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Colorimetric Determination of Some Drugs Containing Phenolic Group Using 3-Methyl -2- Benzothiazolinon Hydra zone Hydrochloride (MBTH)

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الخلاصة

تختص هذه الدراسة بتحليل بعض الأدوية التي تحتوي على مجموعة الهيدروكسيل مثل مثيل دوبا، الليفودوبا و الكاربي دوبا سواء المادة الخام او في شكل المستحضرات الصيدلية.

تعتمد هذه الطريقة على مركبات ملونة مع 3- ميثل 2- بنزوثيازولينون هيدرازوين هيدروكلوريد في وجود كبريتات السيريك الامنيومى او كلوريد الحديديز وتتكون هذه المركبات الملونة في خلال خمس دقائق في درجة حرارة الغرفة وهي ثابتة حتى ساعتين على الاقل وتقاس هذه المركبات عند طول موجي 517، 474 و474 لكل من مثل دوبا, الليفودوبا و الكاربي دوبا على الترتيب.

وجد ان هناك علاقة طردية بين التركيز والامتصاص وأمكن تطبيق قانون بيير على التركيزات من 1-7 مايكرو جرام/مل، 1-11 مايكرو جرام/مل، 1، 2-42 مايكرو جرام /مل لكل من مثيل دوبا، الليفودوبا والكاربي دوبا بالتتابع. قد تم استخدام هذه الطريقة في التحليل الكمي للمستحضرات الصيدلية وأعطت نتائج عالية الدقة و بمقارنة النتائج مع نتائج طرق دستور الأدوية البريطاني ومع الطرائق المرجعية لم تظهر أي فروق جوهرية بينهما.

Abstract

A simple, rapid, sensitive and suitable method for analysis of A methyldopa(I), levodopa (II) andcarbidopa (III) either in pure from or in pharmaceutical formulations is described. The proposed method is based on an oxidative coupling reaction with 3-methyl-2-benzothiazolinon hydra zone hydrochloride (MBTH) in the presence of an oxidant (Ce^{+4} or Fe^{+3}) to give colored species. The resulting colors are well developed within 5 minutes at 20 ± 5°C and stable for at least 2 hours. The λ_{max} were 517, 477 and 474 nm for I, II, both III and IV respectively. A linear correlation was found between the absorbance and the concentration. Beer's law was obeyed for concentration ranges from 1-7 µg/ml, 1-

11 μg/ml, 1-8 μg/ml and 2-24 μg/ml for I, II, III and IV, respectively. The molar absorptiveness and was calculated. The results of analysis of pure drugs by the proposed method are in good agreement with the official BP and USP methods. The results obtained showed recoveries of 99.80 ± 0.27, 100.40 ± 1.26, 99.89 ±0.21 and 99.96 ± 0.36 for I, II, III and IV, in order. The proposed method was successfully applied to determine the studied drugs in their pharmaceutical dosage forms.

Keywords: methyldopa, Levodopa, Carbidopa, colorimetric determination and MBTH.

Introduction

Methyldopa U. S. P. (Aldomet) α- methyl di hydroxyl phenyl phenylalanine is one of the more widely used antihypertensive agents and dopamine HCl, (Intropine) 1-4,3 di hydroxyl phenyl)-2 ethyl ammonium chloride is the precursor in the biosynthesis of nor epinephrine. Levodopa [(-)-3-(3,4- di hydroxyl phenyl)-L- Alanin] a naturally occurring amino acid is the immediate precursor of the neurotransmitter dopamine and used in the treatment of Parkinson's disease, which is associated with depletion of dopamine in the brain[1,2].

Carbidopa [(-)-L- α -hydrazmo-3,4-dihydroxy- α - methyl hydro cinnamic acid monohydrate] is a peripheral decarboxylase inhibitor which inhibits the peripheral decarboxylation of levodopa to dopamine. Carbidopa is used in admixture with levodopa in the treatment of Parkinsonism to decrease the dose of the later, to obtain a more rapid response and to decrease side-effects[3,4].

Various spectrophotometric methods were applied for determination of carbidopa alone or for levodopa Levodopa and carbidopa were analyzed by column chromatography, capillary electrophoresis and high performance liquid chromatography (HPLC) methods[5,6,7].

Spectrophotometric methods applied for determination of methyldopa. are time consuming (heating at 100°C for 25 minutes and at 80°C for 20 minutes), which make them not suitable for routine analysis[8]. Capillary electrophoresis, gas chromatography— mass spectroscopy (GC-MS), high performance liquid chromatography (HPLC),[9,10] proton magnetic resonance (PMR)[11].

Spectroscopy, glass capillary column gas chromatography and ion exchange methods were also used for Methyldopa[12.13.14].

This work aimed to find such method, suitable for routine analysis of the named drugs using the oxidative coupling reaction of MBTH with them.

Material and Methods

Apparatus:

SHIMADZU uv-1201 uv-vis spectrophotometer with 10 mm matched quartz cell was used for all spectral measurements.

Materials and Reagents:

Pharmaceutically pure methyldopa was used as working standards.

The following pharmaceutical preparations were analyzed:

Aldomet tablets: Each tablet contains 25 mg of methyldopa, Levodopa tablets (250 mg/tab.) and carbidopa (25 mg/tab.) were prepared in laboratory and contain the usually included excepients such as starch, talc, avicel (micro crystalline cellulose) and magnesium stearate. The MBTH solution 0.1 % w/v in distilled water was freshly prepared from material obtained from Sigma (USA). Ammonium ceric sulphate solution 0.2 % w/v in 0.2 % H_2SO_4,v/v, in distilled water was freshly prepared. Ferric chloride hex hydrate solution 0.4 % w/v was freshly prepared in distilled water.

Stock solutions:

Stock solutions, 100 μg/ml in distilled water, were prepared for methyldopa while for levodopa 100 μg/ml and carbidopa 200 μg/ml in 0.2 N HC1 were used.

General procedure:

For methyldopa, levodopa and carbidopa:

Into a 10-ml calibrated flask, 1-7 μg of methyldopa or 1-8 μg of levodopa or 2-24 μg of carbidopa were Transferred, followed by the addition of 1ml of MBTH-solution (for etilefrine. HC1 and carbidopa), and 0.4 ml (for levodopa) and then the oxidant (2 ml of ceric ammonium sulphate $Ce(NH_4)_2$ $(SO_4)2$ for methyldopa and 1.5 and 2 ml of $FeCl_3$ solution for levodopa and carbidopa respectively) was added, mixed well and standed for 5 minutes at 20 ± 5°C, diluted to 10 ml with distilled water. The absorbance was measured at 517nm (for methyldopa) and 474 nm (for levodopa and carbidopa) against a reagent blank (Fig.1).

Procedure for pharmaceutical preparations:

For methyldopa, levodopa and carbidopa tablets:

20 tablets were weighed and finely powdered and a portion of the powder equivalent to 10 mg of levodopa and 20 mg of carbidopa was weighed, extracted twice with 0.2 N HC1, filtered and the residue was washed with 0.2 N HCl, then, the filtrate and the washings were completed to 100 ml with the mentioned solvent.

A aliquots of the tablets solution equivalent to 1-8 μg/ml (for levodopa or methyldopa) and 2-24 μg/ml (for carbidopa) were transferred into 10 ml calibrated flasks and proceeded as directed in the general procedures.

Results and Discussion

It has been reported that MBTH loses a proton and two electrons to form the electrophilic intermediate, which is the active coupling species[15]. It is suggested that the intermediate undergoes electrophilic substitution with phenols in the ortho and para positions to produce the colored product[16]. The absorption spectra of the colored reaction product at the optimum conditions recorded in the general procedures show a characteristic λmax at 517, 477 and 474 nm for methyldopa both levodopa and carbidopa in order (Scheme 1).

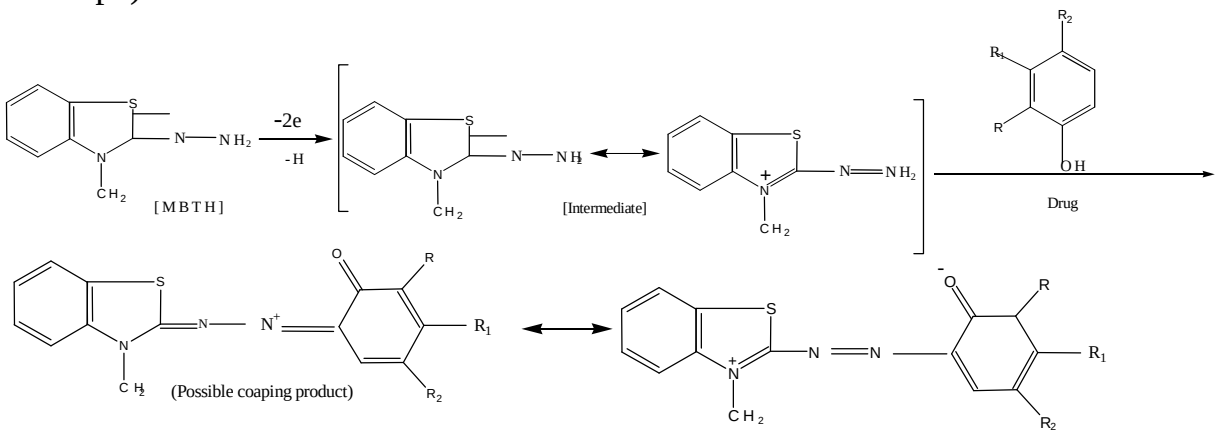
Effect of reaction variables:

Various parameters affecting the reaction processes for the production of the most intense and stable color were studied[17,18]. When different volumes of 0.1 % MBTH- solution were added to a fixed concentration of the drug, 1 ml (for methyldopa and carbidopa) and 0.4 ml (for levodopa) were found to be sufficient for maximum color intensity. Increasing the reagent concentration and volume did not affect the color intensity.

Several oxidizing agents were investigated[19] e.g iron ammonium sulphate, hydrogen peroxide, potassium dichromate, potassium chromate, ceric ammonium sulphate and ferric chloride. Ceric ammonium sulphate (in 0.2 % H_2SO_4 v/v in distilled water) 2 ml was found to be optimum for the maximum color prediction (for methyldopa) whereas ferric chloride hex hydrate 0.4 % w/v solution, 1, 1.5 and 2 ml were the optimum concentration, levodopa and carbidopa respectively). Higher concentrations of oxidant did not affect the color intensity.

It was found that 5 minutes at 20 ± 50C were optimum for maximum absorption intensity of the coloured product. It was also found that addition of drug solution, reagent solution then the oxidant solution is the proper sequence for the determination of methyldopa, levodopa and carbidopa.

Job's method of commons variation is applied to study the steochemetry of the reaction [20.21]. Results revealed a 1:1 ratio (for methyldopa, levodopa and carbidopa).



R-OH	R_1-O	R_2-CH_2-$C(CH_3)(NH_2)$-COOH	Methyldopa
R-OH	R_1-O	R_2-CH_2-$CHNH_2$-COOH	Levodopa
R-OH	R_1-O	R_2-CH_2-CH(NH-NH_2)COOH	carbidopa

Scheme 1

Quantification:

Applying the optimum experimental conditions attained the standard calibration curves are developed for MBTH reaction with the cited drugs. Linear correlation are obtained between the absorbance's and the concentration in the range of 1-7 μg/ml, 1-11 μg/ml, 1-8 μg/ml and 2-24 μg/ml for methyldopa, levodopa and carbidopa respectively with correlation coefficient of 0.9999 for all the cited drugs(Tab.1). The concentration of different.

Unknown samples can be calculated from such calibration graph's or by using the corresponding regression equations:- abs. = b x C + a

Where C is the unknown concentration.

The apparent molar absorbtivities of the resulting colored products ranging from 0.97 x 10^4 to 4.317 x 10^4 L.mol^{-1}. cm^{-1} for carbidopa HCl. The sensitivities were 0.0093, 0.0083 and 0.025 μg cm^{-2} for methyldopa, levodopa and carbidopa respectively.

The results obtained showed recoveries of 99.8 ±0.27, 99.89 ±0.21 and 99.96 ±0.36 and carbidopa respectively(Tab.2). The limit of detection (LOD) was l μg/mi for methyldopa and levodopa and ⋮. 2μg/ml for carbidopa(Tab.1)., while the limit of quantification (LOQ) was 0.33 μg/ml for methyldopa and levodopa and 0.66 μg/ml for carbidopa .

Conclusion

The proposed method was advantageous over the other reported visible colorimetric method; methods with respect to higher sensitivity which permits the determination of up to 0.5 μg/ml, simplicity, reproducibility, precision, accuracy and stability of the colored products more than 2 hours. The interference of the associated excipients and additives was not observed. The proposed method can be applied for routine analysis and in quality control laboratories for the quantitative determination of the studied drugs in pure form and in their pharmaceutical formulations depending on the availability of the chemicals.

Table 2 shows the statistical analysis of the results obtained for the determination of methyldopa, levodopa and carbidopa in pure form by means of the proposed method compared with the BP-XXI method [22].

To approve the applicability of the proposed method it was applied for the determination of the cited drugs in their pharmaceutical preparations applying the standard addition technique and the results obtained demonstrate good precision and accuracy as shown in Table 3.

	λ max nm	Є max 10^4/mol^{-1} cm^{-1}	Linear range mg/ml	Quantitative parameters			Sensitivity mg. cm^{-2}	L.O.D. mG/ML	L.O.Q. mG/ML
				intercept	slope	Correlation coefficient			
Methyldopa	517	$2.33x10^4$	1-7	0.005	0.103	0.9999	0.0093	1	0.33
Levodopa	474	$2.36x10^4$	1-8	-0.00035	0.118	0.9999	0.0083	1	0.33
Carpidopa	474	$0.97x10^4$	2-24	-0.0006	0.04	0.9999	0.025	2	0.66

Colorimetric

* Limit of detection
**Limit of quantification

Table 1: Spectral characteristics of the products from the Colorimetric reaction between MBTH and the studied drugs.

	Proposed method				Official method	
	Mean recovery%	**RSD%**	**T**	**F**	**Mecovery Recovery %**	**RSD**
Methyldopa	99.8	0.27	1.42(2.228)	1.7(5.05)	100.06	0.357
Levodopa	100.4	1.26	0.068(2.228)	2.2(5.05)	99.9	0.299
Carpidopa	99.96	0.36	0.27(2.228)	2.02(5.05)	100.03	0.512

*Average of 6 determinations

Table 2: Statistical analysis of results obtained by the proposed method compared with the official and reference methods [22].

	*Mean recovery %	SD	RSD %	SE	V
Aldomet tab.	100.2	0.29	0.289	0.117	0.0841
Levodopa tab.	100.18	0.476	0.475	0.194	0.226
Carpidopa tab.	99.94	0.124	0.124	0.05	0.015

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Table 3: Statistical analysis of results by the proposed method for determination of the cited drugs in their pharmaceutical preparations.

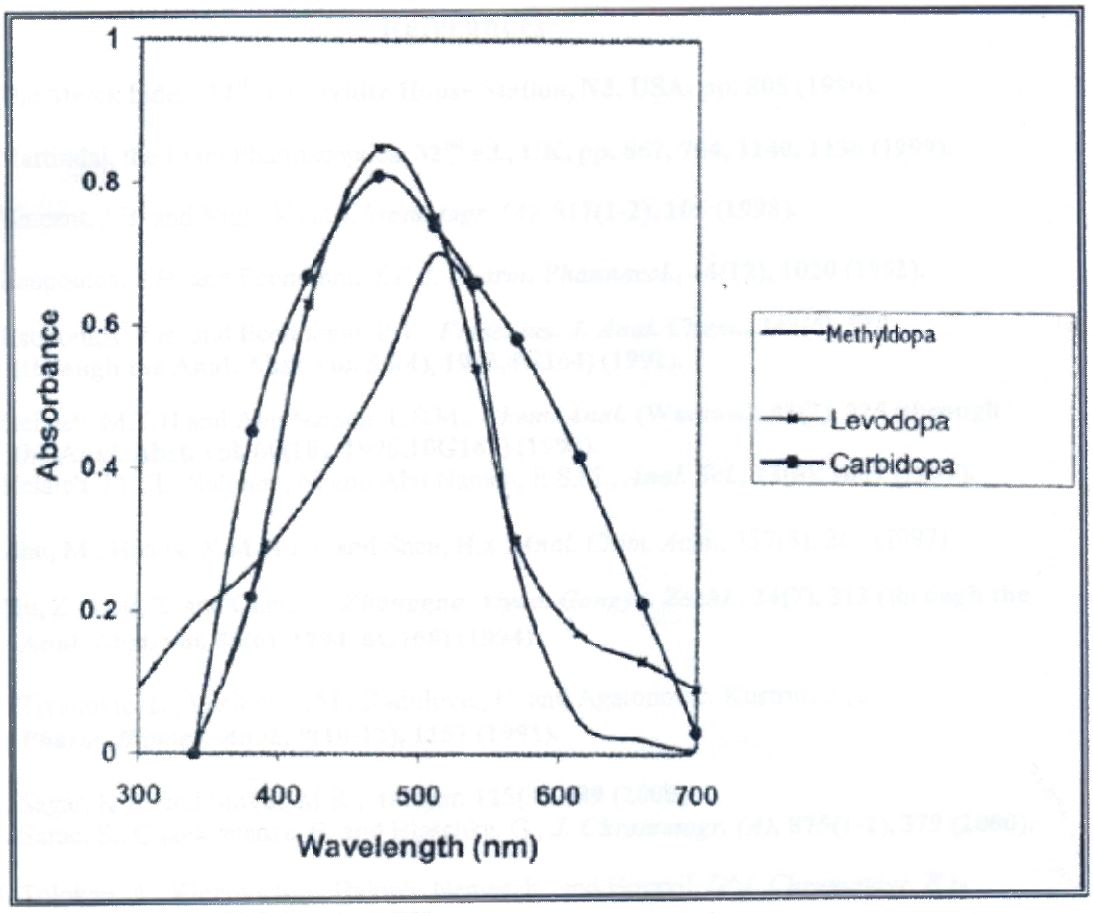

Fig. l: The absorption spectra of the colored reaction product for methyldopa (7㎎/ml), levodopa (7㎎/ml) and carbidopa (20㎎/ml) with MBTH.

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