Determination of Cefsulodin, Cefmenoxime, and Cefadroxil as Residues on Surfaces[1]

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Cefsulodin, cefmenoxime, and cefadroxil are degraded instantaneously in aqueous sodium hypochlorite, sodium hypochlorite-detergent, or alkaline detergent solutions. These alkaline solutions are used to clean surfaces that have been exposed to the cephalosporins. The cleaned surfaces are monitored for residual drug levels (microgram) using a wet swab–dry swab technique. After extraction from the swabs, the content of the respective cephalosporin is determined in the solution by high-performance liquid chromatography. The limit of detection for each of the compounds is 0.1 μg/ml. Recoveries from nonporous surfaces ranged from 56 to 102%.

KEY WORDS: cefsulodin; cefmenoxime; cefadroxil; residues on surfaces; high-performance liquid chromatography.

INTRODUCTION

Cefsulodin (Abbott-46811, SCE-129) and cefmenoxime (Abbott-50912, SCE-1365) are semisynthetic cephalosporin antibiotics that have undergone clinical investigation. Cefadroxil, the active component in a marketed product, has been used as a positive control in clinical investigations. Many individuals are allergic to cephalosporins. In areas where bulk drug and finished products are used, care must be exercised to minimize exposure to these compounds. Internal quality assurance procedures require that all areas exposed to any cephalosporin be cleaned and monitored for residual drug levels (1–3).

To monitor the amount of residual drug remaining after cleaning, a procedure for determining drug residues on surfaces is necessary. Here, high-performance liquid chromatography (HPLC) is used for this purpose because of the specificity and sensitivity required. For the experimental cephalosporins, procedures were developed based on the stability indicating formulation methodology (4,5). The work of Wouters *et al.* (6) on cephalosporins was modified to separate cefadroxil from its degradation products. The chemical structures for these compounds are shown in Fig. 1.

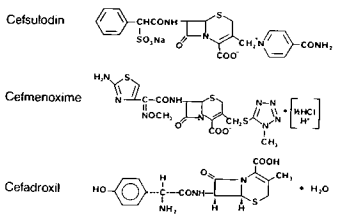

Fig. 1. Chemical structures for the cephalsporins determined.

MATERIALS AND METHODS

Instrumentation and Reagents

The HPLC system used in this work typically consisted of a pump (Model SP-8700, Spectra-Physics, San Jose, CA), an autosampler (WISP 710, Waters Associates, Milford, MA), and a UV detector (Schoeffel 770, ABI, Foster City, CA). Chromatograms were processed using a computing integrator (Model SP-4100, Spectra-Physics, San Jose, CA). Different columns were used for each of the analyses. A Waters μBondapak C18 column (3.9 mm × 30 cm, Milford, MA) was used for cefmenoxime. A Zorbax C8 column (4.6 mm × 25 cm, MAC-MOD Analytical, Chadds Ford, PA) was used for cefsoludin. A Nucleosil C18 (5 μm, 4.6 mm × 15 cm, Alltech Associates, Deerfield, IL) was used for cefadroxil. The HPLC eluent was filtered through a 0.45-μm nylon membrane (Cuno, Inc., Meridan, CT). Chemicals and solvents were reagent grade and HPLC grade, respectively. Characterized in-house standards for cefsulodin and cefmenoxime and USP reference standard cefadroxil were used for quantitation. The surfaces were swabbed with sterile cotton swabs having hollow polyethylene stems (Baxter Healthcare, Scientific Products Division, McGaw Park, IL). Prior to use for swabbing of surfaces, each lot of swabs was checked for potential assay interferences. Sample and standard solutions were filtered through 0.45-μm polycarbonate membranes (Cuno Inc., Meridan, CT).

Chromatographic Conditions

The chromatographic conditions and HPLC eluent for each of the cephalosporin determinations are summarized in Table I.

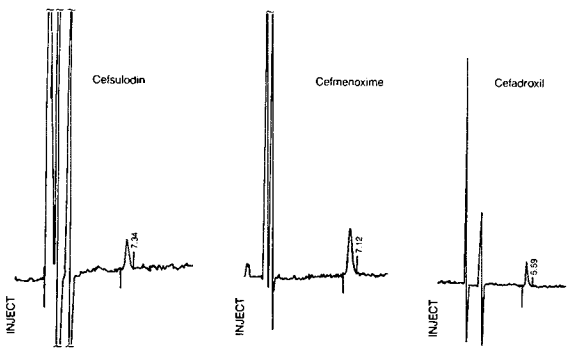

Fig. 2. Typical chromatograms of 0.1-μg/ml solutions of cephalosporins. Conditions stated in Table I.

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Table I. HPLC Conditions and Assay Performance

		Cefsulodin	Cefmenoxime	Cefadroxil
A.	Conditions used			
	Eluent	0.02 *M* $NH_4OAc/CH_3CN/CH_3OH$, 950:15:35	H_2O/CH_3CN/HOAC, 1000:150:10	0.07 *M* KH_2PO_4/CH_3CN, 960:40
	Flow rate (ml/min)	1.5	2.0	1.0
	Detector wavelength (nm)	261	254	254
	Range (AUFS) × attn.	0.04 × 2	0.04 × 2	0.04 × 4
	Injection volume (μl)	100	100	50
B.	Assay performance			
	1. Linearity (upper concentration examined; μg/ml)	25.2	2.0	11.3
	y intercept	60.9	14.7	−12.7
	Slope	599.9	1034	1442
	Corr. coefficient	0.999	0.999	1.000
	2. Repeatability (RSD value) (%)	±18.5	±7.3	±3.4

Swabbing Procedure and HPLC Sample Preparation

For cefsulodin and cefmenoxime analyses, add 10.0 ml of 1% pH 6 phosphate buffer (7) to an appropriate number of test tubes. Water is used as the swabbing solvent for cefadroxil. Moisten a cotton swab in the prescribed swabbing solvent. Using the moistened swab, sample approximately a 10 × 10-cm square of the surface(s) to be analyzed. Swab the area sampled with at least 25 strokes in one direction and then 25 strokes in a perpendicular direction. Remoisten the swab as needed. Place the swab into the test tube containing the swabbing solution. Using the technique described above, dry the area sampled with a second swab. Add this swab to the test tube. Extract the cephalosporins from the swabs with two 45-sec vortex mixings and filter the solutions through polycarbonate membranes.

Quantitation of the residual cephalosporin was performed versus a 0.1-μg/ml standard preparation dissolved in the prescribed swabbing solvent using either peak areas or peak heights. If a sample peak was above the linear range of the cephalosporin, the filtered sample preparation was diluted with swabbing solvent until the peak response was within the linear portion of the curve. The microgram per milliliter values obtained were multiplied by the appropriate dilution factor and the amount of cephalosporin (μg or microgram activity)/100 cm^2 is reported.

RESULTS AND DISCUSSION

Shown in Fig. 2 are typical chromatograms of 0.1-μg/ml solutions of cefsulodin, cefmenoxime, and cefadroxil. The linear working range and repeatability of the methods (as determined by injecting 0.1-μg/ml solutions at least five times) are shown in Table I.

For each cephalosporin, equal portions of a stock solution (approximately 10 μg) were added to three sets of two swabs with a microsyringe. The swabs were allowed to air-dry and each set of swabs was added to a test tube containing the prescribed swabbing solution. Using 45-sec intervals, a tube was vortexed for 45, 90, or 135 sec. The mean recov-

Table II. Recovery of 10 μg of Cephalosporins Added to Various Surfaces

	Percentage recovered[a]					
	Cefsulodin		Cefmenoxime		Cefadroxil	
Surface	Swab	Rinse[b]	Swab	Rinse[b]	Swab	Rinse[c]
Glass	60.8	38.2	101.7	<10.7[d]	76.0	14.0
	98.0	<9.8[d]	73.1	16.3	72.0	22.0
Plexiglas	87.2	12.7	85.6	<9.6	95.9	31.6
	—	—	—	—	85.7	9.2
Floor tile	70.3	37.6	73.7	10.5	77.5	8.5
	105.9	9.8	81.0	10.0	55.9	16.7
Stainless steel	59.4	36.6	78.9	18.9	87.6	7.8
	90.2	<9.8[d]	74.0	14.4	83.3	11.8
Conveyor belt	73.5	21.6	85.0	<10.0[d]	—	—
Lab bench	<9.9[d]	<9.9[d]	<10.5[d]	<10.5[d]	<10.1[d]	<10.1[d]
	<9.9[d]	<9.9[d]	<10.5[d]	<10.5[d]	<10.0[d]	<10.0[d]

[a] When more than one value is reported, the experiment was performed on 2 days.
[b] After the surface was swabbed, it was rinsed with 10 ml of 1% pH 6 phosphate buffer.
[c] After the surface was swabbed, it was rinsed with 5 ml of water.
[d] The sample peak height or area was below that obtained for a 0.1-μg/ml standard solution.

Table III. Amount of Cephalosporin Residue on Surfaces Which Were Exposed to Milligram Quantities of Cephalosporin and Cleaned

Surface	Detergent[a]	μg/100 cm² found		
		Cefsulodin	Cefmenoxime	Cefadroxil
Glass	A	<1, <1	<1	1.2
	B	—	—	1.4
Floor tile	A	2.0, <1, <1	<1	<1
	B	—	—	1.3
Stainless steel	A	<1, <1	<1	<1
	B	<1, <1	<1	<1
	C	<1	<1, <1	<1
Plexiglas	A	<1, <1	1.7, <1, <1	<1
	B	—	—	1.5
	C	<1	5.5, <1	—
Conveyor belt	C	<1	1.7, 1.0	—

[a] (A) One and one-half percent aqueous sodium hypochlorite solution; (B) 1 part sodium hypochlorite (5.25% min) and 3 parts 0.5% aqueous detergent; (C) 2% aqueous CONTRAD 70 (pH 11).

eries for cefsulodin, cefmenoxime, and cefadroxil were 102.9, 99.7, and 87.1, respectively. Since there was no trend in the recovery data for the cephalosporins with the number of extractions used, two 45-sec vortexings are used to ensure complete extraction.

Another set of standard addition samples was prepared to determine how long and under what conditions swab samples can be stored. Samples for these studies were prepared as previously described. Initial values were obtained on two swab sets. The remaining samples were capped and stored either at room temperature or in a refrigerator. Samples were then analyzed on day 1 and day 2 or 3 depending on the results of the day 1 samples. The data obtained show that cefsoludin samples must be stored in the refrigerator and analyzed within 2 days. Cefmenoxime and cefadroxil samples, however, can be stored at room temperature for up to 3 days.

A third set of standard addition experiments was performed to check the recovery of the cephalosporins from a variety of surfaces. Using a microsyringe, approximately 10 μg of a cephalosporin was spread over approximately 100-cm² areas of glass, Plexiglas, floor tile, stainless steel, conveyor belt, and laboratory bench. The solution was allowed to evaporate and the surfaces were swabbed according to the procedure described in the text. Any residual cephalosporin not removed by the swabs was rinsed off the surface with a known aliquot of swabbing solvent. For each surface, the amount of drug in the swab solution and the rinse solution was determined separately. The data are summarized in Table II and show that acceptable mass balances were obtained for all surfaces except the laboratory bench. The poor recovery from this surface was probably due to its porosity.

To validate the cleaning procedures, the surfaces used for the recovery experiments were exposed to 5–10 mg of cephalosporin. A 5-ml aliquot of cleaning solution was placed on the surface and the surface rotated to ensure that the entire area came in contact with the solution. Paper towels were used to remove the solution from the surface. The first towel was used to remove the majority of the solution and a second towel was used to dry the area. The surface was then rinsed with 5 ml of water, dried as described above, and sampled according to the procedure described in the text. This experiment was performed separately for each cephalosporin, cleaning solution, and surface. The data in Table III show that all residual drug levels were below the internal guidelines for surfaces tested.

In conclusion, low-level assays have been developed for monitoring surfaces that have been exposed to cefsulodin, cefmenoxime, and cefadroxil. These cephalosporins can be quantitated at 0.1 μg/ml or 1 μg/100 cm² swabbed surface. Acceptable recovery data were obtained for the three cephalosporins from all of the surfaces examined, except for the porous laboratory bench top.

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REFERENCES

1. D. W. Mendenhall. Cleaning validation. *Drug Dev. Industr. Pharm.* 15(13):2105–2114 (1989).
2. Quality Control Reports. *The Gold Sheet* 24(12):3, 6 (1990).
3. L. Elrod, Jr., and S. G. Spanton. Analysis of o-phenylenediamine by HPLC with electrochemical detection. *J. High Resolut. Chromatogr. Chromatogr. Commun.* 5(11):628–629 (1982).
4. L. Elrod, Jr., L. B. White, D. C. Wimer, and R. D. Cox. Determination of cefsulodin sodium [D(−)-SCE-129] by HPLC. *J. Chromatogr.* 237:515–521 (1982).
5. *Code of Federal Regulations, Title 21,* U.S. Government Printing Office, Washington DC, 1990, Part 436.222.
6. I. Wouters, S. Hendrickx, J. Hoogmartins, and V. Vanderhaege. Selectivity of reversed-phase packing materials in high-performance liquid chromatography of cephalosporins. *J. Chromatogr.* 291:59–80 (1984).
7. *Code of Federal Regulations, Title 21,* U.S. Printing Office, Washington, DC, 1990, Part 436.101.