Determination of Clarithromycin as a Contaminant on Surfaces by High-Performance Liquid Chromatography Using Electrochemical Detection

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Microgram levels of clarithromycin residues on various surfaces are quantitated. After cleaning, any residual clarithromycin remaining is removed from the surface by a wet swab–dry swab technique. High-performance liquid chromatography with electrochemical detection is used for quantitation of the resulting solutions. Recoveries ranged from 93 to 118%.

KEY WORDS: clarithromycin; cleaning validation; high-performance liquid chromatography; electrochemical detection; residue on surfaces.

INTRODUCTION

Clarithromycin is a new broad-spectrum semisynthetic macrolide antibiotic. Internal quality assurance procedures require that when a production or clinical supply area is exposed to an investigational drug, the exposed areas and equipment are cleaned and monitored for any residual drug levels (1,2). A direct assay for clarithromycin bulk substance is possible using high-performance liquid chromatography (HPLC) with either UV (in preparation) or electrochemical detection (3–5). Since clarithromycin contains no major chromophore, UV detection must be done at low wavelengths where interferences from the sample matrix usually occur. Clarithromycin does contain a tertiary amine functional group and is electrochemically active. Electrochemical detection is more selective and affected less by these matrix effects. In addition, the electrochemical system provides greater sensitivity which allows detection at low levels. In this paper, we report a simple procedure to quantitate low levels of clarithromycin residues on various surfaces using HPLC with an electrochemical detector. Clarithromycin is detectable at concentrations >0.3 μg/ml. The assay is rapid, is specific for clarithromycin, and does not require sample concentration. If the residual level is high, dilution of the sample may be required.

MATERIALS AND METHODS

Reagents and Materials

Clarithromycin was obtained from Abbott Laboratories. A working standard of clarithromycin was prepared by dissolving 10 mg of clarithromycin reference standard in 100 ml of 50–50 ethanol–water and then serially diluting 5 to 100 and 20 to 100. The resulting concentration was approximately 1 μg/ml. This solution was filtered through an ACRO LC-13 disposable filter in exactly the same way as the swab sample solutions prior to injection. The working standard solution was stable when refrigerated for 5 days. Ethanol was 190 proof, USP. Other reagents were either HPLC grade or AR grade, while the water was distilled water.

Swabs were made from 100% polyester fiber that was washed with ethanol, 190 proof, USP, prior to use. The polyester fiber was placed into a beaker and covered with 190 proof ethanol and allowed to stand for about 10 min. The ethanol was removed by filtration from the polyester fiber using a fritted glass-filter funnel and the polyester was then air-dried by drawing air through it using a vacuum.

Swabbing Procedure

1. Add 10.0 ml of ethanol–water (50–50) to a test tube.
2. Using a forceps, a tuft of polyester fiber is moistened in the ethanol–water.
3. Select a square of approximately 10 × 10 cm to be swabbed. The surface is swabbed at least 20 strokes in one direction and then 20 strokes in a perpendicular direction.
4. Place the swab in the test tube.
5. Using the technique described above, dry the same area with a dry swab.
6. Add this swab to the test tube.
7. Repeat steps 2–5 in order to give a total of four polyester tufts in the test tube.

Sample Preparations

1. Vortex the test tube containing the swabs for two minutes.
2. Filter the solution through an ACRO LC-13 disposable filter.

Chromatographic Conditions

The HPLC system consisted of an LDC Constametric III pump, a Rheodyne 7125 injector equipped with a 100-μl sample loop, an ESA (Environmental Sciences Associates) Coulochem Model 5100 electrochemical detector with a Model 5010 analytical cell, and a Shimadzu C-R3A integrator for integration of peak heights. The analytical column was a 5-μm Nucleosil octadecyl (C-18) bonded phase (Macherey-Nagel) packed into a 150 × 4.6-mm stainless-steel column (Alltech Associates). A mixture of 400 ml acetonitrile, 600 ml distilled water, and 16.3 g sodium acetate trihydrate with the apparent pH of the solution adjusted to 6.6 with acetic acid was used as the mobile phase. The flow rate was 1.7 ml/min and the retention time of the clarithromycin was approximately 7 min.

RESULTS AND DISCUSSION

Shown in Fig. 1 are typical chromatograms of a swab

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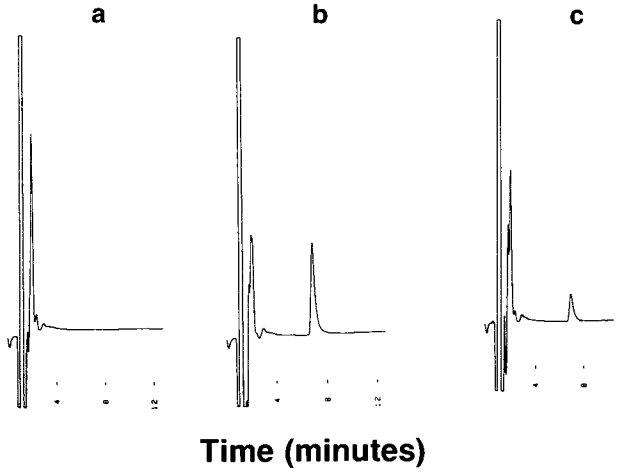

Fig. 1. Chromatograms of swab solution blank, 1.0 μg/ml clarithromycin standard solution, and an authentic sample solution: (a) swab blank (b) 1.0 μg/ml standard; (c) authentic sample.

solution blank, a clarithromycin standard at 1.0 μg/ml, and an authentic sample solution. Using the conditions stated in the text, the total chromatographic assay time was approximately 9 min.

Detector response is linear from 0.3 to 3.0 μg/ml of clarithromycin ($y = 2114x - 26.9$, $r = 0.9999$, where y and x are peak height and concentration, respectively). This corresponds to a range of 3 to 30 μg of clarithromycin per 100 cm^2 of surface for the procedure stated in the text. Repeatability of the method was demonstrated by injecting the 1-μg/ml standard six consecutive times (Table I). The relative standard deviation was 4.9% for manually peak heights and 3.9% for integrated peak heights.

The stability of the sample solution was shown by assaying the solution on 2 separate days. The solution initially contained 5.11 μg/ml. After 5 days at room temperature the clarithromycin content was 5.04 μg/ml (98.6% of the initial).

Standard addition and recovery data for clarithromycin added to different lots of polyester fiber are presented in Table II. The clarithromycin was added from stock clarithromycin solutions using a microsyringe, and the swabs were allowed to air-dry and then extracted as described in the text. The data in Table I show excellent recovery of clarithromycin from the swab material.

A second set of experiments was performed to determine if clarithromycin is removed quantitatively from surfaces in the swabbing procedure. To 100-cm^2 areas of glass, Plexiglas, and stainless steel, clarithromycin was added from stock solutions by microsyringe. The surfaces were air-dried and swabbed according to the procedure described in the text. Any residual clarithromycin not removed by wet swab–dry swab technique was rinsed off of the surface with 10.0 ml of ethanol–water (50–50) in order to provide a mass balance. For each surface, the swab solutions and rinse solutions were assayed separately, the recoveries were calculated separately, and then both recoveries were added to give a mass balance. The results are summarized in Table III. Considering that the levels of clarithromycin on each surface were very low and that the percentage recovery is the summation of two separate analyses at yet lower levels, the range of 93.3 to 118.5% for the mass balance is more than acceptable for validating cleaning procedures.

Swab samples which have concentrations greater than 3 μg/ml are diluted quantitatively using class A glassware with 50–50 ethanol–water to bring them within the established linear range of 0.3 to 3 μg/ml. An example of this is shown in Fig. 2. The undiluted sample was found to contain 46 μg/ml of clarithromycin.

Many times swab samples have to be taken at a remote location where the equipment necessary for the analysis was not available. Because the samples would be in transit for 24–48 hr, an alternative to using test tubes for sample transport was investigated. Screw-cap scintillation vials either with a polyethylene insert or with an aluminum foil liner in the top of the cap were tested. Four polyester swabs were

Table I. Repeatability of 1.0 μg/ml Clarithromycin Standard Preparation Injections

	Measured peak height (mm)	Integrated peak height (counts)
	42	845
	44	895
	45	899
	44	883
	48	933
	47	940
Mean	45	899
SD	±2.2	±34.7
RSD	±4.9%	±3.9%

Table II. Standard Addition and Recovery Data of Clarithromycin from Polyester Fiber

Lot of polyester	Clarithromycin added (μg)	Clarithromycin found (μg)	% recovery
1	0.97	0.93	95.9
1	2.43	2.44	100.4
1	9.89	9.55	96.6
2	4.41	3.89	92.4
2	10.51	10.33	98.3
2	21.03	19.63	93.3
3	20.67	18.52	89.6

Table III. Standard Addition and Recovery Data of Clarithromycin from Surfaces

		Percentage recovered		
Surface	μg added	Swab	Rinse	Mass balance
Glass	9.0	70.0	31.1	101.1
	103.4	95.9	8.9	104.8
	103.4	92.3	26.2	118.5
Plexiglas	9.0	82.2	11.1	93.3
	103.4	97.7	6.8	104.5
Stainless steel	9.0	96.7	13.3	110.0
	100.1	107.4	6.9	114.3
	100.1	100.3	12.4	112.7

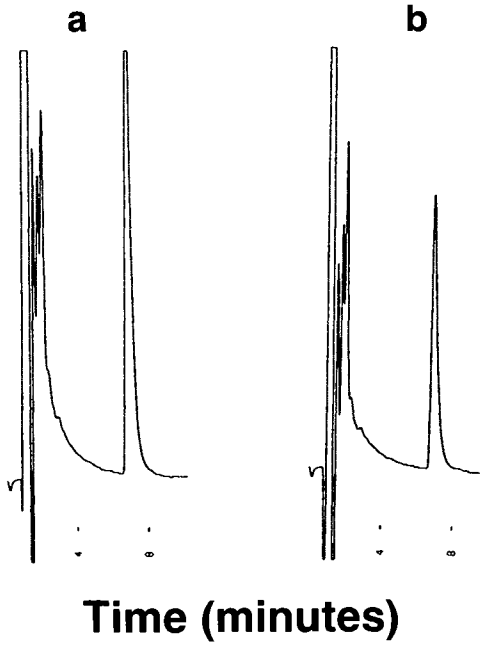

Fig. 2. Chromatograms of a high-concentration authentic sample, undiluted and then diluted 1 to 1, concentration of the undiluted determined to be 46 μg/ml: (a) undiluted sample (b) 1-to-1 dilution of the sample.

placed into each vial along with 10 ml of 1.00 μg/ml 50–50 ethanol–water solution of clarithromycin or 10 ml of 50–50 ethanol–water. The vials were capped and then shaken to wet the surface of the vials. Half the vials were refrigerated and the other half were left at room temperature on the lab bench. Samples were assayed at 24, 48, and 120 hr. The sample blanks showed no peaks that would interfere with the clarithromycin peak. The results are shown in Table IV.

From the data, loss of clarithromycin is obvious in the solutions stored in the scintillation vials with the polyethylene cap inserts at all data points. No loss was seen in the solutions stored in the scintillation vials with the aluminum liners in the caps for 48 hr at any of the storage conditions.

In summary, a sensitive, accurate low-level assay for clarithromycin using electrochemical detection has been developed. This assay has a minimum detectable level for clarithromycin of 3.0 μg/100 cm^2 and a minimum quantifiable level for clarithromycin of 9.0 μg/100 cm^2 when applied to swab solutions used to check for residual clarithromycin. Clarithromycin does not bind to the swabs and is stable in the swabbing solution for at least 5 days. Excellent recovery and mass balance was seen from drug deposited and removed from glass, Plexiglas, and stainless-steel surfaces. This procedure can then be applied to cleaning validation studies to ensure proper cleaning.

Table IV. Stability of a 1.00 μg/ml Solution of Clarithromycin in Scintillation Vials Containing Polyester Swabs at Room Temperature (RT) and Under Refrigeration (Refrig.)

Cap insert	24 hr		48 hr		120 hr	
	RT	Refrig.	RT	Refrig.	RT	Refrig.
Polyethylene	0.88	0.84	0.92	0.92	0.91	0.84
Aluminum foil	1.05	1.00	1.04	1.02	0.94	1.00

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