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

P. Srinivasa Babu¹, SK. Rihana Parveen¹,², K. B. Chandrasekar², B. R. Challa³ and Bahlul Zayed Sh. Awen⁴

¹Vignan College of Pharmacy, Vadlamudi, Guntur, Andhrapradesh, 522213, India.
²Jawaharlal Nehru Technological University Anantapur Andhrapradesh, 515002, India.
³Vagdevi College of Pharmacy, Guntur, Andhrapradesh, 522415, India.
⁴Faculty of Pharmacy, Tripoli University, Tripoli, 13610, Libya.

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.

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.

*Corresponding author: E-mail: rihanaparveen@gmail.com, psbabu1@yahoo.co.in;
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-2-carboxylate. Solifenacin succinate is having C_{23}H_{26}N_{2}O_{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 30°C. 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.

<table>
<thead>
<tr>
<th>% dissolved of Solifenacin</th>
<th>$A_{Spl}$ x standard weight x sample dilution x % P x 100</th>
<th>$A_{Std}$ x standard dilution x 1 x 100 x LC</th>
</tr>
</thead>
</table>

Where,

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

7. PREPARATION OF TABLETS

Tablets were prepared by wet granulation technique (Phaecharomud 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

<table>
<thead>
<tr>
<th>Ingredients in mg/tablet*</th>
<th>Solifenacin succinate</th>
<th>HPMC K4M</th>
<th>SCMC</th>
<th>Guar Gum</th>
<th>Xanthan Gum</th>
<th>Lactose monohydrate</th>
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*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±0.5°C 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 25°C 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 refrigerated 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 20°C. After this centrifugation, supernatant from each sample was removed and added into respective vials and allowed for evaporation under nitrogen at 40°C 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 4°C temperature for 10 min. Plasma samples were taken and stored at −30°C 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

$$f_2 = \frac{500 \log \left\{ 1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - S_i)^2 \right\}^{-0.5} \times 100}{1}$$

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$_{(0-t)}$). The (AUC$_{(0-t)}$) was determined by the trapezoidal method. AUC$_{(0-\alpha)}$ was determined by the following equation

$$AUC_{(0-\alpha)} = AUC_{(0-t)} + \frac{C_{(\alpha)}}{k_e}$$

$k_e$ 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

$$F(t) = C'(t) + k_e AUC_{(0-t)}$$

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

$$\text{% dose absorbed} = \left[ \frac{C_{(\alpha)} + k_e AUC_{(\alpha-0)}}{k_e AUC_{(0-\alpha)}} \right] \times 100$$

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.

![Graph of Solifenacin drug release in vitro](image1)

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

![Graph of Solifenacin drug absorption in vivo](image2)

**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 intervals

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

$y = 1.023x$

$R^2 = 0.998$
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


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