Development of Stability Indicating RP-HPLC Method for Ertapenem in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT: A simple, inexpensive, rapid and novel stability indicating isocratic HPLC method has been developed and validated for quantitative analysis of ertapenem sodium in the bulk drug and in pharmaceutical dosage form. An isocratic separation of ertapenem sodium was achieved on Hypersil BDS C_{18} column (4.6 x 250 mm, 5 µ particle size) as the stationary phase with a flow rate of 1.2 ml/min and using a UV detector to monitor the eluate at 298 nm. The mobile phase consisted of acetonitrile : water (60:40v/v) and pH adjusted 2.9 by othophosphoric acid enabled separation of the drug from its degradation products. The method was validated for linearity, accuracy (recovery), precision, specificity and robustness. The linearity of the method was satisfactory over the range 2-10 µg/ml (correlation coefficient 0.999). Recovery of ertapenem sodium from the pharmaceutical dosage form ranged from 99.97 to 103.7%. Ertapenem sodium was subjected to stress conditions [hydrolysis (acid, base), oxidation, photolysis and thermal degradation] and the samples were analyzed by this method. The forced-degradation study with ertapenem sodium showed that it was degraded under basic condition. The drug was stable under the other stress conditions investigated. Ertapenem sodium was found to be less stable in solution state, whereas it was comparatively much stable in solid state. The degradation products were well resolved from main peak. The forced degradation study prove the stability indicating power of the method and therefore, the validated method may be useful for routine analysis of ertapenem sodium as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Key words: RP-HPLC, Ertapenem sodium, forced degradation, method validated

INTRODUCTION

Ertapenem sodium (INVANZ, Merck, formerly MK-0826 and L-749,345) is chemically (4R, 5S, 6S)-3-[(3S, 5S)-5-[(3-carboxyphenyl) carbamoyl] pyrrolidin-3-yl] sulfanyl-6-(1hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid monosodium. Ertapenem sodium (Figure 1) a parenteral 1-methyl carbapenem (beta-lactam antibiotics), is structurally related to imipenem and meropenem and are widely used against a broad spectrum of aerobic and anaerobic Gram-positive and Gram-negative bacteria. Ertapenem sodium has been suggested as having potential use against *Mycobacterium tuberculosis*.[1,2] Ertapenem sodium (ERP), approved by the FDA in 2001, is one of these carbapenems. Since ertapenem has an approximate half-life of 4 hrs, it can be administered once daily. Therefore, ERP can be favored above other carbapenems.[3] Stability testing forms an important part of the process of drug product development. The purpose of stability testing

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is to provide evidence on how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, light and enables storage conditions, retest periods and shelf life to be recommended.[4,5] The two main aspects of study of the stability of a drug product that play an important role in shelf life determinations are assay of the active drug and the degradation products generated during stability studies. Assay of a drug product in a stability test sample must be performed with stability-indicating method, as recommended by the International Conference on Harmonization (ICH).[6] Literature survey revealed that a few analytical methods have been reported for the determination of ERP in pure drug, pharmaceutical dosage forms and in biological samples using UV visible spectroscopy[7]and liquid chromatography.[8] Several methods of liquid chromatography coupled to either UV absorbance or mass spectrometry detection have been developed for the quantification of ertapenem in human plasma.[9-13]A LC-MS method was developed for the quantification of ERP in human plasma by detecting the deprotonated molecular ion of ERP under negative ionization mode with lower limit of quantification of 0.5 or 1 µg/ml and analytical run time of more than 5 min.[14,15] LC-MS/MS methods have also been developed for the quantification of ertapenem on dried blood spot,[16] microdialysate[17] and human serum.[18] None of the reported procedures enables analysis of the ERP alone in pharmaceutical dosage forms in the presence of their degradation products. This manuscript describes the development and validation, in accordance with ICH guidelines[19] of a rapid, economical, precise and accurate stability-indicating isocratic reversed phase HPLC method for analysis of ERP in the presence of its degradation products. This paper mainly deals with the forced degradation of ertapenem sodium under the stress conditions such as acidic & basic hydrolysis, oxidation, heat, light and validation of the method for accurate quantification of ertapenem in the bulk drug & pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and reagents. Ertapenem sodium bulk drug (purity 98.9%) was purchased from Merck Ltd. Mumbai, India and injection Ertazen (1 gm) was purchased from the local market. Acetonitrile (HPLC grade), othophosphoric acid, sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were obtained from Merck Fine Chemicals Mumbai, India. Double HPLC grade water was used throughout the experiment. Other chemicals used were of analytical or HPLC grade. Standard stock solution (1 mg/ml) of ERP was prepared by dissolving the working standard in HPLC grade water and diluting with the same solvent. Standard calibration solutions (2-10 µg/ml) for assessment of linearity were prepared from this stock solution by dilution with diluent.

Chromatography. A high performance liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and Photodiode array detector (YL 9160 detector) connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used. The chromatographic analysis was performed by using a mobile phase of acetonitrile: water (60:40 v/v), pH adjusted 2.9 by othophosphoric acid. These were filtered through 0.45 µ membrane filter and degassed by sonication before use. The mobile phase was pumped isocratically at a flow rate of 1.2 ml/min during analysis at ambient temperature. The run time was set at 10 min and the volume of injection was 20 µl and eluent was detected at 298 nm on a Hypersil BDS C_{18} column (4.6 x 250 mm, 5 µ particle size).

Analysis of dosage form. A volume of the injection equivalent to 100 mg of ERP were weighed into a 100 ml volumetric flask, 50 ml of diluents was added and shaken thoroughly for about 10 minutes, then the volume was made up to the mark with the diluents, mixed well and filtered. Further dilutions were made and the assay of injections was completed according to general procedure.

Forced degradation study. To study the effect of acid, accurately weighed 10 mg ERPWS was dissolved in 9 ml water (HPLC) and volume was made up to 10 ml with 1N HCl to get a concentration of 1000 µg/ml (i.e conc. of HCl in solution was 0.1 N) and kept on water bath at 80°C for 60 min. Aliquots of above solution was neutralized with 1N NaOH and diluted with diluents to get 6 µg/ml solution. The sample solution was analyzed and chromatogram was recorded. To study the effect of alkali, accurately weighed 10 mg of ERPWS was dissolved in 9 ml water (HPLC) and volume made up to 10 ml with 1N sodium hydroxide to achieve the solution of 1000 µg/ml (i.e. conc. of NaOH in solution was 0.1N). The above mixture was kept on a boiling water bath at 80°C for 60 min. Aliquots of above solution was neutralized with 1N HCl and diluted with diluents to get 6 µg/ml solution. The sample solution was analyzed and chromatogram was recorded. To study the effect of oxidizing conditions, accurately weighed about 10 mg ERPWS was dissolved in 9 ml water (HPLC) and volume was made up to 10 ml with 30.0 % hydrogen peroxide to achieve a solution of 1000 µg/ml (i.e. conc. of hydrogen peroxide in solution was 3%) and kept on water bath at 80°C for 60 min. Aliquots of above solution was neutralized with 1N HCl and diluted with diluents to get 6 µg/ml solution. The sample solution was analyzed and chromatogram was recorded. To study the effect of temperature, accurately weighed about 1.0 g ERP WS was kept at 80°C in oven for 3 days. Sample equivalent to 10 mg of drug was withdrawn after every 24 hour and diluted as per the procedure. Aliquots of 6 µg/ml concentration were prepared and chromatogram was recorded. To study the effect of UV light, accurately weighed about 1.0 g ERP WS was exposed to short and long wavelength UV light (222 and 366 nm, respectively) for 48 hrs, Sample equivalent to 10 mg of drug was withdrawn after every 24 hrs and diluted as per the procedure. Aliquots of 6 µg/ml concentration were prepared and chromatogram was recorded.

METHOD VALIDATION

The method was validated for linearity, specificity, limits of detection (LOD) and quantification (LOQ), system suitability, accuracy, precision, robustness and stability in accordance with ICH guidelines. To assess specificity, peak purity was determined by use of the photodiode-array detector. To check linearity, test solutions of ERP were prepared at six concentrations 2-10 µg/ml. Each solution was injected in triplicate and calibration graphs were obtained by plotting peak area against concentration. Linearity was checked over the same concentration range on three consecutive days. RSD (%) of the slope and Y-intercept of the calibration plot were also calculated. The limits of detection (LOD) and quantification (LOQ) for ertapenem were determined, as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. Precision, as RSD (%) was determined by measuring the concentration of drug in the injection six times. Intermediate (inter-day) precision was evaluated by two analysts on different days in the same laboratory. The accuracy of the method was studied by measurement of recovery after adding known amounts of the drug (80, 100 and 120% of the label claim of 1gm ertapenem sodium per injection) to the placebo. Three samples were prepared for each recovery level and results were calculated by use of the calibration plot. The robustness of the method was assessed by deliberate alteration of the experimental conditions and determining the effect on resolution of ERP from the main product obtained by degradation under basic conditions. The change was made in the ratio of mobile phase, instead of acetonitrile: water (60:40v/v) pH adjusted 2.9 by othophosphoric acid, acetonitrile: 1 water (65:35 v/v) pH adjusted 2.9 by othophosphoric acid was used as a mobile phase and flow rate, instead of 1.2 ml/min, 0.8 ml/min and 1.0 ml/min. During these tests all other conditions were held constant at the optimum values. The stability of ERP and sample solutions (at ambient temperature) were tested by analysis after 24, 48 and 72 hrs, comparison of the results with

those obtained from freshly prepared standard solutions and calculation of RSD.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions. The primary objective in developing this stability indicating HPLC method were to achieve resolution between ERP and its degradation products. To achieve this, Young Lin 9100 with YL 9160 detector and C18 column was employed for envisaged work. Combination of acetonitrile: water (60:40 v/v) pH adjusted 2.9 by othophosphoric acid as mobile phase was attempted for quantitation of ERP with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates and HETP) at 298 nm as detection wavelength. Linearity was found 0-10 µg/ml with correlation coefficient r^2 = 0.9997 having equation as: AUC = 28605Conc.+14629. The column temperature was 25°C. The tailing factor for ertapenem was <2 and retention times were approximately 6.67± 0.5 min for main peak and less than 10 min for the degradation products (Figure 2). This low total runs time resulted in high productivity and low cost of analysis as per sample.

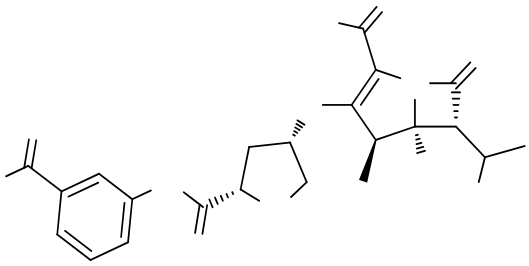


Figure 1. Molecular structure of ertapenem sodium

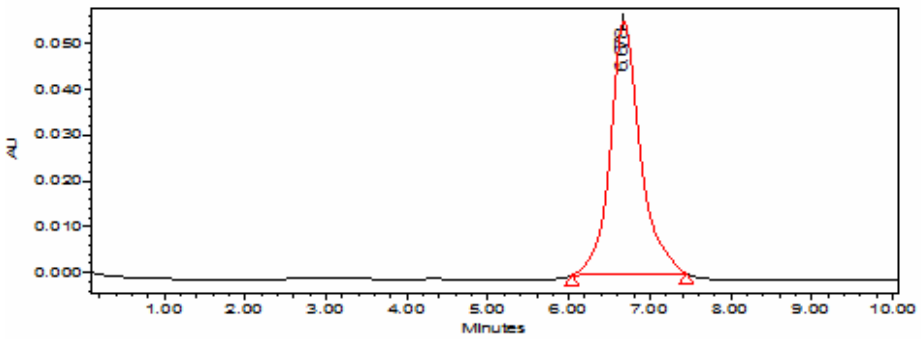
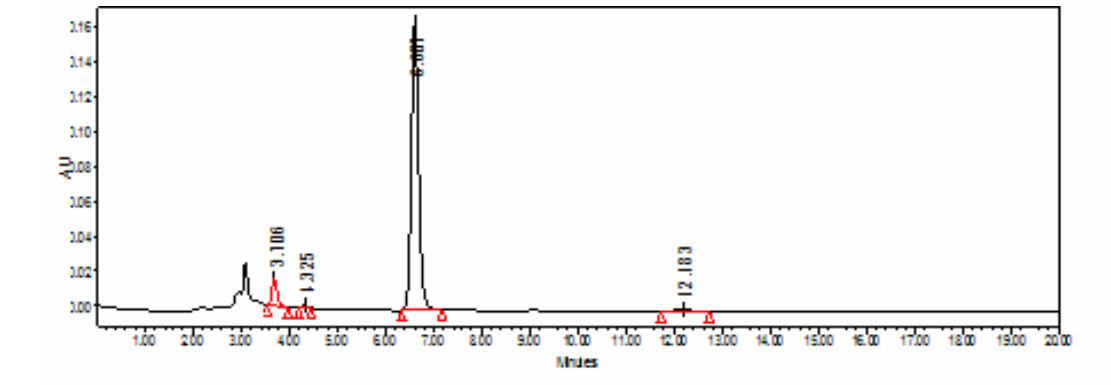
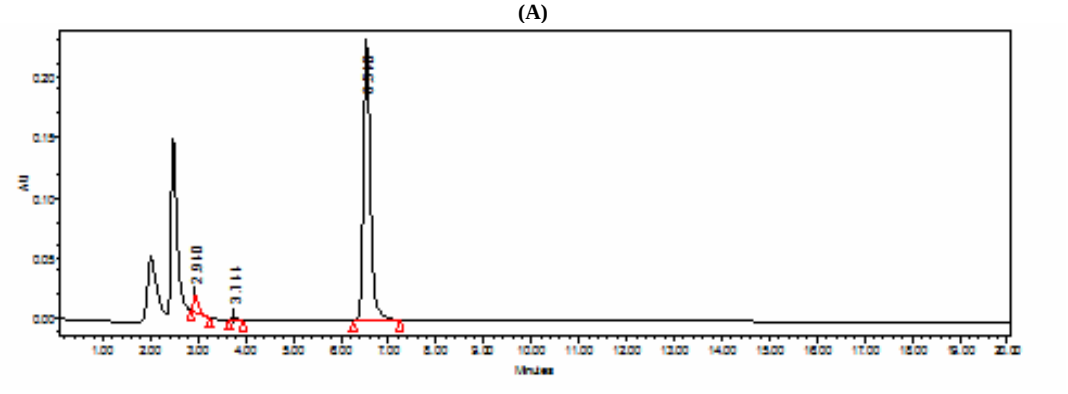


Figure 2. Standard chromatogram of ertapenem sodium

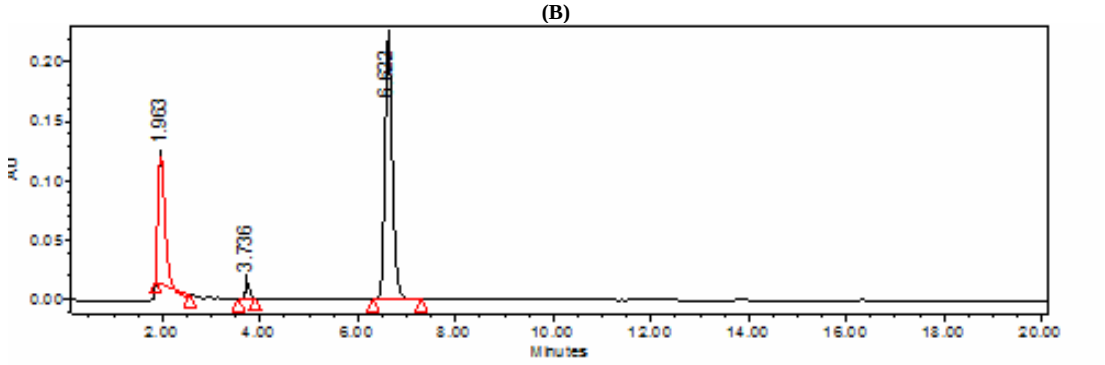
Forced degradation study. Bakshi *et al.*[20] suggested target degradation of 20-80% when establishing the stability-indicating properties of analytical methods, because even intermediate degradation products should not interfere with any stage of drug analysis. Although conditions used for forced degradation were adjusted to achieve degradation in this range, this could not be achieved for conditions other than exposure to acid, base and oxidising agent, even after long exposure. Peak purity test results confirmed that the ertapenem peak was homogeneous under all the stress conditions tested. The mass balance of ERP in stress samples was close to 100% and, moreover, assay of unaffected ERP in the injection confirmed the stability-indicating nature of the method. The results from forced degradation studies are summarised in table 1. Chromatographic peak-purity data were obtained from the spectral analysis report; peak purity greater than 99 is indicative of a homogeneous peak. The peak purity



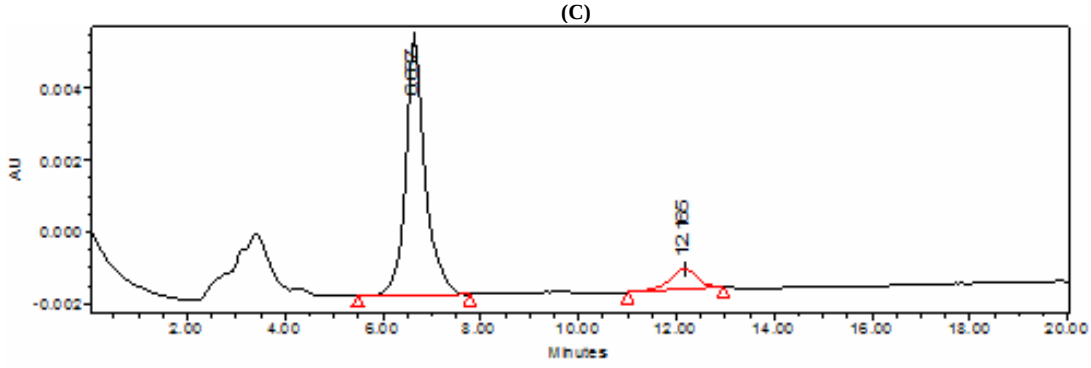
(A)



(B)



(C)



(D)

Figure 3. Typical chromatogram obtained after degradation of ertapenem sodium under (A) acidic conditions, (B) basic conditions, (C) oxidising conditions, (D) photolytic condition

for ERP from degradation studies was in the range 99.9-100.0, indicating homogeneous peaks and thus establishing the specificity of the method. Chromatograms from the solutions obtained after degradation under acidic, basic, oxidising, photolytic conditions are shown in Figure 3, respectively. No peaks co-eluted with the ERP peak, suggesting the method enabled specific analysis of ertapenemin the presence of its degradation products.

Table 1. Results from analysis of samples from the forced degradation study, showing percentage degradation and peak purity of ertapenem sodium.

Stress condition and duration	% degradation	Peak purity*
Acid degradation 0.1N HCl –80°C /60 min	4.8	99.928
Base degradation 0.1N NaOH – 80°C /60 min	88.45	99.983
oxidizing degradation (3% H_2O_2)- 60 min	21.2	99.912
Thermal degradation- 80°C /48 hrs	6.9	99.971
UV light/222nm/48 h	10.3	99.931
UV light/366nm/48 h	9.3	99.910

*Peak purity values in the range of 99-100 indicate a homogeneouspeak

Table 2. Results from regression analysis and system suitability data.

Parameters	Ertapenem
Retention time*	6.67 ± 0.5 min
Tailing factor*	1.13
Theoretical plate*	1406733
Linear range (µg/ml)	2-10
limits of detection (µg/ml)	0.05
limits of quantification (µg/ml)	0.21
Linear equation	28605 conc.+14629
Slope	28605
Intercept	14629
Correlation coefficient	0.9997
S.D. of slope	836.4
% RSD of slope	0.084
S.D. of intercept	13266
% RSD of intercept	0.212

*Mean of six readings

Table 3. Result of precision of test method of ertapenem sodium.

Std. conc. (µg/ml)	Repeatability	Intermediate precision	
		Day to day	Analyst to analyst
2	100.2	99.80	99.26
4	102.7	103.9	100.19
6	99.60	102.3	99.80
8	101.00	100.5	99.02
10	100.19	100.9	100.12
Mean	100.738	101.48	99.678
S.D.	1.205	1.632	0.519
% R.S.D.	1.196	1.608	0.520

*Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 4. Recovery of ertapenem sodium.

Level of addition	Std. drug sol. added (µg/ml)	% mean* recovered
80	2	103.7
100	4	101.1
120	6	99.97

*Average of five determination

Table 5. Results from robustness testing.

Parameter	Percentage (Mean ± S.D*.)	Percentage RSD*
Mobile phase	97.24 ±0.17	0.179
Flow rate	98.40 ±0.09	0.091

*Mean obtained at three concentrations and three replicate

Method validation. Peak purity was >99.9% for drug substance and drug degradation products at 298 nm, which showed that the analyte peaks were pure and that formulation excipients and degradation products were not interfering with analyte peaks. LOD and LOQ for ERP were 0.05 and 0.21 µg/ml, respectively, for 20 µl injection volume. Results from regression analysis are listed in table 2, with system suitability data. When precision was determined by six fold analysis of drug injection, the RSD of ERP peak area was less than 2%, indicating that the method is reliable. Results from assessment of precision are listed in table 3. Results obtained from determination of recovery are listed in table 4 and results from robustness testing are shown in table 5.

CONCLUSION

The method developed for quantitative analysis of ertapenem sodium is rapid, precise, accurate and selective. Peak purity studies under all the stress conditions showed the drug peak to be pure and hence the method is stability indicating. In other words it can be mentioned that the method developed can be utilized for the successful quantification of the drug in presence of its degradation product and excipients. The method was completely validated and satisfactory results were obtained for all the characteristics tested. The method is stability-indicating and can be used to assess the stability of ertapenem sodium in the bulk drug. The method can be conveniently used for routine analysis of ertapenem sodium as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Conflict of interest

The authors declare no conflict of interest.

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