Tropical Journal of Pharmaceutical Research May 2015; 14(5): 845-851
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i5.15

Original Research Article

Quantitation of Solifenacin in Human Plasma using a Specific and Sensitive Liquid Chromatography-Tandem Mass Spectrometry Technique

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Received: 6 January 2015 *Revised accepted: 22 April 2015*

Abstract

Purpose: *The current work validated a high performance liquid chromatography-tandem mass spectrometric (HPLC-MS/MS) bioassay method developed in-house for the quantitation of solifenacin in human plasma.*
Methods: *Solifenacin was extracted from plasma by a liquid-liquid extraction (LLE) technique using tert-butyl methyl ether. The dry extract was then reconstituted with 200 µL of the mobile phase (acetonitrile-water (80:20, v/v)). Solifenacin-d5 was the internal standard (IS). Elution was carried out on a C18 column at a flow rate of 1 mL/min. The MS/MS employed turbo-ion spray ionization in the positive ion mode. Solifenacin and IS were monitored at a mass to charge ratio (m/z) of 363.4 and 368.4, respectively. Bioassay validation followed International Bioanalytical Method Validation Guidelines.*
Results: *The validated calibration curves were linear over a range of 0.5 – 60.0 ng/mL (regression factors ≥ 0.9994). Method specificity was established in 6 different human plasma batches. Intra- and inter-day precision and accuracy were within ± 20 % (for lower limit of quantitation (LLOQ)) and ± 15 % (for low, mid and high quality control (QC) levels). Short- and long-term stability was within accepted range.*
Conclusion: *A specific, accurate and precise HPLC-MS/MS method has been validated for the determination of solifenacin in human plasma.*

Keywords: *Liquid extraction, Mass spectrometry, Solifenacin, Validation*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Overactive bladder (OAB) syndrome affects around 16 % of adult subjects with a prevalence that increases with age [1]. The sudden involuntary contraction of the urinary bladder, caused by the pathophysiological mechanisms of the OAB syndrome, results in the clinical presentation of urgency, frequency and nocturia that may or may not be accompanied with incontinence [2]. The activation of the parasympathetic nervous system, and thus the muscarinic receptors on the smooth muscles of the bladder, mediates these annoying symptoms of the OAB syndrome [3]. Antimuscarinic agents are, therefore, considered the keystone in therapeutically managing this condition [3,4]. Solifenacin succinate is a competitive, specific muscarinic receptor antagonist that has been approved as 5 or 10 mg once daily tablets for the treatment of OAB syndrome [5].

Specific and valid bioanalytical methods of medicines in biological matrices are importantly

needed to conduct various pharmacokinetic, bioequivalence as well as other clinical research studies. Therefore, the objective of the current work was to develop and validate a LLE technique and a high performance liquid chromatography-tandem mass spectrometric (HPLC-MS/MS) analytical method for the determination of solifenacin in human plasma.

EXPERIMENTAL

Standards and chemical reagents

Solifenacin (1-azabicyclo [2.2.2] oct-8-yl (1S)-1-phenyl-3,4-dihydro-1H-isoquinoline-2-carboxylate), with the molecular formula; $C_{23}H_{26}N_2O_2$ and a molecular weight 362.465, was the reference standard (analyte) of the study. It was supplied as solifenacin succinate by Tabuk Pharmaceutical Manufacturer, Jordan, Batch No. RD-WS-224. The internal standard (IS) was an isotopically labelled solifenacin; solifenacin-d5 ($C_{23}H_{22}D_5ClN_2O_2$, molecular weight 403.96), which was supplied by Toronto Research Chemicals Inc. (TRC), Canada, Batch No. 5676702. All used chemicals and reagents were of HPLC analytical grade. These were acetonitrile (Merck, Germany), tert-butyl methyl ether (Pharmaco, USA) and formic acid, 98 - 100 % (Supplico Chemicals, Italy, supplied by S and C Chemicals, Amman, Jordan). Water was deionised and purified using the Milli-Q gradient system (Millipore, MA, USA). The blank human plasma batches were obtained free-of-charge from the Jordan National Blood Bank, Amman, Jordan. These were donated by anonymous, healthy adult subjects. Institutional approval was given for the samples to be used only for drug spiking of anonymous biological matrices without direct enrolment of human subjects.

Instrumentation and chromatographic conditions

The HPLC system used consisted of Waters® 515 HPLC pump, Waters® 717 plus autosampler, Waters® in-line degasser AF (USA). The HPLC analytical column was an Agilent® Zorbax XDB C18 (100 x 4.6 mm ID, 5 µm) (USA). The mass spectrometric detection was performed using Applied BioSystems API 3000 mass spectrometer detector (MDS Sciex, Canada) which was equipped with a turbo V ion spray source in a positive mode. Nitrogen gas (99.99 %) was used (Peak nitrogen generator, Scotland). The collision gas and curtain gas were set at 7 and 10 psi, respectively. The ion spray voltage was set at 4500 V. The entrance and declustering potentials were 10 V and 45 V, respectively. The interface heater was set at 500 ^oC. The collision energy for solifenacin was 41 V and the collision cell exit potential was 11 V. Multiple reactions monitoring (MRM) was the scanning detection mode. The acquisition of data was performed with a dwell time of 200 ms for each transition. The monitored transitions used for solifenacin (m/z) were Q1: 363.4 → Q3: 193.3; and for the IS (m/z) were Q1: 368.4 → Q3: 198.0.

Data processing and statistical analysis

Processing of the chromatograms was performed by the Analyst® 1.4.1 software (MDS Sciex, Canada). The statistical acceptance criteria followed the International Guidelines [6,7]. The required statistical analysis was done using MS Excel 2010.

Preparation of solifenacin and IS stock and working solutions

A diluting solvent was prepared by mixing acetonitrile and deionized water (70:30, v/v). An amount of solifenacin succinate standard powder equivalent to 5.0 mg of solifenacin was used to prepare a stock solution in 5 mL diluting solvent with a final concentration of 1.0 mg/mL. Then, a volume of 0.5 mL of solifenacin stock solution was diluted up to 25 mL with the diluent to prepare a working solution with a final concentration of 20.0 µg/mL. Similarly, the IS stock and working solutions were prepared to give final concentrations of 97.5 and 0.64 µg/mL, respectively.

Preparation of standard calibration curve samples

In labelled volumetric flasks, eight serial dilutions of the solifenacin standard working solution, using the diluting solvent of acetonitrile-deionized water (70:30, v/v), were prepared to give solifenacin concentrations of 10.0, 20.0, 40.0, 100.0, 200.0, 400.0, 800.0 and 1200.0 ng/mL. Then, in eight labelled 5-mLvolumetric flasks a volume of 250 µL of each of these prepared solutions was used to spike 2.5 mL human plasma. The volume of each flask was then made up to 5 mL with plasma to give the following eight calibration curve points with final concentrations equivalent to 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40.0 and 60.0 ng/mL of solifenacin. Additionally, blank and zero samples were prepared to confirm the absence of interferences (i.e. the lower limit of quantitation (LLOQ) response was at least five times the response of the blank) at the retention times of the analyte and the IS. The blank sample was a plasma sample that was prepared and extracted without

being spiked with solifenacin and IS. Whilst, the zero sample was a plasma sample that was spiked with the IS only before being extracted as shown later.

Preparation of quality control (QC) samples

Three serial dilutions of the solifenacin working solution using the diluting solvent were prepared to give solifenacin concentrations of 30.0, 600.0 and 1000.0 ng/mL. Then, in three labelled 5-mL volumetric flasks a volume of 250 µL of each of these solutions was used to spike 2.5 mL human plasma. The volume of each flask was then made up to 5 mL with plasma to give the Low QC, Mid QC and High QC samples with final concentrations equivalent to 1.5, 30.0 and 50.0 ng/mL of solifenacin, respectively.

QC samples for dilution integrity test

Samples with solifenacin concentrations above the upper limit of quantitation (ULOQ) were diluted properly in order to bring the concentrations down into the calibration curve linear range. A volume of 1200 µL of solifenacin working solution was added to prepare a solution with a final concentration of 2400.0 ng/mL. Five 10-mL test tubes were labelled as DF ULOQ. In each of which, a volume of 500 µL of human plasma was placed and spiked with 25 µL of the prepared standard solution (DF ULOQ). Then, a volume of 1500 µL plasma was added and vortexed well to get a final diluted concentration equivalent to 30.0 ng/mL of solifenacin.

Sample extraction and preparation for analysis

Solifenacin was extracted from plasma samples using a LLE technique. A volume of 500 µL of human plasma was spiked with 25 µL of the IS working solution (0.64 µg/mL) except for the blank sample. A volume of 25 µL of the diluting solvent was added to the blank and the zero samples to compensate for the 25 µL of the analyte solution used for spiking the calibration curve and QC samples. Another volume of 25 µL of the diluent solvent was also added to the blank sample to compensate for the 25 µL of the IS solution used for spiking the calibration curve and QC samples. A volume of 4 mL of tert-butyl methyl ether was added to each sample, vortexed and then centrifuged for 5 min at 4000 rpm and 5 ºC. After freezing, the upper organic layer was transferred to an evaporation test tube and evaporated under a nitrogen gas stream at 40 ºC. Finally, the solifenacin dry extract was reconstituted with 200 µL of the mobile phase and vortexed well for analysis.

HPLC-MS/MS analytical method

A volume of 10 µL of the prepared samples was injected into the HPLC-MS/MS system for analysis using the autosampler kept at 5 ± 3 ºC. The samples were eluted on a reversed stationary phase column using the Agilent Zorbax XDB C18 (100 x 4.6 mm ID, 5µm) kept at 40 ^{o}C. The mobile phase consisted of a mixture of acetonitrile-deionized water (80:20, v/v) which was acidified with 1.0 mL of formic acid per litre of the mixture. An isocratic elution was used at a flow rate of 1 mL/min with a total analytical run-time of 2.5 min.

Method validation

International Bioanalytical Method Validation Guidelines [6,7] were followed.

Calibration curve linearity and LLOQ

The linearity of the solifenacin calibration curve was tested over the concentration range of 0.5 - 60.0 ng/mL. The 0.5 ng/mL concentration was investigated as the LLOQ for solifenacin. Six different calibration curves were prepared, extracted and analysed on six different days to check for the linearity and accuracy. The peak area ratios (peak area of analyte/peak area of IS) on y-axis vs. the calibration curve points concentrations on x-axis were plotted and fitted to the equation; $y = bx + a$, where (a) represents the y-axis intercept and (b) represents the slope. The acceptance criteria included not more than ± 20 % deviation at LLOQ (± 15 % at other points) from the nominal concentrations. At least 75 % of non-zero standards should meet the above criteria including the LLOQ and ULOQ with an overall correlation coefficient (r) ≥ 0.95.

Dilution process integrity

The dilution process' accuracy and precision were evaluated at one concentration (120.0 ng/mL) for solifenacin which was diluted to 30.0 ng/mL. The concentrations were calculated by applying the regression equation of the calibration curve and then multiplied by the dilution factor (DF). The deviation of the mean from the true value served as the measure of accuracy. The mean value should be within ± 15 % of the actual value and the precision around the mean value should not exceed 15 % as the coefficient of variation (CV) [6,7].

Intra- and inter-day accuracy and precision

The intra-batch accuracy and precision were investigated by analysing five replicate samples

at four different solifenacin concentrations (ng/mL); 0.5 (LLOQ), 1.5 (Low QC), 30.0 (Mid QC) and 50.0 (High QC) which were prepared from the same plasma batch and analysed on the same day. The inter-day accuracy and precision were evaluated by analysing five replicate samples at each of the previous concentration levels prepared from 3 different plasma batches and analysed on 3 different days. The concentrations were calculated by applying the regression equation of the calibration curve. The deviation of the mean from the true value was used as a measure of accuracy. The precision was the ratio of standard deviation to mean (CV %). The acceptance criteria were an accuracy within ± 20 % at the LLOQ level (± 15 % at other QC levels) and precision of < 20 % at the LLOQ (< 15 % at other QC levels) [6,7].

Specificity

The specificity of the analytical method was evaluated by screening six different batches of healthy human plasma to ensure that the analytical response of the LLOQ was at least five times the response of the blank samples at the retention times (t_R) of solifenacin and the IS.

Recovery

The absolute recoveries were calculated for solifenacin and the IS by comparing the mean of peak areas (detector response) of the samples that were spiked before extraction with the mean of the peak areas of the samples that were spiked post extraction. The recoveries were measured at the three QC concentration levels (1.5, 30.0 and 50.0 ng/mL). The recovery was accepted if the precision (CV %) at each QC level was < 15 % [6,7].

Stability

Sufficient aliquots of human plasma were spiked with solifenacin to reach the final concentrations of Low QC 1.5 ng/mL and High QC 50.0 ng/mL. The aliquots were prepared so as to suffice the conduction of the stability evaluation mentioned under this section. Five determinations were assigned soon after the aliquots preparation for an initial concentration determination for each aliquot. The following allocation for each of the spiked aliquots was labelled as: 5 determinations for the short-term stability, 5 for the freeze and thaw stability, and 5 for each cycle of the long-term stability. The stability acceptance criteria were an accuracy between 85 – 115 % (± 15 %) and a precision (CV %) < 15 %.

Short-term stability

The short-term stability aliquots were frozen at – 70 ± 15 ^{o}C for about two hours (until completely frozen) and were then thawed completely at room temperature (RT) for around 23 h. After that these samples were prepared, extracted and analysed. The concentrations of solifenacin in these samples were compared to the mean of that calculated initially to give the percentage stability.

Long-term stability

The long-term stability aliquots were frozen at – 70 ± 15 ^{o}C for 33 days before being thawed completely at RT, extracted and then analysed. After the analysis, the concentrations of solifenacin in these samples were compared to the mean of that calculated initially at the first day of long-term stability to give the percentage stability.

Freeze and thaw stability

Freeze and thaw stability was studied through subjecting the samples to three freeze and thaw cycles. Five samples from each of the stored plasma aliquots were thawed completely unassisted at RT and refrozen for 12 – 24 h under the same conditions (- 70 ± 15 °C). This cycle was repeated two more times. The samples were then prepared and analysed. The concentrations of solifenacin in these samples were compared to the mean of that calculated initially to give the percentage stability.

Dry extract stability

The dry extract stability was evaluated at the three QC levels (1.5, 30.0 and 50.0 ng/mL). Five replicate samples (at each level) were prepared, however before the reconstitution step was done the dry extract residues were stored at – 70 ± 15 °C for 24 h. After the analysis, solifenacin concentrations were calculated by applying the regression equation of the calibration curve. The stability acceptance criteria were accuracy between 85 – 115 % (± 15 %) and a precision < 15 % [6,7].

Post-preparative stability

The post-preparative stability was studied by preparing enough volume of spiked samples of solifenacin at the three QC levels (1.5, 30.0 and 50.0 ng/mL). Five replicates of the processed samples were analysed initially. The remaining aforementioned processed samples were kept under the autosampler conditions (5 ± 3 ºC) for

26 h and then analysed. The concentrations of solifenacin in these samples were determined as follows. Firstly, the processed sample integrity (PSI) method where the concentration of solifenacin was determined by applying the regression equation of the re-injected calibration curve and compared to their initial values; presented as the percentage stability. Secondly, the autosampler stability evaluation where the concentration of solifenacin was determined by applying the regression equation of the original calibration curve and compared to their initial values; presented as the percentage stability.

RESULTS

Calibration curve linearity

Table 1 shows the area ratios of the six validated solifenacin calibration curves and their linearity equations (y = bx + a) parameters along with their regression coefficients (r). The calibration curves were linear over the range of 0.5 (LLOQ) – 60.0 ng/mL. The mean accuracy of the validated calibration curves ranged between 96.88 and 105.01 % with a precision range of 1.34 – 3.68 %. The LLOQ was identifiable and reproducible with a mean accuracy of 105.01 % and a precision of 3.68 %.

Dilution process integrity

The dilution of samples having solifenacin concentrations higher than the ULOQ provided a rational approach with an acceptable accuracy and precision for the estimation of the analyte concentrations in these samples. The accuracy and precision were 98.24 % and 4.26 %, respectively.

Intra- and inter-day accuracy and precision

The intra-day accuracy and precision results for the LLOQ, Low, Mid and High QC levels met the acceptance criteria. The accuracy (precision) percentages were 91.52 (8.77), 98.91 (3.74), 103.52 (2.79) and 97.40 (2.25) for these levels, respectively. Table 2 shows the inter-day accuracy and precision results for the three analysed human plasma batches which met the acceptance criteria.

Table 1: Representative area ratio of solifenacin calibration curves and linearity functions

Nominal concentration (ng/mL)	Curve 1	Curve 2	Curve 3	Curve 4	Curve 5	Curve 6
0.5	$3.35x10^{-02}$	$3.37x10^{-02}$	$3.11\ x10^{-02}$	$3.80\ x10^{-02}$	$3.59x10^{-02}$	$3.67x10^{-02}$
1.0	$8.04\ x10^{-02}$	$7.20x10^{-02}$	$5.67x10^{-02}$	$6.39x10^{-02}$	$6.12x10^{-02}$	$6.00x10^{-02}$
2.0	$1.13x10^{-01}$	$1.12x10^{-01}$	$1.04x10^{-01}$	$1.29\ x10^{-01}$	$1.21x10^{-01}$	$1.20x10^{-01}$
5.0	$2.48x10^{-01}$	$2.49x10^{-01}$	$2.57x10^{-01}$	$3.21x10^{-01}$	$2.90x10^{-01}$	$2.92x10^{-01}$
10.0	$5.06x10^{-01}$	$5.01x10^{-01}$	$5.20x10^{-01}$	$6.45x10^{-01}$	$5.84x10^{-01}$	$5.83x10^{-01}$
20.0	1.04	1.06	0.996	1.27	1.16	1.17
40.0	1.95	2.00	1.92	2.58	2.40	2.35
60.0	3.09	3.08	3.10	3.85	3.49	3.53
Parameters for the calibration curve functions; *y* = b*x* + a						
a*	$8.01x10^{-03}$	$7.62x10^{-03}$	$5.81x10^{-03}$	$3.25x10^{-03}$	$4.64x10^{-03}$	$4.62x10^{-03}$
b**	$5.04x10^{-02}$	$5.08x10^{-02}$	$5.01x10^{-02}$	$6.40x10^{-02}$	$5.85x10^{-02}$	$5.85x10^{-02}$
r***	0.9996	0.9997	0.9994	0.9999	0.9998	0.9999

a = Calibration curve y-axis intercept; **b = calibration curve slope; * r = Calibration curve regression factor*

Table 2: The inter-day accuracy and precision results for solifenacin in plasma

Plasma batch (n=3)	Solifenacin concentration in human plasma			
	LLOQ[a] (0.5 ng/mL)	Low QC[b] (1.5 ng/mL)	Mid QC[b] (30.0 ng/mL)	High QC[b] (50.0 ng/mL)
Mean	0.493	1.469	30.813	50.301
SD	0.047	0.039	0.642	1.353
Precision (CV %)	9.49	2.69	2.08	2.69
Accuracy %	98.67	97.95	102.71	100.60

a = lower limit of quantitation; b = quality control level

Specificity

For this analytical method to be specific, there should be no analytical response (peaks) exceeding five times that of the solifenacin's LLOQ concentration level at its t_R in the prepared blank samples. Additionally, the analytical peak in each corresponding blank sample at the t_R of the IS should be < 5 % of the IS response in the spiked samples. The results showed no interfering peaks at the t_R of both solifenacin and the IS in the chromatograms of the six analysed blank plasma batches. The ratios of the peak areas of solifenacin and IS in each LLOQ sample of the six validated plasma batches to their corresponding peak areas (not observed) in each blank sample indicated that the LLOQ response was at least five times that in the blank sample.

Recovery

The mean recovery percentages of solifenacin from plasma at the Low QC, Mid QC and High QC levels were 111.45 %, 72.04 % and 85.29 %, respectively. The mean recovery percentage of the IS was 70.18 %. The corresponding precision percentages (CV %) for solifenacin and the IS spiked samples before- and post-extraction were < 15 %. Thus, the recovery of both solifenacin and the IS from plasma was consistent, precise and reproducible.

Short-term, long-term and freeze-thaw stabilities

Table 3 shows the analytical results of the freshly-prepared (initial) samples along with those of the short-term, long-term and freeze-thaw stability tests of solifenacin in human plasma. The results met the stability acceptance criteria under the studied conditions.

Dry extract stability

The mean accuracy (precision) percentages for the solifenacin dry extract at the Low, Mid and High QC levels were 101.59 (4.35), 100.28 (2.62) and 98.77 (2.32), respectively. These results indicated that the extracted solifenacin dry residue was stable for 24 h at – 70 ± 15 °C before being reconstituted for analysis.

Autosampler stability

Post-preparative stability

The autosampler stability results of the Low, Mid and High QC solifenacin samples showed mean stability (precision) percentages of 98.00 (4.32), 99.84 (2.13) and 101.52 (1.75), respectively. The results confirmed that solifenacin in plasma samples was stable for 26 h in the autosampler (at 5 ± 3 ºC).

Processed sample integrity (PSI)

The PSI mean stability (precision) percentages were 100.49 (4.10), 97.39 (2.12) and 98.93 (1.74) for the Low, Mid and High QC levels, respectively. These results also confirmed that solifenacin in plasma samples was stable for 26 h in the autosampler (at 5 ± 3 ºC).

DISCUSSION

Determination of a therapeutic drug in samples of various body fluids is a cornerstone to conducting other drug-related research studies. Solifenacin succinate is a competitive muscarinic receptor antagonist that blocks specifically M3 receptors on the urinary bladder, and thus is indicated to manage the OAB syndrome in adults [2]. The validation process has shown that our assay was specific, accurate, precise and reproducible. Moreover, the linearity for solifenacin determination in plasma was confirmed over a concentration range between 0.5 (LLOQ) and 60.0 ng/mL.

A number of studies describing human systemic-level and pharmacokinetics of solifenacin had been published [8-13].

Table 3: Initial, short-term, long-term and freeze-thaw stability results for solifenacin in plasma

Analytical run (n=5)	Initial analysis		Short-term 23 hours at RT[b]		Long-term 33 days at -70°C		Freeze-thaw after 3 cycles	
	Low QC[a]	*High QC*[a]	*Low QC*[a]	*High QC*[a]	*Low QC*[a]	*High QC*[a]	*Low QC*[a]	*High QC*[a]
Mean	1.441	51.118	1.458	48.199	1.349	54.277	1.403	46.724
SD	0.016	0.371	0.092	0.689	0.063	1.931	0.038	1.134
Precision (CV %)	1.13	0.73	6.30	1.43	4.69	3.56	2.73	2.43
Stability (%)			101.22	94.29	93.67	106.18	97.38	91.40

a = measured quality control (QC) concentrations in ng/mL solifenacin; b; room temperature

However, no specific and sufficient details related to the IS used, sample extraction and preparation procedures and MS/MS detection set-up were given [9-13] to allow the reproduction of the used solifenacin analytical methods. On the other hand, Mistri *et al* had some limitations in their pre-analytical protein precipitation step needed to extract solifenacin from its biological matrix [10]. This led to problems related to the analytical column blockage, ion source cleaning and matrix interferences. The current work described the details of an in-house developed LLE and HPLC-MS/MS procedures for solifenacin in human plasma, along with their detailed validation outcomes. These procedures, therefore, can support other research work of solifenacin.

CONCLUSION

A sensitive and specific developed HPLC-MS/MS method has been validated for the rapid determination of solifenacin in human plasma. This method is robust and can be used for pharmacokinetic, bioequivalence and clinical studies of solifenacin.

ACKNOWLEDGEMENT

The author is immensely thankful to Dr Mohammad Khalil, Executive Director, and Dr. Rabab Tayyem, Technical Director, at ACDIMA Centre for Bioequivalence and Pharmaceutical Studies (ACDIMA BioCenter), Amman, Jordan, for supporting this study. The collaboration of ACDIMA BioCenter has made this work possible.

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