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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF MEROPENEM AND POTASSIUM CLAVULANATE

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Abstract: A stability-indicating LC assay method was developed and validated for a simultaneous determination of meropenem and potassium clavulanate in the presence of degradation products formed during acid-base hydrolysis, oxidation and thermolysis. The isocratic RP-HPLC method was developed with a LiChrospher RP-18 (250 mm × 4.6 mm, 5 µm) column and gradient elution of 12 mmol/L ammonium acetate and acetonitrile. The flow rate of the mobile phase was 1.0 mL/min, the detection wavelength 220 nm and the temperature 303 K. The method was validated with regard to linearity, accuracy, precision, selectivity and robustness, and was applied successfully for the determination of meropenem and potassium clavulanate separately as well as jointly in pharmaceutical formulations.

Keywords: liquid chromatography, meropenem, clavulanate potassium

Meropenem, a β-lactam antibiotic and a member of the carbapenem group (Fig. 1), has a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria (1–4). Similarly to other carbapenems, meropenem is often used as a last resort in the treatment of many complicated bacterial infections. Potassium clavulanate (Fig. 2) is a β-lactamase inhibitor which shows a relevant post-antibacterial effect when administered together with amoxicillin. β-Lactam antibiotics are known to be ineffective against *Mycobacterium tuberculosis*, as they are rapidly hydrolyzed by the chromosomally encoded *blaC* gene product.

Recent *in vitro* and *in vivo* studies have indicated the effectiveness of connecting meropenem and potassium clavulanate in the treatment of *Mycobacterium tuberculosis*, including its most resistant strains (5–7).

The significant instability of meropenem and potassium clavulanate, associated with the presence of 4,5 fused β-lactam and heterocyclic rings, determines the process of preparing and the storage of formulations containing β-lactam analogs (8, 9). Several studies investigated the chemical instability of meropenem in aqueous solutions (10) and in the solid state (11). Depending on affecting factors, degradants of different chemical structures are formed. Similarly to β-lactam analogs, potassium clavulanate is also chemically unstable. Its degradation products have been observed to have a catalytic effect on the rate of degradation of clavulanic acid

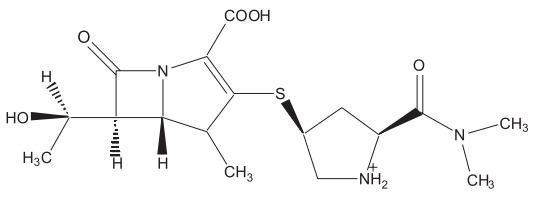

Figure 1. Chemical structure of meropenem

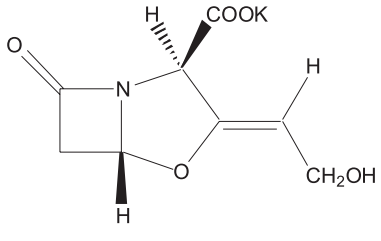

Figure 2. Chemical structure of potassium clavulanate

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and to be determined by pH and the presence of ions (12).

Since the majority of the side effects of β-lactam antibiotics are caused by their degradation products, it is vital to improve analytical methods for the determination of β-lactam analogues. An optimal method is expected to separate and determine the substance to be examined in the presence of related products such as in-process impurities, degradation products and metabolites (13). The current guidelines of the International Conference on Harmonization (ICH) require the development of stability-indicating assay methods (SIAMs) suitable for the determination of drugs based on the analysis of stability test samples (Q1A–R2) (14). It is equally important to develop an appropriate analytical method to assess changes in drug concentrations in intravenous solutions. Furthermore, in the case of labile drugs and those for frequent administration, it is essential to establish whether they are suitable to be mixed in one infusion flow in order to preclude their interaction. Also, it is necessary to develop a selective method for determining their concentration changes in the presence of their degradation products.

Until now, no chromatographic methods for a simultaneous determination of meropenem and potassium clavulanate are available in the literature. The aim of this work was to develop and validate an HPLC method with UV detection suitable for a simultaneous determination of meropenem and potassium clavulanate in the presence of their degradation products.

EXPERIMENTAL

Materials

Meropenem (purity > 98%) and potassium clavulanate were obtained from CHEMOS (Germany). Potassium clavulanate was diluted in ratio 1 : 1 by 50% silicon dioxide and contained less than 2% of impurities.

All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High quality pure water was prepared by using the Millipore purification system, model Exil SA 67120 (France).

Instrumentation

The HPLC system (Dionex UltiMate 3000) consisted of quaternary pump (LPG-3400RS), an autosampler (WPS-3000TRS), a column oven (TCC-3000RS) and diode array detector (DAD-3000). Separations were performed on a LiChrospher RP-18 column, 5 μm particle size, 250 mm × 4 mm (Merck, Germany). The mobile phase consisted of a mixture of solution A (12 mmol/L ammonium acetate) and solution B (acetonitrile) with an initial composition of 4% solution B. From t_0 to $t_{4\ min}$ the concentration of solution B was constant and equal to 4%, then it was changed linearly to reach 25% at $t_{12\ min}$, after which the column was re-equilibrated to starting conditions for 5 min. The flow rate of the mobile phase was 1.0 mL/min. The wavelength of the DAD detector was set at 220 nm. The column and autosampler tray were set at 303 K and 278 K, respectively.

Validation method

The HPLC method was validated according to the International Conference on Harmonization Guidelines (14). It comprised parameters such as selectivity, linearity, precision, accuracy and robustness.

Procedure for forced degradation study of meropenem and potassium clavulanate

Degradation in aqueous solutions

The degradation of meropenem and clavulanate potassium in aqueous solutions was studied at 293 K in hydrochloric acid (0.1 mol/L) and in sodium hydroxide (0.1 mol/L). Degradation was initiated by dissolving an accurately weighed 10.0 mg of meropenem and 10.0 mg potassium clavulanate in 25.0 mL of the solution equilibrated to 293 K in stoppered flasks. At specified times, samples of the reaction solutions (1.0 mL) were instantly neutralized and cooled with a mixture of ice and water. The degradation in condition of an oxidation was initiated by dissolving an accurately weighed 10.0 mg of meropenem and 10.0 mg potassium clavulanate in 25.0 mL of 3% H_2O_2 solution equilibrated to 293 K in stoppered flasks. At specified times, samples of the reaction solutions (1.0 mL) were instantly cooled with a mixture of ice and water.

Degradation in the solid state

Ten milligrams samples of meropenem and potassium clavulanate were weighed into glass vials. In order to achieve the degradation of meropenem and potassium clavulanate in the solid state, their samples were put in the heat chambers at 373 K at RH = 0% and 313 K at RH ≈ 76.4%. At specified time intervals, determined by the rate of degradation the vials were removed, cooled to room temperature and their contents were dissolved in water. The obtained solutions were quantitatively

transferred into measuring flasks and diluted with water to 25.0 mL.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main objective was to develop an HPLC-UV method for the determination of meropenem and potassium clavulanate in the presence of degradation products.

The mobile phase consisted of 12 mmol/L ammonium acetate and acetonitrile (96 : 4 v/v) with isocratic elution. The stationary phase used was a LiChrospher RP-18 column. Although the detection wavelength for meropenem was 300 nm, the absorption maximum of the products of its degradation was at 220 nm, which was also the wavelength at which the absorption maximum of clavulanate potassium occurred. The so-designed mobile phase was proposed for a simultaneous determination of meropenem and potassium clavulanate. However, in order to separate potassium clavulanate from the degradation products of meropenem it was necessary to use gradient elution. By applying an experimentally obtained HPLC gradient program (from t_0 to $t_{4\ min}$ the concentration of solution B was constant and equal 4%, then it was changed linearly to reach 25% at $t_{12\ min}$) it was possible to achieve a satisfying separation of clavulanate potassium (retention time 2.933 min.), meropenem (10.213 min.) and their degradation products (2.073, 4.530 and 11.447 min.) (Fig. 3).

Method validation

The selectivity was examined for non-degraded and degraded samples (the solutions of meropenem and potassium clavulanate after stress conditions: acid and base hydrolysis at room temperature, oxidation H_2O_2 and thermal degradation at 313 K and 373 K). The HPLC method for determination of meropenem and potassium clavulanate was found of selective in the presence of degradation products as shown in the pictures (Fig. 3). Peaks were symmetrical and clearly separated from each other (Fig. 3). Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of meropenem and potassium clavulanate peaks. The calibration plots were linear in the following concentration range 40 – 480 mg/L, n = 7, r = 0.9999 (meropenem), 80–480 mg/L, n = 6, r = 0.9996 (potassium clavulanate). The calibration curve was described by the equation y = ax; y = (0.1257 ± 0.005)x (meropenem) y = (0.1513 ± 0.0044)x (potassium clavulanate). The b value, calculated from equation y = a*x* + b, was insignificant because it was lower than the critical value $t_b = b/S_b$. Statistical analysis using Mandel's fitting test confirmed that linear model is preferred over quadratic regression for the calibration curves. The samples of each solution were injected three times and each series comprised 7 experimental points. Precision of the assay was determined in relation to repeatability (intra-day) and intermediate precision (inter-day) for six samples. Intermediate precision was studied comparing the assays performed on two different

Table 1. Precision data (n = 6) for meropenem and clavulanate potassium.

Analyte	Spiked concentration (mg/L)	Measured concentration ± SD (mg/L)	RSD (%)
Intra-day precision			
Potassium clavulanate	320.00 400.00 480.00	320.84 ± 4.58 393.87 ± 5.57 464.89 ± 7.49	1.36 1.35 1.53
Meropenem	320.00 400.00 480.00	321.27 ± 1.44 395.96 ± 1.54 472.50 ± 3.60	0.43 0.37 0.66
Inter-day precision			
Potassium clavulanate	320.00 400.00 480.00	317.32 ± 9.83 389.98 ± 11.05 462.02 ± 9.71	3.19 2.91 2.16
Meropenem	320.00 400.00 480.00	319.89 ± 3.74 393.96 ± 4.33 470.64 ± 5.61	1.20 1.13 1.14

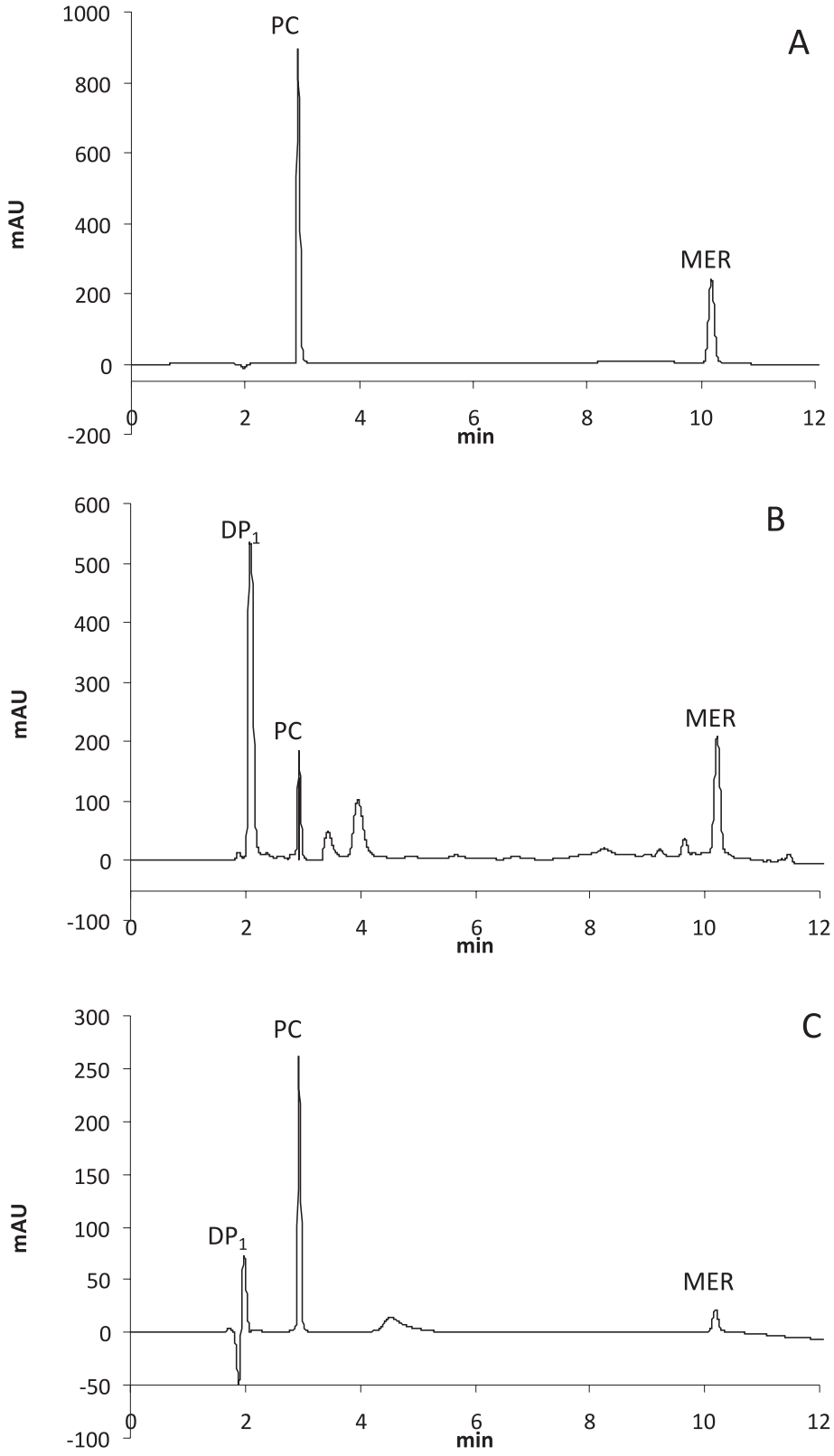

Figure 3. Chromatogram of blank sample of meropenem (MER) and potassium clavulanate (PC) (**A**); chromatogram of meropenem and potassium clavulanate during oxidative forced degradation study (**B**); chromatogram of meropenem and potassium clavulanate during alkali forced degradation study (**C**); DP_1 – meropenem degradation product with an open β-lactam ring

Table 2. Accuracy data (n = 6) for meropenem and potassium clavulanate.

Analyte	Spiked concentration (mg/L)	Measured concentration ± SD (mg/L)	RSD (%)
Recovery studies			
Potassium clavulanate	320.0 (~ 80%) 400.0 (~ 100%) 480.0 (~ 120%)	307.67 ±1.57 375.67 ± 1.87 440.47 ±1.84	96.15 93.92 91.76
Meropenem	320.0 (~ 80%) 400.0 (~ 100%) 480.0 (~ 120%)	316.80 ± 0.54 390.44 ± 0.91 462.67 ± 1.81	99.00 97.61 96.39

Table 3. Results of forced degradation studies.

Stress conditions and time studies	Degradation CP [%]	Degradation MER [%]
Acidic /0.1 mol/L HCl 293 K/ 53 min	23.63	22.73
Alkali /0.1 mol/L NaOH 293 K/ 1 min	98.82	89.86
Oxidizing /3% H_2O_2/293 K/ 115 min	82.55	70.09
Thermal /373 K RH ≈ 0%/ 3 days	69.23	57.91
Thermal /313 K RH ≈ 76.4%/ 28 days	78.25	68.12

CP = potassium clavulanate; MER = meropenem

days. The intra-day and inter-day precision values of measured concentration of meropenem and potassium clavulanate, as calculated for 80, 100 and 120% levels their of initial concentrations, the RSD values were 0.37 and 3.19%, respectively, demonstrating that the method was precise for both of analyzed substances (Table 1). The accuracy of the method was determined by recovering meropenem and potassium clavulanate from placebo. The recovery test was performed at three levels 80%, 100% and 120% of the nominal concentration of meropenem and potassium clavulanate during degradation studies. Six samples were prepared for each recovery level. Values of RSD for meropenem and potassium clavulanate indicate that excipients do not have effect or interference on the determination of tested analytes (Table 2). The LOD and LOQ parameters were determined from the regression equations of meropenem and potassium clavulanate, respectively: LOD = 3.3 S_y/a, LOQ = 10 S_y/a; where S_y is a standard error and a is the slope of the corresponding calibration curve. Under applied chromatographic conditions, the LOD and LOQ of meropenem were 2.57, 7.78 mg/L and of potassium clavulanate 14.51, 43.98 mg/L, respectively.

The robustness of the procedure was evaluated after changing the following parameters: the composition of the mobile phase (concentration of ammonium acetate in the range 12 ± 2 mmol/L), the pH of mobile phase in the range 7.15 ± 0.05, initial and final concentration of acetonitrile in the mobile phase in the range 4 ± 1 and 25 ± 1%, respectively, time of a gradient increase in the range 8 ± 1 min, flow rate in the range 1.0 ± 0.1 mL/min, wavelength of absorption 220 ± 3 nm, temperature 303 ± 2 K. No significant changes in separation, retention time, area and asymmetry of peak were observed when tested parameters were modified.

Results of forced degradation experiments

A comparison of the results of the kinetic studies carried out separately for the potassium clavulante and meropenem under the same degradation conditions with the results obtained for their mixture indicated the absence of any mutual catalytic effect or any impact of their degradation products.

The degradation of potassium clavulanate and meropenem was observed during stress studies in solutions (acidic and basic hydrolysis, oxidation) and in the solid state at an increased relative air

humidity (RH ≈ 76.5%) and in dry air (RH = 0%). No degradation was noted in the solid state during photolysis. Potassium clavulante was found to be the most prone to degradation under oxidizing conditions (3% H_2O_2, RT), whereas meropenem under basic conditions (0.01 M NaOH, RT). Under the influence of an acidic medium (0.1 M HCl, RT) the degradation of meropenem and potassium clavulanate was comparable (approx. 40% after 3 min). The results of forced degradation in various media were summarized in Table 3. The chromatograms of solutions obtained after forced degradation are shown in Figure 3. No peaks of degradation products of potassium clavulanate occurred on the chromatograms of degraded samples. The lack of substituents containing π-bond system chromophores appeared to prevent the application of an LC method with a PDA detector for recording peaks originating from the degradation products of potassium clavulanate. Although during the degradation of meropenem a product with an open β-lactam ring (DP_1) (retention time of 2.15 min) was formed, the presence of potassium clavulanate did not lead to the appearance of any other degradation products.

CONCLUSIONS

The linear gradient RP-LC method for a simultaneous analysis of meropenem and potassium clavulanate in intravenous solutions or in their potential pharmaceutical preparations was developed. Selectivity, accuracy and precision and short run time make this method useful for routine analysis.

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