METHOD DEVELOPMENT AND VALIDATION OF OXYBUTYNIN CHLORIDE BY RP-HPLC ANALYTICAL TECHNIQUE

[1]J. MAMATHA, [2]N. DEVANNA, [3]J. SANDYA RANI

[1]Department of chemistry, G. Pullaiah College Of Engineering And Technology, Kurnool, Andhra Pradesh, India
[2,3]Department of chemistry, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India
E-mail: [1]mamatha@gpcet.ac.in, [2]devanna.chemistry@jntua.ac.in, [3]jasthisandhya@gmail.com

Abstract— A reverse phase high performance liquid chromatography method has been developed and validated for the determination of oxybutynin chloride on dried bases as per USP method in tablet dosage form. Isocratic elution at a flow rate of 1.0 ml / min was employed on a symmetry C8 column (75 × 4.6mm, 3.5µm SS) at 45ºC. The mobile phase consisted of mixture of phosphate buffer and acetonitrle 51:49 (%V/V) and the UV detection wavelength was 210 nm. The Rt value was found to be 13.71min with a run time of 40 min. The developed method was validated for linearity, accuracy, precision, detection limit, quantification limit, ruggedness, specificity, system suitability, and solution stability. Results of all validation parameters were within the limits as per 1CH Guidelines.

I. INTRODUCTION

Oxybutynin chloride (OC) is chemically (racemic) 4 – diethyl amino – 2 – butynyl phenyl cyclo hexylglycolate hydrochloride. (Figure – 1). OC has both antispasmodic action on the smooth muscle of the bladder detrusor muscle and Anticholinergic action inhibiting parasympathetic nerve impulses of smooth muscles present in urinary tract. It inhibits parasympathetic nerve impulses by selectivily blocking the binding of the neuro transmitter acetylcholine to its receptor in nerve cells. OC shows antispasmodic action by causing relaxation of the detrusor muscle of the bladder in patients with an unstable bladder. This increases bladder capacity and reduces the incidence of spontaneous contractions of the detrusor muscle. OC has been used in the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency and frequency. It has also been used in the treatment of pediatric patients with symptoms of detrusor over activity associated with a neurological condition.

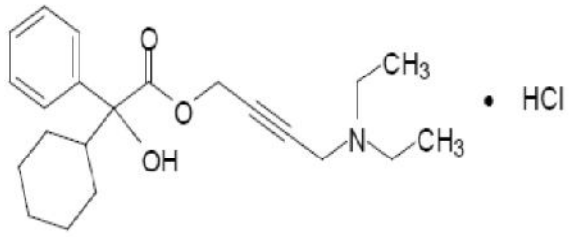


Figure 1 : Oxybutynin chloride (Mol. Wt : 394)

Literature survey reveals that certain UV-Vis spectrophotometric methods and thin layer chromatographic methods have been developed and reported for the determination of the OC. The aim of the present work was to develop a simple, precise, reliable, sensitive and accurate RP – HPLC method for the estimation of OC in bulk and pharmaceutical dosage forms and validate the analytical method in accordance with 1CH guidelines.

II. MATERIAL AND METHODS

2.1. Instrumentation: HPLC column, pH meter, sonicator, vaccum oven,water bath and other glassware were

2.2 Chemicals and Solvents:
The pharmaceutical grade pure sample of OC, OC formulation, milli- Q – water, Analytical grade potassium dihydrogen phosphate,dipotassium hydrogen phosphate, HPLC grade acetonitrile were obtained fromMerck India Ltd.

2.3 Chromatographic conditions:
HPLC equipped with UV/Vis detector (detection wavelength 210nm) was used. The RP-HPLC column consisted of C8 symmetry (75× 4.6 mn, 3.5µmSS column). Mobile phase used was a 51 : 49 (%V/V) degassed mixture of phosphate buffer and acetonitrile. The flow rate of the mobile phase was maintained at 1.0ml/min at a Column oven temperature of 45ºC. The injection volume was 10µl capacity with a run time of 40 min.

III. PREPARATION OF ANALYTICAL SOLUTIONS

3.1 Preparation of phosphate buffer:
Dissolve about 6.67gms of potassium dihydrogen phosphate and 8.55gms of dipotassium hydrogen phosphate in 1000ml of water (HPLC grade) and mixed using an ultrasonicator and filtered through 0.45µ filter paper.

3.2 Preparation of Mobile phase :
The above prepared phosphate buffer and acetonitrile were filtered separately through 0.45µ membrane filters. The filtered solvents were mixed in ratio of (51: 49) (% V/V) making adjustments if necessary and degassed and the resultant solution used as mobile phase.

3.3 Preparation of diluent:

The mobile phase was used as diluent.

3.4 Preparation of standard stock solution:
50 mg of the OC working standard sample was accurately weighed and transferred into a 10ml volumetric flask. 5ml of mobile phase was added. Sonicated to dissolve and diluted to 10ml with mobile phase. 2ml of the resulting solution was pipetted into an 100ml flask and diluted with the mobile phase upto the mark resulting in 100 ppm of OC standard solution.

3.5 Preparation of sample solution:
50mg of OC sample formulation was accurately weighed and transferred into 10ml volumetric flask. 5ml of mobile phase was added to it and sonicated to dissolve it completely and diluted up to the mark with the mobile phase. 2.0ml of this solution was pipetted into a 100ml flask and diluted with the mobile phase upto the mark resulting in 100 ppm of OC sample solution.

3.6 % assay of oxybutynin chloride :
The % assay of OC was calculated by the following procedure. Injecting standard solution in six replicates and checking for the system suitability. Injecting test solution in duplicate.Injecting bracketing standard solution and studying the recorded chromatograms in each case.

Calculations :
The % assay of OC (on dried basis).
% Assay

$$= \frac{T}{S} \times \frac{Ws}{10} \times \frac{2}{100} \times \frac{10}{wt} \times \frac{100}{2} \times \frac{100}{(100 - \%LOD)} \times P$$

Where T = Average peak area of OC peak in test preparation.
S = Average peak area of OC peak in standard preparation.
Ws = Weight of OC standard taken in mg.
Wt = Weight of OC sample taken in mg.
P = % Purity of working standard.
The % Assay was calculated as 99.94 which was well within the acceptance criteria.

IV. METHOD VALIDATION

The method validation was done as per the 1CH guidelines.

4.1 System suitability test:
Six replicates of standard solution were injected into the chromatograph and the chromatograms were recorded. As per the results the % RSD for RT was 0.08 which was well within the acceptance criteria of not more than 2.0. The % RSD for peak area was 0.17 which was also well within the acceptance criteria of not more than 2.0. The obtained results are presented in t**able 1.Fig 2. Fig 3**
The system suitability parameter was validated.Tailing factor for the peak in the standard and sample solution was 1.10 and the numbers of theoretical plates were not less than 5000.

4.2 Specificity:
The specificity of the method was studied by separate injections of OC standard solution, standard solutions of oxybutynin related compounds labeled as A, B and C, OC test sample solution, spiked test solution and the blank. The retention time of the sample solution was found to be 13.71 minutes. Peaks of all the related compounds were separated and there was no interference with the peak of the sample. The results are tabulated and depicted in **Table – 2, Table – 3, Table – 4, Fig - 4, Fig – 5**
The parameter specificity was validated.

4.3 Linearity and range:
Five series of standard solutions of OC were selected for determining the linearity range. The concentration levels of these five series of standard solutions ranged from 80 ppm to 120 ppm (80% to 120%). All linearity level solutions were injected in triplicate. % RSD of peak response in triplicate injections for each level were calculated.Plotted a regression line of average peak areas of the OC versus concentration of the OC and determined the coefficient of correlation r, slope, y – intercept and residual sum of squares. The obtained results are presented and depicted. In **Table – 5, Table – 6, Fig – 6, Table-12.**

4.4 Accuracy:
The Accuracy of the RP – HPLC method was evaluated by selecting three different concentration levels (80%, 100% and 120%). In each concentration a minimum of 3 injections were given and the % Assay, % RSD of Assay were calculated. The obtained results are presented in Table 7.

4.5 Precision:
The precision of the analytical method was demonstrated by system precision, method precision and intermediate precision (Ruggedness).

System precision: Standard solution of OC was injected in six replicate. The % RSD for RT and peak area of six replicate injections values were obtained and were well within predetermined acceptance criteria of not more than 2.0 for RT and not more than 2.0 for peak area.

4.6 Method precision:
Prepared a total of six test solutions.Injected six individual test solutions. Calculated the % RSD of peak areas, % assay of each individual samples and its % RSD.

4.7 Intermediate precision (Ruggedness) In intermediate precision test was performed on a

different day. The results obtained from method precision was considered for the first day analysis. Injected six individual test solutions separately. Calculated the % assay of each individual sample and its % RSD .Also calculated the % RSD of % assay of both the analysis. The obtained results are presented in Table - 8and Table – 9.

4.8. LOD and LOQ:

The limit of detection and limit of quantification were determined by injecting lower concentration of the standard solutions into RP – HPLC column using the optimized chromatographic conditions and calculated using S/N ratio method.

4.9 Solution stability:

Solution stability was calculated in the following manner. Injected six replicates of standard solutions to check the system suitability. Injected standard solution and test solution in duplicate for each time interval that is initial, 6 hours, 12 hours, 18 hours and 24 hours. Calculated the % assay and % variance of assay value at each time interval. The results of solution stability are tabulated in table 10 and table 11.

V. RESULTS AND DISCUSSION

- The Retention time of the OC was 13.71 minutes.
- The % assay of OC on dried basis was calculated as 99.94 which was well within the acceptance criteria of not less than 97.0% and not more than 101.0%.

After ensuring that the system suitability parameter are met, validation was carried for specificity, linearity and range, Accuracy, Precision, LOD and LOQ, and solution stability.

Specificity: The results showed that the method of analysis applied for determination of assay was specific. The retention time of OC and its related compounds are specific and there is no interference observed with each other and peak of the sample. The parameter specificity is validated.

Linearity and Range: The % RSD of peak area of OC at each level was found to be within the acceptance criteria of not more than 2.0 The peak area response of OC at linearity level from 80% to 120% of working level concentration was found to be linear, Response showed linearity with respect to increase in concentration. The correlation coefficient was calculated as 0.9998, slope as 9978.55, y-intercept as 86.20 and Residual sum of squares was 37255468. The correlation coefficient was within the acceptance limit of not less than 0.99. The parameter linearity is validated.

Accuracy: The % assay of OC at accuracy level from 80% to 120% of working level concentration was found satisfactory.

The % RSD of % assay of OC at each level was found to be within the acceptance criteria of not more than 2.0. For level 1 (80%) it is 0.10, for level II (100%) it is 0.24 and for level III (120%) it is 0.09. The parameter accuracy is validated.

Precision:

System Precision: The % RSD for retention time of six replicate injections was 0.08 and for area it was 0.17 which were reproducible and well within the predetermined acceptance criteria of not more than 2.0 for retention time and not more than 2.0 for peak area. This indicated a good degree of precision for the analytical system. The parameter system precision was validated.

Method Precision: Obtained values indicated that the % RSD of peak area was 0.16 and that of % Assay content was 0.16. which were reproducible and within the acceptance criteria. The results indicated a good degree of precision for the analytical method. The parameter method precision was validated.

Intermediate precision (Ruggedness):

Ruggedness is the degree of reproducibility of the results obtained under different conditions. From the chromatograms and values obtained from second analysis it was calculated that the % RSD for peak area was 0.42 and for % assay was 0.48 which was reproducible and within the acceptance criteria for the second analysis.

The cumulative % RSD for the assay of both analysis was 0.35. The intermediate precision between two analysis was reproducible. The parameter intermediate precision was validated.

LOD: Limit of detection was found to be 1.48%.
LOQ: Limit of quantification was found to be 4.93%.

Solution Stability: Theresults from solution stability study indicated that standard solution and test solution were stable over 24 hours of use. The % variance in the results from the initially determined assay values of sample solution are 0.00, 0.00, 0.00 and 0.01 for 6, 12, 18, and 24 hours respectively and for standard solution are 0.02, 0.00, 0.02 and 0.00 for 6, 12, 18, 24 hours respectively at Laboratory conditions.

This variance was well within the acceptance criteria of not more than 10%. The parameter for stability of solution was validated for its use upto 24 hours from time of preparation.

Table 1 : System Suitability

Sr. No.	Injection No	Oxybutynin chloride	
		RT (in min)	Peak area
01	Inj-1	13.67	1016138
02	Inj-2	13.68	1018922
03	Inj-3	13.69	1020135
04	Inj-4	13.69	1019616
05	Inj-5	13.69	1019687
06	Inj-6	13.70	1021370
Average		**13.69**	**1019311**
% RSD		**0.08**	**0.17**

Table 2 : Specificity

Sample	Injection No	Retention time (min.)			
		Oxybutynin chloride	Related Compounds		
			A	B	C
Standard Solution	1	13.70	N.D	N.D	N.D
	2	13.70	N.D	N.D	N.D
Mean		**13.70**	**N.A**	**N.A**	**N.A**
Impurity A Solution	1	N.D	1.93	N.D	N.D
	2	N.D	1.93	N.D	N.D
Mean		**N.A**	**1.93**	**N.A**	N.A
Impurity B Solution	1	N.D	N.D	8.87	N.D
	2	N.D	N.D	8.86	N.D
Mean		**N.A**	**N.A**	**8.87**	N.A
Impurity C Solution	1	N.D	N.D	N.D	10.40
	2	N.D	N.D	N.D	10.40
Mean		**N.A**	**N.A**	**N.A**	**1040**
Test Solution	1	13.71	N.D	N.D	N.D
	2	13.71	N.D	N.D	N.D
Mean		**13.71**	**N.A**	**N.A**	**N.A**
Test solution spiked with impurities	1	13.71	1.93	8.82	10.41
	2	13.71	1.93	8.81	10.41
Mean		**13.71**	**1.93**	**8.82**	**10.41**

N.D : Not detected **N.A : Not applicable**

Table – 3 % Assay of test solution.

Test solution	Oxybutynin chloride	Average peak area	% Assay (ODB)
	Peak area		
1	1) 1002729	1002174	99.94
	2) 1001618		

Table – 4 : % Assay of test solution spiked with impurities.

Test with spiked impurities solution	OC	Average peak area	%Assay (ODB)
	Peak area		
1.	1) 1050806	1051888	100.48
	2) 1052970		

Table – 5 :Linearity of Standard Solution

Level	Volume of Standard Stock solution for linearly (ml)	Conc. Of Oxybutynin chloride (ppm)
80%	4.0	80
90%	4.5	90
100%	5.0	100
110%	5.5	110
120%	6.0	120

Table 6 : Linearity level solutions :

Level	Injection No	Peak area	% RSD
Level I (80%)	Inj – 1	806017	0.48
	Inj – 2	798354	
	Inj – 3	802274	
	Mean	802215	
Level II (90%)	Inj – 1	891472	0.60
	Inj – 2	900344	
	Inj – 3	890760	
	Mean	894192	
Level III (100%)	Inj – 1	991786	0.41
	Inj – 2	999924	
	Inj – 3	996211	
	Mean	995974	
Level IV (110%)	Inj – 1	1097563	0.09
	Inj – 2	1097655	
	Inj – 3	1099311	
	Mean	1098176	
Level V (120%)	Inj – 1	1198038	0.31
	Inj – 2	1196118	
	Inj – 3	1203293	
	Mean	1199150	
Correlation coefficient			**0.9998**
Slope			**9978.55**
Y-Intercept			**86.20**
Residual sum of squares			**37255468**

Table – 7 : Accuracy

Level	Injection No.	Peak area	% Assay	% RSD of Assay
Level I (80)	Inj – 1	807839	100.52	0.40
	Inj – 2	803146	100.58	
	Inj – 3	802602	100.72	
	Mean	804529	100.61	
Level II (100%)	Inj – 1	1009465	100.26	0.24
	Inj – 2	1010293	100.62	
	Inj – 3	1018853	100.16	
	Mean	1012870	100.35	
Level III (120%)	Inj – 1	1184920	99.16	0.09
	Inj – 2	1183496	99.14	
	Inj – 3	1181151	99.31	
	Mean	1183189	99.20	

Table – 8 : % RSD calculation – method precision.

Sr. No.	Test Solution	Wt. of sample (in mg)	Oxybutynin chloride	
			Peak area	% Assay
01	1	50.06	1003745	100.05
02	2	50.08	1006938	100.33
03	3	50.18	1004789	99.92
04	4	5016	1008238	100.30
05	5	50.06	1005047	100.18
06	6	50.08	1006430	100.28
Average			**1005865**	**100.18**
% RSD			**0.16**	**0.16**

Table – 9 : Ruggedness of the method
(Cumulative %RSD)

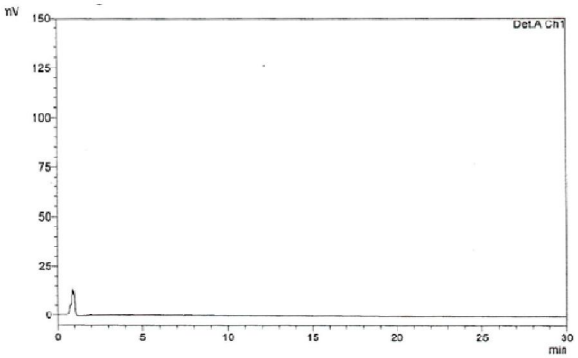
Test	Oxybutynin chloride			
	Day I		Day II	
	Peak Area	% Assay	Peak Area	% Assay
Test – 1	1003745	100.05	1006970	99.37
Test – 2	1006938	100.33	1009228	100.27
Test – 3	1004789	99.92	1020863	99.96
Test – 4	1008238	100.3	1015065	99.74
Test – 5	1005047	100.18	1017920	100.77
Test – 6	1006430	100.28	1011204	99.87
Mean		**100.18**	**Mean**	**100.00**
% RSD		**0.16**	**% RSD**	**0.48**
Cumulative % RSD		**Area**	**100.09**	
		% Assay	**0.35**	

Table : 10 Variance in solution stability of test solution at different interval :

Interval	% Assay of test	% Variance
0 Hrs	99.99	---
6 Hrs	100.22	0.00
12 Hrs	100.10	0.00
18 Hrs	99.81	0.00
24 IIrs	100.58	0.01

Table : 11 Variance in solution stability of standard solution at different Intervals :

Interval	% Assay of standard	% Variance
0 Hrs	99.89	---
6 Hrs	99.50	0.02
12 Hrs	99.78	0.00
18 Hrs	98.46	0.02
24 Hrs	99.68	0.00



Figures : 2 Blank Chromatogram

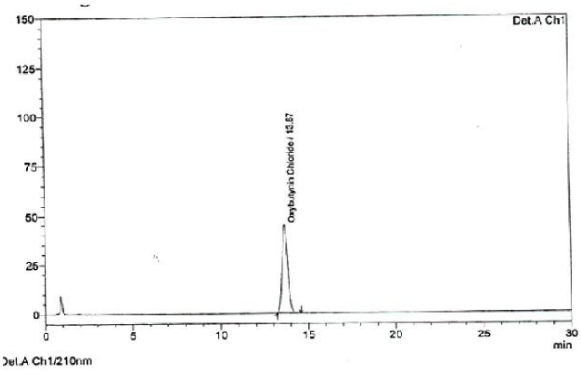


Figure : 3 Standard chromatogram for oxybutynin chloride

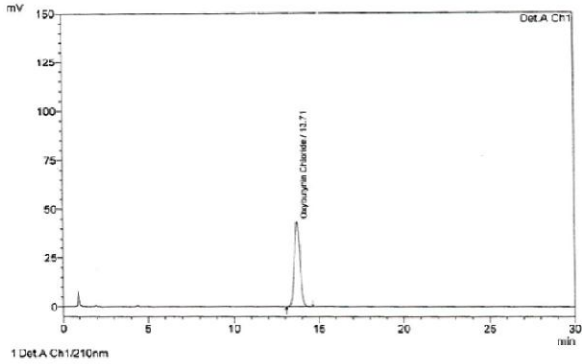


Figure : 4 Chromatogram for test solution of oxybutynin chloride.

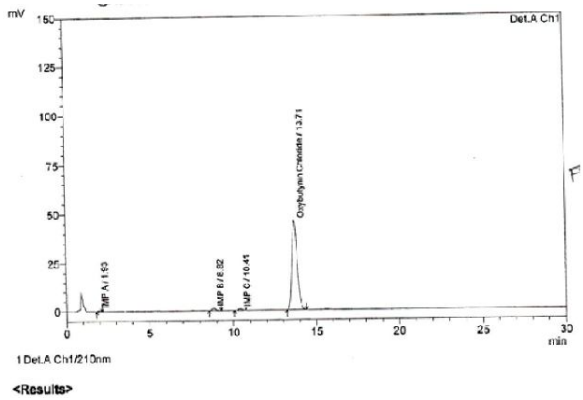


Figure : 5 Chromatogram to depict specificity.

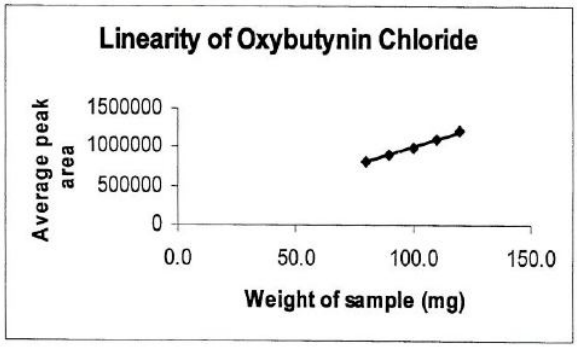


Figure : 6 – Linearity of Oxybutynin Chloride Assay

Table-12 Linearity study of oxybutynin chloride.

	Conc. (ppm)	Average Peak area
	80.0	802215
	90.0	894191
	100.0	995974
	110.0	1098176
	120.0	1199150
Correlation Coefficient		0.9998
Slope		9978.55
Y-intercept		86.20
Residual sum of squares		37255468

SUMMARY AND CONCLUSION

The present RP – HPLC method for estimation of OC was convenient, simple and fast. The method was validated in terms of Accuracy, Precision, Specificity,

linearity and Solution stability, as per 1CHGuidelines and can be applied in pharmaceutical dosage forms and for analysis in quality control.

REFERENCES

[1] https: //www.medicines.org.uk/emc.

[2] http://health.yahoo.com/urinary- medications/oxybutynin-oral/healthwise

[3] http://www.medicinenet.com/oxybutyniner-oral/article

[4] kachur JF : et al (1988). "R and S enantiomers of oxybutynin Pharmacological effects in guinea pig bladder and intestine". Journal of Pharmocology and Experimental Therapeutics. 247:867-72.

[5] Zobrist RH; et al. (2001). "Pharmacokinetics of the R – and S – Enantiomers of Oxybutynin and N – Desethyloxybutynin Following Oral and Transdermal Administration of the Rocemate in Healthy Volunteers" Pharmaceutical Researach. 18 : 1029 -1034.

[6] Official Monographs / Oxybutynin.

[7] NourE.Wagieh, Mahq. A. Hegazy. "Quantitative determination of Oxybutynin hydrochloride by Spectrophotometry, Chemometry and HPTLC in presence of its degradation products and additives in different pharmaceutical dosage forms "Talanta 2010; 80 : 2007 – 15.

[8] PritamKanagale, BrajBhushanLohray, AmbikanandanMisra, PrakashDavadra, and Rajesh Kini. "Formulation and Optimization of Porous Osmotic Pump – based controlled Release System of Oxybutynin". AAPS Pharm SciTech 2007 : 8(3)53

[9] K.Srikanth, K.A.Emmanuel and K. Ramesh Raju. "Spectrophotometric Determination of Oxybutynin chloride through 10N – Association complex formation." RASAYANJ.Chem. 2010; 3 : 179 – 187.

[10] Manthera V.S. Varma, Aditya M. Kaushal, Sanjay Garg "Rapid and Selective UV spectrophotometric methods for dissolution studies of oxybutynin and controlled release formulations. Journal of Pharmaceutical and Biomedical Analysis 2004; 36 : 669 – 74.

[11] Sweetman SC, editor. Martindal, The complete Drug Reference. 34th ed. London : The Pharmaceutical Press; 2005.

[12] Budavari S, editor. The Merck Index. 13th ed. Whitehouse station, NJ : Merck & Co. Inc: 2001. P. 3504

[13] Jenke D.R, Chromatographic method validation : A review of current practices and procedures. 11. Guidelines for primary validation parameters. J. Liq. Chromatogr. 19, 737-757. 1996.

[14] United states Pharmacopoeia, XXIV, United States Pharmacopoeial convention. Inc. Rockville, M.D.

[15] Bakshi M, Singh S, Development of validated stability indicating assay methods – Critical review. Journal of Pharmaceutical and Biomedical Analysis 2002; 28(6) 1011 – 40.

[16] International conference on Harmonization (ICH), ICH quality guidelines. Good manufacturing practice guidance for active pharmaceutical ingredients Q7A, ICH, Geneva, Switzerland, 2001.

[17] International conference on harmonization (ICH), ICH quality guidelines: Validation on analytical procedures: Methodology Q2B. ICH, Geneva, Switzerland, 1996.

[18] US Food and drug administration, Guidance document for industry, Analytical procedures and methods validation, FDA, Rockville, MD, 2000.

[19] H.kim, S.B.Han, "The chiral separation of Oxybutynin enantiomers using an avomucoid column". Journal of Pharmaceutical and Biomedical Analysis. 2003; 31 : 341-49

[20] Tupker RA, Harmsze AM, Deneer VH. "Oxybutynin therapy for generalized hyperhidrosis" Arch Dermatol. 2006; 142 : 1065 – 6.

★★★