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In vitro–In vivo Correlation Studies of Modified Release Solifenacin Tablet Dosage Form

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PSB and SRP designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KBC, BZSA and BRC managed the literature searches, analyses of the study performed the spectroscopy analysis. Author SRP managed the experimental process. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

A modified release formulation of Solifenacin tablet, was investigated in rabbit for pharmacokinetic and *in vitro–in vivo* correlation studies. *In vivo* study was conducted in New Zealand albino male rabbit plasma and In vitro release studies were conducted in simulated gastric fluid and analyzed by using validated HPLC method. The *In vivo–In vitro* correlation coefficients obtained from point-to-point analysis were greater than 99% between concentrations at certain time points obtained from release study in simulated gastric fluid at specific RPMs and HPLC analysis of rabbit's plasma. From the in vitro–in vivo correlation prediction it was evident that the Solifenacin modified release tablet is a good for controlled delivery of Solifenacin.

Keywords: Solifenacin; modified release; In vitro – In vivo correlation.

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1. INTRODUCTION

Solifenacin succinate is a competitive muscarinic acetylcholine receptor antagonist used in the treatment of overactive bladder with or without urge incontinence. Chemically it is 1-azabicyclo [2.2.2] oct-8-yl (1S)-1-phenyl-3, 4-dihydro-1H-isoquinoline-2carboxylate. Solifenacin succinate is having $C_{23}H_{26}N_2O_2$ molecular formulae. The molecular weight is 362.46. Solifenacin is extensively metabolized in the liver. The metabolites observed as one pharmacologically active metabolite (4R-hydroxy solifenacin), and three pharmacologically inactive metabolites (N-glucuronide and the N-oxide and 4R-hydroxy-N-oxide of solifenacin) were observed in human plasma at low concentrations after oral dosing. Peak plasma levels (Cmax) of solifenacin are reached within 3 to 8 hours after administration, and at steady state ranged from 32.3 to 62.9 ng/mL for the 5 and 10 mg vesicare tablets. The $t_{1/2}$ of solifenacin is 45-68 hours. Solifenacin is approximately 98% (in vivo) bound to human plasma proteins, principally to alpha1-acid glycoprotein [1-10].

Literature survey reveals that quantification of solifenacine in Human plasma [11-12], rat plasma [13], Pharmaceutical compounds [14-17], Industrial waste streams [18] were reported. These methods were reported by using LC-MS/MS [11,12,18], HPLC [13-16], HPTLC [17]. Among all, quantification of solifenacin by LC-MS/MS in biological matrices [11-13] were proved best results. The reported methods does not show IVIVC Correlation and application of the method both in *In vivo vs In vitro*. The aim of the present study is to correlate the relation of *In vitro* dissolution and in-vivo absorption of Solifenacin tablet dosage form [19-24].

Controlled release dosage forms are becoming increasingly important either to achieve the desired levels of therapeutic activity required for a new drug entity or to extend the life cycle of an existing drug through improved performance or patient compliance. A fundamental question in evaluating a controlled release product is whether formal clinical studies of the safety and efficacy of the dosage form are needed or whether a pharmacokinetic evaluation will suffice. The *In vitro* dissolution test is important for the purpose of: (a) providing necessary process control; (b) stability determination of the release rate characteristics of the product; and (c) facilitating certain regulation determinations and judgments concerning minor formulation changes [25].

Correlation between *In vitro* testing and *In vivo* performance are encouraged and guide lines were published in the proceedings of a controlled release workshop [26] and a chapter about in vitro and in vivo evaluation of the dosage forms is included in USP [27,28]. In vitro in vivo correlation (IVIVC) is a predictive mathematical model describing the relationship between an in vitro property of a dosage form, usually the rate or extent of drug dissolution or release, and a relevant in vivo response e.g. plasma drug concentration or amount of drug absorbed. This study was designed by fabricating a matrix tablet for modified release of Solifenacin.

In vitro release has been performed at 150 RPMs through HPLC-UV detection in simulated gastric fluid. A simple and suitable HPLC method using UV spectra as detection procedure has been developed and validated for quantification of Solifenacin in rabbit plasma. A point-to-point in vitro/in vivo correlation (IVIVC) model was developed for relating percentage of drug dissolved to percentage of drug absorbed.

2. MATERIALS AND METHODS

Solifenacin succinate was received from Hetero durgs Lt.d, Hyderabad, India., India as a gift sample. HPMC K4M and SCMC were obtained from Aurobindo Pharma, Hyderabad. Xanthan gum 80 mesh SR-2 and Guar gum 100 mesh Food Grade were purchased from SD fine Chemicals, Mumbai. Acetonitrile, Methanol (HPLC grade) obtained from J.T.Baker, Mumbai, Orthophosphoric acid (85%), Potassium Phosphate Monobasic, Triethylamine, obtained from Merck, Mumbai. Milli Q water (HPLC Grade).

3. APPARATUS AND EQUIPMENT

A chromatographic system (Shimadzu Corporation, Japan) model Shimadzu VP, with Chem station (software), Dissolution testing apparatus (Electrolab TDT-08L) model used. Other apparatus used included photo stability chamber, hot air oven: Proto-Tech oven, Analytical balance: AX205, METTLER TOLEDO, pH Meter: Thermo Orion, model 420, Sonicator: Oscar Ultra Sonics OU-72(SPL). Solvent filtration kit used as Millipore 0.45 µm HV membrane filter and Sample filtration kit used as Millipore,Millex-HV,PVDF,0.45 µm,13 mm filter Thermocouple.

4. PREPARATION OF SOLUTIONS

4.1 Mobile Phase Preparation

Solution A: Dissolve about 1.36g of Potassium phosphate monobasic into 1000ml of beaker with water. Add 1 mL of Triethylamine, mix and adjust the pH to 3.5±0.05 with orthophosphoric acid (85%).

Solution B: Acetonitrile Mix 600 ml of solution A with 400 ml of solution B. Filter and degass it.

4.2 Dissolution Medium Preparation

Dissolution Medium: Purified water

Sample solvent Preparation: Dissolution Medium

5. CHROMATOGRAPHIC CONDITIONS

The chromatography was carried out on Purospher STAR RP-18e ([150 mm x4.6mm 5µm) analytical column at 1.0 ml/ min flow rate of mobile phase with isocratic mode. The injection volume was 20.0µL, Column oven temperature was at 30ºC. Detection at 220 nm, and chromatographic run time of 8.0 min was used. Prior to injection of the drug solution, the column was equilibrated for at least 10 min with the initial time gradient mobile phase conditions flowing through the system.

6. PREPARATION OF STANDARD SOLUTION

The standard stock solution of Solifenacin succinate was prepared by dissolving 44.44 mg in 200 mL standard volumetric flask dissolve and dilute to volume with sample solvent and mix.

Further 5 ml of standard stock diluted to 200 ml of with dissolution medium to obtain the concentration of 5.5 µg/ mL. Filter a portion through the sample filtration kit into autosampler vials. Discard a minimum of 3 ml of the filtrate prior to collecting for analysis.

$$\text{\% dissolved of Solifenacin} = \frac{A_{Spl} \times \text{standard weight} \times \text{sample dilution} \times \%P \times 100}{A_{Std} \times \text{standard dilution} \times 1 \times 100 \times LC}$$

Where,

A_{Spl} = Area of sample chromatogram
A_{Std} = Mean Area of standard chromatogram
%p = Percentage potency of standard (as is basis)
LC = Lable claim in mg.
i = Sampling time point

7. PREPARATION OF TABLETS

Tablets were prepared by wet granulation technique (Phaechamud T, 2008). The composition of formulation is given in Table 1. All the powdered were passed through sieved #80. Required quantities of drug and polymer were mixed thoroughly and a sufficient volume of PVP K30 10% w/v solution was added slowly. After enough cohesiveness was obtained, the mass was screened through the sieve #22/44. The wet granules were dried at 40°C for one hour thereafter kept in the desiccators for 12 hours at room temperature. After dry, the granules retained in 44 mesh were mixed with fines (granules that passed through 44 mesh). Lactose monohydrate was used as a diluent. The granules were blended with 2% Magnesium stearate and 2% Aerosil for 2-3 minutes and, which were used as a lubricant and glident respectively to improve flow property. The granules were subjected for evaluation studies to ensure its flowability, followed by compressed into matrix tablets weighing about 180mg using 2.8 mm shallow biconcave punches in Cadmach rotary tablet punching machine to a hardness of 5-6 kg/cm2. The prepared matrix tablets were used for further evaluation studies.

Table 1. Composition of matrix tablets of Solifenacin succinate

Ingredients in mg/tablet*	Solifenacin succinate	HPMC K4M	SCMC	Guar Gum	Xanthan Gum	Lactose mono hydrate
F1	5	15	--	--	--	156.4
F2	5	30	--	--	--	141.4
F3	5	45	--	--	--	126.4
F4	5	60	--	--	--	111.4
F5	5	--	15	--	--	156.4
F6	5	--	30	--	--	141.4
F7	5	--	45	--	--	126.4
F8	5	--	60	--	--	111.4
F9	5	--	--	15	--	156.4
F10	5	--	--	30	--	141.4
F11	5	--	--	45	--	126.4
F12	5	--	--	60	--	111.4
F13	5	--	--	--	15	156.4
F14	5	--	--	--	30	141.4
F15	5	--	--	--	45	126.4
F16	5	--	--	--	60	111.4

**All ingredients were taken in mg, 10% PVP K30 w/v solution was used as granulating agent, 2% w/w of Magnesium stearate and Aerosil were used as a lubricant and glident respectively for all formulations.*

7.1 *In vitro* Study

In vitro release studies were performed using USP II Dissolution Testing Apparatus (Electrolab TDT-08L) in simulated gastric fluid at 150 RPM, as rotating speed of stirrers. 900 ml of dissolution medium was maintained at 37±0.5ºC for 24 h dissolution study [29].

Tablets were placed at the bottom of the baskets. 1 ml of samples were withdrawn at 0.5, 1, 2, 4, 6, 8, 16 and 24 h and the aliquots withdrawn were replaced with fresh dissolution medium. The samples were filtered and assayed spectrometrically at 220 nm. All release studies have been performed in triplicate designated to get the confirmation about release pattern.

7.2 *In vivo* Study

In vivo studies were carried out on six New Zealand albino male rabbits of same age group weighing between 1.25 and 1.5 kg. The animals were kept in individual cages and maintained at 25ºC for 10 days prior to experiment. Standard diet and water ad *libitum* were given to them. All experiments have been performed according to guidelines of the Institutional Animal Ethics Committee, Vignan College of Pharmacy. Solifenacin Tablet (5 mg) was administered orally in a single dose of Solifenacin Tablet. All studies were performed after keeping rabbits for overnight fasting.

8. DRUG STANDARD SOLUTIONS

Standard stock solutions (10 ml) of Solifenacin were prepared in acetonitrile at a concentration of 1 mg/ml and kept at refregirated conditions. Intermediary solutions of Solifenacin were prepared in acetonitrile. All calibration curve samples (non-zero samples), except blank plasma were prepared by spiking three different blank plasma batches. From Solifenacin stock solution of calibration standards were prepared at 10, 20, 30, 40, 50, 60 µg/ mL.

9. SAMPLE EXTRACTION FROM ANIMAL PLASMA

Liquid-liquid extraction was used to isolate drug and IS from rat plasma. For this purpose, 50µL of IS (40.0 ng/mL) and 100 µL of plasma sample (respective concentration) was added into labeled polypropylene tubes and vortexed briefly. Followed by 2.5 mL of extraction solvent (di etyle ether) were added and vortexed for 10 min. Then these samples were allowed for centrifugation at at 4000 rpm for 10 min at 20ºC. After this centrifugation, supernatant from each sample was removed and added into respective vials and allowed for evaporation under nitrogen at 40ºC for 10 min. finally each tube was reconstituted with 1000 µL of reconstitution solution (5 mM ammonium formate pH 3.0: methanol (20:80)) and vortexed briefly. Finally, the extracted sample was transferred into auto sampler vials and injected into LC-MS/MS.

10. *IN VIVO* RELEASE AND PHARMACOKINETIC ANALYSIS

Blood samples of 0.3 ml were collected at the interval of 0 (Predose), 0.5, 1, 2, 4, 6, 8, 16 and 24 h (post-dose) in heparinized Eppendorf tubes after administration. These samples were centrifuged immediately at 3500 rpm and 4ºC temperature for 10 min. Plasma samples were taken and stored at −30ºC until assay. Pharmacokinetic parameters like peak plasma

concentration (Cmax), time to reach peak plasma concentration (tmax), area under the (concentration–time) curve (AUC) and elimination half-life (t1/2) and were calculated following non-compartment model of Win Non-Lin 5.1. All the parameters were calculated for oral administration of Solifenacin 5 mg Tablets.

10.1 *In vitro* Dissolution Data Analysis

The dissolution profiles for each formulation were determined by plotting the cumulative percent of Solifenacin dissolved at various time points. The in-vitro drug release profiles of the two ER dosage forms were compared using the similarity factor, *f*, presented in the following equation

$$f_2 = 50 \log\left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (F_t - S_t)^2 \right]^{-0.5} \times 100 \right\}$$

10.2 *In vivo* Data Analysis

The Solifenacin concentration–time data were evaluated by analysis of variance using SAS version 6.12, GGLM procedure and an *F*-test to determine statistically significant differences (α= 0.05) by Pharmakinetics Laboratories. The measured plasma concentrations were used to calculate the area under the plasma concentration–time profile from time zero to the last concentration time point ($AUC_{(0\text{-}t)}$). The ($AUC_{(0\text{-}t)}$) was determined by the trapezoidal method. $AUC_{(0\text{-}\alpha)}$ was determined by the following equation

$$\mathrm{AUC}_{(0-\infty)} = \mathrm{AUC}_{(0-t)} + \frac{C_{(t)}}{k_e}$$

k_e was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline. The Wagner–Nelson method [29] was used to calculate the % of Solifenacin dose absorbed

$$F_{(t)} = C_{(t)} + k_e \mathrm{AUC}_{(0-t)}$$

where *F* is the amount absorbed. The percent absorbed is determined by dividing the amount absorbed at any time by the plateau value, *ke* $AUC_{(0\text{-}\alpha)}$ and multiplying this ratio by 100

$$\%\ \text{dose absorbed} = \left[\frac{C_{(t)} + k_e \mathrm{AUC}_{(0-t)}}{k_e \mathrm{AUC}_{(0-\infty)}} \right] \times 100$$

10.3 *In-vitro–In-vivo* Correlation

The data generated in the bioavailability study were reported in graphs (Figs. 1-4). Linear regression analysis was used to examine the relationship between percent of drug dissolved and percent of drug absorbed. The percent of drug un-absorbed was calculated from the percent absorbed. The slope of the best-fit line for the semi-log treatment of this data was

taken as the first order rate constant for absorption. The dissolution rate constants were determined from % released vs. the square root of time. Linear regression analysis was applied to the *In-vitro–In-vivo* correlation plots and coefficients of determination (r2), slope and intercept values were calculated.

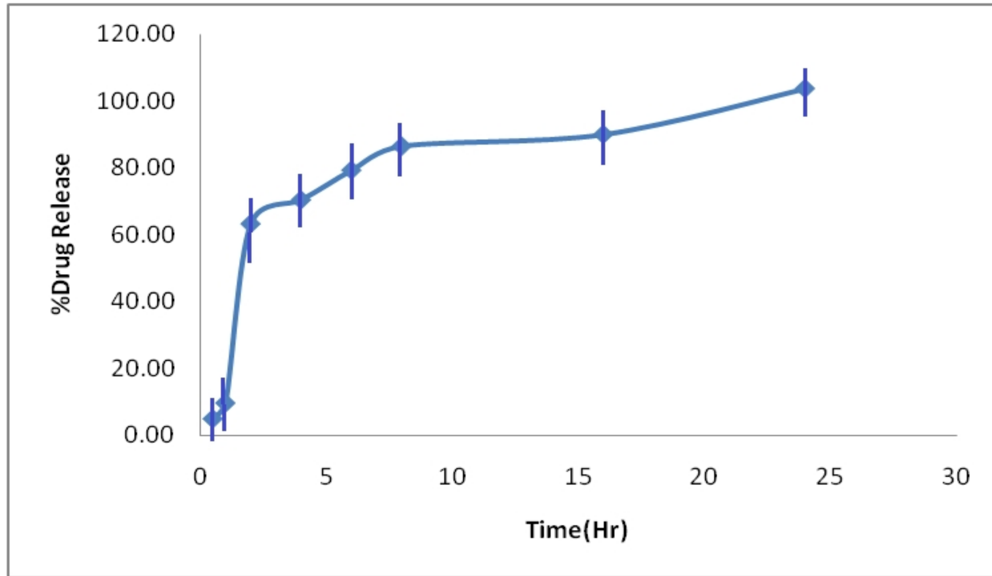

Fig. 1. ***In vitro*** **release of Solifenacin drug in dissolution media (pH6.8) at different time intravels**

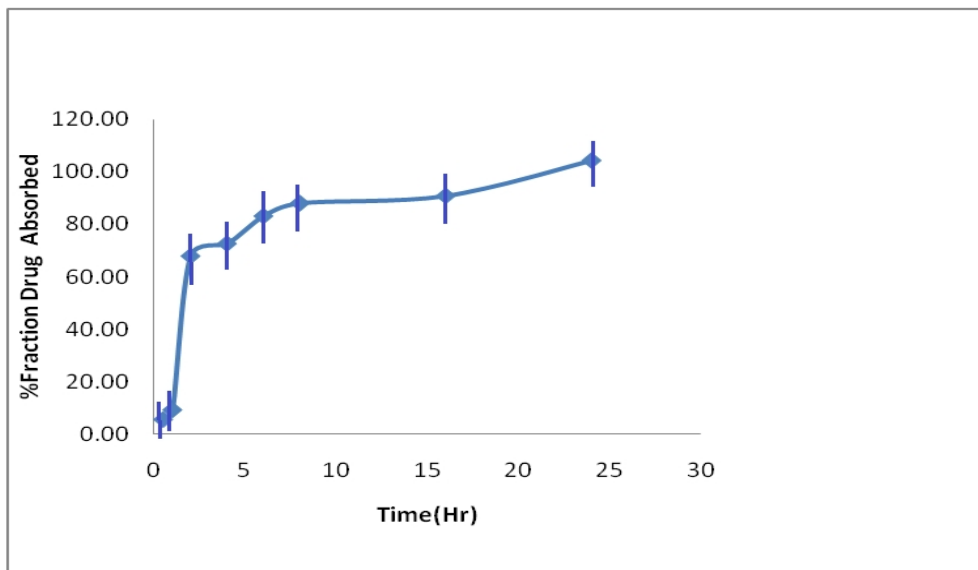

Fig. 2. ***In vivo*** **absorption of Solifenacin drug in rabbit plasma at different time intravels**

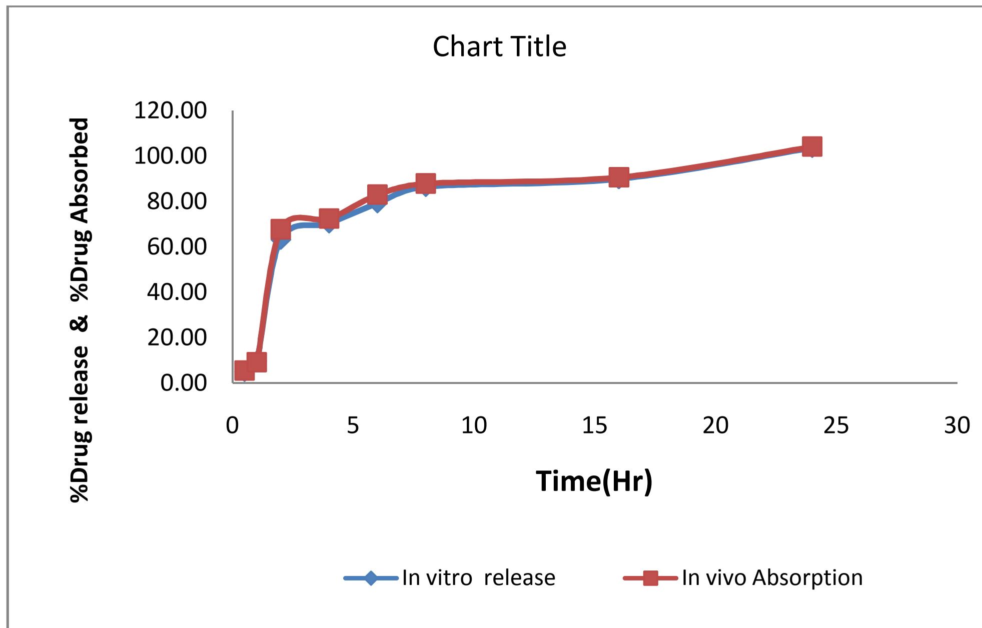

Fig. 3. ***In vitro*** **release and In vivo absorption profile of Solifenacin from modified release tablets at different time intravels**

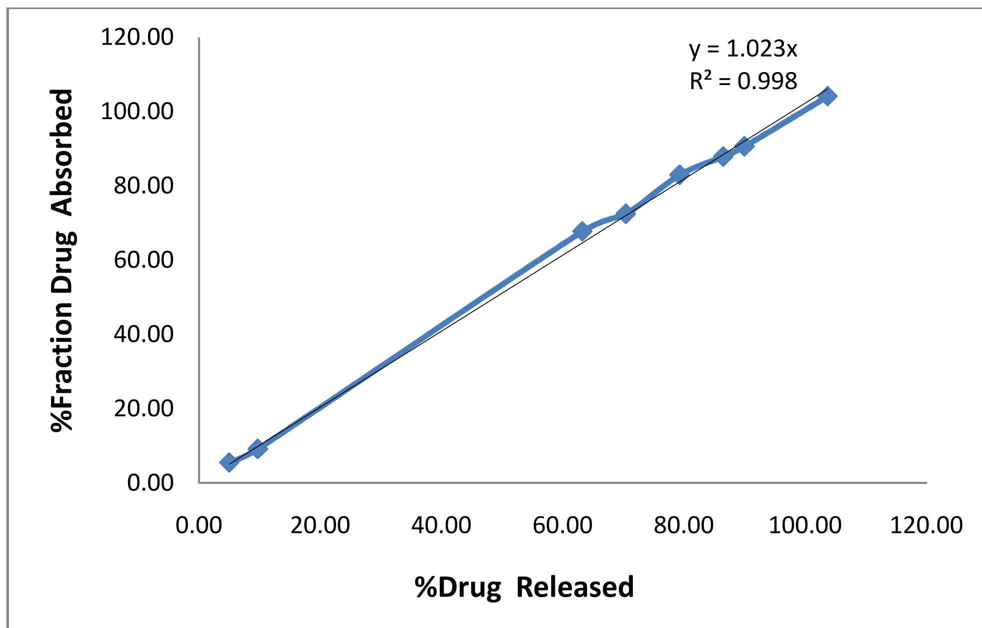

Fig. 4. Mean percentage fraction of dose absorbed ***in vivo*** **versus Mean percent released invitro for Solifenacin from modified release tablets**

11. CONCLUSION

The results indicate that there was good correlation between drug release versus absorption of dissolved drug. The *In vitro–In vivo* correlation coefficients were greater than 0.999 suggesting that a strong correlation between in vitro release and pharmacokinetic effect of a modified release Solifenacin formulation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Morales-Olivas FJ, Estan L. Solifenacin pharmacology. Arch Esp Urol. 2010;63(1):43-52.
2. Doroshyenko O, Fuhr U. Clinical pharmacokinetics and pharmacodynamics of solifenacin. Clin Pharmacokinet. 2009;48(5):281-302.
3. Hoffstetter S, Leong FC. Solifenacin succinate for the treatment of overactive bladder. Expert Opin Drug Metab Toxicol. 2009;5(3):345-50.
4. Kuipers ME, Krauwinkel WJ, Mulder H, Visser N. Solifenacin demonstrates high absolute bioavailability in healthy men. Drugs R D. 2004;5(2):73-81.
5. Leone Roberti Maggiore U, Salvatore S, Alessandri F, Remorgida V, Origoni M, Candiani M, Venturini PL, Ferrero S. Pharmacokinetics and toxicity of antimuscarinic drugs for overactive bladder treatment in females. Expert Opin Drug Metab Toxicol. 2012;8(11):1387-408.
6. Uchida T, Krauwinkel WJ, Mulder H, Smulders RA. Food does not affect the pharmacokinetics of solifenacin, a new muscarinic receptor antagonist: Results of a randomized crossover trial. Br J Clin Pharmacol. 2004;58(1):4-7.
7. Yamada S, Kuraoka S, Osano A and Ito Y. Characterization of bladder selectivity of antimuscarinic agents on the basis of *In vivo* drug-receptor binding. Int Neurourol J. 2012;16(3):107-15.
8. Maruyama S, Tsukada H, Nishiyama S, Kakiuchi T, Fukumoto D, Oku N, Yamada S. *In vivo* quantitative autoradiographic analysis of brain muscarinic receptor occupancy by antimuscarinic agents for overactive bladder treatment. J Pharmacol Exp Ther. 2008;325(3):774-81.
9. Kuipers M, Smulders R, Krauwinkel W, Hoon T. Open-label study of the safety and pharmacokinetics of solifenacin in subjects with hepatic impairment. J Pharmacol Sci. 2006;102(4):405-12.
10. Callegari E, Malhotra B, Bungay PJ, Webster R, Fenner KS, Kempshall S, LaPerle JL, Michel MC, Kay GG. A comprehensive non-clinical evaluation of the CNS penetration potential of antimuscarinic agents for the treatment of overactive bladder. Br J Clin Pharmacol. 2011;72(2):235-46.
11. Macek J, Ptacek P, Klima J. Determination of solifenacin in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2010;878(31):3327-30.

12. Mistri HN, Jangid AG, Pudage A, Rathod DM, Shrivastav PS. Highly sensitive and rapid LC-ESI-MS/MS method for the simultaneous quantification of uroselective alpha1-blocker, alfuzosin and an antimuscarinic agent, solifenacin in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2008;876(2):236-44.
13. Yanagihara T, Aoki T, Soeishi Y, Iwatsubo T, Kamimura H. Determination of solifenacin succinate, a novel muscarinic receptor antagonist, and its major metabolite in rat plasma by semi-micro high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;859(2):241-5.
14. Krishna SR, Rao BM, Rao NS. A validated rapid stability-indicating method for the determination of related substances in solifenacin succinate by ultra-fast liquid chromatography. J Chromatogr Sci. 2010;48(10):807-10.
15. Desai D, Patel G, Shukla N, Rajput S. Development and validation of stability-indicating HPLC method for solifenacin succinate: Isolation and identification of major base degradation product. Acta Chromatographica. 2012;24(3):399-418.
16. Rami Reddy BV, Srinivasa Reddy B, Raman NVVSS, Subhash Reddy K, Rambabu C. Development and validation of a specific stability indicating high performance liquid chromatographic methods for related compounds and assay of solifenacin succinate. Journal of Chemistry; 2013. Available: http://dx.doi.org/10.1155/2013/412353.
17. Diti J Desai, Greeshma Patel, Dipti Ruikar, Reshma A Jain, Sadhana J Rajput. Development and validation of stability-indicating HPTLC method of solifenacin succinate. Asian J Phar Biol Res. 2011;1(3):310-316.
18. Ann-Marie Deegan, Mark Cullen, Michael Oelgemoller, Kieran Nolan, John Tobin, Anne Morrissey. A SPE-LC-MS/MS Method for the Detection of Low Concentrations of Pharmaceuticals in Industrial Waste Streams, Analytical Letters. 2011;44(17):2808-2820.
19. FDA. Guidance for industry: Bioanalytical method validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER); 2001.
20. FDA. Guidance for industry: Food- effect bio availability and Fed Bio equivalence studies. U.S. Department of Health and Human services Food and Drug Administration Centre for Drug Evaluation and research (CDER); 2002.
21. FDA. Guidance for industry Bio availability and Fed Bio equivalence Studies for Orally Administered Drug Products-General considerations U.S. Department of Health and Human services Food and Drug Administration Centre for Drug Evaluation and research (CDER); 2003.
22. ICH, Q1A (R2). Stability testing of new drug substances and products. International Conference on Harmonisation, IFPMA Geneva; 2003.
23. ICH, Q1B. Stability testing of new drug substances and products. International Conference on Harmonisation, IFPMA Geneva; 1996.
24. ICH, Q2B. Stability testing of new drug substances and products. International Conference on Harmonisation, IFPMA Geneva; 2005.
25. Skelley JP, Amidon GL, Barr WJ, Benet LZ, Carter JE, Robinson JR, Shah VP, Yacobi A. Report of the workshop on *In-vitro* and *In-vivo* testing and correlation for oral controlled modified-release dosage forms. J Pharm Sci. 1990;79:849–854.
26. Blume HH, McGilveray JJ, Madhu KK. Bioequivalence and pharmacokinetic studies and pre-conference satellite on *In vitro–In vivo* correlation. Eur J Drug Metabol Pharmacokin. 1995;20:3–13.

27. USP 23. U.S. Pharmacopial convention. Inc., Rockville. 1995;1924–1929.
28. Available:http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070237.pdf.
29. Wagner JG. Biopharmaceutics and relevant pharmacokinetics drug intelligance publishers. Illinois; 1971.

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