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RESEARCH ARTICLE

DESIGN AND EVALUATION OF ZERO ORDER ONCE DAILY ENTERIC EXTENDED RELEASE MATRIX TABLET CONTAINING OXYBUTYNIN HYDROCHLORIDE

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ABSTRACT

Oxybutynin Hydrochloride is antimuscarinic drug used for the management of urinary incontinence. Being a freely water soluble drug, with half-life approximately three hours, making it a good candidate for controlled-release formulations. Enteric Extended-release hydrophilic matrix tablets were prepared by direct compression using a number of hydrophilic polymers. All the compressed formulations were evaluated. Kinetic analysis of the release was performed. Pharmacokinetic parameters of Oxybutynin HCl from the optimized zero order tablet formula (F#24L) was compared to the immediate release innovator "Ditropan®" in human volunteers using a randomized crossover design on a release profile over 48 hours. The optimized tablet formulation was F#24L (containing 50% CMC Na 2000 cps) with sub-coat and enteric coat.

Keywords: Oxybutynin Hydrochloride, Extended Release Tablet, Hydrophilic Matrix Polymer, Carboxymethylcellulose Sodium, Enteric coat, HPMCP-55.

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1. INTRODUCTION

Oxybutynin Hydrochloride, a tertiary amine antimuscarinic, used for the management of urinary frequency and incontinence in bladder disorders and as an adjunct to other therapies for enuresis [1]. Oxybutynin undergoes extensive first-pass metabolism, mainly by the cytochrome P450 and systemic oral bioavailability has been reported to be only 6% [2]. Oxybutynin Hydrochloride is a freely water soluble drug with half-life approximately three hours. The drug is administered three to four times daily. Attempts were made for obtaining controlled release formulations which could give consistent release profile of the drug, reduce the dose dependency, and improve patient compliance [3] as well as to reduce incidence of both local and systemic side effects [4].

Hydrophilic polymers have attracted attention as controlled release excipients for the delivery of water-soluble and water-insoluble drugs [5]. Hydrophilic matrix tablets are monolithic tablets made of swellable, slowly dissolving hydrophilic polymers in which the drug is homogeneously dispersed [6]. Viscosity properties of the polymers are of great importance in determining the final release profile of the matrix tablet [7]. Generally, the drug-release rate is slower for the higher viscosity grade polymer [8].

Tablet Coating is a process used to give specific benefits that mostly ranges from facilitating product identification to modifying drug release from the dosage form [9].

The aim of this study was to prepare extended-release Oxybutynin HCl matrix tablets using hydrophilic polymers like cellulose derivatives as Sodium carboxymethylcellulose (CMC Na), natural polysaccharide as Chitosan, synthetic polymethacrylate copolymer like Eudragit S100, Eudragit L100, Eudragit L 100-55 and carboxyvinyl polymer as Carbomer P940. The optimized tablet formulations were F#24L (containing 50% CMC Na 2000 cps) and F#25L (containing 10% Chitosan). The effect of shelf storage on the optimized tablets formulations after packing in blisters for twelve months at temperature and humidity of 30°C ± 2°C/65%RH ± 5% was performed. Also, accelerated stability study complying with ICH guidelines at temperature 40°C ± 2°C and humidity of 75%RH ± 5% was performed for six months (Muthu, Feng, 2009) [10]. *In-vivo* pharmacokinetic study was carried out to compare the optimized zero order release formula (F#24L) with the market product

2. MATERIALS AND METHODS

Oxybutynin hydrochloride was supplied by Sinochem Jiangsu, China. Carboxymethylcellulose Sodium (2000 cps) was supplied by CP Kelco, USA. Carbomer P940 was supplied by Aqualon, USA. Eudragit S100, Eudragit L100, Eudragit L100-55, Aerosil 200 were supplied by Degussa Ltd, Germany. Magnesium stearate and Calcium Phosphate Dibasic were supplied by Acto-Corp, New York, USA. Hydroxypropylmethylcellulose Phthalate (HPMCP-55) was supplied by Acros Organics, New Jersy, USA. Hydroxypropylmethylcellulose 15 cp was supplied by Hangzhou Zhongbao, China. PEG 6000 was supplied by Basf, Germany. Dibutyl Phthalate was supplied by Spectrum Quality Products, Inc. Gardena, USA. Potassium

dihydrogen orthophosphate, Sodium hydroxide were supplied by Adwic, El-Nasr pharmaceutical chemicals Co., Cairo, Egypt. Hydrochloric acid was supplied by (Merck Kgaa, Damstadt, Germany). Water for HPLC and Acetonitrile HPLC grade was supplied by Merck, Germany. Standard Gabapentin powder 99.3% and Formic acid were supplied by Sigma, Germany. Ultra pure water was supplied by Aquatron, UK. Ditropan® 5mg tablet was supplied by Sanofi-Aventis, France. Universal balance AUY 220, Schimadzu, Japan. High performance liquid chromatography (UFLC Prominence 20 XR), supplied with UV-Vis detector), Schimadzu, Japan. Millipore membrane (0.45 µm), Millipore Company, USA. Tablet hardness tester, Friability tester, Dissolution apparatus, Sieves (250 µm, 180 µm and 710 µm), Erweka, Germany. Korsch XP1, Single punch tablet machine, Korsch, Germany. Magnetic stirrer (PMC), Barcelona, Spain. Mixer, IKA-RW 20 digital, IKA, Germany. pH-meter, Jenway, England. Manual coating pan, Chamunda, India. UPLC-MS-MS: Aquity-Xevo TQD, Waters, USA. Vortex mixer, Stuart, UK. Cooling Centrifuge, Centurion, UK.

FORMULATION

Preparation of Oxybutynin Hydrochloride Hydrophobic Matrix tablets

Different tablet formulations containing of 15 mg oxybutynin Hydrochloride were prepared as shown in table (1-3) [11]. Polymers were used at three concentrations (30, 50 and 70%) except carbomer P940 (5, 10, 15 and 20%) and chitosan (10, 30 and 50%). The formulations were prepared using direct compression technique.

Direct Compression Technique

The drug, hydrophilic matrix polymer (Carboxymethylcellulose Sodium , Carbomer P940, Eudragit S100, Eudragit L100, Eudragit L100-55 and Chitosan) and diluent (Dibasic calcium phosphate dihydrate) were mixed using mortar and pestle to form dry powder mixture. The glidant (0.25% Aerosil 200) and lubricant (1% Magnesium stearate), sieved on sieve 180 µm, were added. Dry blend formulations were compressed using KORSCH XP1 single punch tablet machine with diameter (7 mm round) into 150 mg tablets. The force of compression was kept constant all over the experiment.

COATING

Preparation of sub-coating solution

A protective sub-coat of HPMC 15 cps as film former (0.5% of the tablet core weight) using Polyethylene glycol 6000 as plasticizer was applied.

Preparation of enteric coating solution

Enteric coat of HPMCP-55 (2% of total tablet weight) was subsequently applied after sub-coat using dibutyl phthalate as plasticizer. These coating solutions are applied over tablets using spray gun at appropriate pressure using manual coating pan. The coated tablets are primarily dried using heat blower and secondarily dried in tray drier [11, 12].

Pan coating parameters:

- Speed of coating pan: 12 rpm.
- Spray nozzle: 0.8 mm.
- Spray pressure: 2 bar.
- Type of spraying: continuous.
- Inlet air temperature: 50 ^{O}C.
- Outlet temperature: 35 ^{O}C.
- Spraying time: 60 min.
- Spraying rate: 20 gm/min.

The tablet formulations are shown in table 1 -3.

CHARACTERIZATION OF COMPRESSED TABLETS

Uniformity of Weight

Twenty tablets, from each formula, were individually weighed and the mean tablet weight was calculated. Results are presented as mean value ± standard deviation (SD) [13].

Uniformity of Content

A sample of ten tablets, from each formula was individually assayed for drug content uniformity. Each individual tablet content must be between 90 and 110% of the average content and the tablet formula fails to conform with the test if more than one individual tablet content is outside these limits or if one individual content is outside the limits of 75 to 125% of the average content [12].

Tablet Friability

Ten tablets, from each formulation, were precisely weighed and placed in the drum of friabilator (Erweka type, TAR200, Germany). The tablets were rotated at 25 rpm for a time of 4 min and then detached, de-dusted and accurately re-weighed. The percentage loss in weight was calculated and taken as a measure of friability [14].

In-vitro Release Studies

The dissolution profiles of Oxybutynin hydrochloride in each tablet formulations were determined in a dissolution tester (Erweka DT-700 Dissolution Tester, Germany) following the USP paddle method at a rotation of 50 rpm. . Studies were carried out at 37 ± 0.5ºC in 900 ml of 0.1 N HCl (pH = 1.2) for a phase of two hours and then continued in 900 ml of phosphate buffer pH = 6 (USP) containing 0.2% Sodium lauryl sulfate for twelve hours after shifting the pH from (pH = 1.2) to (pH = 6) [12]. . Ten ml samples were taken after 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 hours. The samples were filtered and analyzed for Oxybutynin Hydrochloride content by high performance liquid chromatographic assay method. The analysis was done on Shimadzu (UFLC. Prominence 20XR) connected with U.V detector; using column ACE (C18/CN) (5µ x 4.6 x 100 mm). The mobile phase was isocratic consisted of phosphate buffer pH 3.2: acetonitrile in ratio of (60: 40 v/v) and was delivered to the system at a flow rate of 1.5 ml/min, with an injection volume of 200 µl and the detection wavelength (λ_{max}) was 220 nm.

Kinetic Analysis of the Release Data

In order to determine the release model, the *in-vitro* release data were analyzed according to zero order, first order and diffusion controlled mechanism according to Higuchi model. The large value of the coefficient of determination (r^2) indicated a superiority of the dissolution profile fitting to mathematical equations [15].

Stability study of the selected Oxybutynin HCl extended-release formulations

Formulations (F#24L) (containing 50% CMC Na) and (F#25L) (containing 10% Chitosan) were selected for further stability studies based on the previous release studies adopting two storage conditions.

Accelerated stability testing

The effect of storage in stability cabinet at high temperature and humidity (40°C ± 2°C/75%RH ± 5%) for a period of six months in the final pack (blisters) was studied. During the storage period, samples were taken periodically after 15 days, 1, 2, 3, 4, 5 and 6 months.

Shelf stability testing

The effect of shelf storage in stability cabinet at (30°C ± 2°C/65%RH ± 5%) for twelve months in the final pack (blisters) was studied. During the storage period, samples were taken periodically after 3, 6, 9 and 12 months.

Evaluation of the stored tablets

Visual inspection

Visual inspection of the stored tablets was carried out at the different time intervals for any change in color or physical appearance.

Quality control tests

Quality control tests as weight variation and friability were carried out to assess the physical stability of the stored tablets.

Effect of storage conditions on content

Chemical analysis of the stored tablets was carried out to determine the amount of Oxybutynin HCl remaining at the end of each time interval as described before.

***In-Vitro* release study**

In-vitro release study has been conducted for the stored tablets at the predetermined time intervals as described before.

***In-Vivo* Performance of Optimized Oxybutynin Hydrochloride Extended-Release Hydrophilic Matrix Tablet**

Human Volunteers

Subjects participating in this study were normal healthy adult male volunteers (n = 6). All subjects were given an appropriate physical examination, as well as clinical laboratory tests (blood and urine).

3. METHODOLOGY

***In-vivo* evaluation for selected Oxybutynin HCl hydrophilic matrix tablet formula (F#24L)**

a) Experimental Design

Cross-over design was adopted for the evaluation of pharmacokinetics parameters for two formulae using 6 human volunteers divided into two groups each time (n = 3/group). The two formulations used were:

- Ditropan ® tablets (reference standard) containing 5 mg Oxybutynin HCl.
- Oxybutynin HCl Hydrophilic enteric extended-release matrix tablet formula (F#24L) containing 15 mg Oxybutynin HCl.

Each treatment was administered to human volunteers after an overnight fast on two periods separated by a one week washout period between treatments. The sample size (n = 6) was selected not based on statistical consideration but rather on economic consideration.

b) Formulations administration

The volunteers were asked to fast for 12 hours with free water access. The drug product is taken with 240 ml of water. Volunteers should continue to fast for at least 4 hours post-dose.

Compound Name	Precursor Ion	Product ion	RT	Cone	Collision energy
Oxybutynin HCl	358.5	142.6	0.97	50	35
Gabapentin	172	67.5	0.41	40	25

c) Sample collection

Blood samples (5 ml) for assay of plasma concentrations of Oxybutynin HCl were obtained via the indwelling cannula at once previous to dosing and at the time of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 and 48 hrs after oral administration. They were put into polyethylene heparinated tubes and were immediately centrifuged at 3000 rpm for 5 minutes. The plasma obtained was frozen at -20^{0}C in labeled polypropylene tubes until LC-MS/MS analysis.

d) LC-MS/ MS assay of Oxybutynin HCl

1. Chromatographic conditions:

- **Mobile phase:** Acetonitrile: 0.1 M formic acid in the ratio of (50:50 v/v).

- **Column:** Acquity UPLC HSS T3 1.8 um 2.1 X 50 mm.
- **Column temperature:** 40°C.
- **Injection Volume:** 10 µl.
- **Detector:** Mass spectrometer: Xevo TQD.
- **Internal standard:** Gabapentin.
- **Flow rate:** 0.3 ml / min.
- **Run time:** 2 minutes.

2. Mass Spectroscopy parameters:

The Xevo TQD operated in positive electrospray ionization mode. General MS parameters:

3. Preparation of Stock and Standard solutions:

4. A. Oxybutynin HCl Stock solutions:

A concentrated stock solution was prepared in methanol, accurately weighed 25 mg oxybutynin HCl were transferred into 50 ml volumetric flask then diluted to volume with methanol (Solution A). This solution contains 500 µg/ ml.

0.5 ml from solution A was transferred into 25 ml volumetric flask and diluted to volume with blank plasma. (Solution B). This solution contains (10 µg/ ml).

0.1 ml from solution A was transferred into 10 ml volumetric flask and diluted to volume with blank plasma. (Solution C). This solution contains (100 ng/ ml).

B. Gabapentin stock solutions

Accurately weighed 25 mg were transferred into 50 ml volumetric flask and the volume was completed with methanol (Solution D). This solution contains 500 µg/ ml.

0.5 ml from solution (D) were transferred to 25 ml volumetric flask then diluted to volume by methanol (Solution E). This solution contains 10 µg/ml.

0.1ml from solution D was transferred into 10 ml volumetric flask and diluted to volume with blank plasma. (Solution F). This solution contains (100 ng/ ml).

C. Preparation of calibrators: Calibration standards in human plasma for constructing the calibration graphs containing Oxybutynin HCl

Solution C was further diluted with blank plasma to obtain concentrations of 0.025, 0.05, 0.2, 0.5,1, 2.5, 5 and 10 ng /ml.

D. Preparation of Quality Control (QC) Samples

QC samples were prepared to cover **low** (0.075 ng/ml), **medium** (4 ng/ml) and **high** (8 ng/ml) Oxybutynin HCl concentrations.

Sample Preparation:

1. 10 µl of Gabapentin as internal standard "containing 100 ng /ml" was added to 500 µl plasma.
2. Samples were extracted using 6 ml (Ethyl acetate: diethyl ether: n- Hexane) (1:1:1 v/v/v), after mixing (30 s), the mixture was centrifuged for 10 minutes at speed of 45 X 10^3 rpm.
3. 5 ml of the clear supernatant was evaporated and the residue was reconstituted with 600 µl mobile phase and injected into the liquid chromatography after filtration through Whatman Filter paper No. 22.
4. Peaks were detected by MS/MS detector and interpreted in the form of reported peaks areas.
5. Responses of Oxybutynin HCl referred to Gabapentin were recorded.
6. Quantitation: Concentrations of Oxybutynin HCl in unknown samples were calculated from the prepared calibration curve.

   Series of standard plasma solutions, previously spiked with Oxybutynin HCl, were employed as calibrators for constructing calibration growth curves covering the concentrations ranging 0.025 – 10 ng/ml. The analytes were extracted using the method described earlier.

E. Assay method validation

I. Selectivity

Plasma samples (six blanks) were chromatographed prior to use to check for endogenous components, which might interfere with Oxybutynin HCl or Gabapentin. Spiked plasma samples were also analysed to verify the selectivity of the method of analysis.

II. Linearity and linear working range

The linearity and linear working range were determined from the constructed standard calibration curve.

III. Intra-day accuracy and precision

The intra-day accuracy and precision evaluations were assessed by repeated analysis of human plasma samples containing different concentrations of Oxybutynin HCl on separate occasions in the same day (3 times on the same day). The analytical precision of the method determined by the relative standard deviation of the percentage drug recovered.

IV. Inter-day accuracy and precision

The day to day reproducibility of the assay for plasma samples was evaluated by comparing the least squares linear regression analysis of three standard plots obtained from spiked plasma standards at three different days.

V. Freeze and Thaw Stability in human plasma

Oxybutynin HCl stability in human plasma was determined after three freeze-thaw cycles. In order to determine the effect of storage on the stability of frozen samples, three concentrations were selected representing the low, medium and high spiked plasma samples of the calibration curve. These concentrations were analyzed when fresh and after storage just prior to analysis (storage was at -20°C for 24 hours). The freeze-thaw cycle were repeated two more times, and then analyzed using the previously validated method.

VI. Extraction recovery

Relative recoveries of low, medium and high concentrations of Oxybutynin HCl used in the plasma standards were evaluated by comparing their peak area ratios with those obtained from direct injection of unprocessed reference solutions of the same concentrations.

F. Pharmacokinetics (PK) analysis

Protocol of this study [Serial number PI (810)] was reviewed and approved by the Research Ethics Committee for Experimental and Clinical Studies at Faculty of Pharmacy, Cairo University, Egypt.

After oral administration of the two treatments of Oxybutynin HCl in two different occasions; pharmacokinetic parameters were determined from plasma concentration time profiles by means of non-compartmental analysis using Kinetica® (version 5, Thermo Fischer Scientific). Maximum Oxybutynin HCl concentration (C_{max} ng/ml), and time required to reach it maximum Oxybutynin HCl concentration (T_{max} hr.) were obtained from the individual plasma concentration time curves. The area under the plasma concentration–time curve $AUC_{(0 \text{ to } \infty)}$ was subdivided into $AUC_{(0 \text{ to } t)}$ (was determined as the area under the plasma concentration–time curve up to the last measured sampling time and calculated by the linear trapezoidal rule), and area under the curve from Ct to infinity $AUC_{(t \text{ to}}$

$_{\infty)}$; where AUC $_{(0 \text{ to } \infty)}$ = AUC $_{(0 \text{ to } t)}$ + Ct/ K (Ct is the last sample concentration at time t). The optimized formulae: Oxybutynin HCl hydrophilic matrix tablet formula (F#24L) and the reference treatment (Ditropan®) were compared with respect to the relevant pharmacokinetic parameters. The pharmacokinetic parameters C_{max}, AUC $_{(0 \text{ to } t)}$ and AUC $_{(0 \text{ to } \infty)}$ were compared between treatments statistically using Two-way analysis of variance (ANOVA) with a level of significance of 0.05 and a p-value smaller than 0.05 was considered statistically significant. Non parametric Mann Whitney test was employed for the evaluation of the median T_{max} and MRT data. The two way ANOVA and the Mann Whitney test were carried out using the software SPSS®17. The percent relative bioavailability (F) of Oxybutynin HCl from the tested formula in comparison to reference formulation (Ditropan® 5 mg tablet) was calculated with respect to AUC $_{(0 \text{ to } t)}$ and AUC $_{(0 \text{ to } \infty)}$.

$$F = \frac{\text{AUC test / Dose}}{\text{AUC reference / Dose}} \times 100$$

4. RESULTS AND DISCUSSION

Characterization of Oxybutynin Hydrochloride Tablets formulations

Enteric extended release matrix tablets of Oxybutynin Hydrochloride were formulated using release retardant hydrophilic polymers. Calcium phosphate dibasic was incorporated in the tablets to improve the compression properties and to further retard the release rate by its hydrophobic properties [16]. The physicochemical properties of all the prepared tablets complied with the pharmacopoeial specifications of weight variation with relative standard deviation ranged from 1-5% for the different formulations. The mean percent of Oxybutynin Hydrochloride content in the compressed tablets was found to be more than 90% for all formulations. The percentage friability for all the formulations was less than 1% indicating a good mechanical resistance. The mean tablet weight, friability and drug content and for the prepared tablets are shown in table 4.

In-vitro Release Studies

The United State Pharmacopeia for extended-release Oxybutynin Hydrochloride tablet state that NMT 10% of the drug would be released after 2 hours; 10-40% after 4 hours; 40-75% after 6 hours and ≥ 85 % after 14 hours [12].

Results of *in-vitro* release as shown in table 5 clarified that the drug release from different formulations was found to be dependent on the type and

concentration of controlled release hydrophilic polymers used [17].

The drug release study of formulations (F#1L–F#19L) showed that the increase in the percentage of the matrix former leading to increase of the tablet binding which lead to high swellability and slow erosion of the diffusion gel layer; causing hindering in the drug release. All the previous formulations failed to give the required amount of the drug in the first two hours. F#2L (50% CMC Na), F#8L (50% Eudragit S100) and F#17L (10% Chitosan) were the most suitable formulations as they could give the required results at 4 and 6 hours but they failed only in the results of the first two hours in the in vitro dissolution test.

Enteric coat (HPMCP-55) was made for the compressed hydrophilic matrix tablets formulations (F#20L–F#22L) to adjust release in the first two hours (N.M.T 10%) [18,19]. The major concern in enteric coating formulations is a risk of premature drug release through the enteric coating film in acidic media. This problem could be solved by an application of a subcoating layer where the coating materials are subject to coating with a small amount of a soluble material, i.e. HPMC prior to enteric coating(F#23L–F#25L). This thin film layer decreases water penetration through the cores and thus prevents the premature drug release. Subcoating is helpful in formulations which contain highly water-soluble drugs [16, 20]. The adopted enteric coating technique hindered the release of the drug from core in the acidic medium as shown in (Fig. 1). The cumulative Oxybutynin Hydrochloride dissolved as a function of time from the compressed tablets was observed. The release profile from F#24L and F#25L complied with the dissolution limits obtained in USP 34 as shown in (Fig. 2).

Results of Kinetic analysis of hydrophobic matrix:

The kinetic analysis of the release data obtained from all tablets formulations showed that the drug release from F#21L, F#22L, F#24L followed zero-order kinetics, which the same amount of the drug is released per unit time independent of drug concentration and this is a method to obtain a prolonged pharmacological action [19]. While the release data from formulae F#1L, F#3L, F#13L, F#20L and F#23L followed first order kinetics. In which the release of highly water soluble drugs from porous

matrices may follow first order kinetics. In this case, the release of the drug is proportional to the amount of the drug remaining in the interior of the matrix and the amount of the drug release by unit of time diminishes [19, 21]. The remaining tablet formulations followed typical Fickian diffusion through the channels of pores and cracks according to Higuchi model.

Stability study of the selected Oxybutynin HCl extended-release tablets formulations:

Visual inspection:

Visual inspection of the stored tablets under accelerated and shelf conditions showed no change in the physical appearance or color of the stored tablets in the final pack (blisters) over all the storage period (for six and twelve months) respectively.

QUALITY CONTROL TESTS

The results of quality control parameters for the stored tablets under accelerated and shelf conditions in the stability cabinet for six and twelve months showed a slight increase in tablets means weight at the end of storage period but values still within the range permitted by the British Pharmacopoeia. The value of friability didn't change during the storage period for the two storage conditions (0%).

Effect of storage conditions on drug content:

Results revealed that the stored tablets at shelf condition showed decrease in Oxybutynin HCl percentage by about 2% from the initial percentage. While tablets stored at accelerated condition possessed Oxybutynin HCl percentage lower than the initial percentage to about 5% and the decrease in the two storage conditions is within the range permitted by the British Pharmacopoeia (90-110%) up to the end of storage period.

***In-Vitro* release study:**

The *in-vitro* release profile of Oxybutynin HCl from the tablets stored under accelerated and shelf conditions of temperature and humidity are shown in (fig. 3, 4). One-way ANOVA test revealed non-significant difference in the release profile of Oxybutynin HCl between fresh and stored tablets at the two conditions of storage ($p > 0.05$) at the different time intervals, So it could be concluded that the in-vitro release profile of Oxybutynin HCl from the stored tablets of formulations (F#24L and F#25L)

was not affected by the two conditions of storage.

Pharmacokinetic analysis:

After oral administration of Ditropan® 5 mg tablet and the optimized Oxybutynin HCl hydrophilic matrix tablet formula (F#24L) by human volunteers. Oxybutynin HCl was determined in all plasma samples. Plasma concentrations of Oxybutynin HCl versus time are graphically represented in (fig. 6). Table 6 clarifies the mean pharmacokinetic parameters of Ditropan® 5 mg tablets and optimized Oxybutynin HCl hydrophilic matrix tablet formula. ANOVA analysis showed that there were significant differences ($p < 0.05$) between the values of C_{max}, $AUC_{(0 \text{ to } t)}$, $AUC_{(0 \text{ to } \infty)}$ and $t_{1/2}$ of the optimized Oxybutynin HCl hydrophilic matrix tablet formula compared to Ditropan® 5 mg tablets . The non-parametric Mann Whitney test on the T_{max} and the MRT showed a significant difference ($p < 0.05$) between the Ditropan® and the extended release formula F#24L with respect to the T_{max} and the MRT. The mean $AUC_{(0 \text{ to } t)}$ and the $AUC_{(0 \text{ to } \infty)}$ of the optimized Oxybutynin HCl hydrophilic matrix tablet formula was found to be 1.31 times and 1.38 times of that of Ditropan® 5 mg tablets; respectively after compensation of the dose. The delayed T_{max} and higher MRT and AUC indicate a slow and prolonged release of the optimized Oxybutynin HCl hydrophilic matrix tablet formula with higher bioavailability (138%) in comparison with the commercially available Ditropan® 5 mg tablets.

TABLES AND FIGURES

Table 1: Composition of Oxybutynin HCl Hydrophobic Matrix Formulations

Formula	Matrix Former (%)		Calcium phosphate dibasic (mg)
F# 1L	CMC Na (2000cp)	30%	88.125
F# 2L		50%	58.125
F# 3L		70%	28.125
F# 4L	EudragitL100-55	30%	88.125
F# 5L		50%	58.125
F# 6L		70%	28.125
F# 7L	Eudragit S100	30%	88.125
F# 8L		50%	58.125
F# 9L		70%	28.125
F# 10L	Eudragit L100	30%	88.125
F# 11L		50%	58.125
F# 12L		70%	28.125
F# 13L	Carbomer P 940	5%	125.625
F# 14L		10%	118.125
F# 15L		15%	110.625
F# 16L		20%	103.125
F# 17L	Chitosan	10%	118.125
F# 18L		30%	88.125
F# 19L		50%	58.125

Table 2: Composition of Oxybutynin HCl Hydrophobic Matrix Enteric Coated Formulations

Formula	Matrix former (%)		Calcium phosphate dibasic (mg)
F# 20L	CMC Na 2000 cps	50%	55.125
F# 21L	Chitosan	10%	115.125
F# 22L	Eudragit S100	50%	55.125

Table 3: Composition of Oxybutynin HCl Hydrophobic Matrix Enteric Coated Formulations with Sub-Coat

Formula	Matrix Former (%)		Calcium phosphate dibasic (mg)
F# 23L	Eudragit S100	50%	84. 375
F# 24L	CMC Na 2000 cps	50%	54. 375
F# 25L	Chitosan	10%	114.375

Table 4: Characterization of Oxybutynin Hydrochloride Tablet Formulations

Formula	Tablet Weight (mg) **	Friability (%) *	Drug content *
F# 1L	149 ± 0.45	0.14 ± 0.0	101.65 ± 1.43
F# 2L	150 ± 0.35	0.14 ± 0.0	102.97 ± 2.64
F# 3L	152 ± 0.55	0	97.80 ± 1.27
F #4L	150 ± 0.52	0	100.1 ± 1.98
F# 5L	150 ± 0.95	0	100.99 ± 3.17
F# 6L	149 ± 0.35	0	98.49 ± 2.35
F# 7L	150 ± 0.54	0	102.74 ± 1.40
F# 8L	151 ± 0.7	0	100.81 ± 1.76
F# 9L	150 ± 0.63	0	99.54 ± 1.46
F# 10L	149 ± 0.72	0	98.94 ± 1.94
F# 11L	152 ± 0.71	0	98.76 ± 1.68
F# 12L	150 ± 0.65	0	98.80 ± 1.75
F# 13L	150 ± 0.25	0	100.1 ± 1.98
F# 14L	150 ± 0.45	0	97.80 ± 1.27
F# 15L	151 ± 0.46	0	100.1 ± 1.98
F# 16L	----	----	----
F# 17L	149 ± 0.24	0.15 ± 0.0	100.49 ± 2.35
F# 18L	150 ± 0.34	0.067 ± 0.0	96.74 ± 1.40
F# 19L	151 ± 0.45	0.034 ± 0.0	98.82 ± 1.46
F# 20L	150 ± 0.66	0	98.88 ± 2.68
F# 21L	149 ± 0.45	0	98.65 ± 1.43
F# 22L	152 ± 0.71	0	98.5 ± 1.46
F# 23L	150 ± 0.75	0	97.45 ± 1.43
F# 24L	150 ± 0.73	0	99.38 ± 1.64
F# 25L	150 ± 0.7	0	97.17 ± 1.27

* *n = 10 tablets*

** *n = 20 tablets*

Table 5: In-Vitro Release of Oxybutynin Hydrochloride from all tablet formulations

	Time (hrs)	0.5	1	2	3	4	6
Percent of Oxybutynin Hydrochloride released (%)	F# 1L	16.8	34	58.3	63.4	70	89.6
	F# 2L	10.2	20.7	33.9	36	41	51.6
	F# 3L	8.9	11.8	21.9	25.6	28.3	34.3
	F #4L	9.8	20.9	30.5	30.9	32.6	36.8
	F# 5L	8.6	12.7	17.6	17.9	18.3	20.5
	F# 6L	4.3	8.5	11.8	12.8	14.9	17.2
	F# 7L	12.7	30.9	61.7	62.9	64.5	72.7
	F# 8L	10	21.6	36.5	37.8	38	42.5
	F# 9L	4	12.7	24.9	26.6	28.3	31.6
	F# 10L	22.6	47.7	67.1	67.2	69.1	71.7
	F# 11L	19.8	26.8	39.2	39.8	40.2	40.9
	F# 12L	7.9	13.4	26.4	26.9	28.7	30.9
	F# 13L	23.5	45	75.9	81.2	90.4	100
	F# 14L	12.6	29.7	40.4	41	43.7	45.7
	F# 15L	9.8	17.8	25.3	30	31.7	34.8
	F# 17L	11.3	21.6	35.8	44	48.5	53.9
	F# 18L	10.8	20.9	30.1	32.7	33.4	36.7
	F# 19L	8.8	17.9	26.6	27	28.4	29.7
	F# 20L	9.8	17.7	26.1	47	59.5	72.6
	F# 21L	5.44	7.89	17.2	33	41.7	48.8
	F# 22L	2.3	6.7	16	29.8	43	46.5
	F# 23L	0	3.7	8	34.5	49	55.5
	F# 24L	0	1.9	4.2	29.7	40	47.2
	F# 25L	0	2.12	6	24.5	33.5	42.5

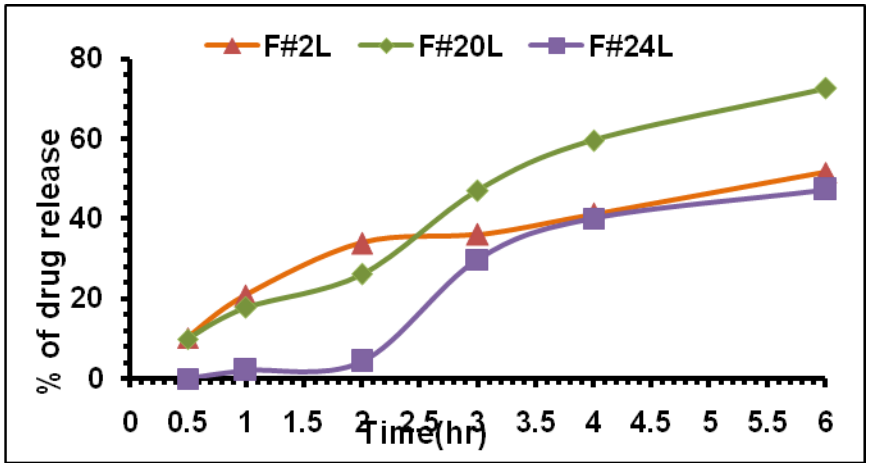
Table 6: Results of Kinetic Analysis of Hydrophobic Matrix

	Zero			First			Diff.			Mechanism of release
Formula	**(r^2)***	**Slope**	**Y-Intercept**	**(r^2)***	**Slope**	**Y-Intercept**	**(r^2)***	**Slope**	**Y-Intercept**	
F#2	**0.839**	13.236	24.032	0.713	0.117	1.394	0.783	0.924	4.982	**Zero**
F#3	**0.912**	9.575	12.759	0.773	0.127	1.173	0.853	0.815	22.881	**Zero**
F#4	**0.873**	11.892	1.374	0.705	0.113	4.873	0.797	0.859	3.901	**Zero**
F#7	**0.838**	11.274	30.781	0.709	0.093	1.493	0.778	0.764	5.589	**Zero**
F#8	**0.906**	8.909	11.1444	0.754	0.133	1.109	0.841	0.802	3.535	**Zero**
F#11	**0.868**	9.477	34.89	0.764	0.073	1.556	0.819	0.628	5.97	**Zero**
F#14	**0.817**	12.573	31.716	0.708	0.098	1.504	0.766	0.83	5.663	**Zero**
F#15	**0.856**	9.333	18.426	0.698	0.113	1.273	0.783	0.761	4.348	**Zero**
F#17	**0.836**	11.932	40.986	0.75	0.078	1.622	0.797	0.727	6.454	**Zero**
F#18	**0.827**	6.988	17.64	0.704	0.098	1.25	0.772	0.617	4.229	**Zero**
F#19	**0.736**	4.076	15.519	0.615	0.08	1.172	0.677	0.427	3.904	**Zero**
F#22	**0.913**	12.525	32.942	0.789	0.089	1.545	0.858	0.793	5.87	**Zero**
F#23	**0.954**	7.358	24.337	0.889	0.073	1.422	0.927	0.555	5.063	**Zero**
F#25	**0.983**	13.898	-3.993	0.922	0.204	0.814	0.976	1.214	2.041	**Zero**
F#26	**0.954**	13.078	-1.602	0.856	0.206	0.856	0.927	1.181	2.148	**Zero**
F#27	**0.957**	17.257	-1.972	0.888	0.198	0.958	0.931	1.342	2.532	**Zero**
F#28	**0.969**	14.154	-3.44	0.844	0.229	0.724	0.934	1.281	1.895	**Zero**
F#29	**0.977**	13.396	5.753	0.886	0.155	1.106	0.945	1.06	3.284	**Zero**
F#30	**0.968**	10.385	0.064	0.918	0.162	0.904	0.938	0.942	2.451	**Zero**
F#31	**0.993**	10.624	5.22	0.882	0.152	1.026	0.954	0.934	3.021	**Zero**
F#32	**0.951**	11.909	-8.278	0.917	0.268	0.384	0.939	1.254	0.877	**Zero**
F#33	**0.919**	15.304	-10.183	0.859	0.314	0.29	0.898	1.507	0.659	**Zero**
F#34	**0.931**	17.343	-13.099	0.855	0.345	0.185	0.904	1.651	1.651	**Zero**
F#35	**0.959**	14.927	-13.916	0.669	0.578	-0.92	0.949	1.643	-0.366	**Zero**
F#36	**0.936**	12.25	-6.586	0.824	0.298	0.307	0.913	1.311	0.926	**Zero**
F#37	**0.963**	7.615	-1.296	0.513	0.186	-0.002	0.864	0.693	1.592	**Zero**
F#38	**0.946**	7.153	-5.714	0.621	0.508	-0.088	0.943	1.09	0.093	**Zero**

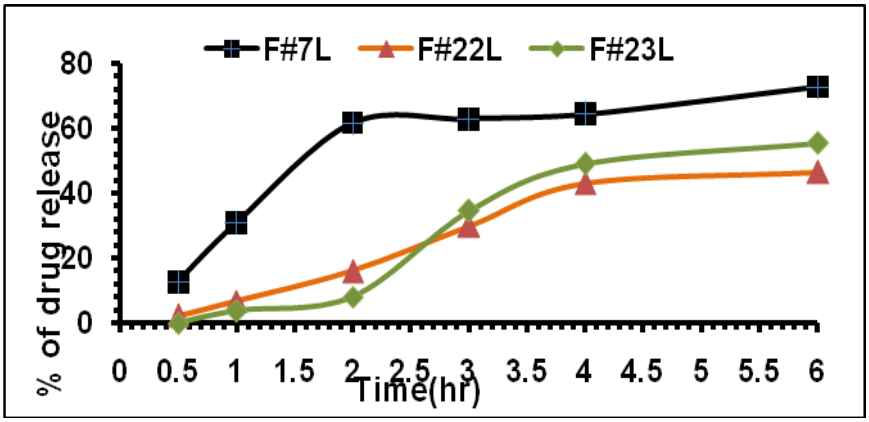
* *Correlation coefficient*

Table 7: Mean pharmacokinetic parameters of Ditropan ® 5 mg tablet and optimized Oxybutynin HCl hydrophilic matrix tablet formula

PK parameter	Ditropan® tab.	Optimized Oxybutynin HCl tab.	Statistical test (p)
C_{max} (ng/ml)	6.072 ± 0.118	5.044 ± 0.408	**0**
T_{max} (hrs)	1 ± 0	5 ± 0.408	**0.001**
AUC $_{0 \text{ to } t}$ (ng. h/ ml)	23.3 ± 1.386	91.86 ± 2.284	**0**
AUC $_{0 \text{ to } \infty}$ (ng. h/ ml)	23.308 ± 1.36	96.625 ± 3.729	**0**
$t_{1/2}$ (hrs)	2.66 ± 0.151	10.538 ±1.2	**0**
MRT(hrs)	3.844 ± 0.22	16.709 ± 1.295	**0.004**
Relative bioavailability		**138.18%**	



(a)



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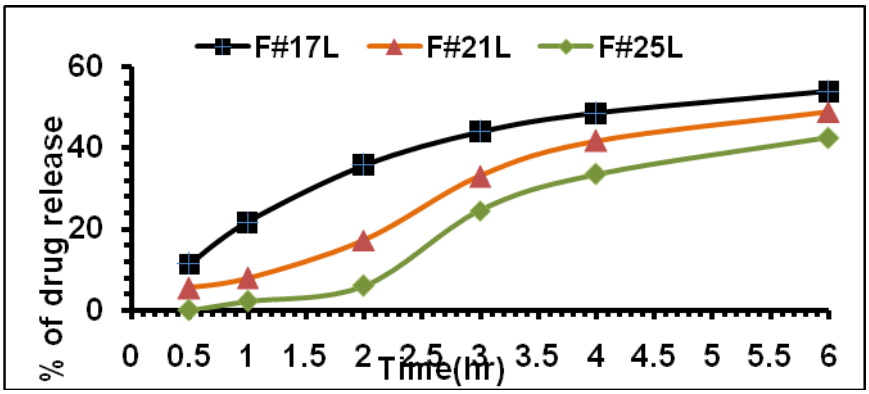

(c)

Fig. 1: In-vitro release profile of Oxybutynin HCl from (uncoated tablet, enteric coated tablet and enteric coated tablet with sub-coat) prepared with (a) 50% CMC Na 2000 cps, (b) 50% Eudragit S100, (c) 10% Chitosan

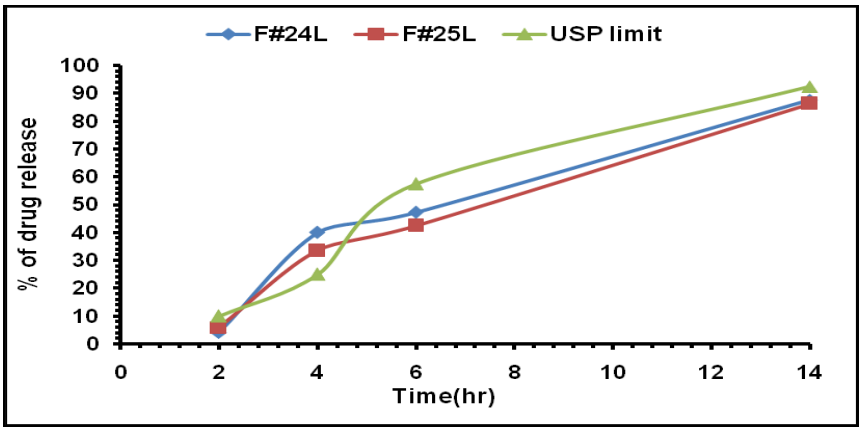

Fig. 2: In-vitro release profile of Oxybutynin HCl (complete dissolution profile for most successful formulations)

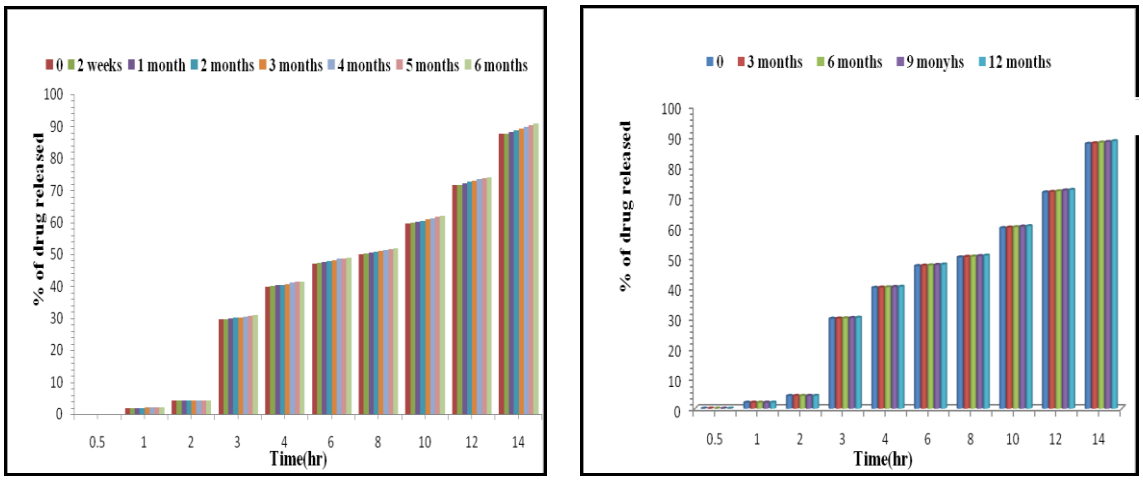

Fig. 3: In-vitro release of Oxybutynin HCl (tablet Formula F#24L) after storage for six months at accelerated condition and for twelve months at shelf condition

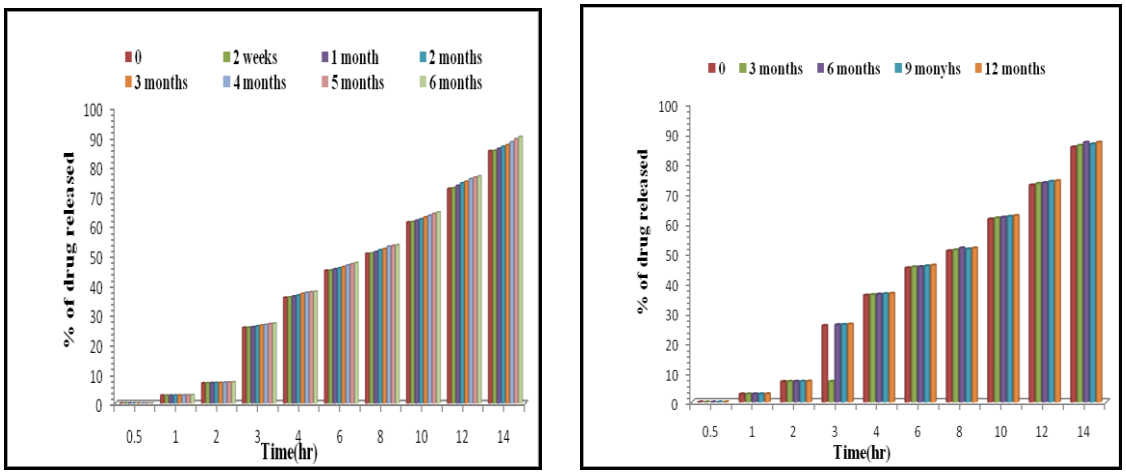

Fig. 4: In-vitro release of Oxybutynin HCl (tablet Formula F#25L) after storage for six months at accelerated condition and for twelve months at shelf condition

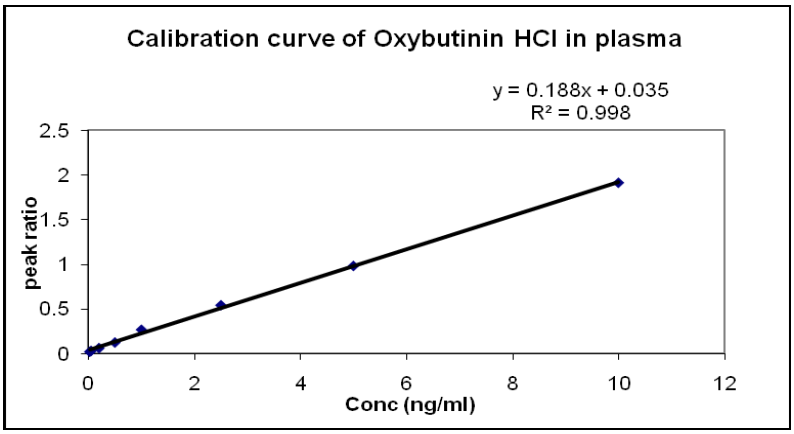

Fig. 5: Calibration curve of Oxybutynin HCl using LC-MS/MS method

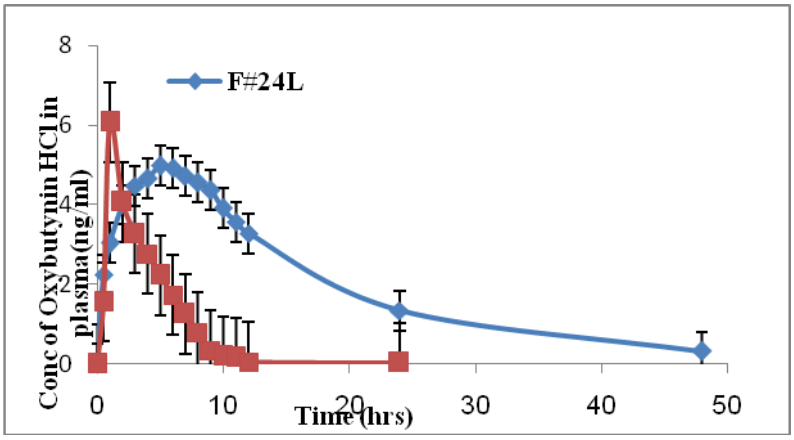

Fig. 6: Plasma concentration-time curve of Ditropan® 5 mg tablets and optimized Oxybutynin HCl hydrophilic matrix tablet formula

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