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Journal of Chromatography B, 859 (2007) 241–245
JOURNAL OF CHROMATOGRAPHY B
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Determination of solifenacin succinate, a novel muscarinic receptor antagonist, and its major metabolite in rat plasma by semi-micro high performance liquid chromatography

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Received 10 August 2007; accepted 4 October 2007
Available online 10 October 2007

Abstract

A sensitive and specific method for the simultaneous determination of the unchanged drug (solifenacin) and its major metabolite (M1, 4*S*-hydroxy solifenacin) in rat plasma was developed and validated. Both solifenacin and M1 were extracted from rat plasma by a two-step liquid–liquid extraction and analyzed by semi-micro HPLC with UV detection at an absorbance wavelength of 220 nm. The chromatographic separations were performed on a TSKgel ODS-80Ts (5 μm, 150 mm × 2.0 mm i.d.) reversed-phase column with a mobile phase of 0.1 M phosphate buffer (pH 3.0):acetonitrile (71:29, v/v). The intra-day precision (expressed as coefficient of variation, CV) ranged from 0.4% to 1.7%, and the accuracy (expressed as relative error, RE) ranged from −5.2% to 2.0% for solifenacin. The corresponding precision ranged from 1.3% to 3.2%, and accuracy ranged from −4.0% to 8.6% for M1. The lower limit of quantitation for both solifenacin and M1 was 2 ng/ml when 1 ml of plasma was used. No endogenous interference was observed in rat plasma.
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Keywords: Solifenacin succinate (YM905); Metabolite; HPLC; UV detection; Rat; Plasma

1. Introduction

Anticholinergic agents are widely used for the treatment of overactive bladder [1,2]. However, they are relatively non-selective and cause frequent side effects such as dry mouth, blurred vision, and constipation [1–4]. Solifenacin succinate ((+)-(1*S*,3′*R*)-quinuclidin-3′-yl 1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate monosuccinate) is a novel muscarinic receptor antagonist, and has higher selectivity for the urinary bladder than for the salivary gland [5,6]. This drug is therefore expected to be useful in the treatment of overactive bladder with reduced severity of adverse reactions, especially dry mouth.

On oral administration to rats, solifenacin succinate is rapidly metabolized to produce the metabolite M1 (4*S*-hydroxy solifenacin, ((+)-(1*S*,3′*R*,4*S*)-quinuclidin-3′-yl 4-hydroxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate)), which possesses pharmacological activity (unpublished data). A highly sensitive method for the simultaneous determination of solifenacin and its active metabolite M1 in rat plasma was required to elucidate the pharmacokinetic profile of solifenacin succinate in relation with its pharmacodynamic and safety evaluation. This paper reports the development and validation of a sensitive method for the simultaneous determination of solifenacin and M1 in rat plasma by high performance liquid chromatography with UV detection (UV-HPLC). Chromatographic separations were performed on a semi-micro column (2.1 mm i.d.) which allowed an increase in sensitivity at a constant injection volume and required less solvent for the mobile phase than a conventional column (4.6 mm i.d.).

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doi:10.1016/j.jchromb.2007.10.005

2. Experimental

2.1. Chemicals and reagents

Solifenacin succinate (assay value 100.1%), M1 (purity 99.9%) and the internal standard (IS) ((−)-[(1*R* or 1*S*),3′*R*]-quinuclidin-3′-yl 1-benzyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate monohydrochloride, purity 100.0%) were synthesized at Astellas Pharma Inc. (Tokyo, Japan). The chemical structures are shown in Fig. 1. This IS was selected as appropriate to its structural resemblance to solifenacin. Heparinized rat blank plasma for the preparation of calibration standards and quality control (QC) samples was obtained from drug-free experimental animals. Milli-Q SP TOC (Millipore Japan, Tokyo, Japan) distilled deionized water was used. HPLC-grade acetonitrile and reagent-grade 0.5 M phosphoric acid were purchased from Kanto Chemical (Tokyo, Japan). HPLC-grade *t*-butyl methyl ether, HPLC-grade distilled water, reagent-grade potassium dihydrogenphosphate and reagent-grade sodium hydrogen carbonate were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a model LC-10AT pump, a model SIL-10A auto-injector, a model SCL-10A system controller, a model CTO-10AC column oven, a model SPD-10AV spectrophotometric detector, a model DGU-12A degasser, and a model C-R7A plus integrator (Shimadzu, Kyoto, Japan). Separation was achieved on a TSKgel ODS-80Ts (5 μm, 150 mm × 2.0 mm i.d.) reversed-phase column (Tosoh, Tokyo, Japan) at the column temperature of 40 °C. The mobile phase consisted of 0.1 M phosphate buffer adjusted to pH 3.0 and acetonitrile (71:29, v/v). The HPLC system was operated isocratically at a flow rate of 0.2 ml/min. The absorbance wavelength of the detector was set at 220 nm.

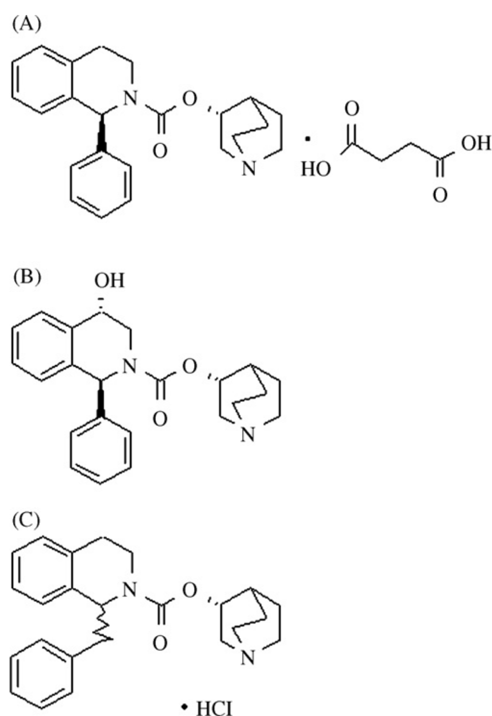

Fig. 1. Chemical structures of (A) solifenacin succinate, (B) M1, and (C) internal standard.

2.3. Preparation of calibration standards and QC samples

In the present study, solifenacin concentrations are expressed in terms of the solifenacin succinate salt unless specified otherwise. Primary stock solutions (1 mg/ml) of solifenacin and M1 were prepared in 0.05 M phosphoric acid for calibration standards and QC samples by separate weighing. The stock solutions of these compounds were further diluted with 0.05 M phosphoric acid to give a series of calibration and QC solutions. Calibration standards over the range 2–1000 ng/ml were prepared by spiking calibration solutions (0.1 ml) into 1 ml of rat blank plasma. QC samples (6, 80 and 800 ng/ml) and limit of quantitation (LOQ) samples (2 ng/ml) were also prepared using blank plasma. The samples were stored at −20 °C prior to analysis.

An IS stock solution (0.5 mg/ml) was prepared in 0.05 M phosphoric acid. A portion of this IS stock solution was diluted with 0.05 M phosphoric acid to give a concentration of 5 μg/ml.

2.4. Sample preparation

Calibration standards (2–1000 ng/ml, 1 ml) and QC samples (1 ml) in glass tubes were spiked with 0.1 ml of IS (5 μg/ml), 1 ml of saturated sodium hydrogen carbonate, and 5 ml of *t*-butyl methyl ether. After shaking for 15 min, the organic layer was separated from the aqueous layer by centrifugation at 1000 × *g* for 10 min. The organic layer was then transferred to another glass tube, and 0.05 M phosphoric acid (0.2 ml) was added. After shaking for 15 min, the organic layer was separated from the aqueous layer by centrifugation at 1000 × *g* for 10 min. The organic layer was removed with an aspirator, and the remaining organic solvent was completely evaporated under reduced pressure. The aqueous layer was transferred to the autosampler vials, and a 0.05 ml aliquot was injected onto the HPLC system.

2.5. Method validation

2.5.1. Calibration curves

Calibration standards (range = 2–1000 ng/ml) at nine concentrations of solifenacin and M1 were extracted and assayed. A linear model was fit to the concentration vs. peak-height ratio (PHR) data using weighted (1/*x*) least-squares regression.

2.5.2. Specificity

Rat blank plasma samples from six drug-free male animals were extracted and assayed. The chromatograms were visually inspected for peaks from endogenous substances which might correspond to solifenacin, M1 or IS peaks.

2.5.3. Accuracy and precision

QC samples at each of three concentrations (6, 80 and 800 ng/ml, $n = 6$) and LOQ samples (2 ng/ml, $n = 6$) of solife-

nacin and M1 were assayed to determine the intra-day accuracy expressed as mean relative error (RE) and precision expressed as coefficient of variation (CV) in rat plasma.

QC samples ($n = 6$) at each of three concentrations were assayed on three different occasions to determine the inter-day accuracy and precision of solifenacin and M1.

2.5.4. Extraction recovery

The recovery of solifenacin and M1 through extraction procedures was assessed at two different concentrations (6 and 800 ng/ml, $n = 3$). The peak height ratios of solifenacin and M1 added to rat blank plasma prior to extraction was compared with those in which solifenacin and M1 was added after extraction (control). The recovery of IS was similarly determined.

2.5.5. Stability

The stability of solifenacin and M1 in rat plasma stored at −20 °C was investigated. QC samples at each of two concentrations (6 and 800 ng/ml, $n = 3$) were assayed after storage for 28 days. The stability, expressed as the residual percentage of control, was determined by comparing the observed solifenacin and M1 concentrations with those obtained from samples which were prepared immediately before analysis (control).

The stability of solifenacin, M1 and IS in stock solutions stored at 4 °C was also investigated. The stock solutions of solifenacin, M1 and IS at 100, 100 and 500 μg/ml ($n = 3$), respectively, were analyzed after storage for 28 days. The residual percentage of control was determined.

2.6. Drug administration

Male F344 rats ($n = 3$) given free access to standard pellet diets and water were orally administered solifenacin succinate at 30 mg/kg following an overnight fast. Rat blood was collected from the inferior vena cava with a heparinized syringe under diethyl ether anesthesia. Plasma was obtained by centrifugation at 1870 × *g* for 15 min and stored at −20 °C until assay.

3. Results

3.1. HPLC chromatograms and specificity

The representative HPLC chromatograms of (A) rat blank plasma, (B) rat plasma spiked with solifenacin (200 ng/ml), M1 (200 ng/ml), and IS, and (C) plasma obtained from rats after oral administration of solifenacin succinate at a dose of 30 mg/kg are shown in Fig. 2. The retention times of solifenacin, M1 and IS in rat plasma were 18.2 min, 7.3 min, and 23.1 min, respectively. No interference peaks of endogenous substances were observed in rat blank plasma. The major metabolite peak corresponding to M1 was observed in the plasma obtained 0.25 h after oral dosing. In addition, another metabolite peak was detected at the retention time of 20 min, which was estimated to be the *N*-oxide of solifenacin (M2), possessing no pharmacological effect.

3.2. Linearity

The calibration curves of solifenacin and M1 in rat plasma were linear over the concentration range of 2–1000 ng/ml.

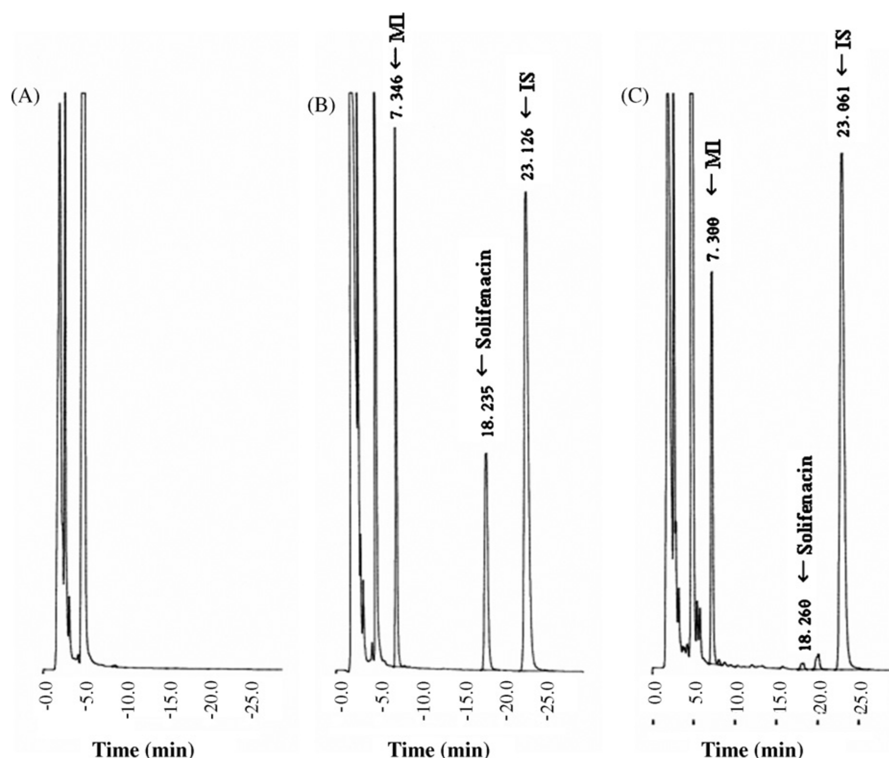

Fig. 2. Representative chromatograms of rat plasma. (A) Rat blank plasma, (B) rat plasma spiked with solifenacin (200 ng/ml), M1 (200 ng/ml) and IS (500 ng/ml), (C) rat plasma obtained 0.25 h after oral administration of 30 mg/kg of solifenacin succinate.

Table 1
Summary of linear regression data for the determination of solifenacin and M1 in plasma

	Analyte	Slope	Intercept	Correlation coefficient
Day 1	Solifenacin	0.002281	0.001727	0.99985
	M1	0.005575	0.005866	0.997848
Day 2	Solifenacin	0.002214	−0.000176	0.9999
	M1	0.005230	0.001752	0.998699
Day 3	Solifenacin	0.002250	0.000007761	0.9999
	M1	0.005407	0.001131	0.99925

The slope and intercept of the calibration curves are listed in Table 1.

3.3. Accuracy and precision

The intra- and inter-day precision and accuracy for solifenacin and M1 in rat plasma are shown in Table 2. The CV and the RE at the LOQ were 6.5% and 2.1% for solifenacin, and 6.5% and −4.1% for M1, respectively. The intra-day CV at 6, 80 and 800 ng/ml ranged from 0.4% to 1.7%, and the RE ranged from −5.2% to 2.0% for solifenacin. The corresponding CV ranged from 1.3% to 3.2%, and the RE ranged from −4.0% to 8.6% for M1. The inter-day CV and RE ranged from 1.1% to 4.8% and −0.3% to 2.6%, respectively, for solifenacin. The corresponding CV and RE ranged from 1.5% to 5.2% and 1.8% to 8.6%, respectively, for M1.

3.4. Extraction recovery

The mean recovery of solifenacin from rat plasma ($n = 3$) was 83.2% at 6 ng/ml and 88.1% at 800 ng/ml. The corresponding values for M1 were 78.0% and 81.4%, respectively. The values for IS ($n = 3$) was 90.6% (Table 3).

Table 3
Extraction recovery of solifenacin, M1 and internal standard (IS) from plasma

Compound	Nominal concentration (ng/ml)	Recovery (%), mean ± S.D.
Solifenacin	6	83.2 ± 3.8
	800	88.1 ± 2.3
M1	6	78.0 ± 2.9
	800	81.4 ± 2.1
IS	500	90.6 ± 5.2

Table 4
Stability of solifenacin and M1 in plasma stored frozen at −20 °C for 28 days

Compound	Nominal concentration (ng/ml)	% of control, mean
Solifenacin	6	107.0
	800	98.9
M1	6	105.9
	800	104.6

3.5. Stability

Solifenacin and M1 were shown to be stable in rat plasma when stored frozen at −20 °C for up to 28 days. The observed values were 107.0% and 98.9% of control at 6 and 800 ng/ml, respectively, for solifenacin, and 105.9% and 104.6%, respectively, for M1 (Table 4).

Solifenacin, M1 and IS were shown to be stable in the stock solutions when stored at 4 °C for up to 28 days. The observed values were 99.7%, 101.2% and 98.2% of control, respectively, for solifenacin, M1 and IS (Table 5).

4. Discussion

Solifenacin succinate possesses no major wavelength of UV absorption maximum although this drug shows UV absorbance. Acetonitrile, the organic solvent used for the mobile phase,

Table 2
Precision and accuracy for the determination of solifenacin and M1 in plasma

	Analyte	Nominal concentration (ng/ml)	Observed concentration (ng/ml), mean ± S.D.	CV (%)	RE (%)
Intra-day ($n = 6$)	Solifenacin	2 (LOQ)	2.08 ± 0.14	6.5	2.1
		6 (QCL)	5.80 ± 0.10	1.7	−5.2
		80 (QCM)	83.22 ± 0.84	1.0	2.0
		800 (QCH)	820.21 ± 3.16	0.4	0.5
	M1	2 (LOQ)	1.94 ± 0.13	6.5	−4.1
		6 (QCL)	5.82 ± 0.18	3.2	−4.0
		80 (QCM)	87.73 ± 1.10	1.3	8.6
		800 (QCH)	835.60 ± 10.65	1.3	3.4
Inter-day[a] ($n = 18$)	Solifenacin	6 (QCL)	6.13 ± 0.30	4.8	0.1
		80 (QCM)	83.69 ± 0.88	1.1	2.6
		800 (QCH)	813.78 ± 9.26	1.1	−0.3
	M1	6 (QCL)	6.23 ± 0.32	5.2	2.8
		80 (QCM)	87.72 ± 1.47	1.7	8.6
		800 (QCH)	822.75 ± 12.62	1.5	1.8

LOQ: Limit of quantitation; QCL: low concentration quality control sample; QCM: medium concentration quality control sample; QCH: high concentration quality control sample.

[a] Six QC samples each at 6, 80 and 800 ng/ml were measured on three different occasions.

Table 5
Stability of solifenacin, M1 and IS in the stock solutions at 4 °C for 28 days

Compound	Nominal concentration (μg/ml)	% of control, mean
Solifenacin	100	99.7
M1	100	101.2
IS	500	98.2

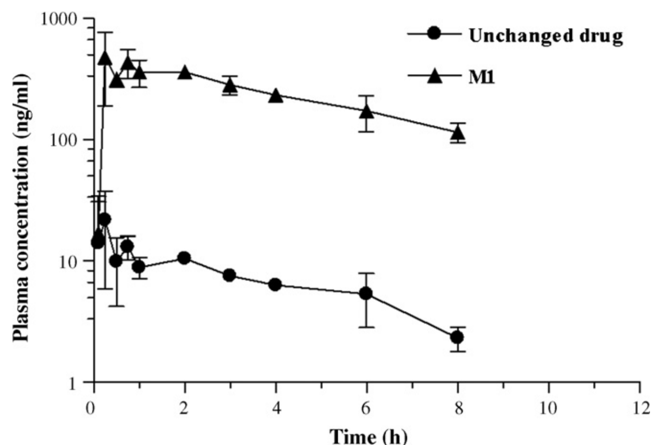

Fig. 3. Plasma concentration–time curves of the unchanged drug and M1 after oral administration of solifenacin succinate to rats at a dose of 30 mg/kg. Values represent mean ± S.D. of three rats. Plasma concentrations of the unchanged drug are expressed as solifenacin free base. Plasma concentrations of M1 are expressed as the equivalent of solifenacin free base.

possess a UV absorption maximum at 210 nm. Taking these findings into consideration, the absorbance wavelength of the UV detector was set at 220 nm, which was higher than the wavelength of the UV absorption maximum for acetonitrile. When the single-step liquid–liquid extraction procedure with *t*-butyl methyl ether under alkali conditions was used in preliminary experiments, many interference peaks of endogenous substances were observed in rat blank plasma with UV detection at the wavelength of 220 nm. The two-step extraction procedure was therefore adapted to improve specificity. When the plasma samples were subjected to extraction with *t*-butyl methyl ether and 0.05 M phosphoric acid in that order, no interference peaks of endogenous substances were observed in rat blank plasma. The LOQ of solifenacin in plasma was approximately 5 ng/ml at the level of S/N = 5 using a conventional column (4.6 mm i.d.). The plasma concentrations of solifenacin after oral administration to rats were found to be very low due to extensive metabolism in the preliminary experiments (unpublished data). Since it is necessary to determine at least several ng/ml of solifenacin in plasma, the chromatographic separations were performed on a semi-micro column (2.1 mm i.d.) to increase sensitivity at a constant injection volume [7,8]. Result showed that the LOQ of both solifenacin and M1 were 2 ng/ml. The use of a semi-micro column also allowed measurement to made with a low flow rate (0.2 ml/min), sparing solvent and contributing to environment protection [9,10]. The composition and pH of mobile phase were selected to achieve the optimal separation and peak shapes of solifenacin, M1 and IS.

The utility of the present methods were demonstrated by monitoring plasma concentrations of the unchanged drug and its metabolite M1 after oral administration of solifenacin succinate at a dose of 30 mg/kg to rats (Fig. 3). Plasma concentrations of the unchanged drug reached a maximum level of 21.7 ng/ml (expressed as solifenacin free base, with the same expression hereafter) at 0.25 h after administration, then declined with a half-life ($t_{1/2}$) of 3.12 h. The area under the plasma concentration-time curve to infinity ($AUC_{0\text{-}\infty}$) was calculated to be 68.5 ng h/ml. The plasma concentrations of M1 reached a maximum level of 478.1 ng/ml at 0.25 h after administration, then declined with a $t_{1/2}$ of 3.93 h. The $AUC_{0\text{-}\infty}$ was calculated to be 2609.5 ng h/ml. The plasma concentrations of both solifenacin and M1 could be determined up to 8 h after administration.

5. Conclusion

A sensitive and specific method for the simultaneous determination of solifenacin and its active metabolite M1 in rat plasma by HPLC was developed and validated using a semi-micro HPLC column. Validation experiments showed that the assay has good precision and accuracy over a concentration range of 2–1000 ng/ml. No endogenous substances which could interfere with the assay were observed. The method is sufficiently sensitive and robust to precisely evaluate the pharmacokinetic profiles of both the unchanged drug and M1 following administration of solifenacin succinate to rats.

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