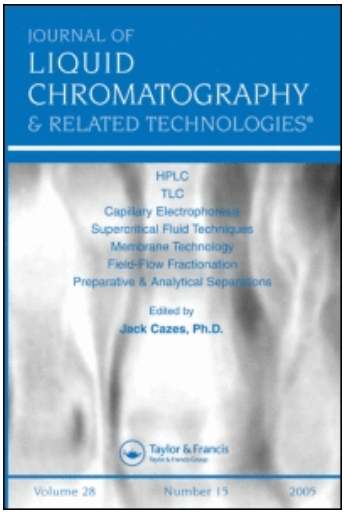


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Analysis of Erythromycin and Related Impurities in Pharmaceutical Formulations

Moheb M. Nasr[a]; Terry J. Tschappler[a]
[a] Division of Drug Analysis, US FDA, St. Louis, Missouri

To cite this Article Nasr, Moheb M. and Tschappler, Terry J.(1997) 'High Performance Liquid Chromatographic Analysis of Erythromycin and Related Impurities in Pharmaceutical Formulations', Journal of Liquid Chromatography & Related Technologies, 20: 4, 553 — 565
To link to this Article: DOI: 10.1080/10826079708010944
URL: http://dx.doi.org/10.1080/10826079708010944

J. LIQ. CHROM. & REL. TECHNOL., 20(4), 553–565 (1997)

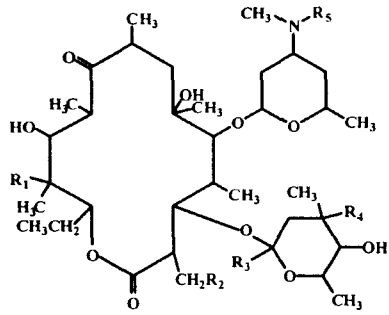
HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ERYTHROMYCIN AND RELATED IMPURITIES IN PHARMACEUTICAL FORMULATIONS

Moheb M. Nasr, Terry J. Tschappler

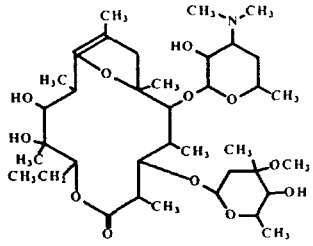
US FDA, Division of Drug Analysis
1114 Market Street
St. Louis, Missouri 63101

ABSTRACT

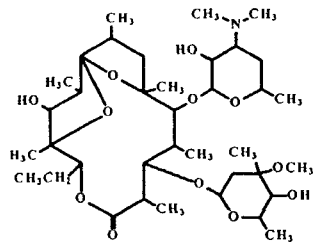
In the last few years, several liquid chromatographic methods for the analysis of erythromycin have been published. Until recently, most of the published methods were either complex or lacked the selectivity needed for the assay of erythromycin in the presence of erythromycin derivatives and impurities. Recently, we developed a significantly improved C_{18} liquid chromatographic method for the assay of erythromycin. In comparison, our method is simpler, more rugged, faster, and more sensitive and selective than other published methods. In this investigation, the developed method was successfully used for the assay of erythromycin in several pharmaceutical formulations.



	R_1	R_2	R_3	R_4	R_5
Erythromycin A (EA)	OH	H	H	OCH_3	CH_3
Erythromycin B (EB)	H	H	H	OCH_3	CH_3
Erythromycin C (EC)	OH	H	H	OH	CH_3
Erythromycin E (EE)	OH	—O—		OCH_3	CH_3
N-demethylerythromycin A (NDEA)	OH	H	H	OCH_3	H



Erythromycin A enol ether (EEEA)



Anhydroerythromycin A (AE)

Figure 1. Chemical Structures of Erythromycin A and related substances.

INTRODUCTION

Erythromycin (free base) is a widely used broad spectrum antibiotic marketed in several pharmaceutical formulations. Erythromycin base can be found in a variety of liquid and solid erythromycin formulations. Solid formulations include delayed-release tablets and capsules, where erythromycin is enteric-coated to protect against degradation by gastric acidity.

Erythromycin topical solutions (2%) are alcoholic solutions of erythromycin base and are indicated for the topical control of *acne vulgaris.*[1] The antimicrobial activity of erythromycin is due mainly to the erythromycin A (EA), erythromycin B (EB), and erythromycin C (EC) forms. Erythromycin E (EE), N-demethyl erythromycin A (NDEA), erythromycin A enol ether (EEEA), anhydroerythromycin A (AE), and other erythromycin derivatives can be present as impurities. Chemical structures of erythromycin A and related substances are illustrated in Figure 1.

There is a considerable interest in developing chemical assay methods of antibiotics. Several methods for the assay of erythromycin and related substances in bulk, biological fluids, and pharmaceutical formulations have been published.[2-9] These methods lack the resolution needed for the separation of several related substances, such as EE, and other common erythromycin impurities. In order to improve chromatographic selectivity, a method utilizing poly(styrene-divinylbenzene) stationary phase, capable of the separation of EE and EA, was developed[10] and adopted by the European Pharmacopoeia.[11] However, the polymer column method lacks the simplicity and ruggedness needed for the routine assay of erythromycin.

Recently, we developed a C_{18} based gradient LC method[12] that is simple, sensitive, rugged, and able to separate and assay erythromycin and most related substances commonly found in commercial erythromycin products. In this manuscript, we are illustrating the application of this method in the assay of erythromycin in different pharmaceutical formulations.

EXPERIMENTAL

Chemicals and Reagents

USP erythromycin reference standard (RS) was used throughout the study. Other erythromycin standards (EB, EC, EE, NDEA, AE, and EEEA) were kindly provided by Abbott Laboratories, North Chicago, Illinois. Commercial samples of erythromycin powder, erythromycin topical solutions, delayed-release capsules, and delayed-release tablets were purchased from available sources. Ammonium hydrogen phosphate, ammonium hydroxide, and tetrabutylammonium hydrogen sulfate (of the highest available purity) were purchased from different sources and used without additional purification. Acetonitrile used was HPLC grade and water was deionized and filtered through a Milli-Q™ water purification system (Millipore, New Bedford, MA).

Solutions

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

Chromatographic Conditions

The HPLC system used in this investigation consisted of Spectra-Physics SP 8800 pump, Spectra-Physics SP 8880 autosampler, Spectra FOCUS Forward Optical Scanning detector set at 205 nm, COMPAQ DESKPRO XL 5100 computer, and PC1000 System Software™ (Ver. 3.0). A Prodigy 5μ ODS-2 (150 Å) 250 x 4.6 mm I.D. column (Phenomenex, Torrance, CA) was used in this study. In addition, a Prodigy 5μ ODS-2 30 x 4.6 mm I.D. guard column was used. The gradient profile and mobile phase compositions are described in Table I. The concentration of both the buffer and tetrabutylammonium sulfate were maintained constant in both mobile phases and the only difference between mobile phase A and B is the percentage of acetonitrile (10 vs 50%, respectively). 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.

Sample Preparation

The sample solvent was prepared by mixing equal volumes of the two mobile phases (50%A-50%B) and used as the sample solvent throughout this study.

Powders

Samples were prepared by dissolving the weighed amount in the sample preparation solvent to give a final concentration of 8-10 mg/mL. Samples were then placed in an ultrasonic bath for approximately 5 minutes to enhance dissolution.

Table 1

Gradient System for the Erythromycin Assay

Time (min.)	%A[a]	%B[b]
0.0	78.0	22.0
39.0	26.0	74.0
47.0	26.0	74.0

[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. This was followed by the addition of 100 mL acetonitrile, diluting to 1L with Milli-Q water, mixing well and filtering through a 0.45μm nylon membrane filter.

[b]Mobile phase B (50% CH_3CN) was prepared as in "A" with the only exception being the use of 500 mL acetonitrile in the mobile phase preparation.

Topical Solutions

Topical solutions were used as such without any additional treatment. In other words, samples of topical solutions were transferred directly into autosampler vials.

Delayed-Release Capsules

The contents of four capsules were transferred into a 100 mL volumetric flask, sample preparation solvent was added to the volume, and the flask sonicated for 15 minutes to ensure complete dissolution of erythromycin. The resulting solution was filtered through a 0.45 μm type HVLP filter. The first few milliliters of the filtrate were discarded.

Delayed-Release Tablets

Ten tablets were weighed in order to determine the average tablet weight, transferred into a mortar, and pulverized to fine powder. A 50 to 70 mg portion of the powdered tablets was placed into a 10-mL volumetric flask,

Table 2

HPLC Assay of Commercial Erythromycin Powders

Commercial Erythromycin Powder[a]	% Erythromycin Found[b,c] ± SD
Product I	101.1 ± 1.0
Product II	98.2 ± 0.7

[a] Samples were dissolved in a solvent made of 50%A and 50%B (as described in the experimental section) at a concentration of 8-10 mg/mL.
[b] Average of 6 runs.
[c] Calculated as the sum of erythromycins A, B, and C. Erythromycins B and C calculations were based on the response factor of erythromycin A.

Table 3

HPLC Assay of Ten Commercial Erythromycin 2% Topical Solutions

Topical Solutions[a]	Erythromycin Found[b,c] ± SD	% of Label Claim[d]
Product I	1.60 ± 0.01	80.0
Product II	1.63 ± 0.01	81.5
Product III	1.70 ± 0.01	85.0
Product IV	1.83 ± 0.02	91.5
Product V	1.69 ± 0.02	84.5
Product VI	1.69 ± 0.00	84.5
Product VII	1.96 ± 0.01	98.0
Product VIII	1.68 ± 0.03	84.0
Product IX	1.84 ± 0.02	92.0
Product X	1.87 ± 0.02	93.5

[a] Solutions were used as such without any prior treatment.
[b] % erythromycin (weight/volume), average of three runs.
[c] Calculated as the sum of erythromycins A, B, and C. Erythromycins B and C calculations were based on the response factor of erythromycin A.
[d] USP limits: 90.0 - 125.0 % of labeled amount.[13]

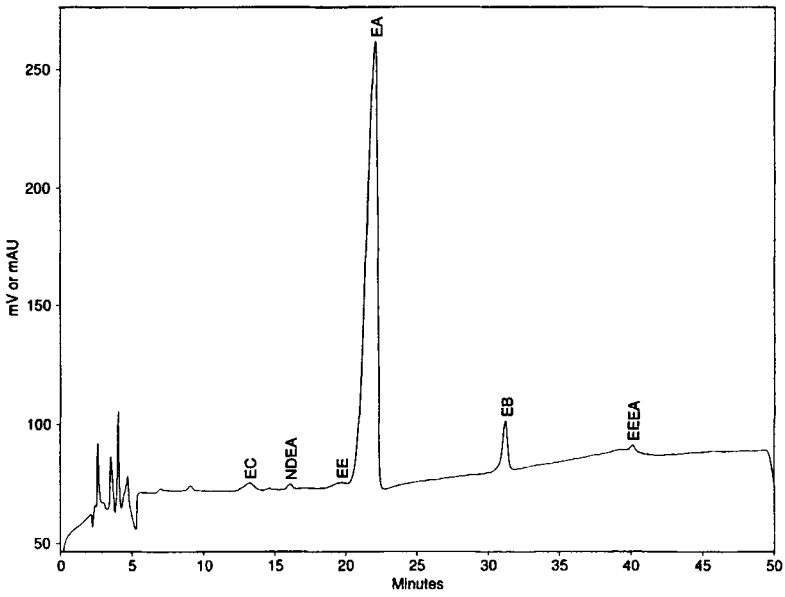


Figure 2. Typical chromatogram (monitored at 205 nm) obtained from the analysis of a commercial sample of erythromycin powder. Details of chromatographic conditions are described in the experimental section and Table 1.

sample preparation solvent was added to the volume, the suspension was sonicated for about 10 minutes, and the resulting solutions were filtered twice through a 0.45μm type HVLP filter. The first few milliliters of the filtrate were discarded.

The effect of changing the sample preparation solvent, its volume, sonication time, and the use of different shakers and blenders was investigated. It was found that the sample preparation method described above was the simplest to provide complete recovery of erythromycin.

RESULTS AND DISCUSSION

A system suitability test mixture was prepared and used to test method performance and ruggedness as described before.[12] Increasing the temperature

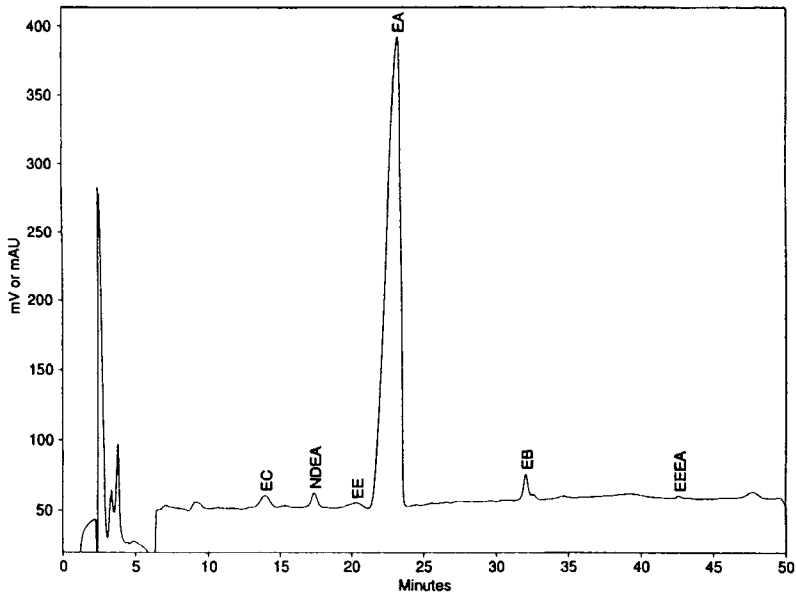


Figure 3. Typical chromatogram (monitored at 205 nm) obtained from the analysis of a commercial sample of erythromycin 2% topical solution. Details of chromatographic conditions are described in the experimental section and Table 1.

to 50°C was the only modification made to the previously described method.[12] The slight increase in temperature enhances the chromatographic selectivity and eliminates interference from additives present in tested pharmaceutical formulations.

Assay of Erythromycin Powders

Two commercial samples of erythromycin powder, suitable for prescription compounding, were obtained and assayed using the developed method. The assay results are summarized in Table 2. The percentage of erythromycin in these samples was calculated as the sum of EA, EB, and EC. The absorptivities of erythromycin isomers and related substances in the sample preparation solution were determined before[12] and in spite of differences in absorptivity, the concentrations of both EB and EC were estimated using the same response factor as for EA. Figure 2 provides an illustration of a typical chromatogram obtained from the analysis of a commercial erythromycin powder.

Table 4

HPLC Assay of Six Commercial Erythromycin 250 mg Delayed-Release Capsules

Delayed-Release Capsules[a]	mg of Erythromycin Found[b,c]	% of Label Claim[d]
Product I	241	96
Product II	246	98
Product III	260	104
Product IV	253	101
Product V	236	94
Product VI	252	101

[a] Samples were obtained and prepared for the assay as described in the experimental section

[b] Average of 6 runs.

[c] Calculated as the sum of erythromycins A, B, and C. Erythromycins B and C calculations were based on the response factor of erythromycin A.

[d] USP limits: 90.0 - 115.0 % of labeled amount.[13]

Assay of Erythromycin Topical Solutions

Ten commercially available samples of erythromycin (2%) topical solutions were obtained and assayed using the developed method (Table 3). The assay was performed directly on the topical solutions without any prior treatment. This is clearly an added advantage of using the developed method for the assay of erythromycin topical solutions. Other solution ingredients present (alcohol, propylene glycol,..etc) had no effect on chromatographic quality or separation (Figure 3).

Assay of Erythromycin Delayed-Release Capsules

Six commercially available erythromycin delayed-release capsules were analyzed as described in the experimental section. The assay worked well and can be utilized as a measure of content uniformity. The percentage of erythromycin in the six tested formulations varied from a lower value of 94 percent of declared to a higher value of 101 percent of declared (Table 4). The chromatographic method used was able to separate inert material interferences from the different erythromycin forms and related substances (Figure 4).

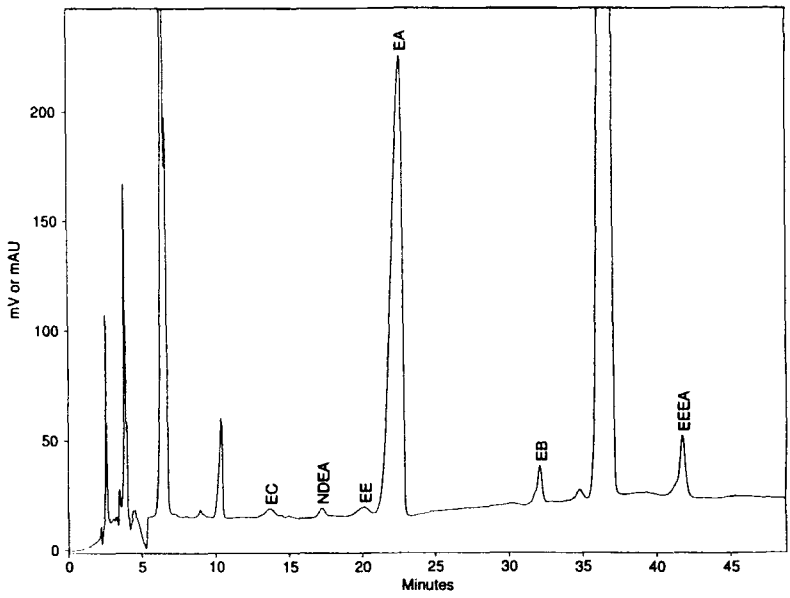


Figure 4. Typical chromatogram (monitored at 205 nm) obtained from the analysis of a commercial sample of erythromycin delayed-release capsule. Details of chromatographic conditions are described in the experimental section and Table I.

Table 5

HPLC Assay of Commercial Erythromycin Delayed-Release Tablets

Delayed-Release Tablet[a]	mg of Erythromycin Found[b,c]	% of Label Claim[d]
Product I, 250 mg	277	111
Product II, 333 mg	359	108

[a] Samples were obtained and prepared for the assay as described in the experimental section

[b] Average of 6 runs.

[c] Calculated as the sum of erythromycins A, B, and C. Erythromycins B and C calculations were based on the response factor of erythromycin A.

[d] USP limits: 90.0 - 120.0 % of labeled amount.[13]

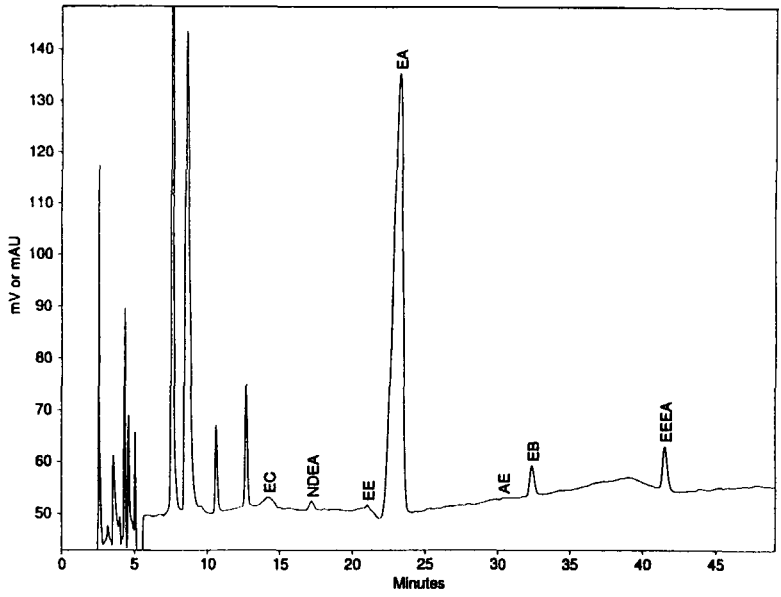


Figure 5. Typical chromatogram (monitored at 205 nm) obtained from the analysis of a commercial sample of erythromycin delayed-release tablet. Details of chromatographic conditions are described in the experimental section and Table 1.

Assay of Erythromycin Delayed-Release Tablets

Two commercially available erythromycin delayed-release tablets were analyzed as described in the experimental section (Table 5, Figure 5).

CONCLUSION

The developed method has been applied successfully for the assay of erythromycin and related substances in different pharmaceutical formulations. The method proves to be simple, versatile, and easy to use. In most cases, the assay results of commercially available products are within the USP[13] specifications for the tested products. However, the assay results of topical solutions (Table 3) shows that most topical solutions tested are not within the USP limit of 90 - 125 % of the label amount or 1.8 - 2.5% erythromycin.[13]

ACKNOWLEDGMENT

The authors are grateful to Jeff York, Jerry Schaar and Ray Cox of Abbott Laboratories for providing needed reference standards and helpful discussions. The editorial review of the manuscript by Yubing Tang, Henry D. Drew, and Walter L. Zielinski, of the Division of Drug Analysis (DDA), US FDA is greatly appreciated. Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the FDA, Division of Drug Analysis, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

REFERENCES

1. **Goodman & Gilman's The Pharmacological Basis of Therapeutics**, 9th edition, J. G. Hardman, L. E. Limbird, P. B. Molinoff, R. W. Ruddon, A. G. Gilman, eds., McGraw-Hill, New York, 1996, pp. 1604-1605.

2. K. Tsuji, J. F. Goetz, J. Chromatogr., **147** , 359-367 (1978).

3. K. Tsuji, M. P. Kane, J. Pharm. Sci., **71** , 1160-1164 (1982).

4. G. S. Duthu, J. Liq. Chromatogr., **7** , 1023-1032 (1984).

5. C. Stubbs, J. M. Haigh, I. Kanfer, J. Pharm. Sci., **74** , 1126-1128 (1985).

6. T. Geria, W. -H. Hong, R. E. Daly, J. Chromatogr., **396** , 191-198 (1987).

7. Th. Cachet, I. O. Kibwage, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Chromatogr., **409** , 91-100 (1987).

8. Th. Cachet, I. Quintens, E. Roets, J. Hoogmartens, J. Liq. Chromatogr., **12** , 2171-2201 (1989).

9. S. Laakso, M. Scheinin, M. Anttila, J. Chromatogr., **526** , 475-486 (1990).

10. J. Paesen, E. Roets, J. Hoogmartens, Chromatographia, **32** , 162-166 (1991).

11. **European Pharmacopoeia** on CD-ROM, Council of Europe, F 67075 Strasbourg Cedex, France,1995, M_r 734.

12. M. M. Nasr, T. J. Tschappler, J. Liq. Chromatogr. & Rel. Technol., **19**, 2329-2348 (1996).

13. **United States Pharmacopeia**, 23rd Rev., United States Pharmacopeial Convention, Inc., Rockville, MD, 1995, pp. 612-614.

Received August 5, 1996
Accepted August 26, 1996
Manuscript 4222