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Quantitative determination of mitiglinide in human plasma by ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry

Shuang Cai[a], Taoguang Huo[a], Wanyu Feng[b], Lingyun Chen[a], Feng Qin[a], Famei Li[a,*]

[a] Department of Analytical Chemistry, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China
[b] The first affiliated hospital of China Medical University, 155 Nanjing Street, Shenyang 110001, PR China

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ABSTRACT

A selective, rapid and sensitive ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) method was developed for the quantitative determination of mitiglinide in human plasma. With nateglinide as internal standard, sample pretreatment involved a one-step extraction with diethyl ether of 0.2 mL plasma. The separation was performed on an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm) with the mobile phase consisting of methanol and 10 mmol/L ammonium acetate (65:35, v/v) at a flow rate of 0.25 mL/min. The detection was carried out by means of electrospray ionization mass spectrometry in positive ion mode with multiple reaction monitoring (MRM). Linear calibration curves were obtained in the concentration range of 1.080–5400 ng/mL, with a lower limit of quantification of 1.080 ng/mL. The intra- and inter-day precision (RSD) values were below 15% and accuracy (RE) was from −3.5% to 7.3% at all QC levels. The method was fully validated and successfully applied to a clinical pharmacokinetic study of mitiglinide in 10 healthy volunteers following oral administration.

1. Introduction

Mitiglinide (MGN), (−)-2(*S*)-benzyl-4-(*cis*-perhydroisoindol-2-yl) butyric acid, is a new insulinotropic agent of the glinide class with rapid onset [1]. It is thought to stimulate insulin secretion by closing the ATP-sensitive K^+ (K_{ATP}) channels in pancreatic beta cells. Its early insulin release and short duration of action could be effective in improving postprandial hyperglycemia [2]. Mitiglinide tablet is commercially available in Japan. Recently it is being developed as a new formulation in China; thus, a pharmacokinetic study of mitiglinide in Chinese people is of great importance. Therefore, the development of a sensitive and specific method to determine mitiglinide in human plasma is necessary and valuable.

Several analytical methods for mitiglinide in biological samples have been reported previously [3,4]. But there is not any report on the quantitative determination of mitiglinide in human plasma by UPLC/MS/MS method.

The ultra-performance liquid chromatography (UPLC) system enabled better chromatographic peak resolution and increased speed and sensitivity. When coupled to mass spectrometer the signal to noise ratio improved while the matrix effect significantly reduced. Therefore, UPLC/MS/MS possesses advantages of high sensitivity and high sample throughput over conventional LC/MS system and is rapidly applied to the analysis of drugs in biological samples [5–7]. In this paper, a UPLC/MS/MS method was developed for the quantitative determination of mitiglinide in human plasma and the validated method was successfully applied to the pharmacokinetic study of mitiglinide in 10 healthy volunteers after oral administration.

2. Experimental

2.1. Chemicals and reagents

Mitiglinide calcium hydrate (99.3% of purity) was kindly provided by Dandong Tongyuan Medicine Co. Ltd. (Dandong, China). Nateglinide (internal standard, 99.2% of purity) was purchased from Sigma Corporation (St. Louis, MO, USA). The structures of mitiglinide and I.S. are given in Fig. 1. Methanol and ammonium acetate (HPLC grade) were obtained from Dikma (Richmond Hill, NY, USA). All other chemicals were of analytical grade. Hydrochloric acid and diethyl ether were purchased from Yuwang (Chemical Reagent Plant, Shandong, China). Water was purified by re-distillation and filtered through 0.22 μm membrane filter before use.

* Corresponding author. Tel.: +86 24 2398 6289; fax: +86 24 2398 6289.
E-mail address: lifamei@syphu.edu.cn (F. Li).

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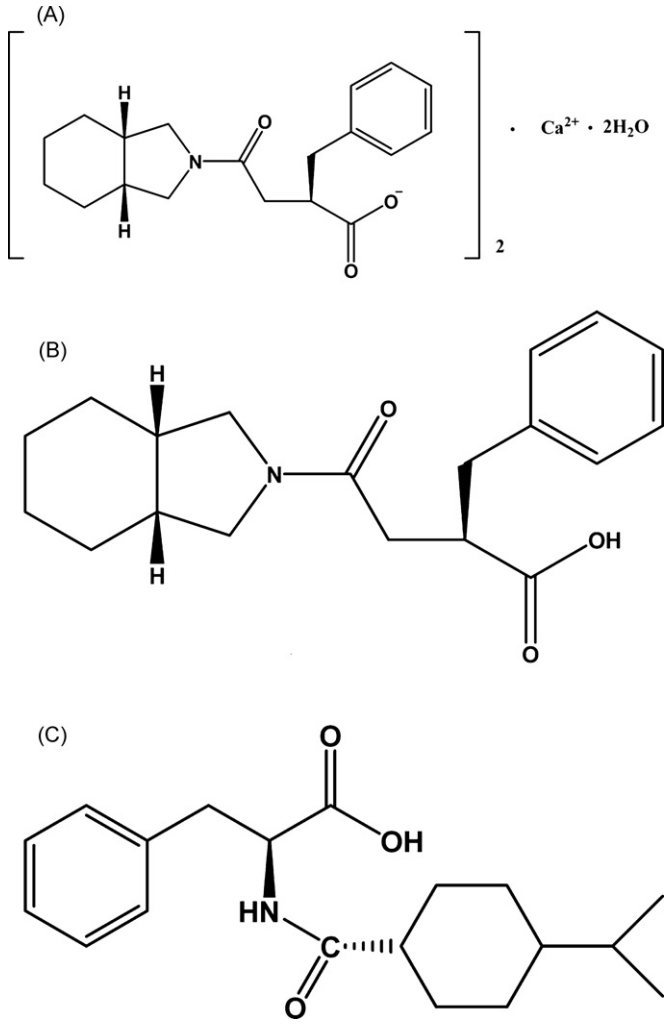

Fig. 1. The structures of (A) mitiglinide (the state of administration), (B) mitiglinide (the state of detection) and (C) nateglinide (I.S.).

2.2. Liquid chromatography and mass spectrometry

Liquid chromatography was performed on ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA). Separation was achieved on an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm) maintained at 40 °C. The mobile phase was composed of 65% methanol and 35% 10 mmol/L ammonium acetate (v/v) with a flow rate of 0.25 mL/min. The autosampler was conditioned at 4 °C and the sample volume injected was 10 μL.

Mass spectrometric detection was carried out on a Micromass Quattro micro™ API mass spectrometer (Waters Corp., Milford, MA, USA) with ESI source set in positive mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 316.2 → 298.2 for mitiglinide, m/z 318.2 → 166.0 for nateglinide (I.S.), respectively, with a scan time of 0.10 s per transition. The following parameters were employed: capillary voltage of 3.0 kV, cone voltage of 30 kV, source temperature of 100 °C and desolvation temperature of 350 °C. Nitrogen was used as the desolvation and cone gas with the flow rate of 400 and 30 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 2.7×10^{-3} mbar. The optimized collision energy for mitiglinide and nateglinide were 15 and 13 eV, respectively.

2.3. Calibration standards and quality control samples

Stock solutions of mitiglinide and nateglinide were prepared in methanol at concentrations of 108.0 μg/mL and 54.00 μg/mL, respectively, and stored at −20 °C. The internal standard solution was further diluted with water to prepare the working solution containing 1080 ng/mL of nateglinide. The mitiglinide stock solution was then serially diluted with methanol daily to provide working standard solutions of desired concentrations.

Calibration standards were prepared by spiking 0.2 mL of blank human plasma with 50 μL I.S. solution (1080 ng/mL) and 50 μL of the mitiglinide working solutions to yield final concentrations of 1.080, 5.400, 10.80, 54.00, 270.0, 1080, 3240, 5400 ng/mL, respectively. One calibration curve was constructed on each analysis day using freshly prepared calibration standards. Quality control (QC) samples were prepared in blank plasma at low, mid and high concentrations of 2.160, 1080 and 4320 ng/mL, respectively, and stored at −20 °C after preparation. The standards and quality controls were extracted on each analysis day with the same procedure for plasma samples. In each run, a blank plasma sample was also analyzed.

2.4. Plasma sample preparation

To a 0.2 mL aliquot of plasma sample, 50 μL of methanol, 50 μL of internal standard (1080 ng/mL) and 200 μL of 1 mol/L HCl were added. The samples were vortexed for 30 s and extracted with 3 mL of diethyl ether by shaking for 10 min. After centrifugation at 3500 × g for 10 min, the upper organic layer was transferred and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The dried residue was then reconstituted in 100 μL of methanol–water (65:35, v/v). An aliquot of 10 μL was injected into the UPLC/MS/MS system for analysis.

2.5. Method validation

Selectivity was studied by comparing chromatograms of six individual human blank plasma samples with corresponding plasma samples spiked with mitiglinide and nateglinide (1080 ng/mL) and plasma samples after oral administration of mitiglinide tablets.

Validation runs were conducted on three consecutive days. Each validation run consisted of a minimum of one set of calibration standards and six sets of QC plasma samples at three concentrations.

Calibration curves were determined by assaying standard plasma samples at eight concentrations of mitiglinide ranged 1.080–5400 ng/mL. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of mitiglinide/I.S. versus the nominal concentration (x) of mitiglinide. The calibration curves were constructed by using weighted ($1/x^2$) least square linear regression.

The method precision and accuracy were evaluated by using replicate analysis of quality control samples at three concentrations. The intra-day precision and accuracy were determined by analysis of six QC samples on the same day. The inter-day precision and accuracy were assayed by analyzing three batches of QC samples on three consecutive days. The precision was expressed as the relative standard deviation (RSD%) and the accuracy as the relative error (RE%).

Extraction recovery of mitiglinide was determined by dividing the mean response obtained from blank plasma samples spiked with analyte before extraction with those from blank plasma samples to which analyte was added after extraction. This procedure was repeated in five replicates for the three QC concentrations.

To evaluate the matrix effect, three concentration levels of mitiglinide (2.160, 1080, 4320 ng/mL) were added to the dried extracts

of 0.2 mL of blank plasma sample from five different lots, then dried and reconstituted with 100 μL mobile phase. The corresponding peak areas (A) were compared with those of the mitiglinide standard solutions dried directly and reconstituted with the same volume of mobile phase (B). The ratio ($A/B \times 100\%$) was used to evaluate the absolute matrix effect. The assessment of relative matrix effect [8] was made by a direct comparison of the analyte peak area values between different lots of plasma. The variability was expressed as RSD (%). The same procedure was performed for I.S.

Freeze and thaw stability was performed by subjecting five aliquots each of low-, mid- and high-concentration unextracted QC samples to three freeze (−20 °C)-thaw (room temperature) cycles. For long-term stability, five aliquots of each low-, mid- and high-concentration QC samples were stored at −20 °C for 21 days. Another five aliquots of QC samples at three concentrations were kept at ambient temperature (25 °C) for 4 h to evaluate the short-term stability of mitiglinide in human plasma. Then the samples were processed and analyzed. To estimate the stability of mitiglinide in the prepared sample, aliquots of prepared QC samples were kept in autosampler maintained at 4 °C for 8 h and then analyzed. The concentrations obtained were compared with the nominal values.

2.6. Application to pharmacokinetic study

The validated method was applied to determine the plasma concentration of mitiglinide from a clinical trial in which 10 healthy volunteers (5 male and 5 female) were selected. Each subject was administrated 5, 10, 20 mg mitiglinide tablets (a formulation made in China) in the first, second and third period, respectively. The pharmacokinetic study was approved by a local Ethics Committee and all the volunteers gave their signed informed consent to participate in the study according to the principles of the Declaration of Helsinki. Blood samples were collected before and 5, 10, 15, 20, 25, 30, 35, 45 min and 1.5, 2, 3, 4, 6 h after administration. The plasma was separated immediately and stored at −20 °C until analyzed.

3. Results and discussion

3.1. Method development

It is necessary to use an I.S. to get high accuracy for the development of LC/MS/MS method. An ideal I.S. in LC/MS analysis is a deuterated form of the analyte. In our laboratory no deuterated mitiglinide was available; thus, a compound that had structure, chromatographic and mass spectrometric behavior similar to the analyte was considered. Nateglinide, a glucose regulator of glinide class, was selected for its similarity to mitiglinide in retention, ionization and extraction efficiency.

Liquid–liquid extraction was chosen as the sample preparation method for it could produce purified as well as concentrated samples. Several extraction solvents such as chloroform, diethyl ether, n-hexane and the mixed solvent of n-hexane-isopropanol (80:20, v/v) were investigated. Diethyl ether was finally adopted for its virtue of easy evaporation and high efficiency with a mean recovery of about 81%. The extraction procedure without and with adding 1 mol/L HCl (200 μL) was also compared, and the latter gave a higher recovery.

The separation and ionization of mitiglinide and nateglinide were affected by the composition of mobile phase. Formic acid and ammonium acetate were added into the mobile phase to improve the response. The response of mitiglinide distinctly increased by adding ammonium acetate. The effect of ammonium acetate with concentrations of 5, 10, 20, 30, 50 mmol/L in aqueous phase on the response of mitiglinide were investigated (Fig. 2), and 10 mmol/L was found to be the best. The reason may be that at lower concentration of CH_3COONH_4, excess charge ions extend into the diffuse layer toward the interior of the droplet leaving fewer ions in the compact layer. A significant fraction of the excess charge ions will be solvated, thus reducing the response by reducing the fraction of the ions that are readily freed into the gas phase and detected by the mass spectrometer. When the concentration of CH_3COONH_4 increased, the fraction of the excess charge ions that were in the compact layer was increased. A smaller fraction of excess charge ions were solvated, thus the response for those ions with higher solvation energy were increased [9]. When the concentration of CH_3COONH_4 was higher than 10 mmol/L, the surface of the ESI droplets became saturated and the electrolytic ions competed with the analyte to reside at the surface of the droplets; therefore, fewer analyte ions were solvated and the response of the analyte was decreased. Furthermore, excess electrolytic ions would also compete with the analyte to get charge and reduce the response of the analyte as well. Finally, methanol-10 mmol/L ammonium acetate (65:35, v/v) was adopted as the mobile phase.

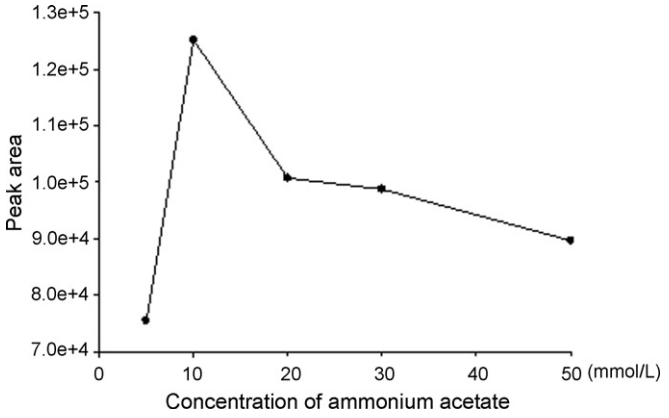

Fig. 2. Effect of the concentrations of ammonium acetate on the response of mitiglinide.

Two channels were used for recording the response, channel 1 for mitiglinide with a retention time of 1.0 min, and channel 2 for the I.S. with a retention time of 1.6 min. Both mitiglinide and I.S. were well separated (Fig. 3) with excellent peak shapes, and no interfering peaks were observed.

3.2. Method validation

Selectivity was assessed by comparing the chromatograms of six individual human plasma with the corresponding spiked plasma. As shown in Fig. 3A–D, there was no interference from endogenous substances at the retention time of mitiglinide and I.S.

The standard calibration curves for mitiglinide were linear in the concentration range of 1.080–5400 ng/mL ($r^2 > 0.99$). Representative regression equation for the calibration curve was $y = 4.898 \times 10^{-3}x + 5.389 \times 10^{-5}$ ($r = 0.9973$). The lower limit of quantification (LLOQ) for mitiglinide was 1.080 ng/mL with precision below 20% and accuracy within ± 20% (Table 1), and a corresponding chromatogram is given in Fig. 3B.

Data for intra- and inter-day precision and accuracy of the method for mitiglinide are given in Table 1. The intra- and inter-day RSDs were less than 8.7% and 14%, and RE was from −3.5% to 7.3% at all QC levels, indicating acceptable precision and accuracy of the present method.

The extraction recoveries of mitiglinide from human plasma were 75.8 ± 3.5%, 83.7 ± 0.7% and 83.2 ± 0.6% at concentrations of

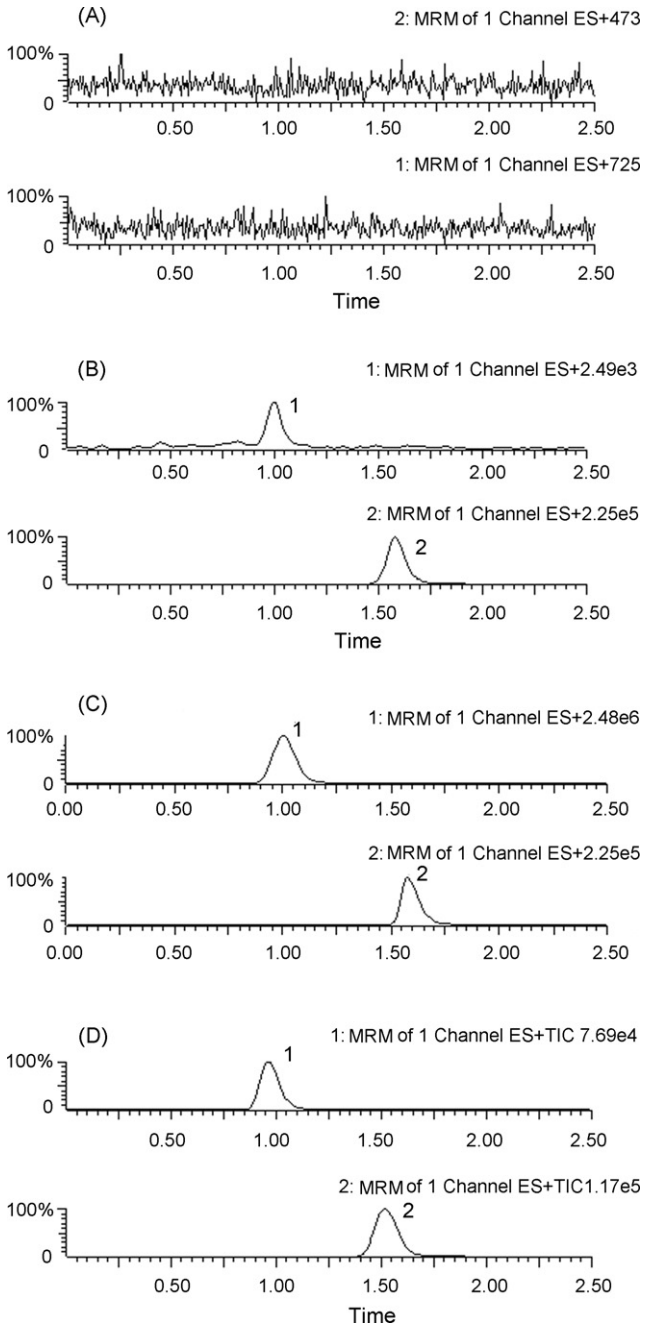

Fig. 3. Representative MRM chromatograms of mitiglinide (peak 1, channel 1) and nateglinide (peak 2, channel 2) in human plasma samples. (A) A blank plasma sample; (B) a blank plasma sample spiked with mitiglinide at the LLOQ of 1.080 ng/mL and nateglinide (I.S.) (1080 ng/mL); (C) a blank plasma sample spiked with mitiglinide at 1080 ng/mL and nateglinide (I.S.) (1080 ng/mL); (D) a plasma sample from a volunteer 10 min after oral administration of mitiglinide. The retention time of mitiglinide and nateglinide were 1.0 and 1.6 min, respectively.

Table 1
Precision and accuracy for the determination of mitiglinide in human plasma (intra-day: $n = 6$; inter-day: $n = 6$ series per day, 3 days)

Added C (ng/mL)	Found C (ng/mL)	Intra-run RSD (%)	Inter-run RSD (%)	Accuracy RE (%)
1.080 (LLOQ)	1.160 ± 0.100	8.7	8.6	7.3
2.160 (Low)	2.140 ± 0.150	5.2	14	−1.1
1080 (Middle)	1087 ± 59.0	4.0	12	0.70
4320 (High)	4171 ± 254	5.0	11	−3.5

Table 2
Stability of mitiglinide in human plasma at three QC levels ($n = 5$)

Stability	Accuracy (mean ± SD) (%)		
	2.160 (ng/mL)	1080 (ng/mL)	4320 (ng/mL)
Short-term stability	98.8 ± 6.67	105 ± 3.07	102 ± 4.53
Freeze-thaw stability	102 ± 8.95	98.7 ± 3.62	93.8 ± 4.79
Long-term stability	94.7 ± 7.64	108 ± 3.08	105 ± 5.90
Postpreparative stability	96.8 ± 10.6	105 ± 2.52	101 ± 4.60

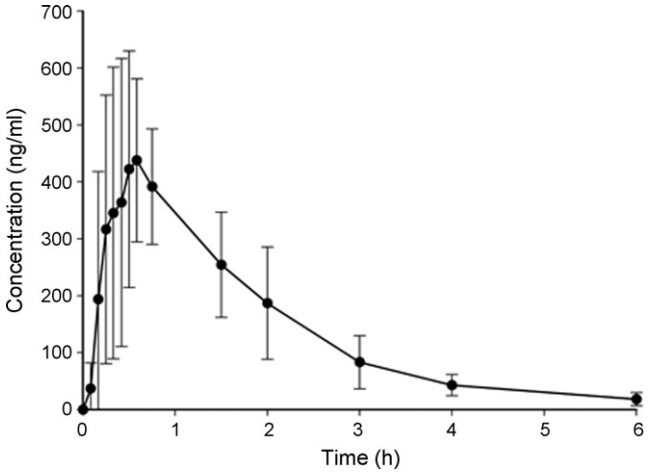

Fig. 4. Mean plasma concentration–time curve of mitiglinide in 10 volunteers after a single dose of mitiglinide of 5 mg.

2.160, 1080, 4320 ng/mL, respectively. The mean extraction recovery of I.S. was 73.2 ± 0.8%.

The absolute matrix effects for mitiglinide at three concentration levels were between 85% and 115%, for I.S. it was from 93.4% to 106.6%. The relative matrix effect for mitiglinide and I.S. were from 5.2% to 8.4% and 2.1 to 4.3%, respectively. The variability was slightly higher than that of standards dried directly and reconstituted in mobile phase (4.3% to 7.1% and 1.3% to 2.5% for analyte and I.S., respectively). Thus, ion suppression or enhancement from the plasma matrix was negligible in this method.

The stock solutions of mitiglinide and I.S. were stable at −20 °C for 21 days and both working solutions were stable at 4 °C for at least 4 h. Table 2 summarizes the results of all stability studies which all well met the criterion for stability measurements.

3.3. Application to a pharmacokinetic study

The present method was successfully applied to the pharmacokinetic study of mitiglinide in 10 healthy volunteers following oral administration of 5, 10 and 20 mg mitiglinide. The profile of the mean plasma concentration of mitiglinide versus time in 10 volunteers after a single dose of 5 mg is shown in Fig. 4, which demonstrated the applicability of the developed method with high sensitivity to pharmacokinetic study with low dosage.

4. Conclusion

A sensitive, selective and rapid UPLC/MS/MS method for the determination of mitiglinide is described for the first time. Compared with the published HPLC/MS method, the present method is featured by higher selectivity and sensitivity with an LLOQ of 1.080 ng/mL due to the increasing column efficiency in UPLC and the high specificity of multiple reaction monitoring. The fast analysis speed with a total analysis time less than 2.0 min (1.0 min for

mitiglinide and 1.6 min for I.S.) enabled high sample throughput to be achieved (5.6 min for mitiglinide in HPLC/MS method). A simpler extraction solvent (diethyl ether) was employed with the extraction efficiency similar to the published HPLC/MS method. The established method was successfully applied to the pharmacokinetic study of mitiglinide in 10 healthy volunteers after oral administration.

References

[1] L.A. Sorbera, P.A. Leeson, R.M. Castańer, J. Castańer, Drugs Future 25 (2000) 1034.
[2] H. Ohnota, T. Kitamura, M. Kinukawa, S. Hamano, N. Shibata, H. Miyata, A. Ujiie, Jpn. J. Pharmacol. 71 (1996) 315.
[3] L. Yu, S. Zeng, J. Chromatogr. B 834 (2006) 204.
[4] J. Liang, Y. Tian, Z. Zhang, S. Feng, Y. Zhao, G. Mao, J. Mass Spectrom. 42 (2007) 171.
[5] X. Sun, X. Li, S. Cai, F. Qin, X. Lu, F. Li, J. Chromatogr. B 846 (2007) 323.
[6] Y. Ma, F. Qin, X. Sun, X. Lu, F. Li, J. Pharm. Biomed. Anal. 43 (2007) 1540.
[7] L. Chen, F. Qin, Y. Ma, F. Li, J. Chromatogr. B 855 (2007) 255.
[8] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[9] T.L. Constantopoulos, G.S. Jackson, C.G. Enke, J. Am. Soc. Mass Spectrom. 10 (1999) 625.