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Investigation of different spectrophotometric and chemometric methods for determination of entacapone, levodopa and carbidopa in ternary mixture

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ABSTRACT

New, simple, accurate and sensitive UV spectrophotometric and chemometric methods have been developed and validated for determination of Entacapone (ENT), Levodopa (LD) and Carbidopa (CD) in ternary mixture. Method A is a derivative ratio spectra zero-crossing spectrophotometric method which allows the determination of ENT in the presence of both LD and CD by measuring the peak amplitude at 249.9 nm in the range of 1–20 μg mL^{-1}. Method B is a double divisor-first derivative of ratio spectra method, used for determination of ENT, LD and CD at 245, 239 and 293 nm, respectively. Method C is a mean centering of ratio spectra which allows their determination at 241, 241.6 and 257.1 nm, respectively. Methods B and C could successfully determine the studied drugs in concentration ranges of 1–20 μg mL^{-1} for ENT and 10–90 μg mL^{-1} for both LD and CD. Methods D and E are principal component regression and partial least-squares, respectively, used for the simultaneous determination of the studied drugs by using seventeen mixtures as calibration set and eight mixtures as validation set. The developed methods have the advantage of simultaneous determination of the cited components without any pretreatment. All the results were statistically compared with the reported methods, where no significant difference was observed. The developed methods were satisfactorily applied to the analysis of the investigated drugs in their pure form and in pharmaceutical dosage forms.

1. Introduction

Parkinson's disease is a progressive, neurodegenerative disorder of the extrapyramidal nervous system. It affects the mobility and control of the skeletal muscular system and is characterized by tremor, rigidity, bradykinesia and postural instability [1].

Levodopa (LD), 3-hydroxy-L-tyrosine (Fig. 1a) is the most effective agent in the treatment of Parkinson's disease. In fact, although LD is able to cross the blood–brain barrier, not more than 1% of the administered dose can reach the central nervous system due to rapid metabolism by two enzymatic pathways, dopa decarboxylase (DDC) and catechol-*O*-methyl transferase (COMT) [2]. In addition, dopamine released into the circulation by peripheral conversion of LD produces undesirable effects, particularly nausea and hypotension. For these reasons, LD is almost always administered in combination with a peripherally acting inhibitor of DDC, such as Carbidopa (CD), (αS)-α-hydrazinyl-3,4-dihydroxy-α-methyl-benzene propanoic acid (Fig. 1b), which increases the amount of LD that reaches the brain and reduces the incidence of peripheral side effects. Unfortunately, the efficacy of chronic administration of LD/DDC inhibitor decreases with time and most patients develop fluctuating responses and dyskinesias [3].

A further improvement in the treatment of Parkinson's disease was obtained during the last decade, with the introduction of COMT inhibitors, such as Entacapone (ENT), (2E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide (Fig. 1c), used to stop the LD metabolism to 3-O-methyldopa, a potentially harmful metabolite of LD.

Nowadays, a formulation containing a combination of LD, CD and ENT is available on the market as oral tablets with the trade name of *Stalevo*®, approved by FDA since 2003. The administration of ENT together with LD and CD leads to greater and more sustained plasma levels of LD than those obtained after administration of LD and CD [4].

Literature review showed that ENT can be determined by spectrophotometry [5,6], chromatography [7,8] and electrochemical detection [9,10]. LD can be determined alone [11–13] or together with CD [14–16] by electrochemical detection, capillary electrophoresis and in plasma using HPLC/MS/MS. Spectrophotometric [17,18] and fluorimetric [19] methods were described for simultaneous determination of LD and CD. Simultaneous determination of ENT, LD and CD was achieved using electrochemical detection [20] and several chromatographic techniques [21–25]. ENT was previously determined through its zero order and first derivative spectra in presence of LD and CD [21].

The aim of our study is to present five new simple methods including; derivative ratio zero crossing (DRSZ), double divisor-ratio derivative (DDDR), mean centering of ratio spectra (MCR), principle

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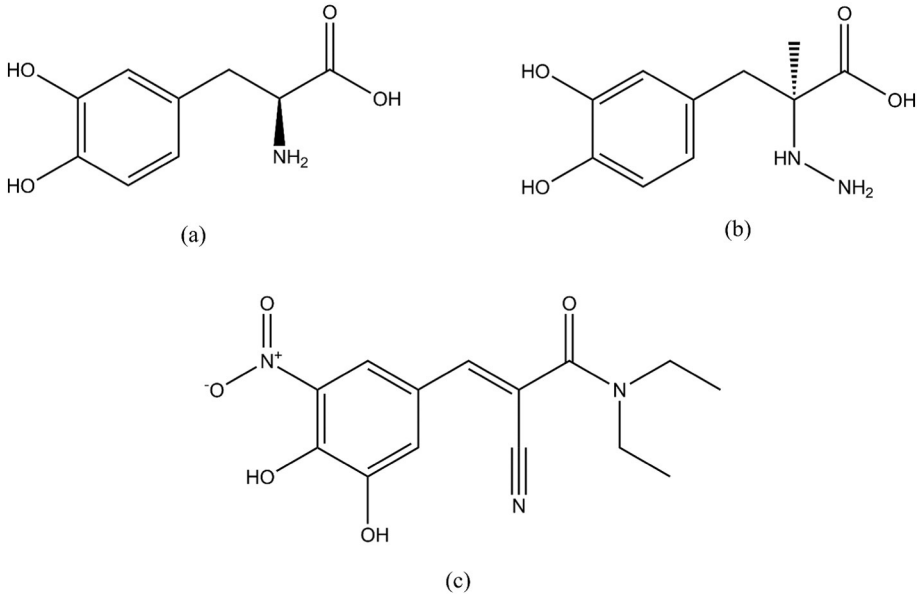

Fig. 1. Chemical structures of LD (a), CD (b) and ENT (c).

component regression (PCR), partial least squares (PLS) spectrophotometric methods which are used for determination of the investigated drugs: ENT, LD and CD in ternary mixture.

The proposed methods are the first spectrophotometric and chemometric methods for the simultaneous determination of this combination in their dosage form. The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, less expensive and less time consuming compared with other published liquid chromatographic (LC) methods.

2. Experimental

2.1. Instrumentation

Double-beam Shimadzu (Japan) 1601 PC UV–Visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used.

The data handling in PCR and PLS-2 methods was done using PLS-toolbox software version 2.1-PC for use with MATLAB® 7.0.1.24704 (R14).

Data analysis in case of MCR method was performed using Minitab® version 14.12.0 software.

The hot plate and stirrer MTopo MS 300HS was used for stirring.

2.2. Pure samples

ENT and LD (with certified purity of 100.00%) were obtained from Cayman Chemical, USA while anhydrous CD (with certified purity of 99.54%) was obtained from Sigma–Aldrich, USA.

2.3. Pharmaceutical formulation

Stalevo® tablets (market sample) nominally containing 200 mg ENT, 150 mg LD and 37.5 mg of CD (as CD monohydrate) per tablet. In addition to the previously mentioned active ingredients, excipients were used to form the tablet core and film-coating namely croscarmellose sodium, magnesium stearate, maize starch, mannitol, povidone, glycerol 85%, hypromellose, magnesium stearate, polysorbate 80, red iron oxide (E 172), sucrose, titanium dioxide (E 171), yellow iron oxide (E 172). Tablets (batch number 1518930) were manufactured by Orion Corporation, Espoo, Finland for Novartis Pharma AG, Basle, Switzerland.

2.4. Reagents

Methanol of analytical spectrophotometric grade was used for all the experiments. Bi-distilled water is used throughout the whole work and is indicated by the word “water”.

2.5. Standard solutions

Stock standard solutions of ENT, LD and CD having concentrations of 100 μg mL^{-1}, 300 μg mL^{-1}, 300 μg mL^{-1} respectively were prepared in methanol/water (70/30, % v/v) which is used as a solvent and then stored at 4 °C.

2.6. Procedures

Aliquots equivalent to 10–200 μg ENT were accurately transferred from its stock standard solution (100 μg mL^{-1}) into a series of 10-mL volumetric flasks. The volume was completed to the mark with methanol/water (70/30, % v/v) to obtain a final concentration range of 1–20 μg mL^{-1}.

The same was done for both LD and CD by diluting aliquots equivalent to 100–900 μg for each of them separately into a series of 10-mL volumetric flasks where a final concentration range of 10–90 μg mL^{-1} was obtained for both drugs.

The zero order absorption spectra (D_0) of the resulting solutions were recorded from 200 to 600 nm versus methanol/water (70/30, % v/v) as a blank and then stored on the computer.

2.6.1. Univariate methods

2.6.1.1. DRSZ. Using the recorded absorption spectra, we will use CD (60 μg mL^{-1}) as a divisor, the amounts of ENT in the ternary mixture were determined by measuring the first derivative ratio amplitudes using ($\Delta\lambda = 4$) at 249.9 nm (zero-crossing point for LD).

2.6.1.2. DDDR. This method is based on the use of the coincident spectra of the derivative of ratio spectra obtained by using a “double divisor” (sum of two spectra) and measuring at either maximum or minimum wavelengths [26,27].

For ENT, the binary mixture solution of LD and CD (60 μg mL^{-1} each), was used as double divisor (DD). The stored spectra of ENT