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Liquid chromatography method for separation of clindamycin from related substances

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Abstract

A reversed-phase liquid chromatography method has been developed for the separation of clindamycin from 7-epiclindamycin, clindamycin B, lincomycin, lincomycin B, 7-epilincomycin and other impurities of unknown identity. The method uses a Hypersil ODS, 5 μm, 250 × 4.6 mm i.d. column maintained at 45°C. The mobile phase comprises acetonitrile–phosphate buffer (1.35% v/v phosphoric acid, adjusted to pH 6.0 with ammonium hydroxide)–water (35:40:25, v/v) at a flow rate of 1.0 ml/min. UV detection is performed at 210 nm. The method was tested on several C-18 columns and showed good robustness. Robustness was further evaluated by performing a full-fraction factorial design experiment. The method showed good selectivity, linearity, and repeatability. It is also suitable for analysis of clindamycin formulations.

Keywords: Clindamycin; Lincomycin; Reversed-phase liquid chromatography

1. Introduction

Clindamycin is an antibiotic effective against Gram-positive aerobes and both Gram-negative and Gram-positive anaerobic pathogens. It is synthesized by chemical modification of lincomycin [1], an antibiotic produced by microbial fermentation, to increase biological activity. Clindamycin hydrochloride is supplied for oral administration in capsules. Further improvement in pharmaceutical properties of clindamycin is obtained by chemical modification to obtain the esters, clindamycin phosphate or clindamycin palmitate. Common impurities (Fig. 1) in clindamycin bulk drug are clindamycin B, 7-epiclindamycin, and a small amount of the lincomycin starting material. Clindamycin B is formed from lincomycin B which is a normal by-product of the fermentation; 7-epiclindamycin is produced during the synthesis of clindamycin [2].

Several methods have been reported for the determination of clindamycin in bulk drug and dosage forms. Microbiological [3,4] and spectrophotometric [5] methods described for assay of clindamycin are non-specific and less accurate.

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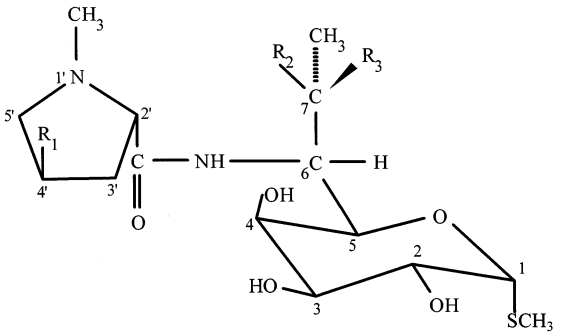

	R_1	R_2	R_3
Lincomycin	CH_3-CH_2-CH_2-	OH	H
Lincomycin B	CH_3-CH_2-	OH	H
7-Epilincomycin	CH_3-CH_2-CH_2-	H	OH
Clindamycin	CH_3-CH_2-CH_2-	H	Cl
Clindamycin B	CH_3-CH_2-	H	Cl
7-Epiclindamycin	CH_3-CH_2-CH_2-	Cl	H

Fig. 1. Structures of clindamycin and related compounds.

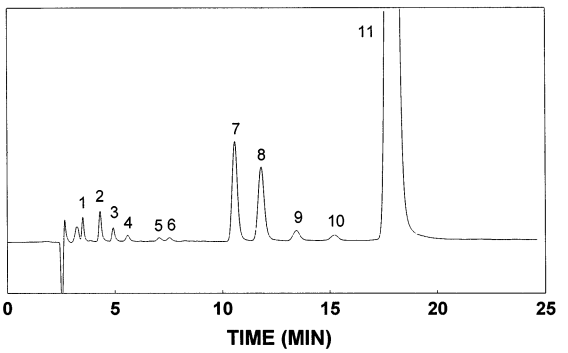

Fig. 2. Chromatogram of 2.0 mg/ml solution of clindamycin bulk drug spiked with 0.002 mg/ml lincomycin B. Conditions: Hypersil ODS column, 5 μm, 250 × 4.6 mm i.d. maintained at 45°C; mobile phase of acetonitrile–phosphate buffer (1.35% v/v phosphoric acid, adjusted to pH 6.0 with ammonium hydroxide)–water (35:40:25, v/v/v) at a flow rate of 1.0 ml/min. UV detection was performed at 210 nm. 1, lincomycin B; 2, lincomycin; 3, 7-epilincomycin; 4, unknown; 5, unknown; 6, unknown; 7, clindamycin B; 8, 7-epiclindamycin; 9, unknown; 10, unknown; 11, clindamycin.

Gas–liquid chromatography (GLC) methods require elaborate extraction and derivatization steps [6,7]. Liquid chromatography (LC) methods have been used in several instances for the analysis of clindamycin hydrochloride. Brown separated clindamycin B from clindamycin on a C-18 column using ion-pair LC with refractive index detection [8]. He used dioctyl sodium sulfosuccinate, formic acid and methanol in the mobile phase. This method was improved by Landis et al. who separated clindamycin from lincomycin, clindamycin B and 7-epiclindamycin [2]. They used camphor sulfonate instead of dioctyl sodium sulfosuccinate as the ion-pairing reagent and replaced formic acid with acetic acid. However, these methods suffered from the instability and poor sensitivity limits associated with refractive index detection. Other investigators used end absorption below 220 nm for the analysis of clindamycin. La Follette et al. analyzed clindamycin in human plasma or serum at 190 nm using an ion-pair system for

Table 1
Separation parameters on different silica-based C-18 columns

Column	Stationary phase (250 × 4.6 mm i.d.)	Acetonitrile in the mobile phase (%)	Symmetry[a]	Resolution[b]	Theoretical plates[a]	k'[a]
1	RSil C18LL, 5 μm	24.7	1.80	0.62	1510	3.49
2	Bakerbond C-18, 5 μm	36.8	2.89	1.09	3390	4.02
3	Bio-Sil C-18 LL, 5 μm	33.9	1.57	0.83	3610	3.14
4	Spherisorb S5 ODS-B, 5 μm	35.9	1.09	1.36	7960	3.75
5	Chromspher B C-18, 5 μm	34.4	1.04	1.50	7790	3.60
6	Hypersil ODS, 5 μm	34.8	1.19	1.13	7770	3.68
7	Hypersil ODS, 5 μm	35.8	1.27	1.08	5470	3.77
8	Hypersil ODS, 5 μm	35.4	1.06	1.51	6780	3.84

[a] Calculated for clindamycin peak according to Ph. Eur. [12].
[b] Resolution between clindamycin B and 7-epiclindamycin.

separation on a C-18 column [9]. Hornedo-Nuñez et al. described a reversed-phase ion-pair LC with electrochemical detection for assay of clindamycin [10]. These workers separated clindamycin from lincomycin and lincomycin B but there was no mention of clindamycin B and 7-epiclindamycin. The current United States Pharmacopeia (USP) method for determining clindamycin hydrochloride also utilizes a reversed-phase LC method with refractive index detection [11], comparable to other methods described in the literature [2,8]. Due to poor sensitivity associated with refractive index detection, UV detection is preferable.

In this study, a reversed-phase LC method was developed using a Hypersil ODS column, 5 μm, 250 × 4.6 mm i.d. maintained at 45°C. The mobile phase comprised acetonitrile–phosphate buffer (1.35% v/v phosphoric acid, adjusted to pH 6.0 with ammonium hydroxide)–water (35:40:25, v/v) at a flow rate of 1.0 ml/min. Detection was by UV at 210 nm. This LC method allows simultaneous determination of clindamycin and its related substances without the addition of ion pairing agents. It was applied satisfactorily for the quantitative analysis of clindamycin capsules (Antirobe®).

2. Experimental

2.1. Reagents and samples

Acetonitrile Grade S was from Rathburn chemicals (Walkerburn, UK). Phosphoric acid solution (1.35% v/v) was prepared from 85% m/m phosophoric acid (Acros Organics, Beerse, Belgium). Ammonia solution 25% was from BDH Laboratory Supplies (Poole, UK). Water was distilled twice from glass apparatus. Clindamycin hydrochloride capsules (Antirobe®) as well as reference samples of clindamycin hydrochloride, 7-epiclindamycin hydrochloride, lincomycin hydrochloride, lincomycin B hydrochloride and 7-epilincomycin hydrochloride were obtained from Pharmacia & Upjohn (Kalamazoo, Michigan). Clindamycin B hydrochloride was from Pierrel S.p.A. (Milan, Italy). Clindamycin hydrochloride European Pharmacopoeia chemical reference substance (Ph. Eur. CRS) was used in quantitative work.

2.2. LC apparatus and operating conditions

The isocratic liquid chromatography system consisted of an L-6200 intelligent pump (Merck–Hitachi, Damstadt, Germany), a Merck–Hitachi model 655A-40 autosampler set to inject 20 μl, an electronic integrator HP 3396 Series II (Hewlett–Packard, Avondale, PA) and a Merck–Hitachi model L-4000 variable UV detector set at 210 nm. The columns (250 × 4.6 mm i.d.) were packed in the laboratory with different stationary phases: Hypersil C-18, 5 μm (Shandon, Runcorn, UK), RSil C-18 LL, 5 μm (Alltech, Laarne, Belgium), Bakerbond C-18, 5 μm (Baker, Phillipsburg, USA), Bio-Sil C-18 LL, 5 μm (Alltech, Laarne, Belgium), Spherisorb S5 ODS-B, 5 μm (Phase Separations, Norwalk, USA) and Chromspher B C-18, 5 μm (Merck, Darmstadt, Germany). The column temperature was maintained at 45°C by means of a Julabo EM thermostat (Julabo, Seelbach, Germany).

2.3. Analytical procedure

Clindamycin hydrochloride bulk substance or reference standard solution was prepared by dis-

Table 2
Nominal values corresponding to −1, 0 and +1 levels

Chromatographic variable	Low value (−1)	Central value (0)	High value (+1)
Acetonitrile (%)	34	35	36
pH	5.5	6.0	6.5
Phosphate buffer (%)	35	40	45
Column temperature (°C)	40	45	50

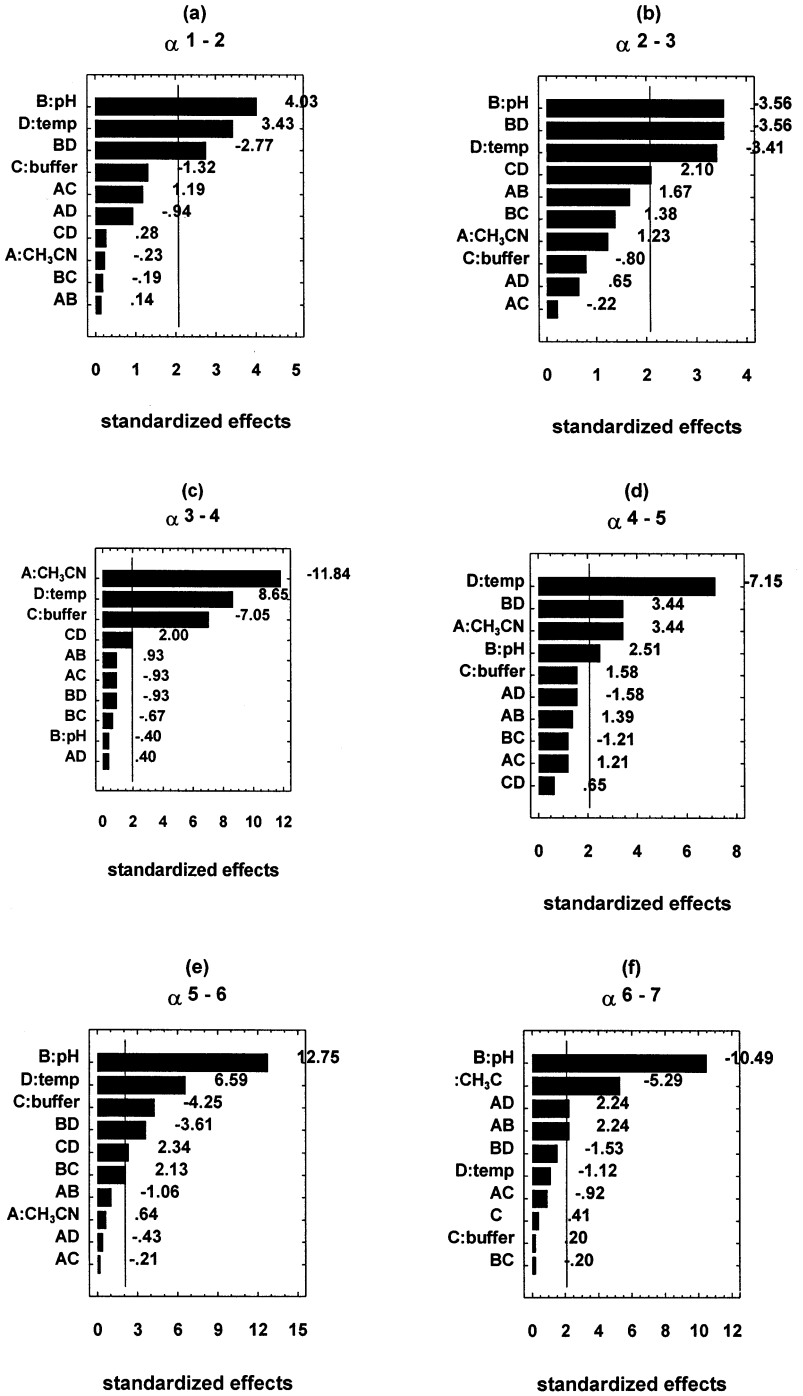

Fig. 3. Standardized pareto charts representing the estimated effects of parameters (A, B, C, D) and parameter interactions (AB, CD, AD, BD, AC, BC) on α1–2 (a), α2–3 (b), α3–4 (c), α4–5 (d), α5–6 (e), α6–7 (f), α7–8 (g), α8–9 (h), α9–10 (i) and α10–11 (j).

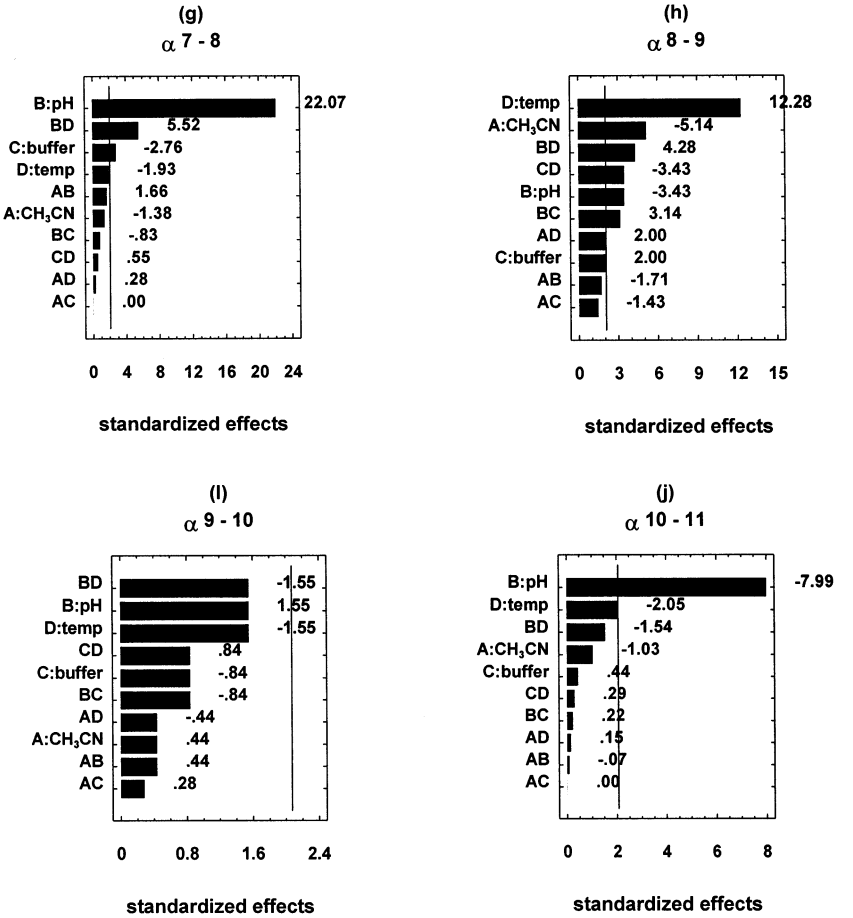

Fig. 3. (*Continued*)

separation on a C-18 column [9]. Hornedo-Nuñez et al. described a reversed-phase ion-pair LC with mg in 10.0 ml of water. The test solution for capsules was prepared by emptying 20 capsules, mixing, and extracting an amount equivalent to 200 mg with 100.0 ml of water.

3. Results and discussion

3.1. Development of chromatographic method and robustness

The methods described in the USP [11] for clindamycin hydrochloride and for lincomycin hydrochloride as well as the method described in Ph. Eur [12] for clindamycin phosphate ester were examined for clindamycin hydrochloride. This Ph. Eur. method resolved clindamycin from clindamycin B but 7-epiclindamycin was not resolved. The USP method for clindamycin hydrochloride, used as described, was not able to give sufficient separation. This method was adapted by using UV detection and a mobile phase containing acetonitrile instead of methanol and phosphate buffer instead of acetate buffer. Sufficient resolution was obtained for clindamycin, 7-epiclindamycin and clindamycin B but the baseline was unstable, probably because the ion-pairing reagent, sodium camphor sulfonate, used in the mobile phase absorbs strongly at 210 nm. Surprisingly, the USP method for lincomycin, which was

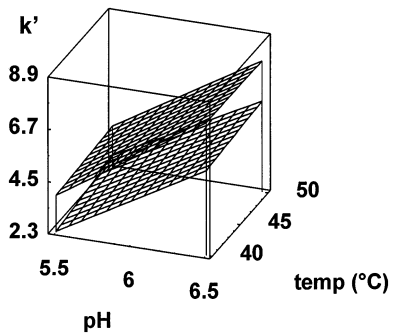

Fig. 4. Estimated response surface plots for peak 10 (lower plane) and peak 11 (upper plane), constructed with the capacity factors as a function of mobile phase pH and column temperature (temp).

not giving very good separation of lincomycin components, seemed to be more suitable for analysis of clindamycin.

In this study, starting from the USP method for lincomycin, a simple and robust method was developed which is able to resolve clindamycin from its related substances within a reasonable run time and which is using UV detection. Acetonitrile was used as the organic modifier because methanol gave poor separations. Chromatography was optimized by varying the composition and the pH of the mobile phase and the column temperature. The reproducibility of the selectivity was examined on different C-18 stationary phases (Table 1). Baseline resolution of clindamycin from its major impurities was obtained on all the columns, examined indicating robustness of the method. Fig. 2 shows a chromatogram of clindamycin bulk drug spiked with lincomycin B, using optimized conditions. Clindamycin was resolved from 7-epiclindamycin, clindamycin B, 7-epilincomycin, lincomycin and lincomycin B. Other impurities of unknown identity were also resolved. Further work was carried out with a Hypersil C-18 column because it gave good resolution between clindamycin B and 7-epiclindamycin, and also good symmetry.

The robustness of the method was further evaluated by performing a full factorial design experiment. The set-up of the full factorial design, together with the analysis of the measured response variable and multivariate regression calculation were supported by the statistical graphic software system STATGRAPHICS Version 6.0 (Manugistics, Rockville, MD, USA). The influence of each of the four chromatographic parameters that governed the separation most was examined by applying a full factorial design at two levels. This involved $2^4 = 16$ different experimental measurements, combining the four parameters examined at two previously fixed extreme levels of each parameter. One central level was included in the design and so 17 measurements were performed as well as duplicate experiments. The chromatographic parameters examined as variables were the concentration of acetonitrile, the pH of the mobile phase, the concentration of the phosphate buffer and the column temperature. The values of the design are given in Table 2. The estimated effects of the four chromatographic parameters with their second-order interactions on the selectivity between the critical pairs of clindamycin components as response variables are presented on the standardized pareto charts in Fig. 3. The bars are displayed in order of the size of the effects, with the largest effects on top. The charts include a vertical line at the critical t-value for an alpha of 0.05. Effects for which the bars are smaller than the critical t-value are considered as not significant. Effects may be positive or negative. A posi-

Table 3
Content (%) of clindamycin and related substances[a] in capsules

	Lincomycin	7-Epilincomycin	Clindamycin B	7-Epiclindamycin	Clindamycin
A[b]	0.10 (5.0)	0.14 (3.6)	0.44 (3.1)	0.72 (1.5)	96.7 (0.5)
B[b]	<LOD	0.09 (12)	0.37 (2.4)	0.95 (0.9)	102.5 (0.6)

[a] Related substances calculated as clindamycin. RSD given in parentheses for $n = 6$.
[b] A and B are samples of clindamycin formulation. Theoretical amount, 150 mg/capsule.

tive effect means an increase in the selectivity with an increase in the chromatographic parameter, while a negative effect means a decrease in the selectivity with an increase in the chromatographic parameter.

Fig. 3 shows that selectivity between most peak pairs is principally influenced by the pH of the mobile phase. An increase in the mobile phase pH increases $\alpha 1-2$ (Fig. 3a), $\alpha 5-6$ (Fig. 3e) and $\alpha 7-8$ (Fig. 3g), while $\alpha 2-3$ (Fig. 3b), $\alpha 6-7$ (Fig. 3f) and $\alpha 10-11$ (Fig. 3j) is reduced. The column temperature has principal influence on $\alpha 4-5$ (negative influence) (Fig. 3d) and $\alpha 8-9$ (positive influence) (Fig. 3h). An increase in the concentration of the organic modifier reduces $\alpha 3-4$ (Fig. 3c), $\alpha 6-8$ (Fig. 3f) and $\alpha 8-9$ (Fig. 3h). Significant effects of buffer pH are observed in Fig. 3c,e,g. Interaction between pH and temperature was also observed to influence selectivity between peak pairs (Fig. 3a,b,d,e,g,h). Interactions established between column temperature and buffer concentration and between pH and buffer concentration influenced $\alpha 8-9$ (Fig. 3h). Other chromatographic parameter interactions had no significant impact on the selectivities between the examined compounds.

Response surface plots were constructed with the capacity factors as a function of the most important chromatographic parameters: mobile phase pH and column temperature. For the major component (peak 11) and the closest component (peak 10), the surface plots are shown (Fig. 4). Except for the unknown peak pairs, 5 and 6, and 9 and 10, in all the conditions examined there was no other overlapping, indicating the robustness of the method.

3.2. Repeatability, linearity and detection limits

The precision of the method was assessed using six replicate injections of a solution of clindamycin hydrochloride (40 μg injected on the column). The relative standard deviation (RSD) of the peak area of the main component was 0.55%. The calibration curve obtained by replicate analysis ($n = 3$) of a series of analyte concentrations corresponding to 20, 30, 40, 50 and 60 μg injected mass was subjected to linear regression analysis: $y = 411753x - 289781$, where y is the peak area, x the mass (μg) injected; correlation coefficient $r = 0.9999$, standard error of estimate $S_{y,x} = 293\,695$. The limit of quantification (LOQ) was 0.12% of the nominal value (40 μg), i.e. 0.048 μg injected mass ($n = 6$; RSD = 18%). The limit of detection (LOD) with a signal to noise ratio of 3 was 0.06% of 40 μg (0.024 μg injected mass).

3.3. Analysis of commercial samples

The method was applied to the assay of two batches of commercial clindamycin capsules. Clindamycin hydrochloride Ph. Eur. CRS was used as an external standard. Replicate injections ($n = 3$) of replicate sampling ($n = 2$) were carried out in each case. Results of the assay of clindamycin and known related substances are presented in Table 3. The unknowns 1–5 as well as lincomycin B were present in quantities between LOD and LOQ. Assay of the samples yielded good precision for the major component. The content of the related substances was calculated as clindamycin, using a diluted solution of the Ph. Eur. CRS corresponding to 2% of the nominal content.

4. Conclusion

The isocratic LC method presented here is suitable for the separation of clindamycin from its potential impurities. This robust method shows good selectivity, repeatability and linearity. It was applied satisfactorily to the quantitative analysis of commercial clindamycin capsule.

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