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RESEARCH ARTICLE

Analytical Method Development and Validation for the Estimation of Related Substances in Oxybutynin HCl Prolonged Release Tablets by Reverse-Phase High-Performance Liquid Chromatographic

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ABSTRACT

Reverse-phase high-performance liquid chromatographic method has been developed for the determination of related compound of oxybutynin hydrochloride in pure form and pharmaceutical dosage forms. Isocratic elution at a flow rate of 1.0 ml/min was employed on a primesil-C_{18} column (150 × 4.6 mm, 3.5 µm SS) at 45°C. The mobile phase consisted of mixture of water:acetonitrile:triethylamine in the ratio of 690:310:2 (%V/V), respectively, and the ultraviolet detection wavelength was 210 nm. The RT value of oxybutynin hydrochloride, impurity-D, and impurity-A was found to be 13.75 min, 19.80 min, and 24.89 min, respectively, with a run time of 60 min. The developed method was validated for linearity, accuracy, precision, detection limit, quantification limit, robustness, specificity, and system suitability. Results of all validation parameters were within the limits as per ICH guidelines.

Keywords: Oxybutynin Hcl, RP-HPLC, Method development

INTRODUCTION

Oxybutynin is chemically 4-(diethylamino)but-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride [Figure 1] and is an anticholinergic medication used to relieve urinary and bladder difficulties including frequent urination and inability to control urination (urge incontinence), by decreasing muscle spasms of the bladder. It competitively antagonizes the M_1, M_2, and M_3 subtypes of the muscarinic acetylcholine receptor. Oxybutynin is also a possible treatment of hyperhidrosis (hyperactive sweating).[1,2] Literature survey reveals complex formation of oxybutynin chloride with acidic dyes TPOOO or ARS,[3] separation and quantitative determination of the enantiomers of oxybutynin hydrochloride,[4] and rapid assay of oxybutynin in tablet dosage form.[5] The aim of the present study is to develop a new, simple, rapid, precise, economic, accurate, sensitive, efficient, reproducible and robust, and stability-indicating analytical method for assay of oxybutynin HCl prolonged release (PR) tablet by reverse-phase high-performance liquid chromatographic (RP-HPLC) and to validate the same as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

HPLC (Shimadzu) with pump autoinjector, Primesil C_{18} (150 × 4.6 mm, 3.5 µ) column detection of drug carried by photodiode array (PDA) detector at data processing was carried out by LC solutions software, weighing balance (Mettler Toledo), pH meter (Thermo scientific), and ultrasonicator (trans-o-sonic).

Reagents and chemicals

The HPLC grade pure samples of O-phosphoric acid, acetonitrile(ACN), triethylamine, water. The solvents were filtered through 0.45 µ polyvinylidene difluoride(PVDF)membrane filter.

Chromatographic conditions

The RP HPLC consists of a primesil-C18 column (150 × 4.6 mm, 3.5 µm SS) at 45°C. The mobile phase consisted of mixture of water: acetonitrile: triethylamine in the ratio of 690:310:2 (%V/V),

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respectively, and the ultraviolet detection wavelength was 210 nm. Flow rate: 1.0 mL/min, run time: 60 min (for Sample, Blank and Placebo) 35 min (for standard), column temperature: 45°C, injection volume: 25 µL and flow rate: 1ml/min.

Solution preparation

Preparation of standard solution

Weighed accurately about 40 mg of oxybutynin HCl working standard into a 100 ml volumetric flask added 50 ml Diluent-01, sonicated to dissolve, and made up the volume with Diluent-01. Further diluted the above 5 ml of oxybutynin HCl working standard solution into 50 ml of volumetric flask and made up with diluent:ACN (50:50). 2 ml of above solution was pipetted into a 100 ml of volumetric flask, 50 mL of Diluent-01:ACN (50:50) was added and made up to the volume with Diluent-02 (0.8 µg/ml).

Preparation of sample solution

Weighed and transfer eight tablets (equivalent to 80 mg) into a 100 ml volumetric flask, added 50 ml of Diluent-01, sonicated for 45 min with intermittent shaking, and make up the volume with ACN. Centrifuge the solution for 3 min at 300 rpm. Transferred 5 ml of above centrifuged solution into 10 ml volumetric flask with Diluent-02. Filter through 0.45 µ PVDF syringe filter (400 µg/mL).

Preparation of impurity A solution

6.09 mg of impurity-A was taken in a 20 mL volumetric flask and made up to the volume with diluents (6.0 µg/mL).

Preparation of impurity D solution

5.01 mg of impurity-D was taken in a 100 mL volumetric flask and made up to the volume with diluents (2.0 µg/mL).

RESULTS AND DISCUSSION

System suitability

The main purpose of the system suitability is to ensure the system including instrument, analyst, chemicals, and electronics is suitable to the intended application. One blank, sample, and placebo injections and six replicative standard injections were injected, and the chromatograms were recorded for the drugs and the chromatogram was shown in Figure 2, and the results were shown in Table 1.

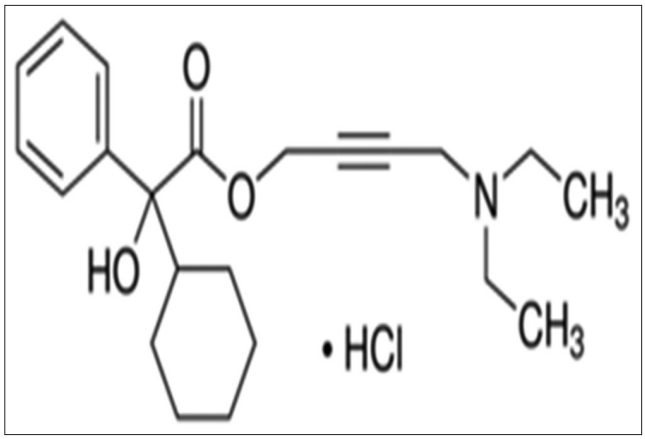

Figure 1: Structure of oxybutynin HCl

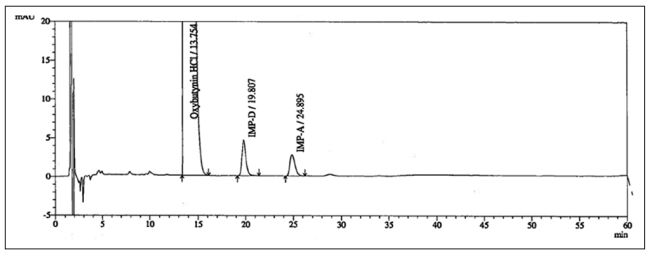

Figure 2: Chromatogram of system suitability

Table 1: System suitability result

Parameter	Result	Acceptance criteria
Theoretical plate count	83821	NLT 2000
Tailing factor	1.1	NMT 2.0
% RSD	1.9	NMT 10.0
System performance	0.7	NMT 10.0%

RSD: Relative standard deviation

Specificity

Blank interference

Blank was prepared and injected as per test method. It was observed that no blank peaks were interfering with analytical peaks.

Placebo interference

Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering with analytical peaks.

Impurity interference

All known impurities solution was prepared at about 1% of the test concentration and analyzed as per test method. It was observed that no coelution

of the all known impurities peaks with analytical peaks. Prepared sample and spiked sample solutions by spiking all known impurities at about 1% of the target test concentration in triplicate and analyzed as per test method.

There was no interference at the retention time of oxybutynin HCl and its impurities in blank, placebo and the impurity mixture solution shown in Figure 3.

Forced degradation studies

Acid degradation study

Weighed and powdered five tablets and weighed accurately a portion of the tablet powder equivalent to 40 mg of oxybutynin HCl and were quantitatively transferred into a 50 ml volumetric flask, filled with 25 ml of Diluent-01, and sonicated for 30 min. 5 ml of 2 N HCl was added to it for 1 h at room temperature. Then, it was neutralized with 5 ml of 2 N NaOH solution and made up to the volume with ACN. 5 ml from the above solution was further diluted to 10 ml with Diluent-02 and direct filtered into the vial with 0.45 µ PVDF filter.

Alkali degradation study

Weighed and powdered five tablets and weighed accurately a portion of the tablet powder equivalent to 40 mg of oxybutynin HCl and were quantitatively transferred into a 50 ml volumetric flask, filled with 25 ml of Diluent-01, and sonicated for 30 min. 5 ml of 2 N NaOH was added to it for 1 h at room temperature. Then, it was neutralized with 5 ml of 2 N HCl solution and made up to the volume with ACN. 5 ml from the above solution was further diluted to 10 ml with Diluent-02 and direct filtered into the vial with 0.45 µ PVDF filter.

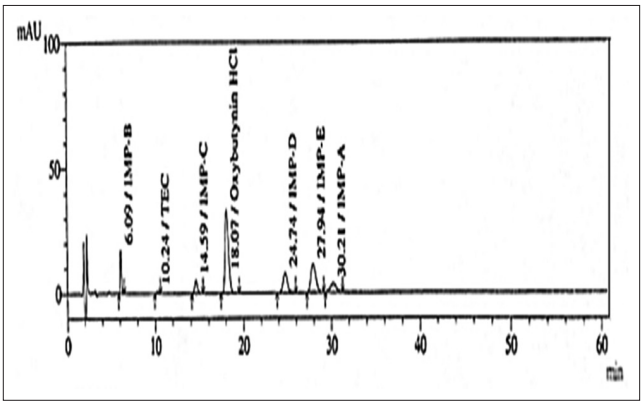

Figure 3: Chromatogram of known impurity mixture with oxybutynin HCl

Thermal degradation study

Sample tablets were taken in a Petri dish and kept in a hot air oven at 60°C for 24 h. Then, samples were crushed and 40 mg equivalent sample was taken in a 50 mL volumetric flask. 25 mL of Diluent-01 was added to it and sonicated for 30 min. The volume was made up to the mark with ACN. 5 mL from the above was taken in a 10 mL volumetric flask and made up to the mark with Diluent-02.

Peroxide degradation study

Weighed and powdered five tablets and weighed accurately a portion of the tablet powder equivalent to 40 mg of oxybutynin HCl and were quantitatively transferred into a 50 ml volumetric flask treated with 5 ml of 30% v/v solution of hydrogen peroxide for 24 h at room temperature. It was filled with 25 ml of Diluent-01 and sonicated for 30 min. It was then made up to the volume with ACN. 5 ml from the above solution was further diluted to 10 ml with Diluent-02 and direct filtered into the vial with 0.45 µ PVDF filter.

Chemical forced degradation was carried out to show the maximum degradation at the extreme condition. The drug was not degraded at the acid treatment. At alkaline condition, 2.6% of the drug was degraded. At hydrogen peroxide oxidation, 7% of the drug was degraded. At thermal treatment, 0.08% of drug was degraded. According to the ICH stability guideline, 5%–20% degradation of drug is allowed in forced degradation condition. Neither known nor unknown impurities were interfered the main peak of oxybutynin HCl and impurity-D. The peak purity was also good (1.000) and was shown in Table 2.

Linearity and range

Weighed accurately about 80 mg of oxybutynin HCl working standard into a 100 ml volumetric flask added 50 ml Diluent-01, to this added required amount of impurity-A and impurity-D sonicated to dissolve and made up the volume with ACN. Centrifuge the solution for 3 min at 300 rpm. Further, transfer 5 ml of above centrifuged solution into 10 ml of volumetric flask and make up to the volume with Diluent-2, then filter through PVDF syringe filters.

Table 2: Forced degradation study

Forced degradation study						
S. No.	**Condition**	**% assay**	**% total impurities**	**Mass balance (% assay+% total impurities)**	**% degradation**	**Remark**
1	Acid degradation	99.68	0.28	99.96	0.04	Almost not degraded
2	Base degradation	81.8	10.6	92.4	6.8	Within limit (5–20%)
3	H_2O_2 degradation	83.2	9.9	93.1	6.9	Within limit (5–20%)
4	Thermal degradation	99.74	0.18	99.92	0.08	Almost not degraded

Table 3: Linearity results

Linearity study			
Interference	**Oxybutynin HCl**	**Impurity A**	**Impurity D**
Slope	25680	10882	43001
Intercept	−74.76	68.88	−42.96
Correlation coefficient	0.9999	0.9999	0.9999
Response	20623.58502	66343.20607	86647.109
Y intercept at 100% level	−0.36	0.10	−0.05
RRF	1.00	0.42	1.60

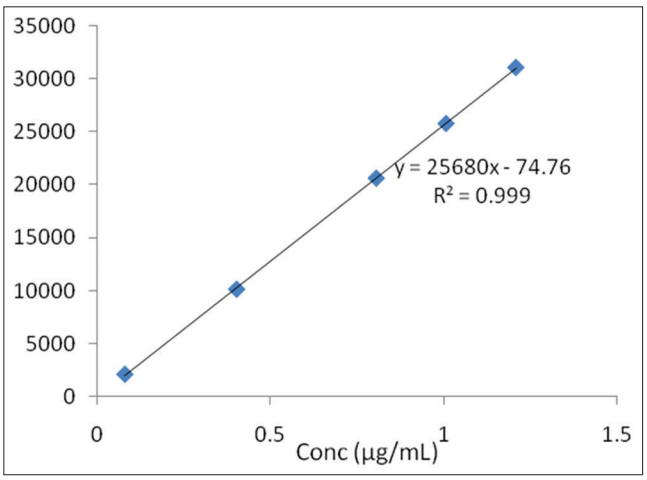

Figure 4: Linearity curve for oxybutynin HCl

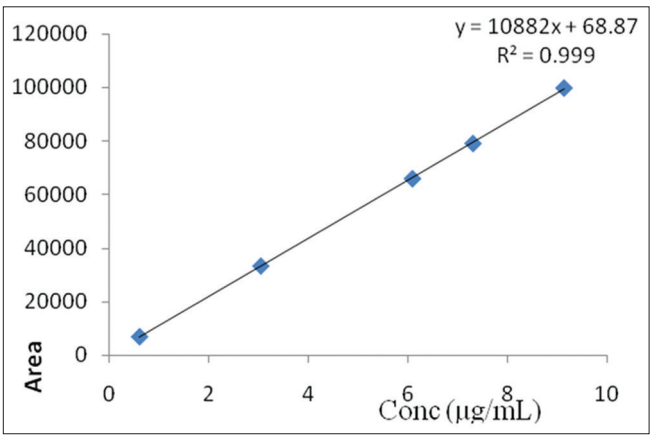

Figure 5: Linearity curve for impurity A

The correlation coefficient values of oxybutynin HCl, impurity-A, and impurity-D were more than 0.999 and were shown in Table 3 and Figures 4-6.

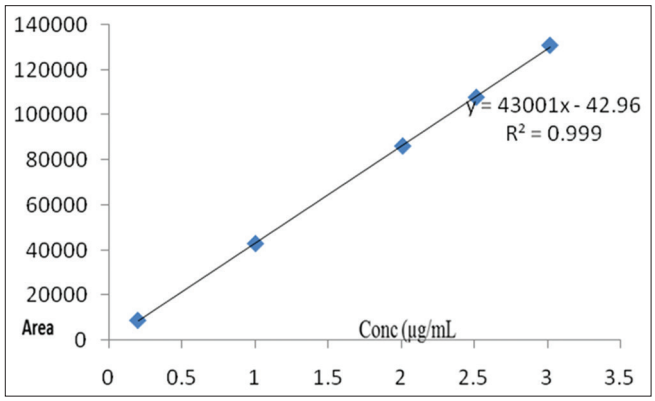

Figure 6: Linearity curve for impurity D

Accuracy/recovery

A series of solutions were prepared by spiking the placebo and API in the range of about 50–150% of test concentration in triplicate and injected into HPLC system and analyzed as per the test method. Calculated individual mean recovery and percentage relative standard deviation (RSD) at each level and the results were found to be within the acceptable limits.

The percentage recovery was found to be between 98 and 102 and percentage RSD should be not more than 2. Hence, the analytical method is accurate and the results were shown in Tables 4 and 5.

Precision

Weighed and transfer eight tablets (equivalent to 80 mg) into a 100 ml volumetric flask, added

50 ml of Diluents-01, sonicated for 45 min with intermittent shaking, and make up the volume with ACN. Centrifuge the solution for 3 min at 300 rpm. Transferred 5 ml of above centrifuged solution into 10 ml volumetric flask with Diluent-02. Filter through 0.45 µ PVDF syringe filter.

The percentage RSD for peak area of oxybutynin HCl was found to be <2%. The resolutions between the peaks were found to be more than 2 and were shown in Table 6.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. 3.3 ml from the 10% solution was taken in a 10 ml volumetric flask and made up to the volume with Diluent-02. It was injected to the system and signal-to-noise ratio was observed. Signal-to-noise ratio for

Table 4: Recovery study of impurity A

Level	Control sample area	Test sample area	Test recovery area	Standard area	% recovery	Mean±SD	%RSD
50%	ND	33425	66624	68032	49.13	49.22±0.21	0.43
	ND	33352	66625	68032	49.02		
	ND	33689	66626	68032	49.52		
100%	ND	66724	66724	68032	98.08	98.22±0.10	0.11
	ND	66846	66546	68032	98.26		
	ND	66893	66893	68032	98.33		
150%	ND	102952	102952	68032	151.33	150.85±0.70	0.46
	ND	101953	101953	68032	149.86		
	ND	102964	102964	68032	151.35		

SD: Standard deviation, RSD: Relative standard deviation

Table 5: Recovery study of impurity D

Level	Control sample area	Test sample area	Test recovery area	Standard area	% recovery	Mean±SD	%RSD
50%	11544	55630	44086	87035	50.65	50.08±0.64	1.27
	11544	54359	42815	87035	49.19		
	11544	55416	43872	87035	50.41		
100%	11544	99261	87717	87035	100.78	100.98±0.19	0.19
	11544	99380	87836	87035	100.92		
	11544	99654	88110	87035	101.24		
150%	11544	141630	130086	87035	149.46	150.32±0.64	0.42
	11544	142532	130988	87035	150.50		
	11544	142963	131419	87035	151.00		

SD: Standard deviation, RSD: Relative standard deviation

Table 6: System precision results

System precision			
S. No.	**Oxybutynin HCl**	**Impurity-D**	**Impurity-A**
1	22605	86651	69170
2	22501	86648	69121
3	22596	86598	69852
4	22654	86656	69123
5	22703	86659	69756
6	22609	86986	69325
Mean±SD	22611.333±61.478	86699.667±129.693	69391.167±301.017
%RSD	0.003	0.001	0.004

SD: Standard deviation, RSD: Relative standard deviation

LOD was found to be 4.1:1 and was shown in Figure 7.

LOQ

Weighed accurately about 80 mg of oxybutynin HCl working standard into a 100 ml volumetric flask added 50 ml Diluent-01, to this added 0.2 ml of impurity-A and 0.4 ml impurity-D sonicated to dissolve and made up the volume with ACN. Centrifuge the solution for 3 min at 300 rpm further transfer 5 ml of above centrifuged solution into 10 ml of volumetric flask and make up to the volume with Diluent-2, then filter through PVDF syringe filters. Signal-to-noise ratio for LOQ was found 22:1 and was shown in Figure 8.

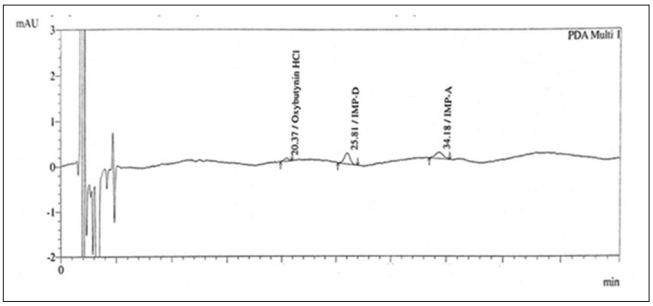

Figure 7: Chromatogram of limit of detection solution

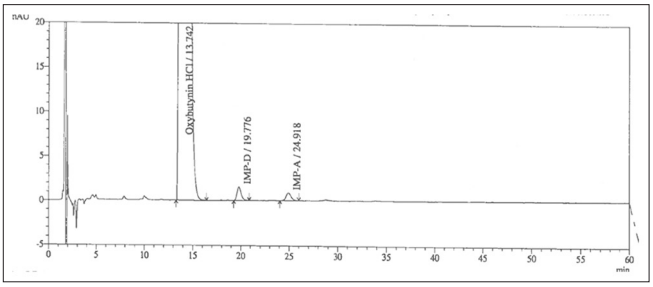

Figure 8: Chromatogram of limit of quantification solution

Robustness

Effect of variation in temperature

During the optimization of chromatographic condition, 45°C was taken as column oven temperature. Here, the operation was performed by taking column oven temperature 40°C, 45°C, and 50°C, and peak areas were observed.

Effect of variation in wavelength

During the optimization of chromatographic condition, 210 nm was taken as wavelength of detection. Here, the operation was performed by taking wavelength 208 nm, 210 nm, and 212 nm, and peak areas were observed.

No significant change was found in peak area and peak shape of oxybutynin HCl and its impurities on changing the temperature and wavelength at specified level. The percentage RSD of the peak area of oxybutynin HCl and its impurities was not more than 2.0. Hence, the analytical method is robust on the change of temperature and wavelength at specified level and was shown in Tables 7 and 8.

Preparation of spiked sample solution (at 100% level)

Weighed accurately about 80 mg of oxybutynin HCl working standard into a 100 ml volumetric flask added 50 ml Diluent-01, to this added 2 ml of impurity-A and 4 ml impurity-D sonicated to dissolve and made up the volume with ACN. Centrifuge the solution for 3 min at 300 rpm. Further, transfer 5 ml of above centrifuged solution into 10 ml of volumetric flask and make up to the volume with Diluent-2, then filter through PVDF syringe filters.

Table 7: Effect of change in temperature

Effect of change in temperature on peak areas of oxybutynin HCl and its impurities						
Name	**Peak area at 40°C**	**Peak area at 45°C**	**Peak area at 50°C**	**NTP**	**Tailing factor**	**Resolu^n**
Oxy.	22605	22596	22596	8220	1.3	-
Impurity-D	86651	86598	86986	11032	1.1	7.3
Impurity-A	69170	69852	69325	10271	1.1	5.4

Table 8: Effect of change in wavelength

Effect of change in wavelength on peak areas of oxybutynin HCl and its impurities						
Name	**Peak area at 208 nm**	**Peak area at 210 nm**	**Peak area at 212 nm**	**NTP**	**T_f**	**Resolu^n**
API	22468	22596	22481	8245	1.2	-
Impurity-D	86559	86598	86486	11452	1.1	7.5
Impurity-A	69037	69852	69345	10271	1.3	5.2

CONCLUSION

A new RP-HPLC method was developed for the estimation of related compounds in oxybutynin HCl PR tablet dosage form.

The chromatographic separation was achieved on Primesil-C_{18}, 150 mm × 4.6 mm, 3.5 µ, column within a run time of 60 min under isocratic elution by mixture of water, can, and TEA at a flow rate of 1.0 ml/min. A PDA detector set at 210 nm was used for detection.

The method was validated according to the ICH guidelines with respect to specificity, precision, accuracy, and linearity and showing satisfactory data for all the method validation parameters tested. The percentage RSD for peak area response was found to be within the limit.

The proposed method for oxybutynin HCl is simple, selective, reproducible, and specific with good precision and accuracy. The method was proved to be superior to most of the reported methods. The proposed method for estimation of RS in selected drug was successfully applied either in bulk or pharmaceutical formulations.

Thus, the purpose of the present investigation was successfully achieved.

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