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LC/MS/MS method for the determination of trace amounts of cefmetazole and cefpodoxime proxetil contaminants in pharmaceutical manufacturing environments

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Abstract

In this study, a selective and sensitive LC/MS/MS method for the determination of trace amounts of cefmetazole (CMZ) and cefpodoxime proxetil (CPDXPR) contaminants in manufacturing environments was developed. The necessary sensitivity of this method was estimated based on the detection limit for Penicillin G required by the FDA and the total surface area and volume of the manufacturing facility. The detection limits of this method were estimated to be 10 pg/ml for CMZ and 5 pg/ml for CPDXPR from the signal to noise ratio and as a result satisfactory sensitivity was achieved. The method was linear in a concentration range from 0.20 to 3.20 ng/ml. The accuracy and precision were verified by the determination of the amount of CMZ and CPDXPR added to the sampling materials, a glass plate and a silica fiber filter. The mean recoveries of nine replicated determinations from the glass plate were 99.1% with 5.58%R.S.D. for CMZ and 97.1% with 3.80%R.S.D. for CPDXPR, and those from the silica fiber filter were 100.7% with 4.50%R.S.D. for CMZ and 95.4% with 2.85%R.S.D. for CPDXPR. This method has been successfully applied to the determination of CMZ and CPDXPR contaminants in samples collected from an actual manufacturing environment.

Keywords: β-Lactam antibiotic; Cefmetazole; Cefpodoxime proxetil; LC/MS/MS; Contamination

1. Introduction

Serious unexpected side effects due to the contamination with diethylstilbestrol were first observed in the 1960's [1,2]. Since then, cross contamination has been considered to be one of the most important issues in pharmaceutical manufacturing. In particular, highly sensitized or highly potent pharmaceuticals contamination is crucial because they can cause serious side effects even in small amounts. β-Lactam antibiotics, as typified by penicillins and cephalosporins, have been known to cause serious anaphylaxis in some cases. Thus, non-β-lactam pharmaceuticals contaminated by β-lactam antibiotics are considered to be potential agents of the induction of unexpected anaphylactic shock. The β-lactam antibiotics contamination should therefore be prevented to the greatest extent possible.

In order to prevent contamination, the FDA requires pharmaceutical manufacturers to establish validated analytical methods for the determination of pharmaceutical residues in manufacturing facilities, as well as adequate cleaning procedures for manufacturing equipment [3]. An analytical method for the determination of sensitizing β-lactam antibiotics contaminants in manufacturing facilities should be sensitive enough to determine trace amounts in order to verify contamination at a very low level. In general, the HPLC-UV [4–8] and the total organic carbon (TOC) [9,10] methods have been used for the determination of pharmaceutical residues in cleaning validations. However, UV detection is not sufficiently sensitive and TOC is not selective for the monitoring of sensitizing β-lactam antibiotic contaminants. As a highly sensitive analytical method, luminol chemiluminescence with flow injection analysis was developed for β-lactam antibiotic residue monitoring [11]. This method gives a quantitative specific response related to the β-lactam structure. In the area of food hygiene, many selective and sensitive LC/MS methods have been reported

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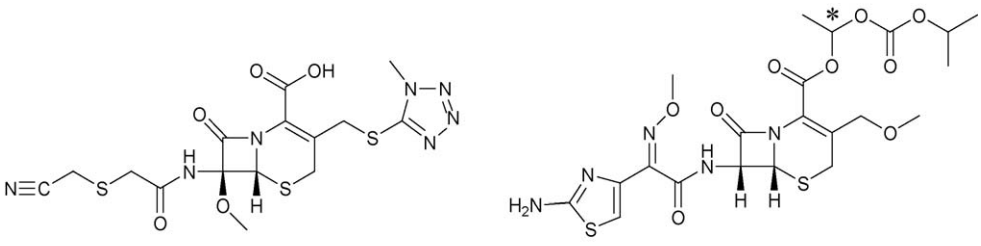

Cefmetazole (CMZ)

Cefpodoxime proxetil (CPDXPR)

* A mixture of *(R)* and *(S)* isomer

Fig. 1. Chemical structures of CMZ and CPDXPR.

for the determination of β-lactam antibiotic residue in bovine milk and tissues [12–15]. The selectivity and sensitivity of the LC/MS method is considered suitable and effective for the determination of trace amounts of β-lactam antibiotic contaminants in pharmaceutical manufacturing environments as well.

The semi-synthetic β-lactam antibiotics cefmetazole (CMZ) and cefpodoxime proxetil (CPDXPR) have a broad spectrum against gram positive and negative bacteria, and are widely used for infectious diseases [16,17]. The pharmaceutical products of CMZ and CPDXPR are manufactured with other non-β-lactam pharmaceutical products in the same plant, though in different manufacturing facilities.

In this study, we therefore developed a selective and highly sensitive analytical method using LC/MS/MS for the determination of CMZ and CPDXPR contaminants. The amount of CMZ and CPDXPR contaminants in a manufacturing environment should be verified at a very low and precise level. In developing this method, the allowable contaminant amounts of CMZ and CPDXPR in the manufacturing environment were estimated based on the FDA requirements [18] and determined the necessary sensitivity of the method.

2. Experiment

2.1. Chemicals and reagents

The cephamycin antibiotic CMZ and the cephalosporin antibiotic CPDXPR used in this study were synthesized by Sankyo Co. Ltd., Japan. The chemical structures of these compounds are shown in Fig. 1. CPDXPR was developed as an esterified prodrug and is a mixture of two diastereomers arising from the stereogenic center at the ester moiety. Manufactured by Sankyo Co. Ltd., the hypertension agent olmesartan medoxomil (OLM) and its drug products were used as the non-β-lactam pharmaceutical.

Acetonitrile and water of HPLC grade and acetic acid of guaranteed grade were purchased from Wako Pure Chemical Industries Ltd., Japan and formic acid of guaranteed grade was purchased from Nacalai Tesque Co., Japan.

2.2. Materials for sampling β-lactam antibiotics contaminant

For sampling CMZ and CPDXPR contaminants, a glass plate (76 mm × 26 mm; 19.76 cm^2, Matsunami Glass Ind. Ltd., Japan) was used after being rinsed with acetonitrile.

For sampling fine CMZ and CPDXPR particles in the air, an ADVANTEC QR-100 silica fiber filter (55 mm in diameter, TyoRoshi Kaisha Ltd., Japan) was used. Before being used, the filters were placed in a furnace at 600 °C for 1 h to reduce the interference of any organic chemicals on the detection of CMZ and CPDXPR.

2.3. HPLC

An Agilent 1100 HPLC system (Agilent Technologies, Japan) consisting of an online degasser G1379A, a binary pump G1312A, an autosampler G1329A, a column compartment G1316A and a diode array detector G1315B, was used.

The separation was performed using a column for reversed phase chromatography with a Develosil C-30-UG-3 (Nomura Chemical Co. Ltd., Japan) as the analytical column. A column of 150 mm in length with an inside diameter of 4.6 mm was used for the optimization of the separation and one with an inside diameter of 2.0 mm was used for the LC/MS/MS analysis. A mixture of water, acetonitrile and formic acid or acetic acid was used as the mobile phase. Aliquots of 20 μl of the analytical solutions were injected into the system and separation was achieved with the analytical column kept at a constant temperature of 40 °C.

2.4. Mass spectrometer

A TSQ 7000 triple quadrupole mass spectrometer (Thermo Electron K.K., Japan) equipped with an electrospray ionisation source was used and operated in positive ESI mode. The capillary applied voltage was set at 4.5 kV. Nitrogen was provided for the sheath gas at a pressure of 70 psi and for the auxiliary gas at a flow rate of 40 arbitrary units. The heated capillary was maintained at 200 °C. Argon was used as the collision gas, and

the magnetic field of the gas cell was adjusted to 2 mT for the MS/MS condition.

2.5. Sampling of actual samples in manufacturing environment

CMZ and CPDXPR contaminants in the manufacturing environment were collected with glass plates and silica fiber filters. The glass plates were placed at sampling points in the manufacturing facility for specified periods to collect any CMZ and CPDXPR contaminants.

The atmosphere of the manufacturing facility was aspirated with an air sampler at a rate of 15 l/min for 3 h (corresponding to 2700 l) to collect fine CMZ and CPDXPR particles in the air.

2.6. Preparation of the sample solutions for the validation study

CMZ of 20 mg and CPDXPR of 10 mg each were dissolved in 100 ml of acetonitrile. The solution was diluted with acetonitrile to 2 ng/ml for CMZ and 1 ng/ml for CPDXPR. 0.1 ml of the solution was deposited onto a glass plate or a silica fiber filter placed in a centrifuge tube. After they had dried completely, a 20 ml mixture of water and acetonitrile (55:45, v/v) was added to the centrifuge tube and the CMZ and CPDXPR were dissolved completely. Then this solution was used for the determination of the detection limit. For linearity, solutions of concentrations in the range of 0.20–3.20 ng/ml were prepared and used. CMZ and CPDXPR of 40 mg were each dissolved in 100 ml of acetonitrile. The solution was diluted with a mixture of acetonitrile and water (55:45, v/v) to obtain the above concentrations. To demonstrate the accuracy, the solutions containing 4, 8 and 16 ng of CMZ and CPDXPR were added to a glass plate or a silica fiber placed in a centrifuge tube. For the precision, the solution containing 8 ng of CMZ and CPDXPR were added to the sampling materials placed in a centrifuge tube. Then, the amounts of CMZ and CPDXPR in the sample solution were determined.

2.7. Preparation of the sample solution for the actual sample

A glass plate was transferred into a centrifuge tube. A 20 ml mixture of water and acetonitrile (55:45, v/v) was added to the centrifuge tube, and the collected CMZ and CPDXPR on the glass plate were dissolved. Then this solution was used as the sample solution.

A silica fiber filter was then transferred into a centrifuge tube. To the centrifuge tube a 20 ml mixture of water and acetonitrile (55:45, v/v) was added. The CMZ and CPDXPR trapped on the silica fiber filter were dissolved by shaking vigorously and then this solution was used as the sample solution.

2.8. Preparation of the standard solutions

CMZ and CPDXPR of 40 mg each were weighed and dissolved in 100 ml of acetonitrile. The solution was diluted with a mixture of acetonitrile and water (55:45, v/v) to obtain a concentration of 0.4 ng/ml.

3. Results and discussion

3.1. Estimation of the allowable contaminant amounts and required sensitivity of the analytical method

The allowable contaminant amounts of CMZ and CPDXPR in the manufacturing environment and the required sensitivity of the analytical method were estimated based on FDA requirements, as stated in Human Drug CGMP Notes (Vol. 7, No. 1), and the total surface area and total volume of the manufacturing facility. Furthermore, the sensitizing potency of both CMZ and CPDXPR were assumed to be the same as that of Penicillin G for this estimation.

According to Human Drug CGMP Notes (Vol. 7, No. 1), the FDA requires the detection of Penicillin G at a level of 0.03 ppm as the violative amount, and that this should be verified with an analytical method as sensitive as 0.006 ppm for the limit of detectability. The facility manufacturing the OLM tablets was 3554 m^2 in cumulative surface area and 3471 m^3 in total volume. The minimum manufacturing amount of the OLM tablets per batch in this facility is about 500 kg. Therefore, in a case where the entire amount of the CMZ or CPDXPR contaminants in the manufacturing environment was taken into the OLM tablets during the manufacturing process, the allowable amount of contaminants in the manufacturing environment was calculated to be 21.2 mg for CMZ and 25.0 mg for CPDXPR, as equivalent to 0.03 ppm of Penicillin G. The sensitivity required for the analytical method to determine the contamination level was calculated to be 4.2 mg for CMZ and 5.0 mg for CPDXPR as equivalent to 0.006 ppm of Penicillin G. The results of the estimated limits are summarized in Table 1. Therefore, we have developed a sensitive analytical method with the ability to detect 0.117 ng/ml of CMZ and 0.139 ng/ml of CPDXPR and for the assay 0.589 ng/ml of CMZ and 0.695 ng/ml of CPDXPR in the sample solution.

Table 1
Estimation of allowable contaminant amounts and required sensitivity of the analytical method

	CMZ	CPDXPR
Violative amount (mg)[a]	21.2	25.0
As glass plate (ng)[b]	11.787	13.900
As silica fiber filter (μg)[c]	16.491	19.447
As sample solution (ng/ml)[d]	0.589	0.695
Required detection limit (mg)[e]	4.2	5.0
As glass plate (ng)[b]	2.335	2.780
As silica fiber filter (μg)[c]	3.267	3.889
As sample solution (ng/ml)[d]	0.117	0.139

[a] Equivalent to 0.03 ppm of Penicillin G, 500 kg × 0.03 ppm × (molecular weight of CMZ or CPDXPR/molecular weight of Penicillin G).

[b] (Violative amount or detection limit/(3554 m^2 × 10000 cm^2/m^2)) × 19.76 cm^2.

[c] (Violative amount or detection limit/(3471 m^3 × 1000 l/m^3)) × 2700 l.

[d] Concentration of sample solution for the glass plate.

[e] Equivalent to 0.006 ppm of Penicillin G, 500 kg × 0.006 ppm × (molecular weight of CMZ or CPDXPR/molecular weight of Penicillin G).

3.2. Optimization of the HPLC condition

The separation of each of the CMZ, CPDXPR and OLM compounds was investigated by using either acetic acid or formic acid and by changing the ratio of acetonitrile in the mobile phase. To a mixture of 500 ml acetonitrile and 500 ml of water, 1 ml of acetic acid or formic acid was added, and the effect of the acids on the separation of each compound was investigated. In the mobile phase containing acetic acid, one of the diastereoisomer peaks of CPDXPR did not separate from the OLM peak. On the other hand, in the mobile phase containing formic acid, good separation for all the peaks was achieved. Further investigation of the separation was performed by changing the ratio of acetonitrile in the mobile phase in order to avoid the influence of an OLM peak on the detection of trace amounts of CMZ and CPDXPR. As a result, the mixture of water, acetonitrile and formic acid at a ratio of (550:450:1, v/v/v) gave sufficient separation for all compounds. The chromatograms obtained by monitoring with a UV detector are shown in Fig. 2.

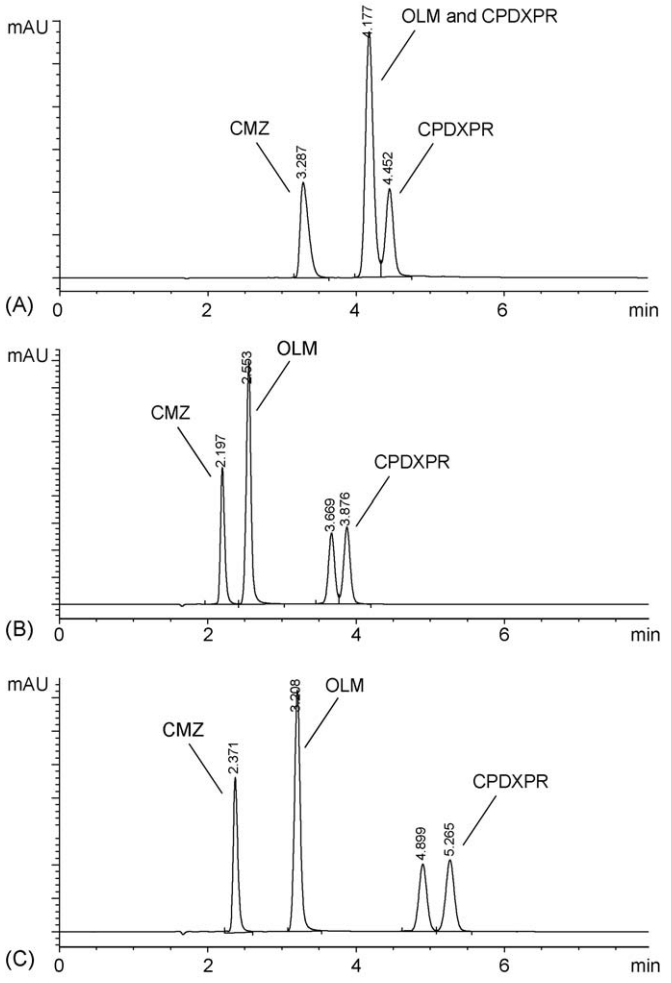

Fig. 2. Effect of the mobile phase composition on the separation of OLM, CMZ and CPDXPR monitored by UV detection. Mobile phase: (A) a mixture of acetonitrile, water and acetic acid (500:500:1, v/v/v), (B) a mixture of acetonitrile, water and formic acid (500:500:1, v/v/v), and (C) a mixture of acetonitrile, water and formic acid (550:450:1, v/v/v).

3.3. Optimization of selected reaction monitoring conditions

A selected reaction monitoring (SRM) method was chosen and investigated to achieve the necessary selectivity and sensitivity of the method. First, the MS and MS/MS spectra of CMZ and CPDXPR were measured, and the precursor ions and product ions to be monitored were selected. In the MS spectrum of CMZ, a protonated molecule was observed at *m/z* 472, and the highest intensity fragment ion was observed at *m/z* 356 in its MS/MS spectrum. Regarding CPDXPR, a protonated molecule at *m/z* 558 was observed in the MS spectrum, and the highest intensity fragment ion was observed at *m/z* 410 in its MS/MS spectrum. The MS/MS spectra of CMZ and CPDXPR are shown in Figs. 3 and 4, respectively. From these results, the protonated molecule at *m/z* 472 for CMZ and that at *m/z* 558 for CPDXPR were selected as the precursor ions. For the product ions, the fragment ions at *m/z* 356 for CMZ and those at *m/z* 410 for CPDXPR were selected for SRM. Since the collision energy

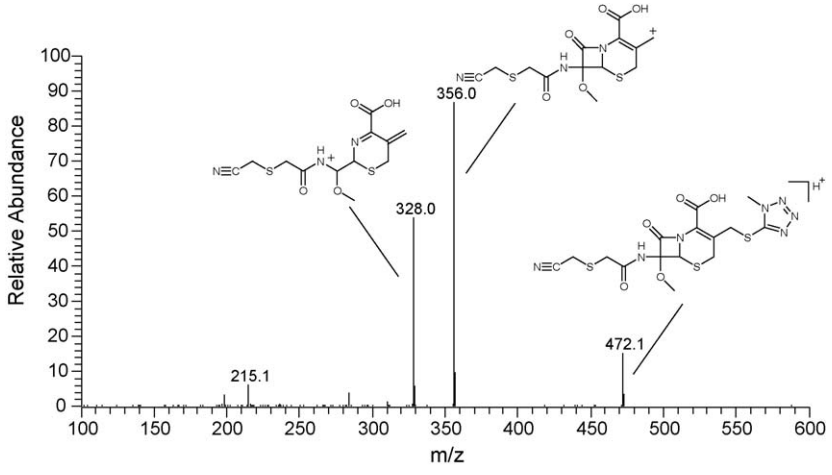

Fig. 3. MS/MS spectrum of CMZ.

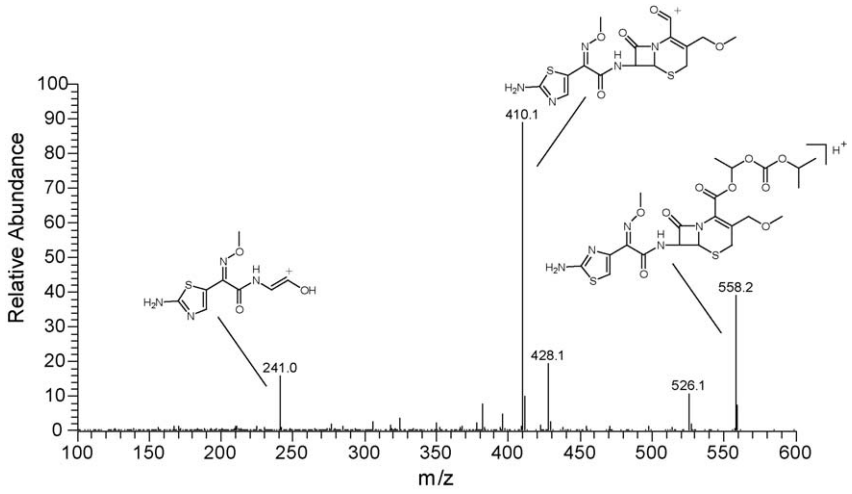

Fig. 4. MS/MS spectrum of CPDXPR.

of 11 eV for CMZ and 17 eV for CPDXPR gave the highest intensity of production signals, those values were selected. The established SRM conditions are presented in Table 2.

3.4. Optimization of sampling procedure

Swab sampling and rinse sampling are generally used as the sampling techniques for collecting pharmaceutical residues on manufacturing equipment in cleaning verification. However, in swab sampling the matrices of the swab material and the adsorption of the analyte to the swab material can sometimes result in poor recovery, whereas in rinse sampling there are technical difficulties in collecting all of the rinsing solvent used for the sampling. Furthermore, these direct sampling techniques are affected by various materials in the manufacturing facilities, such as paint agents on the wall, wax on the floor, etc. Thus, in many cases direct sampling techniques are difficult to utilize efficiently for sensitive residue analysis. Therefore, a clean glass plate was used for collecting the CMZ and CPDXPR contaminants in the manufacturing environment by placing it for specified periods at certain sampling points in the facility. Using this sampling technique, no interference from the matrices of the sampling materials or facilities was noted and a satisfactory recovery of the analyte was achieved. Furthermore, in a manufacturing environment contamination is considered to occur via the air and therefore the monitoring of fine CMZ and CPDXPR particles in the atmosphere was also performed. The air of the manufacturing facility was aspirated by an air sampler at a rate of 15 l/min for 3 h (corresponding to 2700 l), and the fine particles of CMZ and CPDXPR were trapped on the silica fiber sampling filter.

Table 2
SRM parameters for determination of CMZ and CPDXPR

Parameter	Setting
Spray voltage	4.5 kV
Heated capillary	200 °C
Sheath gas	70 psi
Auxiliary gas	40 arbitrary unit
SRM trace for CMZ (segment 0.00–3.50 min)	Precursor ion 472 $m/z \rightarrow$ product ion 356 m/z
	Collision energy 11 eV
SRM trace for CPDXPR (segment 3.50–7.00 min)	Precursor ion 558 $m/z \rightarrow$ product ion 410 m/z
	Collision energy 17 eV

3.5. Validation of the analytical method

3.5.1. Specificity, detection limit and linearity

The interference of the sampling materials, glass plate and silica fiber filter in the detection of CMZ and CPDXPR was verified. As a result, no interference peaks were observed in the detection of the CMZ and CPDXPR peaks from either of the sampling materials. The detection limit was estimated from the signal to noise (*S*/*N*) ratio by the determination of diluted CMZ and CPDXPR solutions. The detection limit (*S*/*N* 3) was 10 pg/ml for CMZ and 5 pg/ml for CPDXPR, and the estimated detection limit was confirmed with spiked samples. The chromatograms obtained are shown in Fig. 5. As shown in Table 1, the detection limit required for the present analytical method was estimated to be 0.117 ng/ml for CMZ and 0.139 ng/ml for CPDXPR (equivalent to 0.006 ppm of Penicillin G). Thus, the detection limit obtained for this method clearly demonstrated satisfactory sensitivity.

The linearity of the peak area responses versus the concentrations was verified in concentrations ranging from about 0.20 to 3.2 ng/ml, which covers the violative amounts of CMZ and CPDXPR (equivalent to 0.03 ppm of Penicillin G). The relation between the peak area response and the concentrations (ng/ml) was subjected to linear regression analysis. As a result, the linear regression curves of $y = 2791.5x - 46.5$ with a correlation coefficient of 0.9992 for CMZ and $y = 18766.5x - 121.0$ with a correlation coefficient of 0.9999 for CPDXPR were obtained.

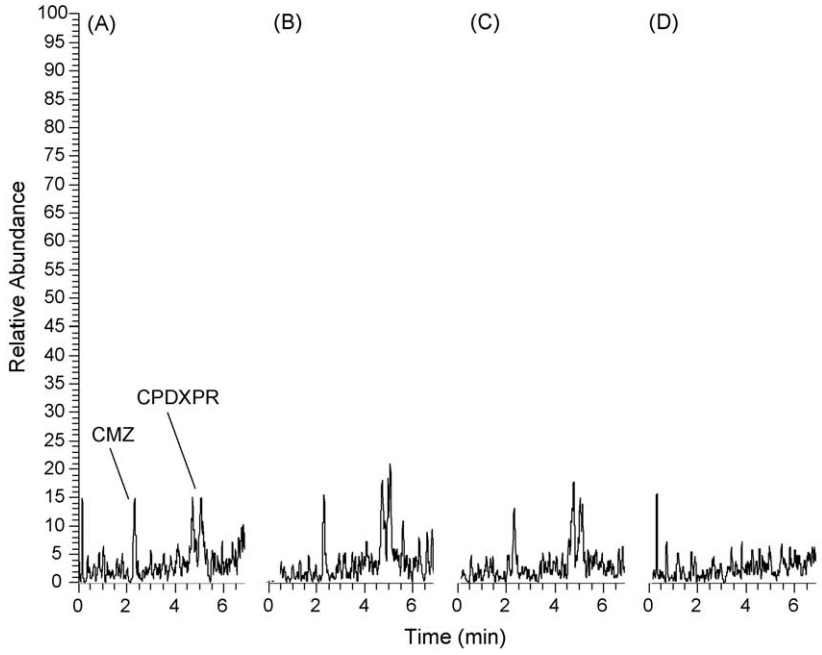

Fig. 5. Chromatograms at the concentration of limit of detection for the standard solution (A), the sample solution of the glass plate (B), the sample solution of the silica fiber filter (C) and the diluent (D).

3.5.2. Accuracy and precision

The accuracy of the method was evaluated by determining the recovery of CMZ and CPDXPR at three concentration levels around the violative amount of the glass plate sample. Although the violative amount of the silica fiber filter sample was higher than that at these concentration levels, the recovery of CMZ and CPDXPR was also evaluated at the same low amount level. The results are shown in Table 3 for CMZ and in Table 4 for CPDXPR.

Separately, solution containing 8 ng of CMZ and CPDXPR was added to the sampling materials and their amounts were determined in order to evaluate the precision of this method. A total of six sets of the sample solution and the standard solution were prepared and determined within a day. The results obtained were 8.07 ± 0.293 ng S.D. for CMZ and 8.03 ± 0.497 ng S.D. for CPDXPR on the glass plate, and 8.00 ± 0.333 ng S.D. for CMZ and 7.98 ± 0.580 ng S.D. for CPDXPR from the silica fiber filter. Consequently, the results demonstrated that the method developed was sufficiently accurate and precise for the determination of CMZ and CPDXPR in the sampling materials.

3.5.3. Stability of the analytical solutions

The sample solution and the standard solution were stored at room temperature and the amounts of CMZ and CPDXPR

Table 3
Recoveries of CMZ from sampling materials

Glass plate (ng/19.76 cm^2)			Silica fiber filter (ng/2700 l)		
Added	Observed	Recovery (%)	Added	Observed	Recovery (%)
4.07	4.07	100.0	4.07	4.45	109.3
4.02	4.28	106.5	4.02	4.17	103.7
4.14	3.73	90.1	4.14	4.34	104.8
8.13	8.40	103.3	8.13	8.15	100.2
8.04	8.33	103.6	8.04	7.77	96.6
8.27	8.51	102.9	8.27	8.03	97.1
16.26	15.43	94.9	16.26	15.53	95.5
16.09	15.70	97.6	16.09	15.77	98.0
16.54	15.40	93.1	16.54	16.74	101.2
Mean (%)		99.1			100.7
Standard deviation		5.53			4.53
R.S.D. (%)		5.58			4.50
95% confidence interval of mean					
Lower limit		94.9			97.2
Upper limit		103.4			104.2

Table 4
Recoveries of CPDXPR from sampling materials

Glass plate (ng/19.76 cm^2)			Silica fiber filter (ng/2700 l)		
Added	Observed	Recovery (%)	Added	Observed	Recovery (%)
4.07	4.22	103.7	4.07	3.97	97.5
4.04	4.01	99.3	4.04	3.83	94.8
4.01	3.68	91.8	4.01	3.89	97.0
8.14	7.62	93.6	8.14	7.99	98.2
8.08	7.77	96.2	8.08	7.53	93.2
8.01	7.66	95.6	8.01	7.21	90.0
16.29	15.48	95.0	16.29	15.58	95.6
16.15	15.80	97.8	16.15	15.21	94.2
16.03	16.12	100.6	16.03	15.75	98.3
Mean (%)		97.1			95.4
Standard deviation		3.69			2.72
R.S.D. (%)		3.80			2.85
95% confidence interval of mean					
Lower limit		94.2			93.3
Upper limit		99.9			97.5

were periodically determined in order to evaluate the stability of the solutions. The deviation in peak area response after storage for 8 h was −4.0% for CMZ and −8.4% for CPDXPR in the standard solution, −0.5% for CMZ and +2.4% for CPDXPR in the sample solution on the glass plate, and +5.3% for CMZ and −0.9% for CPDXPR in the sample solution on the silica fiber filter. These results indicated that the analytical solutions were stable for least 8 h under the above conditions.

3.6. Determination of CMZ and CPDXPR contaminants in a manufacturing environment

Actual samples collected at an environment for the manufacturing of OLM tablets were subjected to a determination of any CMZ or CPDXPR contaminants. Sampling was conducted in the manufacturing facility's change room, pass room, weighing room, pulverizing room, tabletting room, coating room, IPC room, doorway, and air inlet. The amounts of CMZ and

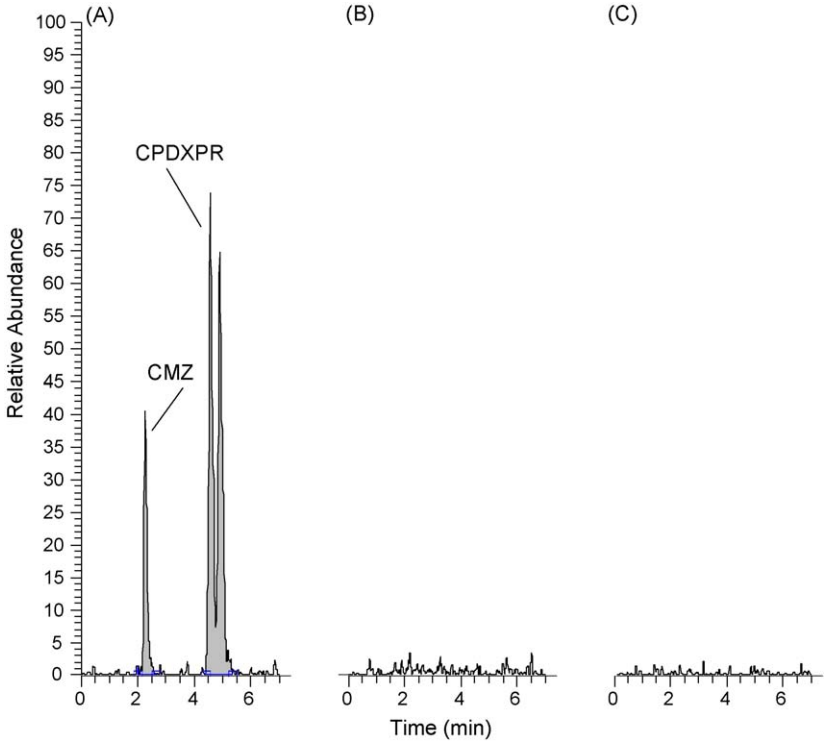

Fig. 6. Typical chromatogram of the standard solution (A), the sample solution of the glass plate (B) and the sample solution of the silica fiber filter (C) in the facility for manufacturing OLM tablets.

CPDXPR in these samples were determined using this validated method. This operation was conducted 5 times and no detectable CMZ or CPDXPR were found in any samples. The typical chromatograms are shown in Fig. 6. Therefore, it was verified that no CMZ and CPDXPR contamination had occurred in the facility for producing OLM tablets.

4. Conclusion

In this study, a selective and sensitive LC/MS/MS method for the determination of trace amounts of CMZ and CPDXPR contaminants in a manufacturing environment was established. The sensitivity of the analytical method required for detecting β-lactam antibiotic CMZ and CPDXPR contaminants in the manufacturing environment was estimated based on FDA requirements and the total surface area and volume of the manufacturing facility. The detection limits of the method obtained were 10 pg/ml for CMZ and 5 pg/ml for CPDXPR and as a result satisfactory sensitivity was achieved. Sufficient accuracy and precision of the method developed were also demonstrated and the method was verified to be efficacious. Utilizing this method, it would be possible to detect trace amounts of β-lactam antibiotics such as CMZ and CPDXPR in a manufacturing environment and to verify contamination in very small amounts.

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