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Research Article

Development and Validation of a RP-HPLC Method for the Simultaneous estimation of Amoxicillin, Omeprazole and Tinidazole in fixed dose combinations

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Abstract

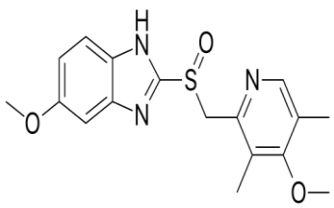
New liquid chromatographic technique was established for the simultaneous estimation of tinidazole, omeprazole and amoxicillin in the fixed dose combination (HP-KIT by Sun Pharma). RP-HPLC elution has performed by using the Phenomenex Luna column (250 mm x 4.6 mm) having internal diameter and the packing material of size 5µm) in isocratic mobile phase of solution A: acetonitrile at a ratio of 80:20 v/v (Solution A consists of Buffer: Acetonitrile: Methanol: Triethylamine in the ratios of 68:22:10:0.01 respectively). The selected flow rate was kept as 1 ml/min and selected wavelength was 230 nm was for detection of the drugs in UV detector. As per the ICH guidelines, the method validation was carried out. Moreover, the different parameters of method such as precision, specificity, linearity, robustness and accuracy were established. The time of retention for the tinidazole, amoxicillin, and omeprazole were 4.021 2.324, and 7.332 minutes respectively. The RP-HPLC approach was robust and accurate, so it is appropriate for repetitive assay of drugs and quality control. This method is effectively used for the assessment of marketable dosage form preparation.

Keywords: RP-HPLC, Amoxicillin, Tinidazole, Omeprazole, Method Development, Method Validation.

INTRODUCTION:

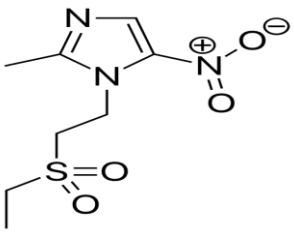
Helicobacter pylorus which is a gram-negative bacterium mostly found in the stomach. It was acknowledged in 1982 by Australian scientists Robin Warren and Barry Marshall. The researchers found that this bacterium was present in people suffering from gastric ulcers and chronic gastritis. Its presence was also noticed in patient of stomach cancer and duodenal ulcers. The stomach and duodenal linings was given much harm by the H. pylori harm due to different mechanisms. These ulcers instigated by the H. Pylori are treated by using the different combination of drugs, like proton pump inhibitor (PPIs) drug combined with two antimicrobials drugs, such as amoxicillin and tinidazole.

Chemical Structure of Omeprazole:



Chemically omeprazole is recognized as 5-methoxy-2-[[(4-methoxy-3, 5- dimethyl-2-pyridinyl) methyl] sulfinyl] benzimidazole. It is formally listed in BP2011 and USPXXXII. Omeprazole is PPIs, mostly employed in treatment of peptic ulcer disorder, ulceration associated with NSAID, in disease related to gastro-esophageal reflux and also in Zollinger-Ellison syndrome. A literature survey shown that estimation of omeprazole performed in pharmaceuticals by using High performance liquid chromatography[1-3].

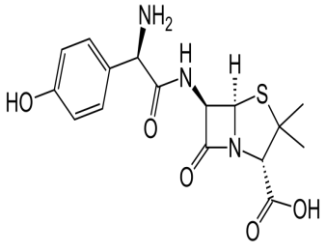
Structure of Tinidazole:



Tinidazole is chemically recognized as 1-(2-ethyl-sulphonyl ethyl)-2- methyl-5-nitroimidazole. It is official published in BP 2011 and USP XXXII. Omeprazole has significant activity

against protozoa anaerobic and bacteria. It is used to eradicate H. pylori in peptic ulcer disease with other proton pump inhibitor and antimicrobials. The estimation can be done through HPLC[4-6].

Structure of Amoxicillin:



Amoxicillin trihydrate chemically recognized as [2S-[2a,5a,6aˆ(S)]]-6-[[Amino(4-hydroxyphenyl)acetyl]amino]-3,3 dimethyl-7-oxo-4-thia-1-azabicyclo is the most extensively used β-lactam antibiotic to treat bacterial infections of ear, nose, throat, skin and lower respiratory tract due to susceptible microorganisms. It has significant absorption ability than other β-lactam antibiotics. The marketed formulations of amoxicillin are capsules, suspensions, tablets and injectable solutions. The combination drugs of Amoxicillin enhance the antibacterial effect and bacterial resistance. In literature, numerous analytical techniques have been employed for quantitative determination of amoxicillin by HPLC[7-10].

Till date only one RP-HPLC method has been reported by Kasnia et al[11] for the simultaneous estimation of these three drugs. This estimation was done for the microsphere formulation. So, it is a prerequisite to create a validated method using RP-HPLC to estimate the three drugs content in tablet/capsule formulation.

MATERIALS AND METHODS

Instrumentation and Equipment:

There is the establishment of analytical method and validation was done on the HPLC (Make & Model: Agilent 1220 with UV Detector) is equipped with degasser, solvent delivery pump, using Software (Openlab®).

The elution of RP-HPLC was performed employing Phenomenex Luna column (internal diameter of 250 x 4.6 mm and the packing material having size of 5µm) and solution A with isocratic mobile phase: acetonitrile at a ratio of 80:20 v/v (The pH was accustomed to 6.7 by the help of orthophosphoric acid). For detection of the drugs, flow rate was 1 mL/min and UV detector wavelength was fixed at 230 nm.

Reagents and Chemicals

Omeprazole, Tinidazole and amoxicillin were the gifted samples obtained from Uttranchal Research and Testing laboratory, Uttrakhand. Infrared Spectroscopy (IR) and Mass Spectroscopy were employed for the characterization of these samples to examine the purity level.

RANKEM Chemicals has supplied the AR grade chemicals which are orthophosphoric acid (OPA), potassium dihydrogen phosphate, triethylamine, methanol and HPLC grade acetonitrile. Milli-Q water purification system was used for generation of water which was used during analysis (Make & Model: MILLIPORE / Integral 5).

Methods

Chromatographic conditions	
Column	Phenomenex Luna C18, column (250 mm x 4.6 mm), 5 µm
Mobile phase	Solution-A: Acetonitrile: Buffer: Methanol: Triethylamine (Ph 6.7) in the ratio of 22:68:10:0.01 Solution A: Acetonitrile (80:20) v/v
Detector	UV detector
Flow rate	1 ml/min
Wavelength	230nm
Injection Volume	20µL
Temperature	35°C
Diluent	Mobile phase

Preparation of Standard Solution:

The stock solutions were formulated by dissolving Omeprazole (25gm) in 25 ml flask, followed by addition of 10 ml of diluent and mix well. Make the volume. Take 1 ml of solution from this solution in 50ml flask. Add 31mg of amoxicillin and 25 mg of tinidazole. Mix well with diluent and finally make the volume to prepare concentrations of 20 µg/mL for Omeprazole and 500 µg/mL for Amoxicillin & Tinidazole respectively.

Preparation of Sample Solution:

The sample solutions were formulated by dissolving 1/10 of average weight of drugs in 50ml flask and mix well with diluents to prepare concentrations of 500 µg/mL for Amoxicillin & Tinidazole and 20 µg/mL for Omeprazole respectively.

Method Validation

As per ICH Q2 guidelines, linearity, suitability, accuracy, specificity, precision, LOD/ LOQ and robustness were considered for validation of developed method[12].

Specificity and selectivity

The established method was found to be suitable for omeprazole, amoxicillin and tinidazole, as the blank solution injection has given the confirmation of absence of interfering peak at RT examined substance at 230 nm wavelength. The outcome obtained demonstrates that no interference was exhibited from other material in the established method and therefore it confirms the established method specificity[12].

RESULTS

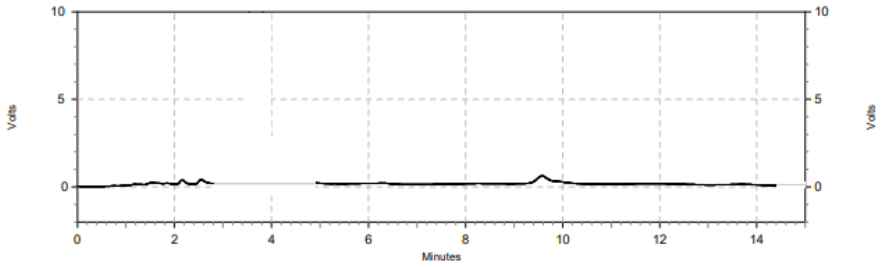
System Suitability

The test performed for suitability of system is an essential part of method development, were employed to confirm acceptable chromatographic system performance. The evaluation for retention time (RT), peak asymmetry, tailing factor, and theoretical plates (T) were performed. The outcome are summarised below in Table 1

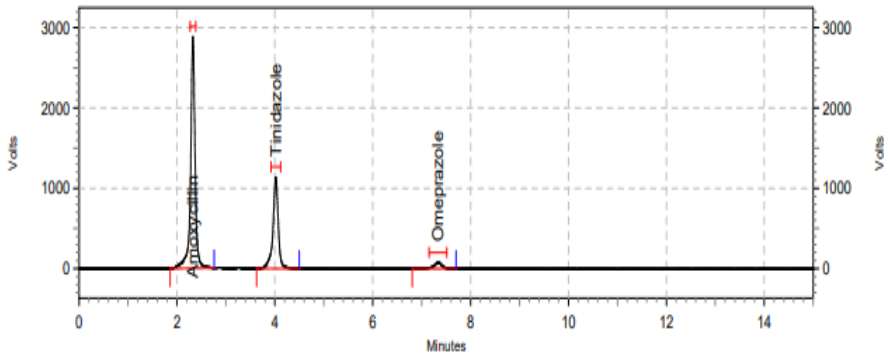
Table 1: **System suitability parameters of Amoxicillin, tinidazole and omeprazole**

Sr. No.	Property	Amoxicillin	Tinidazole	Omeprazole	Acceptance criteria
1.	Retention Time (RT)	2.228	4.112	7.256	-
2.	Tailing factor (T)	1.10	1.21	1.01	NMT 2.0
3.	Theoretical plates (N)	6337	4265	3832	NLT 2000

From the above data it was established that that all the suitability parameters of system were within the limit for developed method.



Chromatogram: Blank



Chromatogram: Standard

Linearity and Range

The developed method linearity proves the method ability to deliver an outcome which is directly proportional to analyte concentration in the sample. The amount of amoxicillin, tinidazole and omeprazole were made for linearity in the 80-120% range. The amoxicillin, tinidazole and omeprazole amount in five dissimilar concentrations are 80%, 90%, 100%, 110% and 120% of working standard respectively. The graph was plotted between area of peak and concentrations. The omeprazole, amoxicillin and tinidazole exhibited good correlation coefficients (R^2= 0.9992, 0.9982, and 0.9995) and the planned method was linear in 80-120 % concentration range.

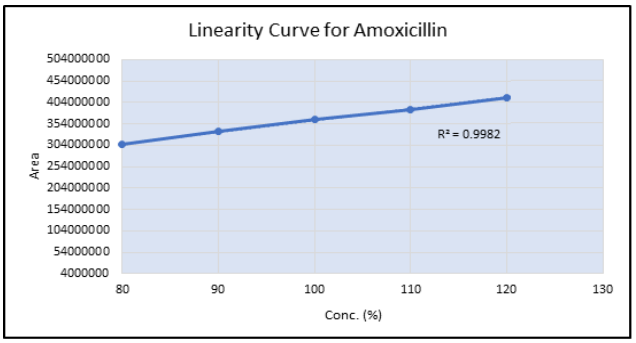
Table 2: **Linearity of Amoxicillin, Omeprazole and Tinidazole**

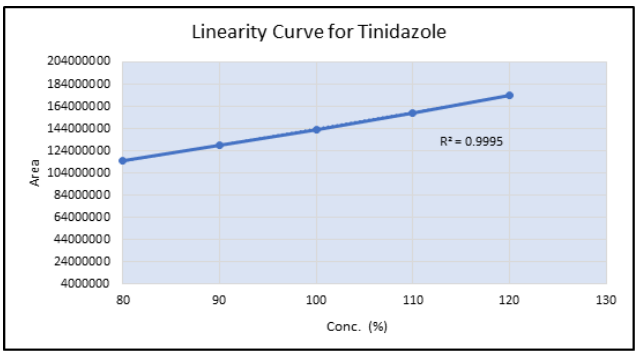
S. No.	Compound	Values of X and Y Variables						Correlation co-efficient
1	Amoxicillin	Variable	1	2	3	4	5	0.9982
		X	400	450	500	550	600	
		Y	306529813	336208695	365098652	388299753	415609155	
2	Tinidazole	X	400	450	500	550	600	0.9995
		Y	114987563	128677481	143320695	158340861	173826962	
3	Omeprazole	X	16	18	20	22	24	0.9992
		Y	11975684	13659318	15030951	16632982	18237528	

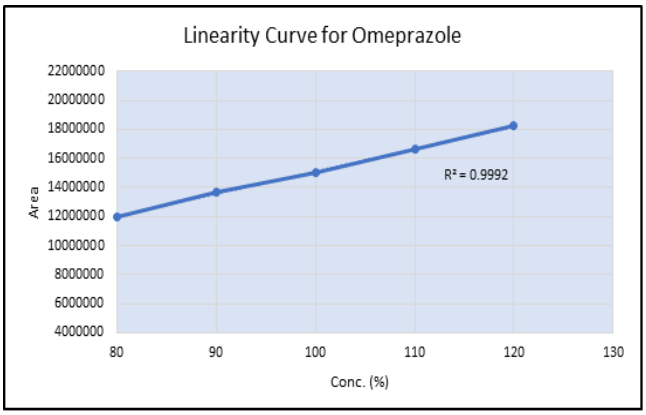
Note: X is the concentration of the respective component in µg/mL . Y is the peak response of the respective component in area counts.

Linearity Curve

Calibration curve was plotted between peak area and different concentrations. The outcomes were noted for equation of line and correlation co-efficient were also calculated.







Precision

The precision exhibits the closeness between the series of measurements. The developed method precision was established by method precision and system precision. A homogenous sample concentration of 20 µg/mL for Omeprazole and 500 µg/mL for Amoxicillin & Tinidazole were prepared under recommended conditions and determination was performed as well. The outcomes are mentioned in the form of standard deviation (SD) and RSD value. Table 3 and 4 discloses the outcome of method precision and system precision respectively and the developed method is extremely precise as the value for % RSD is less than 2%.

Table 3: Calculation of %RSD for Amoxicillin, omeprazole and tinidazole (System Precision)

S. No	Compound	No. of Injections						Mean	S.D.	%RSD
		1	2	3	4	5	6			
1	Reference Standard Amoxicillin	310619562	310645821	310687549	310679852	310705593	310652984	310665227	31474.918	0.010
2	Reference Standard Tinidazole	145596431	145319566	145748241	146032184	146318547	145524718	145756615	363929.37	0.250
3	Reference Standard Omeprazole	15715962	15748752	15705487	15774851	15795822	15739856	15746788	34350.517	0.218

Table 4: Calculation of %RSD for Amoxicillin, omeprazole and tinidazole and (Method Precision)

S. No	Compound	No. of Injections						Mean	S.D.	%RSD
		1	2	3	4	5	6			
1	Sample Amoxicillin	365098652	364895162	365132476	365198411	365862325	365710648	365316279	381069.7	0.104
2	Sample Tinidazole	143320695	143215548	143395682	143486071	143965842	143895243	143546514	311250.46	0.217
3	Sample Omeprazole	15030951	15025476	15036741	15037245	15040887	15039604	15035151	5846.1862	0.039

Mean represents the average values of six replicates analysis. **SD** is the standard deviation calculated on the six replicates. **RSD** is the relative standard deviation.

Table 5: System Precision and Method precision

Precision	Drug	% RSD
System precision	Amoxicillin	0.011
Method precision	Amoxicillin	0.106
System precision	Tinidazole	0.255
Method precision	Tinidazole	0.221
System precision	Omeprazole	0.209
Method precision	Omeprazole	0.045

Accuracy

Accuracy is also known as trueness or recovery. The accuracy was checked by using 80%, 120% and 100% of working strength of amoxicillin, omeprazole and tinidazole. Each level of solution was prepared in duplicate and analysis is done as per the above method. This is usually mentioned in the form of SD and RSD. The outcome expressed that the value of % RSD is less than 2%. The % recovery results are mentioned in **Table 6**.

Table 6: Summary of assay of Amoxicillin, omeprazole and tinidazole

S. No.	Level	Compound	% Average Assay	%RSD
1	80%	Amoxicillin	99.49	0.09
		Tinidazole	99.83	0.16
		Omeprazole	99.12	0.19
2	100%	Amoxicillin	99.96	0.11
		Tinidazole	99.50	0.25
		Omeprazole	99.26	0.10
3	120%	Amoxicillin	99.04	0.15
		Tinidazole	99.01	0.26
		Omeprazole	99.80	0.24

Assay values of tinidazole were found of in the range of 99.11-99.89, amoxicillin in the range of %99.14-99.86 % and omeprazole in the range of 99.09-99.79%. Moreover, amoxicillin % RSD assay values were in the range of 0.08-0.22 %, tinidazole in the range of 0.15-0.24 % and omeprazole in the range of 0.11-0.25%. The study confirms that the developed method is precise for the estimation of amoxicillin, tinidazole and omeprazole assay over the range of 80-120% of target concentration.

LOD and LOQ (Limit of Detection and Limit of Quantification)

Limit of detection (LOD) and Limit of Quantification (LOQ) exhibits information related to the analyte concentration that yields signal-to-noise around 1 to 10. The serial dilutions are fabricated from the amoxicillin, tinidazole and omeprazole solution for estimation of LOQ and LOD values respectively. The prepared samples were injected into the HPLC system and blank and sample signals were compared for LOD and LOQ calculation. As per the parameters mentioned earlier, LOD and LOQ were calculated for amoxicillin, omeprazole and tinidazole detected values were 9 µg/ml, 10 µg/ml, 27 µg/ml 2 µg/ml and 0.7 µg/ml, 25 µg/ml, respectively.

Robustness

The robustness of the method was carried out by doing little deliberate changes in the current developed HPLC method process parameters. These parameters include mobile phase flow rate variations, the minute variation in detector wavelength and variation in the proportion of acetonitrile and buffer. Additionally single concentrations of amoxicillin, omeprazole and tinidazole were employed for verification of robustness parameters. The minor variation of parameters can do some noteworthy changes in the RSD values and peak area. This study recapitulated that the developed method is robust under small changes like ± 2 wavelengths, ± 10% flow rate and ± 10% surge and decline in mobile phase and at the diverse column (Inertsil ODS-3, column (250mmx4.6mm), 5 micron. There is no significant change in recovery of omeprazole, amoxicillin and tinidazole. The % RSD values shown in the **Table 7** exhibited that insignificant changes were seen after doing the deliberate changes. So, this study describes that the developed method is robust in nature.

Table 7: Robustness Data

Drug	Parameters	% RSD
Amoxicillin, tinidazole and omeprazole	Wavelength minus	0.003
	Wavelength plus	0.002
	Flow minus	0.004
	Flow plus	0.002
	Mobile phase ratio change	0.003
	Column Change	0.001
	Temperature minus	0.004
	Temperature plus	0.005

DISCUSSION

Trial and error method was employed and subsequently a random number of trials with different, mobile phases were employed but the finest separation of Amoxicillin, omeprazole and tinidazole was present in the Solution A: Acetonitrile in the ratio of 20: 80 (in Isocratic mode). Finally, best outcomes were attained with the flow rate programming of chosen mobile phase for the estimation purpose of all the three drugs. Mobile phase was continued for 60 seconds to 15 min with a of 1 ml/min flow rate and detection was done at 230 nm by using UV detector.

The validation of fabricated and the optimized method of RP-HPLC was done according to the ICH guidelines with reference to the diverse parameters such as limit of detection (LOD) linearity, limit of quantification (LOQ), accuracy, precision, and specificity. All the results obtained were found in accordance with ICH guideline.

CONCLUSION

Agilent 1220 LC system was used for the validation reason of the fabricated and optimized liquid chromatographic method for simultaneous assessment of amoxicillin, omeprazole and tinidazole in HP kit combination. The approach for simultaneous determination of tablet/capsule dosage form has not been mentioned earlier. So, the recent method is novel for these drugs determination at a single wavelength

which is 230 nm, along with 20µL injection volume and by employing the Phenomenex Luna C18 5µm 4.6*150mm column. The process is very simple, sensitive and fast as well as accurate, precise, linear and robust which is in accordance of guidelines of ICH. The experimentation work demonstrates that the method development of liquid chromatographic approach exhibited good linearity, resolution, and RSD values which is less than 2%, which proves that simultaneous determination of Amoxicillin, omeprazole and tinidazole can be done suitably by this developed method.

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