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Short communication

Determination of solifenacin in human plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A liquid chromatography–electrospray tandem mass spectrometry method was developed and validated to quantitate solifenacin in human plasma. The assay was based on protein precipitation with methanol and liquid chromatography performed on a pentafluorophenylpropylsilica column (50 × 4 mm, 3 μm particles), the mobile phase consisted of methanol – 100 mM ammonium acetate containing 1% of formic acid (90:10, v/v). Quantification was through positive-ion mode and selected reaction monitoring at m/z 363 → 193 and 368 → 198 for solifenacin and the internal standard solifenacin-D_5, respectively. The lower limit of quantitation was 0.47 ng/ml using 0.25 ml of plasma and linearity was demonstrated up to 42 ng/ml. Intra-assay and inter-assay precision expressed by relative standard deviation was less than 11% and inaccuracy did not exceed 11% at all levels. The assay was applied to the analysis of samples from a pharmacokinetic study.

1. Introduction

Solifenacin (Fig. 1) is a competitive muscarinic receptor antagonist with selectivity for the urinary bladder over salivary glands in vitro and in vivo. It is used for the treatment of patients with overactive bladder syndrome [1].

The pharmacokinetic profile of solifenacin is linear over the dose range 5–100 mg. The maximum concentrations of solifenacin are reported to be 10–15 ng/ml after oral administration of 10 mg dose, they are reached in about 4–6 h. The mean oral absolute bioavailability of solifenacin is 88%. Solifenacin is eliminated mainly through hepatic metabolism via cytochrome P450 3A4, the metabolites are unlikely to contribute to clinical solifenacin effects. The drug has a mean terminal elimination half-life of about 50 h [1,2].

A liquid chromatographic method with spectrophotometric detection was published for determination of solifenacin and its major metabolite in rat plasma. The analysis time was long and the limit of quantitation was 2 ng/ml when 1 ml of plasma was used [3]. Only a few published chromatographic methods are available for the determination of solifenacin in human plasma or serum. The liquid chromatographic–tandem mass spectrometric (LC–MS/MS) assays employed liquid–liquid extraction as a preseparation technique and separation of solifenacin from interfering compounds by reversed-phase chromatography [4–6]. Mistri et al. [5] tried protein precipitation, but they were unsuccessful due to problems with matrix effects, column clogging and necessity of frequent cleaning of ion source.

The aim of this study was to simplify sample preparation step using protein precipitation. In order to overcome the drawbacks discussed above, an alternative separation mechanism, mixed-mode ion-exchange/reversed-phase liquid chromatography on a pentafluorophenylpropylsilica column was used, which separated the majority of compounds causing ion suppression. Also a more suitable internal standard, isotopically labelled solifenacin, was used to further improve precision and accuracy of the method. These improvements enabled to develop a rapid, simple and sensitive LC–MS/MS method for determination of solifenacin in human plasma. The method was successfully applied to a pharmacokinetic study.

2. Experimental

2.1. Chemicals

Methanol (HPLC gradient grade) was produced by J.T. Baker (Deventer, Holland). Formic acid (puriss. p.a.) was obtained from Fluka (Buchs, Switzerland). Solifenacin succinate was obtained from Zentiva (Prague, Czech Republic) and the internal standard, solifenacin-D_5 hydrochloride was bought from Toronto Research Chemicals (North York, Ontario, Canada).

2.2. Apparatus and conditions

The LC–MS/MS system consisted of the P4000 pump, AS3000 autosampler, TSQ Quantum Discovery Max triple quadrupole mass

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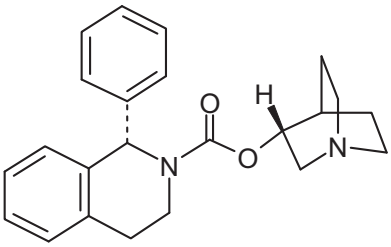

Fig. 1. Chemical structure of solifenacin.

spectrometer with electrospray ion source, data station with Xcalibur software, version 2.0.7 (all from Thermo Fisher Scientific, Waltham, MA, USA). Methanol–water–formic acid (50:50:1, v/v) was used as a washing solution in the autosampler and the injection was performed in a push-loop mode. The temperature of the column oven was 45 °C.

The separation was performed on a Discovery HS F5 column (3 μm, 50 × 4 mm, Supelco, Bellefonte, USA) protected with a silica 4 × 3 mm precolumn (Phenomenex, Torrance, CA, USA). The mobile phase consisted of methanol – 100 mM ammonium acetate containing 1% of formic acid (90:10, v/v), the flow-rate was 0.6 ml/min. The effluent from the column was diverted to waste for the first 1.2 min of the run and then it was directed to the ion source using a switching valve.

The detection of the analytes was carried out using positive electrospray ionization technique and selected reaction monitoring mode to monitor the transitions (precursor → product) m/z 363 → 193 and m/z 368 → 198 for solifenacin and solifenacin-D_5, respectively. The dwell time was 0.4 s for both analytes and scan width was set to 1.0 m/z. Ion spray voltage was set to 4500 V, temperature of the ion transfer capillary was 270 °C. Collision energy was 32 V both for solifenacin and internal standard. The pressure of argon in the collision cell was 0.8 mTorr. The pressure of the sheath gas, sweep gas and auxiliary gas was 70, 6 and 20 arbitrary units, respectively.

2.3. Standards

Stock solution was made by dissolving a suitable amount of solifenacin succinate in 25 ml of methanol–water (1:1, v/v). Further standard solutions were obtained by serial dilutions of stock solution with the same solvent, the concentration of solifenacin in the solution was calculated by multiplying the concentration of solifenacin succinate by 0.75426. The standard solutions were stored at −18 °C and were protected from light; they were stable at least 6 weeks under these conditions.

The calibration and quality control plasma samples were prepared by addition of standard solutions to drug-free plasma in volumes not exceeding 3% of the plasma volume.

The solution of the internal standard was obtained by dissolving 1 mg of solifenacin-D_5 hydrochloride in 1 ml of methanol and the precipitation solution containing 10 ng/ml of solifenacin-D_5 hydrochloride was obtained by further diluting this solution with methanol.

2.4. Preparation of the sample

The plasma samples were stored in the freezer at −18 °C and thawed at room temperature before processing of the sample.

Two hundred and fifty microliters of plasma were pipetted to the polypropylene tube, 1 ml of the precipitation solution containing internal standard was added and the tube was vortex-mixed for 30 s at 2000 rpm. Then the tube was centrifuged for 2 min at 2000 × g and the supernatant was transferred to an 1.8 ml autosampler vial. Four microliters aliquot was injected into the chromatographic system.

2.5. Calibration curves

The concentrations of individual calibration samples were 0.4665, 1.219, 2.867, 6.660, 16.60 and 41.74 ng/ml. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$): the peak area ratio (analyte/internal standard) was plotted vs. the analyte concentration. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

3. Results and discussion

3.1. Method development

Solifenacin was strongly retained on the pentafluorophenylpropylsilica column in the mobile phase containing 90% of methanol and high concentration of ammonium acetate in the aqueous part of mobile phase was required to elute it from the column. Increasing the concentration of ammonium acetate from 10 mM to 100 mM decreases the capacity factor 14 to 3. The nature of organic modifier has not large effect on the retention; using acetonitrile instead of methanol increased the capacity factor from 3 to 4. Decreasing the methanol concentration from 90% to 80% modestly increased the capacity factor from 3 to 3.4. This behavior indicated an ion-exchange between solifenacin and the residual silanol groups as the major retention mechanism in combination with some reversed-phase effects. Formic acid was added to the mobile phase to enhance protonation and improve peak shape, the final mobile phase composition was methanol – 100 mM ammonium acetate containing 1% of formic acid (90:10, v/v).

The sample solvent with high content of methanol is perfectly compatible with the mobile phase and the sample after protein precipitation can be injected directly into the column. Injection of the same sample on the reversed-phase column would cause peak distortion.

The mobile phase with high methanol content had also an additional benefit of increased sensitivity, which was better using methanol in comparison with acetonitrile. This enabled to inject only a small aliquot of the sample (4 μl) and consequently no problems with column clogging were observed.

Typical chromatograms of drug-free plasma (a); spiked plasma at limit of quantitation at 0.47 ng/ml (b) and plasma from a pharmacokinetic study containing 3.39 ng/ml of solifenacin (c) are shown in Fig. 2. The method selectivity was demonstrated on six blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks.

Simple protein precipitation with methanol was found sufficient as a preseparation technique. The recovery of solifenacin was studied in samples with low, medium and high concentration; it was not dependent on concentration and was about 70%.

3.2. Linearity and limit of quantitation

The calibration curves were linear in the studied range. The calibration curve equation is $y = bx + c$, where y represents analyte/internal standard peak area ratio and x represents the analyte concentration in ng/ml. The mean equation (curve coefficients ± standard deviation) of the calibration curve ($N = 6$) obtained from 6 points was $y = 0.0342(\pm 0.0021)x - 0.0001(\pm 0.0030)$ (correlation coefficient $r = 0.998$).

The limit of quantitation was 0.467 ng/ml. The precision, characterized by the relative standard deviation, was 7.8% and accuracy,

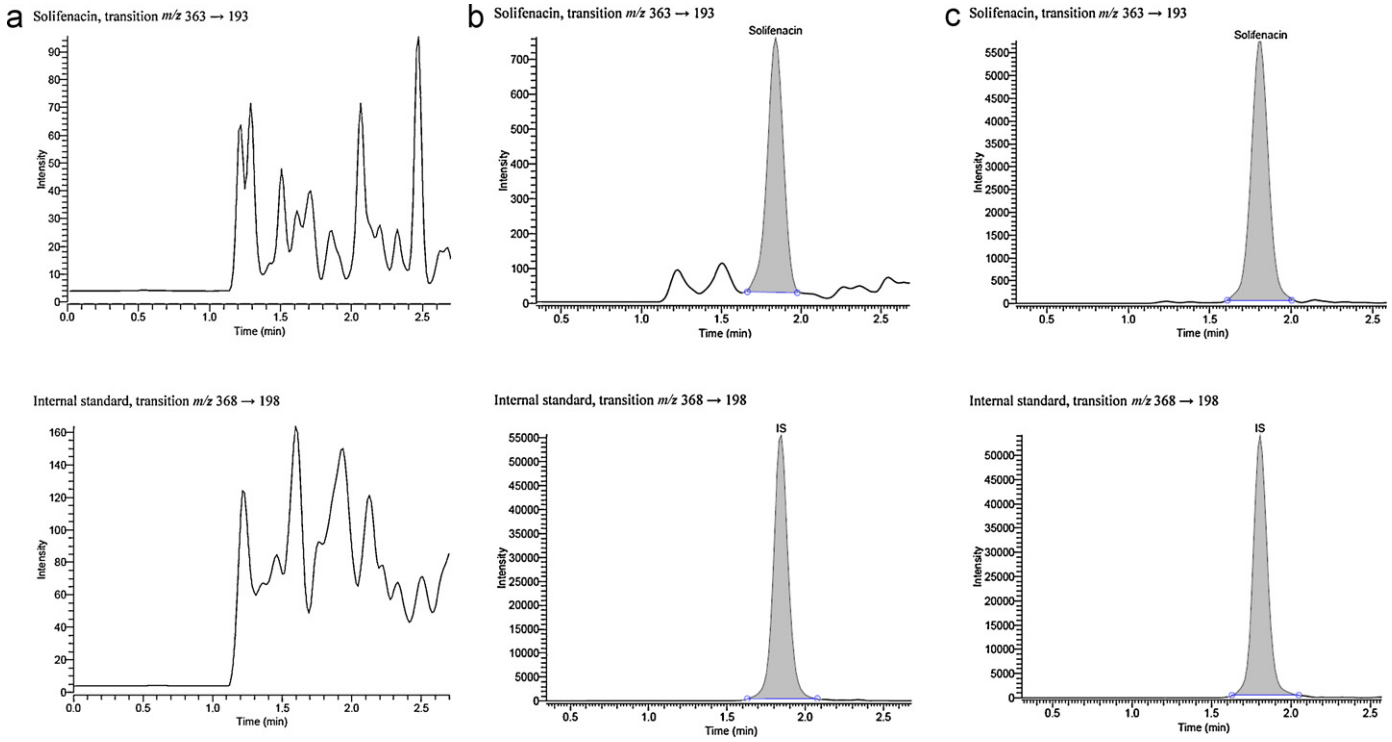

Fig. 2. Chromatograms of (a) drug-free human plasma, (b) spiked plasma at limit of quantitation (0.47 ng/ml), (c) plasma sample from a subject 60 h after administration of 10 mg of solifenacin succinate, the measured concentration of solifenacin was 3.39 ng/ml. The upper panel shows selected reaction monitoring of the transition m/z 363 → 193 (solifenacin) and the lower one the transition m/z 368 → 198 (solifenacin-D_5, internal standard).

defined as the deviation between the true and the measured value expressed in percents, was 1.4% at this concentration ($N = 6$).

3.2.1. Intra-assay precision and accuracy

Intra-assay precision of the method is illustrated in Table 1. It was estimated by assaying the quality control samples (low, medium and high concentration) six times in the same analytical run. The precision was better than 11% and the bias was at most ±11% at all levels.

3.2.2. Inter-assay precision and accuracy

Inter-assay precision and accuracy was evaluated by processing a set of calibration and quality control samples (3 levels analyzed twice) on six separate runs. The samples were prepared in advance and stored at −18 °C. The respective data are given in Table 1. The precision was better than 9% and the inaccuracy did not exceed ±2% at all levels.

3.2.3. Sample stability

Stability was generally concluded if the concentration change was not larger than ±15% compared to freshly prepared samples. The results are shown in Table 2.

Table 1
Precision and accuracy.

	Concentration (ng/ml)			
N	Added	Measured	Bias (%)	RSD (%)
Intra-assay				
6	1.329	1.288	−3.1	11
6	4.332	4.604	6.3	4.8
6	33.04	36.56	11	2.1
Inter-assay				
12	1.329	1.328	−0.1	5.2
12	4.332	4.321	−0.3	8.2
12	33.04	33.64	1.8	7.8

3.2.3.1. Freeze and thaw stability. Plasma samples with a low and high concentration of analyte were prepared. The samples were stored at −18 °C and subjected for 3 thaw and freeze cycles. After the third cycle triplicate 0.25 ml aliquots were processed, analyzed and the results averaged. No significant substance loss during repeated thawing and freezing was observed.

3.2.3.2. Processed sample stability. Two sets of spiked samples with a low and a high concentration of analyte were analyzed and left in the autosampler at ambient temperature. The samples were analyzed using a freshly prepared calibration samples 3 days later. The processed samples were stable at room temperature for this period.

3.2.3.3. Stability of plasma samples. The short-term stability of thawed plasma samples (with a low and high concentration) was studied for period of 24 h at room temperature and ambient light. The long-term stability of frozen plasma samples was examined after 7 weeks storage at −18 °C in the dark. The samples were stable under studied conditions.

3.2.4. Matrix-effects

In order to study matrix effects on the ratio of analyte/internal standard peak areas and on the response of individual compounds the following experiment was performed: six different plasma samples (from 6 different subjects and pooled plasma used for preparation of calibrators and quality controls) were spiked with solifenacin (1.621 and 36.23 ng/ml for samples with low and high concentration, respectively) and internal standard (40 ng/ml), processed and analyzed. The relative standard deviation of peak area ratios was 9.7% and 2.7% at low and high concentration, respectively and the relative standard deviation of peak areas of individual compounds was lower than 11%, indicating no significant relative matrix effects, which could negatively influence quantitation results.

Table 2
Stability of solifenacin.

Conc. (ng/ml)	N	Conc. found (ng/ml)		RSD (%)	Difference (%)
		Fresh	After 3rd cycle		
Freeze and thaw stability					
1.329	3	1.257	1.294	8.9	2.9
33.04	3	30.18	30.54	2.3	1.2

Sample	Conc. (ng/ml)	N	Conc. found (ng/ml)	RSD (%)	Difference (%)
Stability of processed samples					
Fresh	1.329	3	1.375	5.4	
3 days old	1.329	3	1.467	1.9	6.7
Fresh	33.04	3	34.27	3.1	
3 days old	33.04	3	36.32	3.9	6.0

Conc. (ng/ml)	Storage conditions	N	Conc. found (ng/ml)		RSD (%)	Difference (%)
			Fresh	After storage		
Stability of plasma samples						
1.329	24 h/+21 °C	3	1.257	1.071	15	−15
33.04	24 h/+21 °C	3	30.18	32.85	6.0	8.8
1.329	7 weeks/−18 °C	3	1.328	1.282	6.9	−3.5
33.04	7 weeks/−18 °C	3	33.64	33.04	1.9	−1.8

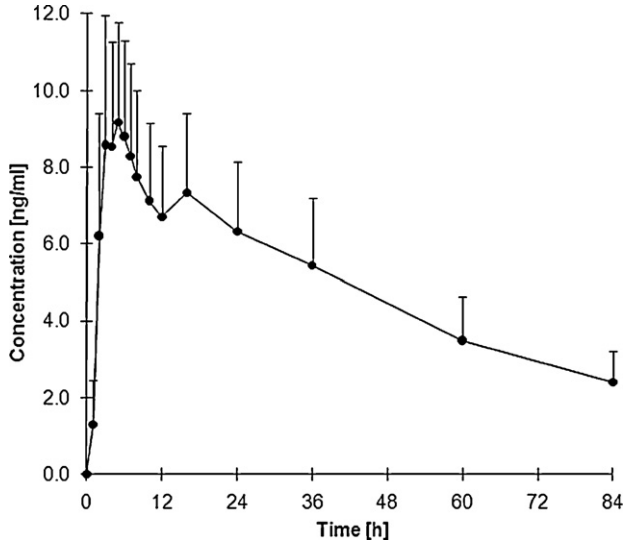

Fig. 3. Mean plasma concentrations (+SD) after a single 10 mg oral dose of solifenacin succinate administered to 26 healthy subjects.

3.3. Application to biological samples

The proposed method was applied to the determination of solifenacin in plasma samples from a pharmacokinetic study, which was approved by the local ethics committee. The plasma samples were collected following a single oral dose of 10 mg of solifenacin succinate (Vesicare tablets, Astellas Pharma Europe) administered to 26 healthy male volunteers: mean age of the group was 29 years (range 20–53), mean weight was 79 kg (range 56–96). Fig. 3 shows the mean plasma concentrations of solifenacin; the error bars indicate standard deviations at individual time points.

Plasma levels of solifenacin reached their maximum 4.5 h (range 2.0–7.0 h) after drug administration and thereafter the plasma level declined with an elimination half-time of 42 h (range 26–74 h). The maximum concentration (C_{max}) was 9.7 ng/ml (range 6.9–20.6 ng/ml) and the mean area under concentration–time curve (AUC) extrapolated to infinity was 565 μg.h/l (range 258–1025 μg.h/l). These values were obtained using noncompartmental analysis. The pharmacokinetic parameters are similar to those published earlier [1,2,4,6].

4. Conclusions

The validated method allows determination of solifenacin in the 0.47–42 ng/ml range. The limit of quantitation and chromatographic run time are better or comparable with the previous methods, but the sample preparation is simpler. About 300 samples can be prepared and analyzed in one working day. The precision and accuracy of the method are well within the limits required for bioanalytical assays. The low limit of quantification permits the use of the method for pharmacokinetic studies.

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