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Simple, rapid and valid thin layer chromatographic method for determining biogenic amines in foods

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Abstract

A simple, rapid and valid thin layer chromatographic (TLC) method for determining biogenic amines in foods is established. Biogenic amines were extracted from foods with 5% TCA, the extract was washed by ethyl ether, the washed extract was dried and the dansyl derivatives were prepared. Separation of the dansylated amines was achieved on silica gel TLC plates by multiple development technique, and quantification was performed by densitometry at 254 nm. The response of the densitometer was linear and highly correlated with the amounts of dansylamines. The calculated determination coefficient (r^2) ranged from 0.997 to 0.970. The sensitivity of the method was 45, 174, 703, 101, 132, 88, 515 and 61 for TRY, PUT, CAD, SPD, HIS, SPM, TYR and PHE, respectively. The relative standard deviation (RSD) of the proposed method (repeatability) ranged from 0.39 to 6.36% for TYR (80 ng) and PHE (20 ng), respectively. The accuracy of the proposed method ranged from 86.2 to 93.2% for PHE and HIS, respectively, and there was no significant differences ($p < 0.05$) between the recoveries obtained for all kinds of food analyzed. The limit of detection of the proposed method was found to be 10ng for TRY, SPM and PHE, and 5 ng for the other biogenic amines tested.

1. Introduction

Biogenic amines have been associated with several outbreaks of food borne illnesses in the world. Histamine toxicity has been reported (Taylor et al., 1984) and its possible potentiators, cadaverine and putrescine, have been suggested (Bjeldanes et al., 1978, Parrot & Nicot, 1966). As well as, a relationship between tyramine content of foods and illnesses noted after ingestion of such foods was established (Blackwell & Mabbit, 1965). A level of 1 g histamine kg^{-1} foods was considered necessary to induce a toxic response in humans (Arnold & Brown, 1978), while 3 mg of phenylethylamine causes migraine headaches in susceptible individuals (Sandler et al., 1974), and 6 mg total tyramine intake was reported to be a dangerous dose for patients receiving monoamine oxidase inhibitors (Blackwell & Mabbit, 1965). On the other hand, amines were also investigated as a possible mutagenic precursor, since some of them may be nitrosated or act as precursors for other compounds capable of forming nitrosamines which are carcinogenic to various species of animals and pose a potential health hazard to humans (Shalaby, 1996a).

The studies of occurrence of biogenic amines, as well as controlling its limits in foods and food products, would not be possible without the supporting analytical methods which should be standardized and harmonized by different analysts (Shalaby, 1996a). The complex sample matrix, the presence of potentially interferring compounds and the occurrence of several biogenic amines simultaneously in the same aliquot of an extract are typical problems encountered in the analysis of food for biogenic amines. However, various analytical methods have been established for determination of biogenic amines in foods. Paper chromatography (Perry & Schroeder, 1963), thin layer chromatography (Shalaby, 1994, 1995), gas liquid chromatography (Staruszkiewicz & Bond, 1981), and high performance liquid chromatography (Luten et al., 1992; Eerola et al., 1993) have all been used for the separation and identification of amines or amine derivatives.

The methods used for analysis of food for amines individually would be extremely difficult and time-consuming, since they require large-scale and laborious purification, as well as, the time required for separation and determination of lot of biogenic amines is also so long (Shalaby, 1996a). On the other hand, carriers

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(trucks, coolers, ships, . . . etc.) which transport foods to processing plants or to markets cannot be detained for a lengthy determination, and the elapsed time will permit to more development of amines, so miss results will be obtained (Shalaby, 1996a). In the same time, the analysts, especially public analysts, have always been accountable to the courts for the quality and reliability of their work, in the time where there has been a steady reduction in resources for enforcement analysis (Harrison, 1995). So, an accurate, economic, and rapid analytical method is urgently needed.

In general, TLC is simple and does not require special equipment, but most of the published methods suffer from the excessive time needed for analysis and/or inaccuracy of the obtained results (semi-quantitative). Therefore, this study is conducted to establish a rapid, sensitive and accurate thin layer chromatographic method for determining biogenic amines in foods.

2. Materials and methods

2.1. Chemicals and supplies

Biogenic amines (tyramine 'TYR', tryptamine 'TRY', β-phenylethylamine 'PHE', histamine 'HIS', cadaverine CAD, putrescine 'PUT', spermine 'SPM', and spermidine 'SPD') as their crystalline hydrochlorides, as well as dansyl chloride (5-dimethylamino-naphthalene-l-sulphonyl) were purchased from E. Merck, Germany. The stock standards were prepared at a concentration of $0.5\,mg\,ml^{-1}$. Thin layer chromatography (TLC) plates (20×20 cm aluminum sheets precoated with 0.25 mm silica gel G 60) were purchased from E. Merck, Germany. All chemicals and solvents were of reagent grade and double distilled water was used.

2.2. Biogenic amines analysis

2.2.1. Extraction of amines

Ground food (50 g) was extracted with 5% trichloroacetic acid (TCA) (3×75 ml) using a Waring Blender. Each blended mixture was centrifuged and the clear extracts combined. The volume was adjusted to 250 ml with 5% TCA. The equivalent of 1 g of sample, as the TCA extract (5 ml), was introduced into a screw-capped tube and washed three times with an equal amounts of diethyl ether to remove the acid, and the ether remaining with the aqueous extract was removed by heating in water bath. Two drops of concentrated hydrochloric acid were added to the washed extract and the solution was evaporated just to dryness using a current of air and hot water bath.

2.2.2. Derivative formation

The dansylated derivatives of the amines were formed by dissolving the residue with 1 ml of saturated sodium bicarbonate solution, and 1 ml of dansyl chloride reagent ($5\,g\,l^{-1}$ acetone) was added. The sealed tube was immediately mixed for 30 s using a Vortex mixer and the reaction mixture was then left for 1 h at 40°C. The dansylamines were extracted by adding water (about 10 ml) and extracting the mixture with several portions of diethyl ether. The combined ether extracts were evaporated to dryness and the residue was dissolved in 2 ml acetonitrile for TLC.

2.2.3. Preparation of standard solution

A mixed standard solution, as dansyl derivatives, was prepared using 100 µl of each amine stock standard solution ($0.5\,mg\,ml^{-1}$). Using a current of air and a steam bath, the prepared solution was evaporated to dryness. The dansyl derivatives were prepared as described above. The residue was dissolved in 10 ml acetonitrile (intermediate standard). Afterwards, 1 ml was diluted to 5 ml using acetonitrile.

2.2.4. Separation of dansylamines

One-dimensional TLC technique described by Shalaby, (1995, 1996b) was used to separate the eight dansylamines under investigation. The standard amines and the dansylated food extracts, indicated below, were applied 2 cm from the base of the TLC plate and at 1 cm intervals using a microsyringe: 10, 20, 30, 40 and 50 µl of dansylamine standards; 10 µl of each dansylated food extract.

The plate was developed in chloroform: benzene: triethylamine (6:4:1, v/v/v) for 15 cm. The plate was removed from the jar and allowed to dry. Then, it was developed in the same direction in benzene : acetone : triethylarnine (10:2:1, v/v/v) for 15 cm. The plate was allowed to dry at room temperature, and then dried with a hair dryer to remove excess solvent before interpretation.

2.2.5. Interpretation of the chromatogram

The chromatogram after the second development was examined under longwave (360 nm) ultraviolet light to establish whether or not the dansylamines of interest are present in the sample.

2.2.6. Quantification of dansylamines

The developed TLC plate was placed under a Shimadzu CS-9000 chromatogram scanner and the absorbance value for each separated spot is recorded at wavelength of 254 nm.

2.2.7. Recovery assay

For the recovery assay, i.e. accuracy, a known amount of biogenic amine was added as a mixture to a sample of fish, meat products, and cheese free from biogenic amine to a level of $10\,mg\,kg^{-1}$ (giving a final solution, to be spotted, with a concentration of $5\,\mu g\,ml^{-1}$). Five replicates were conducted on each

contaminated sample. The recoveries were calculated by comparing the densitometer peak areas of each sample to the areas of the same standard solution.

3. Results and discussion

One-dimensional TLC technique has been successfully used for the separation of the eight biogenic amines under investigation. The quantitative determination of these amines was performed by densitometry at 254 nm. A chromatogram of co-chromatographic standard, where corned beef sample spot (10 μl) and standard spot of biogenic amines (50 ng each) are superimposed, is shown in Fig. 1. The chromatogram obtained shows complete resolution of the eight biogenic amines from each other as a sharp peak, as well as good separation of amines from interfering materials was achieved.

Methods of analysis have scientific and practical characteristics which determine the reliability and applicability of the method, respectively (Shalaby, 1996b). Among the scientific characteristics of the analytical methods are the sensitivity, precision, accuracy and detectability; among the practical characteristics are the applicability, the cost of performance, the time and equipment required, and the level of training needed (Shalaby, 1996b). So, scientific characteristics of the proposed method were considered in this study.

Standard curves of the eight dansylated biogenic amines were prepared in the range of 50–250 ng/spot. After TLC separation, the absorbance of the separated spots was densitometry determined at 254 nm, and the obtained data were subjected to linear regression analysis. The obtained results (Table 1) revealed that the response of the densitometer was linear and highly correlated with the amounts of dansylamines, where the calculated determination coefficient (r^2) ranged from 0.997 to 0.970, and each dansylamine had own linear equation. The sensitivity is defined as the change in analytical signal per unit concentration change and is represented by the slope of the analytical calibration curve (Shalaby, 1996b). Accordingly, the sensitivity of the established method are 45, 174, 703, 101, 132, 88,

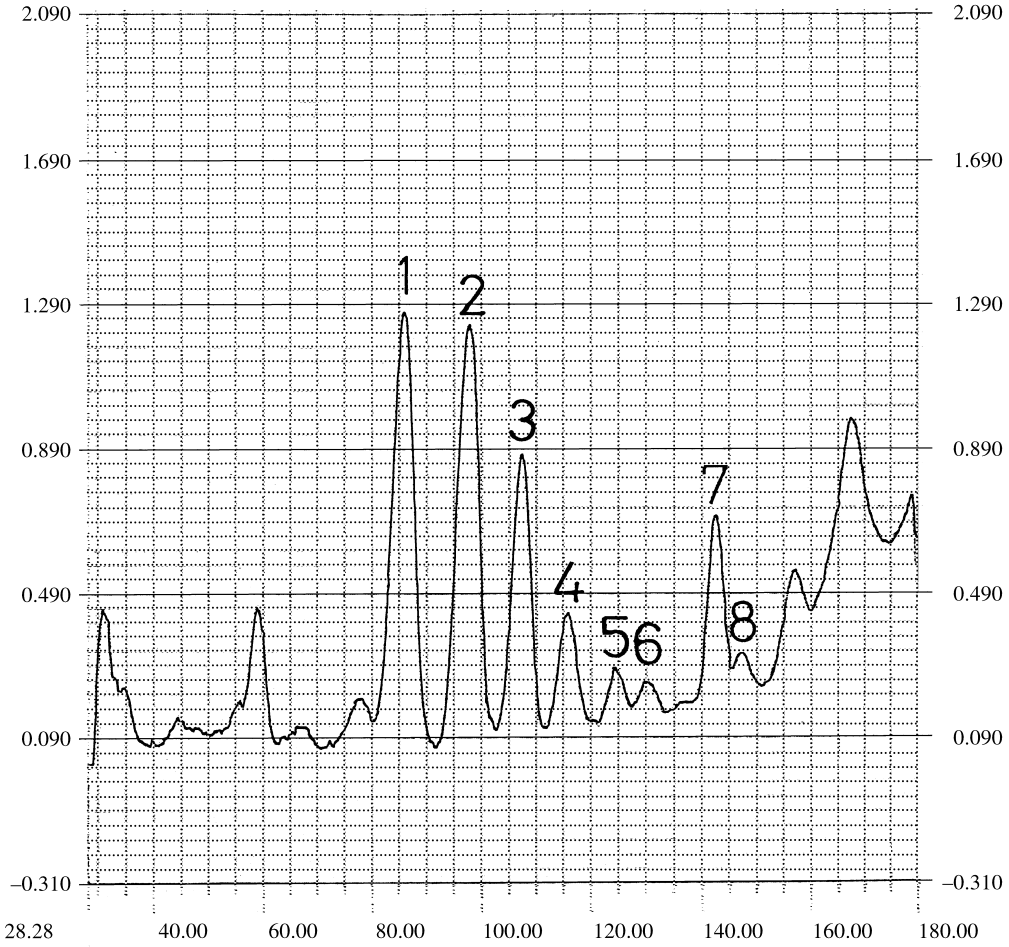

Fig. 1. Densitometric profile of co-chromatographic standard (10 μl of food extract + 10 μl of intermediate standard of biogenic amines) on silica gel TLC plate. (1; TRY, 2; PUT, 3; CAD, 4; SPD, 5; HIS, 6; SPM, 7; TYR, 8; PHE).

Table 1
Linearity of densitometer response for increasing concentrations of dansylamines on TLC

Amines	Determination coefficient (r^2)	Intercept (A)	Slope (B)	Equation
TRY	0.970	38	45	$Y = 38 + 45X$
PUT	0.982	817	174	$Y = 817 + 174X$
CAD	0.993	830	703	$Y = 830 + 703X$
SPD	0.971	−232	101	$Y = -232 + 101X$
HIS	0.986	−344	132	$Y = -344 + 132X$
SPM	0.993	−80	88	$Y = -80 + 88X$
TYR	0.997	−523	515	$Y = -523 + 515X$
PHE	0.991	−11	61	$Y = -11 + 61X$

X = ng of dansylamines on TLC.
Y = response of the densitometer (peak area).

515 and 61 for TRY, PUT, CAD, SPD, HIS, SPM, TYR and PHE, respectively. It is of interest to point out that a very high sensitivity could be obtained by the established method, i.e. a change of 703 units per 1 ng CAD change was obtained indicating that a change of 0.1 ng CAD (20 ng g^{-1}) could easily be detected and determined where the densitometer response will be changed by 70.3 units. Nevertheless, since the sensitivity depends directly on the apparatus used, it will be changed according to the apparatus available, therefore, each TLC plate should contain a standard curve along with the sample spots.

Precision is a measure of variability of the results. A common description of precision is by standard deviation or relative standard deviation (coefficient of variation, c.v.) of a set of replicate results (Shalaby, 1996b). The precision may relate to the within-laboratory error of a method (repeatability) or to the between-laboratory error of a method (reproducibility). The repeatability of the proposed method was estimated and the obtained results are given in Table 2. Three different amounts of each dansylated amine (20, 40 and 80 ng, representing concentrations of 4, 8 and 16 μg g^{-1} sample) were analyzed, where ten replicates of each amount were applied on the TLC plate, developed and determined by densitometry. The obtained results indicate that a relative standard deviation (RSD) of less than 10% was recorded for all amines tested. A maximum RSD of 6.36% was obtained for PHE when 20 ng amine were applied on the TLC, while the minimum RSD (0.39%) was reported for 80 ng TYR applied on the TLC, representing the combined errors of TLC resolution, application and apparatus detection and quantification. Generally, it could be observed that as the amounts of dansylamines on the TLC increased, the calculated RSD value decreased.

Accuracy is a measure of systematic deviation of the obtained results from the true value (Shalaby, 1996b). The smaller the systematic part of the experimental error, the more accurate is the procedure. The accuracy of a method is usually expressed in terms of percentages of recovery. Five replicates 50-g samples of fish, meat products and cheese free of biogenic amines were spiked with sufficient quantities of standard solution to give 10 mg kg^{-1} of each amine in the samples, after extraction, washing and derivatization, the percent recoveries were calculated and the results are given in Table 3. It could be noticed that recoveries were high by the proposed procedure for all samples subjected to analysis. The highest recovery was reported for HIS and the lowest recovery was for PBE in all sample analyzed. The HIS recoveries were 91.8, 93.2 and 94.6% for fish, meat products and cheese, respectively. The corresponding recoveries recorded for PHE were 86.6, 85.2 and 86.8%. The overall recovery was ranged from 93.2 to 86.2% as calculated for HIS and PHE, respectively. It is of interest to point out that statistical analysis of the obtained data indicates that there was no significant differences ($p < 0.05$) between the recoveries of all kinds of food analyzed, which reflect the suitability of this method to determine biogenic amines in foods as general.

Limit of detection of a method is the lowest concentration level that can be determined to be statistically different from an analytical blank. IUPAC states that the limit of detection, expressed as a concentration, is derived from the smallest amount that can be detected

Table 2
Precision of the TLC method for analysis of amines

Amine	Amounts applied (ng)	Detected amounts (ng)		SD	RSD
		Range	Mean		
TRY	20	19.2–20.7	20.02	0.55	2.75
	40	39.2–41.1	39.96	0.80	2.00
	80	78.9–81.6	79.88	1.07	1.34
PUT	20	19.1–20.8	19.90	0.70	3.52
	40	38.3–41.6	40.00	1.31	3.28
	80	77.2–82.0	79.96	1.10	1.38
CAD	20	19.6–20.4	20.00	0.34	1.70
	40	39.7–40.2	39.98	0.19	0.74
	80	78.9–80.5	79.86	0.59	0.48
SPD	20	19.4–20.1	19.82	0.28	2.01
	40	38.4–40.5	39.74	0.80	1.41
	80	78.2–81.0	79.60	1.02	1.28
HIS	20	19.4–20.6	19.84	0.47	4.41
	40	38.5–42.9	40.62	1.79	2.37
	80	78.2–82.7	80.04	1.64	2.05
SPM	20	19.4–20.6	20.00	0.47	2.35
	40	39.4–41.2	40.02	0.70	2.09
	80	78.0–82.3	79.84	1.67	1.75
TYR	20	19.8–20.4	20.00	0.25	1.25
	40	39.8–40.2	39.98	0.15	0.83
	80	79.5–80.4	79.98	0.31	0.39
PHE	20	18.4–21.5	19.96	1.27	6.36
	40	39.1–40.5	40.00	0.62	2.96
	80	77.2–83.4	79.86	2.36	1.55

SD: Standard deviation.
RSD: Relative standard deviation.

Table 3
Recovery (%) of biogenic amines from different food samples obtained by the proposed method

Amines	Fish		Meat products		Cheeses		Overall	
tested	Average	SD	Average	SD	Average	SD	Average	SD
TRY	87.8	4.49	88.8	6.58	88.2	4.71	88.3	5.36
PUT	90.0	3.69	91.2	4.26	88.6	4.45	89.9	4.3
CAD	90.6	3.72	93.0	1.89	90.8	3.76	91.5	3.42
SPD	85.4	3.61	85.2	5.31	88.2	4.83	86.3	4.84
HIS	91.8	4.31	93.2	2.40	94.6	3.93	93.2	3.82
SPM	87.0	4.09	85.6	5.16	87.2	2.99	86.6	4.24
TYR	91.6	2.73	90.8	5.91	90.0	2.09	90.8	4.00
PHE	86.6	3.61	85.2	1.72	86.8	6.11	86.2	4.28

with reasonable certainty for a given analytical procedure (Long & Winefordner, 1983). The limit of detection of the proposed method was determined by spotting increasing amounts of standard biogenic amines on the TLC (i.e. 1, 2, 3,..... 10 µl of working standard solution which represent 1, 2, 3,..... 10 ng of each amine / spot), developed and quantitated. The limit of detection (the lowest amount that can be detected and quantitated by the apparatus used) was found to be 10 ng for TRY, SPM and PHE, and 5 ng for the others biogenic amines tested which is in parallel with previously findings of Shalaby, (1995).

Desired accuracy and precision are quite important since the usability of the method can be excluded or confirmed depending on the accuracy and precision of the method (Shalaby, 1996b). From the aforementioned results, it is clear that the proposed method has good recovery, more precision and high sensitivity. Furthermore, the proposed procedure has several other advantages, among the practical characteristics, over the other methods including clean-up step is omitted, consumption of solvents is much reduced, and the sample-processing time is shorter. On the other hand, the proposed method was successfully used to analyze of different food items and the results will be reported elsewhere. Accordingly, TLC-densitometry can be applied to determine biogenic amines in foods as general, and it can readily be employed in most laboratories because of its simplicity, rapidity and validity.

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