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In vitro- In vivo Correlation Studies of Modified Release Tolterodine Tablet Dosage form in Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PSB and SKRP designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors KBC and BRC performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Tolterodine is an antimuscarnic drug that is used for sympathetic treatment of urinary incontinence. Tolterodine modified release tablet, was investigated in rabbit for pharmacokinetic and *in vitro–in vivo* correlation studies. Tablets were prepared and in vitro release was studied in simulated gastric fluid at 150RPMs. New Zealand albino male rabbits have been used as animal model for in vivo study. A sensitive and simple HPLC method was developed for the determination of Tolterodine content in rabbit plasma. In vitro release studies showed that release patterns followed zero order for around 24h. 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 and HPLC analysis of rabbit's plasma.
From the in vitro–in vivo correlation prediction it was evident that the Tolterodine matrix assisted tablet is a good for controlled delivery of Tolterodine.

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1. INTRODUCTION

Tolterodine tartarate is chemically (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen tartrate. The molecular formula is $C_{26}H_{37}NO_7$ with a molecular weight of 473.58. Tolterodine is a competitive muscarinic receptor antagonist. Both urinary bladder contraction and salivation are mediated via cholinergic muscarinic receptors. After oral administration, tolterodine is metabolized in the liver, resulting in the formation of the 5-hydroxymethyl derivative, a major pharmacologically active metabolite. The 5-hydroxymethyl metabolite, which exhibits an antimuscarinic activity similar to that of tolterodine, contributes significantly to the therapeutic effect. Both tolterodine and the 5-hydroxymethyl metabolite exhibit a high specificity for muscarinic receptors, since either show negligible activity or affinity for other neurotransmitter receptors and other potential cellular targets, such as calcium channels [1-4]. Tolterodine has a pronounced effect on bladder function. The main effects of tolterodine are an increase in residual urine, reflecting an incomplete emptying of the bladder, and a decrease in detrusor pressure, consistent with an anti muscarinic action on the lower urinary tract [5].

Tolterodine and its active metabolite, 5-hydroxymethyltolterodine, act as competitive antagonists at muscarinic receptors. Cmax and area under the concentration-time curve (AUC) determined after dosage of tolterodine are dose-proportional over the range of 1 to 4 mg. Based on the sum of unbound serum concentrations of tolterodine and the 5-hydroxymethyl metabolite ("active moiety"), the AUC of tolterodine extended release 4 mg daily is equivalent to tolterodine immediate release 4 mg (2mg bid). Cmax and Cmin levels of tolterodine release are about 75% and 150% of tolterodine immediate release, respectively. Maximum serum concentrations of tolterodine extended release are observed 2 to 6 hours after dose administration [6-9].

Literature survey shows that, a few analytical methods were reported for quantification of tolterodine by using capillary electrophorsis [10], HPLC [11-14], UPLC [15], GC-MS [16], LC-MS [17-22,30]. Among all, quantification was done in pharmaceutical compounds [10-14], Biological [15-22,30]. As of now, there were no methods reported for *in vitro–in vivo* correlation of tolterodine tablets. The aim of the present study is to correlate the relation of *in vitro* dissolution and *in vivo* absorption of tolterodine tablet dosage form [23-25].

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 [26].

Correlation between *in vitro* testing and *in vivo* performance are encouraged and guide lines were published in the proceedings of a controlled release workshop [27] and a chapter about *in vitro* and *in vivo* evaluation of the dosage forms is included in USP [28,29]. *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. The IVIVC guidelines presents a comprehensive perspective on (1) methods of developing an IVIVC and evaluating its predictability; (2) using an IVIVC to set dissolution specifications; and (3) applying an IVIVC as a surrogate for *in vivo* bioequivalence when it is necessary to document bioequivalence during the initial approval process or because of certain pre or post approval changes (e.g. formulation, equipment, process and manufacturing site changes). This study was designed by fabricating a matrix tablet for modified release of Tolterodine.

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 Tolterodine 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. CHEMICALS AND REAGENTS

Tolterodine tartarate was received from Aurobindo Pharma Lt.d, Hyderabad, India., India as a gift sample. Acetonitrile, methanol (HPLC grade) obtained from J.T. Baker, Mumbai, Phosphoric acid (85%) (HPLC grade), Potassium Phosphate Monobasic, (ACS Grade), obtained from Merck, Mumbai. Milli Q water (HPLC Grade).

3. APPARATUS AND EQUIPMENT

A chromatographic system (Shimadzu Corporation, Japan) model Shimadzu VP, consisted of a system controller (CLASS-VP), on-line degasser (LC 2010C, Shimadzu), solvent delivery module (LC 2010C, Shimadzu), auto injector (LC 2010C, Shimadzu), column oven (LC 2010, Shimadzu), UV-VIS detector (LC 2010C UV PHARMASPEC 1700), Shimadzu and CLAS-VP software version=SPI, low pressure gradient pump, auto injector (SIL-10AD VP, Shimadzu), column oven (CTO-10AS VP, Shimadzu) and PDA detector (PDA-SPD-M10A VP, Shimadzu Diode Array Detector) and chemstation (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 0.45µm HV membrane filter and Sample filtration kit used as Millipore, Millex-HV, PVDF, 0.45µm, 13mm filter thermocouple.

4. PREPARATION OF SOLUTIONS

4.1 Mobile Phase Preparation

Solution A: Dissolve about 2.88g of Ammonium phosphate monobasic into 1000mL of water.

Solution B: Methanol. Mix 400ml of solution A with 600ml of solution B. Add 5ml of TEA. Adjust final pH to 7.0±0.1 with 85% Phosphoric acid. Filter and de-aerate.

Sample solution preparation: Mix of 1N NaOH: Dissolve 4g of sodium hydroxide in 100ml of water.

4.2 Dissolution Medium Preparation

Dissolution Medium: 0.05M Phosphate Buffer, pH 6.8 Dissolve 40.8g of KH_2PO_4 and 5.5g of NaOH in 6L of USP Purified water, adjust pH to 6.8±0.05 with 1N NaOH or 85% H_3PO_4.

De-aeration Mode: Helium gas.

5. CHROMATOGRAPHIC CONDITIONS

The chromatography was performed on Zorbax Eclipse XDB-C18 ([15cm x 4.6mm] 3.5μ) analytical column at 1.0ml/min flow rate of mobile phase with isocratic mode. The injection volume was 10.0μL, Column oven temperature was at 35ºC. Detection at 220nm, and chromatographic run time was 6.0min for each sample. Prior to injection of the drug solution, the column was equilibrated for at least 10min with the initial time gradient mobile phase conditions flowing through the system.

6. PREPARATION OF STANDARD SOLUTION

The Standard stock solution of Tolterodine tartrate was prepared by dissolving 44mg in 200mL standard volumetric flask dissolve and dilute to volume with methanol and mix. Further 2ml of standard stock diluted to 100ml of with dissolution medium to obtain the concentration of 44μg/mL. Filter a portion through the sample filtration kit into autosampler vials. Discard a minimum of 3ml of the filtrate prior to collecting for analysis.

% Dissolved of tolterodine	A_{Spl}x mg StdxfxDx900x100
	A_{Std}x200x100xLC

Where,

A_{Spl} =Area of Tolterodine peak in test sample
A_{Std} =Area of Tolterodine peak in reference standard sample
f =Correction factor for potency, expressed as the percentage of Tolterodine tartrate in Std $x10^{-2}$
LC =Lable claim in mg.
D =Standard dilution 2ml for 4mg Tablet.

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 40ºC for one hour thereafter kept in the desiccators for 12hr at room temperature. The granules retained in 44mesh, after drying these granules were mixed with fines (granules that passed through 44mesh). 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 flow ability. Matrix tablets were compressed into (weighing about 180mg using 2.8mm) shallow biconcave punches in Cadmach rotary

tablet punching machine to a hardness of 5-6kg/cm2. The prepared matrix tablets were used for further evaluation studies.

Table 1. Composition of matrix tablets of tolterodine tartrate

Ingredients in mg/tablet*	Tolterodine tartrate	HPMC K4M	SCMC	Guar gum	Xanthan gum	Lactose monohydrate
F1	4	15	--	--	--	157.4
F2	4	30	--	--	--	142.4
F3	4	45	--	--	--	127.4
F4	4	60	--	--	--	112.4
F5	4	--	15	--	--	157.4
F6	4	--	30	--	--	142.4
F7	4	--	45	--	--	127.4
F8	4	--	60	--	--	112.4
F9	4	--	--	15	--	157.4
F10	4	--	--	30	--	142.4
F11	4	--	--	45	--	127.4
F12	4	--	--	60	--	112.4
F13	4	--	--	--	15	157.4
F14	4	--	--	--	30	142.4
F15	4	--	--	--	45	127.4
F16	4	--	--	--	60	112.4

**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. F=Formulation; F1-F16=Different formulations from 1 to 16*

8. *IN VITRO* STUDY

In vitro release studies were performed using USP II Dissolution Testing Apparatus (Electrolab TDT-08L) in simulated gastric fluid at 150RPM, as rotating speed of stirrers. 900 ml of dissolution medium was maintained at 37±0.5ºC for 24hr dissolution study [29] Tablets were placed at the bottom of the baskets. 1ml of samples were withdrawn at 1, 2, 4, 6, 8, 12, 16, 18 and 24hr and the aliquots withdrawn were replaced with fresh dissolution medium. The samples were filtered and assayed spectrometrically at 220nm. All release studies have been performed in triplicate designated to get the confirmation about release pattern.

9. *IN VIVO* STUDY

9.1 Animals

In vivo studies were carried out on Six New Zealand albino male rabbits weighing between 1.25 and 1.5kg. 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. Tolterodine tablet (4mg equivalent dose) was administered orally. All studies were performed after keeping rabbits for overnight fasting.

9.2 Drug Standard Solutions

Standard stock solutions (10ml) of Tolterodine were prepared in methanol at a concentration of 1mg/ml and kept at -30ºC. Intermediary solutions of Tolterodine were prepared in methanol. All calibration curve samples (non-zero samples), except blank plasma were prepared by spiking three different blank plasma batches. From tolterodine stock solution of calibration standards were prepared at 10.00, 20.00, 30.00, 40.00, 50.00, 60.00µg/ mL.

9.3 Sample Extraction from Animal Plasma

Liquid-liquid extraction was used to isolate Tolterodine from Rabbit plasma. 50µL of IS (50.00µg/mL), and 100µL of plasma sample (respective concentration) were added into labelled polypropylene tubes placed in ice bath and vortex briefly. Then 2.5mL of extraction solvent (methyl t-butyl ether) was added, closed with tight caps and vortexed for 10min followed by Centrifuge at 4000rpm, 10min at 20ºC temperature. Then the supernatant from each sample was transfered into labelled polypropylene tubes. After that, the samples were Evaporated at 40ºC under nitrogen to dryness. Followed by, the dried residue samples were reconstituted with 100µL of reconstitution solution (acetonitrile: 10mM ammonium acetate 80:20v/v) and vortexed briefly. Finally, sample from each tube was transfered into auto sampler vials with microinserts for injection (10µL) for HPLC analysis.

9.4 In vivo Release and Pharmacokinetic Analysis

Blood samples of 0.3ml were collected at the interval of 1, 2, 4, 6, 8, 12, 16, 18 and 24 hours in heparinized Eppendorf tubes after administration. These samples were centrifuged immediately at 3500rpm and 4ºC temperature for 10min. 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) were calculated following non-compartment model of Win Non-Lin 5.1. All the parameters were calculated for oral administration of Tolterodine 4mg Tablets.

9.5 In vitro Dissolution Data Analysis

The dissolution profiles for each formulation were determined by plotting the cumulative percent of tolterodine 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.

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

9.6 In vivo Data Analysis

The tolterodine 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. $AUC_{(0-\alpha)}$ was determined by the following equation k_e was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline.

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

The Wagner–Nelson method [31] was used to calculate the percentage of the tolterodine dose absorbed where *F* is the amount absorbed. The percent absorbed is determined by dividing the amount absorbed at any time by the plateau value $AUC_{(0-\alpha)}$, $AUC_{(0-\alpha)}$ and multiplying this ratio by 100.

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

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

9.7 *In-vitro–In-vivo* Correlation

The data generated in the bioavailability study were used to develop the IVIVC (Figs.1-4). The percent of drug dissolved was determined using the aforementioned dissolution testing method and the fraction of drug absorbed was determined using the method of Wagner–Nelson [30].

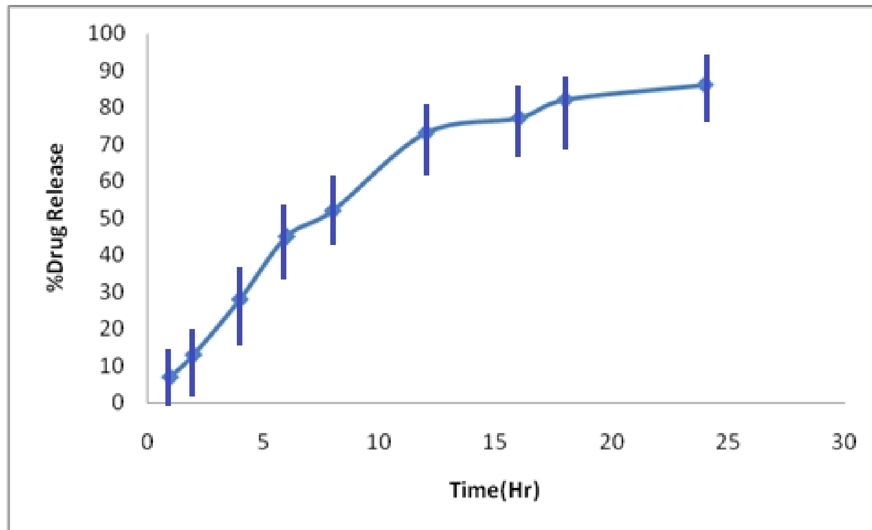

Fig. 1. *In vitro* release of tolterodine drug in dissolution media (pH6.8) at different time intravels

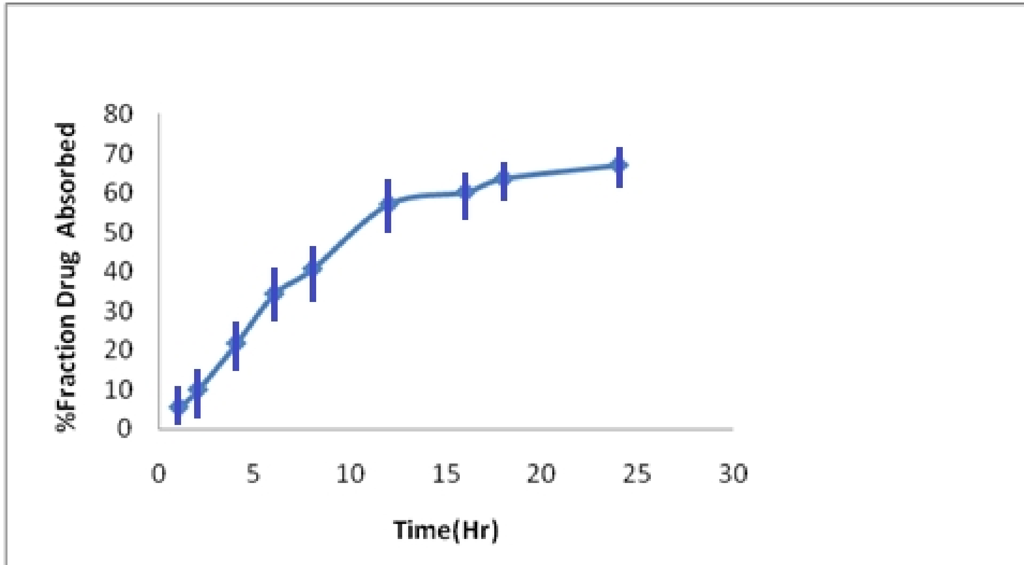

Fig. 2. ***In vivo*** **absorption of tolterodine drug in rabbit plasma at different time intravels**

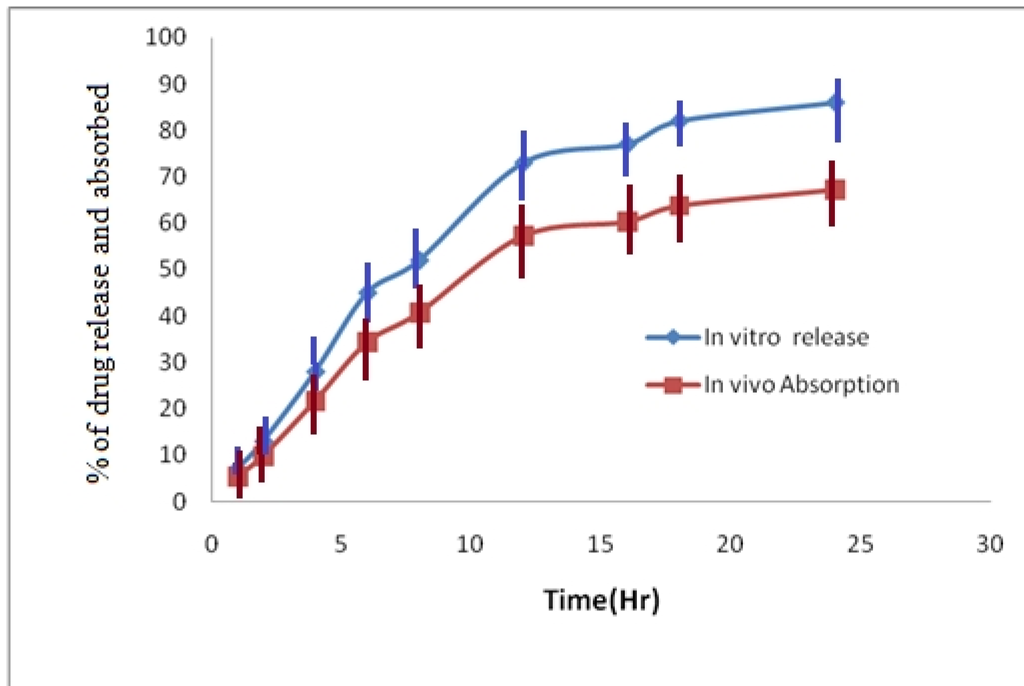

Fig. 3. ***In vitro*** **release and** ***In vivo*** **absorption profile of tolterodine from modified release tablets at different time intravels**

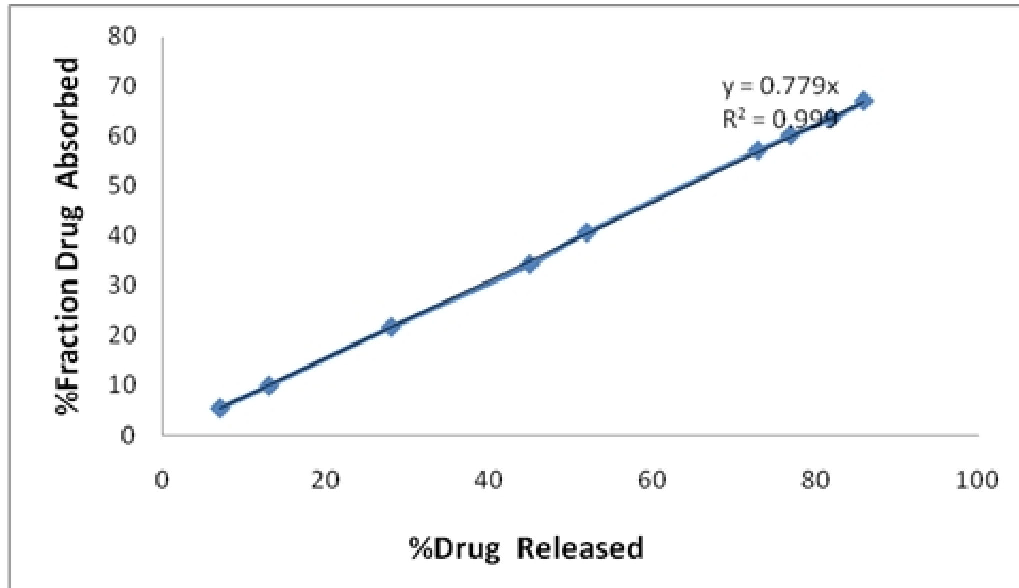

Fig. 4. Mean percentage fraction of dose absorbed *In vivo* versus mean percent released *In vitro* for tolterodine from modified release tablets

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.

10. 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 Tolterodine formulation.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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