Capillary Gas Chromatographic Determination of Dopamine by Pre-column Derivatization Method using Trifluoroacetylacetone as Derivatizing Reagent

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(Received on 13th May 2010, accepted in revised form 23rd June 2010)

Summary: Capillary gas chromatographic separation of putrescine (Pu), cadaverine (Cd), dopamine (DA) and octopamine (OA) as derivatives of trifluoroacetylacetone (FAA) has been carried out using a column HP-5 (30 m × 0.32 mm id), temperature 100 °C for 1 min, followed by heating rate 10 °C/min up to 250 °C using FID detection. Nitrogen flow rate was 4 mL/min and split ratio was 10:1 v/v. The linear calibration graphs were ranging between 2.5-25 μg/mL. The method was applied for the determination dopamine in pharmaceutical preparations with relative standard deviation (RSD) within 1.5-2.5% and recovery of 98% with RSD 1%.

Introduction

Dopamine (DA) is an important biologically active compound and plays a role as neurotransmitter in mammals. It is a member of catecholamines [1]. putrescine (Pu) and cadaverine (Cd) are biogenic amines and are constituent of living cells. Pu may be an indicator of spoilage of fish during storage [2]. Octopamine (OA) is a naturally occurring trace amine and is a major neuromodular with neurotransmitter and neurohormone functions. The determination of dopamine from the pharmaceutical preparations and biological fluids and Pu from food items continue to be of analytical interest. More analytical methods are based on high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) [3, 4]. The sensitive HPLC procedures are based on the electrochemical detection [5] or fluoremetric determination with pre-column derivatization [6-9]. The HPLC procedures indicate sensitivity for their determination from biological fluids, but capillary gas chromatography has higher resolution power for organic compounds with lower running cost as compare to HPLC. GC is also free from the problems of safe disposal of eluting solvent and volatilization of analyte at operating conditions could remove matrix effect. The GC of catecholamines has been carried out using pentafluoropropionic acid anhydride, diazoethane and *n*-butylboronic acid [10, 11]. *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide and 2-phenylbutyl chloride [12], pentafluorobenzyl bromide [13], heptafluorobutyric anhydride [14], pentafluoropropionic anhydride [15] and ethylchloroformate [16]. The fluorinated acid anhydrides are expensive and their use may have damaging effects on GC column. The use of simple and inexpensive derivatizing reagent for GC determination of DA, Pu, Cd, and OA could be of analytical interest. Trifluoroacetylacetone (TFAA) has been used for the determination of Pu, and Cd from food products by GC mass spectrometry (MS) [17], and serum of cancer patients by GC-flame ionization detection (FID) [18]. TFAA has also been used for GC determination of phenylpropanolamine [19], and isoniazid [20] from pharmaceutical preparations. The present work examines the GC separation and quantitation of Pu, Cd, DA, and OA using TFAA as derivatizing reagent and determination of DA from pharmaceutical preparations.

Results and Discussion

TFAA reacts with compounds containing primary amino functional groups Pu, Cd, DA and OA to form Schiff bases (Fig. 1). The product was analyzed by GC. A constant volume (1 μL) with split ratio 10:1 was injected and average GC-FID response

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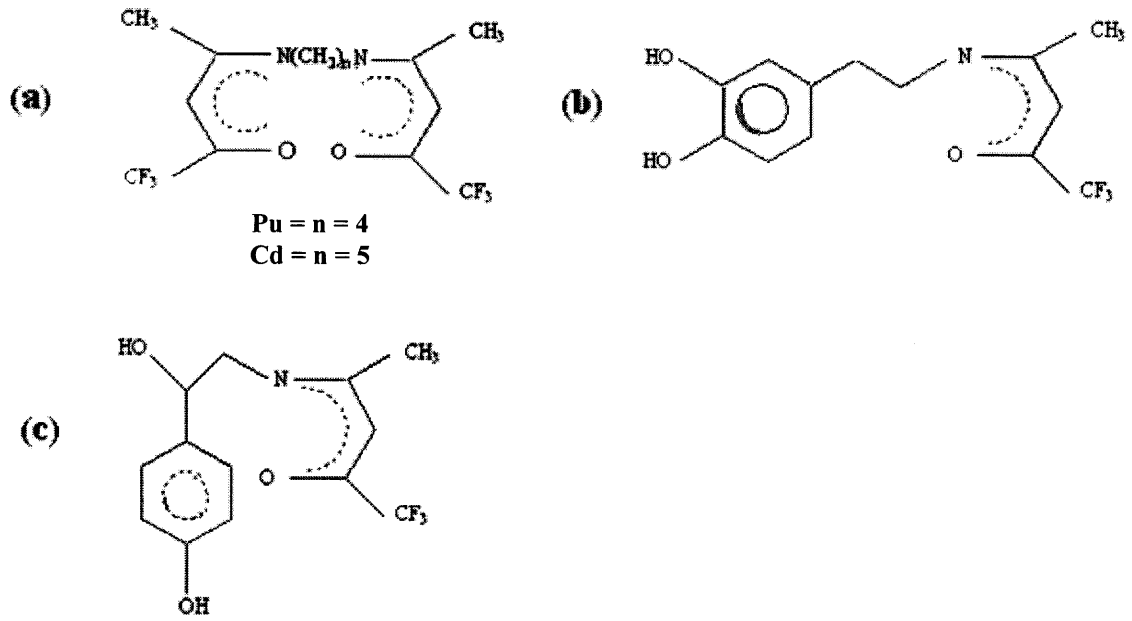

Fig. 1: Structural diagrams of TFAA derivatives of (a) Pu, and Cd (b) DA (c) OA.

(n=4) (peak height/peak area) for each component was noted. All the components eluted separately with good resolution. The effects of pH, derivatizing reagent concentration and warming time on derivatization were also examined. The reaction mixture was warmed at 70 °C for 5-30 min at an interval of 5 min. Maximum response was obtained within 10-20 min and reaction time of 20 min was selected. Different buffer solutions within pH 1-10 at unit interval were examined. The maximum response was obtained at pH 5 to 8 for Pu and DA and pH 7 using sodium acetate buffer proved optimal for the extraction as TFAA derivative and was selected (Fig. 2). The amount of derivatizing reagent (2 % v/v in methanol-water 1:1, v/v) added was varied between 0.5 and 3.0 with an interval of 0.5 mL and similar response was observed above the addition of 1 mL and 2 mL was selected. The solvents chloroform, 1,2-dichloroethane and methyl isobutyl ketone (MIBK) were examined for the extraction of derivatives and each solvent indicated a similar response, but chloroform was used for extraction of the derivatives. The derivatives did not show any change in response up to 24 hours and indicated high solution stability of the derivatives. GC separation of Pu, Cd, DA and OA as derivatives of FAA was next examined from column HP-5 (30 m × 0.32 mm id) using different temperature elution programs and different nitrogen

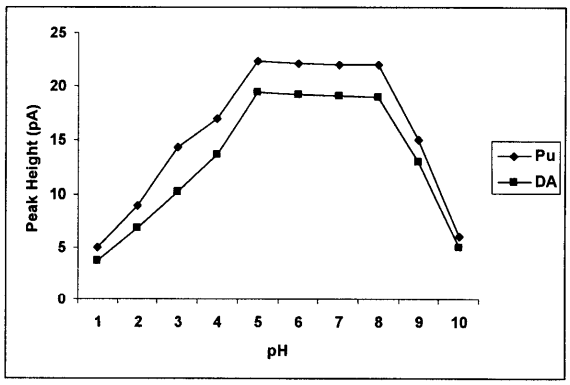

Fig. 2: Effect of pH on the derivatization of Pu, DA, Cd and OA with TFAA. GC conditions: The column HP-5 (30 m × 0.32 mm id) with a film thickness of 0.25 μm at an initial column temperature of 100 °C for 1 min with heating rate 10 °C/min up to 250 °C. Nitrogen flow rate was 4.0 ml/min and split ratio 10:1, v/v. Injection port and detector temperatures were 250 °C and 300 °C, respectively. Nitrogen make up flow rate was 45 mL/min. FID air and hydrogen flow rates were 450 and 40 mL/min, respectively.

flow rates, to obtain complete separation between all the four components with retention time within

acceptable limits. At the optimized conditions, symmetrical peaks with complete separation were obtained within 10 min (Fig. 3). The repeatability of the separation was examined in terms of retention time and peak height/peak area (n=5) and relative standard deviations (RSD) were obtained within 1.5% and 2.5%, respectively. Intra day variations were again measured in terms of retention time and peak height/peak area (n=4) and RSD were obtained within 0.6-1.6 and 2.0% respectively. Linear calibration curves were obtained by plotting average peak height/peak area (n=4) versus concentration corresponding to DA 5-25 μg/mL, OA 6-30 μg/mL, Pu 2.5-15.0 μg/mL and Cd 2.5-15.0 μg/mL. The coefficient of determination (R^2) for five to six points of calibration for Pu, Cd, DA and OA was obtained within 0.9964-0.9988 (Table-1). That limit of detection (LOD) and limit of quantifications (LOQ) measured as signal to noise ratio (3:1) and (10:1) for Pu, Cd, DA and OA were determined as 0.14, 0.16, 0.25 and 0.33 ng, and 0.42, 0.48, 0.75, and 1.0 ng, respectively. DA is available in pharmaceutical preparations and to determine its amount in pharmaceutical preparations consisting of injections Dopamine and Intropin Dupont were analyzed and the results (Table-2) obtained agreed with relative deviation within 1.0 to 2.0 % from labeled values with RSD 0.9-1.1 %. % Recovery of DA from pharmaceutical preparations calculated by standard addition was obtained 98 % with RSD 1.0 %.

Experimental

Materials

Trifluoroacetylacetone (Fluka Switzerland), dopamine (DA), putrescine (Pu), cadverine (Cd), octopamine (OA), ethanol and chlororform (E-Merck

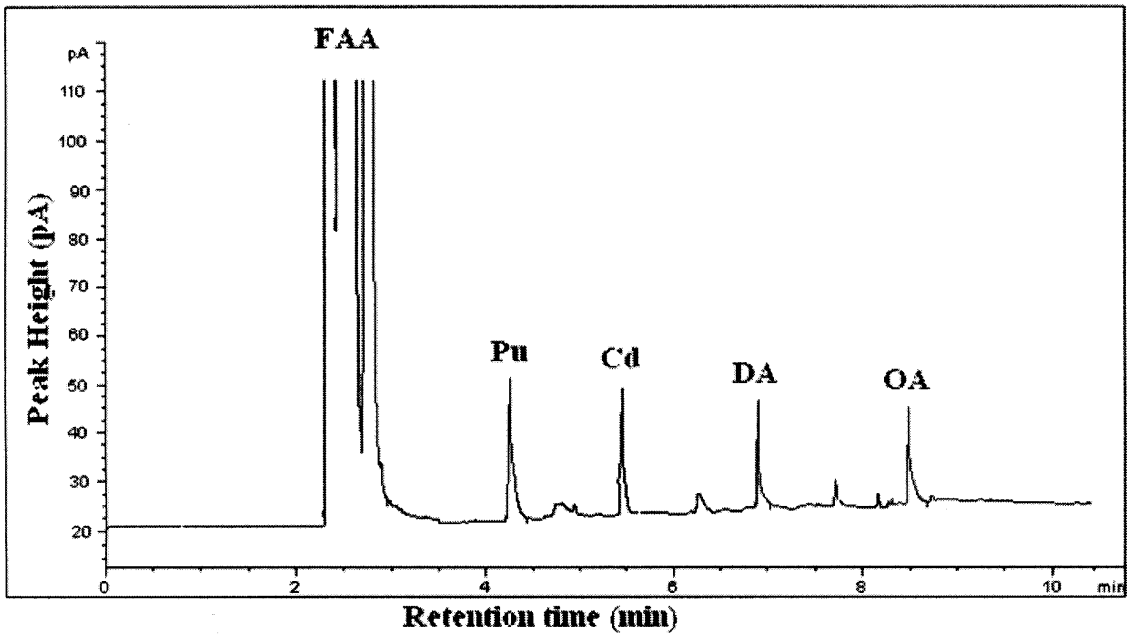

Fig. 3: GC separation of (1) solvent and reagent (2) Pu (3) Cd (4) DA and (5) OA as derivatives of TFAA. GC conditions as described in Fig. 2.

Table-1: Quantitative GC data of TFAA derivatives.

S. No	Compound	Calibration range μg/mL	Coefficient of determination	Linear regression equations	Detection limits μg/mL	Limits of quantitation μg/mL
1	DA	5-25	0.9941	Y=1.0807x+0.5733	2.5	7.5
2	OA	6-30	0.9965	Y=0.5257x-0.7333	3.3	10.0
3	Pu	2.5-15	0.9988	Y=1.5443x-2.4667	1.4	4.2
4	Cd	2.5-15	0.9985	Y=1.4114x-2.3667	1.6	4.8

Table-2: Determination of dopamine from pharmaceutical preparations.

S. No	Name of preparations (Company)	Compounds present	Amount labeled mg/mL	Amount found mg/mL (RSD %)	% Relative deviation (RD)
1	Dopamine HCl	Dopamine HCl, Citric acid, Sodium citrate buffer	40	39.4 (1)	1.5
2	Intopin dupont	Dopamine HCl	40	39.0 (1)	2.5

Germany), sodium hydroxide (Fluka Switzerland), hydrochloric acid (37%), potassium chloride, acetic acid, sodium acetate, ammonium chloride, ammonia (32%), sodium bicarbonate and sodium carbonate (E-Merck Germany) were used. Buffer solutions at unit interval were prepared from the following: hydrochloric acid (0.1M), potassium chloride (1M), acetic acid (1M), sodium acetate (1M), sodium carbonate (saturated) and sodium bicarbonate (1M). Standard solution of Pu, Cd, OA and DA containing 1000 μg/mL were prepared by dissolving their appropriate amounts in 0.1M hydrochloric acid. Further dilutions were made with 0.1M HCl. Injection Dopamine (Abbot lab. Karachi), Intropin Dupont (Knoll Phamaceutical Ltd, Korangi, Karachi) were obtained from local market, Hyderabad, Pakistan.

Instrumentation

All pH measurements were made with Orion 420A pH meter with glass electrode and internal reference electrode (Orion Research Inc; Boston, USA). The gas chromatographic studies were carried out on Agilent model 6890 Net work GC system gas chromatograph (Agilent Technologies Inc; USA) coupled with split/splitless injector operated in split mode, flame ionization detector (FID), hydrogen generator Parker Balson Model H_2-90, Analytical gas system (Parker Hannifin Haverhill, M.A, USA) and pure nitrogen (British Oxygen Company (BOC), Karachi). The gas chromatograph was controlled by the computer with Chemstation software (Agilent Technologies). The capillary column HP-5 (30 m × 0.32 mm id) with film thickness of 0.25 μm (J & W Scientific Corporation, USA) was used throughout the study.

GC-FID Method

Solution (0.1-1.0 mL) containing Pu (2.5-15.0 μg), Cd (2.5-15.0 μg), DA (5-25 μg) and OA (6-30 μg) was added 2 mL of FAA (2% in methanol-water 1:1, v/v) and 2 mL of sodium acetate buffer pH 7. The contents were warmed at 70°C for 20 min and cooled to room temperature, chloroform (1 mL) was added and contents were mixed well. The layers were allowed to separate and an aliquot of organic layer was transferred to screw capped sample vial. The solution (1 μL) was injected and compounds were eluted from GC column HP-5 (30 m × 0.32 mm id) at an initial column temperature of 100 °C for 1 min, followed by heating rate 10 °C/min up to 250 °C. Injection port and detector temperatures were maintained 280 °C and 300 °C, respectively. The nitrogen flow rate was 4.0 mL/min. The split ratio was 10:1, v/v and nitrogen was used as make up gas. The flow rates of hydrogen and air for FID were maintained at 40 and 450 mL/min, respectively. Average peak height (n=4) was measured for each of the component.

Analysis of DA from Pharmaceutical Preparations

The solution (0.1 mL) from Dopamine and Intropin Dupont injections (1 mL) was diluted to 100 mL with aqueous solution (1:1 water/methanol) and again 0.1 mL from Dopamine and Intropin Dupont was diluted to 10 mL. The 1 mL solution from each was transferred to sample vial and *"GC-FID Method"* was followed. The quantitation was carried out using external calibration curves.

% Recovery of DA from Pharmaceutical Preparations by Standard Addition Method

The solution (0.1 mL) from dopamine injection was diluted as *"Analysis of DA from Pharmaceutical Preparations" as described above* and two solutions of 1 mL of each were transferred to sample vials. A solution was processed as *"GC-FID Method"* and the other was added DA (5.0 μg) and again procedure *"GC-FID Method" as described above* was followed. The quantitation was carried out by linear calibration curves and an increase in response with DA was observed.

Conclusion

Analytical procedure based on capillary gas chromatography determination of dopamine using trifluoroacetylacetone as derivatizing reagent has

been developed for the determination of dopamine from pharmaceutical preparations.

Acknowledgement

Pakistan Science Foundation Islamabad is acknowledged for the Grant of Research Project S-SU / chem. (411).

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