

Spectrophotometric Determination of Catecholamines in Pharmaceutical Preparations Via Charge Transfer Complex Formation Using Bromanil Reagent

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of trace amounts of catecholamine drugs (dopamine, levodopa and methyldopa). The method is based on the interaction of these drugs in aqueous medium with tetrabromo-p-benzoquinone (bromanil), in the presence of borate buffer solution of pH 9 to form n- charge-transfer complexes with maximum absorbance at 350, 366 and 368 nm for methyldopa, levodopa and dopamine respectively. Beer's law was obeyed in the range of 1-25, 0.8-30 and 1-30 g/ml with molar absorptivities 8075, 8500 and 3475 l.mol⁻¹.cm⁻¹ for the

above drugs respectively. The accuracy (average recovery%) of method was found better than 99% and the precision (RSD) was less than 3%. The nature and stability of bromanil complexes with the above mentioned drugs were studied. The method was successfully applied for the assay of methyl dopa, levodopa and dopamine in some of their pharmaceutical formulations, and the results compared favorably with British pharmacopoeia and the standard addition technique. The common excipients used additives in pharmaceutical, do not interfere with the proposed method.

INTRODUCTION

Catecholamine drugs are aromatic vicinal-diols that consist of amines attached to a benzene ring bearing two hydroxyl groups (catechol). The catecholamines are primarily synthesized in vesicles of the chromaffin cells in the adrenal medulla. These drugs are widely used in the treatment of bronchial asthma, hypertension, Parkinson's disease, drug abuse, Schizophrenia and myocardial infarction⁽¹⁻⁴⁾.

Several spectrophotometric methods have been applied to assay of catecholamines as cited in the literature including derivatization reaction with organic reagents such as o-phenanthroline⁽⁵⁾, diazodized nitroaniline⁽⁶⁾, ninhydrin⁽⁷⁾, thiosemicarbazide^(8,9), chloramine-T⁽¹⁰⁾, phenylenediamine⁽¹¹⁾, and inorganic reagents like iodine⁽¹²⁾, ammonium metavanadate⁽¹³⁾, metaperiodate^(14,15), cerium(VI) nitrate⁽¹⁶⁾, neotetrazolium chloride⁽¹⁷⁾. Other methods have been adapted to stopped flow technique^(18,19), flow injection analysis⁽²⁰⁻²⁴⁾, ¹HNMR spectroscopy⁽²⁵⁾, coulometric titration and cyclic voltametry⁽²⁶⁾.

The charge transfer complex reaction has been used for determination of catecholamine by using various organic reagents such as chloranil⁽²⁷⁾, DDQ⁽²⁸⁾ and fluoranil⁽²⁹⁾. The present paper describes simple, sensitive, and accurate spectrophotometric determination of dopamine hydrochloride, methyl dopa and L-dopa using charge transfer complex formation reaction in aqueous medium with bromanil in the presence of solution of pH 9 borate buffer.

EXPERIMENTAL

Apparatus:

All absorption measurements were made using Shimadzu UV-210 double beam spectrophotometer with 1-cm matched optical silica cells.

Heating of solutions was carried out on waterbath (Forst Instruments LTD).

The pH readings were made using a PW 9420 pH-meter supplied with an electrode type CE10-12 pH.

Weighing was carried out on a balance type of Sartorius No. 6407.

Reagents:

All chemical used were of the analar grade from BDH and Fluka.

- a. Tetrabromo-p-benzoquinone, (bromanil) solution: A solution of 3×10^{-3} M of bromanil was prepared by dissolving 0.127092 g of bromanil in 100 ml of 96% ethanol.
- b. Borate buffer solution of pH 9 was obtained by preparing 5×10^{-2} M sodium tetraborate in distilled water. Other borate buffer solutions of pHs (6-10) were prepared by the addition of different volumes of 10% boric acid or 0.1 M sodium hydroxide to the sodium tetraborate solution and the pH value of solution was adjusted by pH-meter.
- c. Standard solutions of catecholamine drugs: A stock solution of 100 ppm aqueous solution of each drug was prepared. Diluted solutions were prepared from this solution as needed.

Determination of catecholamines in pharmaceutical:

Aqueous formulation:

Dopamine injection was diluted with distilled water in order to obtain a concentration range of the drug between 4-12 g.ml^{-1} . After this, the procedure described previously was carried out.

Tablets:

The contents of ten tablet were mixed well and from the finely powdered, an accurately weight portion (250 mg of methyldopa or levodopa) was taken and dissolved in a hot distilled water. The solutions were clarified by filtration and appropriate dilutions were made, and finally treated as described in recommended procedure.

Recommended procedure:

Into a series of 25 ml volumetric flask increasing volume (mls) of 100 ppm of each catecholamine (25 g.ml^{-1}) were transfered and followed by addition of optimum amounts of bromanil and buffer solution of pH 9 (Table 1). The solutions were then diluted to the mark with distilled water and allowed to stand in waterbath at 40 °C. The absorbance were measured at appropriate wavelength against reagent blank.

RESULTS AND DISCUSSION

When dilute aqueous solutions of catecholamine drugs were mixed with bromanil reagent in the presence of borate buffer solution of pH 9, a yellow coloured charge transfer complexes were observed with maximum absorption at 350, 366 and 368 nm for dopamine, methyldopa and levodopa respectively in contrast to reagent blank which shows a maximum absorption at 313 nm (Fig. 1).

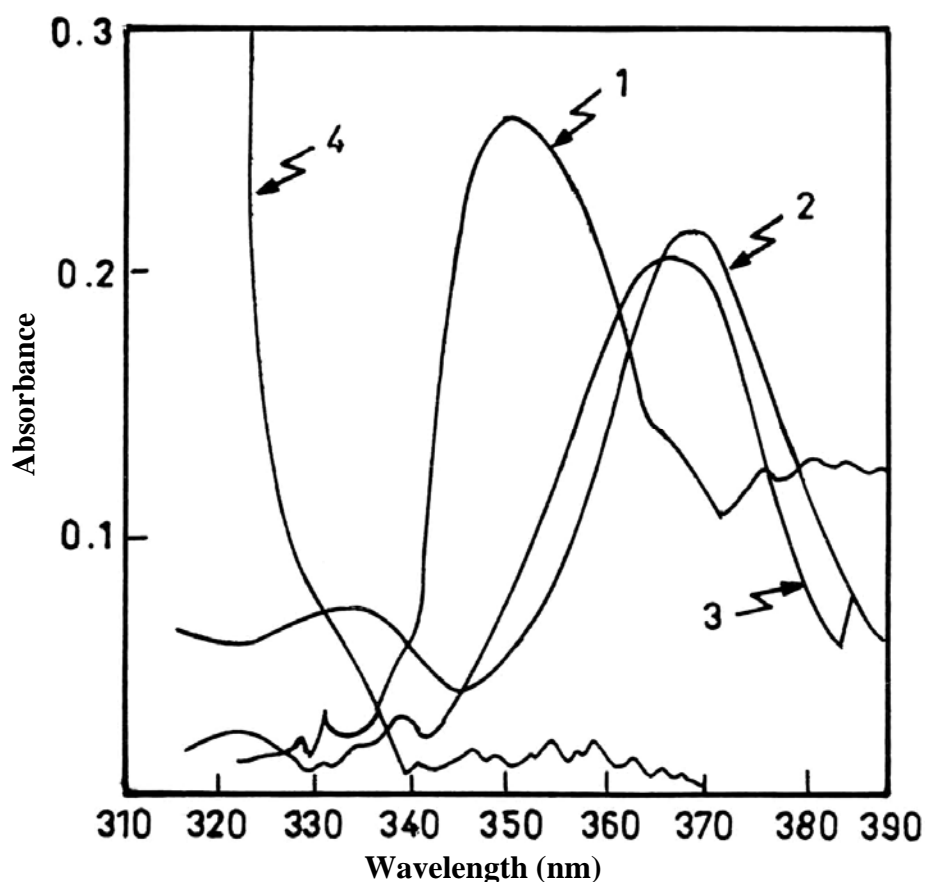


Figure 1: Absorption spectra of (1) 8 g.ml⁻¹ methyldopa, (2) 8 g.ml⁻¹ dopamine, (3) 8 g.ml⁻¹ L-dopa, versus reagent bank, and (4) blank versus distilled water under their optimum conditions

Optimization of conditions:

The effect of various parameters on the absorption of coloured n-charge transfer complex have been investigated and the reaction conditions were optimized for each catecholamines. Table 1 shows the summary of the optimum conditions for the determination of catecholamines. The effect of pH in the range 6-12 on the absorbance of the drugs was studied and found that pH 9 was the optimum.

Table 1: Summary of optimum conditions for the determination of catecholamines with bromanil

Catecholamine drug	λ_{\max} (nm)	Temp. (°C)	Development time (min)	Stability period (min)	pH 9 amount (ml)	Bromanil 3×10^{-3} M (ml)
Dopamine	368	45	30	120	2.0	3.5
Methyldopa	350	40	35	140	2.5	4
L-dopa	366	40	35	110	2.0	4

Quantification:

Under the optimum conditions described above (Table 1) standard calibration curves of charge transfer complexes for catecholamine drugs and bromanil were constructed by plotting absorbance versus concentration (Fig. 2), and the good result of correlation coefficient indicating good linearity (Table 2). Beer's law was obeyed over the concentration range as cited in (Table 2), and the resultant molar absorptivity indicates that the method is sensitive.

Table 2: Linearity range, molar absorptivity, slope, intercept and correlation coefficient of the calibration graphs for catecholamine

Catecholamine	Linearity range (g/ml)	Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	Slope	Intercept	Correlation coefficient
Dopamine	1-30	3475	0.0153	0.0514	0.9965
Methyldopa	1-25	8075	0.0407	0.0284	0.9978
L-dopa	0.8-30	8500	0.0185	0.0281	0.9965

Accuracy and precision:

The recovery and the relative standard deviation (RSD) were estimated at three different concentrations. The results shown in Table 3 indicate high accuracy and precision for the proposed method.

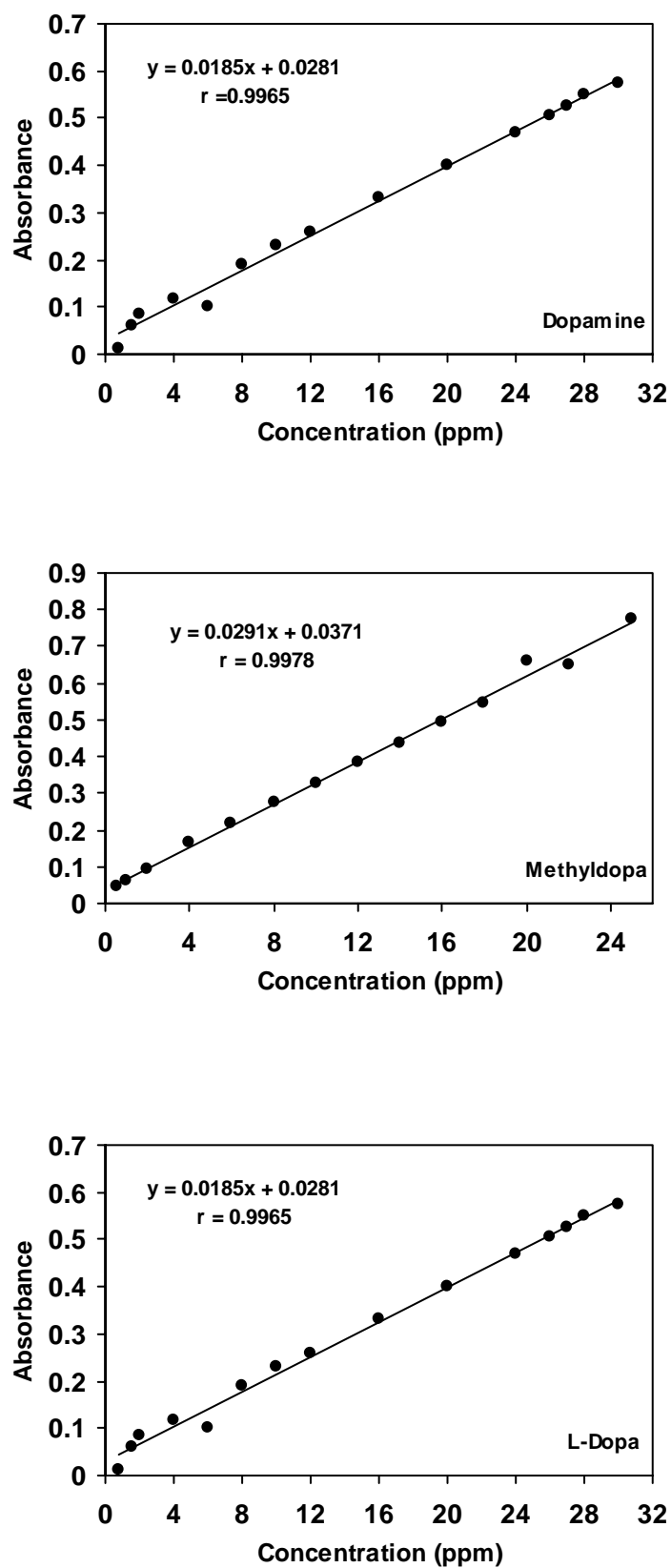


Figure 2. Calibration curve for Dopamine, Methyldopa and L-Dopa

Table 3: Accuracy and precision of the method

Catecholamine drug	Amount added (g/ml)	Recovery* (%)	Average recovery (%)	RSD*
Dopamine	2.0	98.46	99.54	0.45
	4.0	100.35		0.30
	8.0	99.80		0.15
Methyldopa	2	99.33	101.06	1.62
	4	101.53		0.90
	6	99.33		0.39
L-dopa	2.0	97.58	100.42	2.5
	4.0	100.35		1.8
	6.0	103.33		0.97

* Average of five determinations.

Interferences:

To check the selectivity of the method using the recommended procedure. A 100 g of L-dopa in a final solution was determined in the presence of various excipients (foreign organic and inorganic compounds) expected to be present with catecholamine drugs. The results shown in Table 4 indicated that common excipients do not interfere.

Table 4: Effect of interference

Excipient	Fold excess added	Relative error (%)
Fructose	5	+ 0.86
	10	+ 1.21
	20	+ 1.92
Glucose	5	+ 0.99
	10	+ 0.96
	20	- 1.91
Sucrose	5	+ 3.22
	20	+ 3.91
Starch	5	+ 2.47
	20	+ 3.14
Sodium chloride	5	+ 1.36
	10	+ 1.97
	20	+ 2.06
Sodium sulphite	5	+ 1.12
	10	+ 2.34
	20	+ 2.94
Citric acid	1	- 0.29
	5	- 3.51
	10	- 7.96
Nicotinic acid	5	- 1.21
	10	- 1.42
	50	- 4.37
Sodium bicarbonate	10	- 0.66
	20	+ 0.96
	100	+ 2.91

The nature and stability constant of the complexes:

The stoichiometry of the reaction was investigated using Job's method (30). The obtained results showed that the product formed in the ratio of 1:1 (drug : reagent), (Fig. 3).

The stability constant of the complexes were 5×10^4 , 8.5×10^5 and $5.4 \times 10^5 \text{ l}^2.\text{mol}^{-1}$ for dopamine, methyldopa and levodopa respectively.

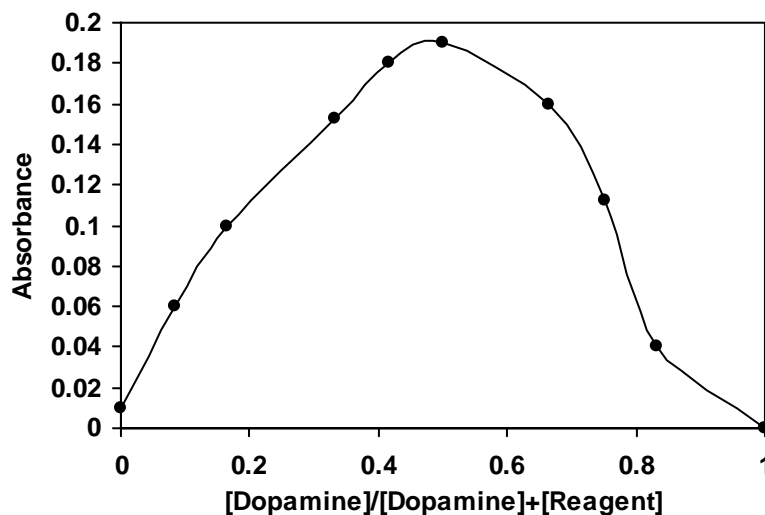


Figure 3. Continuous variation plot of dopamine

Application:

The proposed method was applied satisfactory for the determination of dopamine, methyldopa and L-dopa in some of their pharmaceutical preparations. The concentration of catecholamine drugs were calculated by direct measurement on appropriate standard calibration curve (Table 5). The similar results were obtained by applying the standard addition technique for dopamine injection and L-dopa tablet (Fig. 4) indicating that the method is free from interferences. The method was compared favorably with the British Pharmacopoeia method⁽³¹⁾ for the assay of methyldopa tablets and the results obtained are summarized in Table 6. The assay results were in good agreement with certified values for all the formulations.

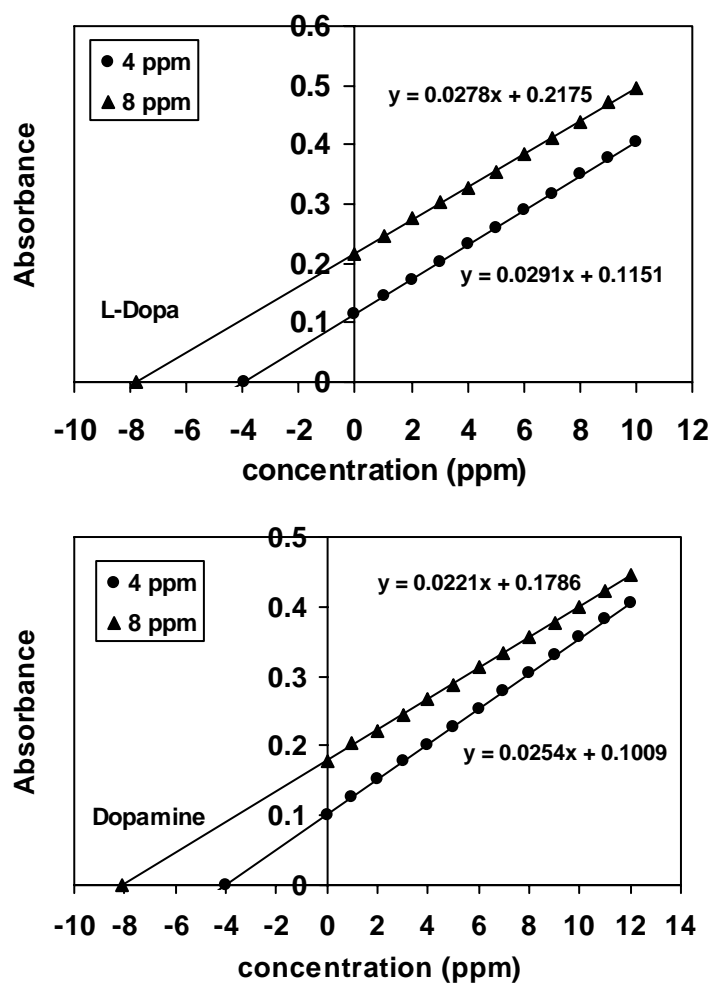


Figure 4. Plots of standard addition technique for determination of L-dopa and dopamine

Table (5): Assay of catecholamines in pharmaceutical preparation using the proposed method

Pharmaceutical preparation	Amount (mg)		Recovery* (%)	Average recovery (%)
	Certified value	Found		
Dopamine.HCl Injection ^a	200 mg/5 ml	196.20 203.50 201.09	98.10 101.75 100.54	100.13
Methyldopa Dopanore tablet ^b	250	257.63 255.40 251.29	103.05 102.16 100.52	101.91
L-Dopa Sinement tablet ^c	250	247.21 248.90 249.20	98.88 99.56 99.68	99.37

* Average of three determination.

a Marked by Biological Italia Lab. Novate-Milano-Italy

b Jordanin Pharm. Mtg. Co. Lid.

c Algorithm S.A.L.

Table (6): Assay of catecholamines in pharmaceutical preparation using standard addition method and British pharmacopoeia

Pharmaceutical preparation	Amount		Found (mg)	Recovery*
	Certified (mg)	Added (g/ml)		
Dopamine Injection	200 mg/5 ml	4	3.97	99.25
		8	8.07	100.87
L-Dopa Sinemen tablet	250	4	3.95	98.75
		8	7.52	97.75
Methyldopa Tablet	250	-	8.05**	100.62

* Average of three determinations.

** British pharmacopoeia.

Conclusions:

A spectrophotometric method was proposed for the determination of dopamine, L-dopa and methyldopa drugs, it could be used in control analysis of the pharmaceutical preparations. The procedure is simple, fast, sensitive, reproducible and not expensive. The method may be suitable for routine analysis.

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