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Short communication

Determination of cefmetazole residue at pharmaceutical manufacturing facilities by chemiluminescence flow injection analysis

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Abstract

Application of a sensitive and rapid flow injection analysis (FIA) method with luminol chemiluminescence detection for determination of trace amounts of cefmetazole (CMZ) in cephamycin antibiotic residue in pharmaceutical manufacturing facilities and on pharmaceutical manufacturing equipment has been investigated. The method was shown to be sensitive at a level of limit of detection of 0.06 ng/ml and for linear concentrations in the range of 0.3–1.5 ng/ml. Average recoveries of CMZ from stainless steel plates and glass plates were 62.1% and 60.1%, respectively, by adding 15 ng/100 cm^2, and that of air sampling filters was 91.9% by adding 3 ng/filter. The proposed method has been successfully applied to the determination of CMZ residue in samples collected from an actual manufacturing facility and equipment. According to the results, no detectable CMZ residue was observed, therefore it was verified that no contamination had occurred to other pharmaceutical products manufactured in the facility.

Keywords: β-Lactam antibiotic; Cefmetazole; Contamination; Flow injection analysis; Luminol chemiluminescence

1. Introduction

As guidelines related to cleaning validation and cross-contamination have been published [1–5], cross-contamination is a critical issue in the manufacturing of pharmaceuticals. In particular, contaminated pharmaceuticals with highly potent or highly sensitized pharmaceuticals may cause unexpected serious side effects [6]. β-Lactam antibiotics, as typified by penicillin and cephalosporin, are known to cause severe anaphylaxis in some cases [7–9]. Therefore, anaphylaxis may be unexpectedly induced by administration of non-β-lactam pharmaceuticals with β-lactam antibiotic contamination.

Since contamination occurs during the manufacturing process with pharmaceutical residue in the manufacturing environment or on equipment, it is important to establish certain cleaning procedures for manufacturing equipment and an adequate analytical method for determination of pharmaceutical residues. For detection of sensitizing β-lactam antibiotic residues, in particular, an effective cleaning procedure and a sensitive analytical method are necessary and have to be employed. Furthermore, many sampling points of the manufacturing facility and the manufacturing equipment have to be tested for verifying occurrence of contamination. For these reasons, an analytical method for residue monitoring should also be rapid and simple analysis.

An analytical method of HPLC with UV detection is a generally used for determination and monitoring of pharmaceutical residues on manufacturing equipment in cleaning validation [10–13]. And for β-lactam antibiotic determination, HPLC with UV or fluorescence detection [14–16] has also been reported with respect to food hygiene. However, for monitoring β-lactam antibiotic residue in manufacturing facilities, UV detection is not sufficient in sensitivity and fluorescence detection is not appropriate for rapid analysis because of its complicated sample preparation. From the point of view of sensitivity, detection with MS and chemiluminescence has an advantage over those methods in general. LC/MS methods [17–19] have been developed for residue analysis of β-lactam antibiotics in bovine milk and tissues. Determination of β-lactam antibiotics with luminol chemiluminescence has also already been reported [20,21]. The mechanism of the chemiluminescence consists of the reaction

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between luminol and oxidized species that are generated during degradation of β-lactam antibiotics in alkaline solution, and its quantitative response has been demonstrated [21]. In those two reports, luminol chemiluminescence was studied with flow injection analysis (FIA). FIA with chemiluminescence detection could be used for sensitive and rapid analysis, and thus application of this method is considered suitable for residue monitoring of trace amounts of β-lactam antibiotics.

Cefmetazole (CMZ), a semisynthetic derivative cephamycin antibiotic, has a broad spectrum of activity against gram-positive, gram-negative and anaerobic bacteria, is widely used for treating infectious diseases [22–24], is a representative β-lactam antibiotic of Sankyo Co. Ltd., Japan, and is manufactured at one of our plants. Analytical methods for determination of CMZ in serum by HPLC with UV detection have been reported [25–28]; a sensitivity of 1.3 μg/ml using a column switching technique [26] and that of 20 ng/ml with microbore HPLC [28] was achieved. However, as described above, a sensitive analytical method should be employed for monitoring of β-lactam antibiotic residues, and the chemiluminescence method is expected to be more sensitive than those methods. In the present study, therefore, application of luminol chemiluminescence FIA as a highly sensitive and rapid method for determination of trace amounts of CMZ residue at manufacturing facilities was investigated. The contamination of CMZ to other pharmaceuticals was also verified with the method developed.

2. Experimental

2.1. Materials

2.1.1. Chemicals and reagents

CMZ was synthesized by Sankyo Co. Ltd., Japan. Its chemical structure is shown in Fig. 1. Luminol sodium salt used for chemiluminescence detection was purchased from Sigma–Aldrich Japan K.K. Potassium hexacyanoferrate(III) was purchased from Merck Ltd., Japan. Potassium hexacyanoferrate(II)·3H_2O, and sodium hydroxide of guaranteed reagent grade were purchased from Kanto Kagaku, Japan. Acetonitrile of HPLC grade was purchased from Wako Pure Chemical Industries Ltd., Japan.

2.1.2. Materials for sampling of CMZ residue in pharmaceutical manufacturing environment

For collecting CMZ residue on equipment surfaces, BEMCOT® 15 cm × 15 cm swabs (Asahi Kasei Corp., Japan) were used. The swabs were washed with a mixture of water and acetonitrile (2:1, v/v) with sonication for 30 min before usage, and the excess solvent was squeezed out. This washing procedure was repeated three times. And then, the washed swabs were filtered with a glass filter to remove any remaining excess solvent by suction filtration.

For sampling CMZ fine particles in air, ADVANTEC QR-100 silica fiber filters (55 mm o.d., Toyo Roshi Kaisha Ltd., Japan) were used. Before usage, the filters were placed in a furnace at 600 °C for 30 min to reduce interference of any organic chemicals for detection of CMZ.

Powder free Saniment polyethylene gloves (AS ONE Corp., Japan) were used and worn for swabbing and handling of washed swabs. The gloves were immersed in a mixture of water and acetonitrile (2:1, v/v) for 30 min to remove any interference from the chemicals of the gloves for detection of CMZ prior to use.

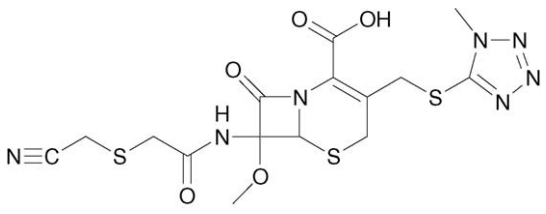

Fig. 1. Chemical structure of cefmetazole.

2.2. Apparatus

A schematic diagram of the FIA system used in this study is shown in Fig. 2. The system consisted of an ERC-3125α online degasser (ERC Inc., Japan), DP-8020 pumps (TOSOH Corp., Japan) for delivering the reaction solutions, a Rheodyne Model 7125 injector, a reaction coil made by PEEK tubing of 300 cm in length, 0.5 mm inside diameter, a 860-CO column oven (JASCO Corp., Japan), and a CL-1525 chemiluminescence detector (JASCO Corp., Japan).

The flow rate of the solutions for the chemiluminescence reaction, degassed with the online degasser, was adjusted to 0.15 ml/min. A portion of 10 μl of each analytical solution was injected into the system and the reaction was carried out with a reaction coil maintained at a constant temperature of 60 °C in the oven. The chemiluminescence of the reaction of the β-lactam antibiotics injected with luminol was measured with the chemiluminescence detector.

2.3. Procedure for sampling of CMZ residue at manufacturing facility

Residue of CMZ on manufacturing equipment surfaces was collected by swabbing with washed BEMCOT® swabs. Each swab was folded into four, and then an area of 100 cm^2 of the manufacturing equipment surface was swabbed horizontally five times and vertically five times. The swabbed sample was

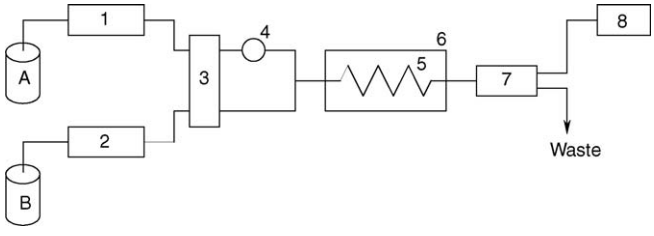

Fig. 2. Schematic diagram of FIA system. (1) Pump 1, (2) pump 2, (3) degasser, (4) injector, (5) reaction coil 300 cm, (6) oven 60 °C, (7) chemiluminescence detector, (8) integrator, (A) a mixture of 0.075 mM potassium hexacyano ferrate(III) and acetonitrile (1:1 v/v), and (B) 0.2 mM luminol solution containing 200 mM sodium hydroxide and 50 mM potassium hexacyano ferrate(II).

transferred into a centrifuge tube. Separately, CMZ fine particles in the air were collected by filtering the air of the facility at a rate of 15 l/min for 3 h (equivalent to 2700 l), as well as CMZ trapped on the air sampling filters. Then, the air sampling filters were transferred into centrifuge tubes.

2.4. Preparation of sample solutions

For the validation study, CMZ was dissolved in a mixture of water and acetonitrile (3:1, v/v). This solution was diluted with a mixture of water and acetonitrile (3:1, v/v) to concentrations in the range of 0.3 to 10.0 ng/ml.

Actual samples of the BEMCOT® swabs and air sampling filters were transferred into centrifuge tubes, respectively. Ten milliliters of a mixture of water and acetonitrile (3:1, v/v) was added accurately. The centrifuge tubes were shaken vigorously for 1 min for extraction of the CMZ. The resultant supernatants were used as the sample solutions for determination of any CMZ residue. Separately, CMZ of 50 mg was weighed and dissolved in acetonitrile. This solution was diluted with a mixture of water and acetonitrile (3:1, v/v) to prepare a 0.3 ng/ml solution as the standard solution.

3. Results and discussion

3.1. Optimization of chemiluminescence reaction

The chemiluminescence between β-lactam antibiotics and luminol is induced by oxidation of luminol with the superoxide species that are generated along with the decomposition of the β-lactam structure in alkaline solution [21]. The β-lactam antibiotic CMZ has a β-lactam structure, and therefore is supposed to shows chemiluminescence with the same mechanism.

In this study, 0.075 mM potassium hexacyanoferrate(III), and 0.2 mM luminol solution containing 200 mM sodium hydroxide and 50 mM potassium hexacyanoferrate(II) were used as reaction solutions, in accordance with a previous report [21]. Due to the solubility of CMZ in aqueous solution, acetonitrile was added to the potassium hexacyanoferrate(III) solution in the ratio of 1:1 (v/v) to avoid precipitation.

It is known that β-lactam antibiotics are hydrolyzed in both acidic and alkaline solutions with decomposition of the β-lactam structure [29]. To obtain strong and reproducible chemiluminescence in the chemiluminescence reaction, it is important to prevent degradation of the β-lactam structure before the chemiluminescence reaction. Therefore, the analytical solutions were injected into the potassium hexacyanoferrate(III) solution.

3.2. Method validation

3.2.1. Limit of detection (LOD), limit of quantitation (LOQ) and linearity

LOD and LOQ were estimated from the standard deviation of the peak area obtained by replicate injections of diluted CMZ solution. An LOD (3.3S.D.) of 0.06 ng/ml and an LOQ (10S.D.) of 0.18 ng/ml were obtained. The sensitivity achieved by this method was better than the reported method for CMZ [25–28] and the HPLC-UV method used for residue monitoring in general [10–13].

The linearity between concentration and peak area response was evaluated using solutions of concentrations in the range of 0.3 to 1.5 ng/ml. According to the results of linear regression analysis, the corresponding linear regression equation was $y = 29.33x + 120.60$, and the correlation coefficient was 0.9990. Consequently, good sensitivity and linearity of the method were demonstrated.

It is known that the intensity of chemiluminescence is affected by the coexistence of other chemicals. In residue monitoring, the method is applied for swabbed samples of manufacturing facilities and equipment, which have been cleaned with a validated appropriate cleaning procedure, and for fine particles in the air of manufacturing facilities trapped in sampling filters. It is considered that no other chemicals would exist other than CMZ residue. Therefore, reproducible sensitivity of the method would be obtained when it is applied for residue monitoring.

3.2.2. Accuracy and repeatability

To evaluate the accuracy and repeatability of the swabbing procedure of determination of CMZ residue in manufacturing facilities and on manufacturing equipment, CMZ was added to stainless steel plates and glass plates, the same materials as those of the manufacturing facilities and manufacturing equipment, and air sampling filters for sampling of any CMZ fine particles in the environment. Then, its recovery was determined to verify the accuracy of this method.

CMZ was dissolved in acetonitrile and an equivalent of 15 ng of CMZ was added to the stainless steel and glass plates (100 cm^2: 10 cm × 10 cm). The solutions were dispersed dropwise so that small droplets of the solution covered each plate. After they had dried completely, the plates were swabbed with the washed BEMCOT® swabs and the amount of CMZ residue was determined. The results of recovery were relatively low and variable from both materials as shown in Table 1, 62.1% ± 25.2S.D. (9.32 ng ± 3.77S.D.) for the stainless steel plates and 60.1% ± 26.7S.D. (9.02 ng ± 4.01S.D.) for the glass plates. The reason for this recovery results was considered to due to swabbing loss of CMZ from the surface. However, this recovery result would be acceptable for the purpose of residue monitoring, because the contamination of CMZ is comprehensively evaluated with the testing of many samples collected from the same facility and equipment. Separately, an equivalent of 3 ng of CMZ acetonitrile solution was added to the air sampling filters directly. The amount of CMZ residue was determined, after they had completely dried. According to the results shown in Table 1, a good recovery of 91.9% ± 8.7S.D. (2.76 ng ± 0.26S.D.) was achieved.

Repeatability of the method was demonstrated based on the standard deviation of the peak area response obtained by six-replicated analysis of 0.3 ng/ml CMZ solution. The resulting standard deviation of the peak area response was 169.7 ± 6.1S.D., with relative standard deviation of 3.6%.

Table 1
Recovery results of CMZ from sampling materials

Repetition	Stainless steel plate		Glass plate		Air sampling filter	
	ng	% Recovery	ng	% Recovery	ng	% Recovery
1	7.35	49.0	4.74	31.6	2.48	82.7
2	6.24	41.6	12.63	84.2	2.79	93.1
3	8.91	59.4	14.61	97.4	3.00	100.0
4	14.70	98.0	5.13	34.2	–	–
5	5.57	37.1	9.48	63.2	–	–
6	13.14	87.6	7.50	50.0	–	–
Mean	9.32	62.1	9.02	60.1	2.76	91.9
S.D.	3.77	25.2	4.01	26.7	0.26	8.7

Added amount: 15 ng for glass plate and stainless plate, 3 ng for air sampling filter.

3.3. Determination of CMZ residue in an actual facility

The samples collected at an actual manufacturing facility were analyzed for any residue of CMZ with this validated method. Areas of 100 cm^2 of the surfaces of manufacturing equipment, reactors, centrifuges, filters, dryers, pulverizers and tanks, were swabbed, and those of a sampling room and a warehouse were also swabbed. The air of the manufacturing room, warehouse and sampling room was filtered. The operation was conducted five times periodically and no detectable CMZ residue was found in any samples. This result indicated that no contamination of CMZ to other non-β-lactam pharmaceuticals had occurred in this facility.

4. Conclusion

Application of a rapid and sensitive FIA method with luminol chemiluminescence detection for determination of trace amounts of CMZ residue to pharmaceutical manufacturing facilities has been investigated. In terms of LOD and LOQ, linearity, accuracy and repeatability were evaluated to verify the method. According to the results of the LOD and LOQ, it was demonstrated that the method was highly sensitive for residue determination in pharmaceutical manufacturing facilities. Regarding linearity, accuracy and repeatability, acceptable results were obtained. Consequently, with this developed method, it is possible to detect very small amounts of CMZ residue in manufacturing facilities.

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