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Chromatographic determination of polymerized impurities in meropenem

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Abstract

A method for the separation of polymerized impurities in meropenem has been developed by gel filtration chromatography (GFC). The chromatographic conditions included a Superdex peptide HR 10/30 column and the mobile phase consisting of 0.01 mol L^{-1} sodium phosphate buffer (pH 7.0), at a flow rate of 0.8 mL min^{-1}. The wavelength of UV detector was set at 220 nm. The linear range was 0.08–10 μg ($r = 0.9998$); relative standard deviation (R.S.D.) = 0.5–1.5%; the LOD and LOQ were 2.4 and 12.4 ng, respectively. Specificity of the GFC was checked by switching the effluent of each peak on the GFC to a C_{18} trap column and analyzing the effluent by LC–MS. The result shows that for the determination of polymers, the gel filtration chromatography is a rather simple separation mode as compared to RPLC.

Keywords: Meropenem-polymerized impurities; GFC; LC–MS

1. Introduction

Meropenem is a semi-synthetic 1β-methylcarbapenem antibiotics, which exhibits an extremely broad spectrum of antibacterial activity and is highly stable against renal dehydropeptidase-I (DHP-I). Formulation of meropenem is stable for long time at room temperature, but degraded by free water [1].The degradation products of meropenem in aqueous solution were the β-lactam hydrolyzed product and the dimer product resulting from intermolecular aminolysis of β-lactam ring by the amine of the second molecule [2]. It is of common knowledge that the polymers of β-lactam antibiotics are important with respect to the quality of the product. The separation of amoxicillin and its related substances containing dimer and trimer amoxicillin by a gradient elution on a C_{18} column and the identification of the polymer peaks by comparison of their relative retention times (RT) between amoxicillin and polymers are described in *British Pharmacopoeia* (BP) and *European Pharmacopoeia* (EP) [3,4], and the ampicillin polymers are controlled by the same way [3,4]. But the high molecular weight polymers of cephalosporins including cefotaxime, ceftriaxone, cefoperazone and ceftazidime are controlled by a gel filtration chromatography (GFC) in *Chinese Pharmacopoeia* (ChP) [5]. In addition, the use of high-performance capillary electrophoresis (HPCE) has been reported for the separation and determination of ampicillin polymers [6]. There are some RP-HPLC methods available for the determination of meropenem [7–10]. Meropenem and its open-ring metabolite UK-1a in plasma and dialysate fluids could be determined by a solid-phase extraction (SPE) combining with a HPLC method [9]. The separation of meropenem and its related substances for quality control (QC) is described in Japanese pharmacopoeia [10]. However, although the reported methods could separate the dimer from meropenem product, it is difficult to identify the dimer peak as usually its reference substance is of difficulty obtained and could not address in detail the polymerized impurities of meropenem. In fact, there are no easy ways for its quality control without using polymerized impurity reference substance of meropenem in RP-HPLC methods.

For the separation of high molecular weight impurities of β-lactam antibiotics, a novel Sephadex G-10 gel filtration chromatographic system for determining cephalosporin

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polymers [11] and a Superdex peptide gel filtration chromatographic method for determining amoxicillin polymers [12] have been developed. It is an easy way to find the polymerized impurities by gel filtration chromatography. The aim of the present study was to develop an optimized routine quality control method for separation and analysis of meropenem-polymerized impurities.

2. Materials and methods

2.1. Materials

Meropenem and meropenem reference standards are provided by National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). The acetonitrile used was HPLC grade. All other reagents were analytical grade and came from different commercial suppliers.

2.2. Gel filtration chromatography

The GFC system consisted of two pumps (Waters 510), a UV detector (Waters 486) and a Superdex peptide HR 10/30 column (Pharmacia). The mobile phase was 0.01 mol L^{-1} sodium phosphate buffer (pH 7.0). The flow-rate was 0.8 mL min^{-1}. The elution pattern was recorded by a UV detector set at a wavelength of 220 nm. The injection volume was 50 μL. Chromatographic data analysis was performed by a Millennium 2010 chromatography manager (Waters).

2.3. RPLC–MS

A Waters Alliance HPLC system with a photodiode-array detector (PDA) was coupled with Waters ZMD2000 mass spectrometer equipped with an electro-spray ionization (ESI) probe. The column was Kromosil C_8 (4.6 mm × 200 mm, 5 μm). The mobile phase was 0.1% triethylamine (adjusted to pH 5.0 with acetic acid) (A) and acetonitrile (B). A gradient ran from 0 to 18 min, isocratic elution with ratio A:B of 93:7; 18–25 min, a linear gradient elution with ratio A:B of 80:20; 25–30 min, isocratic elution with ratio A:B of 80:20; 30–40 min, re-equilibration elution with A:B of 93:7. The injection volume was 30 μL. The flow-rate was 1.5 mL min^{-1} and the splitting rate was 4:1. The MS system was operated in negative mode. Nitrogen was used as drying gas (400 L h^{-1}, 200 °C) and sheath gas (92 L h^{-1}). The source temperature was maintained at 120 °C. The ionization voltage was at 3 kV and cone voltage was optimized at 10 or 30 V. The delay time between PDA and mass spectrometer detector was about 0.1 min.

2.4. Sample preparations

Preparation of meropenem polymerized: An amount of meropenem for injections was opened, and then was put under a condition at 75% relative humidity for five days. The sample concentrations (meropenem and meropenem polymerized) for GFC were 10,000 μg/mL. The sample concentration for meropenem reference substance for assay was 30 μg/mL.

Preparation of GFC peak fractions: A column switching system was used according to reference [12]. The meropenem-polymerized sample was first injected onto the Superdex peptide HR 10/30 column. Each peak eluting from the gel column was collected on a C_{18} preparation trap column and subsequently washed by water to remove the phosphate buffer, and then further washed out by acetonitrile to get the peak fraction by applying corresponding position of a switching valve. The acetonitrile in the peak fraction was volatilized out, then the fraction was freeze-dried, and re-injected onto GFC and RPLC–MS systems to confirm the retention times.

2.5. Specificity experiments of GFC regarding meropenem and its polymerized impurities

RPLC–MS test was used to evaluate the specificity of the GFC. The dry GFC fraction elutes were dissolved in 200 μL of acetonitrile–water (15:85). The solution was centrifuged, and the supernatant was used for HPLC–MS analysis.

2.6. Assay of meropenem-polymerized impurities by GFC

The samples of meropenem were degraded under various stress conditions as described in Table 3 and were tested for the content of meropenem. The content of impurities was calculated using meropenem reference standard substance.

3. Results and discussion

3.1. Separation of meropenem-polymerized sample by GFC

When using 0.01 mol L^{-1} sodium phosphate buffer (pH 7.0) as the mobile phase, meropenem-polymerized sample gave five peaks, with resolution values 0.66, 1.13, 1.45 and 1.22, respectively. The relative retention times of each peak were about 0.77, 0.81, 0.87, 0.94 and 1.00, respectively. The

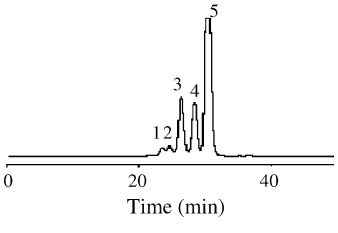

Fig. 1. The chromatogram of meropenem-polymerized sample on Superdex peptide column. Mobile phase: 0.01 mol L^{-1} sodium phosphate buffer (pH 7.0). Peaks 1–4, polymerized impurity; Peak 5, Meropenem.

Table 1
m/z value and retention time of meropenem and its related substances on LC–MS

Peak fraction of GFC	RT on LC (min)	*m/z* value $[M-H]^-$	Possible impurity
1	24.8	1166	Ring-open trimer
	22.5	1166	Ring-open trimer
2	10.5	783	Ring-open dimer
3	10.3	765	Ring-closed dimer
4	2.7	400	Meropenemic acid
5	5.2	382	Meropenem

result indicated that the GFC system might have a good separation for the polymerized impurities (Fig. 1). By series of experiments, it was found that the chromatographic behavior of meropenem on Superdex peptide column mainly depended on size exclusion, and the retention times did not vary significantly with changes in different mobile phase.

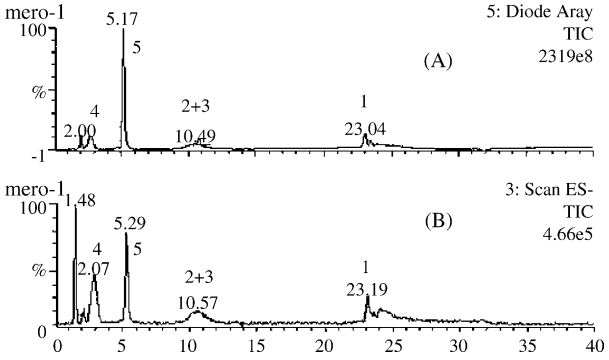

Fig. 2. LC–ESI–MS chromatogram of polymerized meropenem sample. (A) Diode array detection at 220 nm, (B) total ion current detection; the markers of the chromatographic peaks in this figure are the same as in Fig. 1.

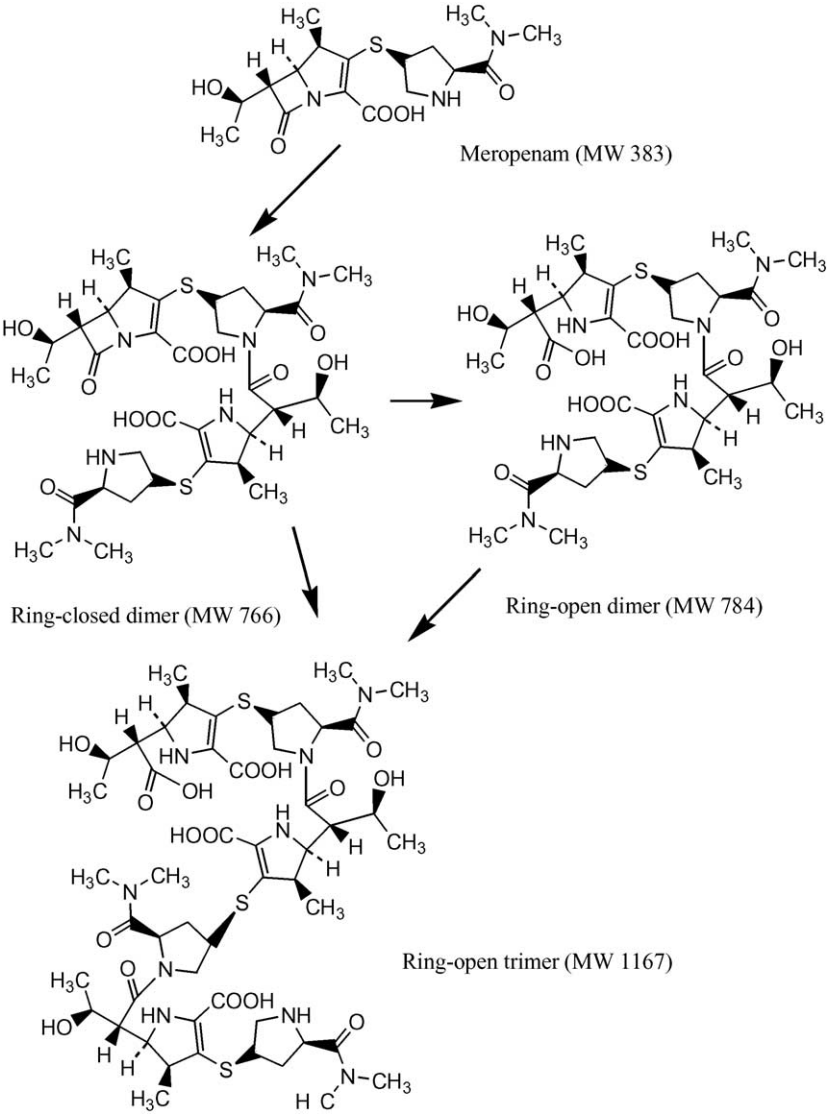

Fig. 3. Possible mechanism of meropenem polymerization.

Table 2
Assay of meropenem-polymerized impurities

Product	Polymerized impurity content (%)	
	GFC	LC–MS
1	0.30	0.38
2	0.23	0.24
3	0.26	0.23
4	0.35	0.38

The analytical procedure was validated. For calculating the linearity, the total number of analyses was 24 in six levels. A calibration curve in the range from 0.08 to 10 μg was tested: a regression equation, $y = 3 \times 10^6 x + 29659$ with y, peak area and x, amount injected in μg, was calculated, with a correlation coefficient $r = 0.9998$ and a relative standard deviation (R.S.D.) = 0.21%; the R.S.D. of slope was 0.78% and the R.S.D. of intercept was about 48% ($n = 6$). For quantification, the detection limit was 0.0024 μg with a signal-to-noise ratio of 3 ($n = 5$) and the quantitation limit was 0.0124 μg with a signal-to-noise ratio of 10 ($n = 5$). The repeatability was tested by analyzing the solution corresponding to 7.5, 0.75 and 0.18 μg; the R.S.D. for the peak area of meropenem was 0.5, 0.6 and 1.5%, respectively ($n = 4$). The R.S.D. for the intermediate precision of the assay of meropenem was 1.6% ($n = 5$). Although ruggedness/robustness of the method has not been evaluated in our laboratory, the Superdex peptide GFC system available in two pharmaceutical quality control laboratories in China were used to carryout these studies. Besides, if a Superdex peptide PE 7.5/300 column was used instead of the Superdex peptide HR 10/30 column, the flow-rate should be changed to 0.4 mL min^{-1} and the injection volume to 20 μL. However, when a TSK G2000SW column was used instead of the Superdex peptide columns, a poor separation was observed.

A 30 μg/mL meropenem reference substance solution was injected serially to the GFC system to check the stability of meropenem solution. After five operations, an impurity peak was found to elute clearly before the meropenem peak in the chromatogram. The result indicates that meropenem solution is unstable. Mendez et al. reported that meropenem in aqueous solution could be stable for 24 h at 4 °C [7]. So all meropenem solutions used for analysis should be freshly prepared and stored at 4 °C before injection.

3.2. Specificity experiments of GFC

As the chromatographic behavior of a compound is affected by size exclusion and adsorption on the gel media [12], the elution order is not exactly according to molecular size. To validate the specificity of GPC, besides to check whether each of the impurity peaks is pure or not, it is important to confirm that all the impurities eluting before the meropenem peak are with a larger molecular size than meropenem. For this purpose, the effluent of each peak on the gel chromatographic system was switched to the C_{18} trap column by column switching trap technology and analyzed by LC–MS. The m/z value and retention times of the effluents of each peak are shown in Table 1. The LC–MS chromatograms of the meropenem-polymerized sample are shown in Fig. 2.

The LC–MS analysis showed the GFC fraction 1 had mainly two peaks at about 24.8 and 22.5 min on the LC system with same m/z value [1166 ($M - 1$)]; the GFC fractions 2 and 3 showed almost same RT at about 10.5 and 10.3 min, respectively. However, these had different m/z values (783 and 765); the RT of GFC fraction 4 was at about 2.7 min with the m/z value of 400; and the GFC fraction 5 was meropenem with a RT of about 5.2 min and the m/z value of 382. Combining the mechanism of meropenem polymerization [2], the possible relative impurities of meropenem found by the GFC are listed in Table 1. The results also showed that in the GFC system, based mostly on size exclusion, all polymer peaks co-eluted in front of the meropenem peak and the polymer peaks are easily identified. Gel filtration chromatography is a versatile separation mode for the determination of polymers. In addition, the gel system is advantageous with regard to environmental protection, as no organic solvents are used as mobile phase components.

As the dimers of meropenem in aqueous solution were resulting from intermolecular aminolysis of β-lactam ring by the amine of the second molecule [2], a possible mechanism of meropenem polymerization was surmised according to the LC–MS analysis (Fig. 3).

Table 3
Assay of meropenem impurities under different stress conditions by GFC

Treated condition	Time (day)	Impurity content (%)					Meropenem content (%)
		Peak 0[a]	Peak 1	Peak 2	Peak 3	Peak 4	
Normal	0	0	0	0.06	0.28	0	99.5
60 °C, 40% RH	10	0	0.18	0.21	0.97	1.36	97.0
	20	0	0.20	0.21	0.82	1.28	97.3
	30	0	0.24	0.30	0.90	1.26	97.1
60 °C, 90% RH	10	33.4	18.5	25.0	5.48	13.3	0
	20	35.0	16.7	23.9	8.48	6.83	0
	30	46.3	8.3	14.7	10.1	5.97	0
60 °C, 40% RH, light	10	0	0	0.02	0.27	0	99.7

[a] Sum of the impurities with a relative RT less than peak 1.

3.3. Assay of meropenem-polymerized impurities by GFC

Meropenem for injections were dissolved in 0.01 mol L^{-1} sodium phosphate buffer (pH 7.0) and its polymerized impurities (sum of the peaks 1–3) were detected by the GFC. The results obtained by the GFC technique are comparable to those obtained with the LC–MS (Table 2).

For understanding the characteristics of the polymerization of meropenem for injection, the samples were kept under different stress conditions, and the polymerized impurities were detected by the GFC (Table 3). The results show the polymerization of meropenem was more facile under high humidity condition indicating the important role played by free water in the samples for polymerization.

4. Conclusion

For analysis of polymerized impurities of meropenem in quality control laboratories, although some RPLC methods [2,7,10] and HPLC–MS technique can be employed, GFC method is highly useful especially in the absence of reference substances. The GFC method, which is simple and environmental friendly, is the best choice for the routine polymer impurity control. The result indicates that the GFC method is superior to RPLC in the analysis of meropenem.

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