



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**  
*'A Bridge Between Laboratory and Reader'*

[www.ijbpas.com](http://www.ijbpas.com)

---

---

**A REVIEW ON ANALYTICAL TECHNIQUES FOR THE ESTIMATION OF  
OXYBUTYNIN HYDROCHLORIDE**

**V.DILLI RANI, M.VIJEY AANANDHI AND R.GANDHIMATHI\***

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences,  
Vels Institute of Science Technology and Advanced Studies (VISTAS)  
Chennai-600117, Tamilnadu, INDIA

\*Corresponding Author: Dr. R. Gandhimathi; E Mail: [drgmthipharm2017@gmail.com](mailto:drgmthipharm2017@gmail.com)

Received 18<sup>th</sup> Jan. 2022; Revised 25<sup>th</sup> March. 2022; Accepted 18<sup>th</sup> April. 2022; Available online 1<sup>st</sup> Oct. 2022

<https://doi.org/10.31032/IJBPAS/2022/11.10.6508>

**ABSTRACT**

Overactive Vesicle is a lower perspiration plot circumstances, expressed along with indications of criticalness, with or without ask indulgence, ordinarily with frequency and dysuria. Antimuscarinic specialists are presently the pharmacology therapy of decision for victims among with uncontrollable vesicle, posses grounded adequacy. Oxybutynin is artificially 4-(diethylamino)but-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride and is an anticholinergic prescription utilised to mitigate urinary and bladder hardships together with successive pee and failure to be in control of pee (encourage incontinence), by diminishing brawn fits of the bladder.

A straightforward, delicate and consistent apparent spectrophotometric technique had been produced for the assurance of oxybutynin chloride in unadulterated structure and in drug definition. The technique includes the particle interrelation compound development of oxybutynin chloride along with acidulous colors TPOOO or ARS. A dispersive fluid microextraction strategy joined with fine electrophoresis/diode cluster discovery was created and adapted to the assurance of oxybutynin and in human pee. The electrophoretic investigations were executed utilizing a melded silica uncoated hairlike with a compelling extent of 36.5cm and a 50 mmolL<sup>-1</sup> arrangement of TEA, pH 3.0, as the foundation electro dialysis electrolysis. Invert stage superior execution fluid chromatographic strategy has been produced for the assurance of related compound of oxybutynin hydrochloride in

unadulterated structure and drug measurements structures. The antecedent→item particle change for the pair of the determinants and Internal Standards were takes place checked on a triad quintuple mass spectrometer, working in the various response checking and certain excitation method. In human Plasma and human serum the drug is assigned with suitable system in LC-MS/MS. The process are used to explore the study of analytical technique for regular use of analysis, the development and validation of the Oxybutynin exhibits the accurate values in all analytical techniques.

**Keywords: Oxybutynin, UV, HPLC, HPTLC, LC-MS/MS, Human Plasma**

## INTRODUCTION:

Antimuscarinic drugs are a gathering of anticholinergic specialists, explicitly known for hindering the movement of muscarinic receptors. These receptors assume a significant part in interceding the elements of the parasympathetic sensory system, which controls numerous compulsory capacities to ration energy, including the withdrawal of smooth muscle, enlargement of veins, expanded in essence emissions, gastrointestinal movement, and pulse [1]. Antimuscarinic specialists are presently the pharmacologytherapy of decision for victims along with, Uncontrolled vesicle, possess grounded adequacy [2, 3]. Overactive bladderis a lower perspiration lot circumstances, portrayed by side effects of earnestness, with or without ask indulgence, typically with frequency and dyduria. This side effect convoluted essentially impact victim's nature of life. Much of the time, the fundamental

pathophysiology is an compulsory distension constriction throughout the capacity stage of the clearing cycle [4]. These specialists prevent the impacts of ACh at muscarinic labyrinth of the vesicle also in this way decrease the recurrence and/or force of the compulsory distension constrictions that assume a significant part in this constant circumstances. Five particular muscarinic receptor subsets (M1–M5) are well to subsist [5]. Antimuscarinic meds can be endorsed for a wide range of reasons. One of the more normal cases is in people with overactive bladder disorder, a lower urinary lot condition that makes compelling impulses pee and at times pee spillage. Antimuscarinics help by diminishing the motility of smooth muscle cells in the urinary plot and expanding the tone of the sphincters controlling pee [1].

Table 1: Arrangement of Antimuscarinic Drugs

Natural Alkaloids	Semi Synthetic derivatives	Synthetic Compounds		
		Mydriatics	Vasoselective	Antispasmodics
Atropine Hyoscine (scopolamine)	Homatropine Atropine- methonitrate Ipratropium- bromide Tiotropium-bromide	Cyclopentolate	Oxybutynin	Propenthaline
		Tropicamide	Flvoxate	Oxyphenonium
			Tolteridine	Dicyclomine
			Pirenzepine	

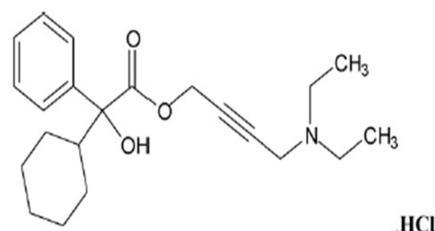
Consequently we will continue our cycle audit with Drug named as “Oxybutynin” which is set under Synthetic compound of Vasoselective.

### Oxybutynin:

Antimuscarinic specialists, with the approach of quick delivery oxybutynin (OXYIR), have taken place the pillar of therapy for Uncontrollable vesicle for being the beyond 30 years lifespan. Despite the fact that numerous patients react well to antimuscarinic specialists, just a little rate of patients accomplish all out dryness [6]. Oxybutynin quick delivery definition was the first treatment for Uncontrollable vesicle endorsed by the United State FDA in 1975. It is a chiral drug managed as a racemic combination; in any case, its antimuscarinic movement dwells transcendentally on the (R)- enantiomer [7]. Oxybutynin is a tertian amine ester with antimuscarini, antispasmodic and nearby sedative effects, that produce a restoratively helpful specialist in the therapy of victims along with perspiration indulgence [8].

Oxybutynin is artificially 4-(diethylamino)but-2-yn-1-yl 2-cyclohexyl-

2-hydroxy-2-phenylacetate hydrochloride [Figure 1] and is an antimuscarinic medicine utilised to alleviate urinary perspiration and vesicle is used together with incessant pee and failure to be control pee, by diminishing brawn fits of the vesicle. It seriously alienates the Muscarinic receptor1, Muscarinic receptor2, and Muscarinic receptor3 subset of the muscarinic acetylcholine labyrinth. Oxybutynin is additionally a potential therapy of hyperhidrosis [9, 10].



**Figure 1: Structure of Oxybutynin Hydrochloride**  
Oxybutynin is quickly assimilated from the gastrointestinal plot following oral organization with greatest plasma fixation came to in under 1 hour. Oxybutynin is widely utilized by the liver, firstly by the cyclin p450 compound system, particularly CYP3A4 establish for the most part in the liver and stomach divider [11].

### Drug Profile:

**IUPAC NAME:** 4-(diethylamino)but-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride

**Class Name:** Anti muscarinic group

**Brand Name:** Cystran, Noctorin Ditropan

**Generic Name:** Oxybutynin

**Frame Work:** Oxybutynin is in a class of Anti muscarinics. It works by loosening up the bladder muscles.

**Type of Molecule:** Tertiary amino compound and a racemate.

**Molecular Weight:** 393.95

**Empirical Formula:**  $C_{22}H_{31}NO_3.HCl$

### Physical Properties

**Solubility:** It is promptly dissolvable in water and acids, but somewhat insoluble in soluble bases.

**Melting Point:** 129-130°

**Appearance:** It appears as though White Crystalline Solid

### Pharmacology

**MOA:** It hinders parasympathetic nerve driving forces at particularly obstructing the limiting of the neurotransmitter ACh to its labyrinth in neurons. Oxybutynin exhibits antispasmodic activity by effecting unwinding of the distension brawn of the vesicle in victims along with an unsound vesicle. This expands vesicle limit and decreases the frequency of unconstrained compressions of the distension brawn [12].

### Pharmacokinetics:

**Absorption :** Oxybutynin was quickly retained, greatest plasma fixations (8 ng·ml<sup>-1</sup>) being reached in under 1 h. Indisputably the foundational accessibility arrived at the midpoint of 6% and the tablet and arrangement structures showed comparative relative fundamental accessibility [13].

**Amount of Circulation:** Oxybutynin has undergo a extensive measure of course of 193 L

**Capsid Binding:** Oxybutynin enantiomers are over 97% obliged to vital proteins.

**Metabolism:** Oxybutynin is heavily processed by the CYP3A4 compounds framework in the pair of hepatic and the mass of the digestive system.

**Passage of Exception:** Oxybutynin is intensely void by the hepatic. Below 0.1% of a controlled portion is established as unaltered medication in the evacuation. Lower than 0.1% of a solitary portion of oxybutynin is excreted as desethyloxybutynin.

**Half-life:** The vital fluid disposal of period of time is around 2 hrs. In the elderly, the end half-life is delayed till 5 hrs

### Various Analytical Techniques for Estimation of Oxybutynin

#### Estimation of Oxybutynin by UV Method:

A basic, delicate and consistent noticeable spectrophotometry strategy was created for being the assurance of oxybutynin chloride

in unadulterated structure and in drug detailing<sup>[14]</sup>. UV spectrometric technique depended on particle set combination of picric corrosive with tertian-amine gathering of Oxybutynin to shape an Ultraviolet dynamic compound. Oxybutynin possess transmittance utmost at 196 nanometer where a part of fixing or dissolvable will meddle with announced measure of Oxybutynin in fluid glasslike congeal along with spectrophotometry technique, examined at 230 nm [15]. The strategy includes the particle alliance compound development of Oxybutynin chloride along with acidulous colours TPOOO or ARS. It possess certain assimilation most extreme at 480 nanometer and 430 nanometer individually and complies with brew's principle in the focus scope of 1.0-7.5 µg.ml-1 and 2.0-15 µg.ml-1 separately [14].

To make spectrophotometric systems prepared to choose the focused on medicament in existence of methyl and propylparabens despite of their more prominent absorptiveness. Digestion spectra of the ensuing game plans were inspected and isolated by the osmosis scope of 20 g/mL degradate course of action. The zenith proportions of the chief subordinate of extent stretches around 216nm were takes place isolated in regards to recurrence along with 4 and were plotted versus the relating concentration, and backslide

condition was handled [16]. Particle twin eradication spectrophotometric has gotten impressive consideration for being determination assessment of numerous drug composite. This strategy relies upon the response of a medicament that has fundamental positron nitrogen and an superoxide anion colour, where a exceptionally shaded particle pair complex is shaped. The proposed technique depends on the response of the chose medicament along with Chromoxane cyanine R in cradled watery arrangement at pH 1.0. The shaped particle pair buildings takes place extricated along with Methylene dichloride and estimated determinatively along with most extreme assimilation at 464 nm [17].

#### **Estimation of Oxybutynin by HPTLC:**

A particular chiral HPTLC method has been produced for the partition and quantitative assurance of the enantiomers of oxybutynin hydrochloride with practically no earlier derivatization and test arrangement. The arrangement and Rf esteems were doled out in comparison with Mass spectra and UV obtained for individual isomers by micro preparative TLC [18]. This strategy offers a straightforward method for evaluating straightforwardly as for Thin Layer Chromatography plate at estimating the ocular thickness of the isolated groups. The sums of mixtures are dictated by contrasting with a constant bend from

recommended substances chromatographed at the constant time under a similar condition. The wellspring of radiation utilized was deuterium lamp emanating a continuous UV spectrum between 190 and 400nm and convergences of the compound not set in stone from the force of diffusely reflected light. A dispersive fluid microextraction (DLLME) methodology joined with fine electrophoresis/diode exhibit location had been created and appeal to the assurance of oxybutynin and Desethyloxybutynin in human pee. The Cataphoretic investigations takes place executed utilizing a 50  $\mu\text{m}$  i.d. intertwined vitreous silica unsheathed narrow with a compelling extent of 36.5cm and a 50 mmolL<sup>-1</sup> arrangement of TEA, pH 3.0, as the foundation Electro dialysis electrolysis<sup>[19]</sup>.

#### Estimation of Oxybutynin for HPLC:

A straightforward and explicit Reverse Phase-High Performance Liquid Chromatography strategy, along with concise run time period was produced for the aspect be in control of examination of Oxybutynin similarity tests, solidness tests and furthermore in disintegration tests. Examine was completed for every one of the details utilizing HPLC strategy. These examples were separated through 0.45m nylon channel and broke down by RP-HPLC. Test gratified of every plan had been take hold of as 100 percent in

disintegration works [15]. The rate recuperation had been viewed as somewhere in the range of 98 and 102 and rate RSD ought to be not more than 2. Subsequently, the logical strategy is exact. The rate RSD for top space of Oxybutynin HCl was viewed as <2%. The goals between the pinnacles were viewed as more than 2 [9, 10]. The framework appropriateness boundary was authenticated. Tailing factor for the top in the norm what's more example arrangement was 1.10 and the quantities of hypothetical plates were at least 5000. The possession season of the Oxybutynin Chloride was 13.71 minutes. The % examine of Oxybutynin Chloride on dehydrated premise was determined as 99.94 that had been adequately inside the acknowledgment standards of at least 97.0% and never over 101.0%<sup>[12]</sup>.

The investigation of medication was done on a PEAK HPLC framework furnished with an opposite stage C18 section. The goal of the technique approval is to show that the technique is reasonable for its planned reason as it is expressed in ICH rules [20]. The created strategy was approved for linearity, exactness, accuracy, recognition limit, measurement limit, roughness, particularity, framework reasonableness, and arrangement steadiness. Aftereffects of all approval boundaries were inside the cut off points

according to ICH Guidelines. The % measure of OC was determined by the accompanying technique. Infusing graded arrangement in six duplicates and examine for the framework appropriateness [12]. Isocratic eluting at a stream pace of 1.0 ml/min was utilized as to a primesil-C18 section. The RT worth of oxybutynin hydrochloride, pollutant D, and contamination A was viewed as 13.75 min, 19.80 min, and 24.89 min, separately, with a run season of 60 min. There was no impedance at the maintenance time of oxybutynin HCl and its contaminations in clear, fake treatment and the contamination combination arrangement [9, 10].

Test arrangement transient solidness was tried at encompassing temperature for three

days. To affirm the soundness of both standard arrangements at 100 percent level and tablet test arrangements. Strategy precision was tried (% recuperation and %RSD of individual estimations) by dissecting test of Oxybutynin at three distinct levels in unadulterated arrangements involving three arrangements for each level<sup>[20]</sup>. Short run time, top balance and pinnacle immaculateness are the benefits that takes place establish along with the existing High Performance Liquid Chromatography strategy over with the current techniques. Concise run time period is quite compelling throughout disintegration work of CR plan afterward countless examples are created in each study [15].

Table 2: Various parameters of HPLC

Drug Name	Method	Column	Mobile Phase	Flow rate	Detector
Oxybutynin	RP-HPLC	C <sub>8</sub> symmetry	Phosphate Buffer, Acetonitrile	1.0mL/min	UV-Detector 210nm
Oxybutynin	RP-HPLC	Premisil-C <sub>18</sub>	Water, Acetonitrile, Triethyl-amine	1.0mL/min	UV-Detector 210nm
Oxybutynin	RP-HPLC	Hypersil BDS CPS precolumn	Water, Methanol, Triethylamine, Acetonitrile	1.5mL/min	UV-VIS Detector 203nm
Oxybutynin	RP-HPLC	C <sub>18</sub> column	1% orthophosphoric acid, Acetonitrile, Methanol	1.0 mL/min	UV-Detector 210nm

### Estimation of Oxybutynin by LC-MS/MS:

Profoundly delicate and quick Liquid Chromatography-Mass Spectrometry/Mass Spectrometry strategy being concurrent assurance of Oxybutynin and significant metabolite N-Desethyloxybutynin in human vital fluid had been created.

Deuteriumnoun marked Oxybutynin D11 and N-Desethyloxybutynin D5 were utilized as Internal principles subsequently, Vital fluid tests are separated through fluid extraction with tert-Methyl Butyl Ether [11]. A chiral Liquid Chromatography-Mass Spectrometry/Mass Spectrometry technique is depicted to examine the

enantiomers of Oxybutynin and N-Desethyloxybutynin and further chirality medicament and their substances consumption from human vital fluid. The confine of determination had been 0.150 and 0.750 ng/mL being Oxybutynin and N-Desethyloxybutynin respectively it considered stereo selective pharmacokinetics of the pair of the determines in human hepatic cytosol. A certain recemic selective High Performance Liquid Chromatography technique has been introduced involving fluid stage extraction for the assurance of Oxybutynin and N-Desethyloxybutynin rodent hepatic cytoplasm [7].

An isotope weakening elite execution fluid chromatography–pair mass spectrometry strategy posses takes place produced for the concurrent assurance of oxybutynin and its

pharmacological dynamic metabolite N-desethyl oxybutynin in human vital fluid. Eradication of oxybutynin, its substance also their deuteriumnoun congener as inside guidelines out of 300 microL human vital fluid was completed by fluid eradication with methyl tertiary-butyl ether-ethyl acetate derivation dissolvable blend [8]. A separator below surface had been infused into a C18 MS segment with 3.5 mm molecule size. The MS was worked in sure particle method utilizing ESI. Nitrogen was utilized as the atomizer and inert gas had been utilized as the impact air. Utilizing MS/MS along with numerous response observing various response monitoring method, oxybutynin and desethyloxybutynin were identified without serious impedances out of vital fluid network [20].

Table 3: Various Parameters of LC-MS/MS

Drug Name	Column	Mobile Phase	Run time
Oxybutynin	Phenomenex Gemini C18 column	Acetonitrile, Ammonium acetate	0.4mL/min
Oxybutynin	Cosmosil C18 column	Acetonitrile, Ammonium acetate	1.2 mL/min
Oxybutynin	C18 XTerra MS column	Methanol	2.0 mL/min
Oxybutynin	column Hypurity C18,	Acetonitrile, Ammonium acetate	3.5 min

### CONCLUSION:

The created UV spectrophotometric technique for the assessment of Oxybutynin is simple, delicate and conseravative. UV spectroscopic technique enjoys the benefits of undervalue and achievability, and as it may be utilized for regular disintegration

works throughout CR definition advancement. spectrophotometric strategy is basic, more advantageous, less tedious and financial steadiness demonstrating technique contrasted with other distributed LC strategies. The strategy was approved by the ICH rules concerning particularity,

accuracy, precision, and linearity and showing palatable information for all the strategy approval boundaries tried. The rate RSD for top region reaction was viewed as inside the breaking point. Chiral HPTLC is a quick, basic however specific methodology that can be effectively utilized for specific division, isolation and quantitative assurance of enantiomers without derivatization of diastereomers. The created achiral and chiral Liquid Chromatography-Mass Spectrometry/Mass Spectrometry method for the determination of Oxybutynin, N-Desethyloxybutynin and their enantiomers in human vital fluid was completely approved and fortunately adapted for a bioequivalence analysis in sound topics. The proficiency of fluid eradication and chromatographic run season of 4.0 min per test delivers the technique helpful in greater-all overbioanalysis.

#### ACKNOWLEDGEMENT:

The authors express our gratitude to Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, TamilNadu, India for providing the facilities to complete this task.

#### CONFLICT OF INTEREST:

No conflict of interest declared by the authors.

#### REFERENCES:

- [1] Athanasopoulos, A., & Giannitsas, K. An Overview of the Clinical Use of Antimuscarinics in the Treatment of Overactive Bladder, 2011; 18(2): 1-8.
- [2] Gary G. Kay, PhD, Mohamed B. Abou-Donia, PhD, W William S. Messer. Antimuscarinic Drugs for Overactive Bladder and Their Potential Effects on Cognitive Function in Older Patients; 2005; 53: 2195–2201.
- [3] Chapple C, Khullar V, Gabriel Z *et al*. The effects of antimuscarinic treatments in overactive bladder: A systematic review and meta-analysis. *EurUrol*, 2005; 48: 5–26.
- [4] H.Hashim and P. Abrams, “Overactive bladder: an update, “Current Opinion in Urology” 2007; 14(4): 231-236.
- [5] Napier C, Gupta P. Darifenacin is selective for the human recombinant M3receptor subtype. Presented as a non-discussion poster at the meeting of the International Continence Society, Heidelberg, Germany, 2002.
- [6] Rebecca J McCreery, Rodney A Appell. Oxybutynin: an overview of the available formulations, Therapeutics and Clinical Risk Management 2006; 2(1): 19 –24.

- [7] Primal Sharma, Daxesh P. Patel, Mallika Sanyal. Parallel achiral–chiral determination of oxybutynin, N-desethyloxybutynin and their enantiomers in human plasma by LC–MS/MS to support a bioequivalence trial; *Journal of Pharmaceutical and Biomedical Analysis*. 2013; 88: 81-91.
- [8] Primal Sharma, Daxesh P. Patel, Mallika Sanyal, Hiren Berawala. Simultaneous analysis of oxybutynin and its active metabolite N-desethyl oxybutynin in human plasma by stable isotope dilution LC–MS/MS to support a bioequivalence study; *Journal of Pharmaceutical and Biomedical Analysis*, 2013; 84: 244-255.
- [9] K. S. Nataraj, A. SrinivasaRao, N. Aishwarya Lakshmi. Analytical Method Development and Validation for the Estimation of Related Substances in Oxybutynin HCl Prolonged Release Tablets by Reverse-Phase High-Performance Liquid Chromatographic, *International Journal of Pharmaceutical & Biological Archives* 2018; 9(2):60-66.  
<http://www.chemicaland21.com/lifescience/phar/OXYBUTYNIN%20HYDROCHLORIDE.html>. [Last accessed on 2018 Jun 03]
- [10] Vivek Anand PA, Sambasiva Rao Puram. Highly Sensitive Simultaneous Determination of Oxybutynin and N-Desethyloxybutynin in Human Plasma by LC-MS/MS, *Journal of chemical and Pharmaceutical Sciences*, 2016; 9(4): 2456-2459.
- [11] J.Mamatha, N. Devanna, J. Sandyarani. Method Development and Validation Of Oxybutynin Chloride By RP-HPLC Analytical Technique; *International Journal of Advances in Science Engineering and Technology*. 2017, 5(1).
- [12] J. Douchamps, F. Derenne, A. Stockis, D. Gangji. The pharmacokinetics of oxybutynin in man, *European Journal of Clinical Pharmacology*, 1988; 35: 515-520.
- [13] K. Srikanth, K.A. Emmanuel and K. Ramesh Raju. Spectrophotometric Determination of Oxybutynin Chloride Through Ion-Association Complex Formation, *Rasayan J.Chem.* 2010; 3(1): 179-187.
- [14] Manthana V.S. Varma, Aditya M. Kaushal, Sanjay Garg. Rapid and selective UV spectrophotometric and RP-HPLC methods for dissolution studies of oxybutynin immediate-release and controlled-

- release formulations, *Journal of Pharmaceutical and Biomedical Analysis*, 2004; 36: 669-674.
- [15] Nour E. Wagieh, Maha A. Hegazy, M. Abdelkawy. Quantitative determination of oxybutynin hydrochloride by spectrophotometry, chemometry and HPTLC in presence of its degradation product and additives in different pharmaceutical dosage forms, *Journal homepage:www.elsevier.com/locate/talanta*;2010
- [16] Akram. M, El-didamony, Monuir Z. Saad, SALEEM. Extractive Spectrophotometric Determination of Some Antimuscarinic Antagonist In Tablet Formulations Using Eriochrome Cyanine R, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2018; 10(4): 22-28.
- [17] P.Sitadevi, P.L.K.M.Rao. Development and validation of a method for the enantioseparation of oxybutynin hydrochloride by HPTLC, *Analytical Chemistry An Indian Journal*, 2010; 9(3): 378-383.
- [18] B. J. Moreira, K. B. Borges, A. R. M. de Oliveira, Analysis of oxybutynin and *N*-desethyloxybutynin in human urine by dispersive liquid-liquid microextraction (DLLME) and capillary electrophoresis (CE), 2013; 1-10.
- [19] Suman Avula, K. Naveen Babu, M.V. Ramana. Validated RP-HPLC Method for the Estimation of Oxybutynin In Formulation, *An International Research Journal*, 2011, Vol. 2 (2), 119-125.
- [20] HohyunKim, Sang Beom Han. Sensitive determination of oxybutynin and desethyloxybutynin in dog plasma by LC\_ESI/MS/MS, *Journal of Pharmaceutical and Biomedical Analysis*, 2003; 31: 341-349