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Liquid Chromatographic Determination of Dopamine, Methyldopa, L-dopa and Tyrosine in pharmaceutical preparations using 4- Dimethylaminebenzalaldehyde as a Derivatizing Reagent

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Abstract: Analytical procedure has been developed for the determination of Dopamine (DA) methyl dopa (MD) L-dopa (LD) and Tyrosine (TY) by High Performance Liquid Chromatography using 4- Dimethylaminebenzalaldehyde as pre-column derivatizing reagent, The separation was achieved from Phenomenex C – 18, 5 µm (150×4.6mm id) column when eluted isocritically with methanol-acetonitrile-water (58;3;39v/v/v with a flow rate of 1ml/min. The detection by UV was at 280 nm. The linear calibration curves were obtained within the range of 2-10 µg/ml with limit of detection within 0.065-0.098, 0.062-0.075 and 0.081 µg/ml for each of the compound. The derivatization , separation and quantitation was repeatable with relative standard deviations (RSDs) within2.3-3.8%(n=5).The method was applied for the determination of dopamine methyldopa, L-dopa and tyrosine from pharmaceutical preparations with RSDs within2.4-4.1% (n=3) and the results agreed with labeled values. The pharmaceutical preparations were also analyzed by standard addition and recovery of analytes was calculated within 95-98% with RSD 4.1%. The pharmaceutical additives did not affect the determination.

Keywords: Dopamine, Methyl dopa, Perkinson’s, Aldomet, Sinmet

1. INTRODUCTION

Dopamine (Dihydroxyphenylethylamine) (DA) is an important neurotransmitter in the mammalian central nervous system and is member of catecholamines. DA is involved in neuropsychiatric disorders such as. Perkinson’s disease . (Hornykiewiez, *et al.*, 1987), Schizophorenia, epilepsy, attention deficit hyperactivity disorder (Ernst, *et al.*, 1998)and drug dependence (Simith, *et al.*, 2006) Levodopa (L - 3, 4 dihydroxyphenylalanine) (L-dopa) is most commonly used for symptomatic treatment for. Parkinson s disease, but is itself is largely inert. L-dopa is able to cross the blood brain barrier and is decarboxylated to dopamine by dopa decarboxylase (Kollor, *et al.*, 1998 Bugamelli, 2011) In the human body L-dopa is synthesized from amino acid Tyrosine by tyrosine hydroxylase (Uchino, *et al.*, 2002). Methyl dopa is an important antihypertensive that take part in catecholamine metabolic pathway and could therefore alter, the entry and metabolism of L-dopa in the brain (Muzzi, *et al.*, 2008),. Dopamine, methyldopa, L-dopa and tyrosine are important biological compounds and are present in pharmaceutical preparations. Their determination individually and in combination in the drugs in dosage form could be of analytical interest for quality control.

The number of analytical procedures have been developed for the determination of dopamine methyldopa, L-dopa and tyrosine individually and in different combinations (Muzzi, *et al.*, 2008), More analytical procedures are based on high performance liquid chromatography (HPLC) (Bugamelli, 2011 (Muzzi, *et al.*, 2008), Baranowska,2008) Sexan,2004) Ragab, *et al.*, 2000) Jeon *et al.*, 1991), gas chromatography (GC) (Khuhawar *et al.*, 2008) and on capillary electrophoresis *et al.*, Way, 2005) Kartsova *et al.*, 2004) Zhu, *et al.*,1997) The analytical procedure based on HPLC are connected with electrochemical (Bugamelli, *et al.*, 2011) Sexan *et al.*, 2004) Letekier *et al.*, 1997) Machida *et al.*, 2006), fluorometric Nikolajsen *et al.*, 2001) Chan *et al.*, 2000) (Muzzi, *et al.*, 2008),. Mell 1978) and mass spectroscopic Tornkvist *et al.*, 2004) Nohta *et al.*, 1997) detections. HPLC with fluorescence or UV detection is more suitable and is therefore more widely used Letekier *et al.*, 1997). The derivatizing reagents for determination of the compounds include 1,2 diphenylethylenediamine Mistsui *et al.*, 1985) 2-phenylglycinonitrile and benzylamine Nohta *et al.*, 1997) 9-fluorenylmethyloxy carbonyl chloride Chan *et al.*,2000) glycylglycine (Seki *et al.*,1990)

The present work examines DMB for the HPLC coupled with commonly available UV detection for the determination of dopamine,l-dopa, methyldopa and tyrosine from pharmaceutical preparations. The conditions are optimized for pre-column derivatization, HPLC separation, quantitative determination and validation of analytical procedure by standard addition.

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2. MATERIALS AND METHODS

Experimental

The standard solutions containing (1mg/ml) of dopamine (DA) methyl-dopa (MD) L-dopa (LD) Tyrosine (TY) (E-Merck, Germany) were prepared in 0.1M hydrochloric acid by dissolving 50 mg each in acid solution. Further solutions were prepared by appropriate dilution as required. 4-Dimethylaminebenzalaldehyde(DMB) (Fluka Switzerland) methanol (E-Merck, Germany) Acetonitrile (J.T Baker Holand) ,sodium hydroxide (Fluka Switzerland),hydrochloric acid (37%), potassium chloride, acetic acid sodium acetate, sodium bicarbonate, sodium carbonate, ammonium chloride and ammonia solution (E-Merck Germany) methanol (E-Merck, Germany) were used. Buffer solutions at unit interval were prepared from the following : Potassium chloride (1M) adjusted to pH 1 and 2 with hydrochloric acid (0.1M), sodium acetate (1M) acetic acid (1M) (pH 3-6) ammonium acetate (1M) (pH 7) sodium bicarbonate (1M) sodium carbonate (saturated) (pH 8-9), and ammonium chloride (1M) –ammonia solution (1M) (pH 10) were used. Injection Dopamine (Abbot lab. Karachi), Intropin Dupont(Knoll Pharmaceutical Ltd, Korangi Karachi) Aldomet tab (MSD) ,Sinmet tab (MSD) and Solger cap (USA)were purchased from local market Hyderabad Sindh Pakistan.

2.1 Instrumentation.

All pH measurements were made with Orion 420A pH meter with glass electrode and internal reference electrode (Orion Research Inc, Boston, USA.).Spectrometric studies were carried out with Perkin Elmer Lambda 35 (Perkin Elmer Singapur) double beam spectrophotometer, controlled by the computer Lambda 35 software. Dual silica cuvettes of 1cm path length were used throughout the study.

Chromatographic studies were carried out on an Agilent HPLC system 1100 series (Agilent technology USA) equipped with G1300A Lc pump, G1315B diode array detector and 7725 Rhedyne injector. Data was obtained by the computer with chemstation data acquisation software. Reverse phase L c was performed isocritically at room temperature using column Phenomenex C18, 5 µm (150×4.6mm id) used throughout the study.

2.2 Spectrophotometric Procedure.

The solution (1-2ml) containing DA, L-dopa, MD or TY was transferred to 10 ml volumetric flask separately to obtain final concentration within 10-60µg/ml each of the component .

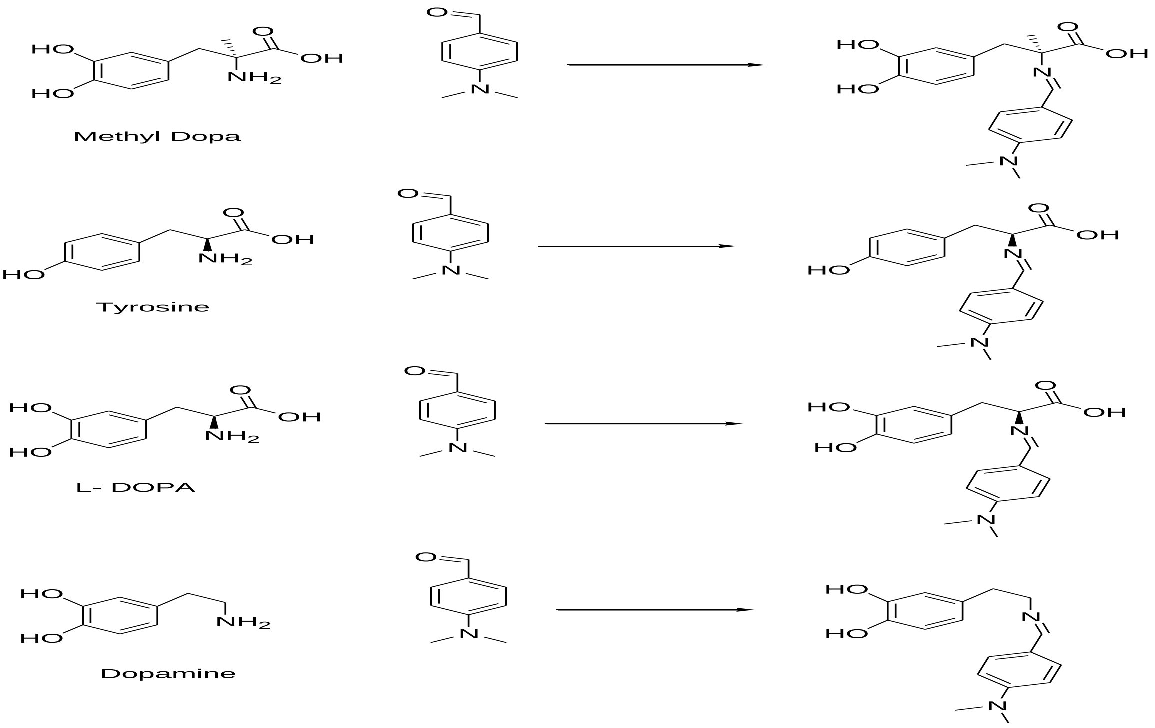

Fig. 1 : Chemical reaction of DMB together with structural formula of MD, L- dopa, TY and DA.

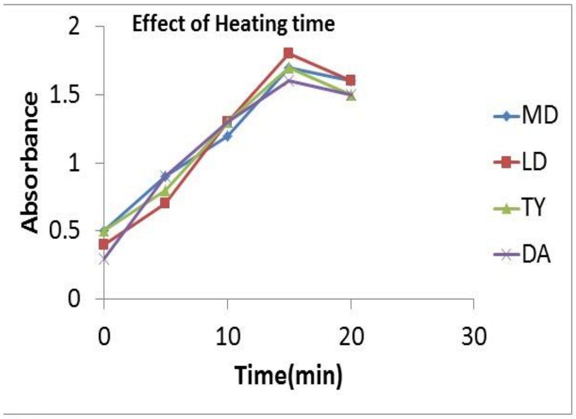

Fig. 2

Fig/ 2: Effect of heating time on the absorbance of MD, L- dopa, TY and DA as derivatives of DMB using heating time at 75 ^{0}C.for 15 min.

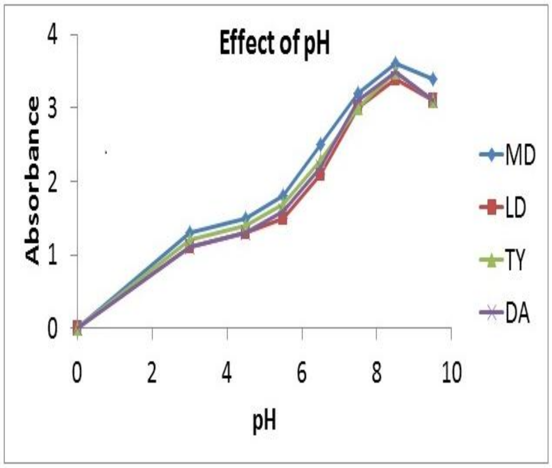

Fig. 3

Fig/ 3: Effect of pH on the absorbance of MD, L- dopa, TY and DA as derivatives of DMB using heating time at 75 ^{0}C.for 15 min.

Effect of heating time at 75 ^{0}C of reagent DMB to analytes MD, L-dopa, TY and DA. Solution was added DMB solution 1ml (3%methanol), boric acid – sodium tetraborate buffer pH 9 (1ml) and contents were heated in water bath at 75 ^{0}C for 15 min. The volume was adjusted to mark with methanol and the absorption spectra were recorded within 500-250 nm against reagent blank. The reagent blank was prepared following the same procedure without addition of analytes.

2.3 HPLC Method

The solution (0.2-1.0 ml) containing DA (10-60µg), L-dopa(10-50µg) MD (10- 60µg) and TY (15-60µg) were transferred to 5ml volumetric flask and added 1 ml of DMB (3%in methanol) reagent and 1ml of boric acid –sodium tetraborate buffer pH 9. The contents were heated for 15 min on water bath at 75 ^{0}C and after cooling at room temperature, the volume was adjusted with methanol to the mark. The solution was mixed well and 20 µl was injected on column Phenomenex C18, 5 µm (150×4.6mm id) and derivatives were eluted with a ternary mixture of methanol-acetonitrile-water (58:3:39v/v/v) with a flow rate of 1ml/min UV detection by photodiode array was at 280 nm. Average peak height /peak area (n=4) was measured for each of the component.

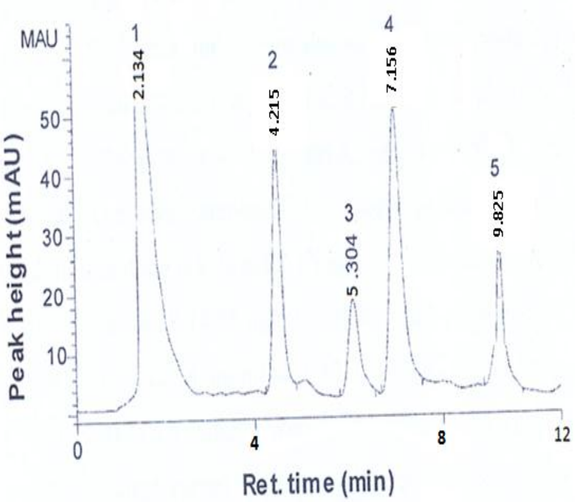

Fig-4. Separation of derivatives of MD, L- dopa, TY and DA together with reagent DMB from the column Phenomenex C-18 column when eluted isocratically with methanol – acetonitrile-water (58:3:39 v/v/v) with a flow rate of 1 ml/min. UV detection by photodiode array was at 280 nm

3. Analysis of pharmaceutical preparations.

Three injections of 5ml each Dopamine and Intropin Dupont containg 200mg /injection of dopamine were mixed and 1ml of composite mixture was diluted to 100 ml with water-methanol (1:1 v/v). The solution (0.1ml) was again diluted to 10 ml. The solutions (0.2 and 0.4) were transferred to 5ml volumetric flask and above HPLC procedure was followed.

Three Aldomet tablets each weighing 0.35gm were ground to fine powder and amount of 0.14g was weighed, and dissolved in methanol-water (1:1v/v). The solution was filtered and final volume was adjusted to 100 ml.

Three Sinmet tablets each containing the constituents 0.37g were mixed thoroughly and 0.073g was weighed and dissolved in methanol-water (1:1v/v).The solution was filtered and volume was adjusted to 50ml. Similarly three Solger capsules, each weighing 0.79g were ground to fine powder and 0.079g was weighed and dissolved in methanol-water(1:1v/v).The solution was filtered and final volume was adjusted to 50ml. Each of the solution (1ml) of Aldomet , Sinmet tablets and Solger capsules was diluted to 10 ml and the solution 0.2ml each was transferred to 5ml volumetric flask and above HPLC procedure was followed. The quantitation was made from linear regression equation Y= ax+b based on external calibration curve.

3.1Analysis of pharmaceutical preparation from linear calibration curve with spiked sample.

The solution of Dopamine injection, Intropin Dupent injection, Aldomet tablets, Sinmet tablets and Solger capsules were prepared as analysis of pharmaceutical preparation. The solution 0.2 and 0.4ml were taken from each separately in duplicate and a solution was added 10-30µg, DA, L-dopa, MD or TY. All the solutions were analyzed following same analytical procedure. The quantitation from linear calibration curve and from an increase in the response (Average peak height/peak area with added standards be noted.

4. RESULTS AND DISCUSSION

The reaction of DMB towards dopamine, methyl dopa, l-dopa and tyrosine were examined spectrophotomatically. The derivatization conditions were optimized in the terms of pH, the amount of derivatizing reagent DMB added per analysis, warming time and temperature for derivatization. The condition which gave maximum absorbance was considered optimum.

The effect of pH was examined within 1-10 at unit interval for maximum values. The reagent DMB (3% in methanol) was varied within 0.2-1.0 ml at the interval of 0.2ml. The warming time and temperature was varied between 5-30min at an interval of 5min and 60-100^{0}C .at an interval of 10^{0}C. The reactions were monitored at 254-280 nm on HPLC. Better absorbance was observed within pH 7-9 within maximum at 9 with sodium tetra borate buffer and was selected. The addition of derivatization reagent was available to reaction mixture. The addition of 1ml to 5ml volumetric flask was considered as optimal. Same response was obtained by warming at temperatures within 70-100 and 10-25 min and heating at 75 ^{0}C for 15 min in water bath was considered as optimal. The derivative once formed was highly stable and did not show any change in absorbance up to 24 h. All the derivatives obeyed the beer's law and indicated as stable derivatives formed.

For the sensitive and simultaneous determination reverse phase HPLC was examined. All the derivatives easily eluted with methanol –water and separated from the excess of derivatizing reagent. Attempt was then made to separate dopamine, methyldopa, L-dopa, tyrosine and octopamine derivative of DMB using different solvent systems. Maximum separation was obtained from phenomenex C-18 column when eluted isocratically with methanol-acetonitrile-water (58:3:39 v/v/v) with a flow rate of 1 ml/min and UV detector was fixed 280 nm. The capacity factor (k') were calculated to 1.3, 1.9,-2.8 and 4.7 for methyldopa, L-dopa, tyrosine and dopamine as derivatives of DMB respectively. The separation was repeatable with relative standard deviation (RSD) within 4.1 % (n=5).

Table: 1-Quantitative HPLC data of derivatives MD, L- dopa, TY and DA

S. No	Compound	Calibration rangeµg /ml	Coefficient of determination	Linear regression equation	Detection limits µg/ml	Limits of quantification µg/ml
1	MD	2-10	0.996	Y=4. 4643x +0.071	0.061	0.457
2	L-dopa	2-10	0.996	Y=6.952x+0.2286	0.091	0.687
3	TY	2-10	0.993	Y=3. 255x+0. 2292	0.071	0.579
4	DA	2-10	0.994	Y=4. 9341x+ 0.79	0.056	0.258

Table: 2 -Determination of bioactive compounds from pharmaceutical preparations

	Name of preparations'	Compound present	Amount labeled mg/ Tablets/ Capsules	Amount found mg/Tablets /Cap (RSDs)	% recovery
1	Dopamine	Dopa amine	40	38.2 (2.91)	98
2	IntopinDupent	Dopa amine	40	38.9 (3.13)	97
3	Aldomet	M-dopa	250	245 (4.1)	98
4	Sinmet	L-dopa	250	244 (4.1)	97.8
5	Solger	Tyrosine	500	490 (3.12)	94

4.1 **Quantitation:**

The linear calibration curves were obtained by plotting average peak height/peak area (n=4) versus concentration within.2-1 µgml with coefficient of determination _R2) within 0.992-0.996. The limits of detection (LoDs) and limits of quantitation measured as signal-noice ratio (3:1) and (10:1) were obtained within 0.061 µg/ml and 0.689 µg/ml respectively. The dervitization,separation and quantition was repeatable in terms of retention time and average peak height/peak area(n=4) inter(n=5 and itera (n=4) day when carried out by the same operation on the same day and different days and RSDs were obtained within 2.61, 2.91, 3.12 and 4.11% respectively.

The pharmaceutical preparations additives methylparaben, Propylparaben, maitol, gum acacia, sodium lauryl sulfate glactose,lactose, talc, magnesium stearate, sucrose, sodium chloride, sodium laurilsulfate (SLS) methyl hydroxyethyl cellulose were added at least twise the concentration of dopamine and the analysises were carried out following HPLC procedures. The result obtained was compared with analytes standards. The addition did not interfere the analysis with relative error or within 4.1%.

4.2 Sample Analysis.

Peak identification was made based on the comparasion of retention times with that of standards. The quantitation was made from external calibrations based on least square regression equation (table1). The analytes dopamine, methyl dopa, l-dopa and tyrosine are available in pharmaceutical preparations and the method was applied for their analysis for the possible use for quality control. The analysis was carried out after extraction of the analytes in methanol-water, followed by the determination as analytical procedure. The results of analysis are summarized in table 2.and agreed with labeled values with RSD within 4.1%. The pharmaceutical preparations were also analyzed be of standard addition. The results of analysis agreed with that of external calibration and the % of recovery of dopamine , methyl dopa, l-dopa and tyrosine in pharmaceutical preparations were calculated9, 98, 96 and 94 % with RSDs 2.61, 2.91, 3.12 and 4.11 respectively.

5. CONCLUSION

Analytical procedure has been developed for the determination of biological active compounds dopamine, methyl dopa, l-dopa and tyrosine from pharmaceutical preparations by HPLC using pre-column derivatization with 4-dimethylbenzaldehyde. The analysis agreed with labeled values with% recovery of analytes from pharmaceutical preparations within 94-98% with RSDs with 2.61-4.11%.

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