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NEW VALIDATED HPLC METHOD FOR THE ESTIMATION OF AMOXYCILLIN TRIHYDRATE IN PHARMACEUTICAL FORMULATION

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Abstract:

A simple, selective, linear, precise and accurate HPLC method was developed and validated for rapid assay of Amoxycillin Trihydrate in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 0.3ml/min was employed. The chromatographic analysis was performed on a C18 5 µm (4.6 mm x 15 cm) or equivalent column at ambient temperature. The mobile phase consisted of Acetonitrile : phosphate buffer in the ratio of 5:95v/v. The UV detection wavelength was 230nm and 100µl sample was injected. Flow rate was found to be 1.0. The retention time for Amoxycillin Trihydrate was identified. The Average percentage recovery of the method was in the range of 0.5. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

Key Words: Amoxycillin Trihydrate, HPLC, UV detection, Recovery & Precise.

1. Introduction:

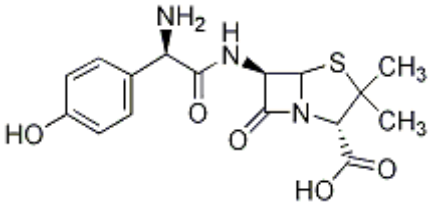

Figure 1: Structure of Amoxycillin Trihydrate

Amoxicillin (INN, BAN), or amoxycillin (AAN), and abbreviated amox, is an antibiotic useful for the treatment of a number of bacterial infections. Amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, which are resistant to a narrow spectrum of β-lactam antibiotics, such as penicillin. This drug combination is commonly called co-amoxiclav. Combining the drugs increases effectiveness by reducing susceptibility to β-lactamase resistance.[1] Side effects include an increased risk of yeast infections and, when used in combination with clavulanic acid, diarrhea.[2] Amoxicillin is one of the most common antibiotics prescribed for children. The drug first became available in 1972. It is on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in a basic health system.[3] Amoxicillin is used in the treatment of a number of infections, including acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, *Salmonella* infections, Lyme disease, and chlamydia infections.[4] It is also used to prevent bacterial endocarditis in high-risk people having dental work done, to prevent *Streptococcus pneumoniae* and other encapsulated bacterial infections in those without spleens, such as people with sickle-cell disease, and for both the prevention and the treatment of anthrax.[4] K. Uma Maheswar .,et.al.,[5] proposed a simple reverse phase – HPLC method was developed and validated for the estimation of diazepam in bulk and tablet dosage forms. Isocratic elution at a flow rate of 1.0 mL/min was employed on BDS Hypersil C18 (250 X 4.6) at ambient temperature. A mixture of 0.05M formic acid,

methanol and acetonitrile in a ratio of 45:20:35 (v/v) was used as the mobile phase. The UV detection wavelength was 239nm and the sample size was 20µL. The validated method was found to be precise and accurate for the estimation of diazepam in tablet dosage forms. Deshpande Ashish R et.al.,[6] developed a HPLC method was validated which shows good separation for Oxazepam, its impurities and degradation products. Chromatographic analysis was performed by using Zorbax Extended C-18 column from Agilent (250 x 4.6 mm, 5ï¿½). The flow rate was kept 1.0 mL.min-1. Detection was performed at 235 nm using PDA detector. The method was found to be reliable for its intended purpose. **Lakshman Raju Badugu[7] derived a** HPLC method for the determination of these compounds in pharmaceutical formulations. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methanol: Water 83:17(v/v) and pH of the mobile phase was adjusted to 4.1 with 0.1% Orthophosphoric Acid . The UV detection wavelength was at 241nm.Linearity was observed in concentration range of 5-30ppm. The retention time for Linagliptin was 5.85min. BEATA STANISZ et.al.,[8] proposed a rapid high performance liquid chromatographic method was developed and validated for determination of atorvastatin in pharmaceutical dosage forms. Separation of atorvastatin was successfully achieved on a C-18 column utilizing water ñ acetonitrile at the volumetric ratio of 48:52, adjusted to pH 2.0 with 80% ortho-phosphoric acid. The detection wavelength was 245 nm. K.Pushpa Latha et.al.,[9] developed a rapid high performance liquid chromatographic method was developed and validated for determination of atorvastatin in pharmaceutical dosage forms. It contains a mixture of buffer, acetonitrile and tetrahydrofuran in the ratio of 70:25:5 v/v/v and mobile phase B contains a mixture of buffer, acetonitrile and tetrahydrofuran in the ratio of 25:70:5 v/v/v. Amir G Kazemifard et.al.,[10] proposed a rapid and sensitive high-performance liquid chromatographic method was developed and validated for determination of oxazepam in serum. The chromatographic separation was accomplished using a 125 x 4-mm (inner diameter) stainless-steel (5 microm) Perfectsil Target ODS-3 reversed phase column with a mobile phase consisting of ammonium dihydrogen phosphate buffer (0.05 mol x L(-1), pH 5.8) and methanol (50:50, v/v), running at a flow rate of 1.5 ml x min(-1). A.V.D.Nagendra kumar et.al.,[10] proposed A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the rapid assay of the Buspirone in tablet dosage form. Isocratic elution at a flow rate of 1.0 mL/min was employed on a symmetry C18 (250 × 4.6 mm, 5 µm in particle size) at ambient temperature. The mobile phase consisted of water: acetonitrile: methanol 45: 35: 20(V/V). The UV detection wavelength was 210 nm, and 20 µL samples were injected. The retention time for the Buspirone was 7.057 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method can be successfully applied for the routine analysis of Buspirone in tablet dosage form. V. D. N. Kumar Abbaraju et.al.,[12] proposed a simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Quetiapine in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of methanol: water: O.P.A 90:10:01 (V/V/V). The UV detection wavelength was 250 nm and 20µl sample was injected. The retention time for Quetiapine was 3.64 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Quetiapine in tablet dosage form.

2. Experimental:

2.1 Instrumentation:

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a C18 5 µm (4.6 mm x 15 cm) or equivalent. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

2.2 Chemicals and Solvents:

The reference sample of Amoxycillin Trihydrate was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Acetonitrile and phosphate buffer used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

2.3 The Mobile Phase:

A mixture of Acetonitrile: phosphate buffer in the ratio of 5:95v/v was prepared and used as mobile phase.

2.4 Preparation of Solutions:

Preparation of Standard Solution:

Accurately weigh 100 mg of Amoxycillin Trihydrate reference standard into a 100 ml volumetric flask. Add 60 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. Dilute 10 ml of this solution to 100 ml with solvent. Further dilute 10 ml of this solution to 100 ml with solvent. Filter sample through a 0.45 µm filter.

Blank Preparation:

Place unused swab in 10 ml of Solvent. Sonicate for 5 minutes. Squeeze swab out well. Filter sample through a 0.45 µm filter.

Sample solution:

Place swab in 10 ml of Solvent (volume accurately determined). Sonicate for 5 minutes. Squeeze swab out well. Filter sample through a 0.45 µm filter.

3. Method Development:

A systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose:

3.1 Detection of Wavelength:

The spectrum of 10 ppm solution of Amoxycillin Trihydrate was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 230 nm was observed.

3.2 Choice of Stationary Phase and Mobile Phase:

Finally the expected separation and peak shapes were obtained on C18 5 µm (4.6 mm x 15 cm) or equivalent

3.3 Flow Rate:

Flow rates of the mobile phase were changed from 0.5-1.5 ml/min for optimum separation. It was found from experiments that 1.0 ml/min flow rate was ideal for elution of analyte.

4. Validation of the Proposed Method:

The parameters studied for validation were specificity, system suitability, detection limit, and method precision.

4.1 Specificity:

Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The results must show that the solvent solution (solution 1) and placebo solution (solution 2) must not contain any components which co-elute with the active compound peak (solution 3) Each of the degradation products and impurities must be well resolved from the active compound peak (at least baseline resolution > 1.5) and must elute within the specified assay run time. Determine the peak purity for each of the active peaks in at least the solutions 3 and 4 above. The purity angle must be less than the threshold angle. The solutions listed below were injected using the conditions specified in the method of analysis. No components are seen to co-elute with the Amoxycillin Trihydrate peak, and the peak Purity results indicate that Amoxycillin Trihydrate peak can therefore be considered spectrally pure. The method employed is specific for the API Amoxycillin Trihydrate in the product. Chromatogram results were shown from Figure 2 to 6 and peak purity results were shown from Figure 7 and 8.

4.2 System Suitability:

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. Six replicate injections of API working standard solution were injected according to the method of analysis. The percentage relative standard deviations (% RSD) for the peak responses were determined. The % RSD of the peak responses due to the Amoxycillin Trihydrate for six injections must be less than or equal to 5.0 %. The analytical system complies with the requirements specified by the system suitability. The results are tabulated in the Table: 1.

4.3 Detection Limit:

The maximum allowable carryover of Amoxycillin Trihydrate is 0.185 mg as determined in the Cleaning Validation Matrix. The range of standard solutions above was also injected twice and the average result was used in treatment of results. Eight solutions containing 0.05, 0.025, 0.0125, 0.00625, 0.00312, 0.001563, 0.00078 and 0.00039 mg/swab of Amoxycillin Trihydrate, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed. The detection limit must be capable of detecting the API at 50% MAC. 50% MAC is equal to 0.0925 mg/swab and the method gives linear response from 0.001563 – 0.05 mg/swab.

Preparation of Standard Solution (Stock Solution):

Accurately weigh 100 mg of Amoxycillin Trihydrate reference standard and quantitatively transfer into a 100 ml volumetric flask (1.0 mg/ml). Add 60 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. Dilute 10 ml to 100 ml with solvent (0.1mg/ml). Filter through a 0.45 µm filter before use, discarding the first few milliliters of filtrate. From 0.1 mg/ml stock solution, a series of standard solutions were prepared as follows: 0.05 mg/swab, 0.025 mg/swab, 0.0125 mg/swab, 0.00625 mg/ swab, 0.00312 mg/ swab, 0.001563 mg/ swab, 0.00078 mg/ swab, 0.00039 mg/ swab. The results were tabulated in Table: 2. and the graph were shown in the Figure: 9.

4.4 Method Precision:

An amount of material (predetermined limit) is placed on a specific surface area (stainless steel) and swabbed as outlined in the Cleaning Validation SOP using the specified solvent and specified material. The precision of the analytical method is determined by assaying the swabs and calculating the % Recovery of the API results.

The precision will entail repeated testing of six samples prepared in the following manner. Six replicate injections of API MAC working standard solutions were injected according to the method of analysis. The percentages Recovery for the peak responses were determined.

Standard Solution:

Accurately weigh 100 mg of Amoxycillin Trihydrate reference standard into a 50 ml volumetric flask. Add 30 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. (Solution 1 to be use for sample preparation). Dilute 10 µl to 10 ml with solvent. Filter sample through a 0.45 µm filter.

Sample Preparation:

Place 10 µl of solution 1 onto a specific surface area of stainless steel plate. Swab the surface area; take the swab stick and place into a 10 ml volumetric flask. Add 10ml of solvent and sonicate for 10 minutes. Filter sample through a 0.45 µm filter. The % recovery should be greater than or equal to 65%. The analytical system complies with the requirements specified by the method precision. The results were tabulated in Table: 3.

5. Results and Discussion:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Methanol : Water in the ratio of 65:35 v/v and 1.0 mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 230nm at which much better detector responses for drug was obtained and it was shown in Figure. The retention times were 5 min for Amoxycillin Trihydrate. The number of theoretical plates was found to be 5557.8, which indicates efficient performance of the column. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in Table. Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 0.2-1.4µg/ml and it was found to be linear. Seven points graphs was constructed covering a concentration range 0.2-1.4µg/ml. The standard deviation of the slope and intercept were low. The data of regression analysis of the calibration curves are shown in Table. The proposed method has been applied to the assay of T commercial tablets containing Amoxycillin Trihydrate. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same author working on the same day on two consecutive days. This indicates good method precision. The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Amoxycillin Trihydrate in bulk and in pharmaceutical formulations without interference and with good sensitivity. The Cleaning Validation method is proven to be valid and the validation test results show that the method complies with the validation requirements. The method is therefore acceptable as valid.

Figure 2: Chromatogram 1: Solvent – no significant peaks detected

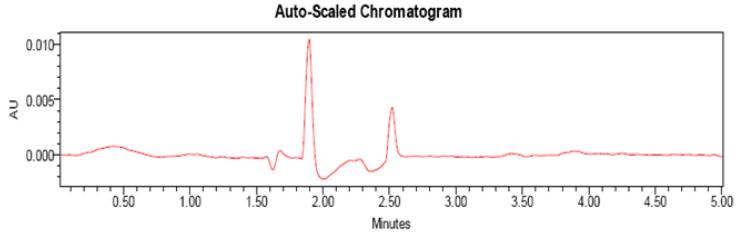

Figure 3: Chromatogram 2: Placebo – no significant peaks detected

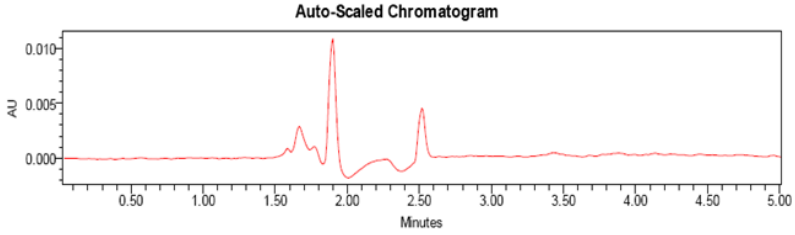

Figure 4: Chromatogram 3: API – peak due to Amoxycillin eluted at about 3 min

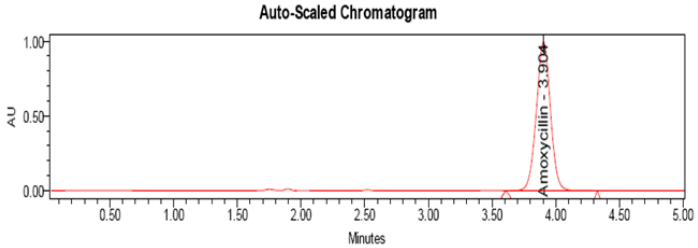

Figure 5: Chromatogram 4: Product - peak due to Amoxycillin eluted at about 3 min

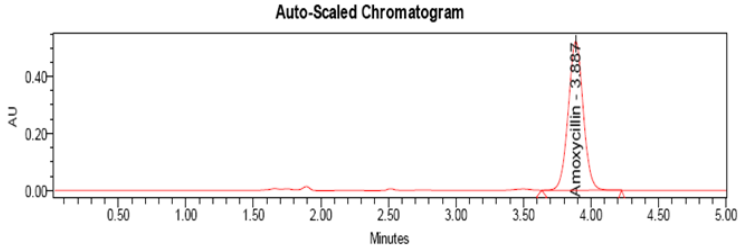

Figure 6: Chromatogram 5: Detergent - no significant peaks detected

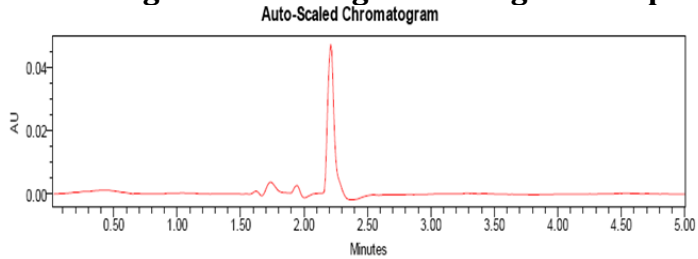

Figure 7: Peak Purity 1: Purity Angle <Threshold: 0.805 < 1.273

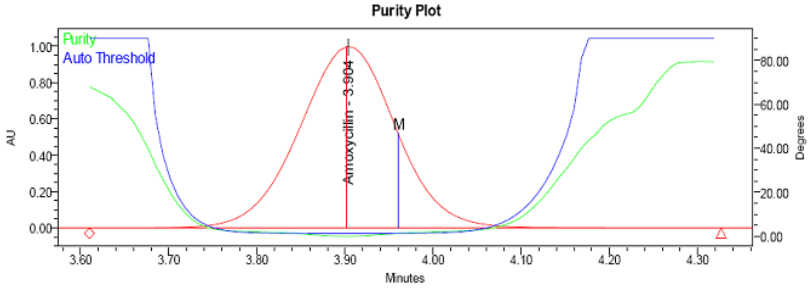

Figure 8: Peak Purity 2: Purity Angle <Threshold: 0.251 < 0.362

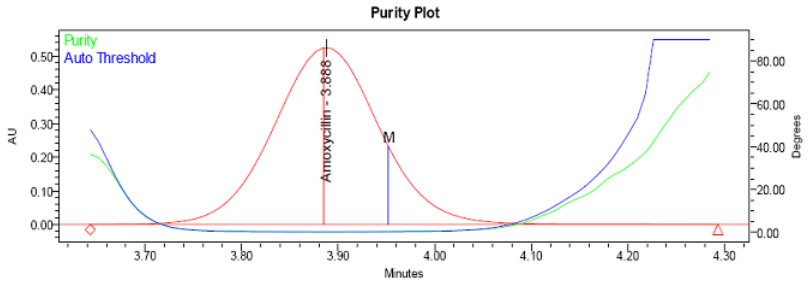

Table: 1 System Suitability results

Sample	Amoxycillin Trihydrate Area
1	137831
2	137630
3	137234
4	136811
5	136555
6	135810
Mean	136978
% RSD	0.5

Table: 2 Detection Limit

Conc (mg/swab)	Area 1	Area 2	Average
0.05	134976	134216	134596
0.025	102588	103783	103186
0.0125	35785	35882	35834
0.00625	17375	17240	17308
0.00312	8445	7998	8222
0.001563	3463	4094	3779
0.00078	1484	1307	1396
0.00039	1062	820	941

Figure 9: Linearity curve

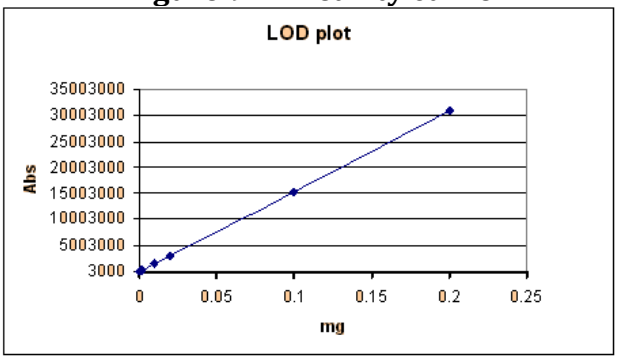

Table: 3 Method Precision Results

Sample	% Recovery
1	99
2	67
3	67
4	108
5	66
6	110
Mean	86

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