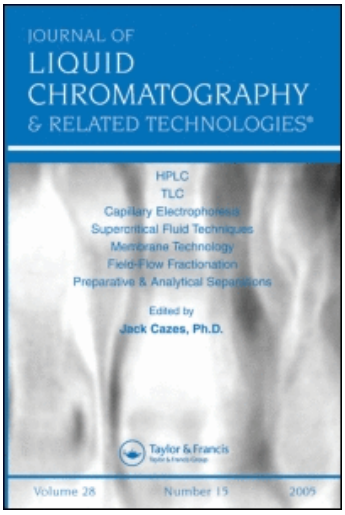

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High Performance Liquid Chromatographic Assay of Erythromycin Salts and Esters in Bulk and Pharmaceutical Dosage Forms

M. M. Nasr[a]; C. M. Stanley[a]
[a] USFDA, CDER, Division of Testing and Applied Analytical Development (DTAAD), St. Louis, MO

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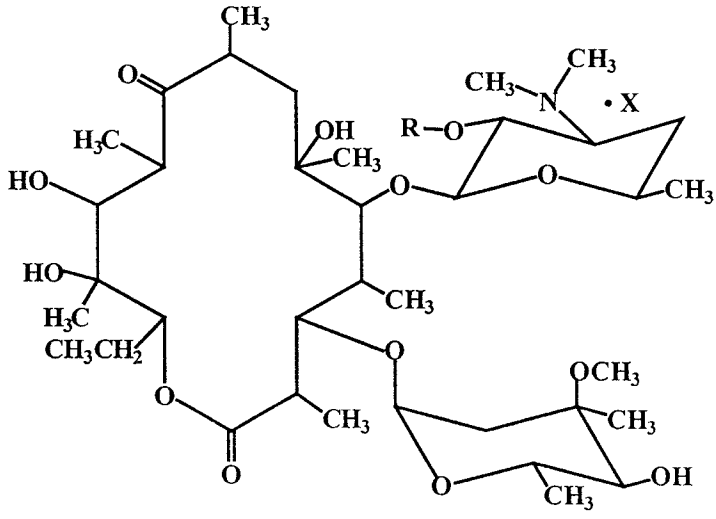
HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF ERYTHROMYCIN SALTS AND ESTERS IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Moheb M. Nasr, Christopher M. Stanley

USFDA, CDER,
Division of Testing and Applied Analytical Development (DTAAD)
1114 Market Street
St. Louis, MO 63101

ABSTRACT

Recently, several liquid chromatographic methods for the assay of erythromycin base were developed and are being considered for the routine assay of erythromycin. In this study, we developed simple and rugged liquid chromatographic methods for the assay of erythromycin salts and esters, and used these methods for the assay of erythromycin estolate, erythromycin ethylsuccinate, erythromycin stearate, erythromycin gluceptate, and erythromycin lactobionate in bulk and pharmaceutical formulations. The HPLC methods proved to be simple, sensitive, versatile, and rugged. The developed methods provide an attractive alternative to current official microbiological methods. The HPLC assay results for all commercial erythromycin products tested comply with the USP Antibiotics-Microbial assay specification.



	R	X
Erythromycin A (E I)	H	---
Erythromycin Estolate (E II)	CH_3CH_2-CO	$C_{12}H_{25}OSO_3H$
Erythromycin Ethylsuccinate (E III)	C_2H_5-COOC-$(CH_2)_2$-CO	---
Erythromycin Stearate (E IV)	H	$CH_3(CH_2)_{16}CO_2H$
Erythromycin Gluceptate (E V)	H	$C_7H_{14}O_8$
Erythromycin Lactobionate (E VI)	H	$C_{12}H_{22}O_{12}$

Figure 1. Chemical structures of Erythromycin (E I) and related esters and salts.

INTRODUCTION

Erythromycin is one of the most frequently prescribed broad spectrum antibiotics. Administration of the free base (E I, [114-07-8]) in its simplest form is complicated because E I is destroyed in the stomach by gastric acid and its absorption is affected by the presence of food. In order to guard against such hydrolytic effects, the free base is formulated with different types of enteric coatings. In addition, acid-stable, water-insoluble, biologically inactive erythromycin esters, and salts are administered, such as erythromycin estolate

(Erythromycin 2'-propionate dodecyl sulfate, [3521-62-8], E, II), erythromycin ethylsuccinate (Erythromycin 2'-ethyl succinate, [41342-53-4], E III), and erythromycin stearate (Erythromycin octadecanoate, [643-22-1], E IV). In the body, these prodrugs (erythromycin salts and esters) are converted into biologically active erythromycin base and therefore exert an identical antimicrobial action.[1,2] Erythromycin gluceptate (Erythromycin mono-glucoheptonate, [304-63-2], E V) and erythromycin lactobionate (Erythromycin mono(4-O-β-D-glactopyranosyl-D-gluconate), [3847-29-8], E VI) are freely soluble in water and administered parenterally when patients cannot tolerate the oral route. Chemical structures of erythromycin, its salts, and esters are illustrated in Figure 1.

Several liquid chromatographic methods for the assay of erythromycin (E I) in bulk and pharmaceutical formulations have been developed and are being used for the routine assay of erythromycin base. [3-6] These methods provide an attractive replacement for tedious microbiological methods. In addition, several liquid chromatographic methods for the analysis of some erythromycin esters have been published. [7-9]

Recently, we have used computerized chromatographic method optimization and newly available high-purity silica columns to develop a rugged and simple C_{18} liquid chromatographic assay method for the assay of erythromycin base (E I) in bulk and pharmaceutical formulations. [5,6] In this paper, we describe the results of using the same strategy in the development of liquid chromatographic methods for the assay of erythromycin salts and esters (E II-E VI) in different dosage forms.

EXPERIMENTAL

Chemicals and Reagents

USP reference standards (RS) of erythromycin (E I), erythromycin estolate (E II), and erythromycin ethylsuccinate (E III) were used throughout the study. Commercial samples of erythromycin estolate, erythromycin ethylsuccinate, erythromycin stearate, erythromycin gluceptate, and erythromycin lactobionate were purchased from commercial sources. Ammonium hydrogen phosphate, ammonium hydroxide, tetrabutylammonium hydrogen sulfate (of the highest available purity), methanol and HPLC grade acetonitrile were purchased from different sources and used without additional purification. The water used was deionized and filtered through a Milli-Q™ water purification system (Millipore, New Bedford, MA).

Table 1

Gradient System (Method I) for the Assay of Erythromycin Estolate (E II) and Erythromycin Ethylsuccinate (E III)

Time (min.)	%A[a]	%B[b]
0.0	90.0	10.0
10.0	0.0	100.0
t[c]	0.0	100.0

[a]Mobile phase A (10% CH_3CN) was prepared by mixing 60 mL stock ammonium phosphate buffer (0.20 M, pH 6.5), 60 mL stock tetrabutylammonium sulfate (0.20 M, pH 6.5), and about 250 mL Milli-Q-water, then adding 100 mL acetonitrile, diluting to 1 L with Milli-Q water, mixing well, and filtering through a 0.45-μm nylon membrane filter.

[b]Mobile phase B (75% CH_3CN) was prepared like mobile phase A; the only exception was the use of 750 mL acetonitrile in place of 100 mL.

[c]t = 18.0 minutes (Erythromycin estolate, E II) and t = 22.0 minutes (Erythromycin ethylsuccinate, E III).

Solutions

Stock 0.20 M ammonium phosphate buffer was prepared by dissolving the calculated amount of $(NH_4)H_2PO_4$ in Milli-Q water, adjusting the pH to 6.5 with ammonium hydroxide, and filtering the solution through a 0.45 μm nylon membrane filter. Stock 0.20 M tetrabutylammonium sulfate (mobile phase additive) was prepared by dissolving the calculated amount of $(C_4H_9)_4NHSO_4$ in Milli-Q water, adjusting the pH to 6.5 with ammonium hydroxide, and filtering the solution through a 0.45 μm nylon membrane filter.

Chromatographic Conditions

The HPLC system used in this investigation consisted of Spectra-Physics SP 8800 pump, Waters WISP autosampler, Spectra FOCUS Forward Optical Scanning detector set at 205 nm, COMPAQ DESKPRO XL 5100 computer, and PC1000 System Software™ (Ver. 3.0). Two chromatographic methods were used in this study:

Method I

This method was developed and used for the assay of erythromycin esters (erythromycin estolate, E II and erythromycin ethylsuccinate, E III). This procedure was optimized by using DryLab software as outlined previously.[5] An Inertsil 5μ ODS-2 (150Å) 150 x 4.6 mm I.D. column (MetaChem Technologies, Torrance, CA) was used in this study with an Inertsil 5μ ODS-2 guard cartridge.

The gradient profile and mobile phase compositions are described in Table 1. The gradient delay volume was determined to be 5.5 mL and no equilibration time was needed between injections. The mobile phase flow rate was set at 1.3 mL/min. The column temperature was controlled at 50°C with a block column heater (Jones Chromatography, Lakewood, CO) and sample injection volume was 50 μL.

Method II

This method was used for the assay of erythromycin salts (erythromycin stearate, E IV, erythromycin gluceptate, E V, and erythromycin lactobionate, E VI). It is essentially the same experimental protocol used for the assay of erythromycin base (E I) in pharmaceutical formulations;[6] with the use of a different C_{18} column.

An Inertsil 5μ ODS-2 (150 Å) 250 x 4.6 mm I.D. column (MetaChem Technologies, Torrance, CA) was used. Both columns (Prodigy and Inertsil) gave similar chromatographic results. In both methods, USP reference standards were obtained and used as external standards.

Sample Preparation

Because of the difference in solubility of the tested salts and esters, different sample preparation solvents were used.

Erythromycin Estolate (E II)

Powder

Samples were prepared by dissolving the weighed bulk powder in mobile phase B (75% CH_3CN) for a final conc'n of 5-6 mg/mL, and were then placed in an ultrasonic bath for approximately 30 seconds to enhance dissolution.

Table 2

HPLC Assay Results of Commercial Erythromycin Estolate (E II) Products

Product Tested[a]	USP Assay Limit[b]	Label Claim	% Found ± Sd[c]
Powder, Product A	≥ 600 μg E 1 per mg	675 μg E 1 per mg	102.8 ± 0.3
Powder, Product B	≥ 600 μg E 1 per mg	672 μg E 1 per mg	99.8 ± 0.2
Capsules, Product A	90- 115%	250 mg	111.2 ± 0.2
Capsules, Product B	90 - 115%	250 mg	113.7 ± 0.7
Capsules, Product C	90 - 115%	250 mg	111.8 ± 0.9
Capsules, Product D	90 - 115%	250 mg	109.2 ± 0.3
Capsules, Product E	90 - 115%	250 mg	107.1 ± 0.4

[a]Samples were prepared as described in the experimental section.
[b]United States Pharmacopeia, 23rd Rev., United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, pp. 615-616.
[c]Average of 3 runs.

Capsules

The contents of 10 capsules were transferred into a mortar, and pulverized to fine powder. An approximately 50 mg portion of the powder was placed in a 10-mL volumetric flask, enough solvent (mobile phase B) was added to make 10 mL, the suspension was sonicated for about 2 minutes, and the resulting solution was filtered through a 0.45-μm type HVLP filter.

The first few milliliters of the filtrate were discarded.

Erythromycin Ethylsuccinate (E III)

Powder

Samples of bulk ethylsuccinate were prepared as described for erythromycin estolate. Samples of powder used for the preparation of oral suspensions were prepared by suspending a representative sample of approximately 500 mg into 100 mL of mobile phase B and sonicating for 15 minutes. The supernatant layer was transferred into a 100 mL volumetric flask, diluted with mobile phase B, and then filtered.

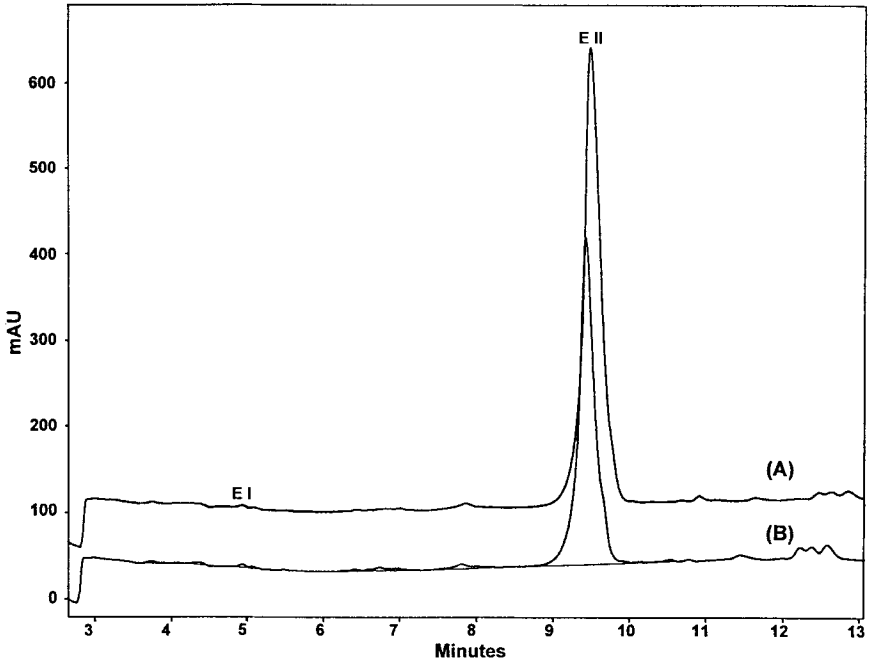


Figure 2. Typical chromatograms (monitored at 205 nm) obtained from the analysis of commercial samples of erythromycin estolate (E II), (A) powder and (B) capsule. Details of chromatographic conditions are described in the experimental section and Table 1.

Tablets

Ten tablets were transferred into a mortar, and pulverized to fine powder. Approximately 125 mg portion of the powder was placed into a 10-mL volumetric flask and enough solvent (mobile phase B) was added to volume. The suspension was sonicated for about 2 minutes and the resulting solution was filtered through a 0.45-μm type HVLP filter. The first few milliliters of the filtrate were discarded.

Erythromycin Stearate (E IV)

Powder

Samples were prepared by dissolving the weighed amount of bulk powder in methanol to give a final concentration of 5-6 mg/mL.

Table 3

HPLC Assay Results of Commercial Erythromycin Ethylsuccinate (E III) Products

Product Tested[a]	USP Assay Limit[b]	Label Claim	% Found ± Sd[c]
Powder, Product A	≥ 765 μg E 1 per mg	837 μg E 1 per mg	92.9 ± 0.3
Powder, Product B	≥ 765 μg E 1 per mg	820 μg E 1per mg	104.3 ± 0.4
Tablets, Product A	90 - 120%	400 mg	92.2 ± 0.4
Tablets, Product B	90 - 120%	400 mg	101.9 ± 0.3
Powder for Oral. Suspension	90 - 120%	400 mg per 5 mL	106.2 ± 0.2

[a]Samples were prepared as described in the experimental section.
[b]United States Pharmacopeia, 23rd Rev., United States Pharmacopeial Covention, Inc., Rockville, MD, 1995, pp. 617-619.
[c]Average of 3 runs.

Tablets

Ten tablets were transferred into a mortar and pulverized to fine powder. Approximately 300 mg portion of powder was placed into a 10-mL volumetric flask, methanol was added to make 10 mL, the suspension was sonicated for about 5 minutes, and the resulting solution was filtered through a 0.45-μm type HVLP filter. The first few milliliters of the filtrate were discarded.

Erythromycin Gluceptate (E V)

Powder

Samples were prepared as described previously for the assay of erythromycin base. [6]

Erythromycin Lactobionate (E VI)

Powder for Injection

Representatives samples were transferred into volumetric flasks and treated as described previously for the assay of erythromycin base.[6]

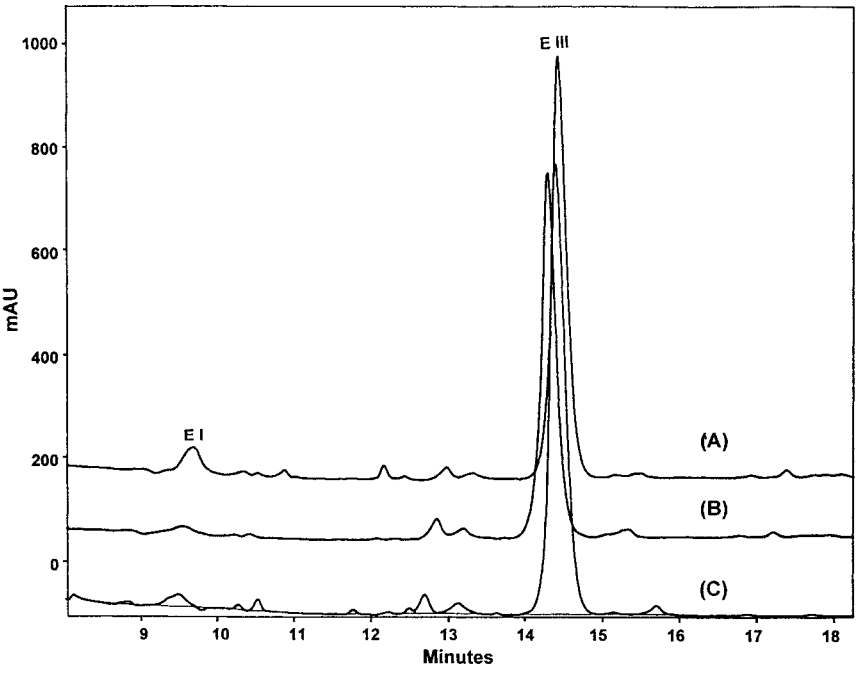


Figure 3. Typical chromatograms (monitored at 205 nm) obtained from the analysis of commercial samples of erythromycin ethylsuccinate (E III), (A) powder, (B) tablet, and (C) powder for oral suspension. Details of chromatographic conditions are described in the experimental section and Table 1.

The stability of erythromycin and some of its esters in methanol and acetonitrile has been reported.[10] To evaluate the stability of tested erythromycin solutions under described analytical conditions, solutions were prepared in sample solvents as described above and stored at different temperatures for extended periods of time. Aliquots were withdrawn, and concentrations of the parent drug and possible degradation products were measured. All tested samples were stable at $\leq$ 0°C. Erythromycin ethylsuccinate (E III) and erythromycin estolate (E II) had the lowest stability in comparison to other salts and esters. Erythromycin ethylsuccinate solution was stable for about 4 hours at 10°C and only an hour at 25°C, whereas, erythromycin estolate solution was stable for 24 hours at 10°C and 4 hours at 25°C. As a result of the stability study, solutions of erythromycin ethylsuccinate and erythromycin estolate were prepared fresh and kept refrigerated prior to injection into the liquid chromatograph.

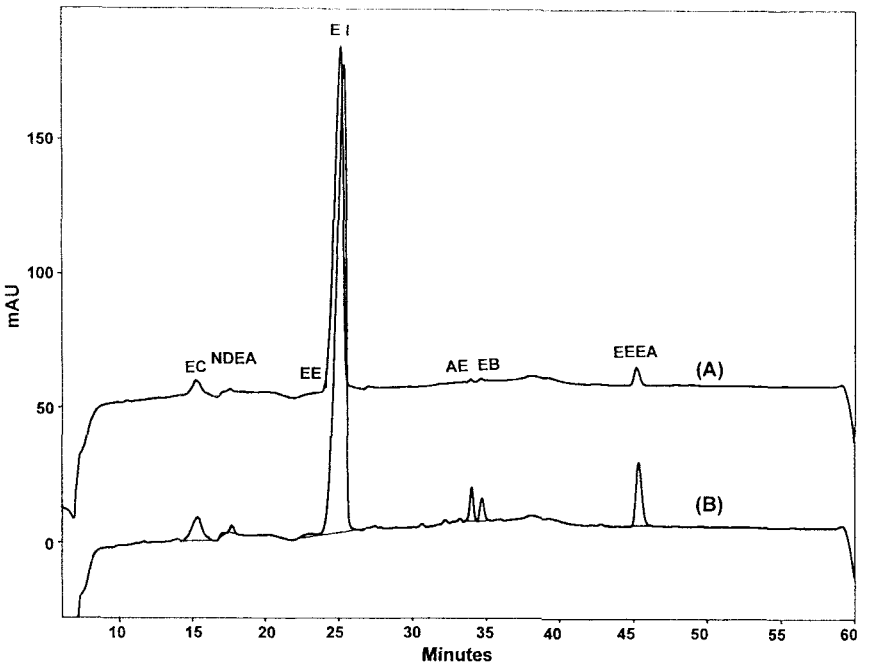


Figure 4. Typical chromatograms (monitored at 205 nm) obtained from the analysis of commercial samples of erythromycin stearate (E IV), (A) powder and (B) tablet. Chromatographic conditions are outlined in the experimental section (Method II, [6]). EC = erythromycinc C, NDEA = N-demethylerythromycin A, EE = erythromycin E, E I = erythromycin A, AE = anhydroerythromycin A, EB = erythromycin B, and EEEA = erythromycin A enol ether.

RESULTS AND DISCUSSION

Assay of Erythromycin Estolate (E II)

The developed method (Method I) was used to assay commercial samples of erythromycin estolate powder and capsules. The assay results are summarized in Table 2, and an illustration of chromatograms obtained from the analysis of commercial samples of powder and delayed-release capsules is provided in Figure 2. The assay results (Table 2 and Figure 2) indicate complete recovery of erythromycin estolate and insignificant degradation of the ester into erythromycin (E I) under described analysis conditions.

Table 4

HPLC Assay Results of Commercial Erythromycin Stearate (E IV) Products

Product Tested[a]	USP Assay Limit[b]	Label Claim	% Found ± Sd[c]
Powder, Product A	≥ 550 μg E 1 per mg	701 μg E 1 per mg	97.1 ± 0.8
Powder, Product B	≥ 550 μg E 1 per mg	660 μg E 1 per mg	99.0 ± 0.15
Tablets, Product A	90 - 120%	250 mg	101.9 ± 1.1
Tablets, Product B	90 - 120%	500 mg	98.8 ± 0.4
Tablets, Product C	90 - 120%	500 mg	97.5 ± 0.9

[a]Samples were prepared as described in the experimental section.
[b]United States Pharmacopeia, 23rd Rev., United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, pp. 621-622.
[c]Average of 3 runs.

Table 5

HPLC Assay Results of Commercial Erythromycin Gluceptate (E V) and Erythromycin Lactobionate (E VI) Products

Product Tested[a]	USP Assay Limit[b]	Label Claim	% Found ± Sd[c]
Erythromycin Gluceptate Powder	90 - 115%	600 μg E 1 per mg	93.9 ± 1.2
Erythromycin Lactobionate For Injection, Product A	90 - 120%	500 mg	100.4 ± 0.1
Erythromycin Lactobionate for Injection, Product B	90 - 120%	500 mg	102.8 ± 0.2

[a]Samples were prepared as described in the experimental section.
[b]United States Pharmacopeia, 23rd Rev., United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, pp. 620-621.
[c]Average of 3 runs.

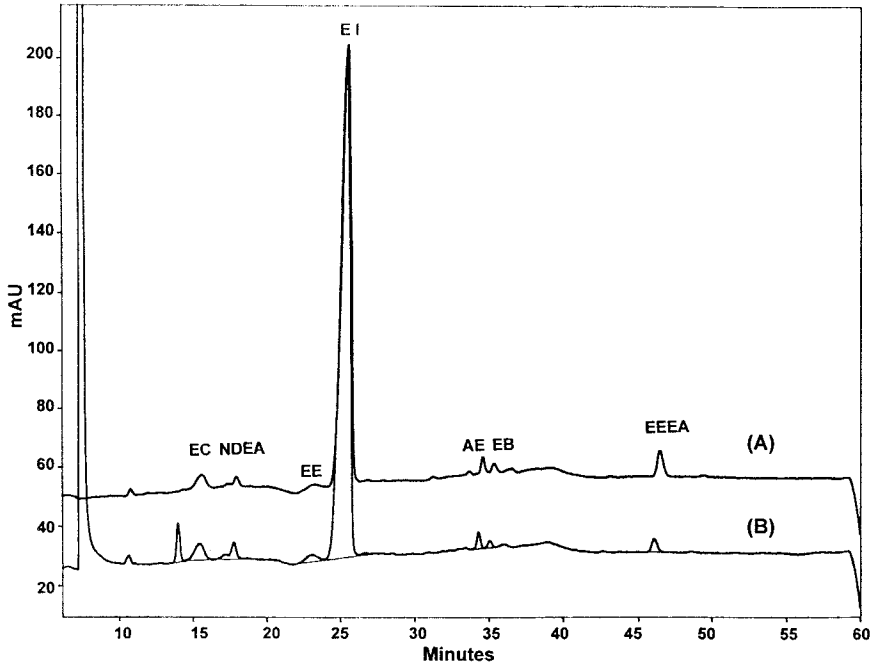


Figure 5. Typical chromatograms (monitored at 205 nm) obtained from the analysis of commercial samples of (A) erythromycin gluceptate powder (E V) and (B) erythromycin lactobionate (E VI) powder for injection. Chromatographic conditions are outlined in the experimental section (Method II, [6]). EC = erythromycin C, NDEA = N-demethylerythromycin A, EE = erythromycin E, E I = erythromycin A, AE = anhydroerythromycin A,EB = erythromycin B, and EEEA = erythromycin A enol ether.

Assay of Erythromycin Ethylsuccinate (EIII)

Three different commercially available products of erythromycin ethylsuccinate were obtained and assayed (Table 3) as described in the experimental section. The results of the stability study indicated that erythromycin ethylsuccinate solutions are the least stable of all erythromycin salts and esters tested in this investigation.

To minimize sample degradation, solutions were prepared fresh and kept refrigerated prior to chromatographic analysis. Other ingredients and/or excipients in the tested formulations had no effect on chromatographic separation (Figure 3).

Assay of Erythromycin Stearate (E IV)

Five commercially available erythromycin stearate products were analyzed by Method II, as described in the experimental section. Because of the low solubility of the stearate salt in acetonitrile, erythromycin stearate samples were prepared in methanol, which had no effect on the chromatographic quality (Figure 4). The percentage of erythromycin stearate in the tested formulations varied from a lower value of 97% of declared drug content to a higher value of 102% of declared label claim (Table 4).

Assay of Erythromycin Gluceptate (E V) and Erythromycin Lactobionate (E VI)

Commercially available samples of the salts were obtained and assayed as described in the experimental section (Method II, Table 5). The presence in the lactbionate formulations of non-erythromycin ingredients, such as benzyl alcohol, did not cause chromatographic interference (Figure 5).

CONCLUSION

The developed chromatographic method has been applied successfully for the assay of erythromycin salts and esters in several solid-dose pharmaceutical formulations. In this study, the assay results of commercially available products were within the USP specifications for the tested products. The developed method displayed ruggedness, precision, repeatability, and short analysis time. It is likely that reliable and validated chromatographic methods can replace tedious and time-consuming microbiological assay of antibiotics.

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