SPECTROPHOTOMETRIC DETERMINATION OF CATECHOLAMINES VIA CHARGE TRANSFER COMPLEXATION WITH BROMANIL, APPLICATIONS TO CATECHOLAMINE DRUG FORMULATIONS

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ABSTRACT

A simple, sensitive, rapid and low-cost spectrophotometric method for catecholamine (levodopa, methyldopa and dopamine) determination in pure and drug formulations is described. This method is based on the complexation reaction of catecholamine with bromanil. Absorbance of the resulting green colored products is measured at 741 nm for levodopa complex and, 738 nm for methyldopa and dopamine. Beer's Law is obeyed in a concentration range of 0.2 – 3.5 µg ml^{-1} for levodopa, 0.1 – 4.0 µg ml^{-1} for methyldopa, and 0.2 – 2.5 µg ml^{-1} for dopamine and the molar absorptivity is 38222, 41474, and 48202 l.mol^{-1} cm^{-1} for levodopa, methyldopa, and dopamine respectively, with an excellent correlation coefficient (r = 0.9974) for levodopa, and (r = 0.998) for both methyldopa and dopamine.. The results show a simple, accurate, fast and readily applied method to the determination of levodopa methyldopa, and dopamine in pharmaceutical products. The analytical results obtained for these products by the proposed method are in agreement with those of the official standard method.

INTRODUCTION

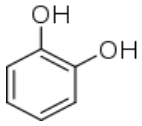
Parkinson's disease is associated with low levels of a chemical called dopamine in the brain. Levodopa is turned into dopamine in the body and therefore increases levels of this chemical. [Madrakain and Afghami atel. (2004)].

Dopamine a simple organic chemical in the catecholamine family is a monoamine neurotransmitter which has a number of important physiological roles in the bodies of animals. Dopamine plays a major role in the brain system that is responsible for reward-driven learning. Every type of reward that has been studied increases the level of dopamine transmission in the brain, and a variety of highly addictive drugs, including stimulants such as cocaine and methamphetamine, act directly on the dopamine system. [Goldman, and Castner, et. al. (2004)], [Davis, K.L., et.al. (1991)].

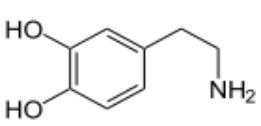
Catecholamine compounds are aromatic vicinal-diols that consist of amines attached to benzene ring bearing two hydroxyl groups (catechol).

Methyldopa, chemically known as α-methyl-3,4-dihydroxyphenylalanine is a catechol derivative (catecholamine) widely used as an antihypertensive agent. Methyldopa is a centrally acting alpha 2-adrenoreceptor agonist, which reduces sympathetic tone and produces a fall in blood pressure. [Hoffman and Lefkowitz, (1996)]

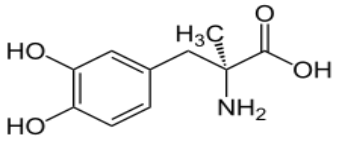
Levodopa (L-DOPA (L-3,4-dihydroxyphenylalanine) is an important neurotransmitter, which has used for treatment of neural disorders such as Parkinson's disease.



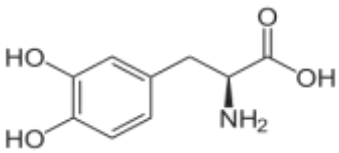
Catechol

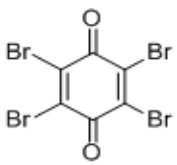


Dopamine



Methyldopa





Levodopa Bromanil

Jenway 3505 pH meter, Denver balance Tp-214 is used in weight measurements.

Reagents:

- All chemicals used are of analytical grade, pure catecholamine compounds from State Company for Drug Industries, Sammara- Iraq.
- Levodopa drug formulation, Sinemet tablets (250 mg + 25 mg carbidopa/ tablet), Merk Sharp and Dohme B. V., Haarlem- Netherlands.
- Methyldopa drug formulation, Aldomethyl tablets (250 mg/tablet), Asia Pharmaceutical Industries- Syria.
- Dopamine drug formulation, Dopamine.HCl Fresenius (200 mg/5 ml), Fresenius Kapi Deutschland GmbH- Germany.
- Pure catecholamine 100 µg.ml^{-1} in distilled water.
- Bromanil solution different molar concentrations in absolute ethanol.
- Hydrochloric acid 0.1 M.
- Sodium hydroxide solution 0.1 M.
- Buffer solutions at pH of 7.5.
- Surfactants solutions 0.1% in distilled water.
- Levodopa and methyldopa drug formulations; 10 tablets of each are finely powdered, weighed, an accurately an amount equivalent to one tablet weighed, 100 µg.ml^{-1} solutions are prepared, filtered and used as stock solutions.
- Dopamine drug formulations; 200 mg/5ml diluted to 100 µg.ml^{-1}.

General procedure:

Appropriate volumes containing 0.2 – 3.5, 0.1 – 4.0 µg ml^{-1} and 0.2 – 2.5 µg ml^{-1} of levodopa, methyldopa, and dopamine standard solutions were added into separated 10-ml volumetric flasks followed by addition 1.0 ml bromanil reagent solution; 0.4 ml of phosphate buffer and 0.5 ml of CTAB respectively. The solutions were diluted to the mark with distilled water, and were left for 5 min at room temperature. A portion of the solution was transferred into a 1cm silica cell to measure the absorbance at 741 nm against the reagent blank.

Results and discussion:

Several methods have been reported for the determination of catecholamines in pure and pharmaceutical preparations:

- Kinetic method for determination of α - methyldopa in pharmaceutical preparations: analytical procedure and reaction mechanism considerations. [Tubino and Debora et .al. 2006]
- Determination of Catecholamines by Flow Injection chemiluminescence method Based on their restraining effects on the luminol–potassium chlorate system.. [Sun and Tanga et. al. 2004.]
- Spectrophotometric for the determination of certain catecholamine derivatives in pharmaceutical preparations. [Nagaraja and Sirinivasa, et. al.1998].
- Separation and determination of levodopa and carbidopa in composite tablets by capillary zone electrophoresis with amperometric detection. [Zhang and Chen et.al. March 2001].
- Simultaneous voltammetric determination of levodopa, carbidopa and benserazide in pharmaceuticals using multivariate calibration. [Urzùa and Ortiz et.al. 2010].
- FIA-Fluorometric determination of adrenaline in pharmaceutical formulations by oxidation with molecular oxygen. [Torres and Romero,. et.al. 1998].
- Simultaneous determination of catecholamines by ion chromatography with direct conductivity detection.[Guan and Ouyang et.al. January 2000].
- Determination of methyldopa in pharmaceutical formulations by combined spot test-diffuse reflectance spectroscopy. [Roberto and Ribeiro et.al 2006].
- A disposable electrochemical sensor for the determination of levodopa. [Bergamini and Santos et.al. 2005].

Experimental:

Apparatus:

All spectrophotometric absorbance measurements are carried out on Jenway 6800 UV-Visible double beam and Jenway 6305 visible single beam spectrophotometers using 1cm quartz cells, pH measurements are done by

the spectrum shows two weak bands at 430 and 460 nm.

Effect of pH:

To study the effect of pH on the spectrum, different amounts of HCl and NaOH (0.1 M) solutions are added to the reaction mixture. HCl has negative effect; the effect of NaOH indicates that there is an increase in the intensity of the two peaks, the optimum pH is found to be 7.5, Table 1.

All optimization conditions are made with 2 $\mu g.ml^{-1}$ of pure levodopa (as an example of catecholamines) with one ml of bromanil (1 x 10^{-3} M) solution in 10 ml volumetric flask, dilutions are made with distilled water, for optimization of reagent (bromanil) different volumes of it were used.

When $2\mu g.ml^{-1}$ allowed reacting with 1 ml of bromanil (1 x 10^{-3} M) solution in 10 ml volumetric flask, after dilution and standing for 5 minutes, a very pale yellow color is produced,

Table 1: Effect of pH on absorption of the complex

pH	Abs at.λ 430 nm	Abs at.λ 460 nm	Notes
6.5	0.0156	0.0205	Complex is pale yellow, blank is colorless
7.0	0.0718	0.0903	
7.5	0.1239	0.1485	Complex is yellow, blank is very pale yellow
8.0	0.1092	0.1370	
9.0	0.0921	0.1044	

1.00	0.2392

Effect of bromanil concentration:

To study the effect of bromanil concentration on the absorption of the complex, different amounts of bromanil (1 x 10^{-3} M) are added to the reaction mixture containing levodopa and phosphate buffer solution, it is found that 1.00 ml of the reagent is sufficient to give maximum absorbance at 460 nm, Table 4.

Table 4: Effect of bromanil concentration:

Bromanil solution (ml)	Abs at 460 nm
0.25	0.2415
0.50	0.2502
0.75	0.2549
1.00	0.2561
1.25	0.2551
1.50	0.2546

Effect of temperature and time:

The reaction time is determined by following the measuring the absorbance of the complex at room temperature 25°C and at controlled water-bath adjusted at 30, and 35 °C. the absorbance are measured at 5 min intervals against regent blank treated similarly, it is found that the absorbance reached maximum after 5 min at

Effect of buffers:

Different buffer solutions at pH 7.5 (0.5 ml) are added to the reaction mixture, it is evident that phosphate buffer gave high absorbance and used in subsequent experiments, Table 2.

Table 2: Effect of buffers on the absorption of the complex

Buffer	Abs at 460 nm
Carbonate	0.2076
Phosphate	0.2558
Borate	0.2089

Effect of phosphate buffer (pH 7.5) concentration:

The effect of phosphate buffer (pH 7.5) concentration on absorption of levodopa-bromanil complex is studied. It is found that the absorbance increased with increasing buffer concentration and reached maximum at 460 nm on using 0.5 ml of the buffer, Table 3.

Table 3: Effect of phosphate buffer concentration on the absorption of the complex

Phosphate buffer (ml)	Abs at 460 nm
0.25	0.2504
0.50	0.2554
0.75	0.2486

(SDS) and tween 80 have no effect on the absorbance intensity.

Effect of CTAB concentration on complex absorbance:

Aliquots of 0.1% CTAB solution are added to reaction mixture; it's observed that 0.4 ml of CTAB has maximum effect, Table 5.

Table 5: Effect of CTAB concentration on complex absorbance

CTAB ml	Abs
0.1	0.1888
0.2	0.2783
0.3	0.4129
0.4	0.4147
0.5	0.4113

room temperature and remain constant for about 10 minutes. Therefore room temperature and reaction time 5 min are chosen for complex formation.

Effect of addition of surfactants:

To study the effect of surfactants on the absorption and the intensity of the complex, different types of surfactants are added to the reaction mixture (0.2 ml 0.1%), it is evident that when acetyl trimethylammonium bromide (CTAB) is added a red shift in the region of the peak happened from 460 to 741 nm Fig 1, the color of the complex is converted to a light green with dilution to 10 ml by addition of distilled water, cetavlone also gave the same shift but with less intensity, while sodium dodecyl sulfate

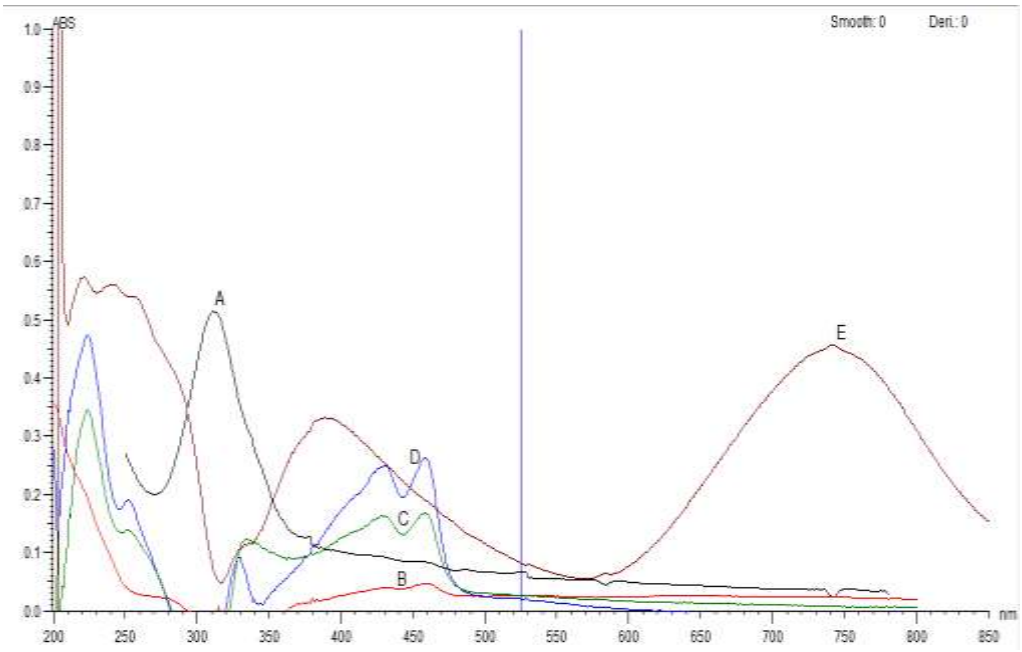


Fig.1: Absorption spectra of levodopa-bromanil complex

It is found that the following order of addition of reaction components has high sensitivity of absorption;

Levodopa + bromanil + phosphate buffer + CTAB then dilution with distilled water.

Optimum conditions of Levodopa-bromanil charge transfer complex formation:

Table 6 summarize the optimum conditions of levodopa-bromanil complex formation, these

A: Blank vrs solvent, **B**: (Levodopa + bromanil) vrs blank, **C**: (Levodopa + bromanil + NaOH) vrs blank

D: (Levodopa + bromanil + phosphate buffer pH 7.5) vrs blank, **E**: (Levodopa + bromanil + phosphate buffer pH 7.5 + CTAB 0.1%) vrs blank

Order of addition:

bromanil complexes.

conditions are also applicable for the other catecholamine (methyldopa and dopamine)-

Table 6: optimum conditions for catecholamines-bromanil complexes

Bromanil ml 1×10^{-3} m	Phosphate buffer pH 7.5 ml	CTAB 0.1%	Final volume ml	Temperature C^o	Time min
1.0	0.5	0.4	10	25	5-10

observed Fig 3, the concentration range; correlation coefficient (r), intercept, slope, limit of detection (LOD), molar absorptivity, and stability constant of catecholamines-bromanil complexes are shown in Table 7.

Quantitation:

Under the proposed experiment conditions a linear relation between the absorbance and concentration of pure catecholamine compounds (levodopa, methyldopa, and dopamine) is

Table 7: Analytical parameters for catecholamines-bromanil complexes

Compound	Linearity $\mu g.ml^{-1}$	LOD, $\mu g.ml^{-1}$	Slope	Intercept	Correlation coefficient (r)	Molar absorptivity, $l.\ mol^{-1}cm^{-1}$	Stability constant, $l.\ mol^{-1}$
Levodopa	0.2-3.5	0.06	0.205	0.0	0.997	38222	4.72×10^3
Methyldopa	0.1-4.5	0.07	0.187	0.0	0.998	41474	4.77×10^3
Dopamine	0.2-2.5	0.04	0.296	0.0	0.998	48202	1.72×10^3

concentrations are determined; the results are shown in table 8 referring a satisfactory accuracy and precision, Table 8.

Accuracy and precision:

To determine the accuracy and precision of the method, different levels of pure catecholamine

Table 8: Quantitative parameters for the spectrophotometric analysis of catecholamines by formation of charge transfer complexes with bromanil

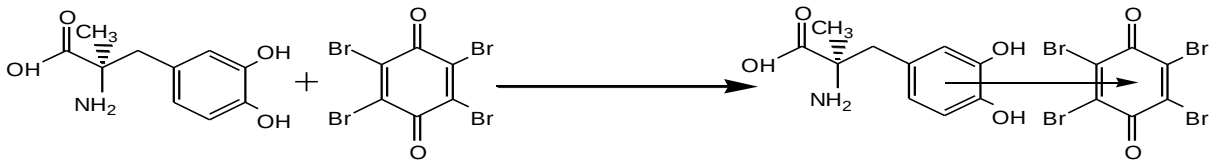
Compound	Amount added $\mu g.ml^{-1}$	Recovery %	Relative standard deviation % (RSD %)
Levodopa	0.8	101.5	1.57
Levodopa	2.0	102.3	2.14
Methyldopa	1.0	98.7	2.97
Dopamine	1.5	99.3	2.08

for both levodopa and dopamine Fig. 3 and mole ratio method, Fig 4 for methyldopa; the results indicate that 1:1 bromanil-catecholamines complexes are formed.

Nature of complexes and reaction mechanisms:

The stoichiometry of the reaction mechanism between catecholamines and bromanil is investigated using continuous variation method

D: + A ⟶ D: → A



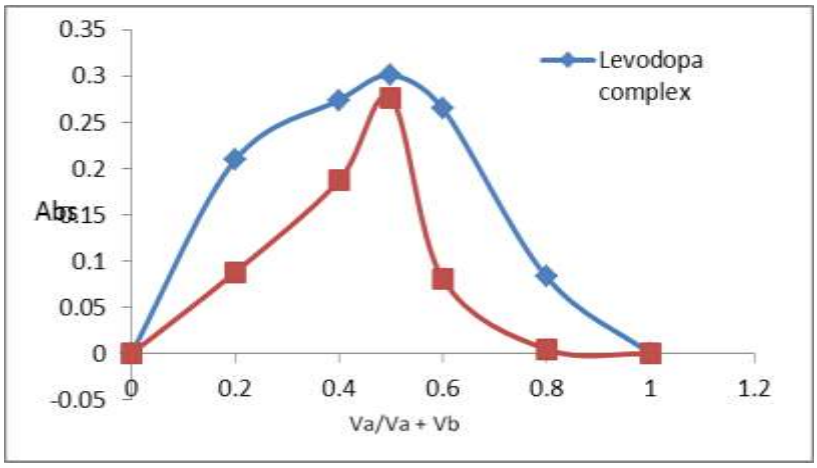


Fig.3: continuous variation plot for levodopa and dopamine complxese with bromanil (Va = volume of bromanil, Vb = volume of catecholamine)

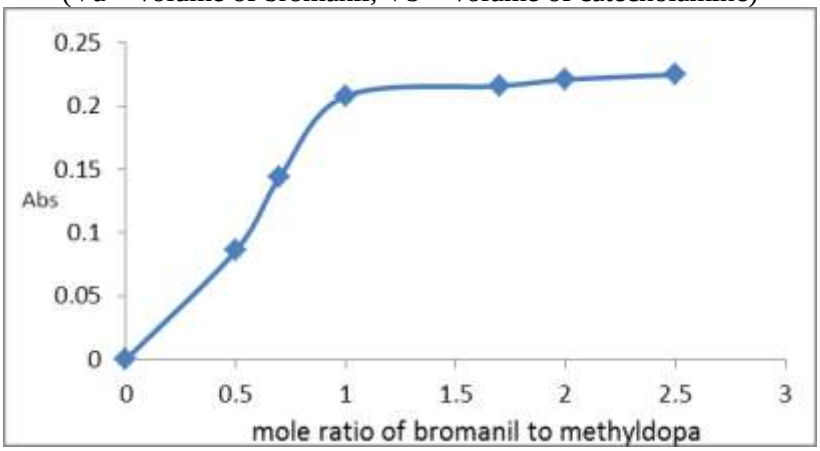


Fig. 4: Mole ratio method for methyldopa-bromanil complex

Application of the method on drug formulations:

The results obtained from the application of the method on catecholamine drug formulations are listed in Table 9. Good recovery is obtained and the results are compared favorably with the official standard method, British Pharmacopeia 2007.

Table 9: Application of the proposed method for determination of catecholamine in drug formulations

Drug formulation	Amount taken $\mu g.ml^{-1}$	Recovery %	Drug found, proposed method	Drug found, official method
Levodopa(250 mg/tablet)	0.75	101.2	253.1	249.5
Levodopa(250 mg/tablet)	1.2	102.2	255.5	256.0
Methyldopa	0.9	102.4	256.0	255.8
Methyldopa	1.8	100.4	251.0	255.0
Dopamine	1.0	100.3	200.6	201.6

Conclusion:

A spectrophotometric method for the determination of catecholamine drugs was developed. The method is simple, reliable, sensitive and less time consuming. The statistical analysis is in good agreement with those of the official British Pharmacopoeia. The colour reaction is selective for the drugs. The method can be successfully applied for the micro determination of levodopa, dopamine and methyldopa either in pure or in pharmaceutical preparations. The advantage of the present procedure is that it does not require many solvents. A significant advantage of a spectrophotometric determination is its application for the determination of individual compounds. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy, since it offers a distinct possibility of quality control in the assay of pharmaceutical dosage formulations.

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الخلاصة

تم وصف طريقة سهلة وبسيطة وحساسة لتقدير كميات ضئيلة من الكاتيكول امينات (ليفودوبا ومثيل دوبا ودوبامين) في الوسط المائي مع كاشف البرومانيل وبوجود بفر الفوسفات وعامل الشد السطحي بتكوين معقد الشحنة المنتقلة ذو اللون الاخضر حيث امكن تقدير كميات (0.2–3.5) و (0.1–4.0) و(0.2–2.5) مايكروكرام لكل ملليليتر من ليفودوبا ومثيل دوبا ودوبامين على التوالي عند طول موجي 741 نانوميتر لليفودوبا و738 نانوميتر لكل من والمثيل دوبا والدوبامين وقد معامل الامتصاص المولاري لكل من الليفودوبا والمثيل دوبا والدوبامين 38222، 41474،و48202 لتر/مول.سم على التوالي كما تم تطبيق الطريقة على هذه المركبات في المواد الصيدلانية الدوائية وبتوافق مع نتائج الطريقة القياسية المعتمدة.

پوختە

ڕێکەکا ئاسان و سادە و هەستیار هاتە بەرهەفکرن بۆ دەرئینانا ڕیژێت کێم ییّت کەتیکول ئەمینات (لیفودوبا ومسیل دوبا ودوبامین) ل ناوەندەکێ ئافێدا دگەل ئاشکراکەرێ بڕومانیل ب هەبونا بەربەستێ فوسفات و فاکتەرێ پەشۆکان ڕووکەشی ب دروست بوونا بارگەیەکا بەلاڤبوی یا ئالوز ب ڕەنگێ کەسك کو بوو ئەکەرێ شیانێن دەرئینانا ڕیژەییّن (0.2-3.5) و (0.1-4.0) و(0.2-2.5) مایکروگرام / ملیلتر ژ لیفودبا و مسیل دوبا و دوبامین ئێك لدویف ئێك ب درێژیا شەبولی 742 نانومیتر بۆ لیفودوبا و 738 نانومیتر بۆ هەر ئێك ژ دوبا و دوبامین. هەلمژینی مۆلاری 38222، 41474،و48202 لتر/مول.سم ئێك لدویف ئێك، لادانی پێوانەیی ڕێژەیی لە 1.57-2.97٪. هەروەسا ئەف ڕێکە هاتە بکارئینان لسەر ڤان ئاوێتەیان د ناف کەرەستەییّن دەرمانێن دەرمانسازی دا ب گونجاندن دگەل ئەنجامێن ڕێکێن پێوانەییّن پاوەرپێکری.