	1.12	0.97	0.06
Cadaverine	Cd	77.20 ± 1.47	0.92	1.08	0.98	0.06
Spermidine	Sd	85.50 ± 1.20	0.90	1.18	0.98	0.06
Agmatine	Agm	98.70 ± 1.54	0.26	1.18	0.99	0.01
Hexamethylenediamine	HDA	96.23 ± 1.47	–	–		
Dopamine	DA	90.03 ± 0.49	−0.28	1.26	0.98	0.05
Spermine	Spm	105.38 ± 1.72	−1.04	1.11	0.99	0.03
Tyramine	TA	109.80 ± 1.15	0.67	1.07	0.99	0.04
β-Phenethylamine	PA	115.20 ± 1.36	1.59	1.13	0.98	0.05
Serotonin	5-HT	126.1 ± 2.01	−0.08	1.35	0.99	0.05
Tryptamine	T	142.50 ± 3.20	1.07	1.21	0.99	0.03

* $\bar{X} \pm$ S.E.

** $y = \log_{10}$ (relative peak area); $x = \log_{10}$ (concentration of amine).

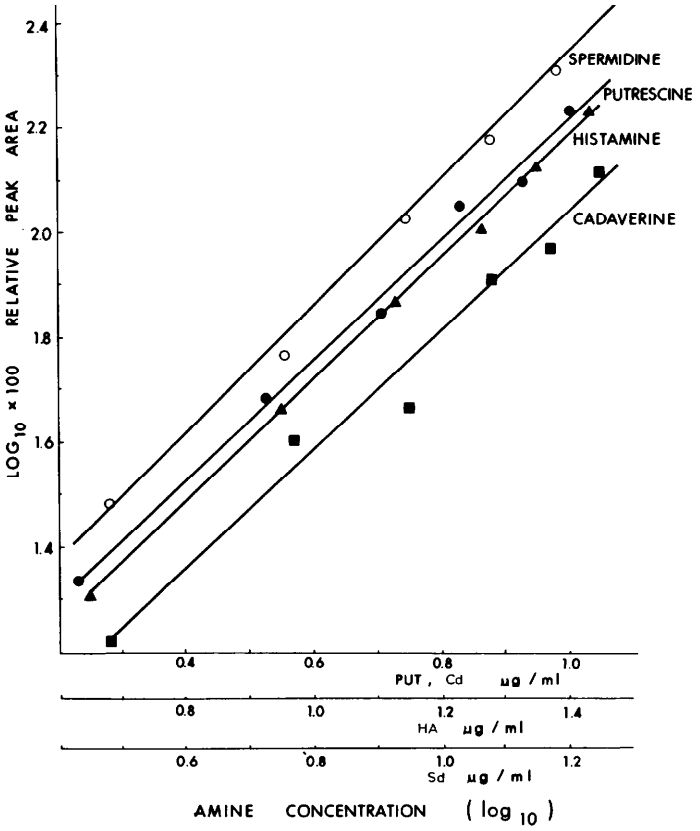

Fig. 2. Standard curves of cadaverine, histamine, putrescine and spermidine. Injection conditions: 1.0 ml of six different concentrations of standard amine solutions; other conditions as in Fig. 1.

was observed, which indicates the high reliability of the method. With increasing concentration of amines the ninhydrin color response was linear over the range from 0.85 µg/ml to 0.92 mg/ml for the pooled biogenic amines. Regression coefficients were calculated on the logarithmic values where the independent variable was the logarithm of concentration in µg/ml and the dependent variable was the logarithm of the relative peak area. Regression and determination coefficients are presented in Table V as well as the retention time and the standard error for each amine. A linear regression equation gave the highest coefficients for all amines tested. Linear regression was used to fit the best straight line to the standard curves for Cd, HA, PUT and Spm currently found in foods. The data and standard curves are shown in Fig. 2.

The versatility of the method is demonstrated by the analysis of ground beef extracts. A typical IEC chromatogram is presented in Fig. 3. In this sample, seven amines were detected: adrenaline, putrescine, histamine, cadaverine, spermidine, spermine and tyramine. As indicated, all amino acids were eluted in the first 30 min, followed by NH_3. Adrenaline, spermidine and spermine were present in relatively high concentrations.

Ripe Cheddar cheese was also analyzed and six amines were detected as shown

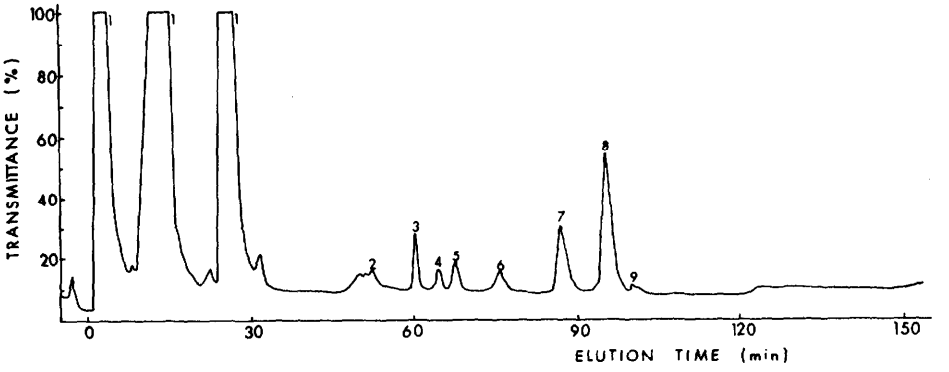

Fig. 3. IEC chromatogram of ground beef extract. Sample and amounts (μg/g of beef sample): 1 = amino acids; 2 = A, 478; 3 = PUT, 8.8; 4 = HA, 1.1; 5 = Cd, 22.7; 6 = Sd, 72.5; 7 = HDA, 4.0; 8 = Spm, 459; 9 = TA, 60.0.

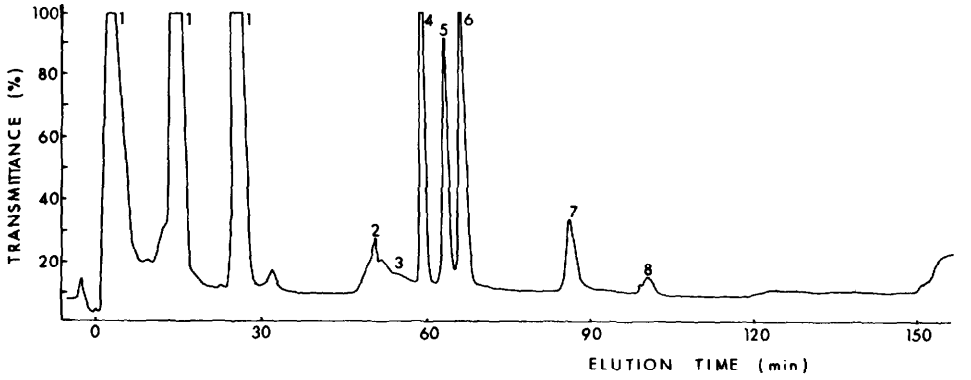

Fig. 4. IEC chromatogram of amines in old Cheddar cheese. Sample, 1.0 ml; other conditions as in Fig. 1. Amounts (μg/g): 1 = amino acids; 2 = A, trace; 3 = NA, trace; 4 = PUT, 661.0; 5 = HA, 28.0; 6 = Cd, 200; 7 = HDA (internal standard), 4.0; 8 = TA, 174.0.

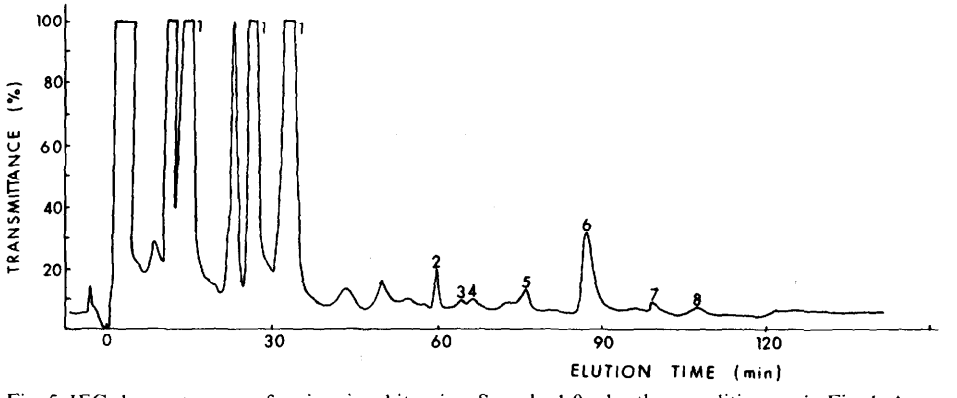

Fig. 5. IEC chromatogram of amines in white wine. Sample, 1.0 ml; other conditions as in Fig. 1. Amounts (μg/ml): 1 = amino acids; 2 = DAP, trace; 3 = HA, 10; 4 = Cd, 1.0; 5 = Sd, 17.0; 6 = HDA (internal standard) 4.0; 7 = TA, 0.2; 8 = PA, 4.0.

in Fig. 4: putrescine, histamine, cadaverine and tyramine were present in high concentration compared to adrenaline and noradrenaline.

For liquid samples, no pre-extraction with $HClO_4$ was necessary; 1.0 ml of the sample was injected directly into the cartridge and the analysis was run without any alteration of the liquid sample. Wine samples were submitted to such analysis, a chromatogram of a wine sample being illustrated in Fig. 5. Six amines were detected: 1,3-diaminopropane, histamine, cadaverine, spermidine, tyramine and phenethylamine. Two components found in wine, 1,3-diaminopropane and phenethylamine, were not found either in cheese or ground beef samples. This observation may reflect the importance of using one method to analyze a large range of different biogenic amines in different products.

CONCLUSION

With this method we were able to analyze the most important biogenic amines in a reduced time, by the use of borate buffer, with a high resolution. Of the fifteen amines studied, ten were found, *viz.*, adrenaline, noradrenaline, 1,3-diaminopropane, putrescine, histamine, cadaverine, spermidine, spermine, tyramine and phenethylamine, in three kinds of foodstuffs. This indicates that the method is sensitive enough to detect these nitrogenous compounds in any biological fluid and solid material. The precision and the reliability of the method have been demonstrated.

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