Journal of Chromatography B, 879 (2011) 2688–2694

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Quantitation of quinapril in human plasma by matrix-assisted laser desorption ionization time-of-flight mass spectrometry with quinolone matrix additives

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ARTICLE INFO

Article history:
Received 24 January 2011
Accepted 19 July 2011
Available online 7 August 2011

Keywords:
Quinapril
Matrix
Time-of-flight
Mass spectrometry
Quinolones

ABSTRACT

The renin–angiotensin–aldosterone system (RAAS) is an essential body fluid maintenance system that controls pressure in the human body. The conversion of angiotensin I to angiotensin II by angiotensin-converting enzyme (ACE) is a key process in the RAAS because angiotensin II causes the vasoconstriction association with hypertension. Because of its effectiveness as an ACE blocker, quinipril is widely used for clinical treatment of hypertension and chronic congestive heart failure. Matrix-assisted laser desorption/ionization coupled with time-of-flight analyzer (MALDI-TOF) is a high throughput instrument for biological sample analysis. This study developed a micro-scale approach for using MALDI-TOF to detect quinapril in biological samples. A micro-liquid-liquid-extraction strategy combined with ion-pair interaction successfully extracted quinapril from aqueous layer to organic layer. Quinolones were then used as matrix additives to suppress undesired substances in plasma produce signals. Several factors affecting extraction efficiency were investigated in a biosample with a volume of only 10 μL. This method is successful to monitor quinapril in the clinical therapeutic range. The proposed method proved effective for monitoring the trace amounts of quinapril typically used for clinical therapy. The relative standard deviation (R.S.D.) and relative error (R.E.) used for evaluating within- and between-day assays of quinapril in plasma consistently remained below 15%.

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1. Introduction

Renin–angiotensin–aldosterone system (RAAS) is a body fluid balancing system that helps to stabilize blood pressure. The RAAS is also involved in all steps of pathways about cardiovascular and cardiorenal disease, including endothelial dysfunction, atherosclerosis, myocardial infarction (MI), stroke, chronic congestive heart failure (CHF), end-stage renal disease and premature death [1]. Angiotensin II, which is converted from angiotensin I by angiotensin-converting enzyme (ACE), is the main effector in RAAS because it induces vasoconstriction by interacting with angiotensin receptors on vascular smooth muscle cells or by stimulating the release of aldosterone from the adrenal cortex [2]. Use of an antagonist for its effects on the RAAS has proven to be an effective hypertension treatment strategy. The ACE inhibitors are also effective for blocking angiotensin II formation in anti-hypertensive drug treatments [3].

Quinapril, a member of the ACE inhibitor family, is widely used for treating hypertension and CHF [4]. Recent studies show that quinapril regulates oxidative stress mechanisms in metabolic syndrome by reducing serum 8-isoprostane, increasing erythrocyte superoxide dismutase activity, and modulating the oxidation of low-density lipoprotein [5]. Combining quinapril with dietary modification resulting in weight loss may help to prevent atherosclerosis-related diseases in metabolic syndrome [6]. In patients with type 2 diabetes, quinapril treatment can increase insulin-stimulated endothelial function [7]. Quinapril also reduces microalbuminuria in both hypertensive diabetics and in patients with essential hypertension [8]. In postmenopausal hypertensive patients, quinapril also improves endothelial function [9]. Other clinical studies of quinapril use after percutaneous coronary intervention show that the benefit of quinapril continues for up to 4 years [10].

Various chromatographic methods coupled with UV detector or mass spectrometry have also been developed for detecting quinapril in biological samples [11–20]. In recent years, matrix-assisted laser desorption/ionization combined with time-of-flight mass spectrometry (MALDI-TOF MS) has been used for analyzing large molecules such as nucleotides, peptides, proteins, saccharides and polymers [21–24]. Unlike liquid chromatography coupled with electrospray ionization LC-ESI-MS, MALDI-TOF MS does not require a mobile phase, so no organic waste is generated by sample

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1570-0232/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2011.07.026

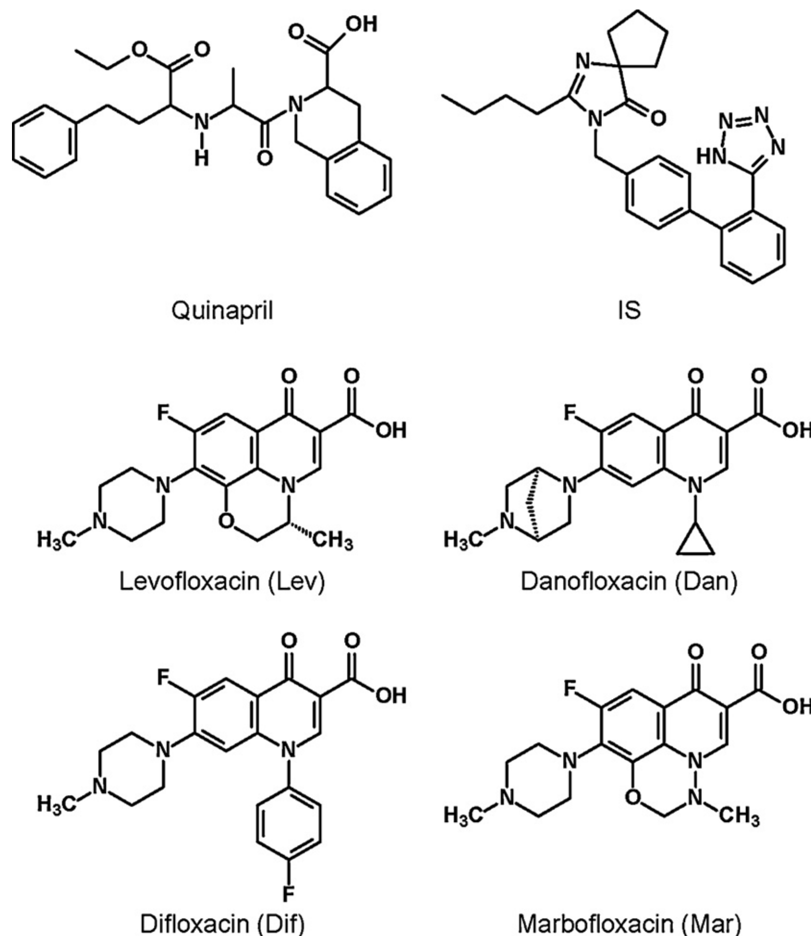

Fig. 1. Structures of quinapril, IS and quinolones.

separation in LC column. For MALDI-TOF MS, samples must be carefully prepared because the high concentration of salts and undesired compounds in the biological matrix may interfere with the target signal.

This study proposes a fast micro-scale MALDI-TOF MS method of monitoring quinapril in human plasma. Micro-liquid–liquid-extraction approach was experimentally combined with ion-pair interaction to isolate quinapril from plasma to organic solvent. Only 10 μL plasma and 20 μL solvent were needed to extract and concentrate the target analyte from a biological matrix. To optimize the analytical process, several factors were evaluated, including ionization assisting matrix selection, extraction solvent, bases and acids employed in ion-pair formation process. To our knowledge, this study is the first to use micro-scale MALDI-TOF MS for measuring quinapril concentration in human plasma. The proposed method provides a simple and fast platform for trace drug detection when the volume of biological samples available for testing is limited (e.g., in research involving small animals or to increase patient tolerability when multiple samples are required).

2. Materials and methods

2.1. Chemicals and reagents

Quinapril hydrochloride, trifluoroacetic acid (TFA), heptafluorobutyric acid (HFBA), 7-mercapto-4-methylcoumarin (7-MMC), levofloxacin (Lev), danofloxacin (Dan), difloxacin (Dif), marbofloxacin (Mar), alpha-cyano-4-hydroxycinnamic acid (CHCA), 2-mercaptobenzoic acid (2-MBA), 2-mercaptobenzothiazol (2-MBT), sinapic acid (SA), 1,8,9-anthracenetriol (1,8,9-AT) and trans 2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (T-BPMPM) were purchased from Sigma–Aldrich (St. Louis, MO). Irbesartan (internal standard, IS) was purchased from U.S. Pharmacopeia (MD, USA). Ammonium bicarbonate (NH_4HCO_3), sodium hydroxide (NaOH), potassium hydroxide (KOH), formic acid (FA), dichloromethane (CH_2Cl_2) acetonitrile (ACN), hexane, ethyl acetate (EtOAc) and toluene were purchased from Merck (Darmstadt, Germany). The water purification system was the Milli-Q system manufactured by Millipore (Bedford, MA, USA).

2.2. Working solutions preparation

Quinapril (1 mg/mL) and IS (1 mg/mL) stock solutions were prepared by dissolving the chemicals in water and CH_2Cl_2, respectively. The aqueous stock solution (1 M) of NH_4HCO_3, NaOH, KOH, HFBA, TFA and FA were prepared in water. The 7-MMC (1 mg/mL) and 200 μg/mL of Lev, Dan, Dif and Mar solution were dissolved in ACN: 0.1% TFA = 50:50 (v/v).

2.3. Instrumentation

Mass spectral analyses were performed with a MALDI-TOF MS system (model Autoflex III Smartbeam) equipped with a 355 nm Nd:YAG laser from Bruker Daltonics (Billerica, MA, USA). For quinapril analysis by MALDI-TOF, 1 μL of the sample solution was spotted on a ground target plate (Bruker Daltonics) then 1 μL of co-matrix (7-MMC: difloxacin = 1:1) was added. Mass spectra were collected for the summing of 2000 laser shots in positive ion reflec-

tor mode. Data processing was performed by FlexAnalysis software (Bruker Daltonics).

2.4. Quinapril extraction from human plasma

Plasma clean-up for quinapril analysis was performed quickly and simply by micro liquid–liquid extraction. Human drug-free plasma samples were spiked with five different quinapril concentrations ranging from 0.05 to 2.00 μg/mL. After human plasma samples (10 μL) were pipetted into Eppendorf vials, 20 mM KOH (pH = 13.0, 10 μL) solution was added. Vials were then vortexed for 30 s before adding 230 mM HFBA (pH = 0.7, 10 μL) solution. After adding 20 μL of CH_2Cl_2 (containing IS 250 μg/mL) for quinapril extraction, the Eppendorf vials were agitated for 30 s. After centrifugation at 10,000 rpm for 2 min, 1 μL of the organic layer was spotted on a target plate, and 1 μL matrix solution was added. For quinapril analysis, the target plate was transferred into MALDI-TOF MS.

3. Results and discussion

Initial experimental attempts to spot the plasma directly in the target plate and to analyze quinapril from plasma with no extraction procedure proved unsuccessful. The MALDI-TOF MS could not detect any quinapril signal. Although liquid–liquid extraction is a simple and fast method for sample preparation, isolation and extraction, the extraction efficiency of desired analytes from the aqueous layer (in this case, the human plasma) to organic layer vary according to the organic solvent used. Hence, human plasma was extracted by micro-scale liquid–liquid extraction in this study. Several parameters, including ionization assisting matrix selection, extraction solvent, different base, amount of KOH, different acid, and amount of HFBA, were optimized for quinapril analysis. Plasma spiked with a 5 μg/mL quinapril (the final concentration of quinapril in a sample) was used for parameter evaluation. The effects of these factors were tested by calculating the peak area ratios of quinapril to IS. Fig. 1 shows the structures of quinapril and IS.

3.1. Selecting a suitable matrix

The matrix is a key factor in MALDI-TOF MS. Several matrices (10 mg/mL) were evaluated for use in quinapril analysis, including 2-MBA, 2-MBT, SA, T-BPMPM, 1,8,9-anthracenetriol, CHCA and 7-MMC. In this study, the signal of quinapril in MALDI-TOF MS could be obtained just by using CHCA and 7-MMC as the matrix. Although the CHCA and 7-MMC are suitable for quinapril analysis, undesired compounds in human plasma interfered with analysis of *m*/*z* 439 in $[M+H]^+$ during the quinapril analysis in this study. Decreasing the concentrations of CHCA and 7-MMC from 10 mg/mL to 1 mg/mL reduced but did not eliminate the interference. When CHCA and 7-MMC were decreased to below 1 mg/mL, no signal (including 5 μg/mL quinapril) was detectable. Hence, the minimum CHCA and 7-MMC (1 mg/mL) concentration was used for quinapril analysis.

To reduce the interference of plasma, another compounds (used as the co-matrix strategy) were added to suppress the unexpected signal. Nitrogen-containing compounds usually suppress the undesired background and enhance the signal of the target analyte, such as triethylamine, butylamine and ionic liquids [25,26]. Quinolones are potent synthetic chemotherapeutic antibiotics, and these compounds contain piperazine moiety. Hence, 1000, 500, 200 and 100 μg/mL concentrations of these compounds (including levofloxacin, danofloxacin, difloxacin and marbofloxacin) were experimentally tested for use as matrix additive for suppressing the undesired signal. Fig. 1 also shows the structures of these compounds. The results indicated that all these quinolones

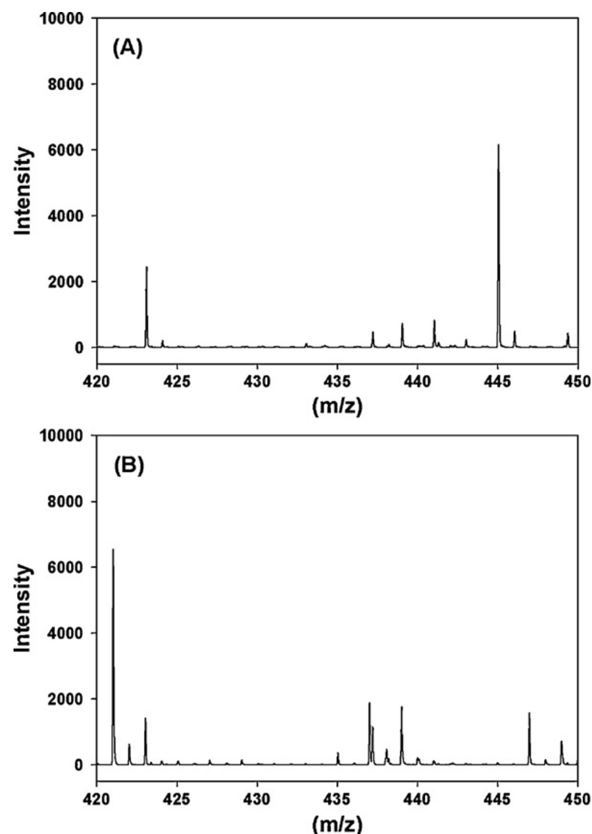

Fig. 2. The MALDI-TOF mass spectra of background produced by plasma constituents at *m*/*z* 439 before adding quinolones by using (A) CHCA and (B) 7-MMC as the matrix. The background at *m*/*z* 439 with undesired signals.

had an ion suppression effect on the background when CHCA or 7-MMC was used as the matrix. At quinolone concentrations exceeding 1000 μg/mL, no signal (up to a maximum of 5 μg/mL quinapril) was detectable, and at concentrations below 100 μg/mL, no ion suppression effect was observed. Finally, when 200 μg/mL of quinolones were used as the matrix additive for quinapril analysis, no signal was detected in the *m*/*z* 439 when CHCA or 7-MMC was used as the matrix. Figs. 2 and 3 show the background resulting from plasma constituents at *m*/*z* 439 before and after adding quinolones, respectively. To select the best matrix additive, the plasma was spiked with 5 μg/mL quinapril to detect a suitable signal. The results in Fig. 4 show that a difloxacin matrix additive combined with a 7-MMC matrix could obtain the best signal for trace analysis of quinapril in human plasma.

3.2. Quinapril extraction by ion pair interaction

Quinapril has amphoteric properties and contains two pK_a values [27]. Quinapril becomes a water-soluble substance in the physiological pH conditions (pH about 7.4) after this drug is orally administered. Quinapril is also soluble in acidic conditions due to protonation of the amino group. To improve extraction of quinapril from plasma to organic layer, organic acid was added to increase the hydrophobicity of quinapril by forming an ion pair. The sample was prepared by adding basic solution (10 μL) to alkalize the plasma and maintain the carboxylic acid of quinapril in the salt form. The plasma was then acidified by adding organic acid (10 μL) to keep the carboxylic acid of quinapril in the free form and to provide the counter ion for the amine groups of quinapril. Finally, organic solvent (20 μL) was added to extract quinapril from plasma by ion pair

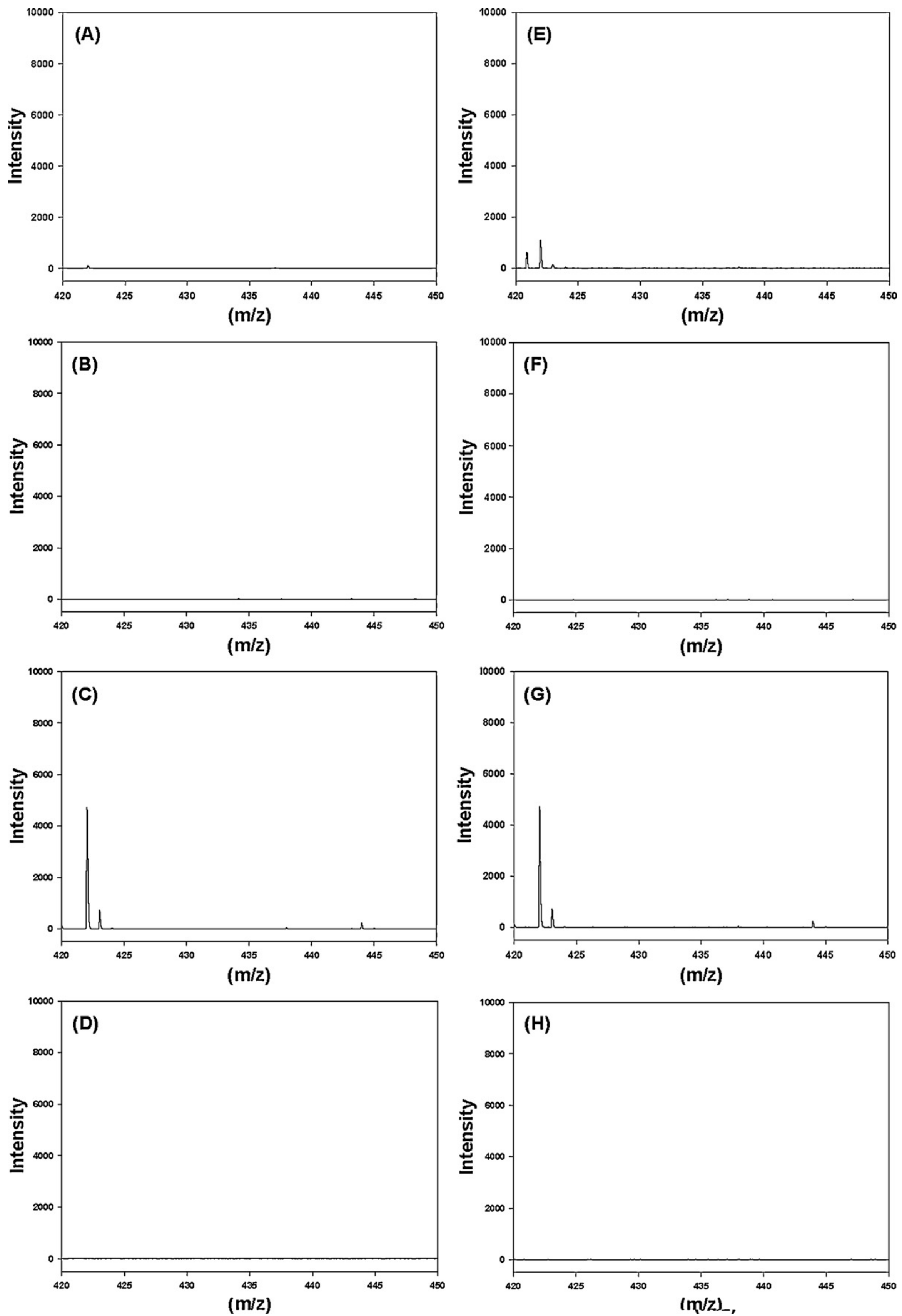

Fig. 3. The MALDI-TOF mass spectra of background produced by plasma constituents at *m*/*z* 439 after adding quinolones by using (A) to (D) CHCA and (E) to (H) 7-MMC as the matrix. The four quinolones (200 μg/mL) Lev (A) and (E); Dan (B) and (F); Dif (C) and (G) and Mar(D) and (H) were used as the matrix additives to suppress the undesired signal at *m*/*z* 439.

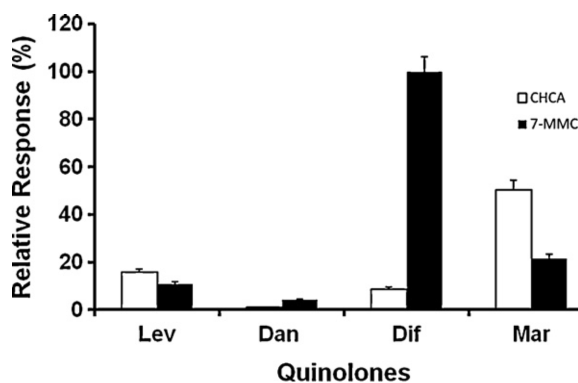

Fig. 4. Comparison of relative response obtained for quinapril versus matrix and matrix additive.

Table 1
Precision and accuracy for the determination of quinapril in spiked human plasma by MALDI-TOF MS.

Quinapril nominal concentration (μg/mL)	Quinapril found concentration (μg/mL)	R.S.D. (%)	R.E.[c] (%)
Within-day[a] ($n=5$)			
0.400	0.405 ± 0.051	12.59	+1.25
0.800	0.805 ± 0.015	1.86	+0.63
1.500	1.471 ± 0.140	9.52	−1.93
Between-day[b] ($n=5$)			
0.400	0.399 ± 0.056	14.04	−0.25
0.800	0.804 ± 0.067	8.33	+0.50
1.500	1.463 ± 0.063	4.31	−2.47

[a] Within-day assay variance from analysis of quinapril at five intervals on a single day.
[b] Between-day assay variance from analysis of quinapril on five consecutive days.
[c] R.E. calculated from (concentration found-nominal concentration)/nominal concentration.

interaction. Fig. 5 shows the sample preparation procedure and ion pair interaction.

3.3. Optimization of the sample preparation procedure

Tests of extraction efficiency using several water-immiscible solvents, including 20 μL of CH_2Cl_2, hexane, ethyl acetate and toluene, revealed quinapril extraction efficiencies (%) of 100, 15, 12 and 7%, respectively. These results indicate that this compound is easily transferred from plasma to the CH_2Cl_2 layer. Fig. 6(A) shows that CH_2Cl_2 is the most effective acceptor solvent.

After experimentally alkalizing the sample solution with 10 μL of three different bases (20 mM) KOH (pH = 13.0), NaOH (pH = 10.2) and NH_4HCO_3 (pH = 10.7), the respective quinapril extraction efficiencies (%) were 100, 72 and 67%, respectively. Fig. 6(B) shows that KOH was the best base for sample preparation. Further tests of KOH (0–80 mM; pH = 6.3–13.7) indicate that KOH 20 mM (10 μL) was optimal for quinapril extraction (Fig. 6(C)).

An ion pair interacting with quinapril was then tested with 10 μL of three organic acids at 230 mM (HFBA pH = 0.7, TFA pH = 0.8 and FA pH = 2.1), these acids could be served as the counter ion for quinapril. The results in Fig. 6(D) indicate that HFBA, the most hydrophobic acid, allowed to obtain the largest increase in quinapril extraction efficiency. The effect of HFBA concentration (50–1000 mM; pH = 1.3–0.5) on ion pair interaction was also evaluated. The results in Fig. 7 show that 10 μL HFBA at concentration of 230 mM can simultaneously acidify the analyte solution and increase the hydrophobicity of quinapril. MALDI-TOF mass spectrum of quinapril, extracted from spiked plasma sample (5 μg/mL) prepared according to the optimal sample preparation procedure, is shown in Fig. 8.

3.4. Analytical calibration curve, precision and accuracy

For quinapril quantitation, the integration of the peak area of quinapril (m/z 439) and IS (m/z 429) for a series of concentrations was carried out. The linear range of calibration for quinapril extended from 0.05 to 2.00 μg/mL. The calibration curve was computed by the peak area ratio (peak area quotient of quinapril divide by IS) as the y coordinate and quinapril concentration (μg/mL) as the x coordinate. The equation for peak area ratio versus concentration plot was $y = (0.0710 \pm 0.0121)\,x + (0.0162 \pm 0.002)$; $r = 0.995$ ($n = 5$). The limit of detection (LOD) for quinapril was 25 ng/mL. The relative standard deviation (R.S.D.) and relative error (R.E.) observed during quinapril analyses were calculated for three concentrations (0.4, 0.8 and 1.5 μg/mL). The results of precision and accuracy expressed as by R.S.D. and R.E. are listed in Table 1. Table 1 shows that the R.S.D. and R.E. used for the evaluation of within- and between-day assays of plasma spiked with quinapril are all below 14.1%. For low quality control (LCQ) analysis, quinapril at 0.1 μg/mL was tested and the R.S.D. and R.E. are all below 15.7%. The recovery of the method is 97–101%. In summary, the proposed method for trace analysis of quinapril in human plasma is characterized by good linearity, precision and accuracy.

3.5. Stability and application

To evaluate the stability of quinapril, three plasma samples were collected in heparin tubes and stored at −20 °C. After 14 days, no significant change in quinapril (5 μg/mL) signal was detected. Therefore, plasma containing quinapril should be stored at −20 °C to ensure sufficient stability before sample preparation and analysis by this method. Developed micro-scale MALDI-TOF MS method was further used for monitoring quinapril in plasma from a healthy

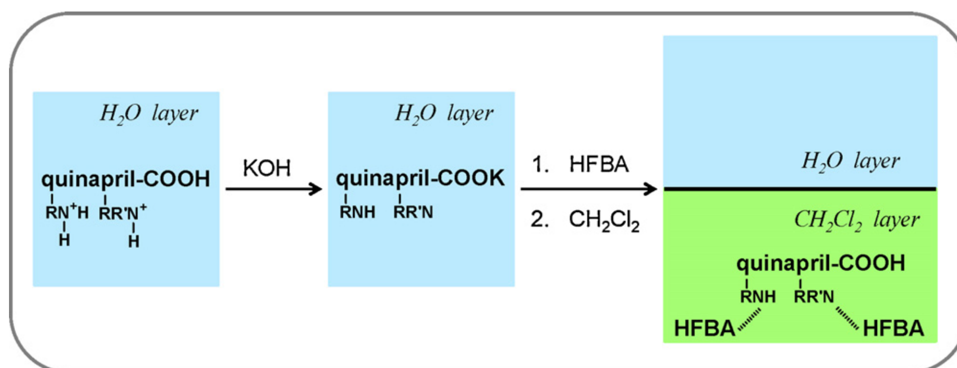

Fig. 5. Flow chart of sample preparation procedure and ion pair formation for quinapril extraction.

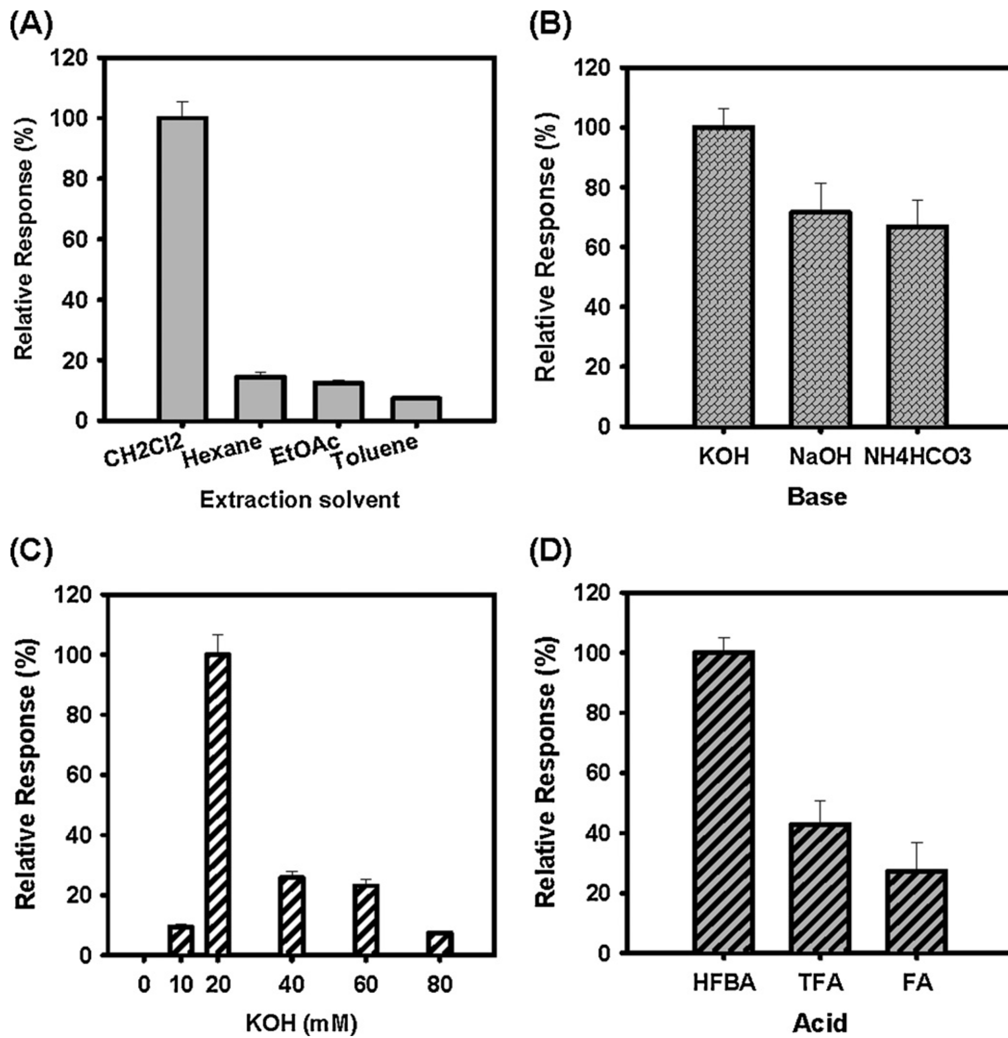

Fig. 6. Effects of different (A) organic solvents; (B) bases; (C) KOH concentrations (0–80 mM) and (D) organic acids on quinapril extraction.

volunteer. Human plasma samples after oral administration of a single dose of 20 mg quinapril (Accupril®) tablet was collected and then extracted (see Section 2.4). Peak plasma concentration (t_{max}) 0.469 ± 0.030 μg/mL (n = 3) was observed 1 h after oral administration of a single 20 mg dose. Fig. 9 shows the MALDI-TOF mass spectra of plasma samples. For clinical purposes, plasma samples collected from patients should be as small as possible to maximize tolerability by patients. The results confirmed that the proposed MALDI-TOF MS method is utilized successfully for trace analysis of quinapril in human plasma samples as small as 10 μL.

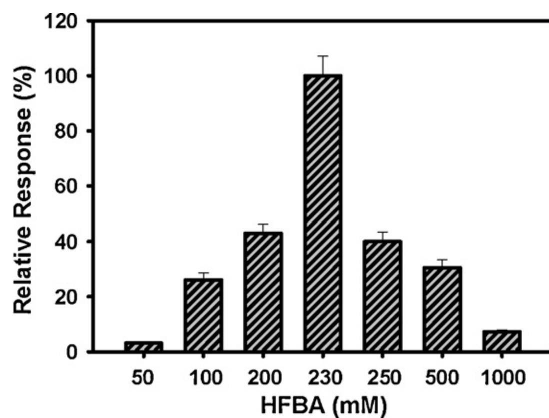

Fig. 7. Effects of different HFBA concentrations (50–1000 mM) on quinapril extraction.

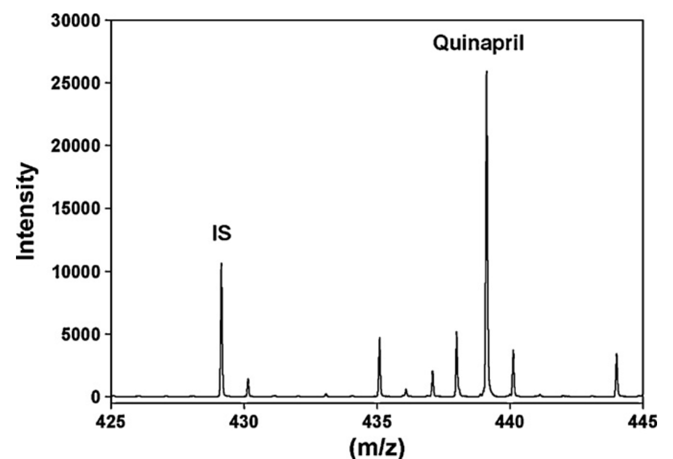

Fig. 8. Optimal MALDI-TOF mass spectrum of plasma sample spiked with 5 μg/mL quinapril and 100 μg/mL IS.

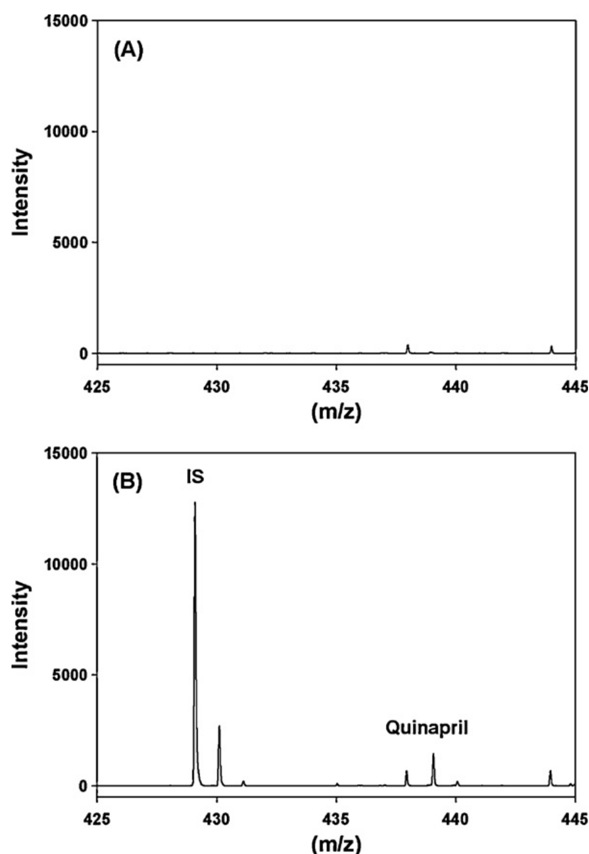

Fig. 9. The MALDI-TOF mass spectra of plasma samples in (A) blank plasma sample before administration of quinapril tablet; (B) plasma sample from a healthy volunteer after receiving 20 mg of quinapril 1 h. The $[M+H]^+$ of IS and quinapril were *m/z* 429 and *m/z* 439, respectively.

4. Conclusion

This study developed a MALDI-TOF method for quinapril detection in human plasma by using quinolones as the matrix additives assisting in ionization process to suppress the undesired substances in plasma produce signals. For quinapril extraction, ion pair formation was utilized by adding HFBA and then CH_2Cl_2 was used as the extraction solvent. This strategy was proved successful in monitoring quinapril in plasma samples as small as 10 μL after administration of a 20 mg pharmaceutical formulation. This micro-scale method is suitable for use in studies involving small experimental animals in which sample volumes are limited.

Acknowledgements

The authors are grateful to the National Science Council (NSC 99-2113-M-037-002 and 99-2113-M-037-004) and the Center of Excellence for Environmental Medicine (Kaohsiung Medical University) for financial support of this work. The Center for Resources, Research and Development (Kaohsiung Medical University) is also acknowledged for instrumental support.

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