Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 551 (2005) 177–183
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Development of a rapid liquid chromatography tandem mass spectrometry method for the determination of lisinopril, applicable for a bioequivalence study, employing a 96-well format solid phase extraction protocol

Constantinos Kousoulos, Georgia Tsatsou, Yannis Dotsikas, Yannis L. Loukas *

Laboratory of Pharmaceutical Analysis and Bioequivalence Services (GLP Compliant), Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimioupoli Zografou GR, 157 71 Athens, Greece

Received 22 March 2005; received in revised form 26 June 2005; accepted 13 July 2005
Available online 25 August 2005

Abstract

A rapid liquid chromatography/tandem mass spectrometry (LC/MS-MS) method was developed for the determination of lisinopril in human plasma. Lisinopril and the internal standard enalaprilat (IS) were extracted from human plasma by semi-automated solid phase extraction (SPE) using a 96-well format extraction plate. Initially, a 1:1 plasma: acetonitrile (ACN) mixture was prepared and vortexed to achieve protein precipitation. After centrifugation, an aliquot of the supernatant water/ACN solvent mixture was evaporated. The residue was dissolved in a certain volume of a reconstitution solution, which was passed through the extraction plate and the eluent was analyzed by combined reversed phase liquid chromatography tandem mass spectrometry with positive ion electrospray ionization using multiple reactions monitoring (MRM). The method was proved to be sensitive and specific for both drugs and its statistical evaluation revealed excellent linearity for the range of concentrations 2.0–200.0 ng mL^{-1} and very good accuracy, and inter- and intraday precisions. The proposed method enables the rapid and reliable determination of lisinopril in pharmacokinetic or bioequivalence studies after per os administration of 20 mg tablet formulations of lisinopril and it was applied in such study from our laboratory.

Keywords: Lisinopril; Bioequivalence; 96-Well format tubes; Electrospray

1. Introduction

Nowadays, the liquid chromatography/mass spectrometry combination is a powerful means for the study of a series of biomedical applications, such as the characterization of host-guest interactions [1,2] and complicated macromolecules [3], but mainly for the determination of organic molecules from complex biological matrices [4–6]. The selectivity and sensitivity of LC/MS-MS has allowed for analysis times to be reduced such that sample preparation time often exceeds the analysis time of samples. To solve this problem automation techniques like solid-phase extraction and liquid phase extraction in 96-well plate formats have been applied. Lisinopril, 1-[*N*-[(*S*)-l-carboxy-3-phenylpropyl]-L-fysyl]-L-proline dihydrate, is one of the most effective drugs for hypertension (high blood pressure) and secondly for heart failure. It belongs in a group of drugs, known collectively as angiotensin-converting enzyme (ACE) inhibitors and the result of their binding with the active sit of the ACE is the blockage of the hydrolytic elimination of angiotensin-I to the potent vasoconstrictor angiotensin-II [7].

Quite a few researchers have dealt with the development of methods that quantify lisinopril in biological media. Methods that include polarografic [8,9] determination of the drug are rather complicated for most analysts, while they are not so sensitive. LC methods were also described for the determination of lisinopril with UV [10] or flu-

* Corresponding author. Tel.: +30 210 7274224; fax: +30 210 7274224.
E-mail address: loukas@pharm.uoa.gr (Y.L. Loukas).

0003-2670/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2005.07.006

orescence detection [11,12]. These methods are mostly applicable in relatively high concentrated samples, require pre-column derivatization reaction and have complicated chromatograms with a run time of several minutes. Assays [13,14] based on gas chromatography/mass spectrometry (GC/MS) are extremely sensitive, but required a two-step derivatization procedure, which resulted in the conversion of the thermolabile and ionic lisinopril into a suitable for GC derivative. Lately, a series of LC-MS/MS methods [l5–l7] were developed which were sensitive and specific for lisinopril.

The target of the present study was to improve these methods regarding two parameters: (i) decrease of the sample preparation time and (ii) minimization of the blood plasma volume. Both factors are of great significance in pharmacokinetic/bioequivalence studies. While previous studies allow the simultaneous solid phase extraction of a few samples, in the present one, a 96-well format extraction plate was utilized. Since, the collection plate had also a 96-well format, the whole procedure kept a few minutes per plate and hence it can be automated. In addition, while other studies require 1 mL plasma samples, here only 500 μL were needed. This is a big advantage in cases of inadequate plasma volumes and furthermore it enables the re-examination of miscalculated samples, e.g. in a plate that its measurements were not acceptable, for any reasons.

In the present study, all plasma samples were initially diluted and vortexed with ACN. After centrifugation, an aliquot of the relatively clean supernatant mixture was evaporated and the residue was dissolved with an aqueous hydrochloric solution and then passed through a universal resin extraction plate. After washing the wells, the molecules were eluted with mobile phase into a 96-well format collection plate, which was immediately transferred into the autosampler for direct injection. An aliquot of this solution is analyzed by combined reversed phase liquid chromatography tandem mass spectrometry with positive ion electrospray ionization, using multiple reactions monitoring (MRM).

2. Experimental

2.1. Chemicals and reagents

Lisinopril and enalaprilat were donated from ILS (Athens, Greece). Formic acid, ammonium acetate and hydrochloric acid were obtained from Sigma–Aldrich (Athens, Greece) and were of analytical grade. Methanol and acetonitrile were of HPLC grade and were also bought from Sigma–Aldrich. All aqueous solutions and buffers were prepared using water de-ionized and doubly distilled (resistivity > 18 MΩ cm) from a Millipore Mlli-Q Plus System, Malva (Athens, Greece). All plasma samples for method validation were prepared with plasma purchased from Scandibodies (France).

2.2. Instrumentation

Blood samples were transferred using a hand-held pipette from Labsystems (Viodynamiki, Athens, Greece). IS was added into 1.2 mL 96-well format micro-tubes plates obtained from Abgene (Epsom, UK) with a multichanell pipette (50–300 μL) purchased from Labsystems. ACN was dispensed into the micro-tubes via a Tomtec Quadra 96 robotic liquid handling system (Bidservice, NJ, USA). Eight-strip polyethylene caps for the microtubes racked in 96-well format were purchased from E&K Scientific (CA, USA). An Eppedorf 5810 R (Bacakos, Athens, Greece) centrifuge that could accommodate 96-well plates was also utilized to cause separation of the protein precipitant and the supernatant liquid mixture. The latter was removed from the microtubes and transferred to 2.2 mL 96-well collection plates, which were purchased along with their piercable rubber caps from Eppedorf (Bacakos, Athens, Greece). These plates were inserted into a Zymark TurboVap 96-well format plate evaporator (Malva, Athens, Greece) that applies nitrogen for solvent evaporation. This nitrogen is produced by an Agilent nitrogen generator (Duratec, Hockenheim, Germany) that receives air from an Atlas Copco SF4 air compressor (Athens, Greece). The whole SPE procedure was performed by using a Supelco (Sigma–Aldrich, Athens, Greece) SPE system and a universal resin membrane 96-well format extraction plates obtained from 3M Bioanalytical Europe (Neuss, Germany).

The HPLC system included a Waters Alliance HT 2795 pump (Malva, Athens, Greece) accompanied with an autosampler, a degasser and a column oven/cooler. The autosampler could accommodate four 96-well plates allowing an automated measurement of a big amount of samples. A Micromass Quattro Micro MS/MS system (Hellamco, Athens, Greece) equipped with an electrospray ion source, operating under MassLynx 4.0 software, was used.

2.3. Chromatographk conditions

The isocratic HPLC elution mobile phase was composed of 75% MeOH, 25% 10 mM formic acid and 5 mM ammonium acetate in H_2O (v/v). A flow rate of 0.7 mL min^{-1} was used for sample analysis on a YMC-Pack-Octyl (Schermbeck, Germany) analytical column (50 mm × 4.0 mm i.d.). The column was maintained at room temperature (∼22 °C), whilst the autosampler temperature was 15 °C. The injection volume was 50 μL and the total run time was set for 2.0 min.

2.4. Mass spectrometric conditions

The mass spectrometer was operated in the positive ion mode, using the electrospray source and interface. The tuning parameters were optimized for both lisinopril and the IS enalaprilat by infusing a solution containing 500 ng mL^{-1} of both analytes at a flow rate of 10 μL min^{-1} via an external syringe pump. The source temperature was 100 °C, desol-

vation temperature was 300 °C, desolvation gas flow was 700 L h^{-1} and cone gas flow was 70 L h^{-1}. The capillary voltage was set at 3.5 kV, while optimized cone voltage values for lisinopril and enalaprilat were 30 V in both cases. The multiplier was set at 650 V and argon was used as the collision gas. Quantitation was performed using selected reaction monitoring (SRM) of the transitions m/z 406.1 → 83.6 for lisinopril and m/z 349.1 → 205.8 for the IS, respectively, with a dwell time of 0.5 s per transition. The optimized collision energy of 25 eV was used for the analyte and 20 eV for the IS.

2.5. Preparation of standard and quality control/method validation samples

Initially, the stock solution of lisinopril was prepared by dissolving the accurately weighed reference compound of lisinopril in M_3OH/H_2O 50/50 (v/v) to yield final concentrations of 100 μg mL^{-1} (SL_1) and 2000 ng mL^{-1} (SL_2). These solutions were then serially diluted with the same diluent mixture to achieve standard working solutions of 2000, 1000, 500, 200, 100, 50, and 20 ng mL^{-1} for lisinopril. A 400 ng mL^{-1} IS working solution was also prepared in the same solvent mixture.

Two quality control-method validation (QC-MV) stock solutions (100 μg mL^{-1} and 2000 ng mL^{-1}, respectively) were prepared from a separate weighing. Dilutions were used to prepare four levels of QC working solutions, 1500, 300, 60, 20 ng mL^{-1}. All these solutions were stored at 4 °C and were brought to room temperature before use.

Calibration standads, QC and MV samples were prepared in the same biological matrix (human plasma) as the samples to be analyzed. Calibration curve consisted of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard) and non-zero samples covering the expected range of concentrations to be quantified. Calibration standards were prepared by diluting 10 times with human plasma the working solutions of lisinopril, obtaining final standard concentrations of 200, 100, 50, 20, 10, 5 and 2 ng mL^{-1}.

QC and MV samples have two distinct purposes: the results for QC samples provide the basis for accepting or rejecting analytical runs, while the results for MV samples are used to calculate bias and precision of the assay methodology. QC and MV samples were prepared by diluting 10 times with human plasma the respective working solutions. They were prepared in bulk and dispensed in 1.5 mL aliquots into properly labeled Eppendorf tubes and stored at −20 °C until required for assay. The following concentration levels of QC/MV samples were prepared: MV_L (2 ng mL^{-1}), MV_1/QC_1 (6 ng mL^{-1}, low), MV_2/QC_2 (30 ng mL^{-1}, medium) and MV_3/QC_3 (150 ng mL^{-1}, high).

2.6. Sample extraction and preparation

Initially, 50 μL of the IS working solution were placed into the appropriate 1.2 mL tubes of a 96-well plate rack. Then, 500 μL of each of the calibration, QC/MV sample were added and the samples were vortex-mixed for 10 min. Next, 500 μL of ACN were dispensed and the racks were vortexed for 10 min to achieve protein precipitation. After centrifugation at 3500 rpm and 4 °C for 10 min, 500 μL of the supernatant mixture were transferred into a 2.2 mL 96-deepwell plate according to the assay proforma sheet pre-generated. Samples were evaporated using a flow of nitrogen at 50 °C and then the residue was dissolved with 500 μL of the washing solution (HCl, 10 mM). After vortex-mixing for 5 min the SPE procedure followed.

Firstly, the waste tray, the manifold collar and the SPE plate were placed accordingly. Then, vacuum was applied and the SPE 96-well extraction disk plates were conditioned with 100 μL of MeOH per well. Next, the wells were washed with 250 μL of the washing solution mentioned above (rinse). Five hundred microliters of the reconstituted samples were added into the corresponding positions of the universal resin plate and vacuum was applied until all wells had drained (load). Five hundred microliters of the washing solution were added three times (wash) and then a collection plate (2.2 mL, 96-well) replaced the waste tray. The molecules were eluted by dispensing 200 μL of the mobile phase and applying vacuum until all wells had drained. Finally, the plate was transferred into the autosampler, immediately, for direct injection.

3. Results and discussion

The application of the specific SPE protocol allowed the development of a rapid method for lisinopril, since a big amount of samples can be determined daily. The high performance well extraction disk plates from 3M Empore provided a simple route to high throughput SPE. The whole procedure may last less than an hour, requires reduced solvent and elution volumes due to the nature of the structure: each well has a unique particle-loaded membrane, instead of a packed bed sorbent mass and furthermore a special prefilter is placed just above the membrane. Its sorbent consists of a terpolymer that contains polar functional groups, enabling high retention of a wide range of drugs, by applying a general approach to sample preparation and thus reducing the method development time dramatically. Due to these characteristics, the plasma sample volume required for analysis is relatively low (much below 1 mL). In previous methods for lisinopril [17] and enalapril/enalaprilat [18] other SPE plates or catridges were used. In these methods, 500 μL plasma samples volumes were also required, but the elution volumes were 500 and 1000 μL, respectively, and therefore an additional solvent evaporation/reconstitution step was required. In the present study, the elution volume, thanks to the properties of the sorbent, was only 200 μL and thus the collection plate was capped and immediately placed into the autosampler for analysis. The new sorbent and the 96-well format constitute a very promising combination for the analysis of a wide range of drugs rapidly and in a less expensive way.

As for the extraction itself, the selected method proved to be very simple and reproducible. The protein precipitation procedure, added as an additional part of the sample preparation, led to very clear plasma samples. This fact enabled us to use the sorbent plates for a second extraction in a raw, following an additional washing step after the first elution. The recovery results from the second elution had no statistically significant variation with the respective from the first one. Subsequently, one can use half of the required plates and reduce the cost of a pharmacokinetic/bioequivalence study in which very often the number of samples exceeds 1000. Further application of the same SPE extraction plate for a third or fourth consecutive elution, gave a recovery value smaller than the respective in the first two, without affecting the linearity of the method, but only the detection limit. Therefore, the same plate was not utilized more than twice in the present method, but this interesting issue will attract our attention and probably we will return with an upcoming report dealing with it.

The protein precipitation, caused by the addition of ACN, reduced the possibility of transferring plasma sample elements into the final solution. The latter is well known [19–21], for having a great impact on the signal intensity and therefore on the precision and accuracy of the developed method.

Along with the extraction procedure, the LC/MS-MS system was used to separate and monitor lisinopril and the IS from the extracted samples. The MS spectra for both molecules are dominated by the $[M+H]^+$ ions: m/z 406.1 for lisinopril and m/z 349.1 for enalaprilat, while the MS/MS daughter ion spectra of the protonated molecules produced major product ions at m/z 83.6 and 205.8, respectively. Representative SRM LC/MS-MS chromatograms are shown in Fig. 1. As shown, the retention times of lisinopril and the IS were 0.85 and 0.94 min, respectively.

3.1. Standard curves

After the definition of the extraction procedure and the LC/MS-MS conditions, a full validation was performed by our GLP-compliant laboratory, according to currently presented US Food and Drug Administration (FDA) bioanalytical method validaton guidance [22].

To test the specificity of the method, blank samples of human plasma were obtained from six individual sources from Aretaieio Hospital. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic and mass spectrometric conditions and no significant interferences at the retention time of the drug or internal standard were found.

To define the relationship between concentration and response, a calibration curve, containing seven non-zero standards ranging from 2.00 to 200 ng mL^{-1}, for each analytical run, was prepared. This range was suitable for a pharmacokinetic study after per os administration of a 20 mg tablet of lisinopril. In addition, a zero and a blank plasma samples were also analyzed to confirm absence of probable interferences and these samples were not used to construct the calibration function. Peak area ratios of lisinopril to IS were used for regression analysis. A linear regression model ($y = ax + b$) was evaluated, where x is the concentration of lisinopril and y corresponds to the areas ratio. Individual standard curve data from five runs met all of the preset criteria: (i) <20% deviation from nominal concentration at the limit of quantitation (LOQ), which was defined as the lowest standard (and MV_L as well); (ii) <15% deviation of standards of their back-calculated concentration, other than LOQ from nominal concentrations; (iii) at least five out of seven non-zero standards of each nominal concentration meeting the above criteria, including the LOQ and the calibration standard at the highest concentration.

The regression coefficients (R-squared) for the five runs were greater than 0.9950, average linear slope was 0.9930 ($S_a = 0.0170$) and average intercept was 0.3370 ($S_b = 1.503$). The experimental values of F-test (Mandel) were smaller than 3.299, when the (theoretical) threshold value of F-distribution (5%, one-sided) was 4.1700. Based on the presented data, it was concluded that the calibration curves used in this method were linear in the operating range. The test of proportionality was also successful: the t-test experimental value of 0.224 was greatly smaller than the theoretical value of 2.042 (5%, two-sided). In conclusion, the 96-well SPE extraction procedure used in this method was capable of producing satisfactory concentration data for lisinopril standard samples.

3.2. Accuracy and precision

During the method validation, the precision and accuracy were also assessed by analyzing MV samples that were defined above, in five runs on three separate days. The % accuracy was determined by calculating the deviations of the predicted concentrations from their nominal values (Table 1). In Table 1 the experimental values from Wilcoxon test for accuracy are also presented. In all cases the values were within the acceptable range, certifying that the nominal concentration was, actually, observed and therefore a systematic error was not detected.

The intra-day precision was assessed by analyzing six replicates at each MV level, while inter-day precision was determined over 3 days by analyzing 30 samples. Data for both types of precision (expressed as % R.S.D.) are presented in Table 1. These results, as well as the respective for % accuracy, were within the acceptance criteria for precision and accuracy, which establish that the deviation values should be within 15% of the nominal values for MV_1, MV_2 and MV_3 (tolerance until 20% for MV_L).

3.3. Extraction recovery

The extraction efficiency of the analytical method was assessed using data from five runs. Three QC samples of each type (low, medium and high), obtained by the usual extraction process were compared with three samples for

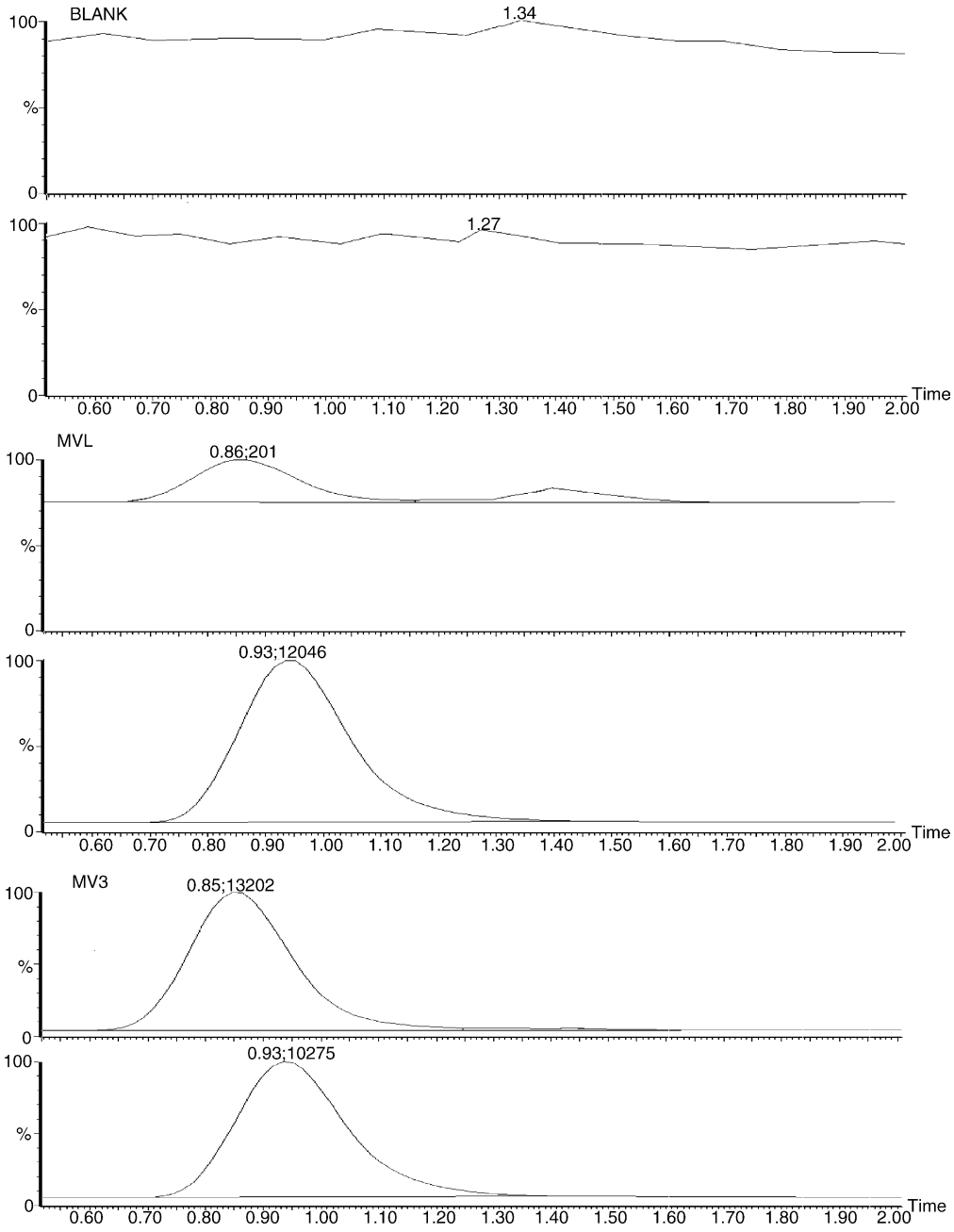

Fig. 1. Representative SRM chromatograms of lisinopril (top) and enalaprilat obtained from a Blank, MV_L and MV3 sample, respectively.

Table 1
Summary of % accuracy and precision of lisinopril from MV plasma samples

% Intra-run accuracy		% Inter-run accuracy		Intra-run precision	Inter-run precision
MV sample	% Accuracy[a]	% Accuracy[b]	Wilcoxon-test[c]	% R.S.D.[d]	% R.S.D.[b]
MV_L (2 ng mL^{-1})	95.10	92.70	2.00	4.96	7.25
MV_1 (6 ng mL^{-1})	103.37	101.35	8.00	7.42	6.37
MV_2 (30 ng mL^{-1})	96.56	103.27	9.00	9.01	8.32
MV_3 (150 ng mL^{-1})	98.78	101.81	8.00	3.36	8.26

[a] ($n = 6$), expressed as 100 × (mean calculated concentration)/(nominal concentration).
[b] Values obtained from all five runs ($n = 30$).
[c] Experimental values: lower and upper theoretical values were 0.00 and 14.00, respectively (5% two-sided).
[d] ($n = 6$).

each concentration mentioned above, obtained by diluting working solutions directly in mobile phase (unextracted samples). Recovery was evaluated by calculating the mean of the area of each concentration and dividing the extracted sample mean by the unextracted sample mean of the corresponding concentration. Mean values of extraction recovery for lisinopril in QC_1, QC_2 and QC_3 were 37.7, 36.1 and 37.8%, respectively, while mean enalaprilat recovery was estimated as 47.3%. These values were relatively small, but absolutely justified considering the very small volume of the solvent used for elution. Furthermore, the rapid elution of both molecules ($t_r < 1$ min) may be accompanied with the co-elution of plasma elements (endogenous compounds) or most probably nonvolatile or less volatile solutes (salts). The presence of these molecules, change the efficiency of droplet formation, which in turn affects the amount of charged ions in the gas phase that ultimately reaches the detector [23]. However, recovery values were consistent and the current extraction procedure was evaluated to be more than adequate for the specific concentration range. It is worth mentioning that the signal-to-noise ratio had a value of >20 in the lowest concentration standard sample.

3.4. Stability studies

As a part of the method validation, data were also generated to ensure that lisinopril was stable at distinct timing and temperature conditions, as well as the stability of the analyte in stock solution. Plasma samples containing two concentration levels of lisinopril were used for the stability experiments: Low–medium (S-l) 10 ng mL^{-1} and medium–high (S-h) 100 ng mL^{-1}.

To evaluate freeze-thaw stability, a freeze and thaw cycle was defined as the storage of S-l and S-h samples at −30 °C followed by thawing at room temperature. Samples were analyzed after the fourth cycle, along with fresh reference samples of the same concentration. Table 2 shows the corresponding results (back-calculated concentrations) of four freeze-thaw cycles versus one (fresh), which should not vary more than 10%.

To evaluate short-term stability, six aliquots of S-l and S-h were maintained, immediately after preparation, at room temperatures for 6 h, which exceeds the time that samples remain at room temperature, before they were analyzed. Results are presented in Table 3 and their variation is ≤10%.

As for long-term stability, aliquots of the two sample-types were initially frozen at −30 °C for at least 20 days, thawed and analyzed. The 20-days period is by far adequate for the analysis of samples from a bioequivalence study, since the current method allows the measurement of hundreds of samples per day. The results (Table 4) indicate that lisinopril samples are stable under these conditions.

Table 2
Summary of freeze-thaw stability results (mean concentrations ± S.D., $n = 6$)

Sample type	S-l (10 ng mL^{-1})	S-h (100 ng mL^{-1})
One cycle (C, ng mL^{-1})	10.35 (± 0.62)	104.64 (± 7.80)
Four cycles (C, ng mL^{-1})	10.93 (± 0.67)	105.36 (± 6.95)
Mean variation %	+5.60	+0.69

Table 3
Summary of short term stability results (mean concentrations ± S.D., $n = 6$)

Sample type	S-l (10 ng mL^{-1})	S-h (100 ng mL^{-1})
Fresh value (C, ng mL^{-1})	10.21 (± 0.61)	98.01 (± 5.83)
After 6 h (C, ng mL^{-1})	10.87 (± 0.58)	96.34 (± 6.24)
Mean variation%	+6.46	−1.70

Table 4
Summary of long term stability results (mean concentrations ± S.D., $n = 3$)

Sample type	S-l (10 ng mL^{-1})	S-h (100 ng mL^{-1})
Fresh value (C, ng mL^{-1})	10.75 (± 0.68)	109.03 (± 7.54)
After 20 days (C, ng mL^{-1})	11.21 (± 0.61)	106.28 (± 7.23)
Mean variation %	+4.28	−2.52

Table 5
Summary of autosampler stability results (mean concentrations from all runs)

Sample type	Beginning (C, ng mL^{-1})	Half-way (C, ng mL^{-1})	Completion (C, ng mL^{-1})
QC_1	5.69	6.24	6.04
QC_2	32.71	33.41	28.14
QC_3	154.07	152.42	146.63

Lisinopril stock solution stability was estimated by comparing fresh and old dilutions in mobile phase of this solution (stored at 4 °C). The measurements proved that lisinopril concentration in stock solution remains intact.

Autosampler stability was another part of the method validation that should have been confirmed. It was assessed by comparing QC samples included at the beginning, at half way and at completion of each of the five analytical runs. Results for the stability of lisinopril in the autosampler (15 °C) are presented in Table 5 and were within the acceptance criteria, which establish that the mean result at completion of the runs should be ≥90% of the mean result at the start of the runs for at least two out of three levels tested.

4. Conclusions

Through the use of liquid handling systems and a 96-well plate format, including individual tubes, a rapid semi-automated SPE extraction method was developed for the quantification of lisinopril in human plasma. The simultaneous SPE approach greatly simplified the preparation process and decreased the time required for sample preparation till measurement versus existing methods with manual sample treatment. Therefore, hundreds of samples can be analyzed daily. Another advantage over these methods was the relatively small quantity of human plasma used for analysis. The developed method was validated over the concentration range of 2.00–200 ng mL^{-1} for glimepiride. This range

is suitable for measuring lisinopril in plasma samples after per os administration of a 20 mg tablet in a pharmacokinetic or bioequivalence study. The validation results demonstrated that this approach worked very well in a 96-well format and this was verified in a bioequivalence study that our laboratory executed. The method possessed excellent precision and accuracy and proved to be reliable. It is expected that this approach can be applied to the extraction and analysis for other pharmaceutical compounds from biological samples.

References

[1] Y. Dotsikas, Y.L. Loukas, J. Am. Soc. Mass Spectrom. 14 (2003) 1123.
[2] M.J. Greig, H. Gaus, L.L. Cummins, H. Sasmor, R.H. Griffey, J. Am. Chem. Soc. 117 (1995) 10765.
[3] M. Adamczyk, J.C. Gebler, P.G. Mattingly, Bioconjugate Chem. 7 (1996) 475.
[4] L. Zhong, R. Eisenhandler, K.C. Yeh, J. Mass Spectrom. 36 (2001) 736.
[5] X. Chen, D. Zhong, D. Liu, Y. Wang, Y. Han, J. Gu, Rapid Commun. Mass Spectrom. 17 (2003) 2459.
[6] Y.J. Xue, J. Pursley, M.E. Arnold, J. Pharm. Biomed. Anal. 34 (2004) 369.
[7] M.A. Ondetti, B. Rubin, D.W. Cushman, Science 196 (1977) 441.
[8] N. El-Enany, F. Belal, S. Al-Ghannam, Microchim. Acta 141 (2003) 55.
[9] O. Abdel Razak, S.F. Belal, M.M. Bedair, N.S. Barakat, R.S. Haggag, J. Pharm. Biomed. Anal. 31 (2003) 701.
[10] Y.C. Wong, B.G. Charles, J. Chromatogr. B 673 (1995) 306.
[11] A.A. El-Amam, S.H. Hansen, M.A. Moustafa, S.M. El-Ashry, D.T. El-Sherbiny, J. Pharm. Biomed. Anal 34 (2004) 35.
[12] O. Sagirli, L. Ersoy, J. Chromatogr. B 809 (2004) 159.
[13] H.J. Leis, G. Fauler, G. Raspotnig, W. Windischhofer, Rapid Commun. Mass Spectrom. 12 (1998) 1591.
[14] H.J. Leis, G. Fauler, G. Raspotnig, W. Windischhofer, Rapid Commun. Mass Spectrom. 13 (1999) 650.
[15] A. Tsakalof, K. Bairachtari, M. Georgarakis, J. Chromatogr. B 783 (2003) 425.
[16] B.M. Tashtoush, F.Q. Alali, N.M. Najib, Pharmazie 59 (2004) 21.
[17] A.A.F. Padua, R.E. Barrientos-Astigarraga, V.M. Rezende, G.D. Mendes, G. De Nucci, J. Chromatogr. B 809 (2004) 211.
[18] J. Lee, J. Son, M. Lee, K.T. Lee, D.-H. Kim, Rapid Commun. Mass Spectrom. 17 (2003) 1157.
[19] R. Dams, M.A. Huestis, W.E. Lambert, C.M. Murphy, J. Am. Soc. Mass Spectrom. 14 (2003) 1290.
[20] C.R. Mallet, Z. Lu, J.R. Mazzeo, Rapid Commun. Mass Spectrom. 18 (2004) 49.
[21] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass Spectrom. 11 (2000) 942.
[22] Guidance for Industry, Bioanalytical Meihod Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2001.
[23] T.M. Annesley, Clin. Chem. 49 (2003) 1041.