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Development and Validation of Stability Indicating RP-HPLC Method for Amoxicillin and Clavulanate Potassium Related Impurities in Pharmaceutical Dosage Forms

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A B S T R A C T

A simple, cost effective, stability indicating reversed-phase High Performance Liquid Chromatography method was developed for separation and quantification of seventeen specified known related impurities in Amoxicillin and Clavulanate Potassium pharmaceutical dosage form using simple gradient method. The chromatographic conditions comprised a reversed-phase Agilent eclipsed C18 column and UV detection at 210 nm with 1.0 mL/min flow rate at 45 ºC column oven temperature. In gradient mobile phase composition-A contains 0.05 M potassium dihydrogen phosphate buffer of pH 3.4 and Composition-B contains mixture of Acetonitrile and 0.05 M potassium dihydrogen phosphate of pH 4.2 in 80:20. The method validation data showed excellent results for Precision, Linearity, Specificity, Accuracy, Limit of detection, Limit of Quantification and robustness.

Keywords: Amoxicillin, Clavulanate Potassium, Method development, Validation, Related impurities.

A R T I C L E I N F O

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1. Introduction

Clavulanic acid [1] is produced by the fermentation of Streptomyces clavuligerus [2]. The Chemically, clavulanate potassium is chemically named as potassium (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1- azabicyclo[3.2.0]-heptane-2-carboxylate the Clavulanate potassium molecular formula is C8H8KNO5, and the molecular weight is 237.25. It is a β-lactam structurally related to the penicillins and possesses the ability to inactivate a wide variety of β-lactamases by blocking the active sites of these enzymes. Clavulanic acid is particularly active against the clinically important plasmid-mediated β-lactamases frequently responsible for transferred drug resistance to penicillins and cephalosporins. In the presence of Clavulanic acid beta-lactamase labile penicillins are protected from degradation by cell-free beta-lactamase preparations and by whole bacterial cultures [4]. Amoxicillin [3] belongs to a class of antibiotics called penicillin's and is chemically named as (2*S*,5R,6R)- 6-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)-acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptanes–carboxylic acid. It is a moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. This drug acts by inhibiting the synthesis of bacterial cell walls. It inhibits cross linkage between linear peptidoglycan polymer chains that make up a major component of the cell walls of both gram positive and gram negative bacteria. It has two ionizable groups in the physiological range, the amino group in alpha-position to the amide carbonyl group and the carboxyl group. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicillin is one of the most common antibiotics prescribed for children. Amoxicillin and Clavulanate Potassium, is a widely used oral antibiotic [5]. Amoxicillin-Clavulanic acid has been widely used as a prophylactic antibiotic in abdominal and gynecological surgery [6]. It is effective in the prevention of wound infections in operations in which the most likely pathogens are gram-negative, an aerobic or mixed bacteria [7]. Both of these drugs are official in pharmacopeias such as USP, EP and BP etc. Several Amoxicillin and Clavulanic acid combinations are available in market in the form of suspensions, tablets and injections. Chemical structures of Amoxicillin as Trihydrate and Clavulanate potassium are shown in figure 1.

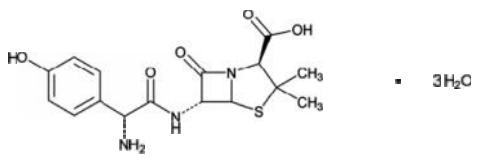

Figure 1: Amoxicillin Trihydrate

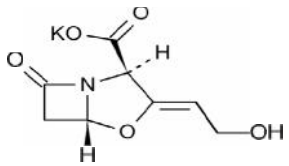

Figure 2: Clavulanate Potassium

In literature survey different analytical methods were reported for determination of Amoxicillin [8-10] and Clavulanic acid [11] individually. In combination there were some HPLC / LCMS assay methods reported [12] and there is one impurity method [13] reported in which only two Amoxicillin impurities were estimated. No simultaneous method was reported to separate these numbers of impurities in oral suspension formulation of Amoxicillin and Clavulanic acid. In the present work, a specific stability indicating HPLC method is developed for simultaneous estimation of seventeen impurities in Oral suspension formulation of Amoxicillin and Clavulanic acid. The product is subjected to stress studies and the degradation products formed were separated from the main peaks. The developed method was validated according to ICH guidelines [14].

High Performance Liquid Chromatography (HPLC) has been considered novel and cost effective development in liquid Chromatography. It is specifically designed with stand high pressure during chromatography analysis so it enables significant decrease in separation time than other conventional methods.

2. Materials and Methods

Chemicals and reagents:

LC-MS grade Acetonitrile (ACN) was purchased from GFS chemicals (USA). Ultra pure water produced from water purification unit (Elga Ltd, England). Potassium dihydrogen phosphate manufactured by Alfa Aesar, Phosphoric acid manufactured by Acros, 0.45µm nylon filter manufactured by Restek and 0.45µm PVDF filter manufactured by Microliter. Amoxicillin and Clavulanate potassium for Oral suspension samples were taken from market.

System suitability solution preparation:

System suitability parameters were measured in order to verify the system performance. The system suitability solution was prepared in diluent consist Water and Acetonitrile mixture in ratio of 100:3 to obtain 15 and 3.75 µg/mL concentrations of Amoxicillin and Clavulanic Acid. Six replicate injections of the system suitability solution were performed to measure the Tailing factor and Relative standard Deviation [15].

Sample preparation:

The Amoxicillin and Clavulanate potassium For Oral Suspension sample was prepared using Water and Acetonitrile mixture in ratio of 100:3 at the concentration 1500 µg/mL for Amoxicillin and 107.25 µg/mL for Clavulanic Acid [16].

Equipment:

Analysis was performed on Shimadzu 2010 HPLC system consisting Quaternary pump manager, sample manager and UV and Photodiode Detector. Boekl water bath used for Acid and Base Hydrolysis studies. Photo stability studies were carried out in a UVP photo stability chamber. Thermal studies were carried out in Yamato DX 600 dry air oven. Intermediate precision study was performed on different Shimadzu HPLC system consists Quaternary solvent manager, a sample manager and UV Detector. The software

used for processing the chromatograms is Class VP 6.1 version.

Liquid Chromatographic conditions:

The mobile phase consists a mixture of A and B, Mobile phase A was a mixture of 0.05M Potassium Dihydrogen phosphate pH adjusted to 3.4 with Ortho Phosphoric acid, Mobile phase B was a mixture of 0.05M Potassium Dihydrogen phosphate pH adjusted to 4.0 with acetic acid and Acetontrile (ACN) in the ratio of 80:20 respectively. The chromatographic separation was achieved on Agilent Eclipse XDB, C18, 250x4.6 mm, 5µm column by performing gradient program with flow rate of 1.0 mL/min. the gradient program performed for separation was shown in Table 1. The detection wavelength was set at 210 nm and the injection volume was 15 µL.

Table 1: Gradient program of Mobile phase A & B

Time	Flow	% Mobile phase-A	% Mobile phase-B
0	1.0	92	8
8	1.0	92	8
39	1.0	65	35
64	1.0	0	100
69	1.0	92	8
75	1.0	92	8

Specificity: Forced degradation studies were performed to demonstrate selectivity and stability-indicating capability of the proposed method. The Oral suspension powder sample and Placebo were exposed to acid (0.5N HCl, 30 min at 60ºC), base (0.5 NaOH, 30 min at 60ºC), strong oxidation (5% H2O2 for 30 min at room temperature), thermal (105ºC, 7days), humidity (90% RH, 25ºC, 7 days) and photolytic (1.2million luxh, 200wh/m2, 7 days) degradation conditions. Samples were withdrawn at appropriate times and subjected to HPLC analysis after dilution equal to sample solution concentration to evaluate the ability of the proposed method to separate Amoxicillin and Clavulanic acid and its impurities from placebo. Photo diode array detector was employed to check and ensure the homogeneity and purity of Amoxicillin and Clavulanic acid peak in all the stressed sample solutions [17].

Explanation and justification for selection of impurities in Validation: The known impurities available in Amoxicillin and Clavulanic acid were listed in table 2. All the impurities listed in table 2 were used in validation except three, these three impurities were not available in market with good purity for procurement in order to perform the method validation [18]. However, available impurities with minimum purity were used for identification and Relative retention time calculations.

Table 2: List of impurities, usage information in validation and type impurity details

Compound name selected for	Method validation (Yes/No)
p-Hydroxyphenylglycine (p-HPG)	Yes / Process impurity
6-APA (or) USP Related compounds-A	Yes / Process impurity
Alfa, Penicilloic acid (or) Alfa-Amoxicilloic Acids	Yes / Degradent
Beta Penicilloic acid (or) Beta-Amoxicilloic Acids	Yes / Degradent
L-Amoxicillin	Yes / Process impurity
p- Hydroxyphenylglycine methyl ester	Yes / Process impurity
Penilloic acid-1 (or) Amoxilloic acid diastereomer-1	Yes / Degradent
Clavu impurity-G	Yes / Degradent
p-Hydroxyphenylglycyl Amoxicillin	Yes / Process impurity
Penilloic acid-2 (or) Amoxilloic acid diastereomer-2	Yes / Degradent
Amoxi 2(R) Piperazine-2,5 dione	Yes / Degradent
Dimer closed beta lactam	Yes / Degradent
Trimer open beta lactam	Yes / Degradent

Linearity:

Linearity test solutions of targeted related impurities were prepared from the impurities stock solution at six different concentration levels from LOQ to 160% and LOQ to 320% of specification level for Amoxicillin and Clavulanate potassium respectively [19]. The calibration curves were constructed by plotting average peak areas from one injection versus its corresponding concentrations. The slope, Y-intercept and correlation coefficient of the calibration curve were calculated for all the impurities.

Limit of Detection (LOD) and limit of Quantification (LOQ): The LOD and LOQ of Amoxicillin, Clavulanic acid and its impurities were determined by using the signal to noise approach as defined in ICH guidelines.

Precision:

System Precision:

System precision was evaluated by injecting 6 replicates injections of the system suitability solution and calculated the % RSD for each analyte.

Method Precision: Method precision was evaluated by injecting the 6 different sample preparations spiked with specified impurities at specification level and calculated the % RSD for each impurity [20].

LOQ Precision:

Impurities, Amoxicillin and Clavulanic acid were spiked to placebo at LOQ level concentration and calculated the % RSD for each targeted peak.

Accuracy:

Recovery experiments were carried out to confirm the accuracy of the proposed method. The accuracy of the impurity method was evaluated at three concentration levels i.e. LOQ, 100 and 150% of Amoxicillin, LOQ, 100 and 300% for Clavulanic acid. The samples were analyzed by the proposed method and the percentage recoveries were calculated [21].

Robustness:

The robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing

influence of small changes in mobile phase composition (10% absolute change), column temperature (± 5 ºC) and flow rate (± 0.1 mL/min).

Stability of sample solution:

Stability of sample solution was established by storage of sample solution at refrigeration condition (2-8ºC) on hourly basis, at room temperature (25 ± 2.0ºC) for three days [22].

3. Results and Discussion

The present work was focused to develop stability indicating HPLC method for determination of seventeen impurities of Amoxicillin and Clavulanic Acid. Due to large number of impurities to be separated, the method development started using 250*4.6, 5µm HPLC column. Plenty of experiments were performed by changing the pH of the mobile phase A between 3.0 to 5.5, Mobile phase-B between 4.0 to 5.5, the optimum separation was obtained at pH 3.4 for Mobile phase-A and 4.2 for Mobile phase-B. It was observed that the "p-Hydroxy methyl Ester" peak is sensitive to pH. The detector wavelength was optimized based lambda maximum of majority of impurities. All the impurities were fairly resolved from one another at 1.0 mL/Min mobile phase flow. The concentration and injection volume of sample finalized based on S/N ratio of Amoxicillin and Clavulanic acid and its impurities. The Retention Times (RT) and Relative Retention Times (RRT) of all the interested analytes with respect to Amoxicillin were shown in Table 3.

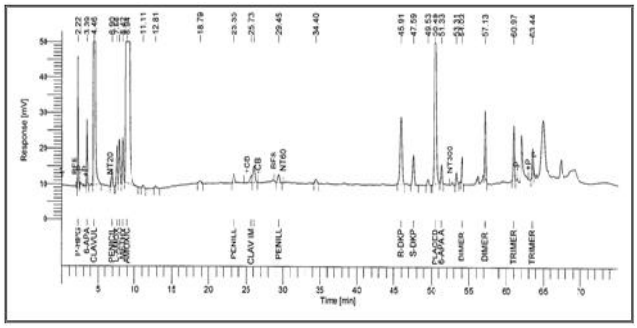

Figure 2: Typical chromatogram showing the separation of all the peaks

Table 3: Retention and Relative Retention times for different compounds in minutes

Compound Name About RRT	About RT (min)
p-Hydroxyphenylglycine, 0.25	2.222
6-Amino penicillanic acid, 0.38	3.390
Clavulanic acid, 0.50	4.457
Alfa-Amoxicilloic Acid, 0.77	6.902
L-Amoxicillin, 0.85	7.562
Beta-Amoxicilloic Acid, 0.89	7.972
p-Hydroxyphenylglycine methyl ester, 0.94	8.425
Amoxicillin, 1.0	8.940
Amoxilloic acid diastereomer-1, 2.61	23.350
Clavu impurity-G, 2.88	25.726
p-Hydroxyphenylglycyl Amoxicillin,2.92	26.070
Amoxilloic acid diastereomer-2, 3.29	29.449
Amoxi 2(R) Piperazine-2,5 dione, 5.14	45.915
Amoxi 2(S) Piperazine-2,5 dione, 5.32	47.585
6-APA Amoxicillin amide, 5.74	51.331
Dimer closed beta lactam, 6.39	57.133
Trimer open beta lactam, 6.82	60.972
Trimer closed beta lactam, 7.10	63.442

Analytical Parameters and Validation:

After satisfactory development of method Amoxicillin and Clavulanate Potassium For Oral Suspension was subject to method validation according to ICH guidelines [14]. The method was validated to demonstrate its suitability for intended purpose using the standard procedure and the validation characteristics including specificity, accuracy, precision, robustness, LOD, LOQ, linearity and stability have been evaluated.

Forced Degradation study results:

The degradation study revealed that product is very sensitive to Oxidation compared to other conditions. The drug substances Amoxicillin and Clavulanate potassium and its placebo were treated with different degradation conditions including acid, base, peroxide, photolytic, humidity and thermal degradations, the respective distinct chromatograms were shown in Figure 3. The major degradation products in peroxide conditions are Beta Amoxicilloic acids impurity, in Thermal and Photolytic conditions is Dimer closed impurity. The forced degradation results were shown in table 4, indicate that Amoxicillin is considerably stable in other conditions. All the spectra of known impurities are matching with its parent peak spectra indicating that there was no co-elution of unknown degradation peak. Spectral purity of Amoxicillin and Clavulanic acid and its impurities in the chromatogram of all the exposed samples are obtained from PDA and found to be spectrally pure. The max plot chromatogram of degradation sample was also checked to ensure that no degradation peak is missed due to use of wavelength of 210nm.

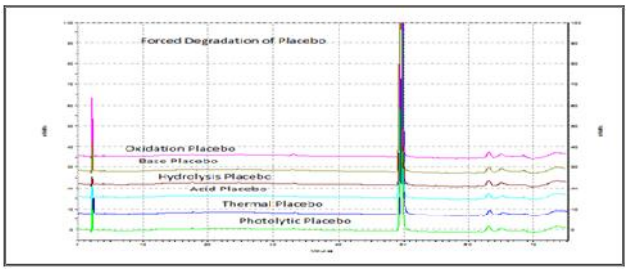

Figure 3a: for Placebo degradation Chromatograms

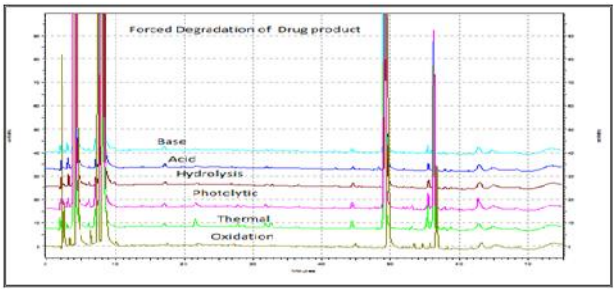

Figure 3b: for Sample degradation chromatograms

System suitability: The system suitability was performed by checking the % Relative Standard Deviation for average area of six standard replicate injections and was found below 1.2 for both Amoxicillin and Clavulanic acid, the Tailing factor was less than 1.2 and Theoretical Plates were more than 7500.

LOD and LOQ: The Concentration with signal to noise ratio of at least 3 was taken as Limit of detection (LOD) and concentration with signal to noise ratio of at least 10 was taken as Limit of Quantification (LOQ), which meets the criteria defined by ICH guidelines [14]. The LOD and LOQ results of Amoxicillin, Clavulanic acid and known impurities were presented in table 5.

Linearity: To demonstrate the linearity of detector response for Amoxicillin, Clavulanic acid, and its related impurities, injected the solutions of concentrations mentioned in table 5 for Amoxicillin and Clavulanic acid and related impurities respectively. Plotted a graph between peak area and concentration, and linearity results are summarized in table 6.

Precision

System Precision: The RSD of area count of 6 replicate injections was below 0.3%. Low values of RSD indicate that the system is precise.

Precision at LOQ: The results of LOQ precision were presented in table 7. The RSD of area count of 6 replicate injections for each impurity was below 10.1 %, which indicate that the method is precise.

Method precision: The percentage RSD for the area of impurities from the method precision were within 3.1%, confirms good precision of the method. The calculated percentage RSD values for method precision were presented in table 7.

Intermediate precision: The percentage RSD of Chemist 1 and 2 data indicates the ruggedness of the method, further the t-test as performed on the data and the difference was found to be not significant, the overall %RSD is not more than 6.1%. The compiled data of method precision and Intermediate precision are given in table 7.

Accuracy:

The recovery results for impurities were expressed in terms of mean percentage. The percentage recoveries obtained for impurities including Amoxicillin and Clavulanic acid were within 79 to 121% and the results presented in Table 8. The recovery results indicate that the method is accurate and also found that there was no interference due to the presence of excepients in the Oral suspension formulation.

R**obustness:**

In all robustness conditions, the system suitability, % RSD found less than 2.5 and Tailing factor found not more than 1.3 and theoretical plates were not less than 7600. The estimation of impurities was within ± 6.5% and proved the method is robust in all conditions.

Stability of solutions: The solution stability results of Amoxicillin and Clavualante Potassium For Oral Suspension indicate that it is stable up to 8 hours at refrigeration conditions i.e. 2-8ºC and the system suitability solution is stable up to 65 hours at room temperature i.e 25 ºC ± 2.0ºC.

Filter Study: The results of filter study revealed that 0.45µm PVDF and 0.45µm Nylon filters are suitable for filtration of sample and system suitability solutions.

Table 4: Forced degradation results of Amoxicillin and Clavulanate Potassium

Condition	%Total degradation	Peak Purity (Not less than 0.99)	
		Clavulanic Acid	Amoxicillin
Acid-Hydrolysis	2.13	0.999967	0.999766
Base-Hydrolysis	2.22	0.996894	0.9999759
Peroxide-Oxidation	10.22	0.999965	0.999935
Water hydrolysis	2.31	0.999443	0.99973
Thermal Degradation	3.68	0.999903	0.999799
UV Degradaton	3.64	0.995798	0.99981

Table 5: LOD and LOQ data

Parameter	LOD (µg mL^{-1})	S/N ratio	LOQ (µg mL^{-1})	S/N ratio
Amoxicillin	0.051	3	0.156	9
Clavulanic acid	0.022	3	0.068	9
p-Hydroxyphenylglycine	0.035	3	0.109	11
6-APA	0.067	3	0.203	11
Alfa Amoxicilloic acids	0.085	4	0.259	11
L-Amoxicillin	0.088	3	0.265	10
Beta Amoxicilloic acids	0.071	3	0.212	10
p-Hydroxyphenlglycine methyl ester	0.115	3	0.347	11
Penilloic acids peak	0.261	3	0.792	11
Clavulanate Impurity	0.025	2	0.084	10
p-Hydroxyphenylglycine Amoxicillin	0.158	3	0.480	10
Penilloic acids peak 2	0.253	3	0.767	11
Amoxi 2(R) Piperzine-2,5 dione	0.062	4	0.188	10
Amoxi 2(S) Piperzine-2,5 dione	0.047	3	0.145	10
Dimer closed	0.091	3	0.305	10

Table 6: Linearity data

Parameters	Range (µg/mL)	Slope (b)	Intercept (a)	Correlation coeffficient (r)
Amoxicillin	0.26 - 22.95	25046.6	2367.8	0.9999
Clavulanic acid	0.07 – 6.52	28049.2	255.0	0.9999
p-Hydroxyphenylglycine	0.11 – 10.89	25447.2	3614.4	0.9996
6-APA	0.20 – 10.15	13068.5	445.7	0.9999
Alfa Amoxicilloic acids	0.26 – 22.95	12823.7	128.9	0.9999
L-Amoxicillin	0.27 – 9.95	19318.8	1273.5	0.9998
p-Hydroxyphenlglycine methyl ester	0.35 – 6.95	12869.2	39.8	0.9997
Penilloic acids peak 1	0.79 – 25.19	8602.4	989.9	0.9999
Clavulanate Impurity G	0.08 – 1.69	21639.8	185.6	0.9995
p-Hydroxyphenylglycine Amoxicillin	0.44 – 13.10	14109.4	554.3	0.9998
Penilloic acids peak 2	0.77 – 24.40	8936.8	150.3	0.9997
Amoxi 2(R) Piperzine-2,5 dione	0.19 – 12.29	28912.8	176.9	0.9999
Amoxi 2(S) Piperzine-2,5 dione	0.14 – 10.87	33881.7	1146.7	0.9999
Dimer closed	0.31 – 25.55	18470.6	1838.8	0.9999

Table 7: Precision data

Parameter	LOQ precision	Method Precision	Ruggedness
Amoxicillin	3.3	Not Applicable	Not Applicable
Clavulanic acid	9.4	Not Applicable	Not Applicable
p-Hydroxyphenylglycine	1.1	1.2	1.5
6-APA	4.8	2.3	0.9
Alfa Amoxicilloic acids	7.0	3.0	4.2
L-Amoxicillin	2.6	0.6	1.6
p-Hydroxyphenlglycine methyl ester	5.8	0.8	3.2
Penilloic acids peak 1	4.8	0.3	1.2
Clavulanate Impurity G	7.6	2.5	3.1
p-Hydroxyphenylglycine Amoxicillin	4.2	2.6	4.3
Penilloic acids peak 2	2.1	0.5	2.5
Amoxi 2(R) Piperzine-2, 5 dione	3.6	3.1	2.1
Amoxi 2(S) Piperzine-2, 5 dione	4.8	0.9	1.3
Dimer closed	10.1	1.5	2.7
Trimer Open	5.7	2.1	2.0
Total Impurities	Not Applicable	0.3	0.8

Table 8: Accuracy data of % Recovery range from LOQ to 150% level

Parameter	% Recovery range from LOQ to 150% level
Amoxicillin	95.3-102.8
Clavulanic acid	96.3 - 103.3
p-Hydroxyphenylglycine	95.8 - 110.1
6-APA	101.6 -115.2
Alfa Amoxicilloic acids	85.6 - 99.9
L-Amoxicillin	104.0 - 111.9
p-Hydroxyphenlglycine methyl ester	107.7 - 114.8
Penilloic acids peak 1	100.0 - 107.2
Clavulanate Impurity G	98.2 - 103.5
p-Hydroxyphenylglycine Amoxicillin	103.2 - 104.6
Penilloic acids peak 2	106.9 - 120.6
Amoxi 2(R) Piperzine-2, 5 dione	102.1-108.0
Amoxi 2(S) Piperzine-2, 5 dione	78.7 - 107.4
Dimer closed	84.0 - 102.1
Trimer Open	90.5 - 96.7

4. Conclusion

A cost effective, simple gradient HPLC method was developed to separate and quantification of seventeen known impurities of Amoxicillin and Clavulanic Acid pharmaceutical dosage forms in routine analysis. Degradation behaviors of Amoxicillin and Clavulanic acid studied under various stress degradation conditions. All the related impurities and the degradation impurities were well separated from the amoxicillin and Clavulanic acid revealed the stability indicating capability of the method.

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6. References

[1] Reading.C, Cole.M, Clavulanic acid A beta lactamase-Inhibiting beta-lactum from Streptomyces Clavuligerus, Antimicorbial agents and Chemotheraphy, 1997, 11(5), 852-857.

[2] Huntur.P.A, Coleman.K, Fisher.J, Tayoler.D, Taylor.E. Clavulanic Acid, a Novel B-lactam with broad Spectrum B-Lactamase inhibitory properties synergistic activity with ampicillin and Amoxicillin, Drugs Under experimental and Clinical research, 1979, 5, 1-6.

[3] Reddy.K.R, Brillant.P, Schiff.E.R. Amoxicillin-Clavulanate potassium Associated cholestasis, Gas troenterology, 1989, 96(4), 135-141.

[4] Hall. F, Curran. D, Burdon.W, Keighley.M.R.B. A Randomized Trial to Compare Amoxicillin and Clavulanate with Metonidazole Plus Gentamycin in Prophylaxis in Elective Colarectl Suregery, Journal of Antimicrobial Chemotherapy, 1989, 24 (B), 195-208.

[5] Durga Mallikarjuna Rao Tippa, Narendra Singh, Development and Validation of stability indicating HPLC method for simultaneous estimation of Amoxicillin and Clavulanic acid in Injection, Scientific Research, 2010, 1, 95-101.

[6] Veer.J.B, Hekster.Y.A, Baars .A.M, Van Der Klerjn.E. Rapid Determination of Amoxicillin and Ampicillin in Body Fluids by Means of High Performance Liquid Chromatography, Journal of Chromatography, 1978, 1145, 496-501.

[7] Lebella.M.J, Wilson.W.L, Lawriault.G. High Performance Liquid Chromatographic Determination of Amoxicillin in Pharmaceutical Dosage Forms, Journal of Chromatography, 1980, 202(1), 144-147.

[8] De Pourcq, Hoebus.J, Roets.E, Hoogmartens.J, Vanderhaeghe. H. Quantiative Determination of Amoxicillin and Its Decomposition Products by High Performance Chromatography, Journal of Chromatography, 1985, 321, 441-443.

[9] Low.A.S, Taylor.R.B, Gould.J.M, Determination of Clavulanic Acid by a Sensitive HPLC Method, Journal of Antimicrobial Chemotherapy, 1989, 24 (B), 83-86.

[10] Haginaka.J, Nakagawa.T, Nishind.Y, Uno.T. High Performance Liquid Chromatography Determination of Clavulanic Acid in Human Urine, Journal of Antibiotics, 1981, 34, 1189-1194.

[11] Abdalla Ahmed; El-Shanawani. HPLC analysis of Clavulanic acid, Amoxicillin, Ticarcillin and their related substances in vials, tablets and suspensions, Acta Poloniae pharmaceutica-Drug Research, 1998, 55(1), 15-19.

[12] ICH-Harmonised Tripartity guidelines: validation of analytical procedures, text and methodology Q2 (R1). IFPMA: Geneva, 2005.

[13] 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.

[14] Jayakar.B, Kumudhavalli.M.V, Margret Chandira.R, Kumar.M, Saravanan.C. Method development and validation of RP-HPLC method for simultaneous determination of Amoxicillin and Potassium Clavulanate, International Journal of PharmTech Research, 2010, 2(1), 906-909.

[15] Hoizey. G, Frances. C, Kaltenbach. M, Lamiable. D, Trenque. T, Denis. J, Millart. H. Simultaneous determination of amoxicillin and clavulanic acid in human plasma by HPLC with UV detection, Jpharm Biomed Anal, 2002; 30(3): 661-6.

[16] Rajesh. K.S, Yadav.A.B, Patel.A. Development and Validation of Stability Indicating HPLC Method for Estimation of Co-Amoxyclav Injection, Pharmagene , 2013,1(2), 60-64.

[17] Nwokoye Peace, Oyetunde Olubukola, Akinleye Moshood. Stability of reconstituted amoxicillin clavulanate potassium under simulated in-home storage conditions, Journal of Applied Pharmaceutical Science, 2012, 02 (01), 28-31.

[18] Mei-Chich hsu, Pei-Wen hsu, High-Performance liquid chromatographic method for potency determination of amoxicillin in commercial preparations and for stability studies, Antimicrobial agents and chemotherapy, 1992, 36(6), 1276-1279.

[19] Ashnagar.A, Gharib Naseri. N, Analysis of three penicillin Antibiotics (Ampicillin, Amoxicillin and cloxacillin) of several Iranian pharmaceutical companies by HPLC, E-Journal of Chemistry, 2007, 4(4), 536-545.