DETERMINATION OF ANTIPARKINSONISM DRUG ENTACAPONE

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ABSTRACT

Spectrophotometric procedures are presented for the determination of the antiparkinsonism drug entacapone. The first method based on the formation of a coloured complex of entacapone with ferric chloride in ethanolic solutions. The coloured product is quantified spectrophotometrically at 665nm.

The other Spectrophotometric method determine entacapone in presence of levodopa and carbidopa (which formulated with entacapone in stalevo tablets) by measuring the UV absorbance of entacapone either in zero order at 391nm or in the first order by measuring the amplitude between 360nm and 402nm without any interference from levodopa and carbidopa.

HPLC method is described for the separation and determination of entacapone, levodopa and carbidopa on Luna CN 150 x 4.6 column with UV detection at 390nm for entacapone and at 280nm for both levodopa and carbidopa

INTRODUCTION

Molecular formula: - C₁₄H₁₅N₃O₅

Relative molecular mass: - 305.29

Chemical name: - (E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide

Entacapone is a nitrocatechol structured compound. Therapeutically classified as a selective, reversible and peripheral inhibitor of catechol-O-methyl transferase. It is given as adjunctive therapy to patient with parkinson's disease. Entacapone may be given as combination preparation with carbidopa and levodopa, this will extend the effect and duration of levodopa in the brain and allows levodopa to be given less often and in lower doses¹.

The reported methods for the determination of entacapone include, a column-switching LC-electrospray ionization-tandem MS method for the direct analysis of entacapone glucuronide in plasma using C18 column, and a mixture of 20mM-ammonium acetate/acetonitrile (17:3) pH 7 as a mobile phase²

Two different micellar electrokinetic capillary chromatography methods were introduced for direct determination of glucuronides of entacapone and its (Z)-isomer in human urine. In the first method good separation of glucuronides was achieved with 20mM-ammonium acetate as separation solution at pH 6.84³. In the second method detection was achieved at 335nm⁴

Three HPLC methods for the determination of entacapone were reported, the first one determine entacapone and its Z-isomer in human plasma and urine on LiChrosorb C18 column fitted with a micro Bondapak C-18 guard column⁵. The second method determine it in plasma using internal standard rofecoxib. Separation was achieved by an isocratic mobile phase of 30 mM phosphate buffer (pH 2.75)/ acetonitrile (62/38, v/v). The used column was a reversed phase C18 column⁶.

The third method detect entacapone at 305nm⁷. Another HPLC method was described for the determination of the drug release of levodopa, carbidopa and entacapone from tablet formulation⁸.

Spectrophotometric method for the determination of entacapone in tablet was reported, the method used acetonitrile as solvent. The results were validated and compared with HPLC method⁹.

EXPERIMENTAL

Chemicals and reagents

Standard entacapone, levodopa and carbidopa with claimed purity 99.81, 99.76 and 99.65 respectively. Comtapone 200mg, Kemapharm company, batch

number RD07037. Ferric chloride (ElNasr company), ethanol analytical grade (Analar), ammonium acetate (El Nasr company), and acetonitrile HPLC grade (Analar).

Preparation of the reagent and mobile phase.

1-preparation of ethanolic solution of ferric chloride (0.4%w/v)

2-the mobile phase was prepared by mixing 20mM ammonium acetate solution pH 7.5 and acetonitrile in ratio 15:5 v/v. The mobile phase was filtered using 0.45µ nylon filters (Milipore, USA) and degassed by sonication before use.

Preparation of standard solutions

A- Stock standard solution for ferric chloride method.

An accurate weight of entacapone (25 mg) was transferred into 100ml volumetric flask dissolving and raising the volume up to the mark with ethanol to produce solution of 0.25 mg/ml concentration

B-Stock standard solution for the spectrophotometric methods and HPLC method

An accurate weight (25mg) of entacapone was transferred into 250ml volumetric flask dissolving and raising the volume up to the mark with distilled water to produce solution of 0.1mg/ml concentration.

C- Preparation of laboratory prepared mixture

Aqueous solutions of each of entacapone, levodopa and carbidopa was prepared by transferring 25mg of each into three separate 250ml volumetric flasks, dissolving and raising the volume up to the mark with distilled water to produce solution of 0.1mg/ml concentration of each.

Into 100ml volumetric flask aliquots of 50ml, 37.5ml and 9.5ml of each of entacapone, levodopa and carbidopa were transferred respectively and the volume was made up using distilled water. The final concentration of the prepared mixture is similar to the concentration ratio of the mentioned drug in stalevo tablets

Apparatus

-Ultraviolet/ visible spectrophotometer shimadzu, Japan 1601 pc
-Ultrasonic crest model 575T Cortland, New York13045, USA.
-pH meter 211 Hanna, Portugal.
-HPLC instrument, Hewlett Packard series 1100 equipped with quaternary pump, diod array detector, manual injector 20µl loop.

RESULT AND DISCUSSION

The aim of the present study was to develop simple, accurate and sensitive methods for the determination of entacapone either alone or in combination with levodopa and carbidopa which formulated with entacapone in stalevo tablets.

The first method was a colorimetric method suitable for the determination of ethanolic solution of entacapone in single form using ferric chloride.

The second method depends on measuring the UV absorbance of aqueous solution of entacapone either in zero or first order. The method was found to be suitable either for entacapone determination alone or in combination with levodopa and carbidopa.

The third method is an accurate, simple and sensitive isocratic HPLC method for the simultaneous determination of entacapone, levodopa and carbidopa in their aqueous solution.

Ferric chloride method.

Procedure and optimization of conditions

The optimum conditions of the colored complex formation between entacapone and ferric chloride were studied including the used solvent, concentration and volume of ferric chloride solution as well as the wavelength of maximum absorbance.

Many solvents were tried including distilled water, ethanol, methanol and acetonitrile. It was found that quantitative results and complex stability were achieved on using ethanol. The optimum ferric chloride concentration and volume which gave higher sensitivity and best result reproducibility were 5ml 0.4%W/V ethanolic ferric chloride solution. The spectrum of the formed complex gave maximum absorbance at 665nm $\pm$ 2nm (Fig. 1).

Fig. 1 Absorbance spectrum of ferric chloride-entacapone complex

Linearity.

Into a series of 10ml volumetric flasks transfer aliquots (0.3-4.5ml) of the stock standard solution prepared for ferric chloride method, to each flask 5ml of 0.4% w/v ethanolic ferric chloride solution was added. The volume was completed using ethanol. The flasks were left to stand for 5 minutes and measured spectrophotometrically at 665nm. Each concentration was measured in triplicate. Good linearity was observed over the concentration range 10 μ g/ml-100 μ g/ml. The linear regression analysis data is given in table1.

Stability of the colored complex was assessed by measuring the formed complex each 10 minutes over 10 hours, the complex was found stable over 4 hours

Table 1. Results of linearity of the proposed ferric chloride method and spectrophotometric methods

method	Linearity range (μ g/ml)	intercept	slop	r ²
Ferric chloride	10-100	-0.0095	0.0094	0.9994
Zero order spectrophotometric	1-12	-0.0043	0.0842	0.9993
first order spectrophotometric	2-16	-0.0011	0.0035	0.9992

Stoichiometry

Job's method of continuous variation indicates the Fe- entacapone complex had a 1:1 stoichiometry. The complex may be formed via binding of Fe to the 3OH group which has more electron density, as the electrons at 4OH group are less available due to the presence of the electron withdrawing nitro group in the 5 position. The formed complex might had more complicated explanation due to the presence of more chemical species (different ions) in the solution as explained by Frank and Murry ¹⁰ in the study of aluminium complexation with catechols.

Spectrophotometric methods

Procedure and optimization of condition

The reported spectrophotometric method for the determination of entacapone in acetonitrile ⁹ is only valid for its determination in single form. The present paper introduce a simple spectrophotometric methods for entacapone determination either in single form or in combination with levodopa and carbidopa depending on the variation of entacapone spectra

on using different solvents.

Entacapone shows variation in color and UV spectrum according to the solvent, acidity or alkalinity. Its methanolic or aqueous solution appears yellow with λ_{max} at 309nm and 391nm respectively. Addition of 0.1N HCl to an aqueous solution of entacapone turns the solution from yellow to colorless with λ_{max} at 305nm, while addition of 0.1NaOH makes the solution red with λ_{max} at 392nm and a shoulder at 425nm (fig.2).

As the catechol structure of entacapone resemble some color indicators as bromocresol green ¹¹ and phenl red ¹² so by studying the mechanism of color changes of these indicators, entacapone behaviour could be explained as follows:-

In water entacapone ionizes by losing one proton from one of its phenolic OH forming an anion, on addition of NaOH a second proton was lost forming dianion. The change of color and maximum wavelength of absorption between entacapone, its anion and dianion may attributed to the increase of the molecule conjugation so it absorb lower energy (longer wavelength) ¹³. The discharge of the yellow color on addition of HCl may be due to the protonization of the keto group breaking the conjugation of the molecule and makes it colorless.

It was found that dissolving entacapone in water gives a maximum absorbance at 391nm at which the drug could be measured in presence of levodopa and carbidopa without any interference, on the other hand dissolving entacapone in methanol or acetonitrile gave a λ_{max} around 306nm $\pm$ 3nm at which levodopa and carbidopa will interfere. So distilled water was chosen as the solvent of choice for this method. The wavelength of maximum absorbance was determined by plotting the spectra of aqueous entacapone solutions of different concentrations in both zero and first order. It was found that optimum wavelength of measuring entacapone in zero order is 391nm, while in first order it is better to measure the amplitude between 360nm and 402nm (Fig 3,4).

Fig.3. Zero order spectra of 1- entacapone 2- levodopa 3- carbidopa

Linearity.

Into a series of 10 ml volumetric flasks aliquots of (0.1-2ml) were transferred and volumes were raised up to mark using distilled water. Absorbance of each solution was measured in triplicates in zero and in first order by measuring the absorbance at 391nm and the amplitude between 360nm and 402nm respectively. Linear relation between the concentration and absorbance was given in the range 1 µg/ml-12 µg/ml on using the zero order and 2 µg/ml-16 µg/ml on using the first order. The linear regression analysis data is given in table 1

Application of the method for the determination of entacapone in mixture with levodopa and carbidopa.

Aliquots (0.5-3 ml) of laboratory prepared mixture were transferred into a series of 10 ml volumetric flasks, the volumes were raised up to mark with distilled water. Each solution was measured in triplicates in zero and in first order by measuring the absorbance at 391nm and the amplitude between 360nm and 401nm respectively. Good results were obtained as shown in table 2.

Table 2. Results of analysis of laboratory prepared mixture using the proposed spectrophotometric and HPLC methods.

drug	Zero order method			first order method			HPLC method		
	Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery
Entacapone	2.5	2.545	101.81	5	4.89	97.80	10	10.11	101.10
	5	4.897	97.90	7.5	7.48	99.50	30	30.19	100.64
	7.5	7.55	100.67	10	10.03	100.30	40	39.99	99.97
		99.90		15	14.89	99.30	47.5	47.52	100.04
	10	9.99	100.07±1.65			99.23±1.04			100.44±0.53
mean±SD									
Levodopa	1.875	—	—	3.750	—	—	7.5	7.55	100.66
	3.750	—	—	5.625	—	—	22.5	22.16	98.51
	5.625	—	—	7.500	—	—	30	30.11	100.38
		—	—	11.25	—	—	35.625	35.85	100.63
	7.500	—	—						100.05±1.03
mean±SD									
carbidopa	0.475	—	—	0.475	—	—	1.900	1.897	99.82
	0.950	—	—	0.950	—	—	5.700	5.79	101.58
	1.425	—	—	1.425	—	—	7.600	7.55	99.37
		—	—	1.900	—	—	9.025	9.023	99.98
	1.900	—	—						100.19±0.96
mean±SD									

HPLC method.

Procedure and optimization of conditions

The reported HPLC method⁸ concerned with the determination of the percent drug release of levodopa, carbidopa and entacapone from its combination dosage form. The present work introduce a HPLC method for the determination of each of entacapone levodopa or carbidopa in its aqueous solution or thier simultaneous determination in laboratory prepared mixture making the method convenient for quality control determination of formulations containing one or more substances of the studied drugs.

Optimization of the chromatographic conditions were carefully tested. Initially various mobile phases and stationery phases were tested to obtain the best separation and resolution between entacapone, levodopa and carbidopa, which were obtained on using Luna CN 150x4.6 mm column as a stationery phase and 20mM ammonium acetate solution pH 7.5 and acetonitrile 15:5 as a mobile phase at flow rate 1ml / min and detection was carried out at 390nm for entacapone and 280nm for both levodopa and carbidopa Fig 5.6.

Fig. 5. HPLC chromatogram of 1-entacapone 2- levodopa 3- carbidopa at 280nm

Fig. 6. HPLC chromatogram of 1-entacapone 2- levodopa 3- carbidopa at 390nm

Linearity

From the stock standard solution 0.1mg/ml in distilled water of each of entacapone, levodopa and carbidopa, 50, 37.5ml and 9.5ml respectively were transferred into three separate 100 ml volumetric flasks and the volumes were made up with distilled water. From these solutions aliquots (0.5-24ml) of each drug were transferred into 25ml volumetric flasks and the volumes were raised to the mark using distilled water. Each flask was measured in triplicate using the mentioned optimum conditions. Results indicated linear relation between the concentration and the peak area within the range 1 µg/ml-48 µg/ml of entacapone, 1.5 µg/ml-36 µg/ml for levodopa and 0.76 µg/ml-9.12 µg/ml for carbidopa. The linear regression analysis data is given in table 3

Table 3. Results of linearity of the proposed HPLC method

drug	Linearity range (µg/ml)	intercept	slop	r ²
entacapone	1-48	-2.6629	165.37	0.9999
levodopa	1.5-36	5.1292	170.85	0.9999
carbidopa	0.76-9.12	-0.6875	175.41	0.9995

Application of the method for the simultaneous determination of entacapone , levodopa and carbidopa.

Aliquots (2.0- 9.5 ml) of laboratory prepared mixture were transferred into a series of 10ml volumetric flasks, volumes were raised up with distilled water. Each solution was injected in triplicate using the mentioned optimum chromatographic conditions, results are illustrated in table 2.

Accuracy

The accuracy of the three methods was tested for the determination of different concentrations of entacapone either alone or in mixture with levodopa and carbidopa. Recovery range and standard deviation are given in table 4, 5.

Table 4. Accuracy of ferric chloride method and spectrophotometric methods.

Ferric chloride method			Zero order method			First order method		
Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery
15	14.84	98.94	3	3.02	100.67	6	6.029	100.45
30	30.48	101.60	6	5.99	99.83	9	9.170	101.90
60	60.48	100.80	9	9.15	101.65	12	12.030	100.24
90	89.94	99.94	12	11.9	99.20	15	14.886	99.24
mean±SD	89.95	100.32±1.143			100.34±1.062			100.46±1.097

Table 5. Accuracy of HPLC method.

Entacapone			Levodopa			carbidopa		
Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery
6	6.033	100.55	4	3.97	99.25	1	1.01	101.00
12	11.950	99.58	8	8.08	101.00	2	2.01	100.50
24	23.810	99.21	16	15.92	99.50	4	3.97	99.34
48	48.380	100.8	32	32.2	100.64	6	6.03	100.50
mean±SD		100.035±0.76			100.1±0.853			100.335±0.704

Application of the proposed methods on pharmaceutical preparation

Entacapone in comet tablet was determined using ferric chloride method, spectrophotometric methods and HPLC method. Results show good agreement between found and claimed amount of entacapone as shown in table 6 , confirming the suitability of the proposed methods for the quality control determination of entacapone in pharmaceutical formulation. The validity of the methods was assessed applying the standard addition technique, results are given in table, 7, 8.

Table 6. Determination of entacapone in comptan tablets using the proposed methods.

Ferric chloride method			Zero order method			First order method			HPLC method		
Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery	Taken (µg/ml)	Found (µg/ml)	% recovery
20	20.37	101.85	4	3.95	98.75	6	6.03	100.50	10	9.90	99.00
40	39.63	99.10	6	6.02	100.33	8	8.03	100.38	20	20.2	101.00
60	60.16	100.27	8	8.59	100.74	10	10.03	100.30	40	40.22	100.55
mean±SD		100.41±1.38			99.94±1.05			100.39±0.10			100.18±1.05

Table 7. Application of standard addition technique for the determination of entacapone in comptane tablets using ferric chloride method and HPLC method.

Ferric chloride method					HPLC method				
Taken of tablets (µg/ml)	Found of tablets (µg/ml)	Standard added (µg/ml)	Found of added standard (µg/ml)	% Recovery of added standard	Taken of tablets (µg/ml)	Found of tablets (µg/ml)	Standard added (µg/ml)	Found of added standard (µg/ml)	% Recovery of added standard
20	20.37	10	9.90	99	10	9.90	5	5.05	101.00
		30	30.11	100.36			20	19.95	99.73
40	39.63	20	19.89	99.50	20	20.2	15	14.82	98.80
		60	60.74	101.20			25	25.06	100.24
mean±SD				100.015±0.97					99.94±0.92

Table 8. Application of standard addition technique for the determination of entacapone in comptane tablets using spectrophotometric methods.

Zero order method					First order method				
Taken of tablets (µg/ml)	Found of tablets (µg/ml)	Standard added (µg/ml)	Found of added standard (µg/ml)	% Recovery of added standard	Taken of tablets (µg/ml)	Found of tablets (µg/ml)	Standard added (µg/ml)	Found of added standard (µg/ml)	% Recovery of added standard
4	3.95	2	3.03	101.37	6	6.03	6	5.999	99.98
		4	4.06	101.50			10	10.280	102.80
6	6.02	4	3.98	99.60	8	8.03	2	1.999	99.93
		6	5.92	98.70			5	5.113	102.26
mean±SD				100.29±1.37					101.24±1.50

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