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A bio-analytically validated HPLC-UV method for simultaneous determination of doripenem and ertapenem in pharmaceutical dosage forms and human plasma: a dual carbapenem regimen for treatment of drug-resistant strain of *Klebsiella pneumoniae*

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The emergence of strains resistant to certain antibiotics is turning into an important issue worldwide that threatens global health with the increasing incidence of carbapenemase-producing *Klebsiella pneumoniae* (KPC). Thus, successful doripenem–ertapenem (DOR–ERTA) combination is highly recommended in treatment of bacteremic ventilator-associated pneumonia due to *Klebsiella pneumoniae*. Hence, a fast and highly-sensitive HPLC-UV method was developed for the estimation of the cited drugs simultaneously in their pure form, pharmaceutical dosage forms and in simulated synthetic mixtures. The DOR–ERTA mixture was successfully separated within 6 min on a reversed-phase ODS column using an isocratic elution; a mobile phase mixture consists of 0.05 M phosphate buffer (pH 3.0 adjusted by 85% ortho-phosphoric acid) : acetonitrile : methanol (86 : 12 : 2; % v/v/v). The proposed method was optimized and validated according to ICH guidelines, where the calibration graph was constructed from 0.04 to 50 μg mL^{-1} and from 0.05 to 50 μg mL^{-1} with low detection limits reached 1.7 and 1.4 ng mL^{-1} for DOR and ERTA respectively. The proposed method showed higher sensitivity than several previous methods, which allowed an effective estimation of the DOR and ERTA in human plasma after a simple extraction method with high recovery results ranged from 96.30% ± 1.55 to 97.90% ± 1.45 and without any interference from plasma components.

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1. Introduction

Doripenem (DOR, Fig. 1a) and ertapenem (ERTA, Fig. 1b) are β-lactam antibiotics which belong to carbapenem subclass. The cited drugs inhibit bacterial cell wall synthesis by inactivating essential penicillin-binding proteins (PBPs) which ultimately causes cell death. Carbapenem antibiotics play an extremely important role in the treatment of severe infections such as pneumonia and urinary tract infections.[1] However, with the extensive application of carbapenem antibiotics, carbapenemases have been increasingly found in Gram-negative bacteria. Carbapenemases accompanied with drug resistance will limit the use of carbapenem antibiotics and consequently threat the global health. From these Gram-negative bacteria, *Klebsiella pneumoniae*, one of the most common and severe pathogen. Carbapenem-resistant *Klebsiella pneumonaiae* strains have caused numerous outbreaks of hospital infections in many countries and become a serious clinical issue.[2–6] Double-carbapenem therapy (DCT) is highly recommended and its clinical effectiveness is attributed to the inactivation of carbapenemases by one carbapenem mainly ERTA. Hence, DOR is recommended to be co-administrated with ERTA as a dual-carbapenem therapy against carbapenemase-producing *Klebsiella pneumoniae* (KPC).[3–6]

The synergistic activity and enhanced efficacy of the combination between the studied drugs in the treatment of pneumonia attributed to KPC strains is attributed to the interaction between ERTA and carbapenemase enzyme itself.[2–6] As previously reported, utilization of the recommended carbapenem mixture treatment against KPC strains led to an enhancement in the antibacterial activity *in vitro* and *in vivo*.[2–7]

Based on our survey, many analytical methods have been reported for analysis of DOR and ERTA such as spectrophotometric,[8–11] spectrofluorimetric,[12–14] HPLC,[15–18] HPTLC[19] and electrochemical[20] methods.

High performance liquid chromatography (HPLC) technique considers the most suitable and highly sensitive analytical

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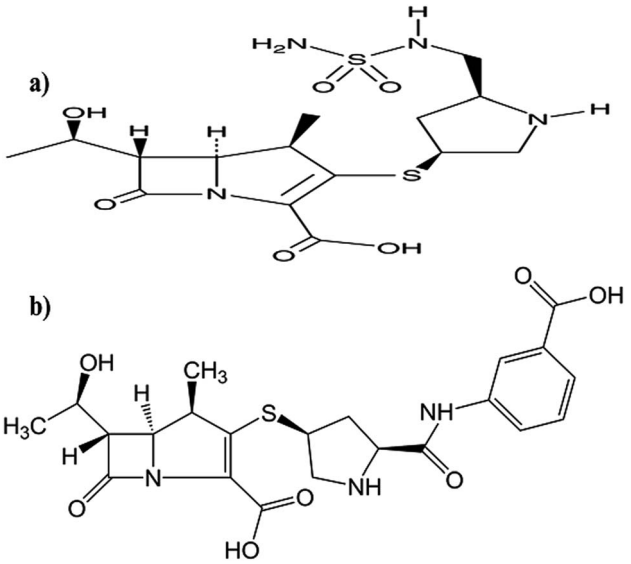

Fig. 1 Chemical structures of: (a) DOR and (b) ERTA.

method for separation of various pharmaceutical drugs.[21,22] Hence, the introduced work was designed to provide simple, low cost and highly sensitive HPLC-UV method for simultaneous determination of DOR and ERTA in their pure form, pharmaceutical products and simulated synthetic mixture. The presented work showed higher sensitivity when compared with previous reported methods.[12,22] Moreover, the presented method considered selective when compared with other spectrometric[8–13] methods. In addition, the proposed HPLC-UV method is rapid and don't consume long analysis time when compared with other methods.[15–18,22]

The pharmacokinetic parameters of the cited drugs were reported, where the maximum concentration of DOR (C_{max}) was observed to be 32.03 ± 2.32 μg mL^{-1} with $t_{1/2}$ 1.12 h.[13,14] On the other hand, the pharmacokinetic parameters of ERTA were found to be 56 and 150 μg mL^{-1} for subcutaneous and intravenous respectively.[23]

The presented method was utilized for bio-analytical validation study and applied for estimation of DOR and ERTA in human plasma after simple extraction method. The method showed high sensitivity and selectivity and considered suitable to be used in quality control and clinical laboratories.

2. Materials and methods

2.1. Instrumentation

All chromatographic separations were performed on Waters 717, plus autosampler with sample thermostat which contains Alltech, 426 LC pump, and UV/VIS (Waters Millipore, USA).

The separation was achieved using on reversed-phase ODS column (25 cm × 4.6 mm id, 5 μm particle size) from GL Science (Japan). The mobile phase composed of a mixture of 0.05 M phosphate buffer (pH 3.0), acetonitrile and methanol (86 : 12 : 2% v/v/v). The flow rate was adjusted at 0.7 mL min^{-1} and UV detector was set at 298 nm. For data interpretation, Kromex (Estonia) software chromatography was used. UV-1601 A Shimadzu spectrophotometer (Tokyo, Japan) with a 2 cm quartz cell was used for obtaining UV spectra of the studied drugs.

2.2. Chemical and reagents

Doripenem (DOR, purity 99.9%) was obtained from Janssen-Cilag CO., Egypt. Ertapenem (ERTA, purity 99.7%) was obtained from Merck Rahway, USA.

Doripax® 500 mg and Invanz® 1 g vials were obtained from the local market of Egypt. Sodium phosphate, acetonitrile (ACN), methanol, 85% orthophosphoric acid were obtained from EL-Nasr Company, Egypt. All the solvents used in chromatographic separation were of HPLC grade.

2.3. Preparation of standard solutions for HPLC analysis

A stock solution of DOR and ERTA (1 mg mL^{-1} for each) were prepared by transferring 25 mg of each drug into 25 mL

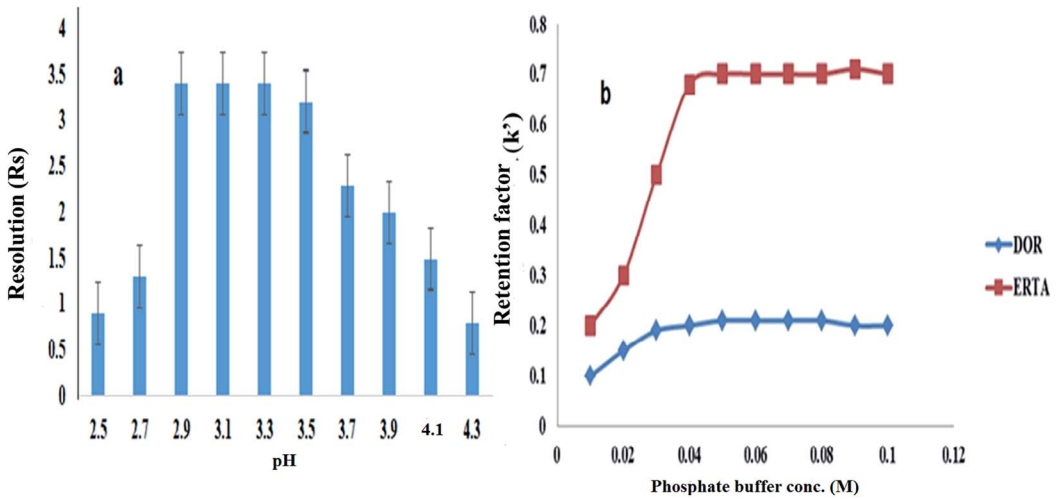

Fig. 2 Effects of: (a) pH value and (b) concentration of phosphate buffer used in the mobile phase composition for chromatographic separation of DOR and ERTA mixture (30 μg mL^{-1} each).

calibrated flask and dissolving with ultra-pure water. To obtain the working solution of the studied drugs the stock solutions were diluted using the mobile phase 0.05 M phosphate buffer (pH 3.0 with 85% ortho-phosphoric acid)- : acetonitrile : methanol (86 : 12 : 2, v/v/v) to obtain the required concentrations.

2.4. Chromatographic procedure

Twenty microliters of the working solution for DOR and ERTA in the concentration range (0.04–50 μg mL^{-1}) and (0.05–50 μg mL^{-1}) for DOR and ERTA respectively were injected into the HPLC-UV system after filtration through 0.45 μm cellulose acetate membrane filter. The chromatographic separation was carried out using ODS column (250 mm × 4.6 mm) with an isocratic elution. The mobile phase consists of a mixture consists of 0.05 M phosphate buffer (pH 3.0 with 85% ortho-phosphoric acid) : ACN : methanol (86 : 12 : 2, v/v/v), with flow rate set at 0.7 mL min^{-1} using UV detector set at 298 nm.

2.5. System suitability parameters

Various system suitability parameters as retention time (t_R), number of theoretical plates (N), resolution (R_s), capacity factor (k') and separation factor (α) were studied to check the system performance and the method repeatability using DOR and ERTA (30 μg mL^{-1} each). All the parameters of chromatographic system were calculated using different equations

$$k' = (t_R - t_0)/t_0$$

The separation factor was calculated using ($\alpha = k'_2/k'_1$). Moreover, chromatographic peak resolution is given by

$$R_s = 2\frac{t_{R_2} - t_{R_1}}{W_{b_1} + w_{b_2}}$$

where, t_R is the retention time and w_b is the peak width at baseline. Here compound **1** elutes before compound **2**.

2.6. Application of the proposed HPLC-UV method

2.6.1. Analysis of the studied drugs in their pharmaceutical vials. An accurate weight of Doripax® (500 mg DOR) and Invanz® (1 g ERTA) equivalent to 25 mg of the studied drugs were placed into 25 mL calibrated flask containing ultra-pure water. Working solutions in the concentration range (0.04–50 μg mL^{-1}) and (0.05–50 μg mL^{-1}) for DOR and ERTA respectively, were diluted with the mobile phase.

2.6.2. Analysis of the studied drugs in human plasma samples. Five hundred microliters from human plasma were spiked with 1.0 mL of the stock solutions of the studied drugs.[14] One milliliter of methanol was added for protein precipitation, vortex mixed and the supernatant solution was isolated and diluted to 10 mL with distilled water. Afterward centrifuged at 4000 rpm for 20 min. Further, the supernatant was filtered through 0.45 μm cellulose acetate membrane filter where 20 μL was injected into the HPLC-UV system.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Egyptian Network of Research Ethics Committees (ENREC) and informed consent was obtained for any experiments with human volunteers.

3. Results and discussion

The increased rate of mortality and long durations of hospitalization due to bacterial resistance is considered a global issue. Carbapenem-resistant *Klebsiella pneumonaiae* strains

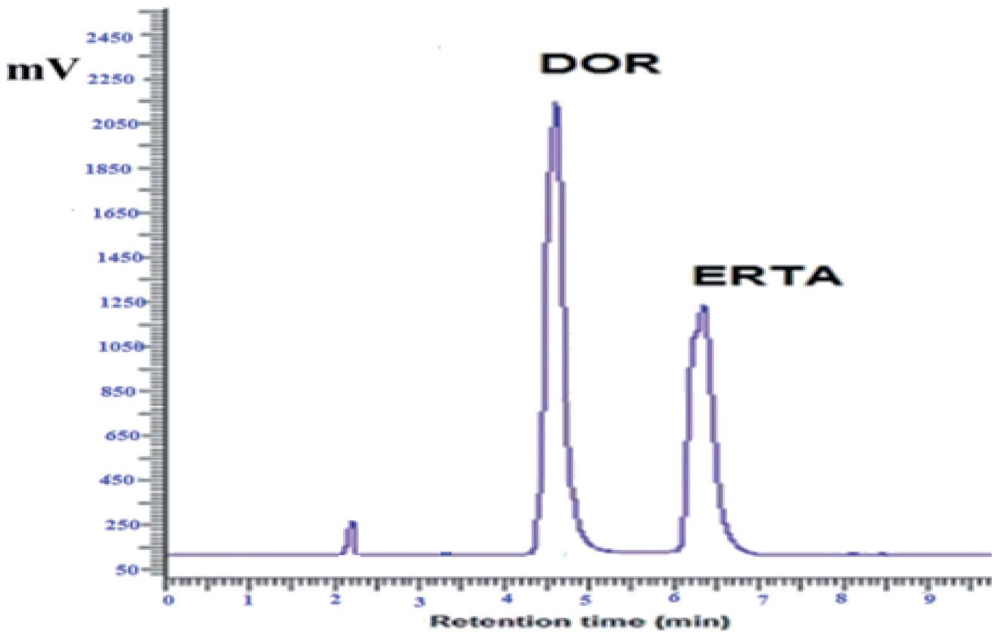

Fig. 3 HPLC chromatogram for DOR and ERTA mixture (30 μg mL^{-1} each) under optimum chromatographic conditions.

have caused numerous outbreaks of hospital infections in many countries and become a serious clinical issue.[2–6] The resistance of these *Klebsiella* to the current antimicrobials, resulted in thinking of a combination therapy of two or three antimicrobial drugs. Based on previous studies, DOR–ERTA combination therapy showed a great effectiveness in treatment of pneumonia attributed to these KPC strains.[2–7]

Hence, the presented method was designed to develop a fast, simple and highly sensitive HPLC-UV approach for simultaneous estimation of DOR and ERTA in their pure form, pharmaceutical vials, synthetic mixture and in human plasma. The synergistic effect of this dual-carbapenem regimen is highly recommended for certain medical cases in order to reduce the time of treatment.

The simultaneous separation of the investigated dual-regimen was achieved using a reversed-phase ODS column. The mobile phase was composed of a mixture of 0.05 M phosphate buffer (pH 3.0 with 85% ortho-phosphoric acid), acetonitrile and methanol (86 : 12 : 2% v : v : v). The flow rate adjusted at 0.7 mL min^{-1}, and UV detection was set at 298 nm.

3.1. Optimization of different chromatographic conditions

For achievement of good resolution within short analysis time and to obtain symmetric peaks of the studied drugs, various chromatographic conditions were investigated.

3.1.1. Mobile system decomposition. To find the most suitable mobile phase for separation, different mobile phases composition and flow rates were studied. Phosphate buffer and ACN were tried firstly as two components eluent, it was observed that DOR and ERTA peaks overlapped and not separated well. So, three components eluent composed of phosphate buffer, ACN and methanol with different ratios were tested. In addition, different pH value of phosphate buffer in the range from 2.5 to 4.3 were tested to obtain good separation for DOR and ERTA. It was found that phosphate buffer (pH = 3.0 ± 0.1), ACN and methanol (86/12/2; v/v/v) was the most suitable mobile phase with good resolution as shown in Fig. 2a. Moreover, the phosphate buffer concentration used in the mobile phase was tested from 0.02–0.1 M, it was found that the highest and stable results were obtained using concentration ranged from 0.04 to 0.09 M, after that a decrease in the response was observed. Hence, 0.05 M phosphate buffer (pH = 3.0 ± 0.1) was selected as optimum one as shown in Fig. 2b.

3.1.2. Mobile phase flow rate. The flow rate was further studied to achieve symmetric sharp peaks of the studied drugs with complete separation and within reasonable time. It was found that flow rate from 0.6–0.9 mL min^{-1} showed good separation between the studied drugs with sharp peaks, hence 0.7 mL min^{-1} was the most suitable flow rate.

3.1.3. Detection wavelength. The optimum detection wavelength for the studied drugs was investigated using UV spectrophotometer, and the UV spectra of DOR and ERTA were recorded at 298 nm.

As represented in Fig. 3, the investigated drugs were completely separated under the optimum chromatographic conditions without any interference or overlapping, DOR was detected at 4.7 min while ERTA was at 6.3 min. The time required for separation of DOR and ERTA was less than 7 min, hence the developed method considered a fast one when compared with various analytical methods used previously for determination of the cited drugs.[10–12,15]

3.2. System suitability parameters

Different system suitability parameters such as retention time (t_R), capacity factor (k'), separation factor (α), resolution (R_s), number of theoretical plates (N) and height equivalent to theoretical plates (HETP) were studied to check the system performance and the method repeatability for separation of DOR and ERTA (30 μg mL^{-1} each). The results obtained using six replicate samples under the optimal chromatographic conditions as summarized in Table 1. The summarized results refer to good separation between the cited drugs; where DOR and ERTA were detected at 4.7 and 6.3 min respectively. The capacity factor (K') was found to be 0.9 and 1.8 for DOR and ERTA respectively. Moreover, chromatographic peak resolution was calculated and it was found to be 3.4 ± 0.5 which ensures good separation between the studied drugs.

3.3. Validation of the developed HPLC-UV method

3.3.1. Linearity range and sensitivity limits. The chromatographic method was validated according to ICH guidelines[24] and US-FDA recommendations[25] for a bio-analytical validation study.

Table 1 System suitability parameters for the studied drugs using the proposed HPLC-UV method[a]

Parameters	DOR	ERTA
Retention time (min), t_R	4.7 ± **0.11**	6.3 ± **0.20**
Void time (min)	2.2	2.2
Adjusted retention time (min), t_R^-	2.5	4.1
Capacity factor, K'	1.14	1.86
Separation factor, α	1.63 ± 0.2	
Resolution, R_s	3.4 ± 0.5	
Column characters	250 mm × 4.6 mm id, 5 μm particle size	
Number of theoretical plates (N, plates)	3155 ± **1.16**	4211 ± **1.16**
Height equivalent theoretical plate (HETP, cm per plate)	0.079	0.059

[a] The bold data are the standard deviation of three injections.

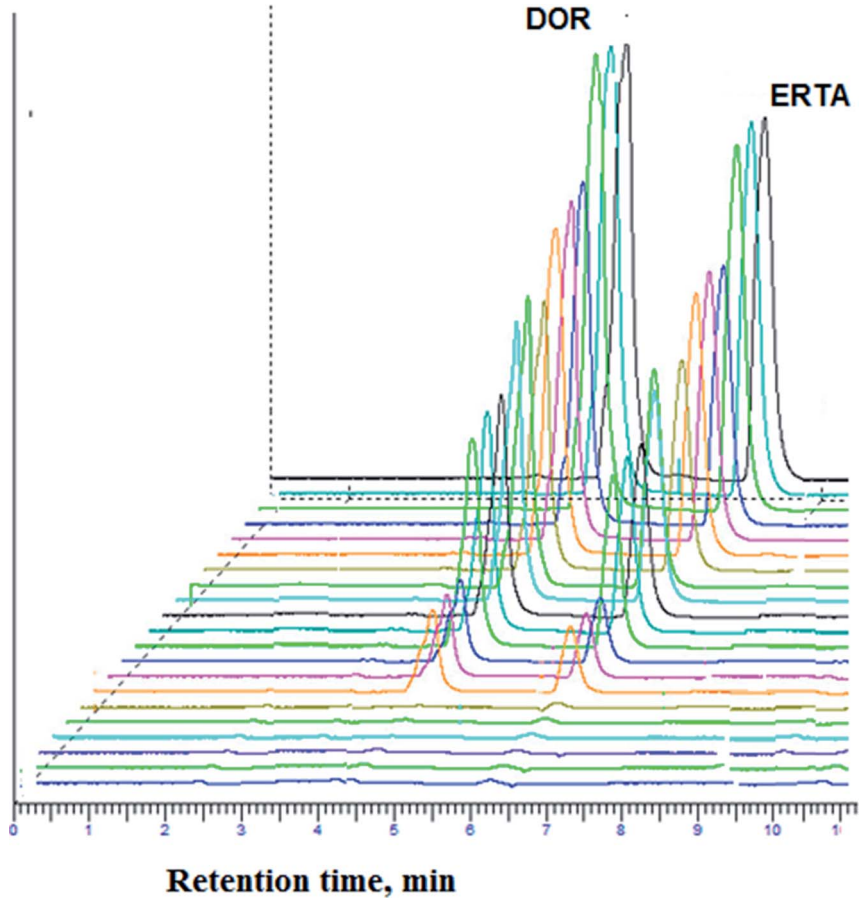

Fig. 4 Three-dimensional chromatogram for determination of DOR and ERTA mixture using five concentrations (0.1, 0.5, 10, 25 and 40 μg mL^{-1}) within calibration range under optimum chromatographic conditions.

Linearity range of the method was obtained by plotting the peak area of the standard solutions of the studied drugs against their corresponding concentration range (0.04–50.0 μg mL^{-1}) and (0.05–50.0 μg mL^{-1}) for DOR and ERTA respectively, Fig. 4 represented chromatograms of the studied drugs in different concentrations (0.1, 0.5, 10.0, 25.0, 40.0 μg mL^{-1}) within calibration range.

The limit of detection (LOD) was expressed as $3.3\sigma/S$ while limit of quantitation (LOQ) was expressed as $10\sigma/S$, where (σ) is the standard deviation of intercept and (S) is the slope of calibration curve. It was observed that LOD values were found to be 1.7 and 1.4 ng mL^{-1}, while LOQ values were found to be 5.0 and 4.2 ng mL^{-1} for DOR and ERTA respectively. The proposed method showed higher sensitivity over several previous

Table 2 Analytical parameters for HPLC-UV method for determination of DOR and ERTA[a]

	HPLC method	
Parameters	DOR	ERTA
Mobile phase system	Phosphate buffer (pH = 3.0) : ACN : methanol (86/12/2; v/v/v)	
Wavelength (nm)	298	298
Flow rate (mL min^{-1})	0.7	0.7
Linearity range (μg mL^{-1})	0.04–50.0	0.05–50.0
Correlation coefficient (r)	0.9998	0.9996
Determination coefficient (r^2)	0.9997	0.9994
Intercept ± SD	8298 ± 33.56	68 350 ± 20.22
Slope ± SD	$67 \times 10^3 \pm 2291.12$	$48 \times 10^3 \pm 1201.10$
LOD (ng mL^{-1})	1.7	1.4
LOQ (ng mL^{-1})	5.0	4.2

[a] The data expressed are the mean of six replicates.

Table 3 Evaluation of accuracy of the proposed method for determining the studied drugs in three different concentration levels within the calibration range[a]

	DOR			ERTA		
Sample no.	Taken (μg mL^{-1})	% Recovery*	% RSD	Taken (μg mL^{-1})	% Recovery*	% RSD
1	0.5	99.57	0.49	0.5	100.02	1.20
2	25.0	101.32	0.63	25.0	100.70	1.51
3	40.0	100.94	1.41	40.0	101.05	0.87

[a] *: average of three determinations. RSD: relative standard deviation.

methods.[10–12] The ultra-sensitivity of the developed HPLC-UV method ensures its ability for trace monitoring of the studied drugs in biological samples. All statistical parameters were summarized in Table 2.

3.3.2. Accuracy and precision. Accuracy of the presented method was checked using three different concentrations levels (0.5, 25.0 and 40.0 μg mL^{-1}) for the studied drugs by injecting each concentration six replicates on the HPLC-UV system. These results indicate good accuracy of the chromatographic method for assay of DOR and ERTA mixture as represented in Table 3.

On the other hand, the intra-day and inter-day precision study was performed using three concentrations 0.5, 25.0 and 40.0 μg mL^{-1}. In case of the inter-day precision study, the analysis was repeated for three successive days. It was observed that the recovery results were ranged from 99.57 to 101.32% with % RSD values not exceeding 1.51.

Table 4 Robustness of the proposed HPLC-UV method for determination of the studied drugs (30 μg mL^{-1} each)

	DOR	ERTA
Parameters	% Recovery ± SD[a]	% Recovery ± SD[a]
No variations	101.20 ± 0.34	101.07 ± 0.65
Mobile phase		
84/14/2 v/v/v	99.04 ± 0.81	98.90 ± 0.39
88/10/2 v/v/v	97.43 ± 0.65	98.61 ± 1.01
Wavelength (nm)		
296	99.65 ± 0.72	97.99 ± 0.90
300	98.51 ± 1.02	98.80 ± 0.73
Flow rate (mL min^{-1})		
0.6	98.11 ± 0.68	99.01 ± 0.94
0.8	97.90 ± 0.85	98.04 ± 0.53
Phosphate buffer pH		
2.9	99.48 ± 1.50	98.55 ± 0.85
3.1	99.30 ± 0.73	98.29 ± 1.21
Phosphate buffer conc. (M)		
0.04	99.80 ± 1.22	98.93 ± 0.43
0.06	99.88 ± 0.55	99.30 ± 0.32

[a] Average of three determinations.

3.3.3. Robustness. The effect of minor change from optimal chromatographic parameters such as (mobile phase system ratio, pH value, mobile phase flow rate and detection wavelength) were studied in order to examine the robustness of the presented HPLC-UV method. It was found that this minor change in these experimental parameters had no significant influence on the performance of the developed method as represented in Table 4, which ensures the reliability of the method.

3.4. Validation of the proposed method in human plasma

3.4.1. Accuracy and precision in human plasma. The investigated method was bio-analytically validated according to US-FDA[25] guidelines, where the accuracy and precision were studied in human plasma. Three concentration levels were measured intra-daily ($n = 6$) and inter-daily on three successive days ($n = 9$) using low quality control sample (LQC), medium quality sample (MQC) and high quality control sample (HQC) for DOR and ERTA. The results summarized in Table 5, refer to good repeatability in human plasma with % RSD not exceeding 2.02 and 2.31 for DOR and ERTA respectively, with percent of recovery ranged from 97.00 to 98.92%. The percentage recovery was calculated following this equation: (found amount/spiked amount × 100).

3.4.2. Matrix effect and selectivity. Matrix effect was tested for the investigated drugs using three levels of quality control

Table 5 Accuracy and precision of the proposed method for determining DOR and ERTA in human plasma

	Intra-day assay ($n = 6$)		Inter-day assay ($n = 9$)	
Taken (μg mL^{-1})	Accuracy (% recovery)	Precision (% RSD)	Accuracy (% recovery)	Precision (% RSD)
DOR				
0.04	97.16	1.33	97.01	1.88
25	97.01	2.02	97.00	1.87
50	98.92	1.90	98.30	1.86
ERTA				
0.05	97.55	2.00	97.12	2.31
25	98.30	1.60	97.94	2.08
50	97.53	2.11	97.33	1.74

samples for the investigated drugs low quality control sample (LQC), medium quality control sample (MQC) and high quality control sample (HQC) (0.05, 25.0 and 50.0 μg mL^{-1}) for DOR and ERTA. The percentage of recovery ± % RSD was found to be between 96.30 ± 1.55 and 97.90 ± 1.42. The outcomes refer to the absence of interference from plasma matrix with the examined dual regimen (DOR–ERTA mixture), this approves the high selectivity of the proposed method as shown in Fig. 5.

3.4.3. Incurred sample reanalysis (ISR). The parameter used to check the accuracy and precision of incurred sample in bio-analytical validations based on FDA guidelines. ISR calculated as (% of difference between incurred samples and initial samples/mean) × 100. In the HPLC study, the percentage difference between the samples was found in the range 1.08–4.66%. The results of incurred samples met the accepted criteria of FDA guidelines.

3.4.4. Stability of the studied drugs in human plasma samples. Different factors were applied to study the stability of the cited drugs in human plasma using three concentrations, low quality control (LQC), medium quality control (MQC) and high quality control (HQC) levels as (three freeze–thaw cycle stability (−24 °C), long-term stability (1 month at −24 °C), short-term stability (12 h at −24 °C), post-preparative stability (6 h at room temperature 25 °C), post-preparative stability (12 h at room temperature 25 °C)). The percent of recovery was found in the range from 95.44 to 96.32%, which refers to good stability of the studied drugs under different conditions with high percentage of recovery.

3.5. Applications of the proposed HPLC-UV method

3.5.1. Determination of the studied drugs in their pharmaceutical vials. The proposed method was successfully used for determination of DOR and ERTA in their dosage forms (Doripax® 500 mg and Invanz® 1 g vials). The percentage of recovery was found to be 101.77 ± 1.05 compared with that of the reported method[9] (100.04 ± 1.80). ERTA also successfully

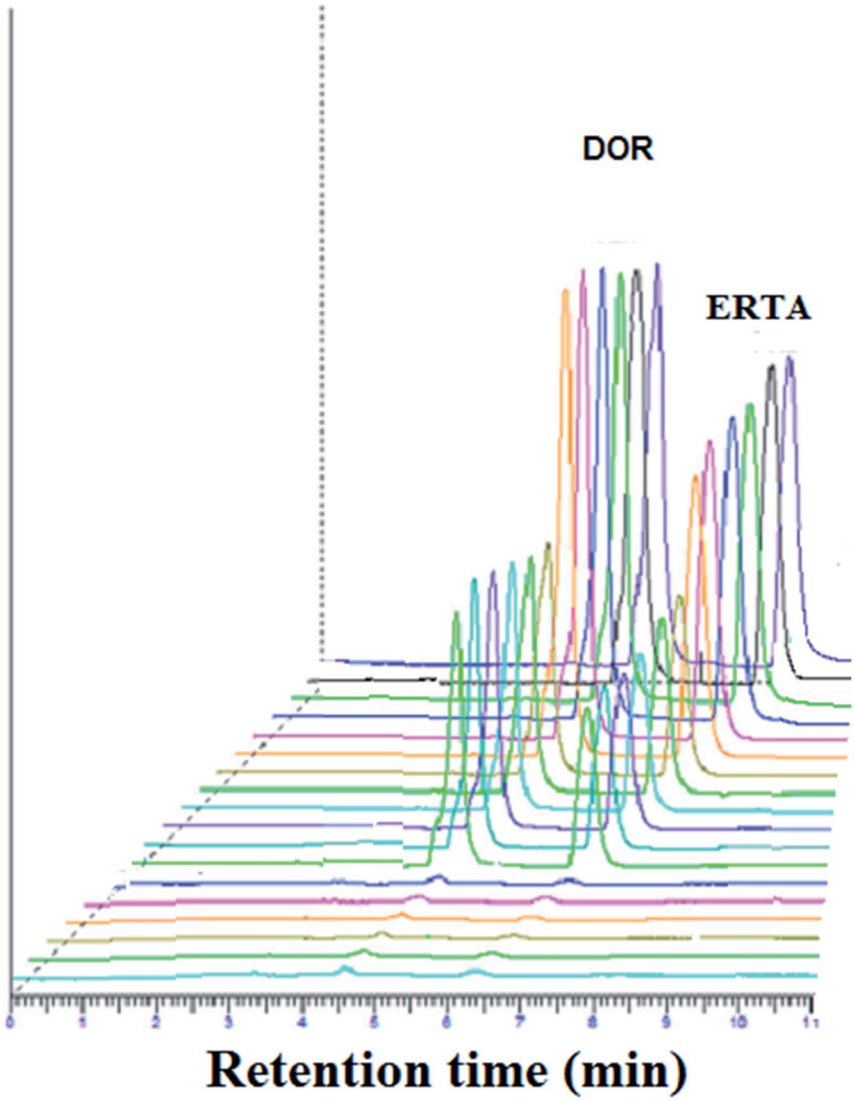

Fig. 5 Accuracy expressed by three dimensional HPLC chromatograms for DOR and ERTA mixture using three concentration levels (0.05, 25.0 and 50.0 μg mL^{-1} for each) under optimum chromatographic conditions.

Table 6 Comparison between the proposed and reported methods for determination of DOR and ERTA in their pharmaceutical vials

	% Recovery ± SD[a]			
Dosage form	Proposed method	Reported method[b]	*t*-Value[c]	*F*-Value[c]
Doribax® (500 mg DOR/vial)	101.77 ± 1.05	100.04 ± 1.80	1.52	2.90
Invanz® (1 g ERTA/vial)	101.52 ± 1.11	100.21 ± 1.54	1.50	3.01

[a] Mean of five determinations. [b] The reported methods ref. 9 and 18. [c] The tabulated *t*- and *F*-values at 95% confidence limit are 2.78 and 6.39, respectively.

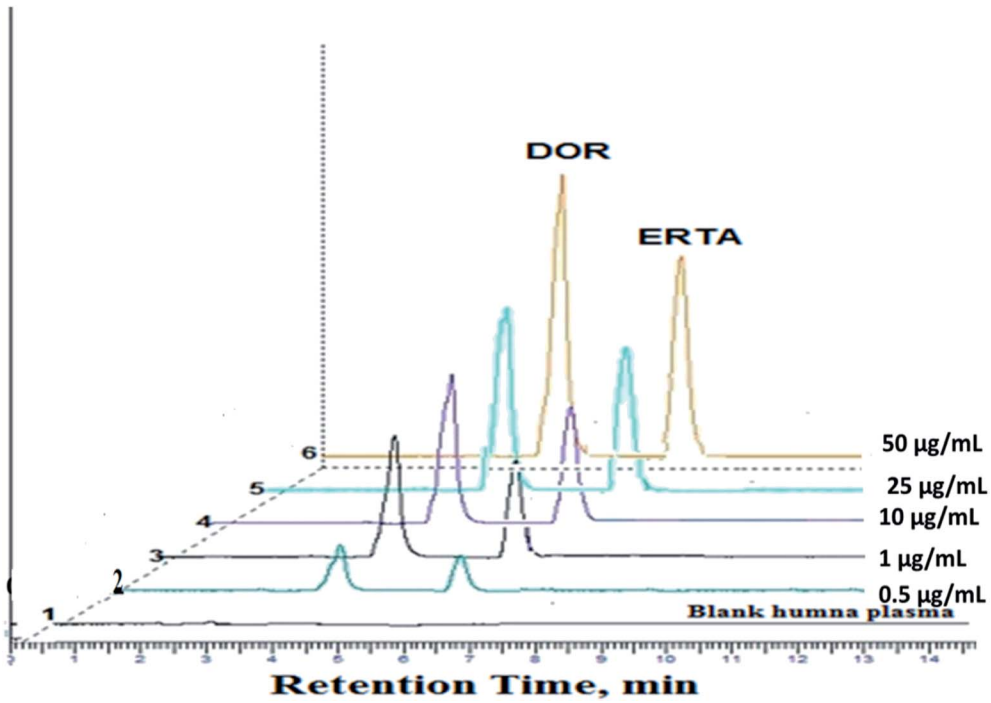

Fig. 6 Three dimensional chromatograms for assay of the studied drugs in human plasma, (1) is blank human plasma and chromatograms from (2) to (6) represents different concentrations of the studied mixture from (0.5, 1.0, 10, 25 and 50 µg mL^{-1}).

determined using the proposed method with percentage of recovery 101.52 ± 1.11 against that of the reported method[18] (100.21 ± 1.54). The *t*-value and *F*-value were calculated and found to be 1.52 and 2.90 and 1.50 and 3.01 for DOR and ERTA respectively as described in Table 6.

3.5.2. Determination of the studied drugs in simulated synthetic mixture. The aim of this study is to determine DOR with ERTA simultaneously in simulated synthetic mixture. As we mentioned, this binary mixture is highly recommended for treatment of pneumonia attributed to KPC strains.[2–6] The proposed method was successfully applied for separation of five synthetic mixtures of DOR and ERTA in human plasma using different concentrations of both drugs within the calibration range (0.5, 1.0, 10.0, 25.0 and 50.0 µg mL^{-1}) as represented in Fig. 6.

3.5.3. Determination of the studied drugs in human plasma. The ultra-sensitivity of the developed method allows determination of DOR and ERTA drugs in human plasma without matrix interference. The percentage of recovery was found to be in the range from 96.30% to 97.90% for the investigated methods at five different concentration levels applied as shown in Table 7. The % RSD values were in the range of 1.45–2.04 and 1.12–1.93 for DOR and ERTA respectively. These

Table 7 Analysis of DOR and ERTA in human plasma using the proposed HPLC-UV method

Added conc. (µg mL^{-1})	DOR		ERTA	
	% recovery[a]	% RSD	% recovery[a]	% RSD
0.5	96.49	2.04	97.89	1.49
1.0	96.30	1.60	96.37	1.81
10.0	97.87	1.66	96.80	1.12
25.0	97.90	1.45	97.04	1.93
50.0	96.96	1.65	96.33	1.55

[a] Average of five replicates.

results fall within the acceptable limits of the analytical method variability arising from different matrix effects and compatible with other reported method.[14]

4. Conclusion

Many combination therapies have shown better survival and mortality reduction for patients suffering from a drug-resistant strain of *Klebsiella pneumoniae* compared with monotherapy regimens. The dual-carbapenem based combination regimen exert good synergistic results and low resistance. Hence, a fast, sensitive and low-cost LC/UV approach was created for simultaneous investigation of DOR and ERTA in pure forms and pharmaceutical vials. The method was validated according to ICH guidelines under optimum chromatographic conditions, and good linearity was obtained in the range from 0.04 to 50.0 μg mL^{-1} and from 0.05 to 50.0 μg mL^{-1} with low detection limits reached 1.7 and 1.4 ng mL^{-1} for DOR and ERTA. The high efficiency of the developed method permits the determination of DOR in human plasma simultaneously with ERTA without matrix interference. The method was utilized to estimate DOR and ERTA in human plasma after a simple extraction method. The method is highly recommended to monitor DOR and ERTA in quality control and clinical laboratories.

Conflicts of interest

There are no conflicts to declare.

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