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Ultra-performance liquid chromatography/tandem mass spectrometry method for the determination of lercanidipine in human plasma

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A simple, sensitive and rapid ultra-performance liquid chromatography/positive electrospray ionization tandem mass spectrometry (UPLC/ESI-MS/MS) method has been developed and validated for the determination of lercanidipine in human plasma. Lercanidipine and the internal standard, nicardipine, were extracted from plasma by liquid-liquid extraction using *tert*-butyl methyl ether as the extraction solvent. UPLC analysis was performed isocratically on an AcQuity UPLC™ BEH C_{18} analytical column (2.1 × 50.0 mm i.d., particle size 1.7 μm). The mobile phase consisted of 70% acetonitrile in water containing 0.2% v/v formic acid and pumped at a flow rate of 0.30 mL/min. ESI in positive ion mode, with multiple reaction monitoring (MRM), was chosen for the detection of the analytes. The assay was linear over a concentration range of 0.05–30 ng/mL for lercanidipine with a limit of quantitation of 0.05 ng/mL. Quality control samples (0.05, 0.15, 15 and 25 ng/mL) in five replicates from five of analytical runs demonstrated intra-assay precision (% CV ≤7.3%), inter-assay precision (% CV ≤6.1%) and an overall accuracy (% relative error) of less than 6.2%. A run time of less than 1.0 min for each sample made it possible to analyze a large number of human plasma samples per day. The method can be used to quantify lercanidipine in human plasma covering a variety of pharmacokinetic or bioequivalence studies. Copyright © 2006 John Wiley & Sons, Ltd.

Lercanidipine, [1-(3,3-diphenylpropylmethylamino)-2-methyl-propan-2-yl]methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride, is a vaso-selective dihydropyridine calcium channel antagonist that causes systemic vasodilation by blocking the influx of calcium ions through L-type calcium channels in cell membranes.[1] It is a highly lipophilic antihypertensive drug that is effective and well tolerated in patients with mild to moderate hypertension.[2] Furthermore, it maintains adequate blood pressure control for more than 24 h with once-daily dosing, without invoking unfavorable hemodynamic or sympathetic activity.[3]

Lercanidipine is administered orally as lercanidipine hydrochloride racemate tablets. It is well absorbed after oral administration with an elimination half-life of 2 to 5 h.[4] The drug exhibits non-linear kinetics and is subject to extensive hepatic first pass metabolism generating mainly inactive metabolites. After single oral administration of 10, 20 or 40 mg of the drug, peak plasma concentration levels were in the ratio 1:3:8 and areas under the plasma concentration–time curves were in the ratio 1:4:18. Single oral administration of 20 mg of the drug leads to peak plasma concentration levels, C_{max}, of 7.6 ng/mL, occurring approximately 1.5 to 3 h after dosing.[5]

Only a few methods have been developed that focus on the detection and determination of lercanidipine in biological matrices. In particular, a clinical pharmacokinetic study on lercanidipine which was based on data obtained by a liquid chromatography (LC) method combined with UV detection has been published.[6] Lercanidipine has also been quantified in human plasma by LC coupled to mass spectrometry using an ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface.[7] The chiral separation and determination of the two enantiomers of lercanidipine in human plasma have also been accomplished by normal-phase LC coupled to tandem mass spectrometry (MS/MS) using an amylase-based chiral stationary phase.[8,9] Enantiomeric separation of lercanidipine enantiomers and other 1,4-dihydropyrines derivatives has been successfully accomplished by chiral high-performance liquid chromatography (HPLC) with vancomycin as chiral selector on a chirobiotic V column[10] and by capillary electrophoresis with neutral and negatively charged β-cyclodextrin derivatives.[11] Several LC methods combined with UV[12,13] and diode-array[14] detectors have been developed for the determination of lercanidipine in pharmaceutical preparations. A differential pulse

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polarographic method has also been described for the determination of lercanidipine in tablets.[15] Recently, lercanidipine and its photodegradation products were separated by reversed-phase LC with subsequent UV detection and the main photoproducts were characterized by LC/MS/MS using an ion trap mass spectrometer equipped with an ESI interface.[16] Lercanidipine, along with other 1,4-dihydropyridine calcium channel antagonists, has been quantified in human plasma using LC coupled to MS/MS.[17] Although the above described methods include some successful approaches, there has been no report of the determination of lercanidipine in human plasma by ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS).

An ever-increasing need exists within bioanalytical laboratories to improve the sensitivity and reduce the total analysis time of the assays. Recently, UPLC separations have been run using columns packed with smaller particles (1.7 μm), mobile phases at high linear velocities and instrumentation that operates at high pressures.[18] UPLC thus enables high speed of analysis, superior resolution and increased sensitivity for analytical determinations, particularly when coupled with mass spectrometry.[19,20]

The high sensitivity and selectivity that are attained by the use of MS/MS detection make MS/MS suitable for the analysis of biological samples where the matrix is complex. UPLC technology when combined with MS/MS analysis greatly improves the sensitivity and selectivity and causes a significant increase in sample throughput capacity over traditional LC/MS systems.[21] In the present work, we developed and fully validated a UPLC/MS/MS method for the determination of lercanidipine in human plasma, which allows the analyst to benefit from the high throughput of UPLC and the identification power of MS/MS. The method described here provides a useful insight into the quantitation of lercanidipine in human plasma as it increases sample throughput and offers higher sensitivity than existing methods.

EXPERIMENTAL

Chemicals and reagents

Acetonitrile Far-UV and methanol were of HPLC grade and were purchased from Lab-Scan Analytical Sciences Ltd. (Dublin, Ireland). Formic acid and sodium hydroxide of analytical reagent grade were obtained from Panreac Quimica SA (Barcelona, Spain). *tert*-Butyl methyl ether of analytical reagent grade was obtained from Fisher Scientific (Loughborough, UK). Water was deionized and further purified by means of a Milli-Q Plus water purification system (Millipore, Molsheim, France) and was filtered through a 0.22 μm filter prior to use in the LC procedure. Lercanidipine hydrochloride was obtained from Glenmark Pharmaceuticals Ltd. (Slough, UK). Nicardipine hydrochloride (internal standard, IS) was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

Liquid chromatographic and mass spectrometric conditions

UPLC analysis was performed on an AcQuity UPLC system (Waters, Milford, MA, USA) with cooling autosampler and column oven enabling temperature control of the analytical column. The analytical column was an AcQuity UPLC™ BEH C_{18} with pressure-tolerant 1.7 μm bridged ethylsiloxane/silica hybrid particles and dimensions 2.1 × 50.0 mm i.d. (Waters). The mobile phase consisted of 70% acetonitrile in water containing 0.2% v/v formic acid and pumped at a flow rate of 0.30 mL/min. Chromatography was performed at 40 ± 1.0°C with a chromatographic run time of 1.0 min. The autosampler temperature was set at 5.0 ± 1.0°C.

Mass spectrometry was performed using a Quatro Micro API triple quadrupole mass spectrometer (Waters) equipped with an ESI interface. The detection of lercanidipine and nicardipine was performed by ESI in positive ion mode with multiple reaction monitoring (MRM). The optimum operating conditions for the mass measurements are summarized in Table 1. Data acquisition and analysis were performed using the MassLynx™ NT 4.0 software with the QuanLynx™ program.

Stock and working standard solutions

Stock standard solutions of lercanidipine and nicardipine, 1.0 mg/mL, were prepared by dissolving the appropriate amounts of the compounds in methanol. These solutions, when stored in the dark at −20°C, were found to be stable for 4 weeks.

A series of working standard solutions of lercanidipine was prepared by subsequent dilution of the above mentioned stock standard solution in methanol/water (50:50, v/v) to reach concentration ranges of 5.0–3000 ng/mL. A working standard solution of the IS, nicardipine, 500 ng/mL, was also prepared in methanol/water (50:50, v/v). The working standard solutions were freshly prepared every week and stored in the dark at −20°C.

Calibration spiked plasma standards and quality control samples

Drug-free human plasma was screened prior to use to ensure that it was free of endogenous interference at the retention times of the analyte and the IS. Calibration spiked plasma standards of 0.05, 0.10, 1.00, 5.00, 10.00, 20.00, and 30.00 ng/mL for lercanidipine were prepared over the concentration range by appropriate dilutions of the above-mentioned working standard solutions in 10.0 mL of drug-free human plasma.

Table 1. Optimum operating mass spectrometric parameters for lercanidipine and nicardipine (IS)

Parameter	Value
Source temperature, °C	100
Desolvation temperature, °C	300
Cone gas flow rate, L/h	10
Desolvation gas flow rate, L/h	400
Transition dwell time, s	0.1
Capillary voltage, kV	3.0
Cone voltage, V	30.0
Collision energy voltage, eV	30.0
Collision gas, mbar	0.0023
Mode of analysis	Positive ion
Ion transition for lercanidipine, *m/z*	612.2 → 280.2
Ion transition for nicardipine, *m/z*	479.9 → 315.1

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Quality control (QC) samples were also prepared in human plasma at four concentration levels (0.05, 0.15, 15.00 and 30.00 ng/mL). Separate stock standard solutions of lercanidipine were used for the preparation of calibration standard solutions and QC samples. All calibration standards and QC samples were dispensed in aliquots (600 μL) into brown Eppendorf tubes and stored at −30°C until the assay.

Sample preparation procedure

The sample preparation procedure was performed in a special room protected from light in order to avoid the photodegradation of lercanidipine and nicardipine (IS). Extraction and cleanup of human plasma samples were carried out by liquid-liquid extraction (LLE) according to the following procedure. The frozen plasma samples required for each assay were thawed at room temperature and 500 μL aliquots were transferred to glass test tubes. Each calibration and QC sample was spiked with 50 μL of the 500 ng/mL nicardipine working standard solution prior to the extraction. The samples were made basic by the addition of 200 μL of 1.0 M sodium hydroxide, vortex-mixed for 30 s and 4.0 mL of *tert*-butyl methyl ether were added. The mixture was gently shaken at 150 *g* for 30 min and centrifuged at 2500 *g* for 10 min. The aqueous phase was frozen using dry ice for 5 min and the organic layer was transferred to a 10 mL glass tube. The contents were evaporated under a gentle stream of nitrogen at 30°C. The residue was reconstituted in 100 μL of mobile phase and vortex-mixed for 30 s. The samples were transferred in the 96-well plate capped vials, to the autosampler rack, and allowed to stand for 20 min at 5°C before starting the sequence. Aliquots of 20 μL were injected into the chromatographic system.

Validation procedures

Spiked plasma calibration standards at seven different concentration levels ranging from 0.05 to 30.00 ng/mL for lercanidipine were prepared and analyzed in triplicate in five different analytical runs. Calibration curves, based on the peak area ratio of the analyte to that of the IS versus the theoretical concentration, were prepared for each run. Weighted ($1/y^2$) least-squared linear regressions were used to obtain the equation of the calibration curves. QC samples were processed in five replicates at each concentration (0.05, 0.15, 15.00 and 30.00 ng/mL) for five different analytical runs in order to evaluate the intra- and inter-assay accuracy and precision.

The recovery of the LLE procedure was evaluated at three concentration levels (0.15, 15.00 and 25.00 ng/mL) of the analyte and at 50.00 ng/mL for the IS. It was determined by comparing the peak areas obtained from the QC samples after the extraction procedure, with the peak areas obtained from the analysis of methanolic standard solutions in equivalent concentrations.

The stability of lercanidipine in spiked human plasma samples was investigated under various storage conditions. In particular, plasma samples were spiked at two concentrations (1.00 and 20.00 ng/mL) of the analyte and were stored at ambient temperature for 4 h and at −20°C for 3 weeks. Freeze/thaw stability was also evaluated by successive cycles of freezing and thawing; three complete freeze/thaw cycles were performed with samples frozen at −20°C for 7 days per cycle and thawed at room temperature. Absolute peak area measurements obtained from the analysis of the stored samples were compared with the absolute peak area measurements obtained from the analysis of freshly prepared spiked plasma samples. The analyte was considered stable in the biological matrix when 80–120% of the initial concentration was found.

RESULTS AND DISCUSSION

Optimization of mass spectrometric conditions

Mass spectrometric parameters were optimized so as to achieve the maximum abundance of the product and fragmented ions of lercanidipine and nicardipine (IS). Acquisition parameters were determined by direct infusion into the tandem mass spectrometer of a 200 ng/mL solution (in mobile phase) of each of the compounds at a flow rate of 20 μL/min. Full scan and product ion mass spectra of lercanidipine and nicardipine obtained in positive ESI mode are presented in Figs. 1 and 2, respectively. Lercanidipine gave a protonated molecule $[M+H]^+$ at *m/z*612.2 which was chosen as the precursor ion. The $[M+H]^+$ ion fragmented under collision-induced decomposition to produce product ions at *m/z* 315.0 and 298.3. The ion at *m/z* 298.3, by loss of water (18 Da), generates the product ion at *m/z* 280.2. The protonated species $[M+H]^+$ at *m/z* 479.9 was the predominant ion in the full scan spectrum of nicardipine (IS) and it was chosen as the precursor ion. The $[M+H]^+$ ion fragmented to produce a less abundant product ion at *m/z* 359.1 by loss of α-methylbenzylamine (121 Da). The ion at *m/z* 359.1, by loss of ethylene oxide (44 Da), gives the major product ion at *m/z* 315.1. The chemical structures of the analyte and the IS along with the masses of the product ions are presented in Scheme 1.

The MRM transitions of *m/z* 612.2 → 280.2 and *m/z* 479.9 → 315.1 were chosen for the quantitation of lercanidipine and nicardipine, respectively. The optimum mass spectrometric conditions along with mass transitions in MRM are presented in Table 1.

Ultra-performance liquid chromatography

UPLC was performed by the use of an AcQuity UPLC™ BEH C_{18} (1.7 μm) column and optimized to achieve adequate retention of the analytes. Various combinations of acetonitrile and water and a number of acidic modifiers (trifluoroacetic acid, formic acid and glacial acetic acid) were investigated to identify the optimal mobile phase composition that produced the best sensitivity and peak shape for all the analytes. It was found that an increase in the content of acetonitrile as organic modifier in the mobile phase could improve peak shape, whereas an increase in water content broadened the peak. The use of formic acid as an acidic modifier in the mobile phase gave excellent positive ESI response for lercanidipine and the IS. A mobile phase consisting of 70% acetonitrile in water containing 0.2% formic acid was finally used. Each chromatographic run was completed within 1.0 min.

A representative MRM UPLC/MS/MS chromatogram obtained from the analysis of a sample spiked with 5.0 ng/mL

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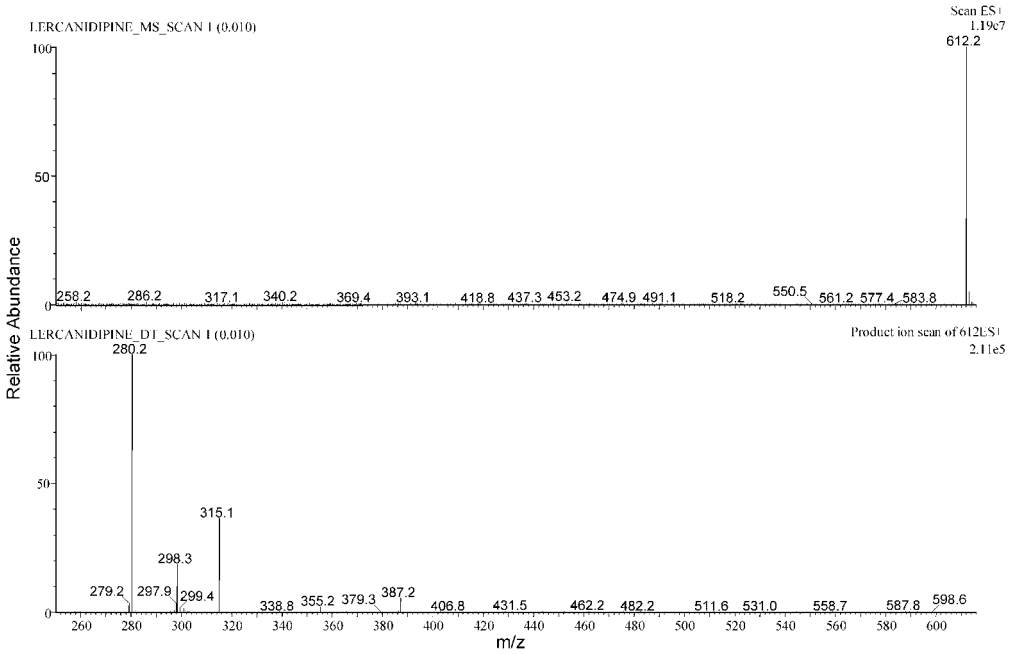

Figure 1. Full scan mass spectrum of a 200 ng/mL lercanidipine standard solution in a mixture of 70% acetonitrile in water containing 0.2% formic acid, along with product ion scan spectra of its protonated molecule $[M+H]^+$ at *m/z* 612.2. MS conditions: positive ESI mode; source temperature 100°C; desolvation temperature 300°C; capillary voltage 3.0 kV; cone voltage 30.0 V; cone gas flow rate 10 L/h; desolvation gas flow rate 400 L/h; collision energy 30 eV.

of the analyte and the IS is presented in Fig. 3. Under the current chromatographic conditions lercanidipine and nicardipine were eluted at 0.41 and 0.38 min, respectively.

Statistical analysis of data

Spiked plasma calibration standards of lercanidipine were analyzed in triplicate in five analytical runs for the calibration procedure. Linear relationships between the ratios of the peak area signals of lercanidipine to that of the IS and the corresponding concentrations were observed, using a weighting factor of $1/y^2$. The regression equations of the calibration curves, the standard deviation values of the slopes and intercepts along with the correlation coefficients are also presented in Table 2; the correlation coefficient

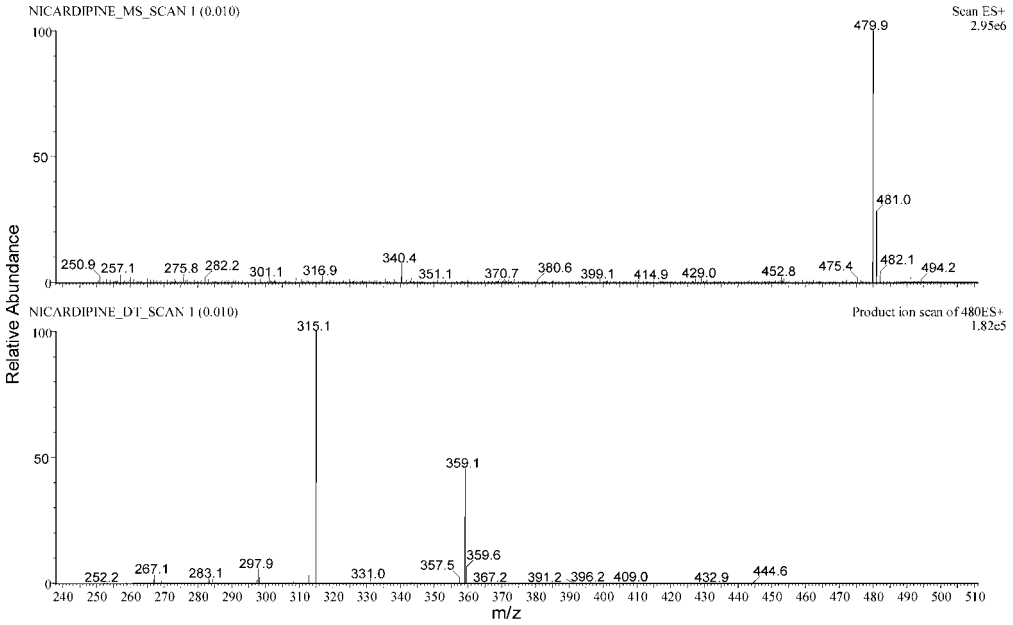

Figure 2. Full scan mass spectrum of a 200 ng/mL nicardipine standard solution in a mixture of 70% acetonitrile in water containing 0.2% formic acid, along with product ion scan spectra of its protonated molecule $[M+H]^+$ at *m/z* 479.9. MS conditions: positive ESI mode; source temperature 100°C; desolvation temperature 300°C; capillary voltage 3.0 kV; cone voltage 30.0 V; cone gas flow rate 10 L/h; desolvation gas flow rate 400 L/h; collision energy 30 eV.

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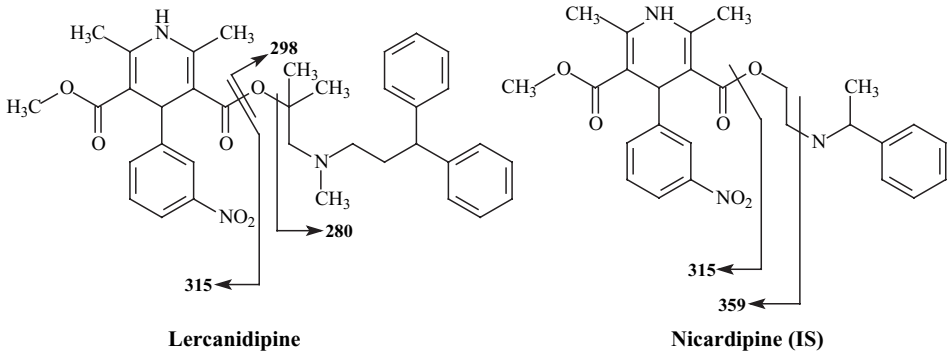

Scheme 1. Chemical structures and masses of the product ions for lercanidipine and nicardipine (IS).

invariably exceeded 0.995. The average slope and intercept of the five calibration curves generated during the validation of the method was found to be 0.0983 ± 0.0092 and 0.00082 ± 0.00055, respectively, with a % coefficient of variance (CV) of 9.3 and 66.0 %, respectively. In all cases, back-calculated concentrations in the calibration curves were within 15% of the nominal values. These results are in agreement with international guidelines[22] and indicate that the linear model acceptably describes the relationship between concentration and response.

A Student's t-test was performed to determine whether the experimental intercepts (α) of the above-mentioned regression equations were significantly different from the theoretical zero value. The test is based on the calculation of the quantities $t = \alpha/S_\alpha$, where α is the intercept of the regression equations and S_α is the standard deviation of α, and their comparison with tabulated data of the t-distribution. The calculated t-values are also presented in Table 2. These values do not exceed the 95% criterion of $t_p = 2.57$ for $f = 5$ degrees of freedom (df), indicating that the intercepts of all regression lines are not significantly different from zero.

The limit of detection (LOD) and the limit of quantitation (LOQ) for lercanidipine were determined according to the definitions of ICH Topic Q2B.[23] Thus, the LOD was calculated using the equations $y - \alpha = 3.3 \times S_\alpha$ and $y - \alpha = b \times LOD$ (where b is the slope and S_α is the standard deviation of the intercept of the regression line) and was found to be 0.02 ng/mL. The LOQ was determined using the equations $y - \alpha = 10 \times S_\alpha$ and $y - \alpha = b \times LOQ$ (where b is the slope and S_α is the standard deviation of the intercept of the regression line). The LOQ was found to be 0.05 ng/mL. A representative MRM chromatogram obtained from the analysis of a blank plasma sample and a chromatogram obtained from the analysis of a sample spiked with 0.05 ng/mL of the analyte (LOQ level) and the IS are presented in Fig. 4.

One-way analysis of variance (ANOVA) was used to evaluate the intra- and inter-assay precision. Results presented in Table 3 indicate that the intra-assay coefficients of variations (% CVs) were between 3.6 and 7.3%, while the

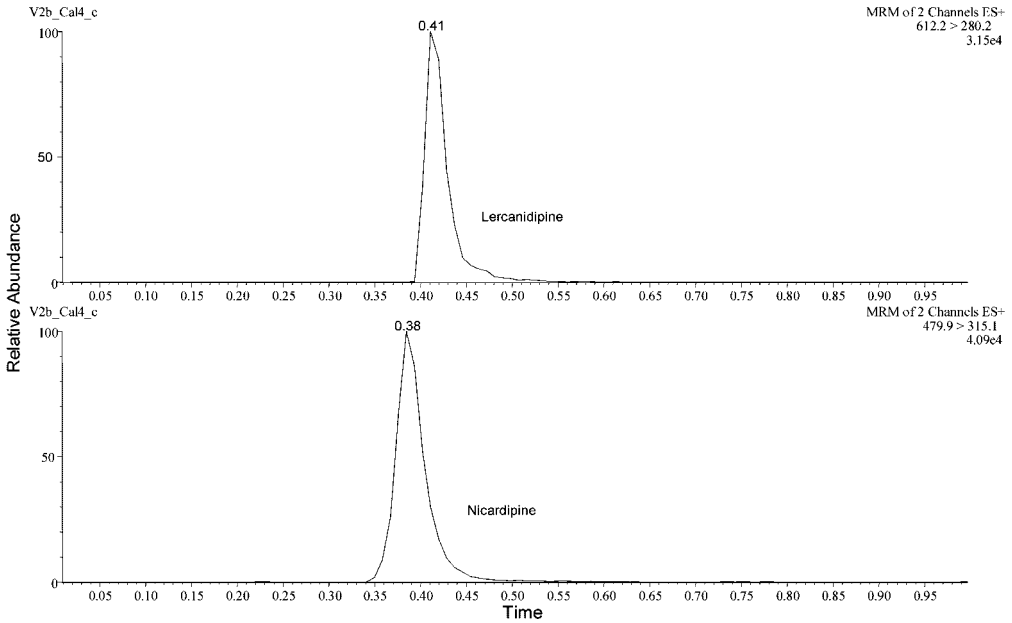

Figure 3. A representative UPLC/MS/MS chromatogram (positive ion ESI MRM mode) of a calibration plasma sample spiked with 5.0 ng/mL of lercanidipine and 50.0 ng/mL of the IS; the retention times of lercanidipine and nicardipine are 0.41 and 0.38 min, respectively.

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Table 2. Analytical parameters of the calibration equations for the determination of lercanidipine by UPLC/ESI-MS/MS

Concentration range (ng/mL)	Regression equations[a]	r[b]	SD[c] Slope	SD[c] Intercept	S_r[d]	α/S_α[e]
Run 1						
0.05–30.00	$R_{Lrp} = 0.1065 \times C_{Lrp} - 0.00029$	0.995	0.0046	0.00052	0.097	0.56
Run 2						
0.05–30.00	$R_{Lrp} = 0.0875 \times C_{Lrp} + 0.00116$	0.998	0.0022	0.00051	0.057	2.29
Run 3						
0.05–30.00	$R_{Lrp} = 0.0998 \times C_{Lrp} + 0.00158$	0.9991	0.0020	0.00069	0.044	2.29
Run 4						
0.05–30.00	$R_{Lrp} = 0.0904 \times C_{Lrp} + 0.00060$	0.9992	0.0022	0.00031	0.041	1.90
Run 5						
0.05–30.00	$R_{Lrp} = 0.1076 \times C_{Lrp} + 0.00045$	0.996	0.0041	0.00051	0.086	0.87

[a] Ratio of the peak area amplitude of lercanidipine to that of the IS, R_{Lrp}, vs. the corresponding concentration, C_{Lrp}.
[b] Correlation coefficient.
[c] Standard deviation of slope and intercept.
[d] Standard error of the estimate.
[e] Theoretical value of t at $P = 0.05$ level of significance, for $f = n - 2 = 5$ df, 2.57.

inter-assay % CVs were lower than 6.1%. The overall accuracy was assessed by the relative percentage error (absolute % E_r), which ranged from −5.0 to 6.2% for the analyte.

The selectivity towards endogenous plasma compounds was tested in six different batches of drug-free human plasma by analyzing blanks (non-spiked plasma samples) and plasma samples spiked with lercanidipine at a concentration level of 0.15 ng/mL. Mass chromatograms of six batches of drug-free plasma contained no co-eluting peaks greater than 20% of the area of lercanidipine at the LOQ level, and no co-eluting peaks greater than 5% of the area of nicardipine (IS). The concentration of lercanidipine obtained after the analysis of the six different lots of human plasma was 0.1620 ± 0.0091 ng/mL with a relative percentage error (% E_r) of 8.0%. The results indicate that the matrix effect does not appreciably affect the assay.

To evaluate the ion suppression effect, six samples of drug-free human plasma were processed according to the sample preparation procedure and then spiked with lercanidipine at 1.0 ng/mL. The corresponding peak areas of lercanidipine were then compared with those of aqueous standard solutions at equivalent concentrations. Ion suppression recovery was found to be $98.0 \pm 6.8\%$, indicating that there was no appreciable matrix suppression.

The recovery of the proposed LLE procedure was determined by calculating the ratio of the absolute peak areas of extracted spiked plasma samples to the absolute peak areas of aqueous standard solutions containing equivalent concentrations of lercanidipine (unextracted standards) that represent 100% recovery. The data presented in Table 4 indicate average recovery of 95.8%. The recovery for nicardipine (IS) was found to be 94.5% at 50.0 ng/mL.

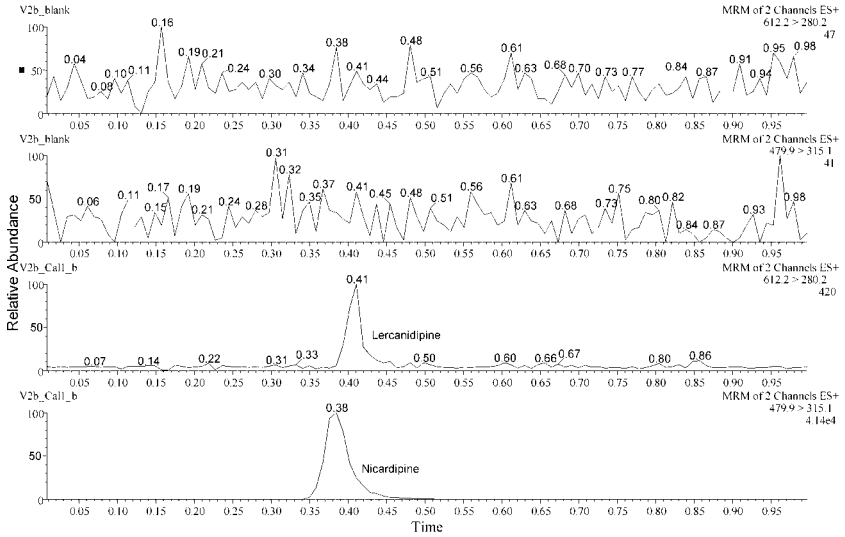

Figure 4. A representative UPLC/MS/MS chromatogram (positive ion ESI MRM mode) of a blank plasma extract (top), along with a UPLC/MS/MS chromatogram (MRM mode) of a calibration plasma sample spiked with lercanidipine at the LOQ level (0.05 ng/mL) and 50.0 ng/mL of the IS; the retention times of lercanidipine and nicardipine are 0.41 and 0.38 min, respectively.

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Table 3. Accuracy and precision evaluation of QC samples for lercanidipine (in five validation days, five replicates per day)

	Concentration (ng/mL)			
	0.05	0.15	15.00	25.00
Run 1				
Mean ± SD	0.0556 ± 0.0044	0.1583 ± 0.0086	16.40 ± 0.29	22.66 ± 0.75
(% E_r)[a]	11.1	5.5	9.3	−9.3
Run 2				
Mean ± SD	0.0570 ± 0.0028	0.152 ± 0.021	16.31 ± 1.70	23.0 ± 1.5
(% E_r)[a]	14.1	1.4	8.7	−8.0
Run 3				
Mean ± SD	0.0545 ± 0.0031	0.146 ± 0.014	15.17 ± 0.63	25.17 ± 0.54
(% E_r)[a]	8.9	−2.7	1.1	0.4
Run 4				
Mean ± SD	0.0499 ± 0.0047	0.1632 ± 0.0068	15.09 ± 0.21	24.31 ± 0.35
(% E_r)[a]	−0.1	8.8	0.6	−2.8
Run 5				
Mean ± SD	0.0485 ± 0.0042	0.1501 ± 0.0046	14.74 ± 0.28	23.68 ± 0.65
(% E_r)[a]	−2.9	0.7	−1.7	−5.3
Overall mean	0.0531	0.1539	15.52	23.76
Overall accuracy				
(% E_r)[a]	6.2	2.6	3.5	−5.0
Intra-assay % CV[b]	7.3	6.5	5.4	3.6
Inter-assay % CV[b]	6.1	2.9	4.4	3.9

[a] % E_r: Relative percentage error.
[b] % CV: coefficient of variation; intra- and inter-assay CV was calculated by ANOVA.

Table 4. Recovery data for the liquid-liquid extraction procedure for lercanidipine and nicardipine (IS) (n = 5)

Compound	Lercanidipine		Nicardipine	
Concentration (ng/mL)	0.15	15.00	25.00	50.00
Mean ± SD	0.142 ± 0.016	14.7 ± 1.2	24.1 ± 1.5	—
%Recovery ± SD	94.7 ± 11.1	96.1 ± 7.3	96.6 ± 5.8	94.5 ± 2.4

The stability results presented in Table 5 indicate that the analyte can be considered stable under the various conditions investigated. In particular, lercanidipine concentrations deviate by no more than −6.8% relative to the reference, while no degradation products were observed. Calibration plasma samples containing lercanidipine may therefore be kept for up to 4 h at ambient temperature, for 20 days at −20°C, and after three freeze/thaw cycles (7 days per cycle) at −20°C without any significant degradation.

Actual measurements of the analytes in human plasma after oral administration

The method was applied to the analysis of plasma samples obtained after oral administration of lercanidipine. Three patients (two males and one female) were used in the study, aged between 50 and 60 years old. The patients were receiving 10 mg of lercanidipine once daily in the morning (Zanidip® 10 mg, Galenica SA) for a long-term treatment.

Blood samples were collected 3 h after dosing of lercanidipine in Venoject® tubes containing lithium heparin as

Table 5. Stability data for lercanidipine in human plasma under various storage conditions (n = 5)

Storage conditions/time	Concentration levels (ng/mL)	Calculated concentration (ng/mL) (mean ± SD)		% E_r[a]
		Freshly prepared samples	Stability samples	
Ambient temperature/4 h	1	0.977 ± 0.037	1.017 ± 0.026	4.1
	20	19.84 ± 0.09	19.80 ± 0.21	−0.2
−20°C/20 days	1	0.998 ± 0.035	0.981 ± 0.038	−1.7
	20	18.65 ± 0.62	18.94 ± 1.05	0.1
−20°C/1 freeze/thaw cycle	1	0.890 ± 0.030	0.9551 ± 0.0028	−6.8
	20	18.42 ± 0.43	18.93 ± 0.62	−2.7
−20°C/3 freeze/thaw cycles	1	0.990 ± 0.033	0.933 ± 0.022	−5.7
	20	18.80 ± 0.60	18.23 ± 0.68	−3.1

[a] Relative percentage error = [(overall mean assayed concentration of stability sample – overall mean assayed concentration of fresh sample)/(overall mean assayed concentration of fresh sample) × 100].

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anticoagulant. Immediately after drawing, the samples were shaken gently and centrifuged at 4000 rpm for 10 min at 4°C. All plasma samples were analyzed one day after storage at −20°C. The samples were analyzed in triplicate, using plasma aliquots of 500 μL, according to the sample preparation procedure (IS was added) in order to calculate the concentration of the analyte. Lercanidipine plasma concentration was found to be 3.63 ± 0.24 ng/mL in the plasma sample obtained from the female patient. In the plasma samples obtained from the males the concentrations of the drug were found to be 2.55 ± 0.34 and 3.19 ± 0.14 ng/mL.

CONCLUSIONS

The proposed UPLC/ESI-MS/MS method takes advantage of the benefits of two highly selective and sensitive techniques and it enables the rapid, accurate and selective assay of lercanidipine in human plasma. The LOQ for lercanidipine was 0.05 ng/mL and the chromatographic run time was less than 1.0 min. The method offers significant improvement in sensitivity and total analysis time and proved to be appropriate for the quantification of the analyte in the plasma of patients receiving lercanidipine. Thus, it is suitable to support a wide range of pharmacokinetic or bioequivalence studies.

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