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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING ASSAY (RP-HPLC) METHOD FOR QUANTITATIVE ANALYSIS OF OXYBUTYNIN IN BULK DRUG AND EXTENDED RELEASE FORMULATION

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ABSTRACT

A novel stability-indicating reversed-phase (RP) HPLC method has been developed and validated for quantitative analysis of oxybutynin in the bulk drug and formulation. Use of a 250 mm × 4.6 mm, 5-µm particle size, C18 column with 40:60 (*v*/*v*) 0.1M phosphate buffer: acetonitrile (pH adjusted to 4.5 with orthophosphoric acid) as isocratic mobile phase enabled separation of the drug from its degradation products. The flow rate and detection wavelength were 1 ml/min and 203 nm respectively. The method was validated for linearity, limits of detection and quantification, accuracy, precision, selectivity, ruggedness and system suitability. The linearity of the method was excellent over the range 8–12 µg/ml. The mean values of slope, intercept and correlation coefficient were 1363, 3607 and 0.999 respectively. RSD in intra-day and inter-day precision studies was < 2 %. Recovery of oxybutynin from bulk drug ranged from 99.05 and 100.40 %. Oxybutynin was subjected to stress conditions (hydrolysis (acid, base), oxidation, photolysis and thermal degradation) and the stressed samples were analyzed by use of the method. Maximum degradation was observed in acid and base hydrolysis and oxidation. The drug was stable to degradation under photolytic and thermal conditions. The degradation products were well resolved from main peak thus proving the stability indicating nature of the method.

KEYWORDS: oxybutynin; stability indicating; degradation products.

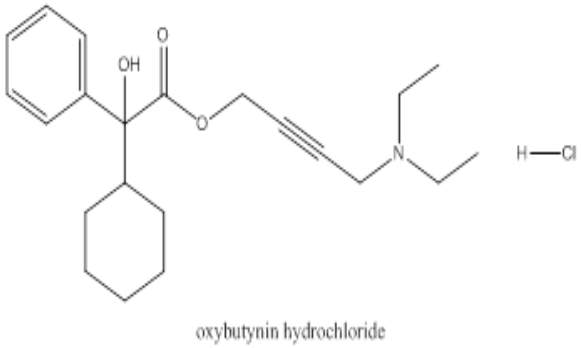
INTRODUCTION

Oxybutynin, is (±)-Diethyl amino-2- butynyl phenyl cyclohexyl glycolate. Oxybutynin HCl is a racemic mixture of R (-) and S (+). Both the enantiomers are therapeutically active and are stable and there is no R (-) to S (+) conversion *in vivo*. Oxybutynin hydrochloride exerts a direct antispasmodic effect on smooth muscle and inhibits the muscarinic action of acetylcholine on smooth muscle. Oxybutynin hydrochloride exhibits only one fifth of the anticholinergic activity of atropine on the rabbit detrusor muscle, but four to ten times the antispasmodic activity. No blocking effects occur at skeletal neuromuscular junctions or autonomic ganglia (ant-inicotinic effects).Oxybutynin hydrochloride relaxes bladder smooth muscle. In patients with conditions characterized by involuntary bladder contractions, cystometric studies have demonstrated that oxybutynin hydrochloride increases bladder (vesical) capacity, diminishes the frequency of uninhibited contractions of the detrusor muscle and delays the initial desire to void.[1-4]

Oxybutynin hydrochloride thus decreases urgency and the frequency of both incontinent episodes and voluntary urination. Oxybutynin hydrochloride is indicated for the relief of symptoms of bladder instability associated with voiding in patients with uninhibited neurogenic or reflex neurogenic bladder (i.e., urgency, frequency, urinary leakage, urge incontinence, dysuria).[5-7]

Stability-indicating assay methods (SIAM's) can be specific one, which evaluate the drug in the presence of its degradation products, excipients and additives, or selective one which is able to measure the drug and all the degradation products in the presence of excipients and additives. The International Conference on Harmonization (ICH) guidelines requires performing stress testing of the drug substance, which can help identify the likely degradation products. Moreover, validated stability-indicating method should be applied in the stability study. Stability is considered as one of the most important criteria in pharmaceutical quality control. Only stable preparation would promise precise delivery of the drug to the patients. Expiration dating on any drug product is based upon scientific studies at normal and stressed conditions.[8]

Hence an attempt has been made to develop and validate a sensitive stability indicating high performance liquid chromatographic method for determination of oxybutynin from bulk drug and formulation which is specific, precise and accurate.



oxybutynin hydrochloride

Figure: 1. Structure of Oxybutynin HCl

EXPERIMENTAL

Reagents and Materials

HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). Deionised and ultra pure water used in all experiments was obtained from Milli-Q System (Millipore). The 0.45-µm Nylon pump filter was obtained from Advanced Microdevices (Mumbai, India). Orthophosphoric acid used for adjusting the pH of buffer solution was of AR grade (Merck, Darmstadt, Germany). Sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were purchased from Qualigens Fine Chemicals (Mumbai, India).

Preparation of Mobile Phase

Potassium dihydrogen orthophosphate (3.4 gm) and dipotassium hydrogen orthophosphate (4.36 gm) were dissolved in 1000 ml volumetric flask containing 500 ml of HPLC grade water. The final volume was made with HPLC grade water. The solution was filtered through 0.45 micron membrane filter. In 200 ml of volumetric flask, 80 ml of buffer solution and 120 ml of acetonitrile was added. The solution was mixed well and sonicated for 10 min. The pH of the mobile phase was adjusted to 4.5 with orthophosphoric acid.

Preparation of Standard Solution

An accurately weighed quantity of about 25 mg Oxybutynin hydrochloride standard was transferred into a 50 ml volumetric flask. Methanol (40 ml) was added to the flask and solution was sonicated for 15 min. The volume was made up to 50 ml with methanol. This solution (1ml) was further diluted up to 50 ml with mobile phase to give final solution of 10µg/ml.

Chromatographic conditions

A Jasco Liquid Chromatographic system with PDA Detector was used for the method development and forced degradation studies. The chromatographic separation was achieved on LICROSPHER® 100 RP-C_{18} column (4.6 mm × 250 mm i.d. 5-µm particle size), 40:60 (*v*/*v*) 0.1M phosphate buffer: acetonitrile (pH adjusted to 4.5 with orthophosphoric acid) as mobile phase pumped at a flow rate of 1.0 ml min^{-1}. Before use it was filtered through a 0.45-µm Nylon filter and degassed in an ultrasonic bath. Analysis was carried out at room temperature. The injection volume was 20 µL and ultraviolet (UV) detection was at 203 nm.

Forced Degradation Studies[9]

- **Acid hydrolysis**

The drug was subjected to forced degradation under acidic condition (2N HCl). Oxybutynin HCl (25 mg) was weighed accurately and was transferred to 50 ml volumetric flask containing 20 ml mobile phase. 2ml of acid solution (2N) was added in it and heated at 60°C for 3 hrs in water bath. This solution was cooled to room temperature and was neutralized by using 2N NaOH solution to pH 7. Volume was made up to 50ml with mobile phase to get 500 µg/ml solution of Oxybutynin HCl. This solution (1 ml) was further diluted up to 50 ml with mobile phase to give 10 µg/ml solution of Oxybutynin HCl and was injected to chromatography to obtain chromatogram.

- **Alkaline hydrolysis**

The drug was subjected to forced degradation under basic condition (2N NaOH). Oxybutynin HCl (25 mg) was weighed accurately and was transferred to 50 ml volumetric flask containing 20 ml mobile phase. 2ml of base solution (2N) was added in it and heated at 60°C for 3 hrs in water bath. This solution was cooled to room temperature and was neutralized by using 2N HCl solution to pH 7. Volume was made up to 50ml with mobile phase to get 500 µg/ml solution of Oxybutynin HCl. This solution (1 ml) was further diluted up to 50 ml with mobile phase to give 10 µg/ml solution of Oxybutynin HCl and was injected to chromatography to obtain chromatogram.

- **Oxidative degradation**

The drug was subjected to forced degradation under oxidation (30% v/v H_2O_2 solution). Oxybutynin HCl (25 mg) was weighed accurately and was transferred to 50 ml volumetric flask containing 20 ml mobile phase. 5ml of (30% v/v H_2O_2 solution) was added in it and heated it at 70°C for 10 minutes in water bath. This solution was neutralized with sodium metabisulphate solution and volume was made up to 50 ml with mobile phase to get 500 µg/ml solution of Oxybutynin HCl. This solution (1 ml) was further diluted up to 50 ml with mobile phase to get 10 µg/ml solution of Oxybutynin HCl and was injected to obtain chromatogram.

- **Thermal degradation**

To study the effect of temperature, Oxybutynin HCl (25 mg) was exposed to dry heat (100°C) in a convection oven for 8 h. The drug was then removed from the oven, dissolved in 10 ml acetonitrile and volume was adjusted upto 50 ml with mobile phase to get 500 µg/ml solution of Oxybutynin HCl. This solution (1 ml) was further

diluted up to 50 ml with mobile phase to get 10 µg/ml solution of Oxybutynin HCl and was injected to obtain chromatogram.

➢ **Photolytic degradation**

To study the effect of UV light Oxybutynin HCl (25 mg) was exposed to short and long wavelength UV light (254 and 366 nm, respectively) for 24 h, then dissolved in 10 ml of acetonitrile and made up to the volume by mobile phase in 50 ml volumetric flask to get 500 µg/ml solution of Oxybutynin HCl. This solution (1 ml) was further diluted up to 50 ml with mobile phase to get 10 µg/ml solution of Oxybutynin HCl and was injected to obtain chromatogram.

Method Validation

The method was validated for specificity, system suitability, linearity, accuracy, precision, robustness, stability in accordance with ICH guidelines.

➢ **Specificity**

The following injections were injected – mobile phase, placebo, reference standard (10µg/ml) and placebo plus reference standard (10µg/ml). There should not be interference of any inactive ingredient from the placebo.

➢ **System suitability**

Six injections of standard solution were injected to the chromatographic system and chromatograms were recorded. The % Relative Standard Deviation (RSD) of peak area and retention times of six injections should not be more than 2%.

➢ **Linearity**

Linearity was performed on standard solution at 5 levels – 80%, 90%, 100%, 110%, and 120% of the working concentration of Oxybutynin hydrochloride (10µg/ml). Each level was injected in triplicate to the chromatographic system and the chromatograms ware recorded.

A linearity curve for 8µg/ml, 9µg/ml, 10µg/ml, 11µg/ml and 12 µg/ml of oxybutynin hydrochloride versus respective area of peak was plotted.

➢ **Accuracy**

To validate, that the test method can accurately quantify oxybutynin HCl within extended release formulation, three samples were prepared, each by spiking oxybutynin HCl raw material to equivalent amount of placebo at 80%, 100% and 120% of the working concentration (10µg/ml of oxybutynin HCl). Each level was weighed thrice and injected. Percentage Recovery (% Assay) for the drug was calculated for each level.Six injections of standard solution (8µg/ml, 10 µg/ml, 12 µg/ml) were injected to the chromatographic system and the chromatogram was recorded. Percent recovery should be between 98% and 102% and the RSD of percent recovery of Oxybutynin hydrochloride should not be more than 2%.

➢ **Precision**

Standard solution at working concentration 10µg/ml was injected six times. The RSD of the peak area of the six injections should not be more than 2%.

Repeatability

Standard solution at working concentration 10µg/ml was injected thrice. Sample solution at working concentration was prepared thrice and was injected. The RSD of the area values of the three injections of sample solution should not be more than 2%.

Intra-day Precision

A standard drug solution of 10µg/ml in mobile phase from three different weighing was injected in triplicate. Relative standard deviation of the data gave a measure of accuracy of the method. The RSD of the area values the six injections should not be more than 2%.

Inter-day Precision

Variability test was conducted on the HPLC system by a different analyst on a different day as per test method. Standard solution at working concentration (10µg/ml) was injected three times. The procedure was repeated on Day 2 by Chemist 2. The RSD of the % area values of the injections should not be more than 2%. The difference in the area values on Day 1 should not be more than 3% from Day 2.

➢ **Robustness**

Change in flow rate

The system suitability parameters were checked by injecting the standard and sample preparation with the flow rate of ± 0.2 ml/min viz. 0.8 ml/min and 1.2ml/min.

Change in mobile phase composition

To study the robustness, the system suitability parameters were checked by injecting the standard and sample preparation by changing the mobile phase composition by 10%, buffer: acetonitrile, 36:64 and 44: 56.

Change in mobile phase pH

To study the robustness, the system suitability parameters were checked by injecting the standard and sample preparation at mobile phase composition ± 0.2 units, with change in pH of mobile phase to pH 4.3 and pH 4.7 by using orthophosphoric acid.

Six injections of standard solution were injected to the chromatographic system and the chromatogram was recorded. Three injections of sample solution were injected to the chromatographic system and the chromatograms were recorded. The percent assay of each sample solution was calculated. The % relative standard deviation of peak area, retention time of three standard solutions, the percent assay and retention times of two sample injections should not be more than 2%.

➢ **Solution stability**

The stability of oxybutynin HCl sample solution and standard preparation were determined. The assay was performed on test preparation as per the test method. The standard solution as well as sample solution was kept at room temperature and as well as in the fridge (Below 10 ºC) for 24 hrs and the sample solution and standard solution were injected at initial, after 12 hrs and after 24 hrs. Six injections of standard solution were injected to the chromatographic system and the chromatogram was recorded. Three injections of sample solution were injected to the chromatographic system and the chromatogram was recorded. The percent assay of each sample solution was calculated. The percent relative standard deviation of area and retention time of three standard solutions and the percent assay and retention times of two samples injections should not be more than 2%. The absolute difference in the % assay values of initial, 12 hrs and 24 hrs should not be more than 2%.

Assay of Oxybutynin HCl in-house extended release pellets

Capsules (20) were randomly sampled and were emptied in mortar and pestle. The pellets were crushed to a fine powder. Powder equivalent to 5 mg of oxybutynin HCl was weighed accurately and was transferred to 50 ml volumetric flask. About 40 ml of methanol was added and the solution was sonicated for 15 minutes. The volume was made up to 50 ml with mobile phase and solution was mixed. Solution was filtered through 0.45µ membrane filter, about 2ml of initial filtrate was discarded and remaining filtrate was collected. This solution (1 ml) was further diluted to 10 ml with mobile phase and was injected in to chromatography.

Accelerated stability studies

Pellets were filled in hard gelatin capsule (Size 3) (orange body, black coloured cap) and was packed in strips of 0.04-mm thick aluminum foil laminated with polyvinyl chloride (PVC) and stored in stability chambers maintained at 25^{o}C /65% RH, 30^{o}C/65% RH and 40^{o}C/75% RH for 6 months. The pellets filled capsules were withdrawn periodically and evaluated for drug content and *in-vitro* release studies.

RESULTS AND DISCUSSION

Stress Testing

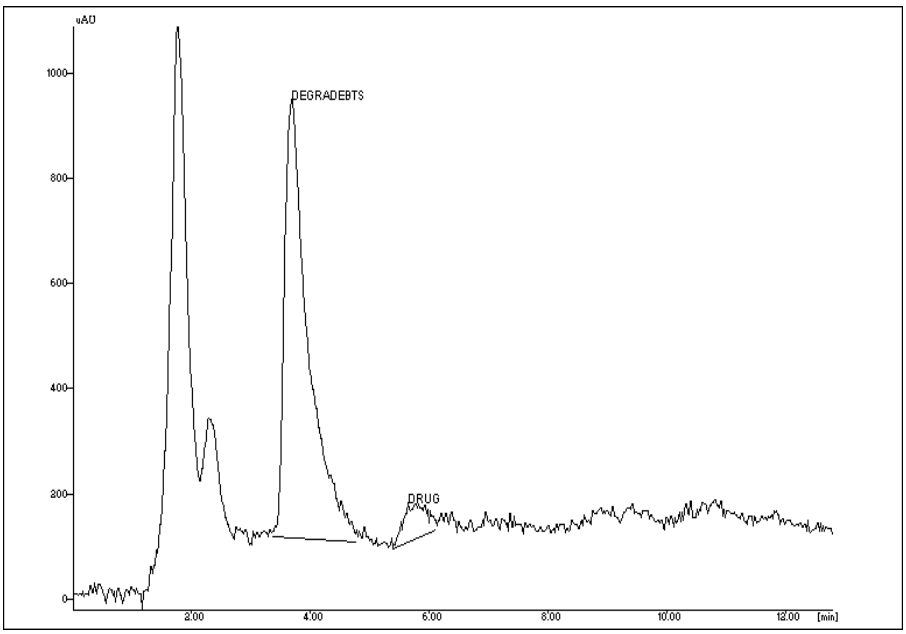


Figure: 2. Chromatogram of acid degradation of Oxybutynin HCl

Oxybutynin HCl and its degradation products (impurities) were separated on a stainless steel LICROSPHER® 100 RP-18e (5 µm, 250 x 4.6 mm) column. Maximum degradation was observed in acid and base hydrolysis. The degradation products are shown in the following chromatograms. Acid degradation solution was prepared as per proposed method and the solution was injected to the chromatographic system. There was no degradation peak interfering with Oxybutynin hydrochloride peak. The degraded peak was well separated from Oxybutynin hydrochloride peak.

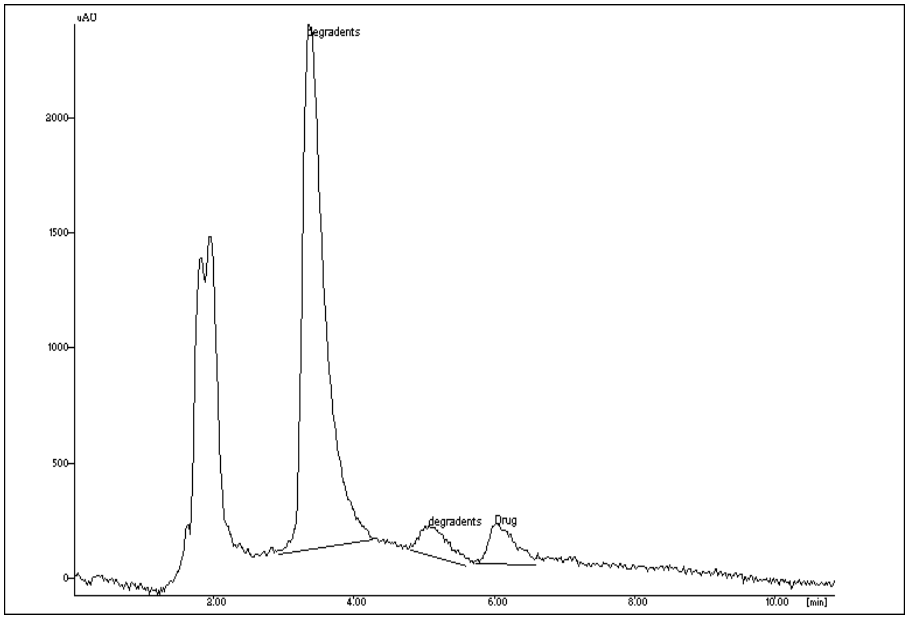


Figure: 3. Chromatogram of base degradation of Oxybutynin HCl

Base degradation solution was prepared as per proposed method and the solution was injected to the chromatographic system. The degraded peak was well separated from Oxybutynin hydrochloride peak.

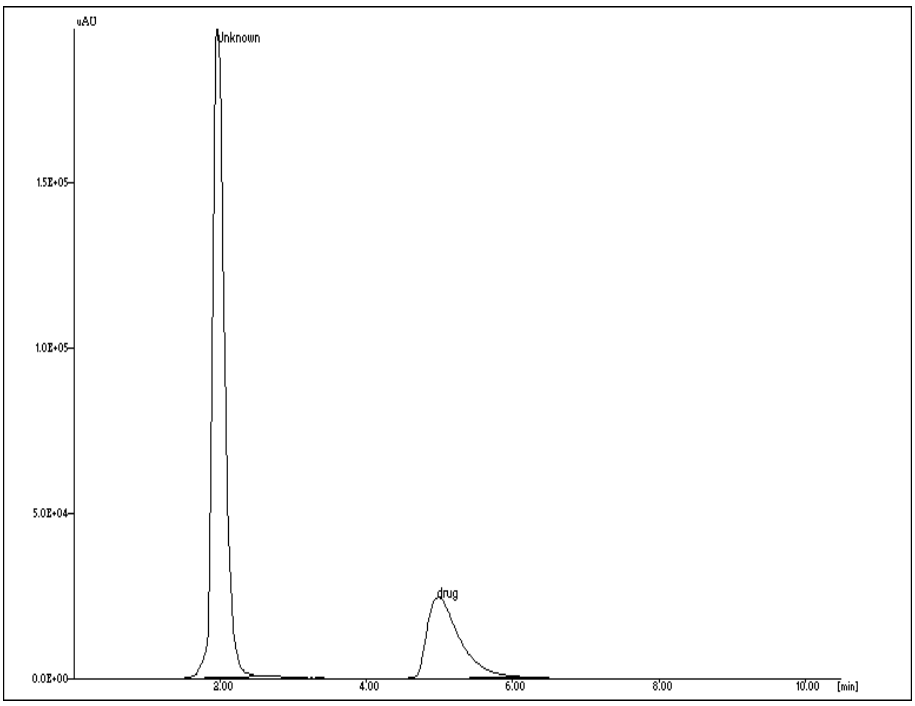


Figure: 4. Chromatogram of peroxide degradation of Oxybutynin HCl

Peroxide degradation solution was prepared as per proposed method and the solution was injected to the chromatographic system. Degradation peaks were not observed in the chromatogram of peroxide degradation of oxybutynin HCl but the peak was reduced indicating degradation.

No degradation was observed in thermal and photolytic stress testing indicating the drug to be thermostable and photostable.

Validation of the Method

Specificity

There was no interference observed at the retention time of drug peak as shown in Figure 5. Thus method was found to be specific for oxybutynin hydrochloride.

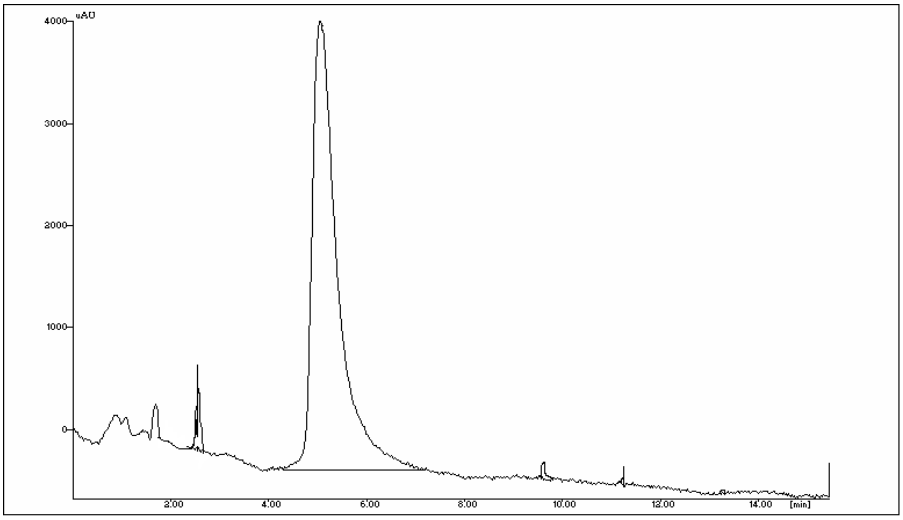


Figure: 5. Chromatogram of Oxybutynin HCl and placebo solution

System Suitability

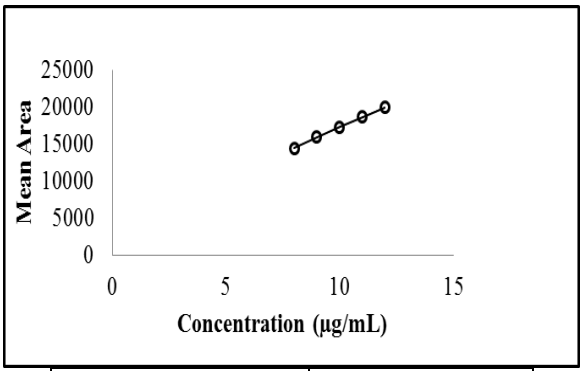
The RSD of the peak area and retention time of six injections of Standard Solution was found to comply with the acceptance criteria as shown in Table 1.

Table: 1. System suitability parameters for Oxybutynin

Concentration	Peak area	RSD	Rt	RSD
10 µg/ml	17664	1.17	4.90	1.04
	17890		4.89	
	18023		4.96	
	17445		5.02	
	17596		4.95	
	17689		4.89	

Linearity

The polynomial regression data for calibration plots (n=3) showed a good linear relationship over concentration range of 8 to 12 µg/ml. Coefficient of correlation (R^2) was 0.999 with slope of 1363 and intercept of 3607. No significant difference was observed in the slopes of standard curve. Figure 6 represents the linearity of oxybutynin HCl response.



Correlation	0.999
Slope	1363
Y intercept	3607
Straight line equation	y = 1363x+3607

Figure: 6. Linearity of Oxybutynin HCl response by HPLC

There was a good correlation of data over the range 8

µg/ml to 12µg /ml of oxybutynin HCl.

Accuracy

Table: 2. Accuracy of HPLC method for estimation of Oxybutynin HCl

Theoretical Content (µg/ml)	Actual Content found (µg/ml)	Recovery %
8.00	7.96	99.5
8.00	8.01	100.2
8.00	8.01	100.1
10.00	9.90	99.0
10.00	9.99	99.9
10.00	10.04	100.4
12.00	11.90	99.2
12.00	11.98	99.8
12.00	12.02	100.1

The accuracy of the method was checked by recovery of drug from sample preparations, accurately spiked with different concentrations of the drug. The results indicated that there was no significant difference between the calculated percent recovery and actual value drug. Percent RSD was found to be less than 2.

Precision

System Precision

Table: 3. Oxybutynin HCl Standard solution 10 µg/ml (n=6)

Sr. No	Peak area
1	17955
2	18143
3	18214
4	18005
5	18286
6	18382
%RSD	**0.90**

Repeatability

Table: 4. Precision Study: Day 1

Concentration µg/ml	Peak Area	Mean	Std. Dev	% RSD
8	17965	18138	164.94	0.90
	18156			
	18294			
10	18006	18284	251.89	1.37
	18496			
	18352			
12	18234	18197	48.01	0.26
	18143			
	18214			

Table: 5. Precision Study: Day 2

Concentration µg/ml	Peak Area	Mean	Std. Dev	% RSD
8	18086	18239	225.05	1.23
	18134			
	18497			
10	18430	18291	207.77	1.13
	18392			
	18053			
12	18254	18178	80.87	0.44
	18187			
	18093			

The results along with the percent RSD, assay of drug shown indicates an acceptable level of precision for the analytical system for each day less than 2%.

Robustness

The method was found to be robust in terms of small change in flow rate, change in mobile phase pH and change in composition of mobile phase as %RSD between normal conditions and altered conditions was not more than 2.0%.

Table: 6. Robustness of HPLC method for estimation of Oxybutynin HCl

Condition	Retention time (min)	Mean area	% RSD	Tailing factor
Unaltered Condition				
Flow rate 1.0 ml/min	7.855	17776	1.02	1.29
Altered condition				
Flow rate 1.2 ml/min	6.615	16256	0.84	1.27
Flow rate 0.8 ml/min	9.501	19101	0.72	1.23
Un-altered Condition				
Buffer (40): Acetonitrile (60)	7.855	17767	1.01	1.29
Altered condition				
Buffer (36): Acetonitrile (64)	7.77	17895	0.49	1.32

Buffer (44): Acetonitrile (56)	10.97	17941	1.14	1.23
Un-altered Condition				
pH 4.5	7.855	18765	0.81	1.25
Altered Condition				
pH 4.3	7.972	18254	0.78	1.28
pH 4.7	7.969	18481	0.83	1.27

The system suitability parameter like the percent RSD of area and tailing factor was not significantly changed with altered conditions. The method was found to be robust in terms of small change in composition of mobile phase and flow rate of mobile phase.

Solution stability

Table: 7. Solution stability of Oxybutynin HCl

Time (Hour)	Test sample			
	Room temperature		Below 10 ^{o}C	
	%Assay	% Assay Difference w.r.t. to initial	%Assay	% Assay Difference w.r.t. to initial
Initial	100.588	-----	100.588	-----
12	100.711	0.123	99.7914	-0.796
24	99.9481	-0.639	100.264	-0.324

The % assay difference in test solution was found to be -0.796 % and -0.324 % at 12 hr and 24 hr respectively when stored at below 10 ^{0}C while 0.123 % and -0.639% at 12 hr and 24 hr respectively when stored at room temperature which is well within the acceptance criteria of not more than 2.0%. Based on the above data it can be concluded that the test solution stored at below 10 ^{0}C and at room temperature showed better similar results. Test solution can be used up to 24 hr after preparation when stored in refrigerator.

Assay of Oxybutynin HCl extended release pellets and Accelerated stability studies

No significant change was observed in *in-vitro* release profile and drug content of the formulations after three months and six months of accelerated stability studies. Thus, it was concluded that the in-house developed formulation was stable during six month accelerated stability studies.

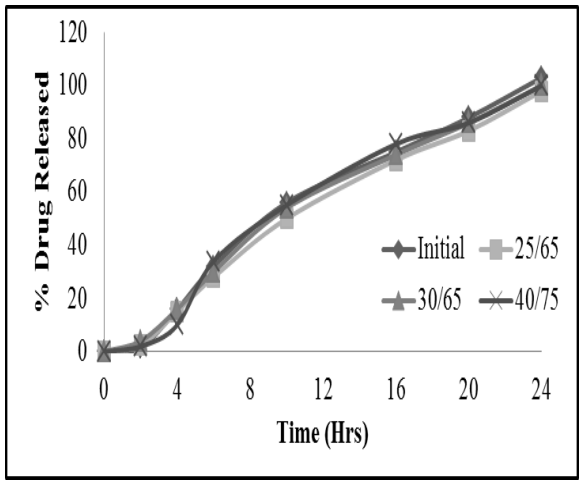


Figure: 7. Three month stability studies: *In-vitro* release profile

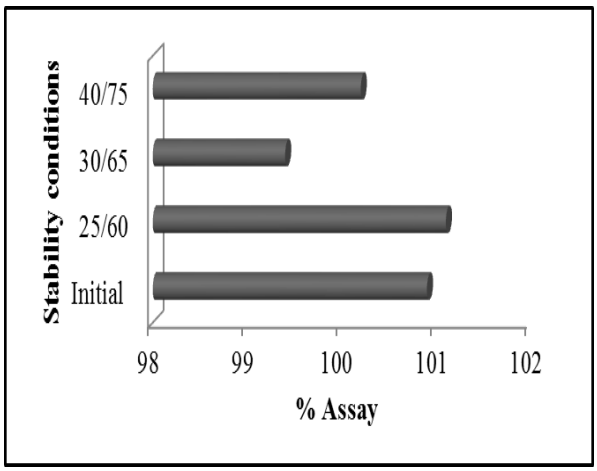


Figure: 8. Three month stability studies: Content analysis

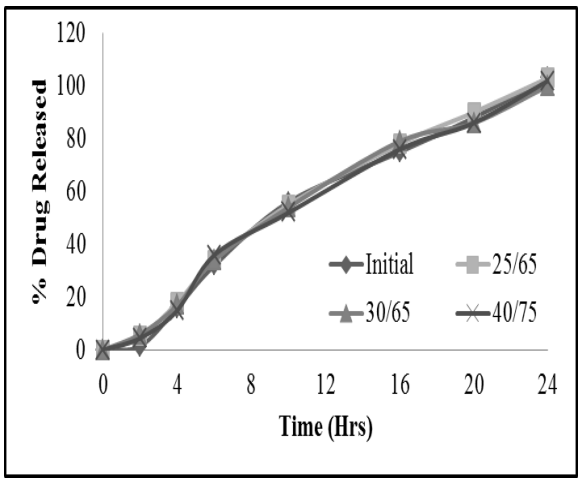


Figure: 9. Six month stability studies: *In-vitro* release profile

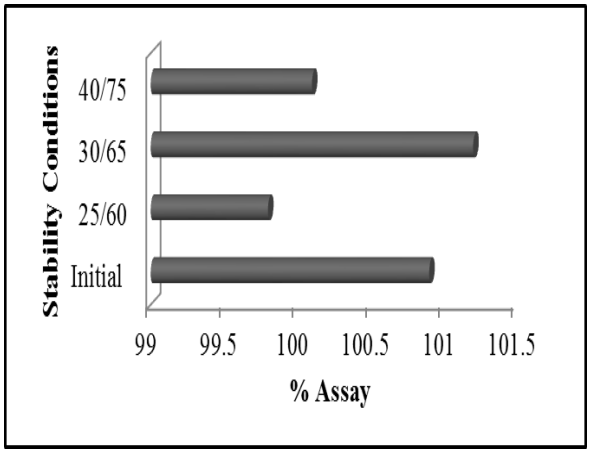


Figure: 10.Six month stability studies: Content analysis

CONCLUSION

Thus the method developed for determination of Oxybutynin Hydrochloride is simple, precise, accurate and selective. The method was successfully validated for all the validation parameters as per ICH guidelines. The method is stability-indicating and can be used to assess the short term and accelerated stability of oxybutynin in the bulk drug as well as formulation. The method can be conveniently used for assay of oxybutynin in the bulk drug and in pharmaceutical dosage forms in pharmaceutical industry.

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