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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF OLEANDOMYCIN IN SERUM AND URINE

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SUMMARY

The determination of oleandomycin in serum and urine by high-performance liquid chromatography using erythromycin as internal standard is described. The separation was achieved on a reversed-phase C_{18} column employing acetonitrile–0.05 *M* phosphate buffer (30:70), adjusted to pH 7.0, as the mobile phase with UV detection at 200 nm. A solid-phase extraction procedure, combined with a simple phase-separation step was used prior to chromatographic analysis. Linear calibration curves were obtained in the concentration ranges 0.25–5.0 μg/ml (serum) and 1.0–25.0 μg/ml (urine). Precise quantitative analysis has been achieved at these levels with relative standard deviations of < 5%.

INTRODUCTION

Oleandomycin is a macrolide antibiotic (Fig. 1) with an antimicrobial spectrum similar to that of erythromycin[1]. Although considered to have weaker antimicrobial activity, this compound has been successfully used in the treatment of susceptible infections[2].

Very few analytical methods for the analysis of oleandomycin have been reported[3–5]; none of them involve high-performance liquid chromatography (HPLC). The objective of the present study was to develop a sensitive, precise and rapid quantitative method for oleandomycin in serum and urine by HPLC with UV detection. The HPLC method described here has a detection limit of 0.25 μg/ml in serum and 0.5 μg/ml in urine. The success of this method was largely due to the efficiency of the solid-phase extraction procedure employed, which resulted in a clean sample extract enabling the utilization of a detection wavelength of 200 nm. In view of the fact that there is currently a dearth of pharmacokinetic data on oleandomycin[6], the present method should provide a valuable tool for future studies in this area.

MATERIALS AND METHODS

Apparatus

Analysis was performed with a high-performance liquid chromatograph consisting of an M6000A pump and a WISP 710B automated sample injector (Waters

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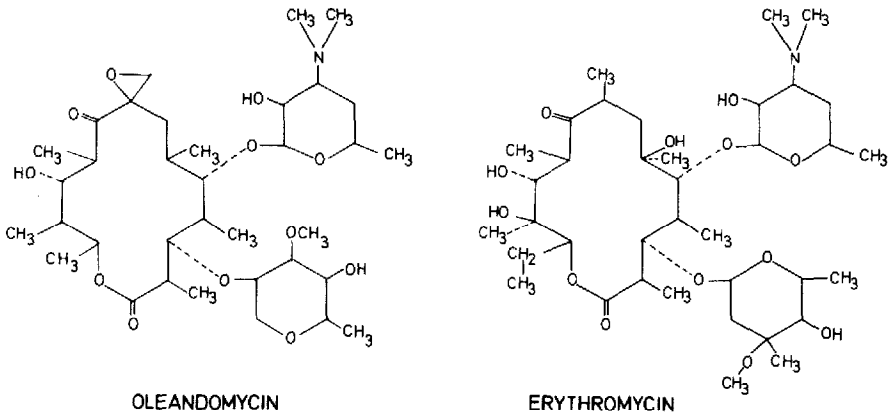

Fig. 1. Molecular structures of oleandomycin and erythromycin.

Assoc., Milford, MA, U.S.A.), a variable-wavelength UV absorbance detector (Model 769, Kratos Analytical Instruments, Ramsey, NJ, U.S.A.) and a strip chart recorder (Model 561, Hitachi, Tokyo, Japan). The temperature of the column was maintained at 35°C with the aid of a Model LC-22 temperature controller (Bioanalytical Systems, W. Lafayette, IN, U.S.A.).

Reagents

All reagents were of at least analytical grade. The acetonitrile was distilled-in-glass UV grade (Burdick & Jackson, Muskegon, MI, U.S.A.). A 15 cm × 3.9 mm I.D. column, containing microparticulate-bonded (5 μm) octadecylsilane (C_{18}) material (Novapak, Waters Assoc.) was used. Oleandomycin phosphate was obtained from Pfizer Labs., Pietermaritzburg, South Africa, and erythromycin base was obtained from Abbott Labs., Johannesburg, South Africa. The HPLC-grade water used in the mobile phase was purified through a Milli-Q system (Millipore, Bedford, MA, U.S.A.).

Mobile phase

The mobile phase was prepared by mixing acetonitrile (300 ml) with 0.05 *M* phosphate buffer (700 ml). The phosphate buffer was prepared by adding 3.2 ml phosphoric acid to 1 l of water. Sodium hydroxide was then used to adjust the solution to pH 6.3. The solvent mixture (pH 7.0) was degassed and filtered through a 0.6-μm filter (Millipore, Type BD). The mobile phase was constantly degassed using an in-line vacuum degassing unit (Model ERC-3510, Erma Optical Works, Tokyo, Japan) during analysis.

Chromatographic conditions

The mobile phase was used at a flow-rate of 1.0 ml/min for the analysis of both serum and urine samples, with a resulting pressure of 100 bar. The detection wavelength was 200 nm, with a detector sensitivity of 0.01 a.u.f.s. and a time constant of 2 s.

Extraction

Aliquots of serum (2 ml) or urine (1 ml) were mixed with 0.25 ml of an aqueous internal standard solution, containing 40 μg/ml erythromycin (Fig. 1). Addition of either 1 ml of acetonitrile (urine) or 2 ml of acetonitrile (serum), followed by vortex-mixing for 1 min and centrifugation for 5 min at 1600 *g*, resulted in deproteinization of the biological samples. The supernatant was transferred to a culture tube (Kimble Glass, Toledo, OH, U.S.A.) containing 8 ml of water for direct loading onto a 1-ml disposable C_{18} extraction column (J. T. Baker, Phillipsburg, NJ, U.S.A.) that had been pre-washed under a 10–15 mmHg vacuum (Baker, No. 10 Extraction System) with 3 ml of acetonitrile, followed by 3 ml of water. The diluted sample was then added to the extraction column with the aid of a 15-ml sample reservoir. The extraction column was washed with 5 ml of water and then with 5 ml of acetonitrile–water (1:1) with vacuum being maintained until dry. Oleandomycin and the internal standard were eluted into 2-ml tapered collection tubes with two successive 500-μl aliquots of acetonitrile–0.05 *M* phosphate buffer (1:1). The sample was then taken to dryness under vacuum in a rotary vacuum centrifuge (Savant Instruments, Hicksville, NY, U.S.A.) and the residue in the collection tube was reconstituted in 20 μl of water and vortex-mixed for 1 min. On addition of 25 μl of acetonitrile, two layers formed due to the high concentration of salts in the aqueous layer. This mixture was vortex-mixed for 1 min and then centrifuged for 1 min at 1600 *g* to ensure complete separation of the two layers. An aliquot of the top acetonitrile layer (15–20 μl) was then transferred to a WISP limited-volume insert (Waters Associates) using a microsyringe. Aliquots (1–5 μl) of this sample were injected into the column. Relevant chromatograms are depicted in Figs. 2 and 3.

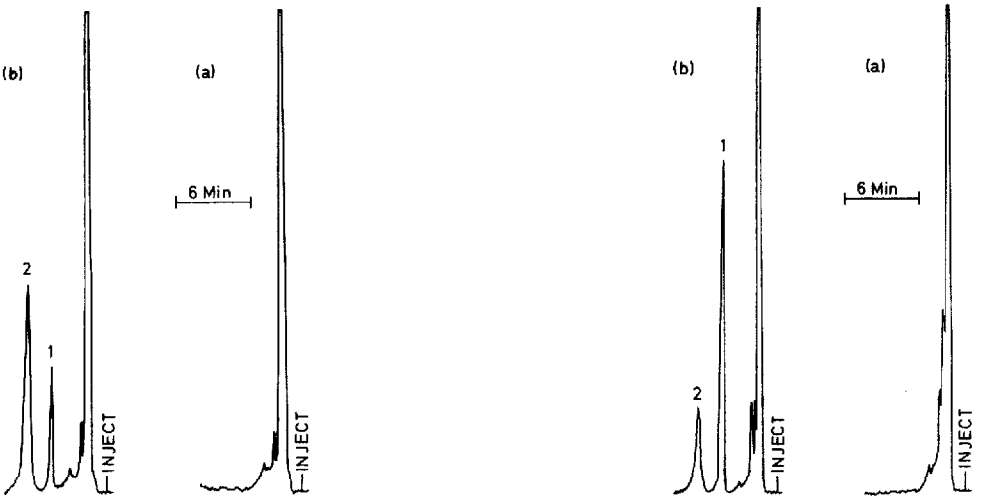

Fig. 2. (a) HPLC profile of blank serum extract; (b) HPLC profile of an extract of serum containing oleandomycin (1) and internal standard (2).

Fig. 3. (a) HPLC profile of blank urine extract; (b) HPLC profile of an extract of urine containing oleandomycin (1) and internal standard (2).

RESULTS

Linearity

Calibration curves with five different concentrations of oleandomycin in serum and urine, obtained by plotting the ratio of the peak height of oleandomycin to that of the internal standard *vs.* their respective concentrations, were linear over the concentration ranges studied. The calibration curve in serum (0.25–5.0 μg/ml) had a slope of 0.5394 and a *y*-intercept of 0.0202 with a correlation coefficient of 0.9999, while the curve in urine (1.0–25.0 μg/ml) had a slope of 0.2582 and a *y*-intercept of 0.0321 with a correlation coefficient of 0.9999.

Precision and accuracy

Within-run precision was assessed by extracting spiked serum and urine samples, over the concentration ranges studied. The results are summarized in Table I.

Extraction efficiency

Spiked serum and urine samples were assayed in triplicate at two different concentrations. All samples were extracted as previously described, except that the internal standard was incorporated in the 25-μl aliquot of acetonitrile used in the final reconstitution and phase-separation step. The mean recovery values of 85% and 87% thus obtained for serum and urine, respectively, represent the recovery from the solid-phase extraction procedure alone. Partition studies of the distribution of oleandomycin during the subsequent phase-separation step indicate that approximately 10% of the drug remains in the aqueous layer.

Sensitivity and detection limit

Under the conditions of this assay and based on a signal-to-noise ratio of 3, the detection limit for oleandomycin was 0.25 μg/ml in serum and 0.5 μg/ml in urine. The detection limit in serum may be increased by using a larger injection volume of 8–10 μl.

TABLE I

ACCURACY AND PRECISION OF THE METHOD FOR THE DETERMINATION OF OLEANDOMYCIN IN HUMAN SERUM AND URINE

	Oleandomycin added (μg/ml)	*Number of samples*	*Oleandomycin found (μg/ml)*	*R.S.D. (%)*
Serum	0.25	3	0.24 ± 0.01	4.4
	0.50	6	0.52 ± 0.01	2.7
	1.00	3	1.00 ± 0.04	4.0
	2.00	3	1.98 ± 0.04	1.8
	5.00	6	5.00 ± 0.11	2.1
Urine	1.00	6	0.98 ± 0.03	3.4
	5.00	3	5.17 ± 0.11	2.0
	10.00	3	9.90 ± 0.14	1.4
	15.00	3	14.90 ± 0.66	4.5
	25.00	6	25.08 ± 1.02	4.1

DISCUSSION

The method described involves a rapid, precise and accurate system for the determination of oleandomycin in serum and urine. During studies on various macrolide antibiotics, it was found that the elution behaviour of oleandomycin from a C_{18} reversed-phase column was highly dependent upon the buffer concentration in the mobile phase. This phenomenon was utilized in the development of the solid-phase extraction procedure, whereby the compounds of interest, oleandomycin and its internal standard, could be selectively retained on the C_{18} extraction column, while unwanted components were washed off with water and acetonitrile–water mixtures. In view of the relatively low concentrations of oleandomycin found in serum and urine during clinical use of this drug[6], as well as the relatively low UV absorptivity of the compound at wavelengths greater than 220 nm, it was necessary to use highly concentrated sample extracts as well as a detection wavelength of 200 nm, where the absorbance of oleandomycin is considerbly enhanced. In view of the non-selectivity associated with the use of 200 nm as the detection wavelength, it was further necessary to develop an extraction procedure that would result in a sufficiently clean and concentrated extract to allow unhindered quantitative analysis. This was achieved by evaporating the extraction column eluate to dryness, adding a 20-μl aliquot of water to dissolve the drug and residual buffer salts and then selectively concentrating the oleandomycin and internal standard into a 25-μl acetonitrile layer. This layer could then easily be removed for injection into the HPLC system as a result of successful phase separation.

In summary, the HPLC method presented here has the necessary precision, sensitivity and accuracy to allow the determination of oleandomycin in serum and urine, and should prove extremely useful for the phamacokinetic characterization of this drug in human subjects.

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