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Short communication

Simultaneous quantitation of levodopa and 3-*O*-methyldopa in human plasma by HPLC–ESI-MS/MS: Application for a pharmacokinetic study with a levodopa/benserazide formulation

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ABSTRACT

A sensitive and simple method was developed for the quantitation of levodopa and its metabolite 3-*O*-methyldopa, in human plasma, after oral administration of tablet formulations containing levodopa (200 mg) and benserazide (50 mg). The analytes were extracted by a protein precipitation procedure, using carbidopa as an internal standard. A mobile phase consisting of 0.2% formic acid and acetonitrile (94:6, v/v) was used and chromatographic separation was achieved using ACE C_{18} column (50 mm × 4.6 mm i.d.; 5 μm particle size). Selected reaction monitoring was performed using the fragmentation transitions m/z 198 → m/z 107, m/z 212 → m/z 166 and m/z 227 → m/z 181 for levodopa, 3-*O*-methyldopa and carbidopa, respectively. Calibration curves were constructed over the range 50.0–6000.0 ng/mL for levodopa and 25.0–4000.0 ng/mL for 3-*O*-methyldopa. The method shown to be specific, precise, accurate and provided recovery rates higher than 85% for all analytes. No matrix effect was detected in the samples. The validated method was applied in a pharmacokinetic study with a levodopa/benserazide tablet formulation in healthy volunteers.

1. Introduction

Parkinson disease is an age-related neurodegenerative disorder that affects around 1–2% of persons aged 60 years and older [1]. Current therapy is largely based on a dopamine replacement strategy, primarily using the dopamine precursor levodopa [2]. Combination therapy of levodopa with an inhibitor of extracerebral decarboxylase such as benserazide (Fig. 1) leads to a marked reduction in both the required levodopa dose and the incidence of undesired side effects [3,4].

Fixed dose combinations of levodopa (200 mg) and benserazide (50 mg) in tablet formulation have been developed [5,6]. For pharmacokinetic and bioequivalence studies of levodopa associated with benserazide, it is recommended to perform the quantitation of levodopa and its major metabolite, 3-*O*-methyldopa, in plasma, since the metabolism of levodopa is shifted towards this metabolite in the presence of a descarboxylase inhibitor [3].

Some methods have been reported for determining levodopa and its metabolite, 3-*O*-methyldopa, in plasma, by high performance liquid chromatography. However, the majority of levodopa and 3-*O*-methyldopa quantitation in plasma was performed by electrochemical detection [7–10], and few works detected the analytes by ultraviolet spectrophotometry [11–13]. In the last years, liquid chromatography combined with atmospheric pressure ionization mass spectrometric detection has almost completely replaced ultraviolet, electrochemical or fluorescence detection in the bioanalytical field, mainly due to the un-matched sensitivity and extraordinary selectivity of the detection [14,15]. The quantitation of levodopa in plasma using liquid chromatography coupled to mass spectrometric detection is described in some works [16–20].

The aim of this work was to develop and validate a rapid HPLC–ESI-MS/MS method for the simultaneous quantitation of levodopa and 3-*O*-methyldopa in human plasma. The method was applied to a pharmacokinetic study with a levodopa/benserazide tablet formulation in healthy volunteers.

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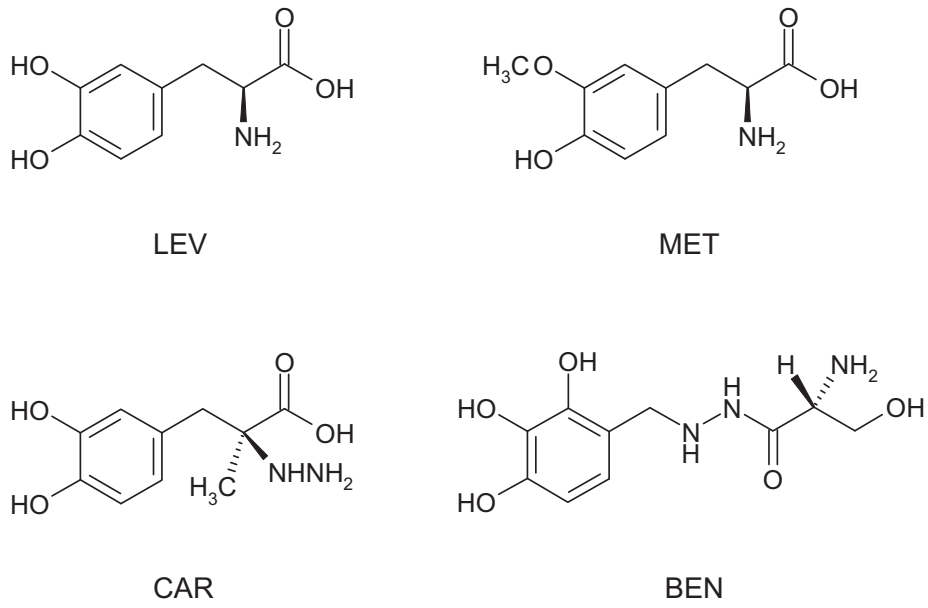

Fig. 1. Chemical structures of levodopa (LEV), 3-*O*-methyldopa (MET), carbidopa (CAR) and benserazide (BEN).

2. Experimental

2.1. Chemicals and reagents

Levodopa and carbidopa (internal standard) reference standards were purchased from the United States Pharmacopoeia (Rockville, MD, USA). 3-*O*-Methyldopa reference standard was purchased from Purity Grade Standards (Carrboro, NC, USA). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Acetonitrile and methanol (HPLC grade) were purchased from Tedia (Fairfield, OH, USA) and formic acid, perchloric acid, hydrochloric acid and sodium metabisulfite (analytical grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation and analytical conditions

The HPLC–ESI-MS/MS analyses were carried out on an Agilent 1200 system (Santa Clara, CA, USA), composed of a quaternary pump, an autosampler, a column oven and an API 5000 triple quadrupole mass spectrometer (MDS-SCIEX, Concord, Ontario, Canada), equipped with an electrospray ion source. Analyst v.1.4.2 software was used for data acquisition and analysis. LC separation was performed on an ACE C_{18} column (50 mm × 4.6 mm i.d.; 5 μm particle size) from ACT (Aberdeen, Scotland), at 18 °C. The mobile phase consisted of 0.2% formic acid and acetonitrile (94:6, v/v), at a flow rate of 0.2 mL/min. The run time was 7.5 min and the injection volume was 10 μL.

Mass spectrometric detection was performed using electrospray ion source in positive ionization mode. The turbo-gas temperature was 500 °C, with an ion spray voltage of 4500 V and declustering potential of 96 for levodopa, 91 for 3-*O*-methyldopa and 116 for carbidopa. Nitrogen was used as nebulizer gas. Curtain gas setting was 10 and collision gas setting was 6. The collision energies were optimized at 35 V for levodopa, 21 V for 3-*O*-methyldopa and 17 V for carbidopa. Selected reaction monitoring (SRM) was employed for data acquisition. The SRM fragmentation transitions were m/z 198 → m/z 107, m/z 212 → m/z 166 and m/z 227 → m/z 181 for levodopa, 3-*O*-methyldopa and carbidopa, respectively. The scan dwell time was set at 0.5 s for each channel.

2.3. Preparation of standard solutions

Stock solution of levodopa was prepared by dissolving the accurately weighed reference substance in methanol and water (7:3) containing 0.5% hydrochloric acid and 0.1% sodium metabisulfite. Stock solution of 3-*O*-methyldopa was prepared by dissolving the accurately weighed reference substance in methanol and water (7:3) containing 0.1% sodium metabisulfite. Stock solution of carbidopa was prepared by dissolving the accurately weighed reference substance in methanol and water (7:3). The working solution of carbidopa (IS) was prepared by diluting the stock solution with methanol and water (1:1) to a final concentration of 1000 ng/mL.

2.4. Preparation of calibration and QC samples

Six calibration samples were prepared by spiking appropriate amounts of the stock solutions of levodopa and 3-*O*-methyldopa in blank plasma. The volume of the stock solutions added did not exceed 1% of the total plasma volume. Serial dilutions were performed to obtain the concentrations of the calibration samples in plasma: 50, 100, 500, 1500, 3000 and 6000 ng/mL for levodopa and 25, 100, 500, 1000, 2000 and 4000 ng/mL for 3-*O*-methyldopa. Quality control (QC) samples in plasma were prepared in a similar way, at low, middle and high concentrations: 150, 2400 and 4800 ng/mL for levodopa and 75, 1600 and 3200 ng/mL for 3-*O*-methyldopa.

2.5. Sample preparation

A 50 μL aliquot of the IS solution (1000 ng/mL of carbidopa) and a 50 μL aliquot of 2 M perchloric acid were added to 250 μL of plasma sample. The sample was vortex mixed for 20 s and centrifuged at 17,500 × *g* for 10 min, at 5 °C. Then, 100 μL of the supernatant were transferred to a vial containing 500 μL of methanol and water (1:1). After vortex mixed for 20 s, a 10 μL aliquot was injected into the chromatographic system.

2.6. Method validation

The validation process was carried out according to Guidance for Industry – Bioanalytical Method Validation, recommended by US Food and Drug Administration [21].

Selectivity was evaluated by assaying human blank plasma samples from six different donors, including one lipemic and one hemolyzed plasma. In addition, plasma samples spiked with caffeine (1 μg/mL), chlorpheniramine (50 ng/mL), metamizole (5 μg/mL) and acetaminophen (20 μg/mL) were also evaluated to ensure no interference in the method.

Linearity was assessed by six-point calibration curves in human plasma in duplicate in three consecutive days. The concentration range evaluated was 50.0–6000.0 ng/mL for levodopa and 25.0–4000.0 ng/mL for 3-*O*-methyldopa. The LLOQ was established as the lowest concentration of calibration curve at which precision was within ±20% and accuracy was within ±20%, by means of the analyses of six replicates. In addition, the analyte response at this concentration should be at least 5 times the baseline noise.

To evaluate the precision and accuracy of the method, QC samples at three concentration levels (150, 2400 and 4800 ng/mL of levodopa and 75, 1600 and 3200 ng/mL of 3-*O*-methyldopa) were analysed in six replicates on three different days.

The extraction recovery of the method was determined by comparing the peak areas obtained from the plasma samples with those of direct injected standards, at the same concentration. It was evaluated by analyzing six replicates of each QC samples of levodopa and 3-*O*-methyldopa. The recovery of IS was determined in a similar way, at the working concentration (1000 ng/mL of carbidopa).

For the matrix effect evaluation, the peak areas of levodopa, 3-*O*-methyldopa and IS from the spike-after-extraction samples were compared to those of the standard solutions in the mobile phase, at the same concentrations. This experiment was carried out with blank plasma samples from six different donors, at low and high QC concentrations of levodopa and 3-*O*-methyldopa and working concentration of carbidopa (1000 ng/mL).

The stability of the analyte in plasma was evaluated using the low and high QC samples, in six replicates. Stability was evaluated after three complete freeze/thaw cycles (−70 to 23 °C) on consecutive days, after storage of plasma samples at ambient temperature (23 °C) for 4.5 h and after storage of extracted samples in the autosampler (18 °C) for 36 h. The stability of the working solution of IS at 4 °C for 7 days was also evaluated. The analytes were considered stable when 85–115% of the initial concentrations were found.

2.7. Application to a pharmacokinetic study

The validated method was applied in a pharmacokinetic study with levodopa (200 mg) + benserazide (50 mg) tablet formulation in 12 healthy volunteers (6 males, 6 females), with normal biochemical parameters. After an overnight fasting, all the subjects received a single oral dose of the tablet formulation (Prolopa®, Roche S.A.). The blood samples were collected into amber tubes, using EDTA as anticoagulant, at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 48, 72, 96 and 144 h after drug administration. Immediately after the withdrawal, 0.2 mL of 10% sodium metabisulfite was added to each 5 mL of blood. Plasma samples were obtained by centrifugation and frozen at −70 °C until analyses. The study protocol was approved by the Ethics Committee of Universidade Estadual de Campinas.

3. Results and discussion

3.1. Conditions for HPLC–ESI-MS/MS

MS spectra of the analytes and IS were recorded in positive ion mode. $[M+H]^+$ was the predominant ion in the Q1 spectrum, and was used as the precursor to obtain product ion spectra. The most sensitive and selective mass transitions were m/z 198 → m/z 107, m/z 212 → m/z 166 and m/z 227 → m/z 181 for levodopa, 3-*O*-methyldopa and IS, respectively, obtained after the optimization of collision energy. Fig. 2 illustrates the fragmentation of each compound.

Different mobile phases containing usual modifiers and buffers (formic acid, ammonium formiate, ammonium acetate) and organic solvents (acetonitrile, methanol) were evaluated. The percentage of formic acid was optimized to provide adequate peak shape and to improve the signal intensities in positive electrospray ionization. The best results were achieved with a mobile phase composed of 0.2% formic acid and acetonitrile (94:6). The flow rate was evaluated and optimized to 0.2 mL/min, aiming to improve the separation between the analytes and matrix components that lead to ion suppression. The retention times of levodopa, 3-*O*-methyldopa and carbidopa were 4.1 min, 6.4 and 6.7 min, respectively, and the total run time was 7.5 min (Fig. 3).

The most of previous reported HPLC with electrochemical, ultraviolet or mass detection present long run time and high solvent consumption. The developed method using tandem mass spectrometry presents the advantage of short run time and high selectivity, so that it may be applied to high-throughput analysis.

3.2. Sample extraction

A liquid–liquid extraction was initially considered for sample preparation. However, the extraction recovery was considerably low using different organic solvents, besides a significant matrix effect was detected in the samples. Different solvents were evaluated as precipitating agents, such as methanol, acetonitrile and acetone. Perchloric acid was shown to be the more appropriate solvent, since recovery rates higher than 85% were achieved for both analytes and IS. The developed protein precipitation method is simple, robust and efficient, resulting in a fast and easily-handled analysis. To overcome the matrix effect detected in the samples, a dilution step of the supernatant was adopted. Thus, after protein precipitation with 2 M perchloric acid, 100 μL of the supernatant were diluted with 500 μL of methanol and water (1:1). This procedure, along with the optimized chromatographic conditions, was important to assure no potential ion suppression due to the matrix components.

3.3. Method validation

No significant interference was detected at the retention times of the analytes, in the six different blank plasma chromatograms (Fig. 3). The plasma samples spiked with caffeine, chlorpheniramine, metamizole or acetaminophen did not interfere at the ion transitions selected for the analytes quantitation.

Calibration curves were shown to be linear over the range 50.0–6000.0 ng/mL for levodopa and 25.0–4000.0 ng/mL for 3-*O*-methyldopa. Typical standard curves were $y = 9.24 \times 10^{-4}x + 5.56 \times 10^{-3}$ and $y = 2.64 \times 10^{-3}x + 6.65 \times 10^{-2}$ for levodopa and 3-*O*-methyldopa, respectively, with a weighted factor $1/x$. Regression coefficients were higher than 0.998 for both analytes, in all calibration curves. Linearity data are presented in Table 1. The obtained LLOQ were 50 ng/mL and 25 ng/mL for levodopa and 3-*O*-methyldopa, respectively.

The obtained data for intra-run and inter-run precision and accuracy are shown in Table 2. The mean R.S.D. values in the intra-run precision were 2.8% and 2.0%, and the inter-run precision were 2.4% and 2.5% for levodopa and 3-*O*-methyldopa, respectively. The mean accuracy values in the intra-run assay were 100.7% and 102.4%, and in the inter-run assay were 100.7% and 101.9% for levodopa and 3-*O*-methyldopa, respectively.

The mean recovery rates of levodopa and 3-*O*-methyldopa ($n = 18$), determined at three concentrations, were 88.7% and 90.0%

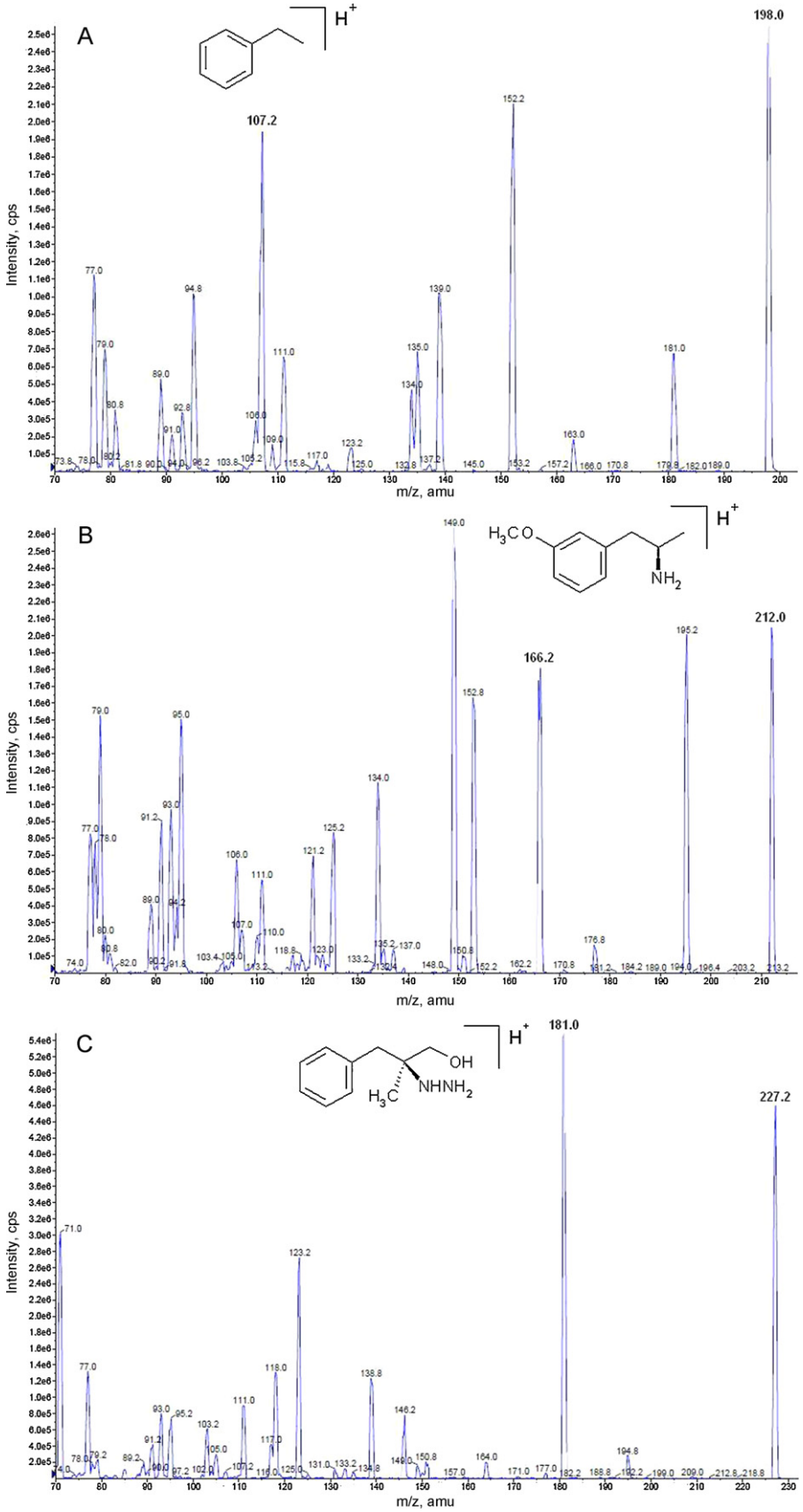

Fig. 2. Product ion spectra and fragment chemical structures of (A) levodopa (*m*/*z* 107), (B) 3-*O*-methyldopa (*m*/*z* 166) and (C) carbidopa (*m*/*z* 181), obtained by electrospray ionization in positive ion mode.

Table 1
Precision and accuracy data of back-calculated concentrations of calibration samples for levodopa and 3-*O*-methyldopa in plasma.

Analyte	Nominal concentration (ng/mL)	Observed concentration (ng/mL, mean ± S.D.)	Precision (%R.S.D.)	Accuracy (%)
Levodopa	50	45.0 ± 2.4	5.2	89.9
	100	96.9 ± 2.2	2.2	96.9
	500	543.3 ± 21.9	4.0	108.7
	1500	1590.9 ± 47.2	3.0	106.1
	3000	3034.4 ± 86.5	2.9	101.1
	6000	5839.6 ± 135.1	2.3	97.3
3-*O*-Methyldopa	25	23.5 ± 1.1	4.8	94.0
	100	100.0 ± 2.8	2.8	100.0
	500	507.5 ± 15.0	3.0	101.5
	1000	1036.5 ± 24.3	2.3	103.7
	2000	2075.8 ± 46.3	2.2	103.8
	4000	3881.8 ± 35.3	0.9	97.0

S.D. = standard deviation; R.S.D. = relative standard deviation.

Table 2
Precision, accuracy and recovery data for the assay of levodopa and 3-*O*-methyldopa by LC–MS/MS.

Validation parameters	Levodopa quality control concentration (ng/mL)				3-*O*-Methyldopa quality control concentration (ng/mL)			
	50	150	2400	4800	25	75	1600	3200
Precision (R.S.D.%)								
Intra-run (n = 6)	5.4	2.8	3.1	2.6	3.6	2.3	1.9	1.9
Inter-run (n = 18)	7.8	2.9	2.2	2.1	8.0	3.7	1.9	1.8
Accuracy (%)								
Intra-run (n = 6)	93.8	97.4	103.3	101.5	100.3	102.1	103.5	101.7
Inter-run (n = 18)	92.1	98.0	103.0	101.0	100.2	102.6	102.1	101.0
Recovery (%) (n = 6)		88.8	90.9	86.3		89.3	90.7	90.0

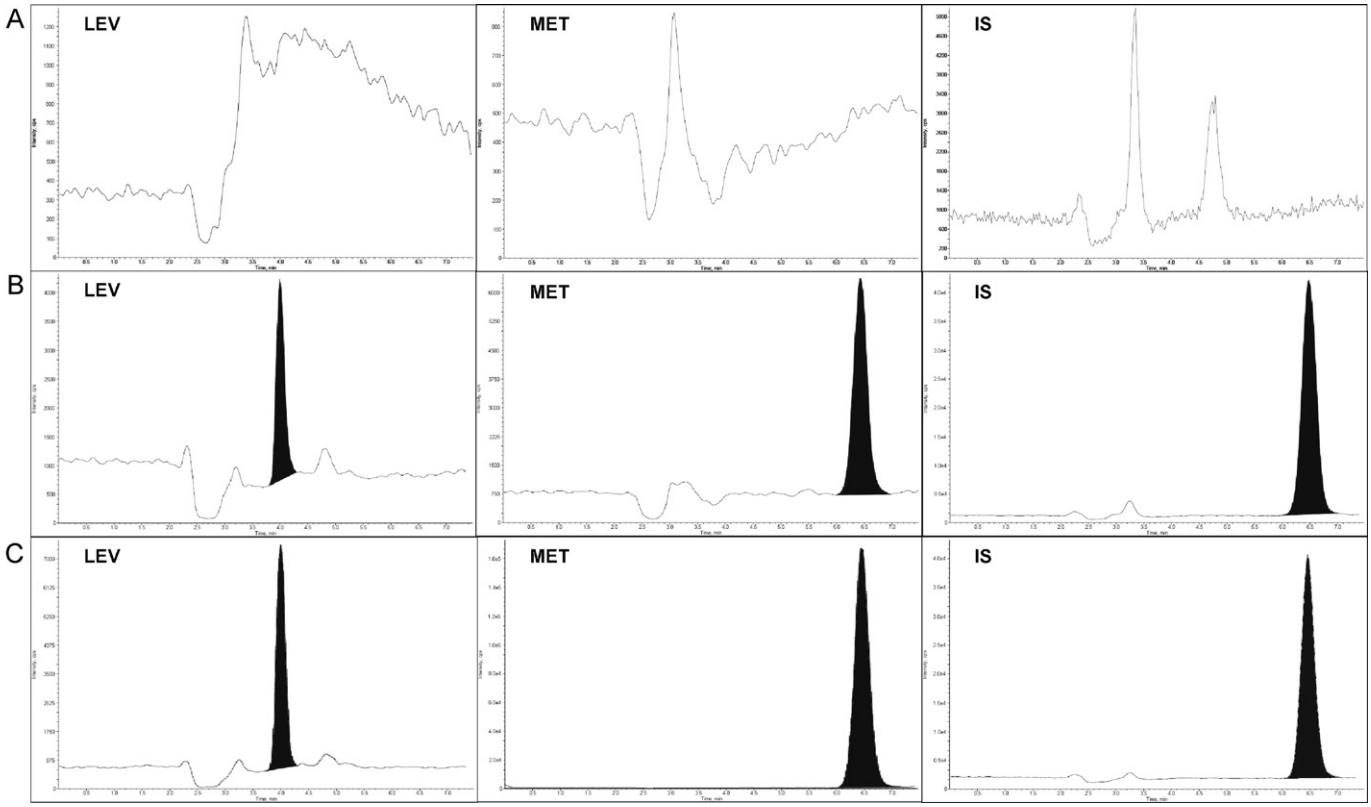

Fig. 3. SRM chromatograms of (A) blank plasma sample, (B) blank plasma spiked with levodopa (LEV) at LLOQ (50 ng/mL), 3-*O*-methyldopa (MET) at LLOQ (25 ng/mL) and carbidopa (IS) at 1000 ng/mL and (C) volunteer plasma collected 7 h after the oral administration.

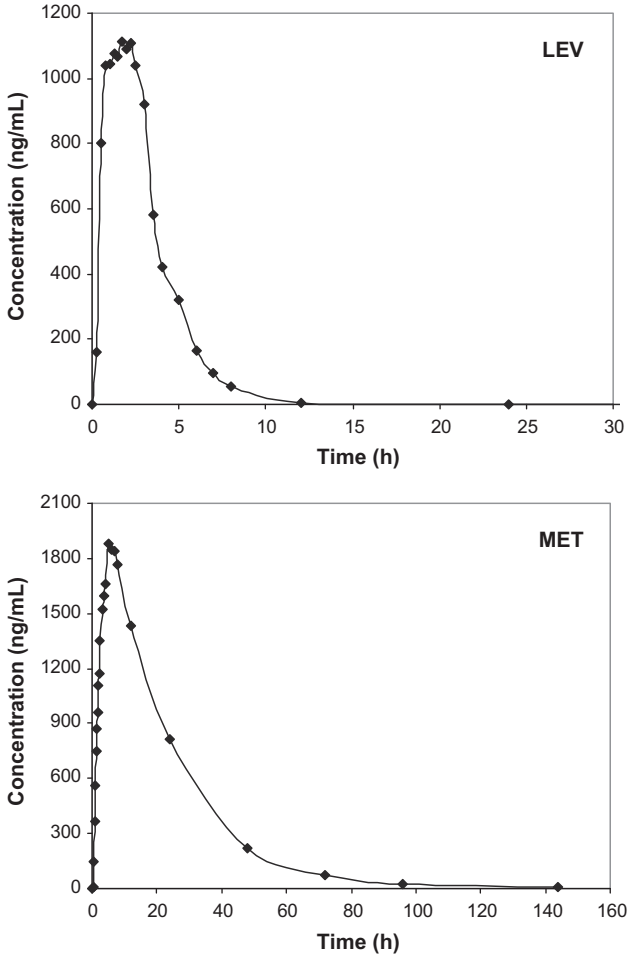

Fig. 4. Plasma concentration–time curve of levodopa (LEV) and 3-*O*-methyldopa (MET) after the oral administration of tablet formulation (levodopa 200 mg + benserazide 50 mg) in 12 healthy volunteers.

(Table 2). The recovery of the IS was shown to be 89.8% ($n = 18$). In matrix effect evaluation, the observed variation did not exceed the range 85–115%, so that in the present HPLC–MS/MS method, ion suppression or enhancement was not significant.

The results of stability experiments showed that levodopa and 3-*O*-methyldopa plasma samples were stable for up to 4.5 h at 23 °C, for 36 h after extraction in the autosampler and after three complete freeze/thaw cycles on consecutive days, as the mean changes in analyte content were within ±15% of initial concentration, at low and high QC. Working solutions of IS were stable for at least 7 days at 4 °C.

3.4. Application to a pharmacokinetic study

The validated method was applied to a pharmacokinetic study in twelve healthy volunteers. The main pharmacokinetic parameters of the drug were calculated, using WinNonLin 5.1 software. The mean plasma concentration–time curves are shown in Fig. 4. The mean C_{max} for levodopa, 2132 ng/mL, was reached 2.1 h (T_{max}) after drug administration, whereas for 3-*O*-methyldopa a C_{max} of 1954 ng/mL was reached after 5.3 h. The mean values of AUC_{0-t} obtained were 4164 and 47,388 ng h/mL for levodopa and 3-*O*-methyldopa, respectively. The mean values of $AUC_{0-\infty}$ were 4315 and 48,496 ng h/mL for levodopa and 3-*O*-methyldopa, respectively. The elimination half-life of levodopa and 3-*O*-methyldopa were 1.2 and 14.4 h, respectively. The extrapolated area of plasma concentration versus time was not higher than 20% of $AUC_{0-\infty}$ for both analytes, demonstrating the suitability of the method and experimental design, as required by Brazilian regulatory agency [22].

4. Conclusion

An HPLC–ESI-MS/MS method for the simultaneous quantitation of levodopa and 3-*O*-methyldopa in human plasma after oral administration of tablet formulation containing levodopa and benserazide was developed and validated. The mass spectrometric detection provided high sensitivity and selectivity, and the simple protein precipitation procedure resulted in a method with short sample preparation time and high sample throughput, besides appropriate precision and accuracy. Thus, the developed method may be successfully applied to clinical pharmacokinetic and bioequivalence studies of fixed dose combinations of levodopa and benserazide in pharmaceutical formulations.

References

[1] C.W. Olanow, M.B. Stern, K. Sethi, The scientific and clinical basis for the treatment of Parkinson disease (2009), Neurology 72 (2009) S1–S136.
[2] W. Poewe, Treatments for Parkinson disease – past achievements and current clinical needs, Neurology 72 (2009) S65–S73.
[3] J. Dingemanse, C.H. Kleinbloesem, G. Zurcher, N.D. Wood, C. Crevoisier, Levodopa with benserazide or carbidopa in Parkinson disease, Br. J. Clin. Pharmacol. 44 (1997) 41–48.
[4] H. Shen, K. Kannari, H. Yamato, A. Arai, M. Mtsunaga, Levodopa with benserazide or carbidopa in Parkinson disease, Tohoku J. Exp. Med. 199 (2003) 149–159.
[5] M. Da Prada, R. Kettler, G. Zurcher, R. Shaffner, W.E. Haefely, Inhibition of decarboxylase and levels of dopa and 3-*O*-methyldopa: a comparative study of benserazide versus carbidopa in rodents and of Madopar standard versus Madopar HBS in volunteers, Eur. Neurol. 27 (1987) 9–20.
[6] U.K. Rinne, P. Molsa, Levodopa with benserazide or carbidopa in Parkinson disease, Neurology 12 (1979) 1584–1589.
[7] I. Rondelli, D. Acerbi, F. Mariotti, P. Ventura, Simultaneous determination of levodopa methyl ester, levodopa, 3-*O*-methyldopa and dopamine in plasma by high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B 653 (1994) 17–23.
[8] K.A. Sagar, M.R. Smyth, Simultaneous determination of levodopa, carbidopa and their metabolites in human plasma and urine samples using LC-EC, J. Pharm. Biomed. Anal. 22 (2000) 613–624.
[9] C. Saxer, M. Niina, A. Nakashima, Y. Nagae, N. Masuda, Simultaneous determination of levodopa and 3-*O*-methyldopa in human plasma by liquid chromatography with electrochemical detection, J. Chromatogr. B 802 (2004) 299–305.
[10] M. Karimi, J.L. Carl, S. Loftin, J.S. Perlmutter, Modified high-performance liquid chromatography with electrochemical detection method for plasma measurement of levodopa, 3-*O*-methyldopa, dopamine, carbidopa and 3,4-dihydroxyphenyl acetic acid, J. Chromatogr. B 836 (2006) 120–123.
[11] C. Muzzi, E. Bertocci, L. Terzuoli, B. Porcelli, I. Ciari, R. Pagani, R. Guerranti, Simultaneous determination of serum concentrations of levodopa, dopamine, 3-*O*-methyldopa and a-methyldopa by HPLC, Biomed. Pharmacother. 62 (2008) 253–258.
[12] M. Chamsaz, A. Safavi, J. Fadaee, Simultaneous kinetic-spectrophotometric determination of carbidopa, levodopa and methyldopa in the presence of citrate with the aid of multivariate calibration and artificial neural networks, Anal. Chim. Acta 603 (2007) 140–146.
[13] S.F. Li, H.L. Wu, Y.J. Yu, Y.N. Li, J.F. Nie, H.Y. Fu, R.Q. Yu, Quantitative analysis of levodopa, carbidopa and methyldopa in human plasma samples using HPLC–DAD combined with second-order calibration based on alternating trilinear decomposition algorithm, Talanta 81 (2010) 805–812.
[14] G. Hopfgartner, E. Bourgogne, Quantitative high throughput analysis of drugs in biological matrices by mass spectrometry, Mass Spectrom. Rev. 22 (2003) 195–214.
[15] S. Zhou, Q. Song, Y. Tang, W. Naidong, Critical review of development, validation and transfer for high throughput bioanalytical LC–MS/MS methods, Curr. Pharm. Anal. 1 (2005) 3–14.
[16] W. Jiang, L. Lv, S. Zhou, X. Huang, X. Shi, C. Lv, L. Wu, C. Xu, Simultaneous determination of L-dopa and its prodrug (S)-4-(2-acetamido-3-ethoxy-3-oxopropyl)-1,2-phenylene diacetate in rat plasma by high-performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study, J. Pharm. Biomed. Anal. 53 (2010) 751–754.
[17] K. Igarashi, K. Hotta, F. Kasuya, K. Abe, S. Sakoda, Determination of cabergoline and L-dopa in human plasma using liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 792 (2003) 55–61.
[18] W. Li, D.T. Rossi, S.T. Fountain, Development and validation of a semi-automated method for L-dopa and dopamine in rat plasma using electrospray LC/MS/MS, J. Pharm. Biomed. Anal. 24 (2000) 325–333.

[19] L. Lv, W. Jiang, S. Zhou, X. Huang, X. Shi, C. Lv, L. Wu, C. Xu, LC–MS–MS simultaneous determination of L-dopa and its prodrug L-dopa *n*-pentyl ester hydrochloride in rat plasma, Chromatographia 72 (2010) 239–243.
[20] A.P.J.M. De Jong, R.M. Kok, C.A. Cramers, S.K. Wadman, E. Haan, A new method for the determination of L-dopa and 3-*O*-methyldopa in plasma and cerebrospinal fluid using gas chromatography and electron capture negative ion mass spectrometry, Clin. Chim. Acta 171 (1988) 49–61.
[21] Food and Drug Administration. FDA Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, 2001.
[22] Agência Nacional de Vigilância Sanitária. Manual de Boas Práticas em Biodisponibilidade e Bioequivalência, vol. 1, Gerência Geral de Inspeção e Controle de Medicamentos e Produtos, Brasília, 2002.