Estimation of solifenacin and tamsulosin in rat plasma by LC-MS/MS: Application to a pharmacokinetic study

Chandramohan Kandasamy*¹, Kumar Mohan¹, Alexandar S², Sivaselvakumar Muthusamy³
¹Department of Pharmaceutical Chemistry, Vinayaka Mission’s Research Foundation (Deemed to be University), Salem - 636008, Tamilnadu, India
²Department of Pharmaceutical Analysis, Vinayaka Mission’s Research Foundation (Deemed to be University), Salem - 636008, Tamilnadu, India
³Department of Molecular Medicine & Therapeutics, PSG Institute of Medical Sciences & Research, Coimbatore - 641004, Tamilnadu, India

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ABSTRACT
Effectiveness tests using a mixture of solifenacin and tamsulosin include the implementation of a research procedure for precise plasma evaluation of both drugs. For the direct estimation of solifenacin and tamsulosin, high quality liquid chromatography-tandem mass spectrometry in rat plasma (LC-MS / MS) methodology was designed. Extraction during solid phase technique used to obtain solifenacin, tamsulosin, and solifenacin D5, tamsulosin D4 (internal standards; IS) from 100 µL rat plasma. On the Thermo BDS Hypersil C8 (100 X 4.6 mm, 3.5 µm) column under isocratic elution with acetonitrile, chromatographic separation of analytes was done:10mM ammonium formate (80:20 v/v) 1 mL/min mobile phase flow rate. For both analytes and various IS, precursor ion and production transitions were tracked on a triple quadrupole mass spectrometer, controlled with positive ionization mode in the specific reaction monitoring. The model was employed for solifenacin over a range of concentrations of 0.100 to 30.014 ng/mL and for tamsulosin between 0.050 and 15.176 ng/mL. Mean recovery of solifenacin, tamsulosin, solifenacin D5 and tamsulosin D4 was 84.7%, 87.5%, 86.1% and 82.6%, was consistent at low, medium and high levels of QC. For both analytes, precision and accuracy were less than 15 percent at low, medium and high QC with good performance levels of operation. Specific stabilities have been tested for all analytes, bench-top, wet extract, freeze-thaw and long-term stability. To help a pharmacokinetic analysis in Wistar rat, this analytical method for simultaneous estimated use of solifenacin and tamsulosin was applied for rat plasma.

INTRODUCTION
Benign prostatic hyperplasia (BPH), which is not a cancer growth in prostate gland rates, also known as prostate enlargement. It's a natural male aging disorder, but the exact cause is unclear. Alterations in ageing male sex hormones can be an element. It happens commonly in middle-aged and elderly men, and mostly contributes to obstruction of the bladder outlet (BOO) and overactive bladder (OAB), which severely impact the lives of the patient and raises psychological and economic pressure on patients.
The clinical manifestations of BOO and OAB include increase in frequency of urination, nocturia, urinary urgency, incontinence (Sakalis et al., 2018; Dimitropoulos and Gravas, 2015).

Use of antimuscarinics helps to eliminate spontaneous intrusive operation during storage time by combining the duration of contractions and their strength. In fact, antimuscarinics have also been found to improve bladder power by impeding afferent bladder pathways. Succinate solifenacin salt is a comparatively recent antimuscarinic agent compared with antimuscarinic substances. Solifenacin is tangentially the most common of bladder; thereby eliminating other side effects pertaining to specific organs. Solifenacin has been used clinically as a new type of M3 receptor antagonist, its mechanism is to act on the M3 receptors in the bladder and urethra, therefore the contraction of bladder smooth muscle can be suppressed and the symptoms of OAB can be alleviated (Nazir et al., 2015; van Kerrebroeck et al., 2013).

Solifenacin is a selective antagonist to the muscarinic acetylcholine receptor which is used with or without urge incontinence in enlarged prostate treatment. It is a phenyl substituted dihydroisoquinoline analog (Doroshyenko and Fuhr, 2009; Smulders et al., 2004). The basic nitrogen is involved in a carbamate linked with a bicycle piperidinol. Solifenacin is administered orally due to its excellent oral bioavailability (approximately 90%). Metabolism mainly happens due to CYP3A4 enzymes and excreted through the kidney (70%) and feces (20%) (Kim et al., 2018; Yamada et al., 2018).

Previously alpha1 blocker tamsulosin was used commonly in the treatment of BPH. It has been proposed that noradrenaline-mediated contraction of flexible prostate muscles be concerned as a consequence of BPH in the pathogenesis of BOO. Alpha1 blockers have long been enough to relieve BPH-related symptoms by preventing prostatic smooth muscle contraction and decreasing BOO induced (Franco-Salinas et al., 2010; Wolzt et al., 1998).

Tamsulosin can be used for treating symptoms in the lower urinary tract (Lepor, 2007), it relaxes the muscles in the prostate and bladder neck thereby facilitating easier urination. The structure contains a basic secondary amine containing a phenol ring and benzene sulphonamide ring (Puttagunta et al., 2014; Rao et al., 2011).

It has been clinically proven that a combination of alpha1 blocker drugs such as tamsulosin and antimuscarinic drugs such as solifenacin work more efficiently with patients having symptoms of BOO and OAB (Yanagihara et al., 2007; Mistri et al., 2008). Very few LC-MS/MS conventional techniques for the measurement of solifenacin in plasma are reported to the best of knowledge (Ptáček et al., 2007). Methods for evaluating tamsulosin in biological fluids were also recorded using LC-MS/MS (Choi et al., 2012; Lin et al., 2008).

Nevertheless, reports for routine analysis of solifenacin and tamsulosin in plasma that identify an approach based on LC-MS/MS are not available (Upreti et al., 2013). In animal models, simultaneous identification of solifenacin and tamsulosin in plasma will help to develop a pharmacokinetic and pharmacodynamics correlation requiring the management of both drugs to gain full effectiveness (Ramakrishna et al., 2005; Shakya et al., 2010).

In this review, we identify a highly responsive, specific and fast LC-MS/MS methodology built and fully validated in rat plasma for simultaneously solifenacin and tamsulosin estimation. This technique generally offers a short time to test and involves just 100 μL of the plasma of rat to obtain samples that use processing of solid-phase extraction.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

Solifenacin Succinate, Tamsulosin hydrochloride, Solifenacin D5 (IS for solifenacin) and Tamsulosin D4 (IS for tamsulosin) (Figure 1) were obtained from Simson Pharma, India. Methanol and Acetonitrile (HPLC-grade) that were obtained from J.T. Baker, Phillipsburg, USA. Ammonium formate buffer, ammonia hydroxide solution and Formic acid of HPLC grade were obtained from Merck, Mumbai, India. Ultra-pure water is collected from Millipore, MA, USA, Milli-Q purification system.

**Chromatographic liquid and mass spectrometry**

A Shimadzu Nexera X2 (Shimadzu Corporation, Japan) SIL-30 ACMP autosampler, SIL-30 AD pump, for the reverse phase chromatographic study of analytes, CTO-10 ASVP column oven, and DGU-20A3R degasser were used. On the Thermo scientific BDS Hypersil C8 column (100 mm X 4.6 mm, 3.5 μm) analytes separation and IS was performed at 30°C. Mixture of Ammonium formate and acetonitrile 10mM (80:20, v/v) for isocratic conditions at flow rate of 1.0 mL/min was employed as the mobile phase. Five microliters of samples were collected and were injected into autosampler, separating an isocratic elution with an at 1.0 mL/min flow rate with post-column flow split of 70%. Directed to flow into
the ion-spray interface. An auto sampler temperature (SIL-30 ACMP) has been held at 4 °C. Mass spectrometric identification of reagents and IS was performed on the complete quadrupole mass spectrometer (API-4000 Applied Biosystems, Toronto, Canada), fitted for electrospray positive ionization mode. For analytes and IS, configured mass parameters and SRM configurations with turbo spray voltage of 5500 V, Source temperature of 500 °C, Entrance potential of 10 V. Nitrogen served as nebulizing gas (40 psi), curtain gas (40 psi), and collision gas (8 psi). The triple compound dependent variables, respectively declustering potential, collision energy & cell exit potential, were tuned for Solifenacin and solifenacin D5 at 85, 40 and 15V although for Tamsulosin and tamsulosin D4 they were configured at 50, 40 and 12 V. The quadruples 1 and 3 had been held at the unit resolution. Multiple Reaction Monitoring (MRM) mode had been used for data processing. Peak integration and optimization is performed using version 1.6.3 of the Analyst® software.

Calibration standards and quality control samples

Measured stock solution (1.0mg/mL) for solifenacin, tamsulosin, solifenacin D5 and tamsulosin D4 freshly weighed and dissolved in methanol. Sparkling blank plasma was used to prepare Samples of calibration curve (CC) & quality control (QC) standards with sufficient amounts of working solutions combining of the two analytes. Final CC concentrations for solifenacin/tamsulosin were 0.100/0.050, 0.200/0.101, 0.603/0.304, 1.507/0.759, 3.014/1.518, 6.028/3.035, 12.056/6.070, 24.112/12.141 and 30.014/15.176 ng/mL. The final QC as follows, 23.102/11.551, 55.000/27.780, 11.551/5.780ng/mL (MQC), 0.300/0.150ng/mL (LQC, Low Quality Control) and 0.100/0.050ngs/mL (LLOQ QC, lower limits of quantification quality control). The storage of Standard stock and working solutions were done at 2-8°C until they are used further.

Procedure of Extraction

Analytes that were extracted from the rat plasma, by solid phase extraction method. Approximately, 100 μL rat plasma was pipetted to a 1.5 mL polypropylene tube, and 25 μL of ISDs (solifenacin D5 2.500 ng/mL, tamsulosin D4 5.000ng/mL) were vortexed after addition. To this 0.200 mL of 1% formic acid in water was vortexed after addition. The sample mixture was loaded on to a pre-conditioned Strata X-CW μm weak cation mixed mode cartridge (30mg/mL) with 1mL of methanol which is followed by 1 mL of 1% formic acid in water. 1 mL of 1% formic acid in water followed by 1 mL of acetonitrile was used to wash the cartridge and dried for approximately 1-2 minutes. The analytes were eluted with 1mL of 5% ammonia in acetonitrile solution into another polypropylene tube and the eluate was evaporated under nitrogen using evaporator at 50°C for 20 min. After evaporation, reconstitution of residue with 0.100 mL of mobile phase and sample was transferred into the vial. The sample (5 μL) was injected into LC-MS/MS system.

Validation of the method

The system performance was verified with injecting replicate samples of mixture of water containing analytes & internal standards and area ratio was within relative standard deviation of 2%. The system performance was performed on daily basis. The carryover test was performed with injecting blank sample followed by high concentration and re-injecting blank sample twice and results were compared with blank response with LLOQ sample. The interference at the analytes area should be less than 20% of area in the blank compared with LLOQ area. The selectivity experiment was carried out with preparing six different lots of plasma was processed with finalized method and evaluated with potential interference of endogenous compound in the processed plasma lots. The interference of analytes should be less than 20% of LLOQ area compared with different lots and less than 5% of area for internal standard. The correlation coefficient of method with linear regression with 1/x² was selected for both analytes. The correlation coefficient was determined with three different days of analytical run of calibration curve standards. The Re-injection reproducibility of method was established with re-injecting calibration curve standards, LQC and HQC after 24 hrs of initial analysis at desired autosampler temperature. The re-injection was compared with initial calibration curve standards. The intra-inter run accuracy and precision were performed by analysing six different samples of LLOQ, LQC, MQC and HQC samples with three different days and acceptance criteria was mean accuracy was ±15% except for the LLOQ where can be ±15% and precision should be less than 15%. To prove the dilution integrity experiment, the concentration was spiked above standard curve and diluting with (1:10) analyte free matrix and analysed. The acceptance criteria of dilution integrity should be accuracy and precision and accuracy should be ±15% and ≤15% of coefficient variation respectively. The Stability experiments were evaluated by preparing freshly prepared stock solution of analyte and stability QCs was compared against with freshly...
prepared calibration curve standard curve. The following stability was assessed for the method, freeze-thaw stability, autosampler stability, stock/working solution stability, processed sample stability, benchtop stability, and long-term plasma stability. The stability experiment data was accepted if the % accuracy ±15% of the nominal value and all the stability experiments should be mimic the actual study sample handled during analysis (Paliwal et al., 2013).

Development of pharmacokinetic analysis of Rat

Wistar model rats weighing 180 ± 30 g which are healthy and 6-8 weeks of age were purchased from Palamur Bioscience Pvt Ltd, Hyderabad and housed at Radiant Research Services Pvt Ltd, # 99 / A, 8th Street, III Phase Peenya Industrial Zone, Bangalore, India in suitable cages. Under normal laboratory conditions, animals were housed in rooms with good ventilation with an overall temperature of 24-27°C and 40-60 percent relative humidity with a daily day-night period of 12 hours. Rats are certified for normal diet of pellet laboratory chow and water ad libitum (Provimi Animal Nutrition India Pvt. Ltd., Bangalore, India). Studies procedure at Radiant Testing Services Pvt Ltd has been endorsed by the Committee on Institutional Animal Ethics, national and international ethical criteria for the treatment and research of Wistar rats have been followed.

Oral specimens were formed by triturating in suspension, in a gravimetric dilution pattern, a precisely weighed volume of powdered compounds (solifenacin and tamsulosin) in methyl cellulose (0.5%, water w / v). Oral doses of 0.06 mg/kg (solifenacin) and 0.005 mg/kg (tamsulosin) were administered to rats overnight (12 h) that used a 10 mL/kg gavage needle. Rats were fed 4 hours after doses. Blood samples of 0.200mL from the retro-orbital sinus were collected in K+EDTA (di-potassium ethylenediaminetetraacetic acid) tubes at Predose, 1, 2, 3, 3.5, 4, 6, 12, 24, 36 and 48 h post-dose and samples were kept on ice till centrifugation. Collected blood samples were centrifuged at 4°C for 10min at 4000 rpm. After cultivation of plasma samples stored at -20°C until further analysis.

The following Pharmacokinetic parameters was evaluated for statistical analysis, Concentration maximum in plasma (C_{max}), the area of curve under concentration-time (AUC_{0–48h}), maximum concentration reaching time (T_{max}), drug half-life (t_{1/2}) and the elimination constant (K_{el}) were estimated by using non-compartmental analysis using SAS® version 9.4.

RESULTS AND DISCUSSION

Mass spectrometry

ESI + and - were tested with several combinations mobile phase, i.e. acetonitrile/methanol and water 2mM – 10 mM ammonium acetate with 0.1% acetic acid / 2 mM – 10mM ammonium formate with formic acid strengths with different ratios of mobile phase. Signal intensity for [M+H]+ ions in ESI +ion mode were 3-5-times more for the solvents analyzed using acetonitrile: ammonium formate buffer. The optimization of the Precursor and product ions were done by infusing 100ng/mL solutions individually in the mass spectrometer between m/z50-700 range. The Q1-MS full spectral scan for the analytes and IS contained protonated precursor predominantly [M+H]+ ions at m/z 362.3, 409.2, 368.2 and 413.2 for solifenacin, tamsulosin, solifenacin D5 and tamsulosin D4, respectively. The most predominant and reliable product ions in the product ion spectra were observed at m/z 193.2, 228.1, 193.2 and 228.1 for solifenacin, tamsulosin, solifenacin D5 and tamsulosin D4, respectively, by applying 40 eV of collision energy for all four compounds (Figure 2).

Liquid chromatography

Due to the successful resilience, acetonitrile was chosen as the organic additive and not as methanol, increased peak sensitivity and resolution. An ammonium formate buffer (10 mM) was needed to achieve an appropriate peak shape and good ionization. A reverse phase column C8 (100 mm × 4.6 mm i.d., 3.5 μm) (BDS Hypersil, ThermoScientific) with acetonitrile:10mM ammonium formate in isocratic mode was used to run samples.

Solifenacin and solifenacin D5 eluted at 2.52 min while tamsulosin and tamsulosin D4 eluted at 1.77 min and. Typical blank rat plasma chromatograms, blank plasma with LLOQ and IS and rat plasmas sample 3 hr after oral administration dose are shown in (Figures 3, 4 and 5).

Accuracy & precision and correlation coefficient

The calibration curves range of 0.100-30.140 ng/mL for solifenacin and 0.050-15.176 ng/mL for tamsulosin, with a correlation coefficient (r^2) ≥ 0.9980 for both the analytes. Mean (+ SD) linear equations obtained for solifenacin and tamsulosin were y = (0.259799 ± 0.002948) x + (0.000451 ± 0.001825) and y=0.148609 ± 0.002051) x+(0.000144 ± 0.000396), respectively.

Precision and accuracy (% CV) for calibration curve of standards ranged from 96.7 to 102.2% and 1.03 to 3.59% for solifenacin, 96.4 to 101.3% and 0.59 to 4.10% for tamsulosin.
Figure 1: Chemical structure of (a) solifenacin succinate (b) tamsulosin hydrochloride (c) solifenacin D5 (d) tamsulosin D4

Figure 2: Product ion chromatogram of (a) solifenacin (b) tamsulosin
Figure 3: Chromatograms of (a) solifenacin (left) and solifenacin D5 (right) (b) tamsulosin (left) and tamsulosin D4 (right) for blank plasma sample

Figure 4: Chromatograms of (a) solifenacin (left) and solifenacin D5 (right) (b) tamsulosin (left) and tamsulosin D4 (right) for extracted rat plasma at LLOQ level
Figure 5: Chromatograms of (a) solifenacin (left) and solifenacin D5 (right) (b) tamsulosin (left) and tamsulosin D4 (right) for rat plasma at 3 hr after a single dose of 0.06 mg/kg of solifenacin and 0.005 mg/kg of tamsulosin.

Table 1: Summary of Accuracy and Precision using rat plasma QC samples

| Drug      | Spiked concentration | Intra batch (n=6) | Inter batch (n=18) |
|-----------|----------------------|-------------------|--------------------|
|           | Estimated concentration (ng/mL) mean ± SD | % Accuracy | % C.V. | Measured concentration (ng/mL) mean ± SD | % Accuracy | % C.V. |
| Solifenacin | 0.100 0.093±0.00          | 93.2           | 4.9       | 0.101±0.01          | 100.8       | 8.7   |
|           | 0.300 0.285±0.01          | 94.9           | 5.0       | 0.289±0.01          | 96.4       | 4.8   |
|           | 11.551 10.998±0.17        | 95.2           | 1.5       | 11.212±0.34         | 97.1       | 3.1   |
|           | 23.102 22.365±1.27        | 96.8           | 5.7       | 22.533±0.77         | 97.5       | 3.4   |
| Tamsulosin | 0.050 0.047±0.00          | 93.7           | 5.6       | 0.049±0.00          | 97.0       | 7.3   |
|           | 0.150 0.142±0.01          | 94.8           | 7.4       | 0.143±0.01          | 95.4       | 5.6   |
|           | 5.780 5.592±0.12          | 96.7           | 2.1       | 5.732±0.28          | 99.2       | 4.8   |
|           | 11.561 11.972±0.18        | 103.6          | 1.5       | 11.657±0.30         | 100.8      | 2.6   |
Figure 6: Plasma concentration versus time profile after a single dose of oral administration of solifenacin and tamsulosin in 6 wistar rats (vertical bars indicate the SD)

Table 2: Stability testing of the rat plasma (n=6)

| Drug      | Nominal concentration (ng/mL) | Stability parameter                      |
|-----------|-------------------------------|-----------------------------------------|
|           |                               | Bench-top stability\(^a\) | Autosampler stability\(^b\) | Freeze-thawing stability\(^c\) | Long-term (30 days) storage stability\(^d\) |
|           |                               | % Accuracy | % CV | % Accuracy | % CV | % Accuracy | % CV | % Accuracy | % CV |
| Solifenacin | 0.300                         | 104.06     | 2.7  | 104.11     | 3.4  | 105.06     | 4.0  | 104.28     | 1.8  |
|            | 23.102                        | 101.33     | 4.9  | 101.74     | 4.0  | 104.65     | 2.6  | 103.04     | 3.4  |
| Tamsulosin | 0.150                         | 96.89      | 4.0  | 96.89      | 4.0  | 94.78      | 1.9  | 94.67      | 3.0  |
|            | 11.561                        | 101.72     | 3.6  | 102.30     | 1.1  | 106.08     | 2.4  | 100.64     | 3.3  |

\(^a\)Exposed at room temperature at 25°C for 2 h, \(^b\) Kept at autosampler temperature of 4°C for 72h, \(^c\) After 6 cycles of freeze-thaw, \(^d\) Stored at -20°C

Table 3: Extraction recovery of solifenacin and tamsulosin in rat plasma (n=6)

| Drug      | Concentration (ng/mL) | Recovery (%) | % C.V |
|-----------|-----------------------|--------------|-------|
| Solifenacin | 0.300                 | 88.9         | 2.7   |
|            | 11.551                | 84.4         | 1.5   |
|            | 23.102                | 80.8         | 2.1   |
| Tamsulosin | 0.150                 | 87.5         | 3.9   |
|            | 5.780                 | 88.8         | 4.4   |
|            | 11.561                | 86.4         | 4.6   |
Table 4: Pharmacokinetic parameters after a single dose of oral administration of solifenacin and tamsulosin simultaneously that are expressed (Mean ± S.D.)

| Parameters | Unit        | Solifenacin       | Tamsulosin        |
|------------|-------------|-------------------|-------------------|
| Cmax       | ng/mL       | 16.887 ± 3.8313   | 11.110 ± 2.0971   |
| AUC0-t     | ng.hr/mL    | 434.889 ± 84.2933 | 217.731 ± 41.7245 |
| AUC0-inf   | ng.hr/mL    | 775.696 ± 178.6960| 227.660 ± 43.5082 |
| Tmax       | hr          | 4.0 ± 0.00        | 6.0 ± 0.00        |
| t1/2       | hr          | 41.548 ± 14.0961  | 8.932 ± 2.0349    |
| Kel        | 1/hr        | 0.019 ± 0.0070    | 0.081 ± 0.0192    |

**Intra-inter run precision and accuracy**

The establishment of intra-inter run precision and accuracy from validation runs were performed at four QC levels (Table 1). The intra-batch precision (%CV) ranged from 1.5 to 5.7 for solifenacin, 1.5 to 7.4 for tamsulosin while accuracy was within 93.2-96.8% for solifenacin and 93.7-103.6% for tamsulosin. For between-run experiments, the precision ranged from 3.1 to 8.7 for solifenacin and 2.6 to 7.3 for tamsulosin while the accuracy remained within 96.4-100.8% for solifenacin, 95.4-100.8% for tamsulosin.

**Stability results**

The room temperature of Stock solutions of analytes and IS were noticed to be stable up to 6 h (short-term stability). The bench-top stability was established for 24 h at ambient temperature. Wet extract stability was stable at 72 h at 4°C. Long-term stability of plasma was stable till 30 days at -20°C. The stability test results are represented in Table 2.

**Recovery and Matrix effect**

Solifenacin and tamsulosin recovered from plasma were estimated at the respective LQC, MQC and HQC levels. Plasma samples (in six aliquots) containing all the analytes at quality control concentration were also elevated in comparison with the internal standard. Results that compare the peak responses (analyte to IS peak area ratio) of the extracted samples with corresponding extracts of blanks spiked with the analytes (post-extraction) at high, medium and low-quality control levels, indicated that the peak responses ratios were within the acceptable limits. Relative recoveries varied from 80.8 to 88.9% and 86.4 to 101%, for solifenacin and tamsulosin, respectively (Table 3). The recovery of solifenacin D5 at 2.500 ng/mL was 86.1% and tamsulosin D4 at 5.000 ng/mL was 82.6%. The determination of the Matrix effect (IS normalised) was done by comparing ratios of internal standard area and analyte ratios of the QC in matrix with their post-spike equivalent amount. Percent CV of the area ratios at LQC and HQC levels were lesser than 15% for all the analytes.

**Results of pharmacokinetic study**

The LC-MS/MS method developed and validated for simultaneous estimation of solifenacin and tamsulosin was successfully applied to generate the plasma concentration versus time profiles of solifenacin and tamsulosin in rat plasma following oral administration.

Absorption was rapid and with maximum plasma concentrations of 16.887 and 11.110 ng/mL at 4.0 and 6.0 hr for solifenacin and tamsulosin, respectively, after oral administration. Solifenacin and tamsulosin were eliminated with a half life of 41.5 and 8.9 hr, respectively. Plasma concentrations were observed up to 48.0 hr for both solifenacin and tamsulosin after oral administration (Figure 6).

Pharmacokinetic variables of solifenacin and tamsulosin are presented in Table 4. Data established successful translation and adaptability of the analytical method for estimation of solifenacin and tamsulosin to an in vivo setting.

**Comparison with the methods recorded**

Documented methods for estimating solifenacin and tamsulosin in plasma are also stated to be focused on the usage of a few LC-MS/MS dependent assays individually. In addition to the low sensitivity, reported technique components have longer run times with a high requirement for plasma volume. The goal of this research was to develop and validate with adequate precision and accuracy a simple LC-MS/MS method to simultaneously estimate solifenacin and tamsulosin and its subsequent usage in pharmacokinetic studies of rats. The present procedure requires solid phase extraction with good sensitivity. An isocratic reverse phase LC-MS/MS analysis for all interest analytes. This process requires the extraction of solid phase plasma by 100 μL.
This solid phase extraction method further reduces matrix effect with high recovery and good assay sensitivity. The conditions of the chromatography were optimized for a 4.0min run time on LC-MS/MS providing faster turn-around time and more number of samples can be analysed. This is a specific method for solifenacin and tamsulosin with desirable linearity, good accuracy and precision.

CONCLUSION

The LC-MS / MS approach emphasizes that the simultaneous quantification of solifenacin and tamsulosin in experimental rats has been thoroughly verified according to the guidelines of the USFDA. Compared to other recorded methods for single analyte in specific biological matrices, the approach suggested is also more robust for both analytes. The solvent extraction efficiency and 4.0 min chromatographic run-time per sample allows the process to be suitable for high-throughput bioanalytical applications. Accuracy values (%CV) for slopes that were measured of calibration curves in different plasma sources clearly show the absence of matrix interference. The validated experiment offers sufficient specificity and selectivity for simultaneous quantification of the findings for all validation parameters and has been used effectively in a preclinical and research. Moreover, it is the first combination of solifenacin and tamsulosin estimation system used in rat plasma.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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