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High-performance liquid chromatography of clindamycin and clindamycin phosphate with electrochemical detection

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SUMMARY

The high-performance liquid chromatographic analysis of clindamycin phosphate and clindamycin hydrochloride was accomplished by using a mobile phase consisting of the ion pair reagent, sodium pentane sulfonate, and a reversed-phase ODS-II column. Detection was performed with a dual electrochemical cell at a screening potential of +0.7 V and a detecting potential of +0.9 V. Limits of detection for clindamycin were 100 pg injected on-column utilizing a pulse-free syringe pump. Bulk preparations of clindamycin hydrochloride were analyzed by high-performance liquid chromatography (HPLC) with electrochemical detection and compared to an HPLC technique utilizing ultraviolet detection at 214 nm.

INTRODUCTION

The official methods of analysis of the antibacterial antibiotics clindamycin (I) and clindamycin phosphate (II) (structures shown in Fig. 1) involve either a gas chromatographic separation after derivatization or a microbiological assay[1,2]. In order to eliminate the time needed for derivatization in the gas chromatographic protocol, the analysis of clindamycin (in the hydrochloride form) and the associated phosphate ester has been performed by high-performance liquid chromatography (HPLC) using a refractive index (RI) detector[3]. The separation of clindamycin by HPLC followed by RI detection was accomplished by using an aqueous solution of dioctyl sodium sulfosuccinate and methanol as the mobile phase, which gave good chromatographic separation, but the quantity at which the samples were analyzed had to be approximately 100 μg injected on-column due to the low detection limits and insensitivity of the RI detector. Extensive sample preparation was also needed, which

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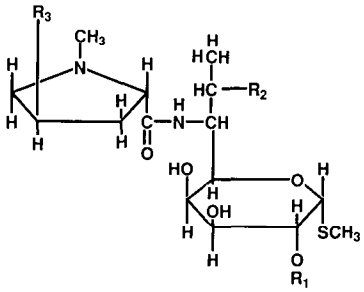

	R_1	R_2	R_3
I	-H	-Cl	$-CH_2CH_2CH_3$
II	$-PO_3H_2$	-Cl	$-CH_2CH_2CH_3$
III	-H	-OH	$-CH_2CH_2CH_3$
IV	-H	-OH	$-CH_2CH_3$

Fig. 1. Structures of clindamycin (I), clindamycin phosphate (II), lincomycin (III) and lincomycin B (IV).

added to the overall analysis time. Landis *et al.*[4] utilized RI and ultraviolet (UV) detection at 214 nm with a reversed-phase ion pair mobile phase consisting of D,L-10-sodium camphorsulfonate or sodium pentane sulfonate for RI or UV detection, respectively. These mobile phases were applied for the analysis of clindamycin and bulk impurities. Minimal sample quantities for RI detection were at levels of 375 μg injected on-column and for UV detection a detection limit of approximately one fourth of RI limits was achieved. Analysis of the clindamycin phosphate ester and its degradation products was accomplished by HPLC with UV detection at 210 nm[5] and a mobile phase containing aqueous potassium phosphate and acetonitrile. The impurities lincomycin (III) and lincomycin B (IV), which may exist in bulk preparations of clindamycin, were also detected. The amount of clindamycin phosphate injected was approximately 4 μg on-column. Recently, determination of clindamycin in plasma or serum was accomplished with UV detection at 198 nm at a detectable level of 0.17 μg/ml after minimal sample preparation[6].

During the course of evaluating the utility of HPLC with electrochemical detection (ED) for the assay of the aminoglycoside antibiotic gentamicin[7], it was found that clindamycin generated an electrochemical response at an oxidizing potential[8]. This report describes the HPLC–ED determination of clindamycin and clindamycin phosphate, as well as its applicability for assaying bulk preparations of clindamycin hydrochloride.

EXPERIMENTAL

Electroanalytical measurements by differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were made on a BAS-100A (Bioanalytical Systems, West Lafayette, IN, U.S.A.) with a glassy carbon working electrode (GCE), a Ag/AgCl reference electrode and a platinum wire as the counter electrode. Electrolyte was 0.04 *M* aqueous phosphate buffer for compound I and 0.025 *M* aqueous phosphate buffer for compound II. Phosphate buffer was composed of $Na_2HPO_4 \cdot 7H_2O$ and KH_2PO_4 at a pH of *ca.* 7. The DPV conditions were scan-rate 5 mV/s and pulse amplitude of 50 mV; CV was performed at scan-rates of 200 mV/s.

HPLC employed an Isco LC-5000 (Lincoln, NB, U.S.A.) syringe pump at a flow-rate of 1.0 ml/min. The mobile phase was similar to that used by Landis *et al.*[4] and consisted of methanol–water (60:40) with 0.1 *M* phosphate buffer (pH 6) and 0.002 *M* sodium pentane sulfonate (Regis, Morton Grove, IL, U.S.A.). The phosphate buffer was prepared by using $Na_2HPO_4 \cdot 7H_2O$ and KH_2PO_4 and adjusted to pH 6, if necessary. A Spherisorb ODS-II C_{18} reversed-phase column (10 cm × 4.6 mm I.D., 3 μm; Custom LC, Houston, TX, U.S.A.) was used. The electrochemical cell was an ESA (Bedford, MA, U.S.A.) high-sensitivity cell, Model 5011, with an ESA Model 5100A Coulochem potentiostat. The cell utilized a dual-electrode configuration whereby the first electrode was set at a screening potential to diminish any electroactive species present in the mobile phase, and the second electrode detected the solute of interest. In the case of clindamycin, the screenig potential was set at +0.7 V *vs.* Pd reference electrode and the detecting potential was +0.9 V *vs.* Pd reference electrode. The output from the detector was connected to a Shimadzu C-R3A integrator (Kyoto, Japan). Injections were made with a Rheodyne Model 7125 (Cotati, CA, U.S.A.) injector with a 20-μl loop.

Clindamycin, as it is referred to in the text, was used as the hydrochloride salt form. A standard for clindamycin with a microbiological potency of 837 mcg/mg was compared with the bulk preparations by HPLC–ED. The nominal concentrations for assay purposes of clindamycin were 0.01 mg/ml.

The HPLC–UV method used for comparison in this study is similar to that reported by Munson and Kubiak[5]. Detection was performed at a wavelength of 214 nm and with a flow-rate of 1.5 ml/min. Nominal concentrations of standard and samples were 0.8 mg/ml utilizing a 20-μl loop. The mobile phase consisted of phosphate buffer–acetonitrile (77:23). The phosphate buffer was prepared from potassium dihydrogen phosphate by dissolving 10.54 g into 1 l deionized water and adjusting the pH to 2.5 with 90% phosphoric acid.

Two mass units were used to describe clindamycin throughout the text. One mass unit, μg, refers to the mass of antibiotic injected on-column. The other mass unit, mcg/mg, where mc stands for micro, refers to the microbiological potency. When the bulk preparations were compared to the standard, the final value is related to the microbiological potency of the standard and has the unit mcg/mg.

RESULTS AND DISCUSSION

The electrochemistry of clindamycin (I), as determined by DPV, is shown in Fig. 2. An initial oxidation is observed at +0.92 V with a subsequent oxidation at +1.59 V, which was near the start of oxidation of the background solvent. In Fig. 3, the DPV of clindamycin phosphate (II) gave an initial oxidation at +0.99 V and a broad hump near the background oxidation at +1.70 V. CV on a GCE for clindamycin and its phosphate ester did not produce sharp waves; however, CV did indicate that the electrochemistry was irreversible in aqueous phosphate buffer.

An example of HPLC–ED of clindamycin is shown in Fig. 4. The chromatogram by HPLC–ED is similar to that reported for UV detection of clindamycin[4,5]. Clindamycin is prepared from lincomycin (III), which is also detectable by HPLC–ED, as illustrated in Fig. 4. It was found that the chromatographic retention time for clindamycin was highly pH dependent, as reported previously[4].

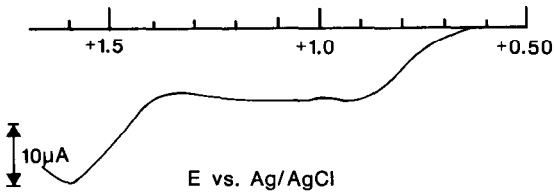

Fig. 2. Differential pulse voltammogram of clindamycin in 0.04 *M* aqueous phosphate buffer.

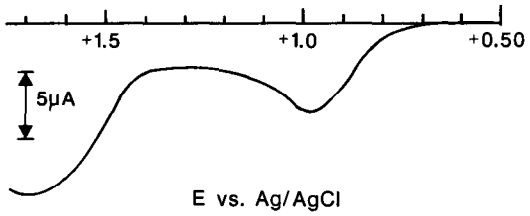

Fig. 3. Differential pulse voltammogram of clindamycin phosphate in 0.025 *M* aqueous phosphate buffer.

The linearity of the electrochemical signal *vs.* concentration of clindamycin as determined by on-column injections and the integrated peak area gave a correlation coefficient (r) of 0.99990 as indicated in Table I. The linear regression produced an equation with the slope being normalized to the y-intercept of $y = 142x + 1$ signal/μg. The dynamic range of this analysis was *ca.* 0.05–1 μg injected on-column. The linearity study for clindamycin phosphate gave an r of 0.9990 for a range of approximately 0.06 to 1.2 μg injected on-column as shown in Table II. The result of linear regression, with normalization to the y-intercept, was $y = 153x + 1$. At approximately 1.5 μg injected on-column and above, the linear relationship for clindamycin and clindamycin phosphate was not maintained.

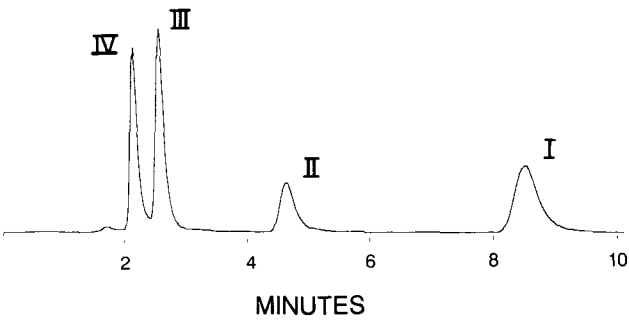

Fig. 4. Typical HPLC–ED of clindamycin and structurally related compounds. Roman numerals refer to structures shown in Fig. 1. Concentrations of all four compounds were 0.01 mg/ml each with 20-μl loop injection.

TABLE I

LINEARITY OF CLINDAMYCIN ANALYSIS BY HPLC–ED PERFORMED FOR TRIPLICATE ASSAYS

A clindamycin standard was used at a potency of 837 μg/mg.

Amount injected on-column[a] *(μg)*	*Mean peak area*	*S.D.*	*R.S.D. (%)*
0.045	$7.6 \cdot 10^5$	$3.70 \cdot 10^4$	4.7
0.090	$1.53 \cdot 10^6$	$3.03 \cdot 10^2$	0.02
0.450	$7.37 \cdot 10^6$	$5.14 \cdot 10^4$	0.6
0.900	$1.43 \cdot 10^7$	$1.17 \cdot 10^5$	0.8
4.50	$3.78 \cdot 10^7$	$6.53 \cdot 10^5$	1.7

[a] Correlation coefficient for the range 0.045–0.900 μg was 0.99990.

The standard deviation for these linearity studies is indicated in Tables I and II. As shown in Table I, the relative standard deviation (R.S.D) in the range of 4.5 to 0.09 μg injected on-column was less than 2% for all levels. At the lowest level of 0.045 μg, R.S.D. for triplicate assays was 4.7%. These values for the R.S.D. compare favorably to those reported previously for HPLC with UV detection at 214 nm[4] where the approximate amount of clindamycin was 90 μg and the R.S.D. was in the 0.5–2% range. For clindamycin phosphate, as shown in Table II, the R.S.D. for triplicate assays was 3.3% or less in the 0.12–5.8 μg range; and R.S.D. of 5.9% was obtained at the 0.058-μg injected level. Again, the R.S.D. values for clindamycin phosphate by HPLC–ED were comparable to those values reported by Munson and Kubiak[5] employing UV detection at 210 nm, where quantities were approximately 4 μg[5]. The linearity for the HPLC–UV analysis of clindamycin as described in this paper is shown in Table III. The R.S.D. values in Table III are comparable to those for the HPLC–ED method (Table I); however, the levels injected are *ca.* 100-fold less for the HPLC–ED technique.

TABLE II

LINEARITY OF CLINDAMYCIN PHOSPHATE ANALYSIS BY HPLC–ED PERFORMED FOR TRIPLICATE ASSAYS

Phosphate ester sample diluted from bulk sample solution rated at 150 mg/ml.

Amount injected on-column[a] *(μg)*	*Mean peak area*	*S.D.*	*R.S.D. (%)*
0.058	$3.33 \cdot 10^5$	$1.98 \cdot 10^4$	5.9
0.12	$6.19 \cdot 10^5$	$1.03 \cdot 10^4$	1.6
0.58	$3.43 \cdot 10^6$	$6.91 \cdot 10^4$	2.0
1.17	$6.42 \cdot 10^6$	$4.13 \cdot 10^4$	0.7
5.84	$2.09 \cdot 10^7$	$6.86 \cdot 10^5$	3.3

[a] Correlation coefficient for the range 0.058–1.17 μg was 0.9990.

TABLE III

LINEARITY OF CLINDAMYCIN ANALYSIS BY HPLC–UV FOR TRIPLICATE ASSAYS

A clindamycin standard was used at a potency of 966 μg/mg.

Amount injected on-column[a] *(μg)*	*Mean peak area*	*S.D.*	*R.S.D. (%)*
4.2	$2.21 \cdot 10^5$	$8.34 \cdot 10^2$	0.4
10.0	$5.04 \cdot 10^5$	$4.83 \cdot 10^3$	1.0
16.0	$7.74 \cdot 10^5$	$4.38 \cdot 10^3$	0.6
20.0	$9.77 \cdot 10^5$	$2.21 \cdot 10^3$	0.2

[a] Correlation coefficient was 0.9997.

As a test for the limit of detection for clindamycin by HPLC–ED, a 100-pg (*ca.* 240-fmol) injection on-column was made. Although the background trace was sloping, the chromatographic peak was easily observed as demonstrated in Fig. 5. This detection limit was highly dependent on the background noise encountered and the preconditioning of the electrochemical detector. The limit of detection for clindamycin phosphate was approximately an order of magnitude higher due to the operating parameters of the cell and potentiostat. As determined by CV and DPV, the oxidation potential for clindamycin phosphate is more anodic than that of clindamycin. In order to attain limits of detection of 100 pg for the phosphate ester, the detection potential would have to be set at an higher potential than +0.9 V. Unfortunately, background current at higher potentials due to the oxidation of the mobile phase, severely limited the analyte current that could be observed. The +0.9 V detection potential was a compromise between a good analyte signal for clindamycin phosphate and low background noise.

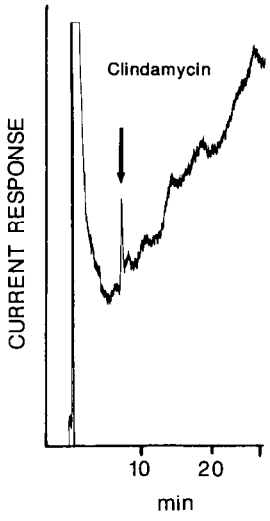

Fig. 5. Test for detection limit of clindamycin (I) by HPLC–ED with 100 pg injected on-column.

Three bulk preparations of clindamycin were assayed by HPLC–ED by comparison to a standard of clindamycin hydrochloride which had a potency of 837 mcg/mg. Nominal sample solutions of 0.01 mg/ml for the bulk preparations were prepared and assayed in triplicate. A linear calibration curve for the standard over the concentration range of the prepared bulk sample solutions was generated. Table IV illustrates the values obtained by HPLC–ED for these bulk preparations of clindamycin on two different days, as well as the average value measured by HPLC using UV detection at 214 nm. On each day, a new calibration curve was generated and new sample and standard solutions were prepared.

The variability of the mean potency from day one to day two for the bulk samples analyzed by HPLC–ED (Table IV) was calculated by taking the difference between the mean potencies and the average mean potencies. This variability ranged from 5.6% for bulk sample A to 2.2% for bulk sample C. The final two columns in Table IV indicate the R.S.D. for six measurements using day one and two combined. The R.S.D. for this HPLC–ED analysis of clindamycin ranged from 4.3% to 1.5%, which compares to an R.S.D. of less than 2% reported for the HPLC–UV analysis of clindamycin phosphate[5]. It should be stated that the typical amount injected on-column for HPLC–ED was *ca.* 20-fold less than the amount injected for the HPLC–UV analysis of clindamycin phosphate[5]. The R.S.D. for the HPLC–UV potencies shown in Table IV was less than 1%. From Table IV, it is noted that all potencies determined by HPLC–ED are less than the HPLC–UV potency. The clindamycin standard used for the evaluation of the bulk samples was not a United States Pharmacopia (USP) standard. It was observed with the non-USP standard that several additional chromatographic peaks, which included compounds III and IV, were observed. Subsequent HPLC–ED of a USP standard rated at a potency of 866 mcg/mg did not have the same quantity or magnitude of these additional chromato-

TABLE IV

COMPARISON OF ASSAY BY HPLC–ED AND HPLC–UV FOR BULK PREPARATIONS OF CLINDAMYCIN ON TWO DIFFERENT DAYS

Potencies in mcg/mg.

Bulk sample	*HPLC–UV*[a] *potency*	*Day one*			*Day two*			*Day one and two combined*[b]	
		Potency	*Mean*	*R.S.D. (%)*	*Potency*	*Mean*	*R.S.D. (%)*	*Mean*	*(%)*
A	820	800 768 735	768	4.2	805 796 835	812	2.5	790	4.3
B	825	765 772 789	775	1.5	755 799 835	803	3.7	786	3.7
C	822	789 785 792	788	0.5	765 768 779	771	0.9	780	1.4

[a] Potency determined by HPLC with UV detection at 214 nm for one day only.

[b] Total of six samples from day one and day two

graphic peaks. The difficulties in assaying antibiotics by HPLC and correlating the assays to the determined microbiological potency have been discussed by Thomas[9]. In this particular study, the compounds associated with the additional peaks observed in the non-USP standard may affect the specific potency indicative of the amount of clindamycin in the non-USP standard.

In the course of developing this analysis for clindamycin by HPLC–ED, several precautions had to be taken. The mixing of methanol and aqueous phosphate buffer generated a sufficient heat of mixing that resulted in retention times for clindamycin that were decreasing continually during the course of the day as the mobile phase cooled. This heating effect also perturbed the reproducibility of integrated peak areas. In order to alleviate this difficulty, the mobile phase was mixed and filtered several hours before use. Thermostating the HPLC–ED system may be helpful in diminishing this heating effect. The retention time for clindamycin, as reported earlier[4], is highly pH dependent above pH 7; care must be taken to be sure that the pH of the mobile phase is 6. The dual electrochemical cell, as is the case for many cells used for HPLC–ED, required cleaning of adsorbed materials from the carbon electrode. This cleaning was performed by flushing the cell with diluted nitric acid. This study used a syringe pump for HPLC. Thorough flushing of the syringe pump and complete filling with the mobile phase is a requirement for reproducible retention times. It was subsequently found that for concentrations of 0.01 mg/ml and above, a conventional dual-head reciprocal HPLC pump may be employed. Furthermore the use of an ion pair reagent in the mobile phase for clindamycin may mot be necessary. Varying the concentration of sodium pentane sulfonate did not have a profound effect on the retention times. As in the case with Munson and Kubiak[5] and the HPLC–UV method used in this study, good reversed-phase HPLC with only aqueous phosphate buffer and acetonitrile was possible. Further work will establish the feasibility and reproducibility of HPLC–ED for assaying injectables of clindamycin phosphate.

CONCLUSIONS

The HPLC–ED method is an effective means to detect and analyze clindamycin and clindamycin phosphate. The low detection limits that are possible with HPLC–ED may make this technique viable for metabolite investigations of clindamycin and its phosphate ester. Because lincomycin and lincomycin B were also detected by HPLC–ED, the HPLC–ED method allowed analysis of several possible bulk impurities. The detection of clindamycin B and 7-epiclindamycin by HPLC–ED was not established due to a lack of representative samples for these compounds, which may also exist as bulk impurities in clindamycin[4]. Future work will develop a similar HPLC–ED procedure for the antibiotic lincomycin.

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