INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 1 | Jan–Mar-2019
Journal Home page: www.ijpar.com

Research article *Open Access*

Method development and validation of amoxicillin in bulk and pharmaceutical dosage form by UV spectroscopy

Divya.Velpula[1], Pallela sowmya lakshmi[2], P.mounika[2], Shaik mallika[1], Pedapudi Umesh Chandra[2], Srividya[1]

[1]*Assistant professor,SIMS college of pharmacy,Guntur*
[2]*Student, SIMS college of pharmacy,Guntur.*

***Corresponding Author: Divya.Velpula**
Email: divyagunturu63@gmail.com

ABSTRACT

A simple, sensitive, specific, rapid, precise and accurate UV spectrophotometric method has been developed and validated for estimation of Amoxicillin in pure drug and dosage form, using 0.1N HCL as solvent .The absorption maxima was found to be 230nm. The method was validated in terms of Linearity, Precision, Accuracy, Repeatability, Sandell's sensitivity according to ICH[Q2(R1)] guidelines. Linear response was observed in the range of 1-5µg/ml with a correlation co-efficient of 0.998.The %RSD for Precision was found to be NMT 2 which indicates the method was precise. The %Recovery was found to be in the range of 99.3-99.8% for amoxicillin which indicates a good accuracy of the method. The percentage recovery for the marketed formulation was found to be 99.37%, which indicates good recovery.Commercial capsule formulation was successfully analyzed using the developed method and the proposed method was applicable to routine analysis for determination of Amoxicillin in capsule dosage form.

Keywords: Amoxicillin, 0.1N HCL, Method development, Validation, UV spectroscopic method.

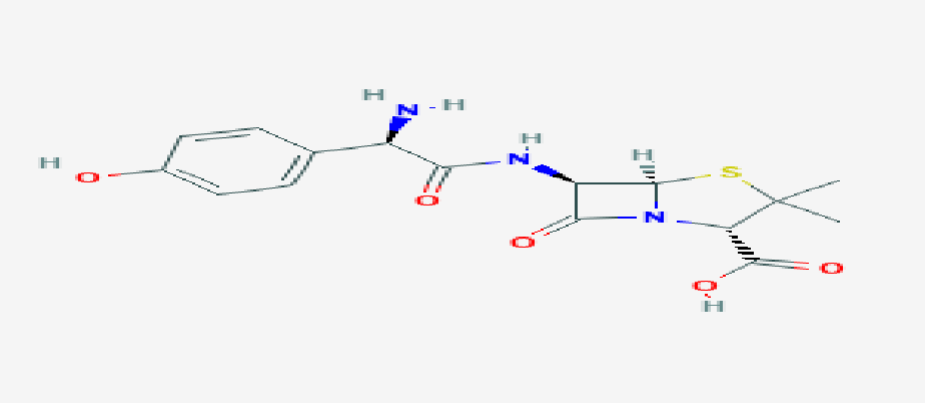
INTRODUCTION

Amoxicillin is considered as a third generation or amino penicillin and is one of the most commonly prescribed antibiotic. Its molecular formula is $C_{16}H_{19}N_3O_5S$[1]. Its IUPAC name is 2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid[2]. Amoxicillin is a Moderate- Spectrum antibiotic active against a wide range of Gram-positive and a limited range of Gram-negative organisms. It is usually the drug of choice within the class because it is better absorbed following oral administration than other beta- lactam antibiotics. Amoxicillin is susceptible to degradation by B-lactamase producing bacteria and so may be given with Clavulanic acid to increase its susceptibility. The incidence of Beta-lactamase producing resistant organisms including E.coli, appears to be increasing. Amoxicillin is sometimes combined with clavulanic acid, a B-lactamase inhibitor to increase the Spectrum of action against

Gram-negative organisms and to overcome bacterial antibiotic resistance mediated through B-lactase Production. Amoxicillin binds to Pencillin-binding protein IA (PBP-IA) located inside the bacterial cellwell

Pencillins acylate the pencillin sensitive transpeptidase C-terminal domain by opening the location ring. This inactivation of the enzyme prevents the formation of a cross link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis, cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins. It is possible that amoxicillin interferes with an autolysin inhibitor. [3-6].

According to literature survey, works had been done on Amoxicillin by HPLC[7-9],uv methods[10,11].



AIM OF THE PRESENT WORK

The main aim of the present study is to develop an accurate, precise, selective, reproducible and rapid analytical technique for cost effective estimation of Amoxicillin in pure drug and capsule dosage form.

The Plan of Work is as follows

- To develop suitable spectrophotometric method for assay of Amoxicillin
- To perform the validation for the developed method.

MATERIALS ANDMETHOD

Amoxicillin was a gift sample from Cipla Pvt. Ltd, Hyderabad. HCL used was of analytical grade and purchased form Merck Chemicals, India. The capsule formulation of Amoxicillin 500mg was obtained from local pharmacy. Spectroscopic analysis was carried out using Double beam T60UV-Visible Spectrophotometer with 1cm path length matched quartz cells used for analytical purpose.

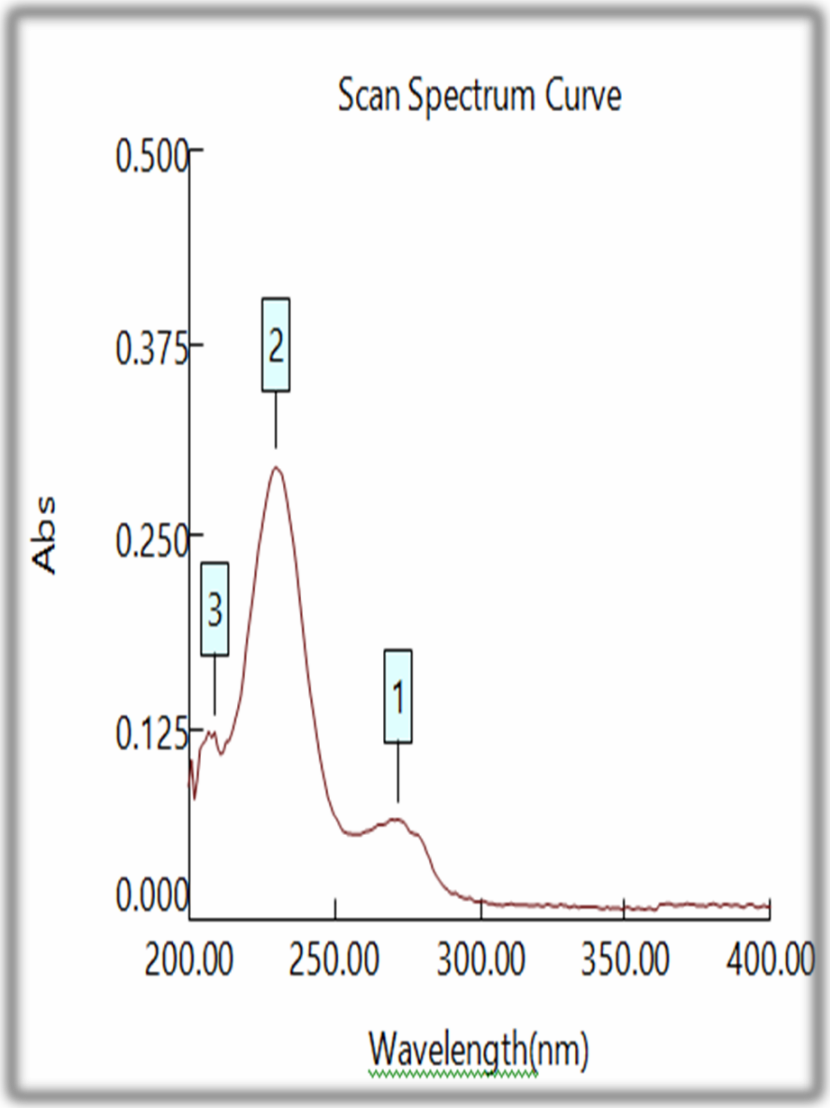
EXPERIMENTAL METHOD

Preparation of standard solution

Weigh accurately10mg of Amoxicillin and transfer it in to a 10ml cleaned and dried volumetric flask, add sufficient quantity of 0.1N HCL to dissolve and finally make the volume to 10ml with 0.1N HCL(1000µg/ml).

Preparation of working standard solution

From the prepared 1000µg/ml of Amoxicillin standard solution,pipette out 1ml into a clean and dried 100ml volumetric flask and finally makeup the volume to 100ml with 0.1N HCL (10µg/m).The resulting solution was scanned in theUVrange(200–400nm). In spectrum Amoxicillin showed absorbance maximum at 230nm.



ABSORBANCE VALUES

S.No	p/v	Wave length Λmax	absorbance
1	Peak	272.00	0.067
2	Peak	230.00	0.296
3	Peak	209.00	0.124

VALIDATION OF THE METHOD [12-14]

The method was validated in terms of Linearity, Accuracy, Precision (Repeatability ,Inter day precision, Intra day precision), Robustness.

LINEARITY

From the prepared 10µg/ml working standard solution, different standard concentrations of 1,2,3,4,5µg/ml solutions were prepared and their absorbance values were measured at 230nm.

PRECISION

Precision study of the method was performed by intraday and inter-day variation study. The intraday precision and interday precision was ascertained by determining absorbance of 3 replicates of a fixed concentration of the drug (3 µg/ml) at three different time period of the same day and on three different days. The result of the precision studies was expressed in terms of % RSD (percentage Relative Standard Deviation).The Repeatability was also done by 3µg/ml solution.

ACCURACY

Accuracy of the developed method was carried out by performing recovery study using standard addition method, in which standard drug was added at three different concentration (30%,40% and 50%) to the pre-analyzed capsule formulation (10 µg/ml).

ROBUSTNESS

Robustness of the proposed method was determined by changing the λ_{max} of the analysis (λ max- 230 nm) by ± 4nm.

ASSAY OF CAPSULE FORMULATION

Preparation of sample solution

Weigh accurately 10 capsules. Powder them in a dry motor and pestle. Weigh the powder equivalent to 10mg and transfer it into a clean and dry 100ml volumetric flask and add sufficient quantity of 0.1N HCL to dissolve and finally make up the volume to 100ml with 0.1N HCL. From the prepared 100ml solution, pipette out 1ml in to a 50ml volumetric flask and make up the volume to 50ml with 0.1N HCL.

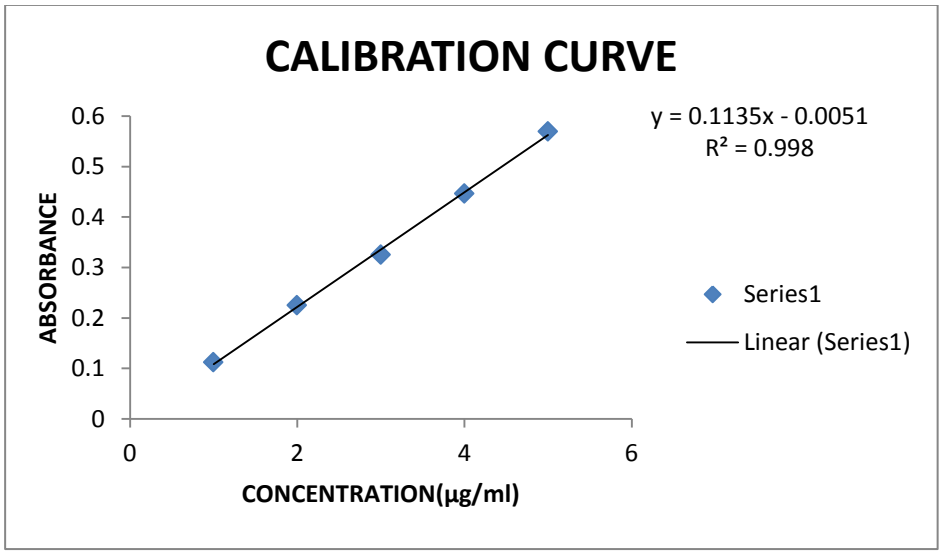
RESULTS AND DISCUSSION

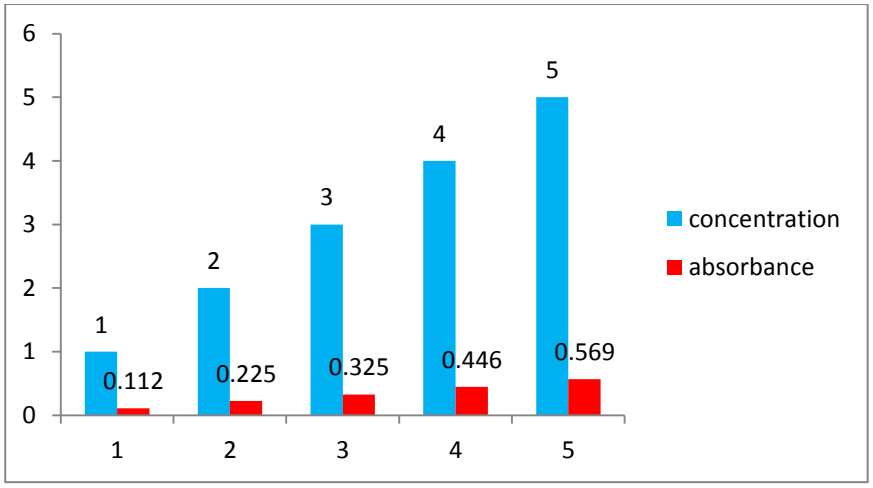
Linearity

Linearity studies were performed at 1-5µg/ml selected drug concentrations, by constructing a calibration curve between concentration VS absorbance. Correlation coefficient was calculated and the data was found within the acceptance criteria of 0.999.So the method was linear over the concentration range 0.998.

Table 1
Linearity result data

Concentration(µg/ml)	Absorbanceat 230nm
1	0.112
2	0.225
3	0.325
4	0.446
5	0.569





Bar diagram of Linearity data of Amoxicillin

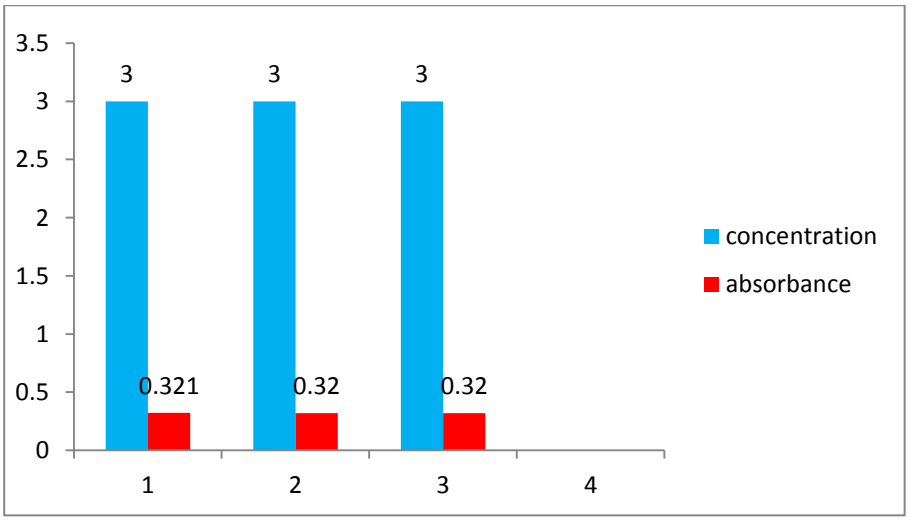
Precision

Precision of drug was verified by Repeatability, Intra day and Inter day by using 3µg/ml of standard solution. %RSD was calculated, and the %RSD is NMT 2 , which indicates the method to be precise.

Repeatability

Table 2
Repeatability data result

Concentration(µg/ml)	λmax at 230nm
3	0.321
3	0.320
3	0.320
Mean	0.3202
SD	0.0005
%RSD	0.1561

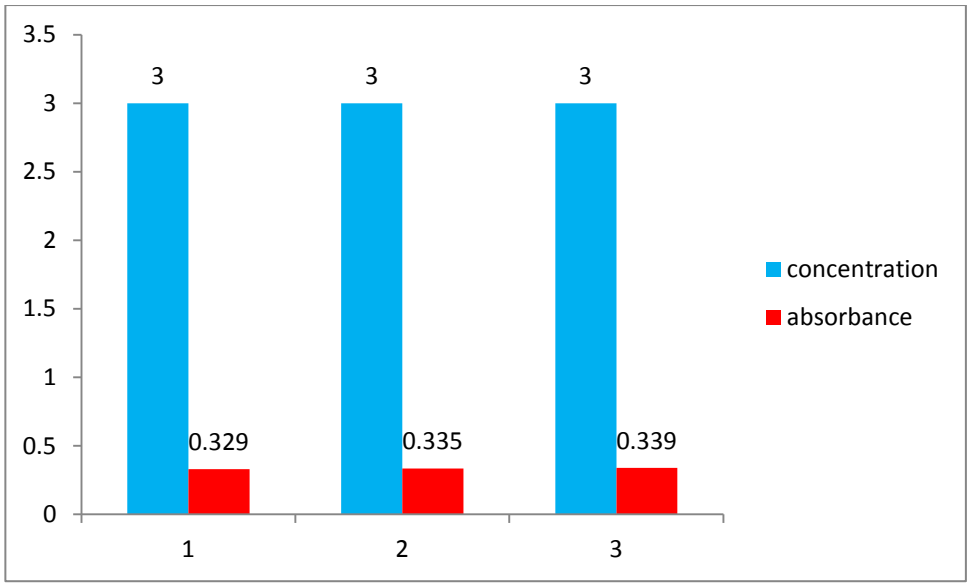


Bar diagram of Repeatability data of Amoxicillin for 3µg/ml solution

INTRA DAY PRECISION

Table-3

Concentration (µg/ml)	Hours	λmax at 230nm
3	Morning	0.329
3	Afternoon	0.335
3	Evening	0.339
Mean		0.331
SD		0.00316
%RSD		0.9553

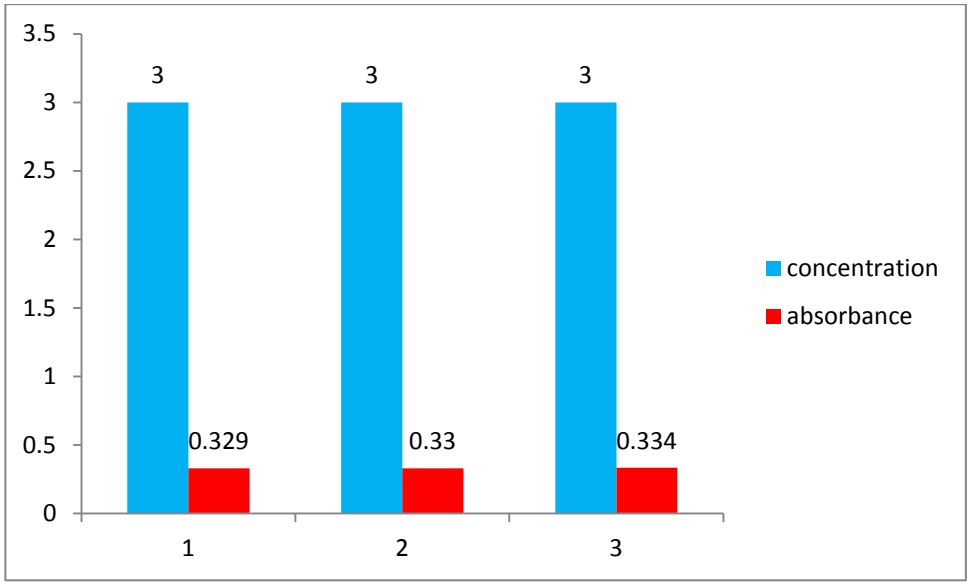


Bar diagram of Intra day precision data for 3µg/ml Amoxicillin solution

INTERMEDIATE PRECISION

Table-4: Intermediate precision data result

Concentration (µg/ml)	Day	λmax (230nm)	SD	%RSD
3	1	0.329	0.00264	0.799
3	2	0.330		
3	3	0.334		



Bar diagram data of Intermediate precision for 3µg/ml of Amoxicillin solution

ACCURACY

Accuracy was measured at 30%, 40%, and 50% level. To the sample solution different concentrations of standard solutions were added. The %Recovery was found to be in the range of 99.3-99.8%for amoxicillin which indicates a good accuracy of the method to that of labeled claim.

Table-5

Accuracy Data	Amount of Drug Added	Amount of Drug Found	%Recovery
30%	3	2.98	99.3%
30%	3	2.98	99.3%
30%	3	2.98	99.3%
40%	4	3.96	99%
40%	4	3.97	99.2%
40%	4	3.97	99.2%
50%	5	4.99	99.8%
50%	5	4.99	99.8%
50%	5	4.99	99.8%

Table-6:Robustness λ_{max} variable changed ±4nm

Concentration(µg/ml)	λ_{max} (235nm)	λ_{max} (227nm)
3	0.226	0.315
3	0.225	0.315
3	0.225	0.315

Sandell's Sensitivity

Table -7

S.NO	Concentration(µg/ml)	Absorbance 230nm	Sandell's Sensitivity
1	1	0.112	0.000112

2	2	0.271	0.000135
3	3	0.325	0.00010
4	4	0.406	0.000101
5	5	0.580	0.000116
Average		**0.000109**	

Assay

Table 8

DRUG	Label claim (mg/capsule)	Amount found (mg for capsule)	%Recovery	Mean Recovery
AMOXICILLIN	500	496.7	99.34	99.37
	500	496.5	99.30	
	500	497.4	99.48	

The percentage Recovery for the marketed formulation was 99.37% which indicates good recovery of the marketed formulation.

OPTICALCHARACTERISTICS

Parameters	Results
λ_{max} (nm)	230nm
Beer's law limit (µg/ml)	1-5(µg/ml)
Molar absorbance, L mole^{-1}cm^{-1}	3942Lmole $^{-1}$ cm^{-1}
Sandell's sensitivity (µg cm^{-2}/0.001 absorbance unit)	0.000109
Regression equation(Y=a+bX)	0.0051+0.1135X
Slope(b)	0.1135
Intercept (a)	0.0051
Correlation Coefficient(r)	0.998

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Amoxicillin in capsules as per ICHQ2(R1)guidelines. It is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of Amoxicillin in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

BIBILOGRAPHY

[1]. US National library of medicine,https://pubchem.ncbi.nlm.nih.gov/compound/amoxicillin
[2]. REKHA RAO*1, SANJU NANDA, SIMAR PREET KAUR1, Amoxicillin: A Broad Spectrum Antibiotic, Int J Pharm Pharm Sci, Vol 3, Issue 3, 2011, 30-37. 5.https://WWW.drugbank.ca
[3]. Bush K. β-lactam antibiotics: Penicillin, and other β-lactam antibiotics. In: Finch RG, Greenwood D, Norrby SR., and WhitleyRJ. Antibiotic and chemotherapy: anti-infective agents and their use in therapy. Philadelphia (USA): Churchill Livingstone, an imprint of Elsevier Science Limited; 8, 2003, 224-78.
[4]. Brogden RN, Heel RC, Speight TM, Avery GS. Amoxicillin injectable: a review of its antibacterial spectrum, pharmacokinetics and therapeutic use. Drugs 18(3), 1979, 169- 184.

[5]. Derek G.Waller BSC*Anthony P.Sampson,Medical pharmacology and Therapeutics,fifth edition,2018,pages:581-629.
[6]. B.Jayakar, M.V.Kumudhavalli*, R.Margret Chandira, M.Kumar, C.Saravanan Method development and validation of RP-HPLC method for simultaneous determination of amoxycillin and potassium clavulanate , International Journal of PharmTech Research,Vol.2, No.1, pp 906-909.
[7]. Durga Mallikarjuna Roan Tippa* and Narendra Singh, Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Amoxicillin and Clavulanic Acid in Injection "American Journal of Analytical Chemistry"., 2010; 1: 95-101.
[8]. Jadhav.S.S, Salunkhe.V.R, Bhinge.S.D. Development and validation of HPLC method for simultaneous estimation of Amoxicillin Tryhydrate and Potassium Clavulanate in pure and marketed tablets dosage form, Current Pharma Research , 3 (4), 2013, 994–998.
[9]. V. K. Pawar*, G. Garg, R. Awasthi, Nitin Kumar and G. T. Kulkarni, Development and Validation of a Simple UV Method for In Vitro Determination of Amoxicillin Trihydrate in New Gastroretentive Dosage Form,International Journal of Pure & Applied Chemistry 5(4), (October-December 2010) pp. 329-333.
[10]. PARTH PATEL1*, PRIYA VARSHNEY1, MINAL ROHIT, Analytical method development and validation for simultaneous estimation of metronidazole and amoxicillin in synthetic mixture by uv-visible spectrophotometry,int j pharm pharm sci, vol 6, suppl 2, 317-319.
[11]. Geneva: ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Methodology, part-I: IFPMA, 1996, Page no 2-15.
[12]. Michael Swartz: Analytical method development and validation, 2009, Page no: 25-27, 60-67.
[13]. ICH:Q2A Text on validation of analytical procedure; 1994.