Simultaneous Estimation of Ampicillin, Amoxicillin, Potassium Clavulanate & Penicillin V Potassium By RP_HPLC Method In Pharmaceutical Waste

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Abstract*— The assay of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium was performed with pharmaceutical waste and the % assay was determined from pharmaceutical waste. The chromatographic Method conditions were done in WATERS HPLC, software: Empower, 2695 separation module, PDA as a analyzer and LABINDIA and UV 3000 UV Spectrophotometer,Inertsil ODSC18 with flow rate 1ml/min, at ambient column temperature, injection volume 20 µl, detection wavelength 295nm and prepared mobile phase of Phosphate buffer pH 3: Acetonitrile (30:70). Assay Result for Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium was performed with pharmaceutical waste and the % assay was found to be 16.21, 15.54, 16.80 and 14.84 from pharmaceutical waste. Stress degradation studies were performed on the Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium using the proposed method. And all the samples degraded within the limits. Concluding sensitive assay method is validated for drugs such as Amoxicillin, Ampicillin, Penicillin V Potassium and Clavulanic Acid. Using a simple buffer and Isocratic programming, an accurate simultaneous determination of these drugs has been made possible at very low level is demonstrated. Author believes that this method is very useful in testing of public sewer waste water and is less expensive.

Index Terms- Pharmaceutical waste, RP-HPLC, PDA, Method validation, Octa decyl silyl carbon

I. INTRODUCTION

Emergence of novel RP-HPLC method for the concurrent analysis of multicomponent in pharmaceutical waste containing Amoxicillin, Ampicillin, Penicillin V Potassium and Clavulanate potassium by Reverse Phase High performance liquid chromatography. However, no method was reported in present combination. Contaminating the public sewer with these Antibiotics drastically affects the public health; hence author considered the importance to develop a single and cost-effective method to estimate these compounds by RP-HPLC and validated according to ICH guidelines.

Amoxicillin and Its Role[1]

Amoxicillin, a penicillin derivative, serves as a vital tool in treating infections caused by gram-positive bacteria. By competitively inhibiting penicillin-binding proteins crucial for cell wall synthesis, Amoxicillin exerts its bactericidal effects, helping to combat upper respiratory tract infections.

Ampicillin and Its Impact[2]

Ampicillin, another penicillin derivative, is crucial in addressing a broad spectrum of infections caused by both gram +VE and gram -VE bacteria. Its stability against various beta-lactamases enhances its efficacy in inhibiting cellwall building through binding to penicillin-attaching proteins, aiding in microbial suppression.

Clavulanic acid[3]

Clavulanic acid[3] a beta-lactamase inhibitor that's persistent combined with Amoxicillin to overcome antibiotic resistance by avoiding their degradation by beta-lactamase enzymes, increasing their spectrum of susceptible bacterial infections. Streptomyces clavuligerus produces Clavulanic acid .

Phenoxymethylpenicillin[4]

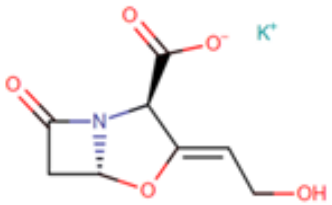
Phenoxymethylpenicillin (Penicillin V) a narrow spectrum antibiotic . It is a phenoxymethyl analogue of Penicillin G, or benzylpenicillin. This drug is used in treatment of infections in the respiratory tract, skin, and soft tissues. As prophylaxis against susceptible organisms it can be used. As controlled clinical

efficacy studies were not conducted, it has been urged by the American Heart Association and American Dental Association it as an oral regimen for prophylaxis for bacterial endocarditis in patients of CHD or relative valvular heart problem.

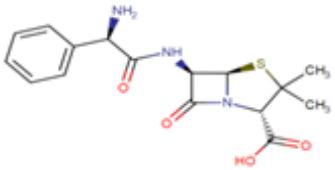
Microbial Antibiotic Resistance[5] which is occurring and increasing drastically in today's era due to improper and irrational use of antibiotics and which is estimated to be silent pandemic and cause global economic loss. Proper regulation of pharmaceutical waste treatment and limiting its production is prior demand.This research studies will provide information the content of antibiotic present in waste which is liable to cause Microbial Antibiotic Resistance.

II. MATERIAL AND METHODS

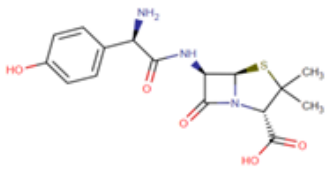
The system composed of WATERS, Hplc Software: Empower, 2695 separation module, PDA Analyser and LABINDIA UV 3000 UV Spectrophotometer, work accomplished in Hyderabad city INDIA. All the steps performed with reference to the International Conference on Harmonization instructions Q1A R2[6-11].



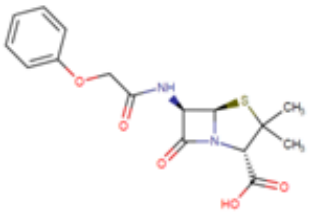
Potassium clavulanate



Ampicillin



Amoxicillin



Penicillin V

Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium was obtained from Pharmatrain Pvt ltd. Potassium hydrogen phosphate from FINER chemicals Ltd, and H_2O Methanol for HPLC LICHROSOLV (MERCK), Acetonitrile for HPLC from MOLYCHEM 8 H_3PO_4 from Acid MERCK.

III. PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of 0.025M Phosphate buffer
Precise 3.4gms potassium dihydrogen ortho phosphate was collected in a 1000ml VF and pH adjusted with Dil. NaOH till 3, later on the solution was strained with help of 0.45 µ membrane filter, sonicated about 10 mins.

Preparation of mobile phase
Following a 10-minute degassing period in an ultrasonic water bath, precisely measured volumes of 300 ml (30%) of the aforementioned buffer and 700 ml of 70% acetonitrile HPLC were filtered through a 0.45 µ filter under vacuum filtration.

IV. PREPARATION OF THE CLAVULANATE POTASSIUM, AMPICILLIN, AMOXICILLIN AND PENICILLIN V POTASSIUM STANDARD & SAMPLE SOLUTIONS:

Standard Solution Preparation:
Weigh 25mg Clavulanate potassium, 100mg of Ampicillin, and 175mg of Amoxicillin and 100mg of Penicillin V Potassium standard in 100ml VF dilute it and sonicate then make volume to the level using exact solvent. Now dropper out 1.5ml up prepared stock solution in 10ml VF and make up to level.

Sample Solution Preparation:

Sample solutions were prepared by collecting the production waste from where it is connected to public sewer. Sample collected of 10mL in every two hours interval for 24hours, the representative 20mL of pooled sample taken and diluted to 100mL with purified water, sonicated for 5 minutes, centrifuged for 10 minutes and used clear solution for testing.

Chromatographic conditions

The chromatographic conditions were achieved by using Inertsil ODS C_{18} with FR of 1ml/min, at column temperature, injection volume was selected 20 µl, detection wavelength 295nm and MP composed of PO_4 buffer pH 3: Acetonitrile (30:70).

Method validation

The Emerged method validated as per stated by 2005 ICH protocols. Validation parameters are succesive to the protocols of ICH which include system suitability, linearity, precision, LOD, LOQ and accuracy, robustness are estimated to be under the instructed limit.

System suitability

All the parameters like theoretical plate count , tailing factor, %RSD of peak Rt and Resolution system suitability tests were examined to make certain optimized chromotographic conditions for the Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium.

Linearity

Weigh 25mg of Clavulanate potassium, 100mg of Ampicillin, and 175mg of Amoxicillin and 100mg of Penicillin V Potassium working standard into a 100ml VF then sonicate it after adding diluet and make volume up to level(Stock solution). Level I-V were prepared with increasing 0.5ml volume from stock and injected.

Precision

From stock solution dropper out 1.5 ml into a 10ml VF and dilute up to the mark with diluent.The standard solution was injected for six times and examine the area for all six injections in HPLC. The % relative standard deviation for the area of 6 reproduce injections were to be within the defined limits factors. The results are summarized for Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium.

Intermediate precision/ruggedness

Determination of ID (also known as Ruggedness) of the method, Precision was conducted on changing days. From stock dropper out 1.5 ml in 10ml VF and dilute up to the level along with diluent. Standard solutions reproduce in the precision was injected on the another day, for six times and examined the area for six injections in HPLC. The %RSD for the area of six reproduce injections were to be inside the set down limiting factors.

Accuracy

weigh 12.5mg of Clavulanate potassium, 50mg of Ampicillin, and 87.5mg of Amoxicillin and 50mg of Penicillin V Potassium standard into a 100 ml VF, dilute make volume up to the level and centrifuge to dissolve it entirely and for 50% solution. Dropper out 1.5ml of prepared stock solution into 10ml VF upto level Certainly, 100%, 150% standard solutions were produced and injected. Amount found and Amount added for Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium were estimated and individual recovery and mean recovery values were determined[-14].

Limit of Detection

0.89µg/ml, 0.8µg/ml, 0.39µg/ml and 12.24µg/ml of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium were prepared from stock solutions.

Limit of Quantification

Lowest conc[n] of the sample was prepared to determined the signal to noise ratio w.r.t the base line noise.

Robustness:

Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium of sample and standard were injected by varying the conditions of chromatography. There was no markable change in the limiting factors like resolution, tailing factor, asymmetric factor, and plate count.

Degradation studies

Hydrolytic degradation under exposure to acidic condition
Measure and take 1.5 ml of stock solution into a 10ml VF and 3 ml of 0.1N HCl was added. Then, the VF was kept at 60ºC for 6 hours and then neutralized with again 0.1 N NaOH and make up to 10ml with diluent. Strain the solution with 0.22 µ syringe filters and place in vials.

Hydrolytic degradation under exposure to alkaline condition
Dropper out 1.5 ml of up prepared solution into 10ml VF , add 3ml 0.1N NaOH in 10ml of VF Then, the VF maintained in 60ºC for 6 hours and then balanced with 0.1N HCl and make up to 10ml with diluting solvent. Strain the solution with 0.22 µ syringe filters and place out in vials.

Degradation under exposure to Thermal conditions
Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium sample was taken in Petri dish and remained in Hot air oven at 110 ºC for 24 hours. Later sample diluted and injected.

Degradation under exposure to oxidative conditions
Dropper out 1.5 ml stock solution in 10ml VF solution , 1 ml of 3% w/v of H_2O_2 VF was then remained for 15 min. Strain the solution with 0.45 µ syringe and placed in vials. Samples degraded under 15%, degradation results are passed.

V. RESULTS

The estimation of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium was achieved by RP-HPLC. The assay of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium was performed with pharmaceutical waste and the % assay was found to be 16.21, 15.54, 16.80 and 14.84 from pharmaceutical waste. The linearity range of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium are 12.5 to 62.6µg/ml, 50 to 250µg/ml, 87.5 to 437.5µg/ml, 50 to 250µg/ml respectively.

And the linearity of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium was estimated to be linear with a correlation coefficient of 0.999, 0.999, 0.999 and 0.999 which shows that the procedure is producing good results .The method show precision 1.0, 0.6, 0.8 and 0.6 for Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium which shows that the method is precise.

The LOD and LOQ for Clavulanate potassium was estimated to be 2.98 and 9.98 and The LOD and LOQ for Ampicillin was found to be 3.02 and 10.02 and LOD and LOQ for Amoxicillin was found to be 3.00 and 10.00 and LOD and LOQ for Penicillin V Potassium was determined to 3.00 and 10.02.

VI. OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	Waters HPLC with auto sampler and PDA analyzer
Temperature	Ambient Temperature
Column	Inertsil ODS C 18 (4.6 x 250mm, 5□m)
Buffer	3.4g potassium dihydrogenortho phosphate was taken in a 1000ml VF and the pH-3was obtained by Dilute NaOH.
P H	3.0
MP	Phosphate buffer Ph- 3: Acetonitrile (30:70)
FR	1 ml/ min
Wavelength	280 nm
Inj volume	20µl
Run time	15 min.

Table -1 showing optimized chromatographic conditions

Fig - 1 Blank obtained Chromatogram

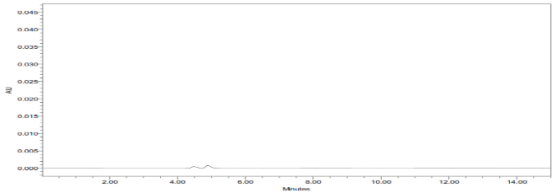


Fig – 2 Standard obtained Chromatogram

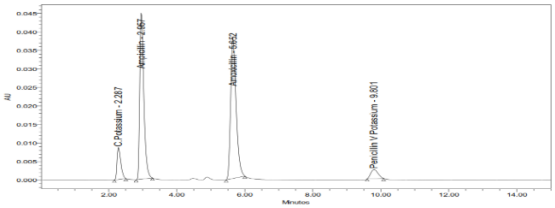
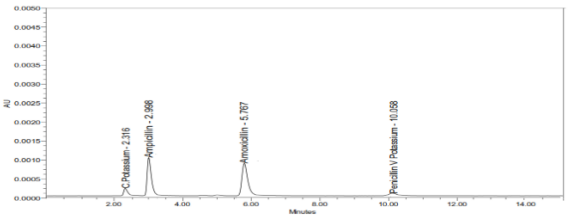


Fig -3 Sample obtained Chromatogram



System suitability

Resolution produced for drugs was not less than 2. Theoretical plates not less than 2000.Tailing factor not more than 2. From mentioned data all system suitability factors were under limiting level.

S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Clavulanate potassium	2.287	62677	8331		1.34	3122.89
2	Ampicillin	2.957	343268	44587	3.19	1.29	2952.03
3	Amoxicillin	5.652	410983	37668	10.49	1.37	5852.32
4	Penicillin V Potassium	9.801	37828	2278	12.25	1.13	4461.23

Table No – 2

Fig -4 System suitability obtained Chromatogram

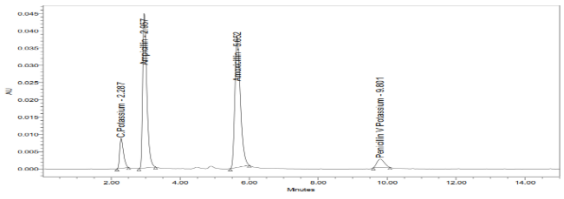


Fig -5 linearity of clavulanate potassium

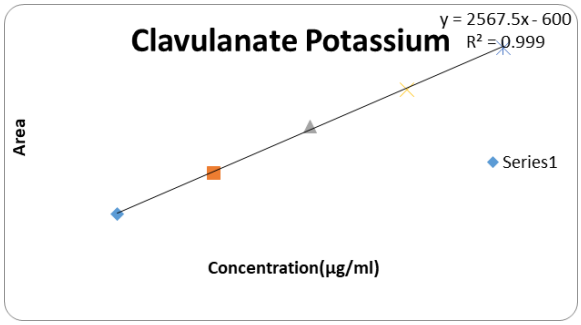


Fig -6 linearity of Ampicillin

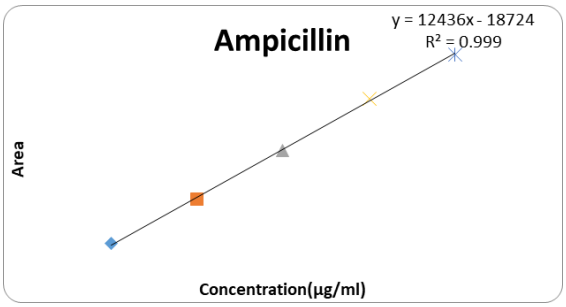


Fig -7 linearity of Amoxicillin

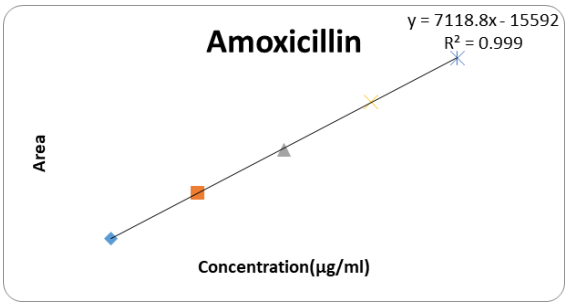
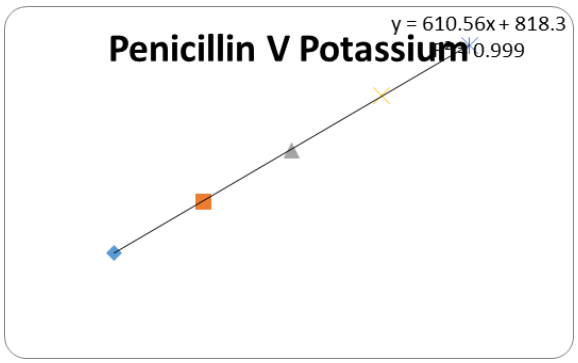


Fig -8 linearity of Penicillin V Potassium



Parameters	Clavulanate potassium	Ampicillin	Amoxicillin	Penicillin V Potassium

Slope (m)	2567.5	12436	7118.8	610.56
Intercept (c)	600	18724	15592	818.3
Correlation coefficient (R^2)	0.999	0.999	0.999	0.999

Table No -3

Linearity Results: (Clavulanate potassium)

S. No	Linearity Level	Conc^n	Area
1	I	12.5	31158
2	II	25	62501
3	III	37.5	98431
4	IV	50	126883
5	V	62.5	159437
Correlation Coefficient			0.999

Table No -4

S. No	Linearity Level	Conc^n	Area
1	I	50	174283
2	II	100	346309
3	III	150	534616
4	IV	200	738393
5	V	250	910971
Correlation Coefficient			0.999

Table No - 5 Linearity Results: (for Ampicillin)

S. No	Linearity Level	Conc^n	Area
1	I	87.5	200028
2	II	175	412844
3	III	262.5	616857
4	IV	350	842986
5	V	437.5	1052774
Correlation Coefficient			0.999

Table No -6 Linearity Results: (for Amoxicillin)

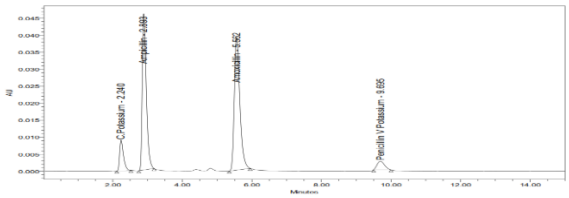
S. No	Linearity Level	Conc^n	Area
1	I	50	19268
2	II	100	37340
3	III	150	55298
4	IV	200	74833
5	V	250	92106
Correlation Coefficient			0.999

Table No– 7 Linearity Results: (for Penicillin V Potassium)

Precision

%RSD NMT 2 produced for sample and below 1 for standard certainly procedure is precise.

Fig - 9 Chromatogram for Precision -6



Injection	Clavulanate potassium	Ampicillin	Amoxicillin	Penicillin V Potassium
Inj-1	62521	340835	412366	37719
Inj-2	63501	345902	417933	37131
Inj-3	63131	340203	418246	37145
Inj-4	62349	343151	410461	37488
Inj-5	64024	341901	413907	37380
Inj-6	63398	341902	416726	37139
Avg	63154	342316	414940	37334
Std Deviat^n	630.252	2028	3188	240
%RSD	1.0	0.6	0.8	0.6

Table No– 8 Results of Precision for Clavulanate potassium, Ampicillin, Amoxicillin Penicillin V Potassium

Intermediate precision %RSD NMT 2 was produced for 5 varying samples which is certainly under limit factor. Hence procedure rugged.

Fig – 10 Chromatogram for ID Precision -6

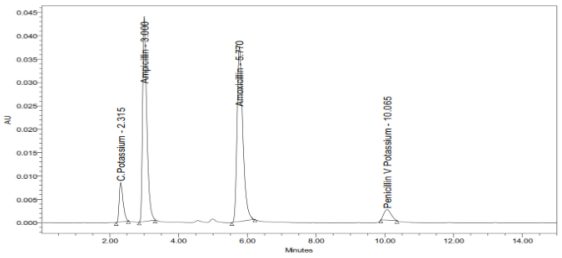


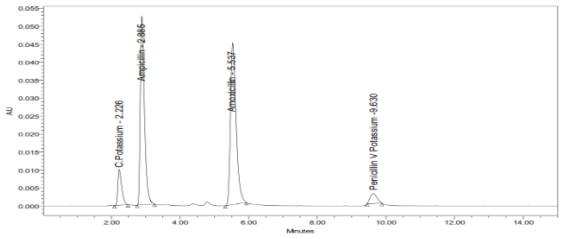
Table No - 9 Results of ID for Clsavulanate potassium, Ampicillin, Amoxicillin & Penicillin V Potassium

Injection	Clavulanate potassium	Ampicillin	Amoxicillin	Penicillin V Potassium
Inj-1	62421	350835	418366	38019
Inj-2	62501	345902	417933	37131
Inj-3	62131	349203	418246	37145
Inj-4	62349	343151	419461	37488
Inj-5	62024	349901	419907	37380
Inj-6	62398	346902	419726	37239
Avg	62304	347649	418939.8	37400.33
Std Deviat^{n}	185.304	2880.244	854.340	333.050
%RSD	0.1	0.8	0.3	0.3

Accuracy

The % recovery shown to be within the limit level (97-103%).The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence technique is accurate.

Fig -11 Accuracy obtained Chromatogram



%Conc^{n} (at specification Level)	Area	Amt Added (mg)	Amt Found (mg)	% Recovery	Mean Recovery
50%	31749	12.5	12.66	101.28	100.11
100%	62401	25	24.88	99.53	
150%	93601	37.5	37.32	99.53	

Table No 10: Clavulanate potassium Accuracy (recovery) data

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	172009	50	50.10	100.20	100.26
100%	346657	100	100.97	100.97	
150%	512952	150	149.50	99.60	

Table 11: Accuracy (recovery) data for Ampicillin

%Conc^{n} (at specification Level)	Area	Amt Added (mg)	Amt Found (mg)	% Recovery	Mean Recovery
50%	207597	87.5	87.97	100.49	100.64
100%	415239	175	175.87	100.50	
150%	625553	262.5	264.94	100.93	

Table No 12: Amoxicillin Accuracy (recovery) data

% Conc^{n} (at specification Level)	Area	Amt Added (mg)	Amt Found (mg)	% Recovery	Mean Recovery
50%	18910	50	50.26	100.52	100.08
100%	37496	100	99.65	99.65	
150%	56478	150	150.10	100.07	

Table No 13: Accuracy (recovery) data for Penicillin V Potassium

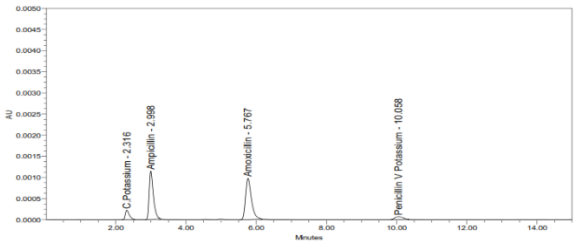
Limit of detection

S/N ratio ought to be 3 for LOD solution. The result produced is under the limit level.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Clavulanate potassium	66	197	2.98
Ampicillin	66	199	3.02
Amoxicillin	66	198	3.00
Penicillin V Potassium	66	198	3.00

Table No 14: LOD obtained Results

Figure -11 Chromatogram of Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium showing LOD



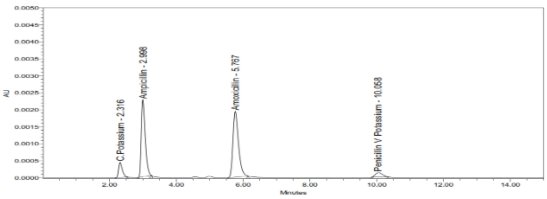
LIMIT OF QUANTIFICATION

S/N ought to be 10 for LOQ solution. The produced result is within the limiting level.

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Clavulanate potassium	66	659	9.98
Ampicillin	66	661	10.02
Amoxicillin	66	660	10.00
Penicillin V Potassium	66	661	10.02

Table No 15 Results of LOQ

Figure -12 Chromatogram obtained for Clavulanate potassium, Ampicillin, Amoxicillin &Penicillin V Potassium showing LOQ



ROBUSTNESS

Clavulanate potassium, Ampicillin, Amoxicillin and Penicillin V Potassium both sample and standard maintained in diverse the conditions and injected. There was no notable difference in resolution, tailing factor, asymmetric factor, and plate count.

S. No	Organic Composition change in the MP	Results obtained by System Suitability	
		USP PC	USP Tailing
1	10% less	2984.61	1.26
2	*Actual	3122.89	1.34
3	10% more	2151.78	1.49

Table No 16 System suitability results obtained by Clavulanate potassium

	Organic Compositio	Results obtained by System Suitability

S. No	n change in the MP	USP PC	USP Tailing	USP Resolution
1	10% less	2736.95	1.28	3.13
2	*Actual	2952.03	1.29	3.19
3	10% more	2787.43	1.50	2.14

Table No 17 System suitability results obtained by Ampicillin:

S. No	Organic Composition change in the MP	Results obtained by System Suitability		
		USP PC	USP Tailing	USP Resolution
1	10% less	5778.93	1.33	10.46
2	*Actual	5852.32	1.37	10.49
3	10% more	4764.64	1.41	6.57

Table No 18 System suitability results obtained by Amoxicillin:

S. No	Organic Composition change in the MP	Results obtained by System Suitability		
		USP PC	USP Tailing	USP Resolution
1	10% less	4761.86	1.17	11.70
2	*Actual	4461.23	1.13	12.25
3	10% more	4652.61	1.24	6.14

Table No 19 System suitability results by Penicillin V Potassium:

Acceptance criteria:

Parameters like Rt, USP plate count, USP tailing factor[13] produced for change of flow rate, difference in mobile phase was found to be under the acceptance level. So, the procedure is robust.

Degradation

All the samples degraded within 15%, so the degradation results are passed. Stability indicating studies[14] were performed in an environment of acid/base/peroxide, thermal, and photolysis. For particular study, samples were made. Maintainence of environment was exact for blank , drug solution and standard solution of Clavulanate potassium, Ampicillin, Amoxicillin &Penicillin V Potassium .

	Clavulanate potassium		Ampicillin	
	Area	% Degraded	Area	% Degraded
Std	62572		342646	
Acid	60297	3.64	331313	3.31
Base	60635	3.10	329952	3.70
Peroxide	60293	3.64	316915	7.51
Thermal	60121	3.92	325902	4.89
Photo	60221	3.76	325462	5.02

Table No 20 Degradation results for Clavulanate potassium and Ampicillin

	Amoxicillin		Penicillin V Potassium	
	Area	% Degraded	Area	% Degraded
Std	412362.7		37550.7	
Acid	393095	4.67	35645	5.08
Base	399441	3.13	35835	4.57
Peroxide	396871	3.76	35209	6.24
Thermal	380461	7.74	35488	5.49
Photo	393431	4.59	35432	5.64

Table No 21 Degradation results for Amoxicillin and Penicillin V Potassium

CONCLUSION

A sensitive assay method is validated for drugs such as Amoxicillin, Ampicillin, Penicillin V Potassium and Clavulanic Acid. Further such HPLC method for simultaneous estimation of Amoxicillin, Ampicillin, Penicillin V Potassium and Clavulanic Acid in effluent waste water is not reported yet. Using a simple buffer and Isocratic programming, an accurate simultaneous determination of these drugs has been made possible at very low level is demonstrated. Author believes that this method is very useful in testing of public sewer waste water and is less expensive.

Stress degradation studies was performed on the Clavulanate potassium, Ampicillin, Amoxicillin and

Penicillin V Potassium using the proposed method.
And all the samples degraded within the limits.