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# Determination of Six Anti-Parkinson Drugs using Cyclodextrin-Capillary Electrophoresis Method: Application to Pharmaceutical Dosage Forms

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## Abstract

A novel capillary electrophoretic method was developed for the assay of two quaternary anti-Parkinson mixtures, entacapone, levodopa, carbidopa, and benserazide (mixture I), and selegiline, levodopa, carbidopa, and benserazide (mixture II), by using α-methyldopa as an internal standard. Furthermore, the method was extended for the determination of another anti-Parkinson drug, lisuride, as well as a psychoactive antihypertensive drug, α-methyldopa, without any modification of the general method. Separation and analyses of all compounds were simply achieved in an untreated fused-silica capillary tube (42.0 cm effective length and 50 μm internal diameter) within 7 minutes under an applied voltage of 20 kV. Optimum separation and analyses were obtained using 25 mM borate buffer (pH 9.5) containing 5 mM β-cyclodextrin as the background electrolyte. The apparatus was equipped with a diode array detector (DAD) to identify lisuride at 240 nm and all other drugs at 200 nm. The addition of 5 mM β-cyclodextrin to the borate buffer has a significant effect on the separation of entacapone and benserazide in mixture I, and on the separation of selegiline and benserazide in mixture II, which cannot be achieved without it. The proposed method was successfully applied to analyse the studied drugs in their multi-component and single-component pharmaceutical dosage forms. The analytical results proved the linearity ($r^2 \geq 0.9997$), accuracy, precision (% RSD < 2), and selectivity of the proposed capillary electrophoretic method.

**Key words:** anti-Parkinson drugs; capillary electrophoresis; β-cyclodextrin; pharmaceutical dosage forms.

## 1. Introduction

Parkinson's disease is a chronic neurodegenerative disease associated with a significant decrease in brain dopamine (DA) levels, leading to motor system disorders such as muscular rigidity and tremors.[1] The first line of treatment for Parkinson's disease is to restore normal DA levels in the brain by taking exogenous DA in the form of its precursor levodopa (LDP), as DA itself cannot cross the blood brain barrier (BBB). Once the LDP prodrug crosses the BBB, it is metabolized to DA by aromatic L-amino acid decarboxylase (AAD).[2] The drawback of using LDP alone to treat Parkinson's disease is its rapid metabolism by AAD and catechol-*O*-methyltransferase (COMT) peripheral enzymes; therefore, only slight amounts of LDP (less than 1 %) make it across the BBB. Furthermore, this leads to an accumulation of DA in the periphery, producing undesirable side effects. Hence, to obtain the highest efficacy and lowest toxicity, fixed dose combinations of LDP with AAD and/or COMT inhibitors are commonly used for treatment of Parkinson's disease.[3] Levodopa is currently combined with either AAD inhibitors, such as carbidopa (CDP) and benserazide (BSZ), or COMT inhibitors, such as entacapone (ENT).[4] Selective monoamine oxidase inhibitors (MAO-B inhibitors), such as selegiline (SGN), are also effective in the treatment of Parkinson's disease since they slow DA catabolism in the CNS, helping to maintain proper DA levels. Selegiline is mainly used as an adjunct to LDP since it has the ability to potentiate and prolong the effect of LDP.[5] Alternative treatments for Parkinson's disease include the use of dopamine agonists such as lisuride (LSD). Lisuride effectively binds to $D_2$ receptors and has a longer duration of action than LDP.

Several analytical methods have been reported for the determination of anti-Parkinson drugs and these methods include spectrophotometric,[6,7] spectrofluorimetric,[8-10] chromatographic,[11-13] electrochemical,[14] and capillary

electrophoretic (CE) methods.[15-19] Capillary electrophoretic methods have several advantages over chromatographic methods owing to their ability to analyse several analytes simultaneously with high separation efficiency. Furthermore, capillary electrophoretic methods are characterized by simplicity, low sample and reagent consumption, and it complies with the green chemistry principles as it avoids the use of organic solvents. Capillary electrophoretic methods have higher selectivity and specificity than spectrometric methods. Capillary electrophoretic methods have the ability to separate and analyse a large number of analytes in different matrices at once without the need for prior tedious separation. Using spectrometric methods, simultaneous analysis can be performed to a some extent but without the ability to separate the analytes, hence lacking the selectivity and specificity.

A literature survey revealed different CE methods for the determination of LDP,[18-20] simultaneous determination of LDP and CDP[21] and simultaneous determination of LDP and BSZ[15,21], but no capillary electrophoretic methods have been applied for simultaneous determination of LDP, CDP, BSZ and ENT or LDP, CDP, BSZ and SGN. Therefore, the development of a general and selective method for the simultaneous analysis of anti-Parkinson drugs in different matrices is highly significant. Although the simultaneous determination of these analytes by a simple CE method is challenging owing to the structural similarity and nearly similar size to charge ratio of most analytes (Fig. 1), modifying the method by using buffer additives could solve such this problem. Addition of β-cyclodextrin (β-CD) to the buffer system drastically improved analyte separation through the formation of inclusion complexes with the different analytes at different rates; therefore, this overcame the overlap issue seen with some of the analytes, and enhanced separation of all the compounds within a reasonable migration time. The versatility of the

proposed method enabled us to quantitatively analyse LSD and MDP in their single component tablets indicating that our method is both a general and selective assay for anti-Parkinson drugs. Furthermore, the method has the ability to separate MDP from the two quaternary mixtures which is one of the most important impurities in LDP/CDP combination dosage forms, as specified by the United State Pharmacopoeia.[22]

## 2. Experimental

### 2.1. Apparatus and conditions

All experiments were achieved using a CAPI-3100 capillary electrophoresis device (Otsuka electronics, Osaka, Japan) equipped with a diode array detector and the analyses were performed in an untreated fused-silica capillary tube (GL Sciences, Japan) with a 0.05 mm internal diameter and an effective length of 42.0 cm (54 cm total length).

Before the first capillary use, the capillary tube was rinsed with 1.0 M sodium hydroxide for 60 min followed by ultra-pure deionized water for 30 min, and finally with the buffer system for 30 min. Everyday, before starting new experiments, the capillary tube was flushed with sodium hydroxide (0.1 M), water, and background electrolyte (BGE) for 10 min each. Between runs, vacuum suction was used to sequentially flush the capillary tube with 0.1 M sodium hydroxide and water for 3 min each, and finally with the BGE for 5 min, to ensure reproducibility of the assay.

Separations and analyses were performed using a BGE of 25 mM borate buffer with pH 9.5 containing 5 mM β-CD under an applied potential of +20 kV at 25 $^{\circ}$C. The analytes were hydrodynamically injected at a height of 25 mm for 15 s and detected at 200 nm for all analytes except for LSD, which was detected at 240 nm. A LAQUA pH–Meter (Horiba, Ltd. Kyoto, Japan) was used for pH measurements after proper device calibration.

**2.2. Materials and reagents**

All chemicals were of analytical grade and used as received without further purification. Levodopa and carbidopa were provided by October Pharma Co. (Egypt). Entacapone was provided by Chemi Pharma Co. (Egypt). Benserazide was purchased from Wako Pure Chemical Industries (Osaka, Japan). Selegiline and lisuride were provided by the National Organization for Drug Control and Research (Cairo, Egypt). α-Methyldopa was purchased from EGIS Pharmaceutical company (Hungary).

Pharmaceutical preparations, including Sinemet® CR 250 tablets (Lot. No: L005234; Merck Sharp & Dohme (MSD) S.P.A., Italy), labelled to contain 200 mg LDP and 50 mg CDP; Stalevo® 325 tablets (Lot. No:1604127; Novartis Pharma AG, Switzerland) labelled to contain 200 mg ENT, 100 mg LDP and 25 mg CDP; Madopar® tablets (Lot. No.:1215230; Roche Pharma AG) labelled to contain 200 mg LDP and 50 mg BSZ; Aldomet® tablets (Lot. No.:1510122; Kahira Pharm. & Chem. Ind. Co., Egypt) labelled to contain 250 mg MDP per tablet; Dopergin® tablets (Lot. No: 2531894; Bayer Schering Pharma AG, Germany) labelled to contain 0.2 mg LSD hydrogen maleate; and Tonus® tablets (Lot. No.: 1327130; ALPHA-CHEM Advanced Pharmaceutical Industries (ACAPI), Egypt) and labelled to contain 5 mg SGN hydrochloride, were purchased from local pharmacies in Egypt.

Sodium tetraborate decahydrate, β-cyclodextrin, and methanol (HPLC grade) were purchased from Sigma-Aldrich. Sodium dihydrogen phosphate, 85 % orthophosphoric acid, sodium hydroxide, hydrochloric acid, and sodium dodecyl sulphate, were purchased from Wako Pure Chemical Industries (Osaka, Japan). *Milli-Q* water (Milli-Q® Gradient A10 system™, Millipore, France) was used to prepare the solutions.

### 2.3. Preparation of background electrolyte

Sodium tetraborate buffer (100.0 mM) and a β-cyclodextrin solution (10.0 mM) were prepared in ultra-pure deionized water and mixed together in a ratio that produced a final BGE containing 25.0 mM borate buffer with pH 9.5 and containing 5 mM β-cyclodextrin.

### 2.4. Standard solutions

Stock standard 1.0 mg/mL solutions of LDP, CDP, MDP and BSZ were prepared in 0.1 M HCl, while 1.0 mg/mL solutions of ENT, LSD and SGN were prepared in methanol. The prepared solutions were kept at 4 °C, away from light, and wrapped in aluminium foil, to avoid photo-oxidation. The stock standard solutions were further diluted with 10 mM borate buffer to get the final concentrations of each analyte in the linear range.

### 2.5. Procedure

#### 2.5.1. Construction of calibration graphs

Accurately measured aliquots of the standard solutions of studied drugs were transferred to a series of 5.0-mL volumetric flasks so that the final concentration of each analyte was in the linear range. To each flask, a specific volume of the standard solution of internal standard (IS) was added to a final concentration was 20 µg/mL and the volume was made up with 10 mM borate buffer, pH 9.3. In the case of LSD, a final MDP (IS) concentration of 40 µg/mL was used.

The samples were then analysed using the optimized BGE composed of 25 mM borate buffer (pH 9.5) containing 5 mM β-CD, adjusting the temperature of the capillary and the sample tray to 25 °C using applied voltage of +20 kV and detecting wavelength at 200 nm for all analytes, except for LSD which was detected at 240 nm. The calibration curves were constructed by plotting the average corrected peak area ratio (analyte/IS) versus the analyte

concentration in µg/mL, followed by a linear regression analysis of the obtained data.

### 2.5.2. Analysis of the studied drugs in their laboratory-prepared quaternary mixtures

Aliquots of the standard solutions of studied drugs were measured in order to keep the ratio of the drugs in their different pharmaceutical combinations (8:4:1:1, ENT/LDP/CDP/BSZ for mixture I and 4:2:1:1, LDP/SGN/CDP/BSZ for mixture II) and were transferred into series of 10 mL volumetric flask. A specific volume (0.2 mL) of 1.0 mg/mL of MDP (IS) was added to each flask followed by dilution of all solutions with 10 mM borate buffer. The above ratios resemble the pharmaceutical ratio of LDP and CDP in Sinemet® tablets (4:1), LDP and BSZ in Madopar® tablets (4:1), and ENT, LDP and CDP in Stalevo® tablets (8:4:1), respectively. The procedure described above under ***"Construction of calibration graphs"*** was then followed. The percentages found were calculated by referring to the calibration graphs, or using the corresponding regression equations.

### 2.5.3. Analysis of the studied drugs in their single and combined tablet dosage forms

For each dosage form (Sinemet®, Stalevo®, Madopar®, Dopergin®, Tonus® and Aldomet® tablets), ten tablets were weighed, finely pulverized, and thoroughly mixed. For Sinemet® tablets, an accurately weighed amount of the powder, equivalent to 200.0 mg of LDP and 50.0 mg of CDP (its pharmaceutical ratio), were transferred to a 100.0-mL volumetric flask wrapped with aluminium foil and diluted with 0.1 M HCl to extract LDP and CDP. The flask was then sonicated for 30 min and filtered to get a clear solution containing 2.0 mg/mL LDP and 0.5 mg/mL CDP. Different working solutions within the linear range of the two drugs were obtained by further dilution with 10 mM

borate buffer. MDP IS (20 μg/mL) was also added to each solution. The relative corrected peak area (the corrected peak area of the analyte/the corrected peak area of IS) was then calculated for each analyte and the concentration was determined using the corresponding regression equation. For the other tablets, the same procedure was followed but the extracting solvent was changed according to the analytes present in the combined or single component tablets (Table 6). In the case of Stalevo® tablets, extraction was challenging since LDP and CDP are soluble in 0.1 M HCl and practically insoluble in absolute methanol, while ENT is soluble in methanol and insoluble in 0.1 M HCl. The two solvents were mixed in different ratios to extract the three drugs, and the results indicated that methanol:1 M HCl (90:10, v/v) is the best extracting solvent, in which the three drugs were efficiently extracted with high percentage recoveries.

## 3. Results and discussion

### 3.1. Method optimization and development

The buffer type, pH, concentration and additives are very important parameters for optimization of the analytical method. Different concentrations of phosphate and borate buffers were studied at different pH values with and without buffer additives. It was found that 25 mM borate buffer at pH 9.5 and containing 5 mM β-CD is the optimum BGE at which complete separation and reproducible quantification of the analytes were successfully achieved. Other influential parameters, such as the applied voltage and the capillary temperature, were also studied regarding their effect on the number of theoretical plates (NTP), resolution, produced current, and total run time (Table 1).

### 3.1.1. The effect of buffer pH

Buffer pH has a great effect on the electromigration of analytes, especially for weakly acidic and basic compounds (weak electrolytes). Regulating buffer pH is very important for stabilizing the migration velocity and electroosmotic flow (EOF) of the studied compounds. The effect of borate buffer pH on the electrophoretic separation was studied over a pH range of 8.0–10.0 with a buffer concentration of 25 mM. Severe overlaps between ENT and BSZ, and MDP and LDP, in mixture I, and between SGN and BSZ, and MDP and LDP, in mixture II, were noticed upon decreasing the pH values close to 8.0. In contrast, increasing the pH values near 10.0 resulted in an increase in the total run time, with good separation between MDP and LDP, but overlaps between BSZ and ENT, and BSZ and SGN, in the respective mixtures were still observed. The pH selected was 9.5 at which high NTP and good resolution were obtained, nonetheless, at this pH, baseline separation of BSZ and ENT, or BSZ and SGN, could not be achieved (Table 1). Therefore, another factor (buffer concentration) was studied to get baseline separation between BSZ and ENT, and BSZ and SGN, in the two quaternary mixtures at pH 9.5.

### 3.1.2. The effect of buffer concentration

Different buffer concentrations were investigated before selecting the best concentration at which complete separation occurred within a reasonable total migration time and with an acceptable produced current. The effect of buffer concentration on separation performance is attributed to its influence on the EOF and on the electric current produced within the capillary. The concentrations of borate running buffer studied varied from 10-50 mM, adjusting the pH to 9.5 for each experiment. As the concentration of borate buffer increased, the resolution and migration times increased (Table 1 & Fig. 2) with an increase in produced electric current (Joule heating), resulting in

peak broadening. The optimum buffer concentration at which the analytes were separated efficiently within a reasonable analysis time, and that produced minimal Joule heating, was 25 mM borate buffer (current; 25-33 μA). Nonetheless, it was noticed that the separation between BSZ and ENT, and BSZ and SGN, was not improved by varying the buffer concentration, so studying buffer additives was the next step.

### 3.1.3. The effect of buffer additives

Several trials were conducted to separate the analytes, especially ENT and BSZ in mixture I, and SGN and BSZ in mixture II, which co-migrated when using the buffer system without any additives, even if the pH or concentration of the buffer were varied, resulting in poor separation. The additives employed in our work included organic modifiers, surfactants, and cyclodextrins.

#### 3.1.3.1. Organic modifiers

The addition of organic solvents to the buffer decreases the EOF due to a decrease in Zeta potential, leading to an increase in the migration time, accompanied by a decrease in the current and in Joule heat production. Two organic solvents (methanol and acetonitrile) were investigated at different concentrations but they only increased the migration time of the analytes without improving the efficiency of the separation. Therefore, no organic modifier was added in this study.

#### 3.1.3.2. Surfactants

One of the effective ways to obtain better selectivity and improve the separation efficiency is the addition of surface active agents to the buffer. The most common additives are anionic surfactants like SDS or cationic surfactants like CTAB. The surfactant selected in our study was SDS at different concentrations over the critical micelle concentration (10-50 mM). The results

indicated that addition of SDS to the buffer system over the critical micelle concentration has no effect on the separation of the overlapped analytes, even if it maintained the good resolution between the other analytes.

#### 3.1.3.3. Cyclodextrins

Cyclodextrins are not only effective for separating chiral compounds but they also have a great effect on the separation of pharmaceutical compounds through host-guest interactions between the drugs and the cyclodextrin cavities. There are different types of cyclodextrins and many derivatives of each type, but the one used in our method is β-CD, which contributed in the effective separation of BSZ and ENT in mixture I, and separation of BSZ and SGN in mixture II. It has also the ability to increase the resolution between BSZ and MDP, while still keeping small migration times for all separated analytes and improving the peak shape of the analytes. As the concentration of β-CD increased, the separation efficiencies increased and high resolutions were obtained. Different concentrations of β-CD were tested (1-6 mM) and it was found that 5 mM is the concentration at which maximum separation and resolution between ENT and BSZ, and SGN and BSZ, were obtained (Fig. 3). It is proposed that β-CD forms an inclusion complex with BSZ and retards its migration throughout the capillary. Furthermore, the addition of β-CD has a great effect in improving the efficiency of the method (high value of NTP) and increasing the resolution between all analytes (Table 1).

### 3.1.4. The effect of applied voltage

The study of the effect of applied voltage on the separation performance of the proposed CE method was investigated by injecting the analyte mixture using the optimized BGE under applied voltages ranging from 15 to 25 kV. In general, as the applied voltage increased, the EOF increased and the migration time decreased, while maintaining efficient separation. However, increasing

the applied voltage over 20 kV resulted in higher currents and increased Joule heat production (Table 1). To overcome Joule heating, the maximum applied voltage selected was 20 kV at which high resolution was obtained within reasonable migration time. It was noticed that the total analysis time decreased to only about 5 minutes with good resolution upon applying a voltage of 25 kV (Fig. 4) but the current produced was high compared with 20 kV.

### 3.1.5. The effect of injection time

The analytes were hydrodynamically injected at a ΔH = 25 mm for 3-30 seconds. The injection time has a significant effect on the peak width and peak height and consequently affects sensitivity, so it is very important to study this parameter. It was found that increasing the injection time increases the sensitivity up to a point, at which the deformation of some analyte peaks, especially that of ENT occurs. Hence, 15 seconds was selected as the optimum injection time.

### 3.1.6. The effect of capillary temperature

The temperature of the capillary should be controlled since it affects the viscosity of the running buffer and the EOF. Different temperatures were investigated in our study, including 20, 23, 25, 27, and 30 °C. The optimum selected temperature was 25 °C since it provided the best resolution and avoided Joule heat production, as the current generated did not exceed 33 µA.

### 3.1.7. Selection of internal standard

Variance in precision is mainly attributed to the minor fluctuations in injection volumes. Therefore, it is recommended to use internal standard (IS) to ensure the accuracy and precision of the method. Selecting a suitable IS will overcome injection errors, and minimize error sources. For this reason, MDP

was selected as the IS in the quantitative analysis of the studied analytes either in combination or in single dosage forms. MDP is an appropriate IS since it was separated completely from the quaternary mixtures, coming after ENT and BSZ, or SGN and BSZ, and before LDP and CDP, and produced a sharp peak at a concentration 20 µg/mL. However, for LSD analysis, the concentration of MDP was increased to 40 µg/mL since the analysis of LSD was performed at 240 nm at which MDP exhibits much lower sensitivity. For MDP analysis, LDP (20 µg/mL) was selected as the IS.

### 3.1.8. Selection of the detection wavelength

Selection of the detection wavelength is very important since it controls the sensitivity of the method. Detections were performed over a range of 200-400 nm and the wavelength at which there was maximum sensitivity of most analytes was 200 nm, but in case of LSD, the maximum sensitivity was obtained at 240 nm, which was selected for its analysis in its tablet dosage form.

## 3.2. Validation

A validation study is performed on analytical methods to ensure that reliable results are always obtained. The proposed method was fully validated according to the ICH guidelines[23]. The validation parameters studied include linearity and range, accuracy, precision, LOD and LOQ, specificity, selectivity, and robustness.

### 3.2.1. Linearity and range

A linear relationship was established by plotting the corrected peak area ratios (the corrected peak area of the studied drug / the corrected peak area of IS) against the drug concentrations in µg/mL. Linear regression data and the main validation parameters are summarized in Table 2.

### 3.2.2. LOD and LOQ

LOQ and LOD were determined according to ICH Q2 (R1) recommendations[23], where LOQ corresponds to 10 $S_a$ /b and LOD corresponds to 3.3 $S_a$ /b ($S_a$ is the standard deviation of the intercept & b is the slope of the calibration curve). The values of LOD and LOQ for each analyte are listed in Table 2.

### 3.2.3. Precision and accuracy

Intraday and interday precisions were assessed using three different concentrations and three replicates of each concentration, for three successive days. The % RSD values were very small (< 2) as shown in Table 3, indicating the precision of the proposed method. To prove the accuracy of the proposed method in pharmaceutical preparations, a standard addition (SA) technique was performed, in which known increasing amounts of standard pure drugs were added to a series of solutions with the same amount of tablet working solution (e.g. 20 µg/mL), starting with a solution with no addition. The response was measured for each solution and the results are listed in Table 4.

### 3.2.4. Specificity and selectivity

The specificity of the proposed CE method was ensured by its ability to determine the studied drugs in their dosage forms (single component and multicomponent tablets) confirming that no interference was encountered in the presence of tablet excipients. Each film-coated tablet of Sinemet® CR 250 contains the following inactive ingredients: hydroxypropyl cellulose, magnesium stearate, hypromellose, FD&C Blue #2/Indigo Carmine AL and FD&C Red #40/Allura Red AC AL. Each tablet of Dopergin® contains lactose monohydrate, microcrystalline cellulose, magnesium stearate, tartaric acid and sodium calcium edetate. Madopar® tablets contain microcrystalline cellulose, magnesium stearate, mannitol, calcium hydrogen phosphate, crospovidone,

ethyl cellulose, iron (III) oxide (E 172), silica, colloidal anhydrous, and sodium docusate as inactive ingredients. It was found that these compounds did not interfere with the results of the proposed method (Fig. 5). In addition, the other formulations (Stalevo®, Tonus® and Aldomet® tablets) did not produce additional peaks upon their analysis, and the results were not different from those of the laboratory prepared mixture electropherograms. The method is also selective since it has the ability to separate each analyte successfully without any interference with the other analysed drugs and also has the ability to separate LDP and CDP from their structurally related compound MDP, which is a major impurity in LDP/CDP mixtures. The selectivity of the method was also studied by injecting SGN with LDP, CDP, and BSZ; SGN was completely separated indicating the ability of the method to determine this quaternary mixture as well (Fig. 6).

### 3.2.5. Robustness

The proposed method is robust since neither small deliberate changes in pH, borate buffer concentration, and β-CD concentration, nor did small changes in the CE conditions, such as applied voltage and capillary temperature, significantly affect the migration times of the analytes or the corrected peak area ratios. The parameters studied to evaluate the robustness of the proposed method include borate buffer (pH 9.5 ± 0.2), buffer concentration (25 ± 3 mM), β-CD concentration (5 ± 0.2 mM), applied voltage (20 ± 2 kV) and capillary temperature (25 ± 2 ˚C).

## 3.3. Application

### 3.3.1. Simultaneous analysis of ENT, BSZ, LDP and CDP (Mixture I) and SGN, BSZ, LDP and CDP (Mixture II) in their laboratory-prepared mixtures

The CE method was successfully applied to the simultaneous determination of ENT, LDP, BSZ, and CDP in their laboratory-prepared mixtures

(Fig. 7A) in a ratio of 8:4:1:1, respectively (Table 5). In addition, the method has the ability to analyse SGN, LDP, CDP and BSZ (2:4:1:1, respectively) in the second mixture (Table 5, Fig. 7B).

### 3.3.2. Analysis of the studied drugs in their single and combined pharmaceutical preparations

The studied compounds were analysed in multicomponent and single component pharmaceutical tablets by the proposed CE method. Simultaneous analysis of LDP, CDP, and ENT in Stalevo® tablets (Fig. 8A), LDP and CDP in Sinemet® tablets (Fig. 8B), and LDP and BSZ in Madopar® tablets (Fig. 8C), were performed successfully with high % recovery (98-102) and good % RSD < 2 as shown in Table 6. Furthermore, the method was applied for the determination of SGN, LSD, and MDP in Tonus®, Dopergin® and Aldomet® tablets, respectively (Fig. 9). The results obtained are listed in Table 6.

## 4. Conclusion

A novel, accurate and precise CD-modified CE method was developed for the simultaneous determination of ENT, BSZ, LDP and CDP, as well as SGN, BSZ, LDP and CDP, for the first time in their quaternary mixtures. The developed method was extended to analyse SGN, LSD, and MDP in their single dosage forms to demonstrate that it is a general and selective method for the analysis of anti-Parkinson drugs. The proposed CE method exhibits good validation criteria, and could be utilized in quality control laboratories. Additionally, our method is superior to the previously published spectrometric, chromatographic and electrochemical methods in terms of selectivity and versatility, and complies with green chemistry concepts by avoiding the use of toxic organic solvents.

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**Figure legends**

**Fig. 1** Structural formulae of the studied drugs.

**Fig. 2** Effect of the borate buffer concentration (10-50 mM) on the electrophoretic behaviour of the studied drugs in mixture I.

**Fig. 3** Effect of β-CD on electromigration of the analytes in mixture I. A: Chemical structure of β-CD, B: Typical electropherogram of ENT, BSZ, MDP, LDP, and CDP using 25 mM borate buffer only, C: Typical electropherogram of ENT, BSZ, MDP, LDP, and CDP after addition of 5 mM β-CD.

**Fig. 4** Typical electropherograms of ENT, BSZ, MDP, LDP and CDP using 25 mM borate buffer containing 5 mM β-CD under an applied voltage of A: 20 kV, B: 25 kV.

**Fig. 5** Typical electropherogram of LDP and CDP in combined Sinemet® tablets, demonstrating the specificity of the method.

**Fig. 6** Typical electropherogram of LDP and CDP in combined Sinemet® tablets after injection of SGN and MDP, demonstrating the selectivity of the method.

**Fig. 7** Typical electropherograms of A: ENT, LDP, BSZ and CDP (80:40:10:10 µg/mL) in their laboratory-prepared mixture, B: LDP, SGN, BSZ and CDP (60:30:15:15 µg/mL) in their laboratory-prepared mixture, using 20 µg/mL MDP IS.

**Fig. 8** Typical electropherograms of: A: ENT, LDP and CDP (80:40:10 µg/mL) in Stalevo® tablets; B: LDP and CDP (80:20 µg/mL) in Sinemet® tablets; C: LDP and BSZ (40:10 µg/mL) in Madopar® tablets, using 20 µg/mL MDP IS.

**Fig. 9** Typical electropherograms of: A: 40 µg/mL SGN in Tonus® tablets using 20 µg/mL MDP IS, B: 40 µg/mL LSD in Dopergin® tablets using 20 µg/mL MDP IS, C: 30 µg/mL MDP in Aldomet® tablets using 20 µg/mL LDP IS.

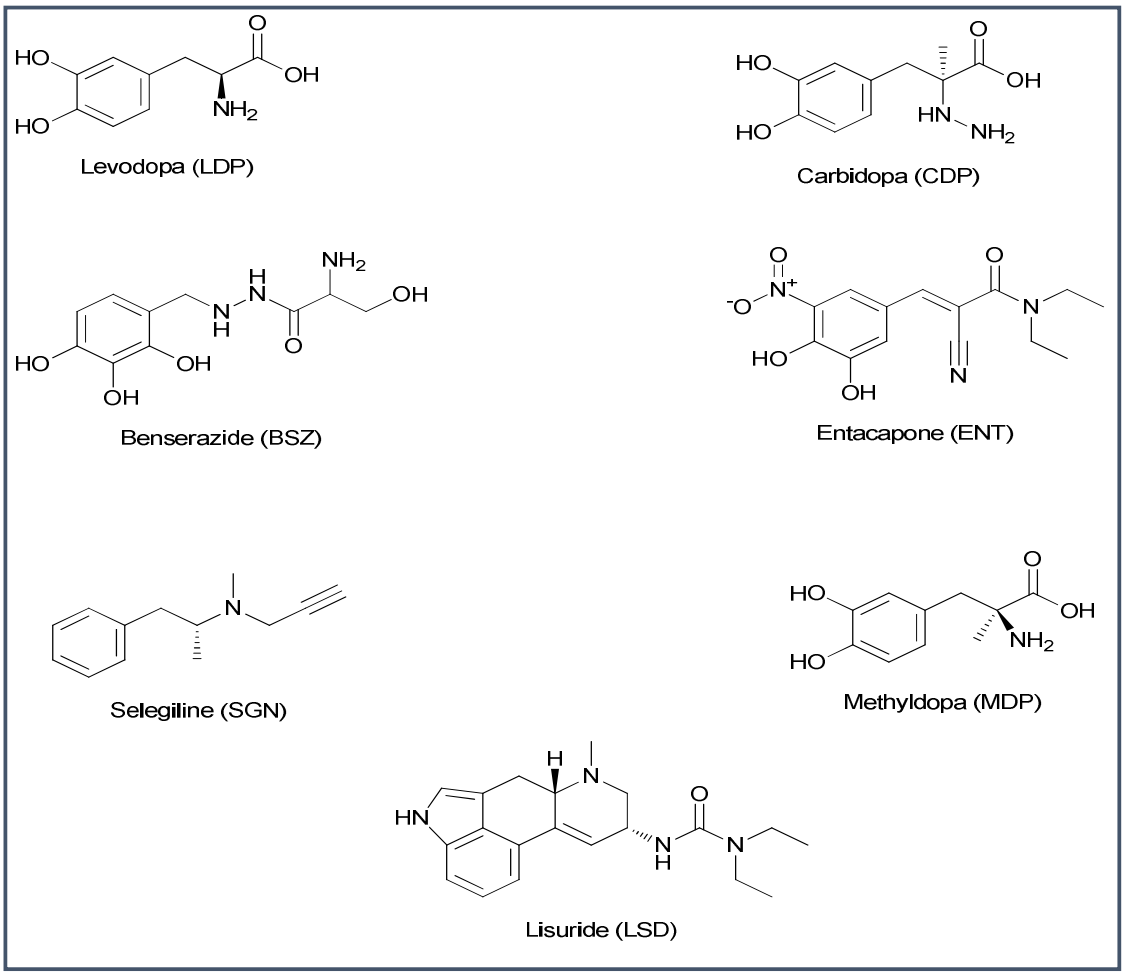


**Fig. 1** Structural formulae of the studied drugs.

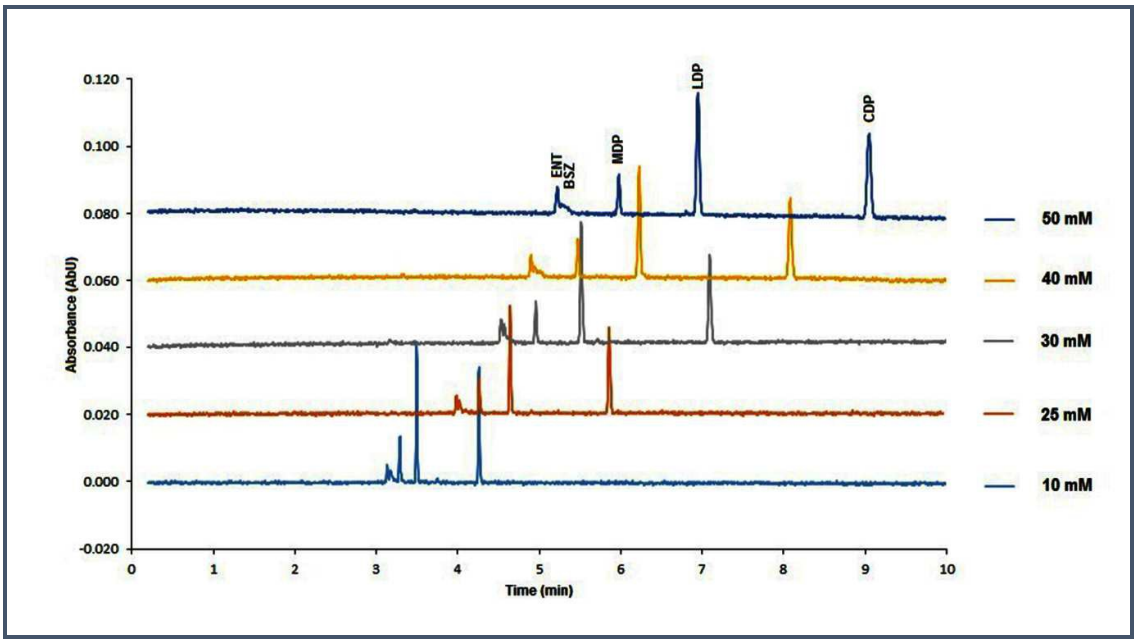


**Fig. 2** Effect of the borate buffer concentration (10-50 mM) on the electrophoretic behaviour of the studied drugs in mixture I.

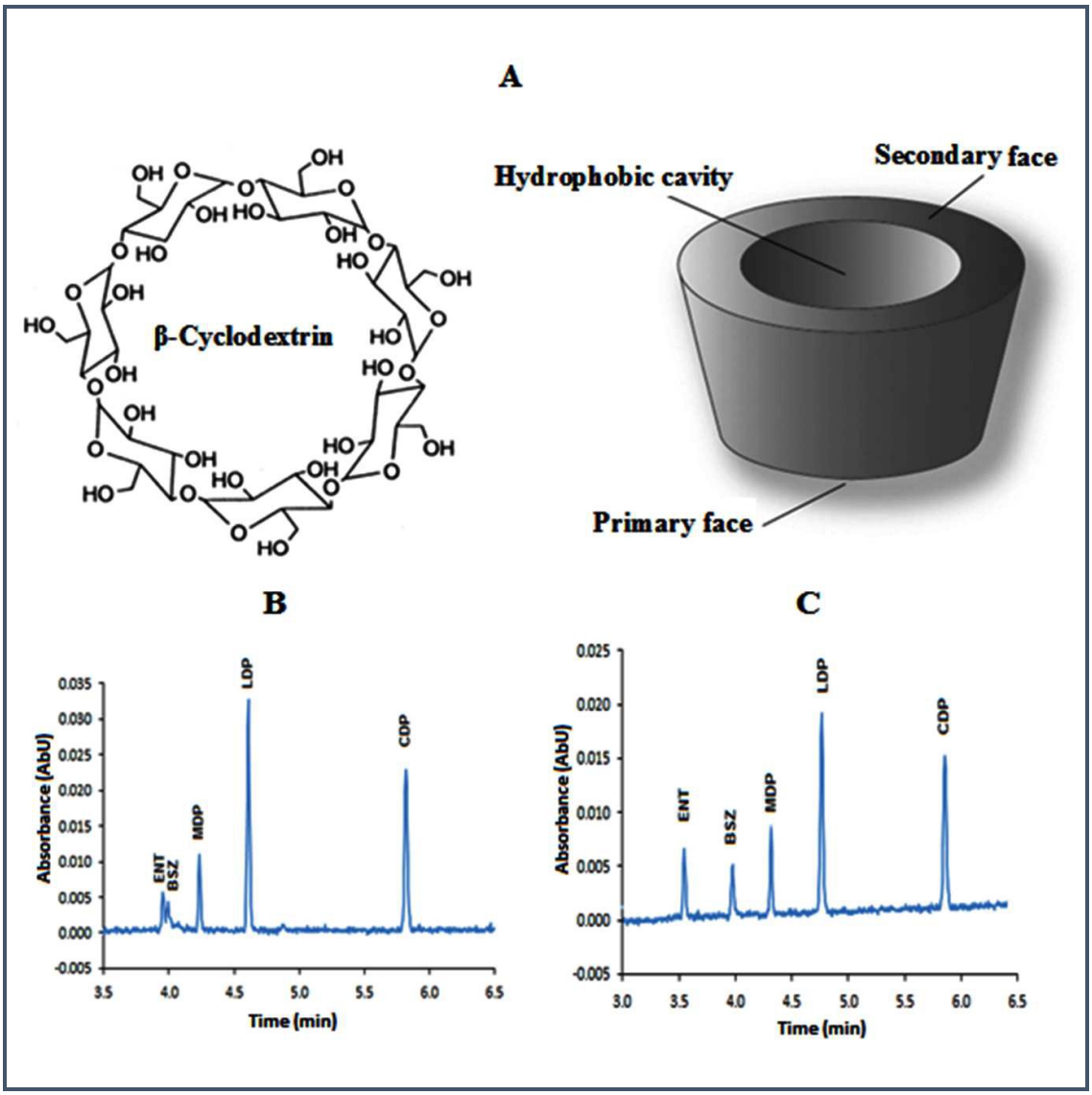


**Fig. 3** Effect of β-CD on electromigration of the analytes in mixture I. A: Chemical structure of β-CD, B: Typical electropherogram of ENT, BSZ, MDP, LDP and CDP using 25 mM borate buffer only, C: Typical electropherogram of ENT, BSZ, MDP, LDP and CDP after addition of 5 mM β-CD.

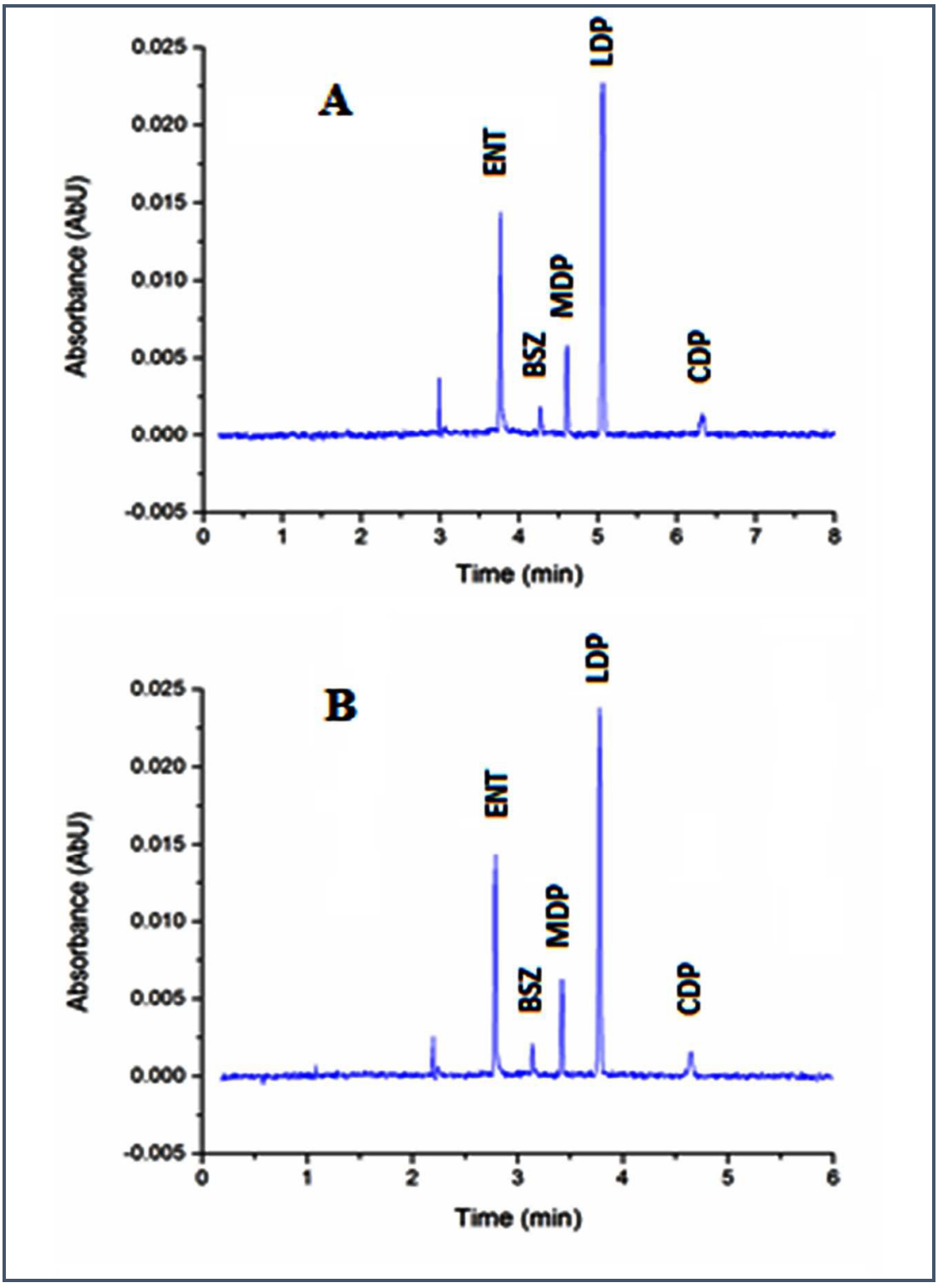


**Fig. 4** Typical electropherograms of ENT, BSZ, MDP, LDP and CDP using 25 mM borate buffer containing 5 mM β-CD under an applied voltage of A: 20 kV, B: 25 kV.

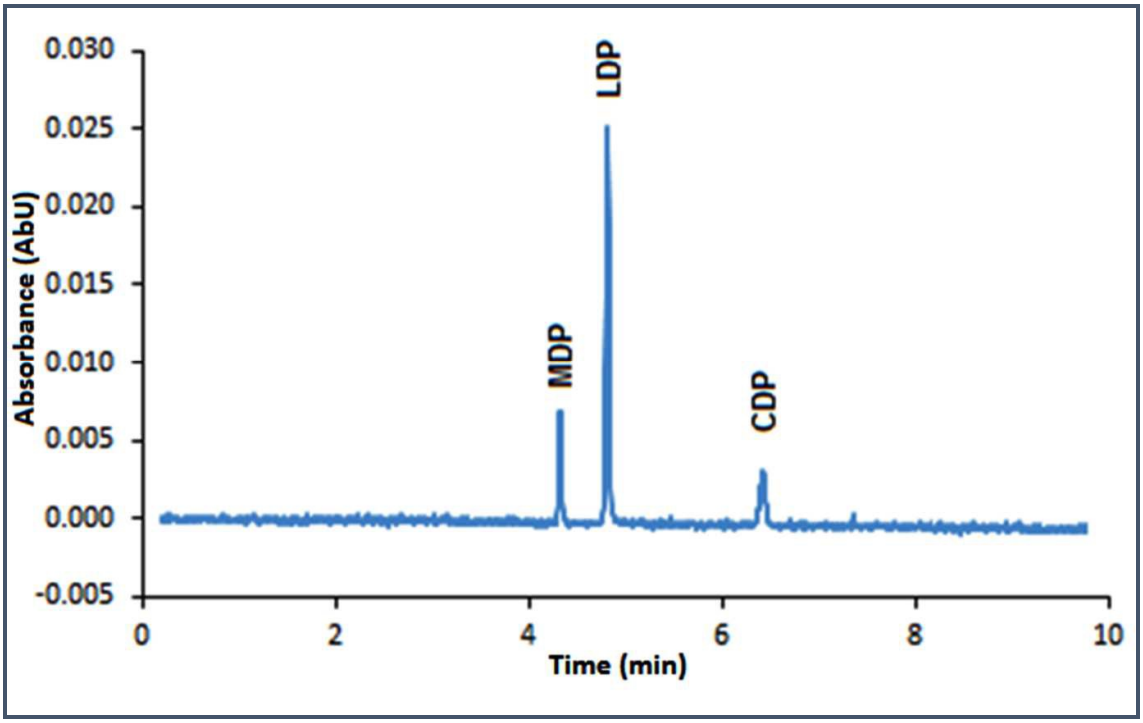


**Fig. 5** Typical electropherogram of LDP and CDP in combined Sinemet® tablets, demonstrating the specificity of the method.

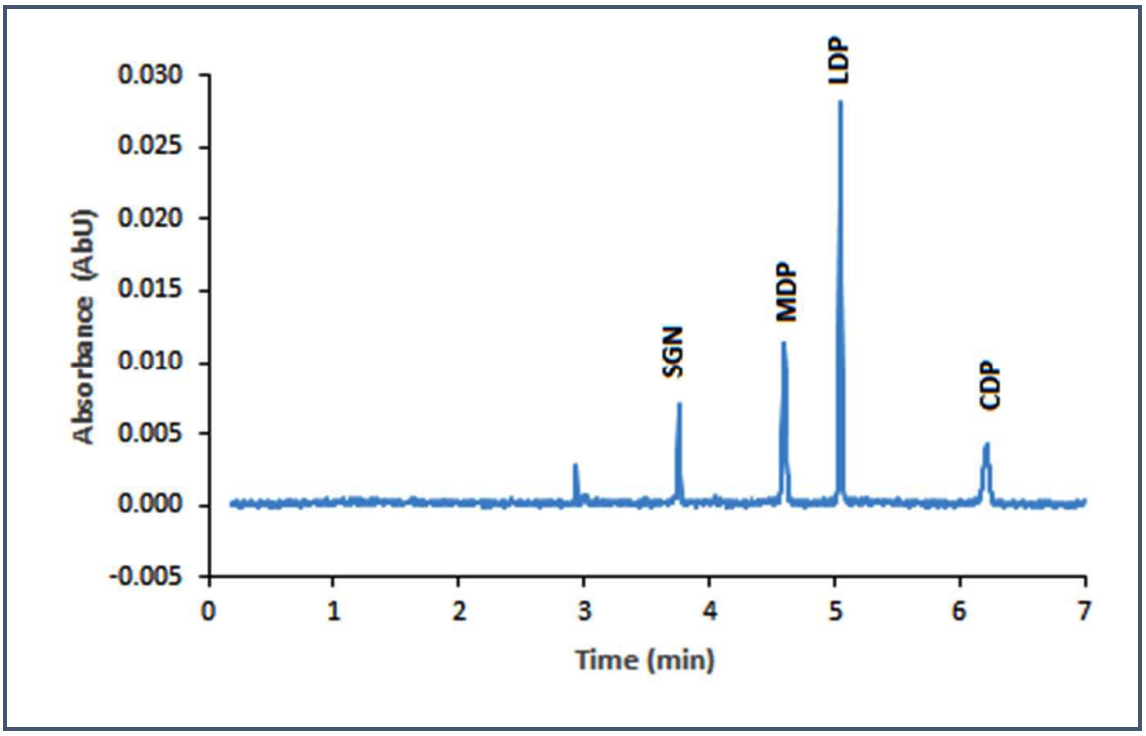


**Fig. 6** Typical electropherogram of LDP and CDP in combined Sinemet® tablets after injection of SGN and MDP, demonstrating the selectivity of the method

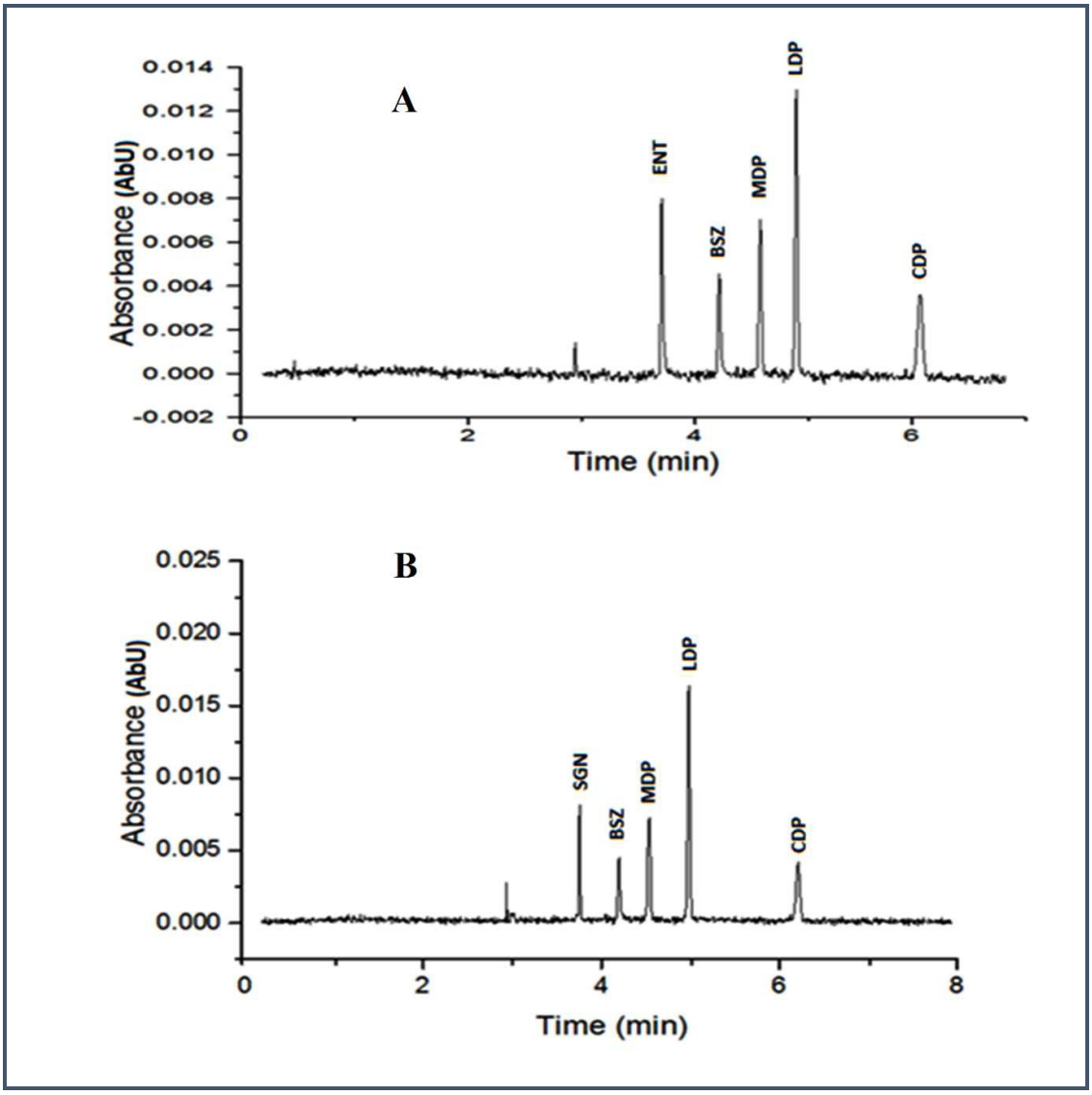


**Fig. 7** Typical electropherograms of A: ENT, LDP, BSZ and CDP (80:40:10:10 µg/mL) in their laboratory-prepared mixture, B: LDP, SGN, BSZ and CDP (60:30:15:15 µg/mL) in their laboratory-prepared mixture, using 20 µg/mL MDP IS.

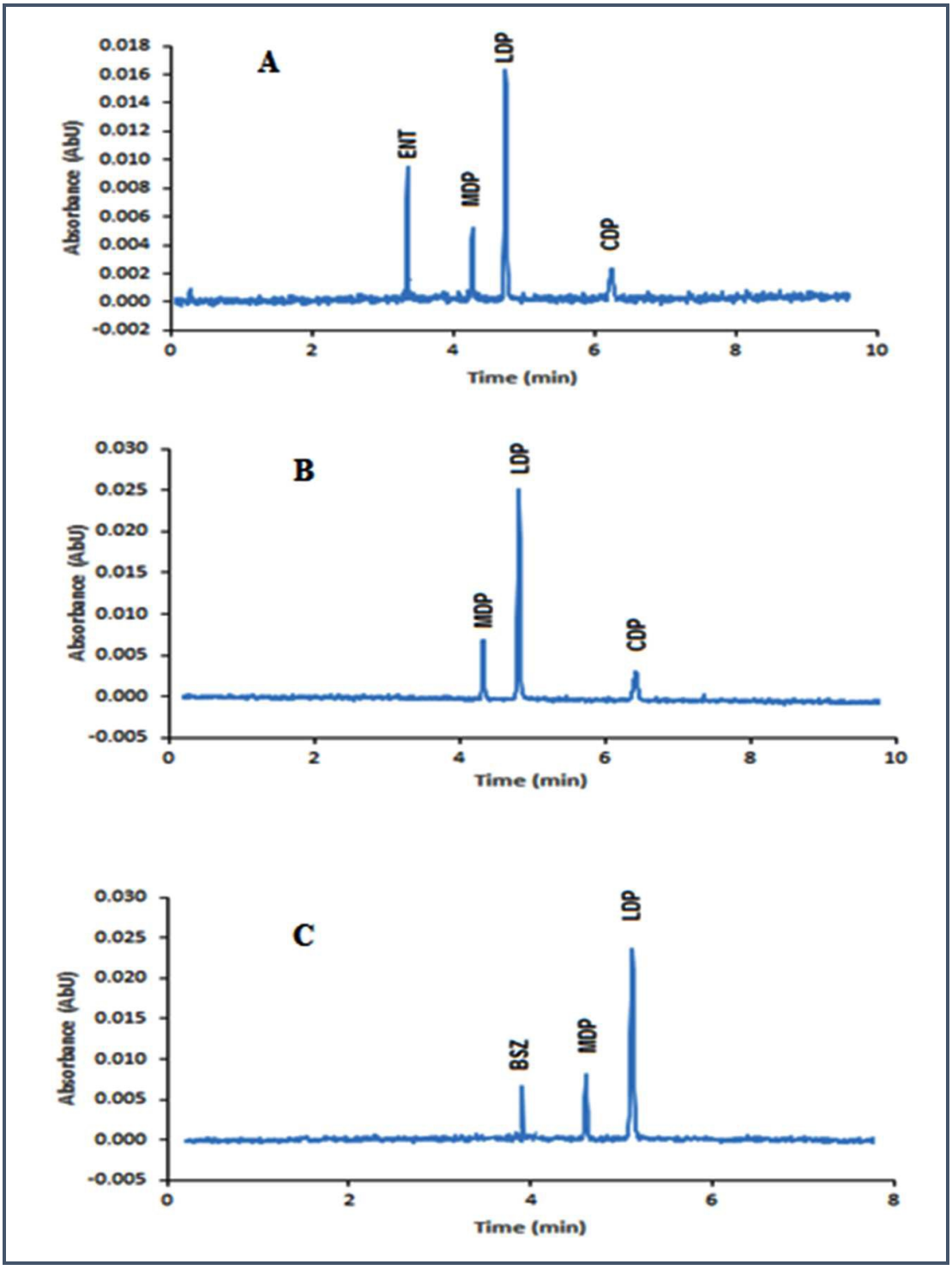


**Fig. 8** Typical electropherograms of: A: ENT, LDP and CDP (80:40:10 µg/mL) in Stalevo® tablets; B: LDP and CDP (80:20 µg/mL) in Sinemet® tablets; C: LDP and BSZ (40:10 µg/mL) in Madopar® tablets, using 20 µg/mL MDP IS.

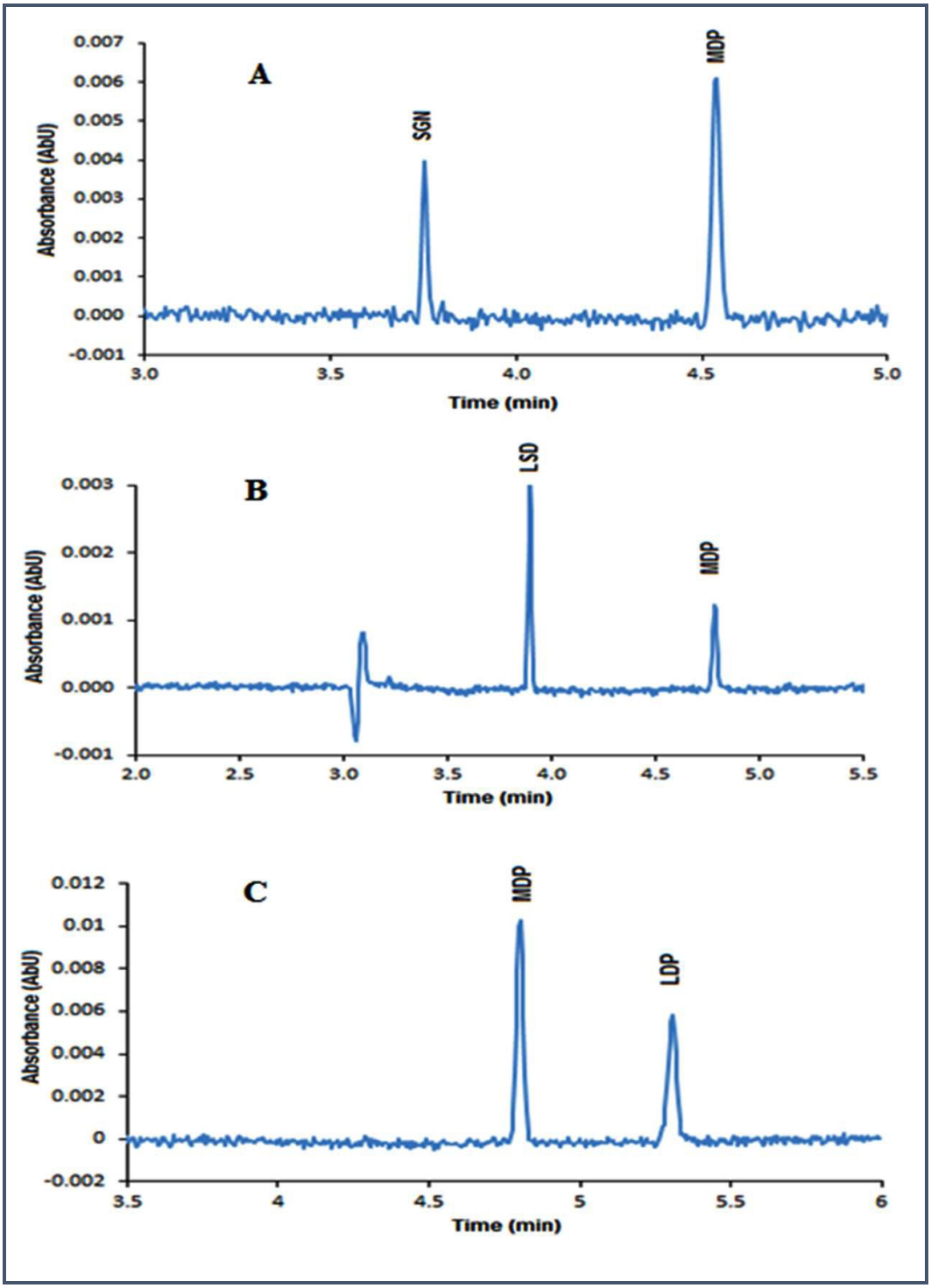


**Fig. 9** Typical electropherograms of: A: 40 µg/mL SGN in Tonus® tablets using 20 µg/mL MDP IS, B: 40 µg/mL LSD in Dopergin® tablets using 20 µg/mL MDP IS, C: 30 µg/mL MDP in Aldomet® tablets using 20 µg/mL LDP IS.

**Table 1** Optimization of the CE conditions for the separation of the studied drugs in mixture I.

| Parameter | | NTP (× $10^5$) | | | | | Resolution ($R_s$) | | | | Total run time (min) | Current (μA) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ENT | BSZ | MDP | LDP | CDP | E&B | B&M | M&L | L&C | | |
| Buffer pH | **8.0** | 2.37 | 2.45 | 5.64 | 6.14 | 8.19 | 0.69 | 1.35 | 1.10 | 6.43 | 5.93 | 48 |
| | **8.5** | 1.81 | 2.74 | 5.09 | 6.17 | 7.79 | 1.01 | 1.45 | 1.61 | 6.94 | 6.86 | 41 |
| | **9.0** | 2.14 | 2.23 | 5.94 | 7.05 | 7.35 | 1.27 | 1.83 | 3.19 | 9.26 | 7.22 | 33 |
| | **9.5** | 2.09 | 4.36 | 6.73 | 7.41 | 6.83 | 1.38 | 2.56 | 4.49 | 12.59 | 7.58 | 29 |
| | **10** | 2.16 | 4.83 | 6.49 | 7.37 | 6.88 | 1.41 | 2.39 | 4.18 | 16.82 | 8.54 | 35 |
| Buffer Conc. (mM) | **10** | 4.22 | 1.24 | 6.61 | 7.45 | 8.43 | 0.59 | 1.31 | 2.67 | 7.10 | 4.26 | 11 |
| | **20** | 5.63 | 3.82 | 7.26 | 7.82 | 8.06 | 1.25 | 2.39 | 8.75 | 9.65 | 5.82 | 21 |
| | **25** | 5.74 | 4.21 | 7.39 | 7.47 | 7.27 | 1.32 | 3.15 | 8.90 | 11.52 | 7.02 | 27 |
| | **30** | 4.01 | 4.34 | 7.53 | 6.62 | 6.80 | 1.19 | 2.25 | 8.48 | 12.28 | 7.48 | 35 |
| | **40** | 3.64 | 1.65 | 5.96 | 6.01 | 5.88 | 0.96 | 3.83 | 9.88 | 14.97 | 8.02 | 48 |
| | **50** | 3.59 | 0.41 | 6.13 | 5.49 | 4.99 | 0.77 | 2.35 | 11.57 | 17.34 | 9.05 | 60 |
| β- CD Conc. (mM) | **1** | 5.10 | 4.67 | 5.05 | 6.38 | 4.18 | 1.41 | 2.43 | 7.15 | 9.52 | 5.95 | 33 |
| | **2** | 5.34 | 5.02 | 4.82 | 7.70 | 4.81 | 1.63 | 3.62 | 10.02 | 10.33 | 6.74 | 30 |
| | **3** | 4.84 | 5.32 | 5.77 | 7.66 | 4.52 | 2.58 | 3.96 | 9.92 | 10.03 | 6.71 | 28 |
| | **4** | 6.21 | 5.07 | 6.01 | 7.75 | 5.41 | 2.86 | 4.50 | 10.28 | 13.67 | 7.15 | 29 |
| | **5** | 6.54 | 5.18 | 6.48 | 8.39 | 6.42 | 5.86 | 6.66 | 11.26 | 15.76 | 6.37 | 26 |
| | **6** | 6.01 | 5.45 | 6.83 | 8.68 | 5.27 | 4.58 | 7.07 | 10.02 | 16.27 | 6.96 | 24 |
| Applied voltage (kV) | **15** | 2.49 | 2.39 | 2.41 | 3.58 | 4.01 | 1.47 | 3.61 | 8.47 | 12.87 | 10.55 | 15 |
| | **17** | 3.17 | 3.88 | 4.19 | 4.41 | 5.35 | 2.83 | 4.48 | 6.37 | 14.73 | 9.71 | 22 |
| | **20** | 5.31 | 4.47 | 6.94 | 5.20 | 5.94 | 5.88 | 6.81 | 12.11 | 16.83 | 6.33 | 29 |
| | **23** | 5.73 | 4.39 | 5.73 | 4.91 | 5.12 | 5.26 | 7.35 | 14.10 | 19.43 | 5.54 | 44 |
| | **25** | 5.26 | 4.50 | 6.84 | 5.77 | 6.64 | 5.77 | 7.00 | 16.64 | 21.24 | 4.65 | 65 |

Abbreviations: B: BSZ; E: ENT, M: MDP, L: LDP, C: CDP.
Each result is the mean average of three separate measurements.

**Table 2** Performance data for the determination of the studied drugs by the proposed CE method.

| Parameter | LDP | CDP | ENT | BSZ | MDP | LSD | SGN |
|---|---|---|---|---|---|---|---|
| **Conc. Range (µg/mL)** | 2.0 – 100.0 | 5.0 – 75.0 | 10.0 - 200.0 | 10.0 – 100.0 | 5.0 -100.0 | 5.0 – 100.0 | 5.0 – 100.0 |
| **Regression Equation** | y=0.0628x – 0.0509 | y=0.0568x – 0.0919 | y=0.0133x – 0.0669 | y=0.0166x - 0.0062 | y=0.0377x – 0.0352 | y=0.0341x – 0.0402 | y= 0.0405+ 0.0186x |
| **Correlation coefficient (r)** | 0.9999 | 0.9999 | 0.9998 | 0.9999 | 0.9998 | 0.9999 | 0.9998 |
| **LOD (µg/mL)** | 0.5900 | 0.6831 | 2.4483 | 1.2337 | 0.7379 | 1.0961 | 1.5116 |
| **LOQ (µg/mL)** | 1.7879 | 2.0700 | 7.4191 | 3.7386 | 2.2360 | 3.3215 | 4.5805 |
| **$S_{y/x}$** | 0.0222 | 0.0188 | 0.0173 | 0.0082 | 0.0143 | 0.0192 | 0.0137 |
| **$S_a$** | 0.0112 | 0.0118 | 0.0099 | 0.0062 | 0.0084 | 0.0113 | 0.0085 |
| **$S_b$** | 0.0002 | 0.0004 | 0.0001 | 0.0001 | 0.0002 | 0.0002 | 0.0001 |
| **% RSD** | 0.9273 | 0.3423 | 1.2032 | 1.319 | 1.0983 | 1.3806 | 1.0028 |
| **% Error** | 0.2922 | 1.0264 | 0.3798 | 0.4980 | 0.3657 | 0.4593 | 0.3546 |
| **Internal Standard (IS)** | MDP (20 µg/mL) | MDP (20 µg/mL) | MDP (20 µg/mL) | MDP (20 µg/mL) | LDP (20 µg/mL) | MDP (40 µg/mL) | MDP (20 µg/mL) |

**y: corrected peak area ratio;**
**x: concentration (µg/mL);**
**$S_{y/x}$, Standard deviation of the residuals;**
**$S_a$, Standard deviation of the intercept;**
**% RSD = Relative standard deviation;**
**$S_b$, Standard deviation of the slope;**
**% Error = %RSD/√n.**

**Table 3** Validation of the proposed method for the determination of the studied drugs in raw materials.

| Concentration added (µg/mL) | % Found [a] | % RSD | % Error |
|---|---|---|---|
| **LDP** | | | |
| Intraday (40, 60, 80) | 98.88, 101.03, 100.81 | 0.82, 1.02, 1.06 | 0.47, 0.59, 0.61 |
| Interday (40, 60, 80) | 99.77, 100.20, 99.50 | 0.88, 0.65, 1.16 | 0.51, 0.38, 0.67 |
| **CDP** | | | |
| Intraday (20, 40, 60) | 101.23, 100.21, 101.53 | 0.44, 0.83, 0.57 | 0.26, 0.48, 0.33 |
| Interday (20, 40, 60) | 100.11, 101.37, 98.54 | 1.29, 0.44, 0.69 | 0.75, 0.26, 0.40 |
| **ENT** | | | |
| Intraday (50, 100, 150) | 98.78, 100.78, 100.05 | 0.92, 0.58, 1.24 | 0.53, 0.33, 0.72 |
| Interday (50, 100, 150) | 99.74, 99.43, 99.57 | 1.79, 0.94, 0.90 | 1.04, 0.54, 0.52 |
| **BSZ** | | | |
| Intraday (20, 40, 60) | 101.28, 101.37, 100.30 | 0.85, 0.45, 0.46 | 0.49, 0.26, 0.27 |
| Interday (20, 40, 60) | 100.78, 99.78, 101.07 | 0.51, 1.00, 0.52 | 0.29, 0.58, 0.30 |
| **MDP** | | | |
| Intraday (20, 40, 60) | 100.79, 101.18, 100.12 | 0.97, 0.69, 0.91 | 0.56, 0.40, 0.52 |
| Interday (20, 40, 60) | 98.15, 98.97, 100.92 | 0.61, 0.89, 0.54 | 0.35, 0.52, 0.31 |
| **LSD** | | | |
| Intraday (20, 50, 80) | 98.69, 101.09, 101.07 | 0.66, 0.43, 0.78 | 0.38 0.25, 0.45 |
| Interday (20, 50, 80) | 99.89, 99.96, 100.45 | 0.88, 0.59, 0.71 | 0.51 0.34, 0.41 |
| **SGN** | | | |
| Intraday (20, 30, 40) | 99.92, 100.16, 100.41 | 0.43, 1.81, 0.55 | 0.25, 1.04, 0.32 |
| Interday (20, 30, 40) | 101.01, 100.69, 101.06 | 0.67, 0.93, 0.54 | 0.38, 0.54, 0.33 |

[a] **Each result is the mean of % of three determinations of three different preparations of the same concentration in three replicate determinations for each preparation.**

**Table 4** Results of the standard addition technique for the determination of the studied drugs in their pharmaceutical tablets.

| Pharmaceutical Preparation | Concentration Added[a] | Concentration (µg/mL) | | % Recovery | |
|---|---|---|---|---|---|
| **Sinemet® CR 250 tablets** | 0, 5, 10, 15, 20 µg/ml | **LDP** | **CDP** | **LDP** | **CDP** |
| | | 40.0 | 10.0 | 98.24 | 98.05 |
| | | 60.0 | 15.0 | 101.80 | 99.15 |
| | | 80.0 | 20.0 | 99.35 | 101.63 |
| **Mean ± SD** | | | | 99.80 ± 1.82 | 99.61 ± 1.83 |
| **% RSD** | | | | 1.8257 | 1.8412 |
| **% Error** | | | | 1.0541 | 1.0630 |
| **Madopar® Tablets** | 0, 5, 10, 15, 20 µg/ml | **LDP** | **BSZ** | **LDP** | **BSZ** |
| | | 40.0 | 10.0 | 101.96 | 100.99 |
| | | 60.0 | 15.0 | 102.07 | 101.76 |
| | | 80.0 | 20.0 | 101.15 | 98.23 |
| **Mean ± SD** | | | | 101.73 ± 0.50 | 100.33 ± 1.86 |
| **% RSD** | | | | 0.4935 | 1.8499 |
| **% Error** | | | | 0.2849 | 1.0680 |
| **Stalevo® 325 Tablets** | 0, 10, 15, 20, 30 µg/ml | **ENT** | **LDP** | **CDP** | **ENT** | **LDP** | **CDP** |
| | | 80.0 | 40.0 | 10.0 | 98.11 | 98.73 | 100.83 |
| | | 100.0 | 50.0 | 12.5 | 99.05 | 98.31 | 98.27 |
| | | 120.0 | 60.0 | 15.0 | 98.02 | 99.01 | 99.19 |
| **Mean ± SD** | | | | | 98.39 ± 0.57 | 98.68 ± 0.35 | 99.43 ± 1.30 |
| **% RSD** | | | | | 0.5793 | 0.3567 | 1.3044 |
| **% Error** | | | | | 0.3345 | 0.2059 | 0.7531 |
| **Dopergin® Tablets** | 0, 10, 15, 20, 30 µg/ml | **LSD** | | | **LSD** | | |
| | | 20.0, | 30.0, | 40.0 | 100.65, | 99.33, | 101.62 |
| **Mean ± SD** | | | | | 100.53 ± 1.15 | | |
| **% RSD** | | | | | 1.1429 | | |
| **% Error** | | | | | 0.6599 | | |
| **Tonus® Tablets** | 0, 5, 10, 15, 20 µg/ml | **SGN** | | | **SGN** | | |
| | | 10.0, | 15.0, | 20.0 | 101.83, | 102.13, | 101.27 |
| **Mean ± SD** | | | | | 101.74 ± 0.44 | | |
| **% RSD** | | | | | 0.4295 | | |
| **% Error** | | | | | 0.2480 | | |
| **Aldomet® Tablets** | 0, 10, 20, 25, 30 µg/ml | **MDP** | | | **MDP** | | |
| | | 20.0, | 40.0, | 60.0 | 101.66, | 99.30, | 101.09 |
| **Mean ± SD** | | | | | 100.68 ± 1.23 | | |
| **% RSD** | | | | | 1.2227 | | |
| **% Error** | | | | | 0.7059 | | |

[a] **Concentrations of each standard pure drug added to the three specific concentrations of tablet extracts.**

**Table 5** Application of the proposed CE method for the determination of LDP, CDP, BSZ, SGN, and ENT in their laboratory-prepared mixtures.

| Item | Concentration taken (µg/mL) | | | | % Found[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | **ENT** | **LDP** | **CDP** | **BSZ** | **ENT** | **LDP** | **CDP** | **BSZ** |
| **ENT, LDP, CDP, and BSZ quaternary mixture I (8:4:1:1)** | 80.0 | 40.0 | 10.0 | 10.0 | 99.36 | 99.52 | 100.72 | 101.64 |
| | 100.0 | 50.0 | 12.5 | 12.5 | 98.19 | 100.77 | 99.40 | 99.82 |
| | 120.0 | 60.0 | 15.0 | 15.0 | 100.78 | 100.12 | 101.82 | 100.28 |
| | 160.0 | 80.0 | 20.0 | 20.0 | 99.06 | 99.46 | 100.93 | 98.19 |
| | 200.0 | 100.0 | 25.0 | 25.0 | 99.82 | 99.27 | 102.17 | 101.02 |
| ***Mean*** | | | | | ***99.44*** | ***99.83*** | ***101.01*** | ***100.19*** |
| ***± SD*** | | | | | ***± 0.96*** | ***± 0.62*** | ***± 1.08*** | ***± 1.32*** |
| ***% RSD*** | | | | | ***0.9611*** | ***0.6163*** | ***1.0714*** | ***1.3144*** |
| ***% Error*** | | | | | ***0.4298*** | ***0.2756*** | ***0.4791*** | ***0.5878*** |
| | **SGN** | **LDP** | **CDP** | **BSZ** | **SGN** | **LDP** | **CDP** | **BSZ** |
| **SGN, LDP, CDP, and BSZ quaternary mixture II (2:4:1:1)** | 20.0 | 40.0 | 10.0 | 10.0 | 100.78 | 98.23 | 100.48 | 100.79 |
| | 25.0 | 50.0 | 12.5 | 12.5 | 99.26 | 98.13 | 100.99 | 98.01 |
| | 30.0 | 60.0 | 15.0 | 15.0 | 99.69 | 102.10 | 98.25 | 98.36 |
| | 40.0 | 80.0 | 20.0 | 20.0 | 101.83 | 99.07 | 101.17 | 100.17 |
| | 50.0 | 100.0 | 25.0 | 25.0 | 98.12 | 98.47 | 99.34 | 98.48 |
| ***Mean*** | | | | | ***99.94*** | ***99.20*** | ***100.05*** | ***99.16*** |
| ***± SD*** | | | | | ***± 1.42*** | ***± 1.66*** | ***± 1.23*** | ***± 1.24*** |
| ***% RSD*** | | | | | ***1.4252*** | ***1.6751*** | ***1.2310*** | ***1.2456*** |
| ***% Error*** | | | | | ***0.6374*** | ***0.7491*** | ***0.5505*** | ***0.5570*** |

[a] **Each result is the average of three separate determinations.**

**Table 6** Application of the proposed CE methods to the determination of the studied drugs in their pharmaceutical preparations.

| Pharmaceutical Preparation | Extracting solvent | Concentration (µg/mL) | | % Recovery[a] | |
|---|---|---|---|---|---|
| **Sinemet® CR 250 Tablets** | **0.1 M HCl** | **LDP** | **CDP** | **LDP** | **CDP** |
| | | 40.0 | 10.0 | 100.75 | 101.76 |
| | | 60.0 | 15.0 | 99.01 | 98.83 |
| | | 80.0 | 20.0 | 100.61 | 100.29 |
| **Mean ±SD** | | | | 100.12 ± 0.97 | 100.29 ± 1.47 |
| **% RSD** | | | | 0.9658 | 1.4607 |
| **% Error** | | | | 0.5583 | 0.8458 |
| **Madopar® Tablets** | **0.1 M HCl** | **LDP** | **BSZ** | **LDP** | **BSZ** |
| | | 40.0 | 10.0 | 98.15 | 100.60 |
| | | 60.0 | 15.0 | 98.70 | 101.65 |
| | | 80.0 | 20.0 | 100.66 | 100.42 |
| **Mean ±SD** | | | | 99.17 ± 1.32 | 100.89 ± 0.66 |
| **% RSD** | | | | 1.3300 | 0.6581 |
| **% Error** | | | | 0.7679 | 0.3799 |
| **Stalevo® 325 Tablets** | **Methanol :1 M HCl (90:10; v/v)** | **ENT** | **LDP** | **CDP** | **ENT** | **LDP** | **CDP** |
| | | 80.0 | 40.0 | 10.0 | 98.76 | 98.32 | 100.27 |
| | | 100.0 | 50.0 | 12.5 | 101.02 | 101.82 | 99.65 |
| | | 120.0 | 60.0 | 15.0 | 99.82 | 99.72 | 100.14 |
| **Mean ± SD** | | | | | 99.87 ± 1.13 | 99.95 ± 1.76 | 100.02 ± 0.33 |
| **% RSD** | | | | | 1.1325 | 1.7628 | 0.3269 |
| **% Error** | | | | | 0.6530 | 1.0173 | 0.1888 |
| **Dopergin® Tablets** | **Methanol** | **LSD** | | | **LSD** | | |
| | | 20.0, | 40.0, | 60.0 | 101.35, | 100.98, | 101.96 |
| **Mean ± SD** | | | | | | 101.44 ± 0.51 | |
| **% RSD** | | | | | | 0.4988 | |
| **% Error** | | | | | | 0.2880 | |
| **Tonus® Tablets** | **Methanol** | **SGN** | | | **SGN** | | |
| | | 10.0, | 20.0, | 40.0 | 99.06, | 101.69, | 99.22 |
| **Mean ± SD** | | | | | | 99.99 ± 1.48 | |
| **% RSD** | | | | | | 1.4831 | |
| **% Error** | | | | | | 0.8563 | |
| **Aldomet® Tablets** | **0.1 M HCl** | **MDP** | | | **MDP** | | |
| | | 20.0, | 40.0, | 60.0 | 99.50, | 98.18, | 101.16 |
| **Mean ± SD** | | | | | | 99.61 ± 1.49 | |
| **% RSD** | | | | | | 1.4988 | |
| **% Error** | | | | | | 0.8653 | |

[a] **Each result is the average of three separate determinations.**

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## Table of Content

**Determination of Six Anti-Parkinson Drugs using Cyclodextrin-Capillary Electrophoresis Method: Application to Pharmaceutical Dosage Forms**

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Addition of β-cyclodextrin to the background electrolyte improves the separation efficiency of multi-component mixtures through inclusion complex formation.