Selective Determination of Ertapenem and Imipenem in the Presence of Their Degradants

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Abstract

Stability-indicative determination of ertapenem (E_{RTM}) and imipenem (I_{MPM}) in the presence of their corresponding open-ring degradation products, the metabolites, is investigated. The degradation products have been isolated via acid-degradation, characterized, and confirmed. Selective quantification of E_{RTM} or I_{MPM} singly in bulk form, pharmaceutical formulations, and/or in the presence of their corresponding degradants is demonstrated. The indication of stability has been undertaken under conditions likely to be expected at normal storage conditions. Among the chromatographic techniques adopted for quantification are coupled thin layer chromatography-densitometry and high-performance liquid chromatography.

Introduction

Carbapenems are a family of antibiotics having the 1-azabicyclo[3.2.0]hept-2-ene system. They have been isolated in the search for inhibitors of bacterial cell wall synthesis and β-lactamases (1). The stability of two model carbapenem drugs, namely ertapenem and imipenem, were investigated. Ertapenem sodium (E_{RTM}) is [4R,5S,6S]-3-[[(3S,5S)-5-[[(3-carboxyphenyl)-amino]carbonyl]-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylic acid monosodium salt (2). Imipenem monohydrate (I_{MPM}) is 1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid,6-(1-hydroxy-ethyl)-3-[[2-[(iminomethyl)amino]ethyl] thio]-7-oxo-, mono hydrate, [5R-[5α,6α(R*)]]-(3). Figure 1 shows the chemical structures of E_{RTM} and I_{MPM}. They are structurally related to β-lactam antibiotics, such as penicillins and cephalosporins, and show activity against a wide range of bacteria (4,5), which exceeds that of several cephalosporins and compares favorably to other carbapenems (6,7).

Carbapenems contain a highly strained ring system that makes them unstable in water at high or low pH values. Hydrolysis of this highly strained ring system leads to the formation of the open-ring degradant (8–10).

Among the several analytical methods for quantitative estimation of E_{RTM} in body fluids and in pharmaceutical formulations is high-performance liquid chromatography (HPLC) (11–17). Preparative HPLC recovery of E_{RTM} from mother liquor stream (18) or from crystallization process stream was achieved as well (19). A chromatographic method for the determination of dimeric degradation products of E_{RTM} was proposed (20). The stability of the drug was studied under various conditions (21–23).

I_{MPM} was determined in different body fluids by many HPLC methods (24–37), and its stability was investigated in aqueous solutions (38) and in infusion systems (39).

Literature survey does not reveal any thin layer chromatography (TLC)-densitometric method for analysis of E_{RTM} or I_{MPM}, either singly in bulk forms or in their pharmaceutical formulations.

In modern analytical laboratories, there is always a need for significant stability-indicating methods in the drug analysis. The present work aimed to develop simple and sensitive chromatographic methods for the selective quantification of E_{RTM} and I_{MPM} in pure forms or even in their pharmaceutical formulations. The methods described here include coupled TLC-densitometry and HPLC.

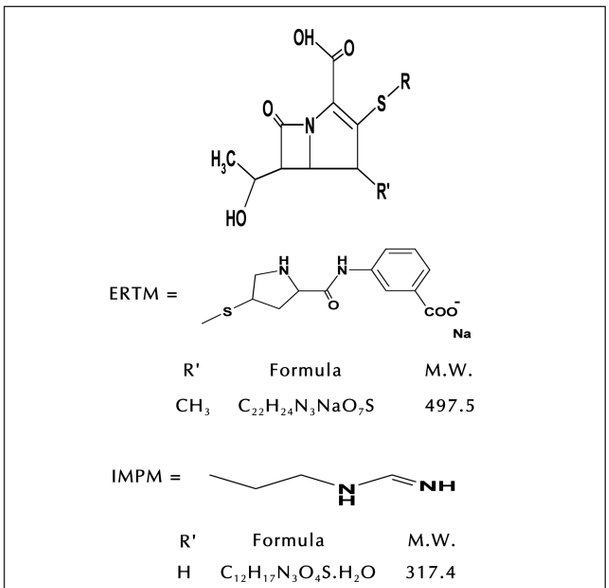

Figure 1. Structural formulae of ertapenem sodium and imipenem monohydrate.

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Experimental

Instruments

The spectrophotometer used was a Shimadzu UV-1601 PC dual-beam UV-visible spectrophotometer (Kyoto, Japan) with matched 1-cm quartz cells connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version 3.7 was used to process the absorption. The spectral bandwidth was 2 nm with wavelength-scanning speed of 2800 nm/min.

The IR spectrophotometer was a Mattson Genesis II FTIR; sampling was undertaken as potassium bromide discs. The gas chromatograph–mass spectrophotometer (GC–MS) was a Hewlett Packard 5988A GC–MS system from Agilent (Santa Clara, CA). The pH meter Digital pH/MV/TEMP/ATC meter, Jenco Model-5005, and Graffin melting point apparatus model SMP1 were obtained from Stuarts Scientific (Staffordshire, England).

Precoated TLC-plates consisted of silica gel 60 F_{254} (20 cm × 20 cm, 0.25 mm) and came from Merck (Darmstadt, Germany).

A Camag TLC scanner 3 S/N 130319 with winCATS software and Camag Linomat 5 autosampler with 100-µL microsyringe were used (Muttenz, Switzerland).

A liquid chromatograph consisted of a dual pneumatic pumping system (Agilent model G1310A), a UV variable wavelength detector (model G1314A, Agilent 1100 Series), and a Rheodyne injector (model 7725 I) equipped with a 20-µL injector loop (Agilent). Stationary phase consisted of a Lichrosorb C_{18} analytical column (250 mm × 4.6 mm i.d., 10 µm) (Alltech, Lexington, KY).

Mobile phase for E_{RTM} was 0.05 M ammonium acetate–acetonitrile–methanol–triethylamine (80:10:10:0.1, v/v/v/v). The final pH value was adjusted to 6.5 ± 0.1 by using *o*-phosphoric acid isocratically at 1 mL/min.

Mobile phase for I_{MPM} was 0.001 M MOPS buffer (pH 7 ± 0.1 by NaOH)–acetonitrile–methanol (80:10:10, v/v/v) isocratically at 1 mL/min.

The mobile phases were filtered through a 0.45-µm Millipore membrane filter (Billerica, MA) and degassed for ~ 15 min in an ultrasonic bath prior to use. UV detection was done at 297 nm for E_{RTM} and at 299 nm for I_{MPM}. The samples were filtered also through a 0.45-µm membrane filter and were injected by the aid of a 25-µL Hamilton analytical syringe.

Materials and Reagents

All chemicals and reagents were analytical-grade, and water was always bi-distilled.

Materials

Reference E_{RTM} standard (sodium salt) was kindly supplied by Merck (Whitehouse Station, NJ). Its potency was found to be 99.85 ± 0.56% (n = 6), according to a reference HPLC method (21).

I_{MPM} pure sample was kindly donated by Merck. Its purity was found to be 100.35 ± 0.39 (n = 6), according to the official HPLC method (3).

Cilastatin sodium (CIL) pure sample was kindly donated by Merck. Its purity was found to be 100.66 ± 0.75 (n = 6), according to the official potentiometric titration method (3).

Authentic cefepime hydrochloride was received from Bristol-Myers-Squibb Pharmaceutical (Cairo, Egypt). Its purity was found to be 100.55 ± 0.54 (n = 6), according to the official HPLC method (3).

Pharmaceutical formulations

To Invanz (1-g vials, BN: NE20790), labeled to contain 1 g ertapenem (equivalent to 1.046 g ertapenem sodium), 175 mg of sodium bicarbonate and sodium hydroxide was added to adjust pH to 7.5. The vials were purchased from a Dubai local market.

To Tienam (500-mg vials, BN: HV 04900), labeled to contain 500 mg imipenem (anhydrous) and cilastatin sodium (equivalent to 500 mg cilastatin) was added to 86.4 mg of sodium bicarbonate to adjust pH to 7.5. Vials were kindly donated by Merck Sharp & Dohme (Cairo, Egypt).

Standard solutions

E_{RTM} standard solution, E_{RTM} degradant standard solution, I_{MPM} standard solution, and I_{MPM} degradant standard solution (each 1 mg/mL) were added in distilled water for the TLC-densitometric method

E_{RTM} standard solution, E_{RTM} degradant standard solution, I_{MPM} standard solution, and I_{MPM} degradant standard solution (each 0.5 mg/mL) were added to the corresponding mobile phase for the HPLC method.

All calculations and samples preparation for reference materials and pharmaceutical formulations were done on the basis of the free drug. Solutions were always freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 h.

Reagents

Acetone, anhydrous sodium carbonate, hydrochloric acid, *n*-butanol, and triethylamine were purchased from El-Nasr Pharmaceuticals (Cairo, Egypt). HPLC-grade HiPerSolv acetonitrile and methanol was purchased from Merck. *o*-Phosphoric acid (85%) came from BDH Laboratory Suppliers (Poole, England). Bi-distilled de-ionized water from "Aquatron" Automatic Water Still A4000 was provided by Bibby Sterillin (Staffordshire, England).

Procedures

Degradation of carbapenem drugs

Accelerated acid degradation was performed by dissolving 25 mg of pure E_{RTM} and I_{MPM} powder separately in ~ 25 mL of 0.1 N hydrochloric acid. The solutions were set aside at room temperature for 2 h, in which time complete degradation was achieved by TLC using *n*-butanol–acetone–water (4:3:3, v/v/v) as a developer solvent. The acid degraded samples were neutralized by adding sodium carbonate powder (pH 7). The solutions were evaporated under vacuum nearly to dryness then re-crystallized from methanol. The obtained degradants were characterized by UV spectroscopy and melting range, and structurally elucidated by GC–MS and IR spectrometry.

TLC-densitometric method

Linearity was measured in the following way: Aliquots of 2–14 µL of E_{RTM} standard solution and 2–12 µL of I_{MPM} (each 1 mg/mL) were applied separately in the form of bands on a TLC plate. The

band length was 4 mm, and dosage speed was 150 nL/s. The bands were applied 11.4 mm apart from each other in case of E_{RTM}, while it was 14 mm apart from each other for I_{MPM} and 10 mm from the bottom edge of the plate for both drugs. Linear ascending development was performed in a chromatographic tank previously saturated with *n*-butanol–acetone–water (4:3:3, v/v/v) for 1 h at room temperature. The developed plates were air-dried and scanned at 297 nm for E_{RTM} and at 299 nm for I_{MPM} using a deuterium lamp with absorbance mode at 3 mm × 0.45 mm slit dimension and a scanning speed of 20 mm/s. Calibration curves relating the optical density of each spot of E_{RTM} and I_{MPM} to the corresponding concentration were constructed. The regression equations were then computed for the studied drugs and used for determination of unknown samples containing them.

Liquid chromatographic method

For linearity, portions of 0.1–2 mL from E_{RTM} standard solution or I_{MPM} standard solution (each 0.5 mg/mL in the mobile phase) were transferred separately into a series of 10-mL measuring flasks and mixed with 0.2 mL (for E_{RTM}) or 0.4 mL (for I_{MPM}) of cefepime-stock solutions (1 mg/mL) as an internal standard (IS). The contents of each flask were completed to volume with the mobile phase to get the concentrations of 5–100 µg/mL of E_{RTM} and I_{MPM}.

The samples were then chromatographed using the following chromatographic conditions: Stationary phase was conducted on a Lichrosorb C_{18} analytical column (250 mm × 4.6 mm i.d., 10 µm); mobile phase for E_{RTM} was 0.05 M ammonium acetate–acetonitrile–methanol–triethylamine (80:10:10:0.1, v/v/v/v). The final pH value was adjusted to 6.5 ± 0.1 with *o*-phosphoric acid using a pH meter. The mobile phase used for I_{MPM} was formed of 0.001 M MOPS buffer (pH 7 ± 0.1 by NaOH)–acetonitrile–methanol (80:10:10, v/v/v). The mobile phases were filtered through a 0.45-µm Millipore membrane filter and were degassed for about 15 min in an ultrasonic bath prior to use. Flow rate was 1 mL/min isocratically at ambient temperature (~ 25ºC) with UV detection at 297 nm for E_{RTM} and at 299 nm for I_{MPM}. The samples were filtered also through a 0.45-µm membrane filter and were injected by the aid of a 25-µL Hamilton analytical syringe. To reach good equilibrium, the analysis was usually performed after passing ~ 50–60 mL of the mobile phase just for conditioning and pre-washing of the stationary phase.

The relative peak-area ratios were then plotted versus the corresponding concentrations of E_{RTM} or I_{MPM} to get the calibration graphs and to compute the corresponding regression equations. Concentrations of unknown samples of both drugs were determined using the obtained regression equations.

Analysis of laboratory-prepared mixtures containing different ratios of ERTM or IMPM and their corresponding degradation products using the suggested methods

Aliquots of intact drugs and the degraded drugs were mixed to prepare different mixtures containing 10–90% (w/w) of the degradation product for each drug, and the procedures mentioned under each method were followed. The concentrations of each one were then calculated from the corresponding regression equations.

Assay of pharmaceutical formulations

The contents of five vials of each E_{RTM} (Invanz) and I_{MPM} (Tienam) were mixed separately, and the average weight of a vial was determined for each case. No sample preparation for vials was required other than dissolving the contents of the vial powder in the appropriate solvent for each method. Vials were dissolved in distilled water to get a drug concentration of 1 mg/mL for the TLC-densitometric method. They were dissolved in the corresponding mobile phase to get a drug concentration of 0.5 mg/mL for the HPLC method. Experimentation proceeded as mentioned under each method.

Kinetic calculations

The stability of E_{RTM} and I_{MPM} in different solutions, namely water, 0.1 N HCl, 0.9% (w/v) saline, and 5% (w/v) dextrose solution, was studied. The degradation rate kinetics was determined for each case by plotting the log of concentration of drug remaining versus time. Each experiment was done in triplicate (analysis by HPLC method), and average values were taken for the analysis.

Results

Degradation of carbapenems

Accelerated acid degradation using 0.1 N HCl was employed for the preparation of both carbapenems'degradants. The melting range (m.r.) of E_{RTM} degradant was found to be 205–208°C (E_{RTM} intact m.r.: 192–195°C), while that for IMPM was found to be 215–219°C (I_{MPM} intact m.r.: 180–185°C). In the GC–MS chart, the parent peak for each degradant spectrum was identified at *m/z* 515 (mol. wt. of E_{RTM} degradant) and that of I_{MPM} at *m/z* 317. This proves that the prepared degradant is the open β-lactam ring metabolite. No other degradation products could be observed under all the different degradation conditions.

TLC monitoring of the degradation of both drugs was done on thin layer plates of silica gel F_{254} using *n*-butanol–acetone–water (4:3:3, v/v/v) as a developing solvent. The developed plates were visualized under short UV lamp and/or by subjecting them to iodine vapors. The open-ring degradant of E_{RTM} (R_f value = 0.39) could be separated elegantly from the intact drug (R_f value = 0.58), while for I_{MPM} the degradant with (R_f value = 0.25) could be separated elegantly from the intact drug (R_f value = 0.44).

TLC-densitometry

A TLC-densitometric method is described for the determination of both E_{RTM} and I_{MPM} in the presence of their corresponding degradants without prior separation. Satisfactory results were obtained by using a mobile phase composed of *n*-butanol–acetone–water (4:3:3, v/v/v), where R_f = 0.58 and 0.39 for E_{RTM} and its degradant, respectively, and R_f = 0.44 and 0.25 for I_{MPM} and its degradant, respectively. The separation allows the determination of each carbapenem drug with no interference from its degradant. The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 297 nm over a range of 2–14 µg/spot for ERTM and measuring at 299 nm over a range of 2–12 µg/spot for IMPM, where a linear

response was obtained. Scanning profile of different concentrations of E_{RTM} at 297 nm is shown in Figure 2. Similar results were obtained for I_{MPM} by scanning at 299 nm. The regression equations were found to be:

E_{RTM}: A = 0.1834C + 0.1529, r = 0.9993;

I_{MPM}: A = 0.3038C + 0.0653, r = 0.9990;

where A = the integrated area under the peak × 10^{-4} for E_{RTM} or I_{MPM}, C = the concentration of the carbapenem drug in µg/spot, and r = the correlation coefficient.

The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be 99.80 ± 0.65 and 99.98 ± 0.68 for E_{RTM} and I_{MPM}, respectively.

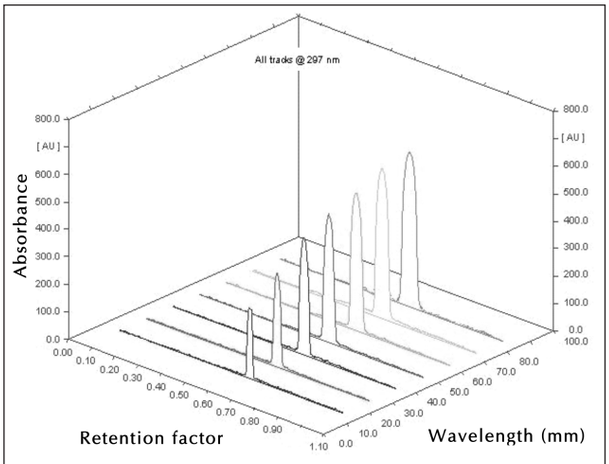

Figure 2. Scanning profile of different concentrations (2–14 µg/mL) of ERTM by the TLC-densitometric method.

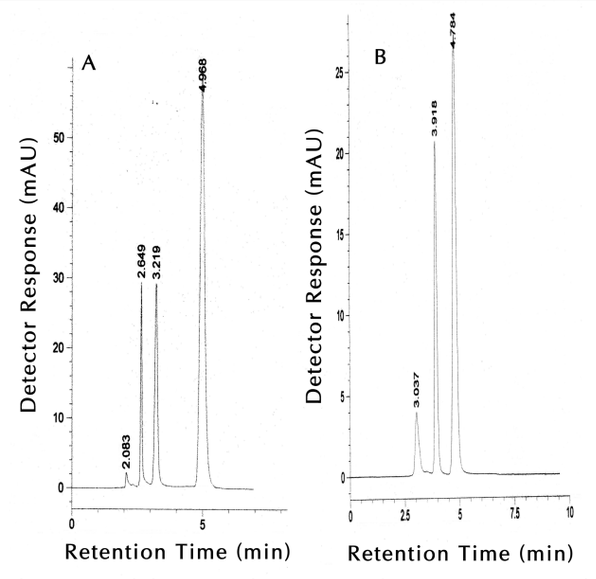

Figure 3. Liquid chromatographic separation of (A) ERTM (3.92 min) and (B) IMPM (3.22 min) from their degradants and cefepime (IS). See Experimental conditions.

High-performance liquid chromatography

Figure 3A shows the average retention times under the conditions described for E_{RTM}. The open-ring degradant elutes after 3.04 min, while intact E_{RTM} elutes after 3.92 min and finally cefepime as IS elutes after 4.78 min. One sample can be chromatographed in 6 min. The average retention times under the conditions described for I_{MPM} are 2.08 min for the open-ring degradant, 2.65 min for cilastatin (a component in the dosage form), 3.22 min for intact I_{MPM}, and 4.97 min for cefepime as IS. One sample can be chromatographed in 6 min (Figure 2B).

Calibration graphs were obtained by plotting the peak-area ratios (drug/IS) against concentrations of E_{RTM} or I_{MPM} (µg/mL). Linearity range was found to be 5–100 µg/mL for both drugs using the following regression equations:

ERTM: A = 0.0754C + 0.0439, r = 0.9999.

IMPM: A = 0.0636C + 0.0174, r = 0.9998.

where A = the relative peak-area ratio, C = the concentration of ERTM or IMPM (µg/mL) and r = the correlation coefficient.

The mean percentage recoveries of pure samples were found to be 100.05 ± 0.31 and 99.96 ± 0.40 for E_{RTM} and I_{MPM}, respectively.

Stability indication

To assess the stability-indicating efficiency of the proposed methods, each degradant of E_{RTM} and I_{MPM} was mixed separately with its intact sample in different ratios and analyzed by the proposed methods. Table I illustrates good selectivity in the determination of both E_{RTM} and I_{MPM} in the presence of up to 90% (w/w) of their corresponding degradants by the densitometric and HPLC methods.

The suggested methods were successfully applied for the determination of E_{RTM} and I_{MPM} in their pharmaceutical formulations, showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique (Table II).

The precision of the suggested methods was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis results (Table III).

Results of the suggested methods for determination of both E_{RTM} and I_{MPM} were statistically compared with those obtained by applying the reference method for E_{RTM} (21) and official HPLC method for I_{MPM} (3). The calculated t- and F-values (40) were found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision (Table IV).

Table I. Determination of ERTM and IMPM in Laboratory-Prepared Mixtures with Their Degradants by the Proposed Methods

Method*	TLC-densitometric method	HPLC method
E_{RTM} (Mean ± SD)	99.99 ± 0.68	100.18 ± 0.54
I_{MPM} (Mean ± SD)	100.18 ± 0.66	100.27 ± 0.54

* In the presence of up to 90% (w/w) degradant content.

The kinetic studies

Estimation of the kinetic order of the acid-degradation of E_{RTM} and I_{MPM} could be done by calculating the percentage of the remaining drug concentration and its logarithmic value in each case at different time intervals during the hydrolysis process. Assay of E_{RTM} and I_{MPM} was carried out by adopting the developed HPLC method. The degradation process of studied carbapenems follows pseudo-first order kinetics during degradation as indicated by the straight-line relationship between the log of the %remaining drug concentration versus time. Lower rate of degradation was observed for the dosage formulation (containing sodium carbonate additive) in water, 0.9% (w/v) saline solution, and in 5% (w/v) dextrose solution. The prepared solutions for E_{RTM} or I_{MPM} were stable in water or saline solutions for more than 4 h at room temperature and in dextrose solution for only 3 h at room temperature.

Table II. Determination of ERTM and IMPM in Their Pharmaceutical Formulations by the Proposed Methods

Preparation	TLC-densitometric method	HPLC method
ERTM: Invanz vials (1 g)* (Mean ± SD)	100.32 ± 0.56	100.22 ± 0.56
IMPM: Tienam vials (500 mg)† (Mean ± SD)	100.28 ± 0.69	100.34 ± 0.50

* BN: NE20790. † BN: HV04900.

Table III. Assay Parameters and Validation Sheet for Determination of ERTM and IMPM by the Proposed Methods

	ERTM		IMPM	
Parameter	TLC method	HPLC method	TLC method	HPLC method
Range*	2–14	5–100	2–12	5–100
Slope	0.1834	0.0754	0.3038	0.0636
Intercept	0.1529	0.0439	0.0653	0.0174
Mean	99.80	100.05	99.98	99.96
SD	0.653	0.314	0.676	0.401
Variance	0.426	0.099	0.457	0.161
CV†	0.654	0.314	0.676	0.401
r†	0.9993	0.9999	0.9990	0.9998
RSD (%)‡	0.641–0.825	0.544–0.557	0.532–0.615	0.743–0.625
RSD (%)‡	0.557–0.743	0.461–0.488	0.435–0.521	0.615–0.622

* The range in µg/spot for the TLC method and in µg/mL for the HPLC method
† CV = coefficient of variation and *r* = correlation coefficient
‡ The inter-day (n = 6) and the intra-day (n = 5) relative standard deviations of (5–10 µg/spot for the TLC method and 10–50 µg/mL for the HPLC method) for both drugs.

Table IV. Statistical Comparison for Results Obtained by the Proposed Method for ERTM and the Official Method for IMPM

	ERTM			IMPM		
Parameter	TLC method	HPLC method	Reference method (21)	TLC method	HPLC method	Official method (3)
Mean	99.80	100.05	99.85	99.98	99.96	100.35
SD	0.653	0.314	0.556	0.676	0.401	0.393
Variance	0.426	0.099	0.309	0.457	0.161	0.154
n	7	11	6	6	11	6
F-test	1.38	3.12		1.95	1.05	
	(4.95)*	(3.33)*		(5.05)*	(4.74)*	
Student's t-test	0.149	0.813		1.159	1.941	
	(2.201)*	(2.131)*		(2.228)*	(2.131)*	

* The values in the parentheses are the corresponding theoretical t- and F-values at P = 0.05 (42).

Discussion

Degradation of carbapenems

The main degradation product of a carbapenem drug is the open β-lactam ring structure (8–10). Both E_{RTM} and I_{MPM} are recommended to be used freshly by intravenous route, so upon degradation, the open β-lactam ring degradant will be formed. Degradation was examined under both acidic and alkaline media at ambient and elevated temperatures for both drugs. It has been confirmed that the main degradant is the open β-lactam ring product, which is also the major metabolite of carbapenems inside the human body. The double bond in the bicyclic structure of the carbapenem nucleus creates a considerable ring strain and increases the reactivity of the β-lactam ring (41). Literature reveals that acid hydrolysis of I_{MPM} followed by neutralization is a convenient method to prepare its metabolite (Scheme 1) (Figure 4) (7). The same principle was used to prepare E_{RTM} degradant using 0.1 N HCl. The higher m.r. of each degradant may be attributed to its increased polarity, leading to increased inter-molecular attraction forces and rise in m.r. In the GC–MS chart, the parent peak for each degradant was identified. This proves that the prepared degradant is the open β-lactam ring metabolite. No other degradation products could be observed under all the different degradation conditions.

TLC monitoring of the degradation of both drugs was done on TLC plates of silica gel F_{254} using *n*-butanol–acetone–water (4:3:3, v/v/v) as a developing solvent. The same method was used for monitoring the degradation of meropenem, a carbapenem drug, by authors (42).

TLC-densitometry

A TLC-densitometric method is described for the determination of both E_{RTM} and I_{MPM} in the presence of their corresponding degradants without prior separation. Different solvent systems were tried for the separation of both drugs and their degradants. Satisfactory results were obtained by using the described mobile phase. The separation allows the determination of each carbapenem drug with no interference from its degradant. The same mobile phase composition was used before by authors for determination of meropenem (42).

High-performance liquid chromatography

A simple isocratic high-performance liquid chromatographic method was developed for the determination of each of E_{RTM} and I_{MPM} in pure

form and in pharmaceutical preparation using a Lichrosorb C_{18} analytical column (250 mm × 4.6 mm, i.d., 10 µm). The mobile phase, in the case of E_{RTM}, consisted of 0.05 M ammonium acetate–acetonitrile–methanol–triethylamine (80:10:10:0.1, v/v/v/v), and the pH was adjusted to 6.5 ± 0.1 using *o*-phosphoric acid. In the case of I_{MPM}, the mobile phase consisted of 0.001 M MOPS buffer (pH 7± 0.1 by NaOH)–acetonitrile–methanol (80:10:10, v/v/v). The mobile phases were chosen after several trials to reach the optimum stationary/mobile-phase matching.

System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor, and resolution. The chromatographic systems described in this work allow complete base line separation of each carbapenem drug from its degradation product and cefepime (IS). Spiking of both intact drugs and their corresponding degradants assured the presence of only one degradant during preparation of the degradation product in each case also by changing the mobile phase ratios; just one peak appeared corresponding to the intact drug and another one for the degradant.

The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions such as small changes in the pH (± 0.1 for each drug), small changes in acetonitrile/methanol ratio (from 10/10 to 12/8) in the mobile phase and changing the column using a Zorbax C_{18} analytical column (250 mm × 4.6 mm i.d., 5 µm) (Agilent). The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified; however, the areas and peaks symmetry were conserved.

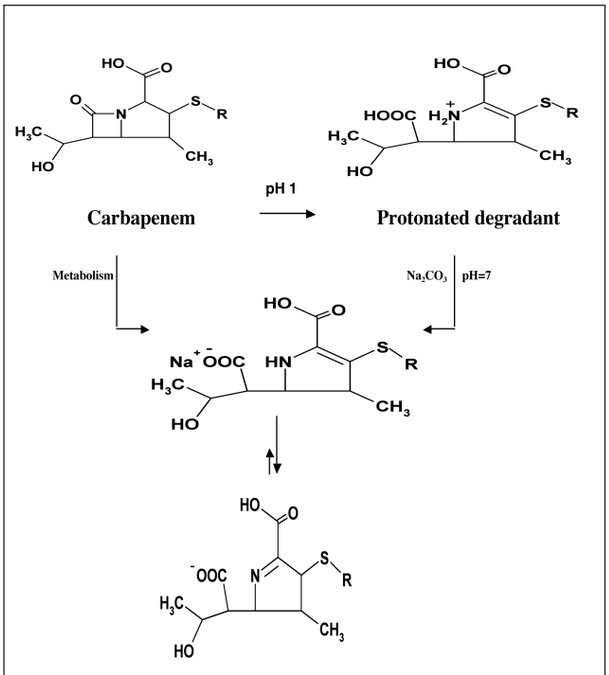

Figure 4. Scheme of carbapenem degradation pathway.

Conclusion

The suggested methods are found to be simple, accurate, selective, and equally sensitive with no significant difference of the precision compared with the reported method for E_{RTM} (21) and the official HPLC method of I_{MPM} (3). Application of the proposed methods to the analysis of both E_{RTM} and I_{MPM} in their pharmaceutical formulations shows that neither the excipient nor the degradation product interferes with the determination.

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