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New Validated RP-HPLC Method For The Estimation of Ertapenem In Pharmaceutical Formulation

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Abstract : *A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Ertapenem in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1.5 ml/min was employed on symmetry Shimadzu LC-20 ATVP Kromasil C-18 Column at ambient temperature. The mobile phase consisted of 0.01M Potassium di-hydrogen phosphate : Water : Acetonitrile (25:50:25 v/v) . The UV detection wavelength was 295nm and 20µl sample was injected. The run time for Ertapenem is 15 min. The Percentage assay of Ertapenem in formulation was found to be 100.01%. The amount of drug present in the human sample was found to be 0.287 mg/ ml The limit of detection for Ertapenem was found to be 20µg/ml and the limit of quantification was found to be 50µg/ml.The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation. The HPLC method can be successfully applied for the routine quality control analysis of Ertapenem formulations.*

Keywords - *Ertapenem, Rp- HPLC, UV detection, Recovery, Precise*

I. INTRODUCTION

Chemistry of Ertapenem reveals that[1] the chemical name is [4R.5S,6S]-3-[[(3S-5S)-5-[[(3-Carboxyphenyl) amino]carbonyl]-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl1]-4-methyl-1-oxo-1-aza bicycle-[3.2.0]hept-2-ne-2-carboxylicacid. Ertapenem is parenteral β-lactam antimicrobial agent[2-7]. Studies have shown that this structurally unique carbapenem has excellent activity against many gram-positive and gram-negative aerobic, facultative, and anaerobic bacteria that, in general, are associated with community-acquired infections. Ertapenem was highly active in vitro[8] against many aerobic and facultative bacterial pathogens commonly recovered from patients with community-acquired bacteremia. M. V. Basaveswara Rao et al.,[9] proposed a RP-HPLC method for the estimation of Stavudine in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Methanol: 0.1 % O.P.A: Acetonitrile (40:50:10) was used. The flow rate was 1.2ml/min. and effluents were monitored at 267 nm. The retention time for Stavudine was 6.8 min. Limit of detection and limit of quantification were found to be 0.5ppm and 3.0ppm respectively and recovery of Stavudine from tablet formulation was found to be 97.2%.. RG Mundkowski etal[10], proposed the HPLC assays for the quantification of ertapenem based on columns of 4.6mm I.D. The assay is rapid for specimen concentrations >/=1mg/l and is easily tuned to achieve low quantification limits at high chromatographic resolution for lower concentrated samples. L.Venkateswara Rao et.al., developed[11] RP-Hplc method for the determination of meropenem. The chromatographic analysis was carried out by RP-technique on an ultra sphere XL-ODS, 75mm x 4.6mm I.D.,5µm column with a mobile phase composed of ammonium acetate buffer of pH =4.0 and acetonitrile in the ratio 95:5 v/v, at a detection wavelength 298nm. In spectrophotometric method meropenem was reacted with oxidized form of 3-methyl 2-benzothia zolinone hydrazone (MBTH) in the presence of ferric chloride (Fe (III)) which forms an orange red colored chromogen giving absorption maximum at 600 nm.

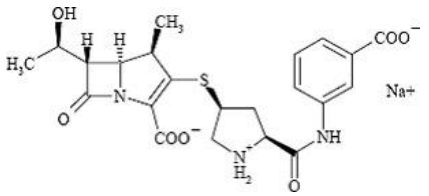

Figure:1 Structure of Ertapenem

II. EXPERIMENTAL

Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable ***PDA detector*** and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size and the column were maintained at ambient temperature. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

Chemicals and Solvents

The reference sample of Ertapenem powder was obtained from Local Market. The Formulation was procured from the local market. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate,Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. The chemicals were procured from E-Merck, India Limited.

The mobile phase

The mobile phase was prepared by mixing 0.01M Potassium dihydrogen phosphate and Water and Acetonitrile in the volume of ratio 25:50:25 v/v/v by ultra bath sonicator for 30 min.

Preparation of solutions

Preparation of mobile phase solution

The mobile phase was prepared by mixing 0.01M potassium dihydrogen phosphate and Water and Acetonitrile (25:50:25 v/v) by ultra bath sonicator for 30 min.

Preparation of standard

Stock solution of Ertapenem was prepared by dissolving accurately weighed 10mg of drugs in 10ml Methanol. The prepared stock solutions were stored away from light. From the stock, standard solutions was freshly prepared during the day of analysis.

Preparation of working standard solution (a.p.i)

From the stock solution 20 µg/ml solution was prepared.

Preparation of working standards for linearity

Solutions in the concentration range of 1-5 mg/ml were prepared from the standard working solution.

Preparation of formulation sample solution

10mg of formulation powder was taken from ERTAPENEM (14 mg formulation) and dissolved in 10ml of mobile phase and injected into HPLC and chromatogram was recorded. The amount of drug present in the 1mg formulation was calculated from linearity graph.

III. METHOD DEVELOPMENT

Detection of wavelength

The spectrum of 10ppm solution of Ertapenem was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 295nm was observed.

Choice of stationary phase and mobile phase

Finally the expected separation and peak shapes were obtained on Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size.

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 1.3 ml/min flow rate was ideal for elution of analyte.

IV. VALIDATION PROCEDURE AND REQUIREMENTS

The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

Linearity And Calibration

Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 1, 2, 3, 4, 5 mg/ml Injection was made at intervals of 15 min. The linearity was tested for the concentration ranging from 1mg/ml to5 mg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

Reproducibility was performed by injecting three replicates concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day. Inter-day variations in the peak area of drug solutions and the amount of drug were calculated in terms of Percentage Relative Standard Deviation. The sample concentration is 20µg/ml.

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate.

Assay

The estimation of drug in pharmaceutical dosage forms. ERTAPENEM 14mg strength was evaluated for the amount of Ertapenem present in the formulation. Each sample was analyzed in triplicate after extracting the drug. The amount of drug present in formulation was calculated by comparing the mean peak area from standard.

Intermediate Precision or Ruggedness

Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Robustness

Robustness was carried out by varying two parameters from the optimized chromatographic conditions.

Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution containing excuse ingredients with that of test results those obtained from standard drug.

System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Ertapenem and it was calculated by determining the standard deviation of Ertapenem standards by injecting standards in five replicates at 6 minutes interval and the values were recorded.

V. FORMULATION ANALYSIS

Ertapenem Analysis In Serum Preparation Of Serum Sample Solution

From a local hospital blood was collected and serum was separated. 1ml of this serum was taken in a test tube and added 100µl of diltizem hydrochloride (1µg/ml) and 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded.

Serum Data:

The amount of drug present in the blood sample was calculated from linearity graph. Drug estimation in human serum by developed protocol:- Amount of ETRPENEM present in serum is 0.906 mg/ ml

VI. RESULTS AND DISCUSSION

The Reverse Phase High Performance Liquid Chromatography method was developed a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate,Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally 0.01M Potassium dihydrogen phosphate and Water and Acetonitrile in the volume of ratio 25:50:25 v/v (P^H: 3.5) and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.83 tailing. Calibration graph was found to be linear at range 1mg/ml to 5mg/ml. five different concentrations of Ertapenem in range given above were prepared and 20µl of each concentration injected in HPLC as shown in the Figure no: 2. The slope (m) and intercept (c) obtained were found to be 38820.93and -0.033801094.The correlation of coefficient (r^2) obtained was found to be 0.9995 as shown in the Table no: 4. It was observed that the concentration range showed a good relationship. The limit of detection for Ertapenem was found to be 20µg/ml and the limit of quantification was found to be 50µg/ml. It proves the sensitivity of the method. The Percentage assay of Ertapenem in formulation was found to be 100.01%.as shown in the Table no: 1 and figure no: 4. The relative standard deviation value obtained was below 1 which indicates the precession of the method. The validation of the proposed method was further verified by recovery studies. The data was presented by in the Table no: 2 and figure no: 3. The percentage recovery was found to be 102.82% which shows a good index of accuracy of the developed method. The amount of drug present in the human serum sample was calculated from the linearity graph was found to be 0.906 mg/ml as shown in Table no: 3 and Figure no: 5.

VII. CONCLUSION

The RP-high performance liquid chromatographic method was developed and validated for the analysis of Ertapenem from their formulations was found to be accurate and precise. Also performed the serum analysis. This method is simple and better than the reported methods in the literature. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Ertapenem formulations

Table :1 Optical chracterisation of ertapenem

PARAMETERS	ERTAPENEM
Linearity range(mg/ml)	1.0 – 5.0
Correlation coefficient (r)	0.9995
Slope(m)	38820.93
Intercept(c)	-0.033801094
Limit of detection (LOD; µg/ml)	20
Limit of Quantification (LOQ; µg/ml)	50

Tailing factor	1.83
Retention time (min)	9.882
Theoretical plates	46721.52
(%)R.S.D	0.145
(%)Accuracy	102.82
(%)Formulation Assay	100.01
Serum (mg/ml)	0.906

Pharmaceutical formulation (brand name)	**Labeled amount (mg)**	**Percentage assay**	**Percentage recovery**
ERTAPENEM	14 mg	100.01	102.82

*Average value of three different levels in triplicate

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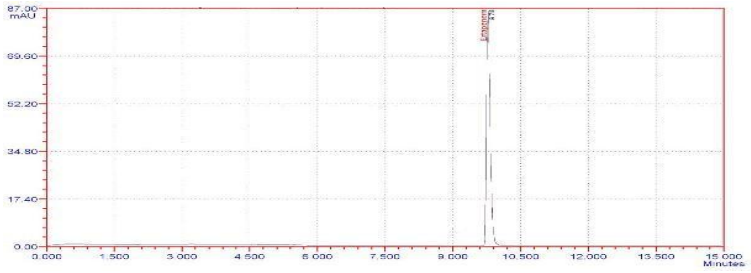
Table :2 Recovery data of ertapenem



S.No	**Name**	**Retain.T**	**Height**	**Area**	**Concentration**	**Tailing Factor**	**Theoretical plate**
1	Ertapenem	9.782	7939	40027.4	100.000	1.08	75020
	Sum		7939	40027.4	100.0000		

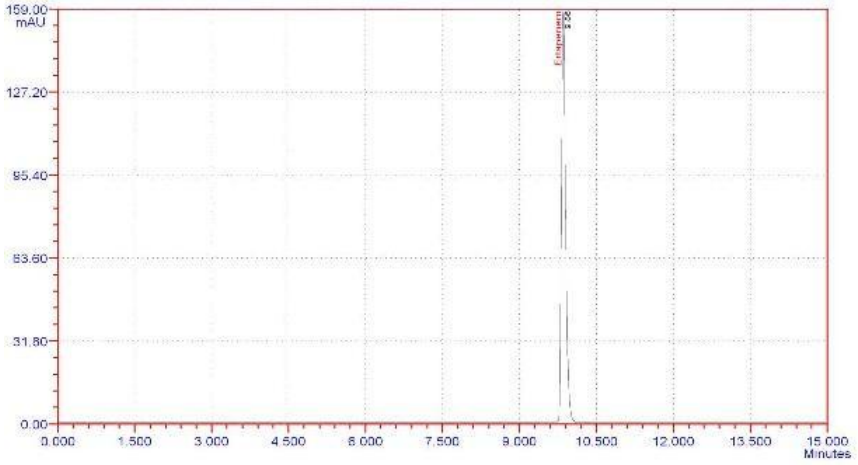
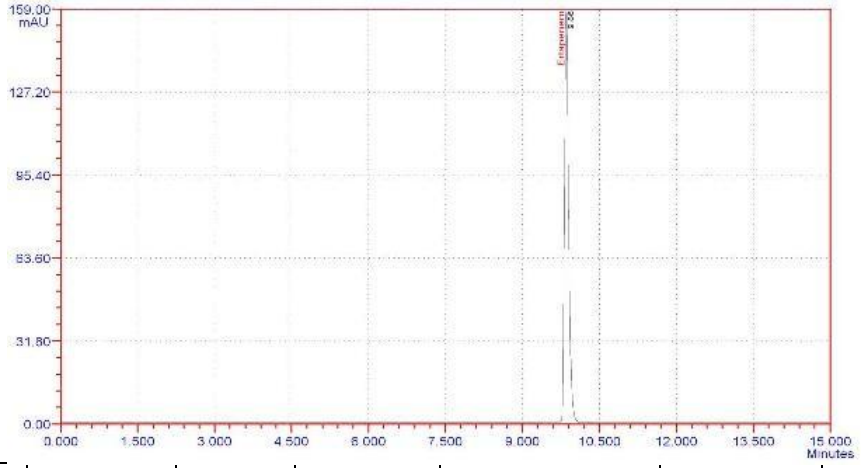
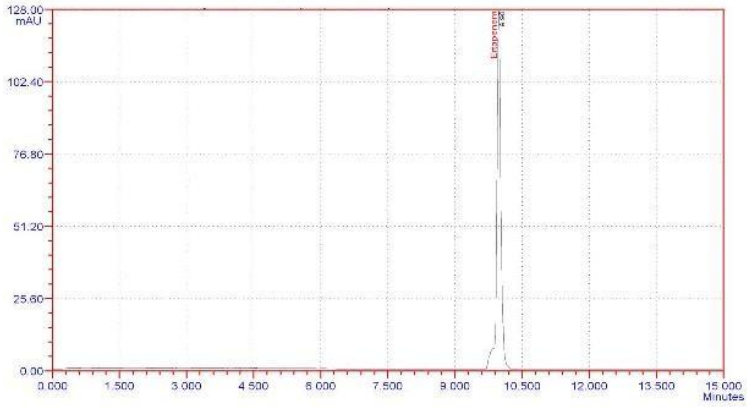


Figure no: 2 Chromatogram of Ertapenem (standard) and their values



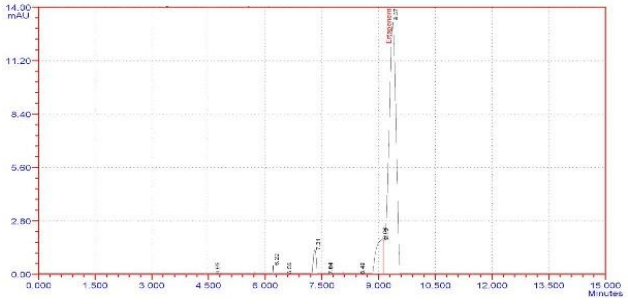
S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
1	Ertapenem	9.857	16672	158492.6	100.000	0.99	21426
	Sum		16672	158492.6	100.0000		

Figure no: 3 Chromatogram of Ertapenem (Accuracy) and their values



S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
1	Ertapenem	9.980	13211	115215.0	100.000	1.34	26101
	Sum		13211	115215.0	100.0000		

Figure: 4 Chromatogram of Ertapenem (Formulation Assay) and their values



S.No	Name	Retain.T	Height	Area	Concentration	Tailing Factor	Theoretical plate
s1	Ertapenem	4.650	566	72714.6	41.908	0.00	26
		6.215	626	6682.4	3.851	0.64	6756
		6.560	448	5684.4	3.276	0.85	5327
		7.313	885	20558.7	11.849	1.30	1975
		7.638	482	196.4	0.113	2.69	7006840
		7.662	240	3576.0	2.061	0.00	5270
		8.493	533	8976.7	5.174	0.78	5069
		9.080	765	19482.9	11.229	0.54	2533
		9.125	757	449.0	0.259	1.73	4717208
		9.372	1874	35188.9	20.281	1.82	4965
	Sum		7176	173509.9	100.0000		

Figure: 5 Chromatogram of Ertapenem (Serum) and their values

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