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Analysis of erythromycin estolate by liquid chromatography*

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Abstract: A method is described for the determination of erythromycin estolate by liquid chromatography. A C18 reversed-phase column (25 × 0.46 cm i.d.) was used with acetonitrile–tetrabutylammonium sulphate (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water [x:5:5:(90–x), v/v/v/v] as mobile phase. The proportion of acetonitrile (x) has to be adapted to the type of stationary phase used. For RSil C18 LL 42.5% (v/v) was used. The column was heated at 35°C, the flow rate was 1.5 ml min^{-1} and UV detection was performed at 215 nm. The main component, erythromycin A propionate, was separated from all other components which were present in commercial samples. The impurities most frequently observed were the propionate ester of erythromycin C and the amide *N*-propionyl-*N*-demethylerythromycin A. Erythromycin A was shown to be present in specialties.

Keywords: *Erythromycin estolate; reversed-phase liquid chromatography.*

Introduction

Soon after the introduction of the antibiotic erythromycin in 1952, a number of esters were developed in an effort to provide an erythromycin derivative with improved acid-stability and uptake characteristics [1]. One of these derivatives is erythromycin propionate (EP), which is often used as erythromycin estolate (EPLS), the lauryl sulphate salt of the 2′-propionate ester. It has been formulated in both liquid and solid pharmaceutical forms and is nowadays still widely used.

Esters of erythromycin are biologically inactive prodrugs and have to be hydrolysed to exhibit antimicrobial activity. At present, pharmacopoeias prescribe a microbiological method for the assay of erythromycin estolate [2, 3]. After hydrolysis of the ester the total activity is determined against an erythromycin standard. On the other hand, liquid chromatography (LC) allows the separation of the ester from erythromycin and other related substances and therefore provides a means for specific and accurate quantitation of the ester.

Papers on LC of erythromycin propionate [4, 5] refer to methods that determine only the main component in pharmaceutical dosage forms [4] and in biological fluids [5]. These methods are not suitable for the assay and purity control of bulk products and preparations. In this paper, an LC method is described for the analysis of EP or EPLS in bulk or pharmaceutical forms.

Experimental

Samples and reference substances

Bulk samples of erythromycin propionate (EP) were kindly provided by Roussel UCLAF (Paris, France). Bulk samples of erythromycin estolate were a gift from Prof. H. Vanderhaeghe (Rega Institute, Katholieke Universiteit Leuven, Belgium). Specialties containing erythromycin estolate were obtained from the Belgium market. The European Pharmacopoeia Chemical Reference Substance (Ph. Eur. CRS) was also available. Pure erythromycin A (EA) was obtained by crystallization of a commercial sample as described [6]. Anhydroerythromycin A (AEA) [7] and erythromycin A enol ether (EAEN) [8] were prepared from EA according to previously described methods. Analogous methods were used to prepare anhydroerythromycin A propionate (AEAP) and erythromycin A enol ether pro-

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pionate (EAENP) from EAP. Details of the preparation of EAENP and AEAP and analytical data will be reported elsewhere. Propionate esters of erythromycin B (EBP), erythromycin C (ECP), erythromycin E (EEP), erythromycin F (EFP), *N*-demethylerythromycin A (dMeEAP), pseudo-erythromycin A hemiketal (psEAHKP) and pseudo-erythromycin A enol ether (psEAENP) were prepared by reaction with propionyl chloride. The following typical procedure was used: to a mixture of 100 mg (*ca* 0.15 mmol) of starting material, dissolved in 10 ml of dry acetone, and 500 mg of anhydrous sodium carbonate, an equivalent amount of propionyl chloride (Janssen Chimica, Beerse, Belgium) was added. The mixture was stirred for 2 h, filtered and evaporated under reduced pressure. Smaller amounts of starting material (up to 10 mg) were used to prepare the derivatives of EB, EC, EE and EF. The starting materials EB and EC were obtained by preparative LC of mother liquor residues from erythromycin production [9]. EE [10] and EF [11] were isolated from commercial erythromycin. dMeEA [12], psEAEN and psEAHK [13] were prepared according to described procedures. After analysis, the derivative obtained from dMeEA turned out to be the amide (*N*-propionyl-*N*-demethylerythromycin A (PdMeEA)) rather than the desired 2′-*O*-ester (dMeEAP). The determination of the structure is discussed below. The structures are shown in Fig. 1.

Instrumentation

The chromatographic system was composed of a Waters M 45 pump (Milford, MA, USA), a Valco (Houston, TX, USA) Model CV-6-UHPa N60 injection valve equipped with a 20 μl loop, a Waters Model 441 detector at 215 nm and a Hewlett–Packard (Avondale, PA, USA) Model 3390 A integrator. The positive-ion liquid secondary ion mass spectrum was recorded on a Concept 1 H mass spectrometer (Kratos, Manchester, UK) fitted with a caesium ion gun. The sample was analysed in a glycerol matrix. The ^{13}C NMR spectrum was taken on a FT 90 MHz FX90Q instrument (Jeol, Tokyo, Japan). The sample was dissolved in $CDCl_3$.

Stationary phases

The stationary phases used are listed in Table 1 and were laboratory-packed in columns of 25 × 0.46 cm i.d. following a classical slurry packing procedure [14]. Partisil materials were obtained from Whatman (Clifton, NJ, USA), RSil from RSL-Biorad (Eke, Belgium), Spherisorb from Phase Separations (Queensferry, UK), LiChrosorb from Merck (Darmstadt, Germany), Zorbax from Dupont (Wilmington, DE, USA) and Nucleosil from Macherey–Nagel (Düren, Germany).

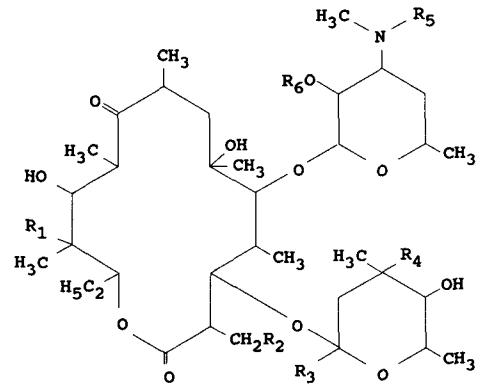
Solvents and mobile phases

Phosphate buffers (0.2 M) were prepared by mixing suitable amounts of 0.2 M solutions of ammonium dihydrogen phosphate and diammonium hydrogen phosphate (analytical reagent grade, E. Merck). Tetrabutylammonium (TBA) hydrogen sulphate (Janssen Chimica) was used to prepare 0.2 M TBA solutions. These solutions were adjusted to the required pH with 40% (m/v) sodium hydroxide solution before the solutions were brought to the final volume. LC-grade acetonitrile was from Rathburn Chemicals (Walkerburn, UK). Water was distilled twice from glass. Mobile phases were degassed by sonication. Acetone was obtained from Janssen Chimica and was purified by distillation after refluxing in the presence of potassium permanganate.

Sample preparation and stability of the solutions

About 150 mg of EP bulk samples were dissolved in a mixture containing 50% (v/v) acetonitrile, 5% (v/v) 0.2 M phosphate buffer (pH 6.5) and water up to 100% (v/v), and diluted to 10.0 ml with the same aqueous mixture. For the analysis of EPLS 200 mg was taken. For dispersible powders and tablets, 10 ml of acetonitrile were added to an amount corresponding to about 300 mg of EP or 400 mg of EPLS. The suspension of finely ground material was sonicated for 5 min in a glass-stoppered test-tube and then centrifuged at 2500 ***g*** for 5 min. An aliquot of 5.0 ml of supernatant was diluted to 10.0 ml with the same aqueous mixture as described for bulk material.

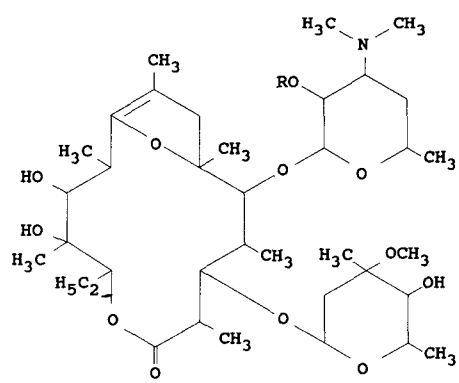
The stability of EP and EPLS in this solution was examined at room temperature using the described LC method. Hydrolysis of the ester to EA was observed, showing pseudo-first order kinetics with $t_{0.90}$ = 170 or 400 min for EP or EPLS, respectively. Samples for analysis were therefore prepared immediately before use. Hydrolysis of erythromycin esters in



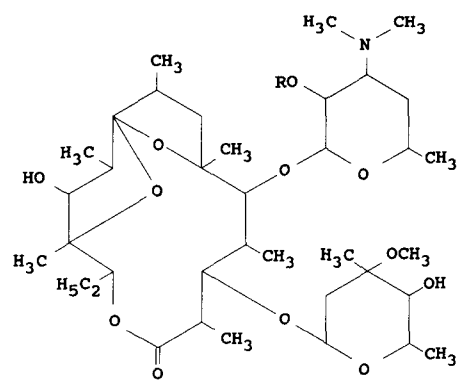
	R_1	R_2	R_3	R_4	R_5	R_6
ERYTHROMYCIN A (EA)	OH	H	H	OCH_3	CH_3	H
ERYTHROMYCIN B (EB)	H	H	H	OCH_3	CH_3	H
ERYTHROMYCIN C (EC)	OH	H	H	OH	CH_3	H
ERYTHROMYCIN F (EF)	OH	OH	H	OCH_3	CH_3	H
ERYTHROMYCIN E (EE)	OH	- O -		OCH_3	CH_3	H
N-DEMETHYLERY-THROMYCIN A (dMeEA)	OH	H	H	OCH_3	H	H

THE CORRESPONDING PROPIONATE ESTERS (..P) HAVE $R_6 = CO\text{-}C_2H_5$

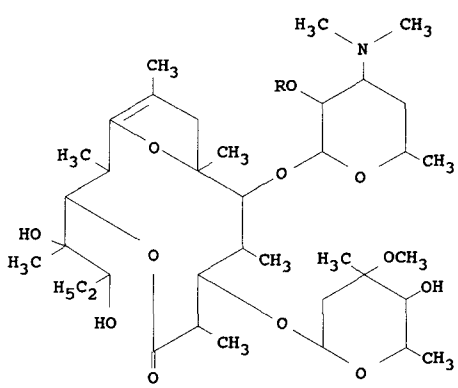
N-PROPIONYL, N-DEMETHYLERYTHROMYCIN A (PdMeEA) CORRESPONDS TO dMeEA WITH $R_5 = CO\text{-}C_2H_5$.



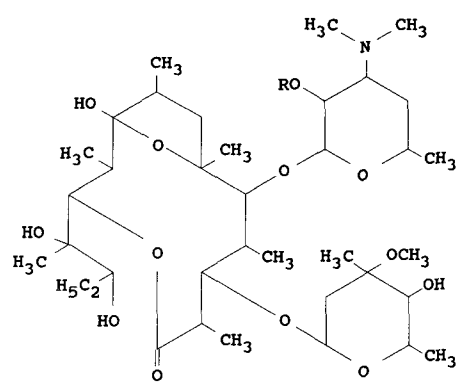
R = H ERYTHROMYCIN A ENOL ETHER (EAEN)
R = $CO\text{-}C_2H_5$ (EAENP)



R = H ANHYDROERYTHROMYCIN A (AEA)
R = $CO\text{-}C_2H_5$ (AEAP)



R = H PSEUDO-ERYTHROMYCIN A ENOL ETHER (psEAEN)
R = $CO\text{-}C_2H_5$ (psEAENP)



R = H PSEUDO-ERYTHROMYCIN A HEMIKETAL (psEAHK)
R = $CO\text{-}C_2H_5$ (psEAHKP)

Figure 1
Structures of erythromycin A propionate and related compounds.

Table 1
Investigated stationary phases

Column no.	Stationary phase	% Carbon content*	Particle shape†	dp (μm)
I	Partisil ODS 2	15	I	10
II	Partisil ODS 3	10	I	10
III	RSil C18 LL	12	I	10
IV	RSil C18 HL	18	I	10
V	Spherisorb ODS 1	7	S	10
VI	Spherisorb ODS 2	12	S	10
VII	LiChrosorb RP-18	22	I	10
VIII	Zorbax C8	15	S	7
IX	Nucleosil C18	14	S	10

*According to the supplier.
†I = irregular; S = spherical.
dp = particle diameter.

neutral aqueous solution has been reported previously [15].

Quantitative analysis

Analysis of the samples was finally performed on an RSil LL C18 (10 μm) column (25 × 0.46 cm i.d.). For the determination of the main component a house standard of EAP was used. The standard was obtained by four consecutive crystallizations from acetonitrile. By LC this standard was found to contain 1.3% of EA and 0.5% of ECP. Titrations with perchloric acid in non-aqueous medium gave a mean value of 95.4% with a relative standard deviation (RSD) of 0.5% for $n = 6$. As contaminating EA is also titrated and EA has a lower molecular mass than EAP, a correction was applied to account for the amount of EA. The water content was determined by means of Karl Fischer titration using a 10% (w/v) solution of imidazole in methanol as the solvent [16]. The result was 3.4% (RSD = 1.0%, $n = 7$). The presence in the house standard of 1.2% of acetonitrile (RSD = 7.0%, $n = 14$) was determined by gas chromatography. The total mass (100%) was well explained by the base titration result (95.4%), the water content (3.4%) and the acetonitrile content (1.2%). Therefore the standard was accepted to contain 100%–1.8% (total impurities by LC)–3.4% water–1.2% acetonitrile = 93.6% EAP.

Regression lines for EAP, EA and EAENP are given in Table 2. Regression lines obtained with EAP were corrected for a purity of 93.6%. Other curves were not corrected. Limits of quantification for an injected amount of 300 μg were 1 μg (0.3%) for EA; 0.15 μg (0.05%) for PdMeEA; 2 μg (0.6%) for AEA and AEAP; 0.5 μg (0.15%) for EAENP. The house standard was analysed 19 times over a period of 5 days. An RSD of 0.6% was obtained on the peak area corresponding to EAP.

Results and Discussion

Structure of N*-propionyl-*N*-demethylerythromycin A (PdMeEA)*

The amide structure of PdMeEA was determined by mass spectrometry and NMR. The liquid secondary ion mass spectrum exhibited a $(M + Na)^+$ ion at *m/z* 798 (11%) and prominent ions at *m/z* 200 (100%, desosamine moiety) and *m/z* 113 (97%, desosamine moiety — $HN(CH_3)COCH_2CH_3$).

The ^{13}C NMR spectrum in $CDCl_3$ clearly showed, as expected, the 21 aglycone and the eight cladinose resonances at essentially the same positions as for the parent compound *N*-demethylerythromycin A (dMeEA) [17]. The remaining seven desosamine signals (104.0, 71.6, 53.7, 35.6, 68.3, 20.8 and 29.2) and the

Table 2
Regression lines

Component	Slope	Intercept	Correlation coefficient	$S_{y,x}$	*R*
EAP	401893	615000	0.999	444483	220–250
EA	416616	10228	0.999	4967	3–40
EAENP	1007681	84956	0.997	83855	1–5

y = peak area; x = mass injected (μg); $S_{y,x}$ = standard error of estimate; R = range of injected mass (μg) examined.

three propionyl peaks (175.2, 27.1 and 9.0) showed an absorption pattern indicative of a 3′-*N*-propionyl moiety, rather than a 2′-*O*-propionyl moiety. Indeed, when compared with the spectrum of dMeEA (102.9, 74.4, 60.0, 36.8, 68.5, 20.9 and 32.7 for desosamine), a 1 ppm downfield shift for C-1′ was observed together with a substantial upfield shift for C-2′ (−2.8 ppm), C-3′ (−6.3 ppm), the 3′-*N*-Me group (−3.5 ppm) and C-4′ (−1.2 ppm). Thus, this clearly contrasts with a characteristic 2–2.5 ppm upfield shift of C-1′ and C-3′ and a *ca* 1 ppm downfield shift of C-2′ upon esterification of the 2′-OH function. Another strong argument in favour of the 3′-*N*-amide structure, as is described also for the 3′-*N*-ethylsuccinyl amide analogue [18] is the appearance of supplementary resonances at a 30% intensity level for almost all desosamine signals (103.5, 70.9, 57.3, 36.2 and 26.6). These are most probably due to the presence of different rotamers in solution, as a result of restricted rotation of the amide bond.

Development of the mobile phase

The development of a suitable mobile phase was based on previous experience with the ethylsuccinate ester of erythromycin (EES) [18]. For the determination of EES a RSil C18 LL (25 cm × 4.6 mm i.d.) was used with acetonitrile–tetrabutylammonium sulphate (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (42.5:5:5:47.5, v/v/v/v). The column was heated at 35°C, the flow rate was 1.5 ml min^{-1} and UV detection was performed at 215 nm. This method was used as the starting point for the investigation of the separation of EAP from EA and its decomposition products AEA and EAEN, which are formed in acid, and from the corresponding esters AEAP and EAENP. A RSil C18 LL column was used throughout. Parameters that

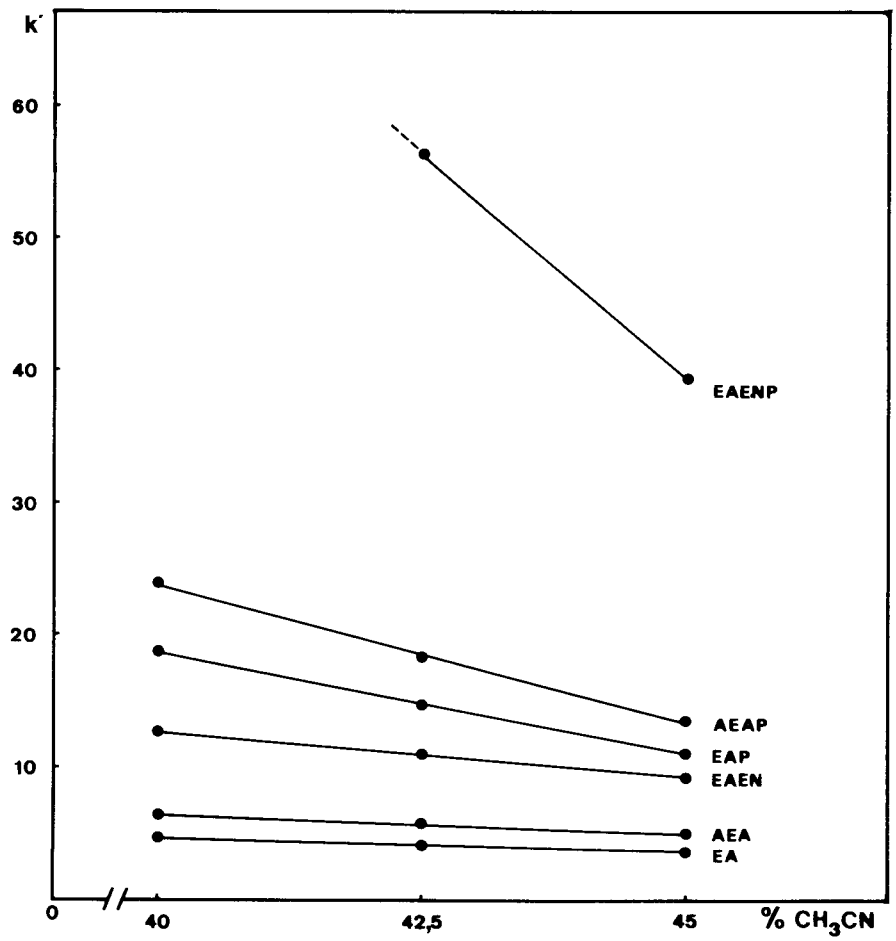


Figure 2
Influence of the acetonitrile content in the mobile phase on the separation. Mobile phase: acetonitrile–TBA (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (x:5:5:90−x, v/v/v/v). Column: RSil C18 LL, 10 μm, 25 × 0.46 cm i.d.; flow rate: 1.5 ml min^{-1}; temperature: 35°C; detection: UV at 215 nm.

were investigated were the acetonitrile content, pH, TBA and buffer concentration of the mobile phase. As expected, the propionate esters of EA, AEA and EAEN do behave similarly to the ethylsuccinate derivatives.

Relatively high acetonitrile concentrations (42.5–45%) were necessary for elution of EAENP (Fig. 2). The same elution profile was observed as with erythromycin ethylsuccinates except that now the anhydro derivative (AEAP) is eluted much closer after the main component (EAP). Therefore, a lower acetonitrile content may be necessary to obtain sufficient separation between AEAP and EAP. This will extend the analysis time. As with erythromycin ethylsuccinate (EES) variation of the pH of the mobile phase between pH 6 and 7 did not much affect the separation of the ester derivatives. At pH 7, EAEN was eluted very close to EAP while at lower pH a complete separation was obtained. A pH higher than 7 was not considered because it lowers the stability of the stationary phase. As with EES, below pH 6 the peak symmetry of the esters was severely affected and on most of the higher loaded stationary phases, fronting was observed. Therefore, pH 6.5 was adopted in subsequent chromatography of EP.

The influence of the TBA content of the mobile phase is shown in Fig. 3. The use of TBA influenced the order of elution and reduced the total analysis time. Beyond 5%, a further increase in TBA content did not substantially change the separation. Therefore, this concentration was adopted.

The influence of the concentration of the buffer in the mobile phase on the chromatography was small. Only the separation between EAEN and EAP was improved slightly on increasing the buffer concentration from 1 to 3%. Surprisingly, by further increasing the concentration of the buffer, the elution order EAP–EAEN switched to EAEN–EAP (Fig. 4). An intermediate value of 5% was adopted.

With the mobile phase acetonitrile–phosphate buffer (pH 6.5, 0.2 M)–TBA (pH 6.5, 0.2 M)–water (45:5:5:45, v/v/v/v), the capacity factors of EA, AEA, EAEN, EAP, AEAP

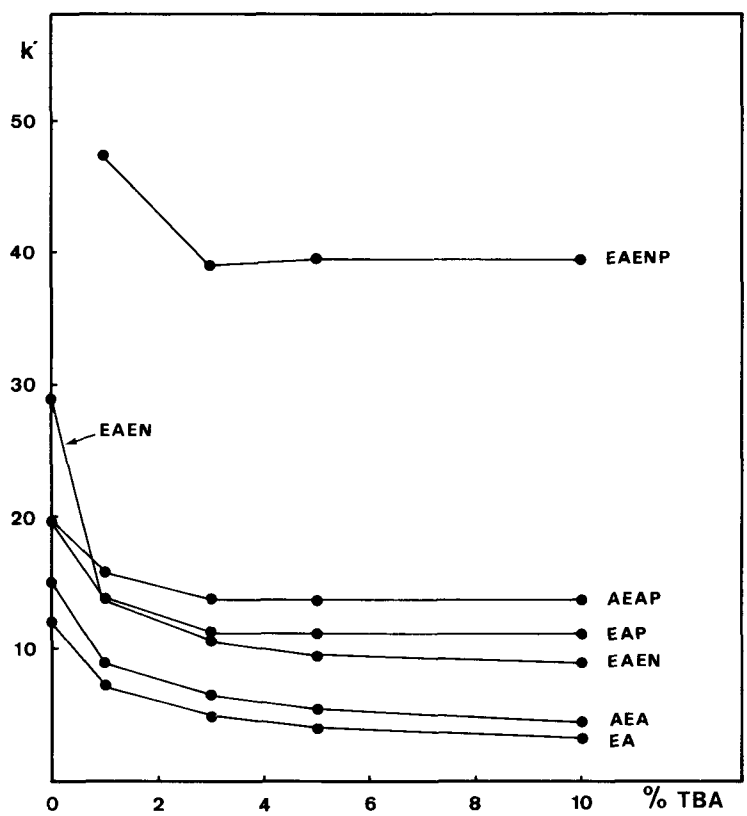


Figure 3
Influence of the concentration of tetrabutylammonium (TBA) in the mobile phase on the separation. Mobile phase: acetonitrile–TBA (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (45:x:5:50−x, v/v/v/v). Other conditions as in Fig. 2.

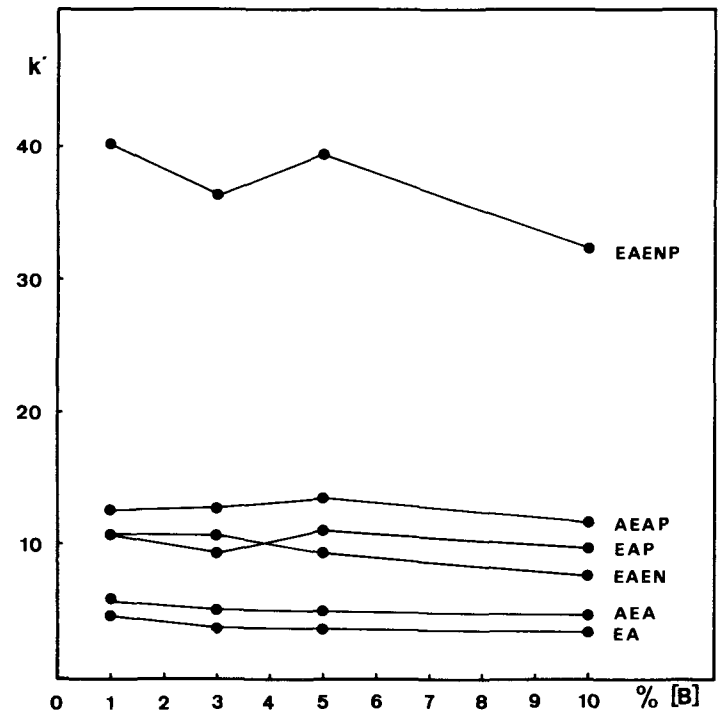


Figure 4
Influence of the buffer concentration in the mobile phase on the separation. Mobile phase: acetonitrile–TBA (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (45:5:x:50−x, v/v/v/v). Other conditions as in Fig. 2.

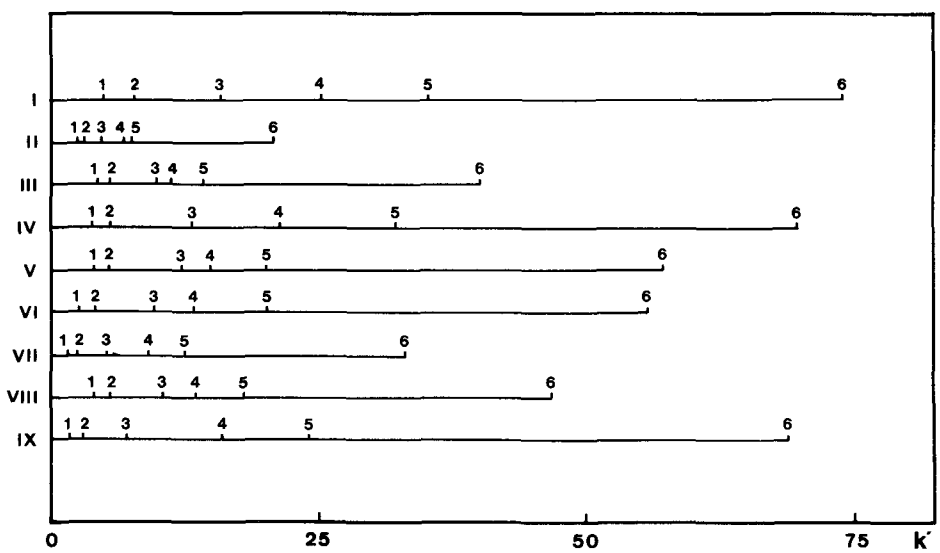


Figure 5
Influence of the stationary phase on the separation. See Table 1, for column identification and characteristics. Mobile phase: acetonitrile–TBA (pH 6.5, 0.2 M)–ammonium phosphate buffer (pH 6.5, 0.2 M)–water (45:5:5:45, v/v/v/v). Other conditions as in Fig. 2. 1 = EA; 2 = AEA; 3 = EAEN; 4 = EAP; 5 = AEAP; 6 = EAENP.

and EAENP were determined on the stationary phases mentioned in Table 1. The results are shown in Fig. 5. The same elution order was observed on all the columns. For this reason, the position of the substances is indicated by their elution number. The elution pattern is dependent on the stationary phase. However, a clear correlation between the column carbon content and the total analysis time was not observed. The total analysis time

can also be adapted by adjusting the acetonitrile content of the mobile phase. As was observed previously with EES [18], the chromatography of the propionate esters was not influenced by the age of the column, as was mentioned previously for erythromycins [19, 20].

Quantitative analysis of erythromycin propionate and estolate

With the mobile phase acetonitrile–TBA (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (42.5:5:5:47.5, v/v/v) and an RSil C18 LL column, a number of commercial EP and EPLS samples of various origin were analysed. The EAP content was calculated by comparison with the EAP standard, which was analysed alternately with the samples. Small amounts of reference substances for EAENP, PdMeEA and EA were injected in order to calculate the percentage of these impurities in the commercial sample. The percentages of the impurities EBP and ECP were calculated against small amounts of EAP standard. Only in a limited number of commercial samples EA was found, at a low level, near the detection limit. AEA, EAEN and AEAP were not detected. Only one sample contained a small amount of EAENP. The chromatograms of most samples showed the presence of other impurities.

It was demonstrated that commercial grade erythromycin may contain appreciable amounts of EB, EC, EE, EF and dMeEA (up to 5%) and small amounts of the decomposition products EAEN and psEAEN. AEA and psEAHK were found in negligible amounts [21]. Therefore one may expect some of these impurities to be present as the corresponding esters in commercial samples. The separation of the esters EBP, ECP, EFP, EEP, psEAENP and psEAHKP and of the amide PdMeEA was examined. The retention times of the various components relative to that of EAP are given in Table 3. There was no separation between EEP and EAP or between EFP and PdMeEA. Except for PdMeEA, the order of elution of the various components was consistent with the order of elution of the parent components [21]. Based on the relative retention times it was possible to identify EBP, ECP and PdMeEA in a number of EPLS and EP samples. As there was no separation between EEP and EAP or between EFP and PdMeEA, the samples were methanolysed and it was possible to show the presence of EF and EE by thin-layer chromatography (TLC). This infers the presence of EFP and EEP in commercial EPLS. TLC of erythromycin has been extensively discussed elsewhere [11]. Further reduction of the acetonitrile content to 40% did not improve the separation between EEP and EAP. On the other hand, separation between EFP and PdMeEA slightly improved. Figure 6 shows a typical chromatogram of a commercial EPLS sample.

Table 4 gives the results obtained in the analysis of commercial samples of bulk erythromycin propionate and estolate. The components of EPLS were calculated as the laurylsulphate salt. The precision of the determination of EAP in commercial EP and EPLS is better than 1% ($n \geq 3$). All the samples contain small amounts of ECP and PdMeEA. EFP, EBP and EEP are also present in a number of samples. The evaluation of EFP and EEP was done by means of TLC, after methanolysis.

Table 3
Relative retention times of the various propionate derivatives

Compound	Relative retention time
N-propionyl-*N*-demethylerythromycin A (PdMeEA)	0.30
Erythromycin F propionate (EFP)	0.31
Erythromycin C propionate (ECP)	0.57
Erythromycin E propionate (EEP)	1.00
Erythromycin A propionate (EAP)	1.00
Anhydroerythromycin A propionate (AEAP)	1.05
Pseudo-erythromycin A hemiketal propionate (psEAHKP)	1.20
Pseudo-erythromycin A enol ether propionate (psEAENP)	1.68
Erythromycin B propionate (EBP)	1.77
Erythromycin A enol ether propionate (EAENP)	4.40

Column: RSil C18 LL, 10 μm, 25 × 0.46 cm i.d. Mobile phase: acetonitrile–TBA (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (42.5:5:5:47.5, v/v/v/v). Temperature: 35°C; flow rate: 1.5 ml min^{-1}; detection: UV at 215 nm.

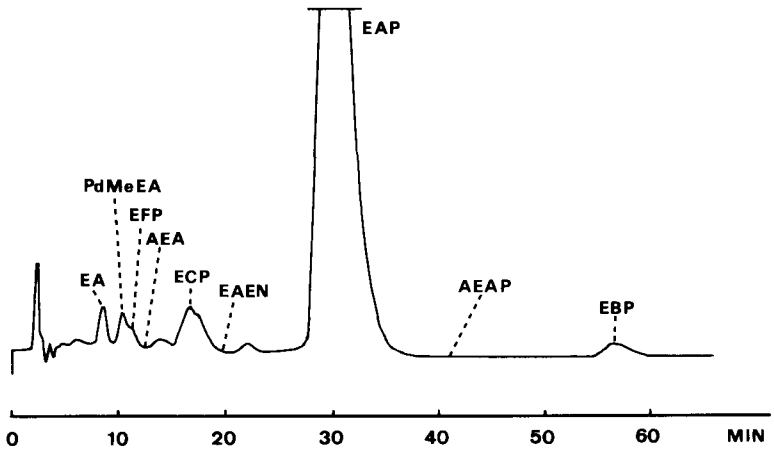


Figure 6
Typical chromatogram of a bulk sample of erythromycin estolate. Mobile phase: acetonitrile–TBA (pH 6.5, 0.2 M)–phosphate buffer (pH 6.5, 0.2 M)–water (40:5:5:50, v/v/v/v). Column RSil C18 LL, 10 μm, 25 × 0.46 cm i.d.; other conditions as in Fig. 2. EA = erythromycin A; PdMeEA = *N*-propionyl-*N*-demethylerythromycin A; EFP = erythromycin F propionate; AEA = anhydroerythromycin; ECP = erythromycin C propionate; EAEN = erythromycin A enol ether; EAP = erythromycin A propionate; AEAP = anhydroerythromycin propionate; EBP = erythromycin B propionate.

Table 4
Composition (% m/m) of bulk samples of erythromycin estolate (samples 1–12) and erythromycin propionate (13 and 14)

Manufacturer	Sample no.	EA	PdMeEA	EFP*	ECP	EAP (RSD %)	EBP	EAENP	H_2O†	Total
A	1	0.4	0.1	<0.5	1.4	93.6 (0.5)	<0.6	<0.15	2.9	98.3
	2	0.3	0.1	<0.5	1.0	94.5 (1.0)	<0.6	<0.15	2.8	98.7
	3	0.4	0.1	0.5	2.1	93.5 (0.5)	<0.6	<0.15	2.9	99.0
	4‡	<0.3	<0.05	1.0	1.6	94.9 (0.6)	<0.6	<0.15	2.8	100.3
B	5‡	<0.3	0.2	1.0	2.6	91.6 (0.4)	0.8	<0.15	2.9	99.1
	6‡	<0.3	0.3	1.0	1.6	90.8 (0.4)	1.3	<0.15	2.9	97.9
	7	<0.3	0.3	1.0	2.1	91.3 (0.7)	1.0	<0.15	2.9	98.6
C	8‡	<0.3	0.2	1.0	1.6	95.2 (0.5)	<0.6	<0.15	3.0	101.0
	9	<0.3	0.5	0.5	0.7	89.0 (0.5)	1.7	<0.15	2.8	95.2
	10	<0.3	0.3	0.5	0.9	94.0 (0.5)	<0.6	<0.15	2.9	98.6
Ph.Eur. CRS	11	<0.3	0.2	<0.5	2.1	93.1 (0.4)	<0.6	<0.15	2.8	98.2
Unknown	12‡	<0.3	0.3	<0.5	3.0	88.0 (0.3)	2.0	<0.15	2.8	96.1
D	13	<0.3	1.4	0.5	0.7	92.0 (0.3)	<0.6	0.4	2.0	97.0
	14	<0.3	1.2	<0.5	0.4	92.6 (0.4)	<0.6	<0.15	1.9	96.1

LC results for the estolate samples are calculated as the laurylsulphate salt.
* Evaluated by TLC.
† KF titration.
‡ This sample contains about 1% of EEP, as evaluated by TLC. This figure is included in the figure for EAP.

Table 5
Composition of specialities as a percentage (m/m) of label claim

Manufacturer	Sample	Presentation	EA	PdMeEA	ECP	EAP (n = 3) (RSD %)	Total
A	EPLS	T	<0.3	0.1	1.5	100.0 (0.6)	101.6
A	EPLS	P	3.4	0.1	1.9	102.2 (0.9)	107.6
A	EPLS	G	<0.3	0.1	2.4	109.2 (0.8)	111.7
A	EPLS	C	0.4	<0.05	1.9	101.2 (0.2)	103.5
D	EP	T	1.6	1.1	1.4	94.2 (0.5)	98.3

LC results for the estolate samples are calculated as the laurylsulphate salt. T = tablets; P = dispersible powder; G = granules; C = capsules.

Results obtained by analysis of specialties are reported in Table 5. The content ranged from 94.2 to 109.2% with ECP and EA as the prominent impurities. The high content of EA in some of the specialities compared with the bulk samples suggest that some EA was formed during processing.

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