Journal of Chromatography, 433 (1988) 149–158
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4395

LIQUID CHROMATOGRAPHIC AND FLOW INJECTION ANALYSIS OF TETRACYCLINE USING SENSITIZED EUROPIUM(III) LUMINESCENCE DETECTION

THOMAS J. WENZEL*, LISA M. COLLETTE, DEIRDRE T. DAHLEN, SUSAN M. HENDRICKSON and LAWRENCE W. YARMALOFF

Department of Chemistry, Bates College, Lewiston, ME 04240 (U.S.A.)

(First received April 19th, 1988; revised manuscript received July 19th, 1988)

SUMMARY

Europium(III) can be used as a luminescent chromophore for detection in the liquid chromatographic and flow injection analysis of tetracycline. Detection is dependent upon an intramolecular energy transfer from the tetracycline to Eu(III). In liquid chromatography, the Eu(III) is added post-column as a complex with ethylenediaminetetraacetic acid. The post-column phase also serves to adjust the pH for optimum sensitivity. The method is highly selective for tetracycline since few compounds are capable of transferring energy to Eu(III). Fluorescent impurities that would otherwise interfere in flow injection analysis can be eliminated through the use of a delay time between the source pulse and the start of data acquisition. The detection limits for tetracycline using sensitized Eu(III) luminescence are better than those obtained using ultraviolet detection. The method is applied to the analysis of tetracycline in urine, blood serum, and gingival crevice fluid.

INTRODUCTION

Liquid chromatography (LC) is the analytical method of choice for measuring tetracycline in a variety of matrices. LC separations of tetracyclines employing reversed-phase packings including C_1 [1], C_8 [2–7], and C_{18} [1–3,8–18] surface groups have been reported. Reversed-phase separations are preferable to those using ion-exchange columns [1]. The effectiveness of these reversed-phase methods is dependent, however, upon the degree of "capping" of surface hydroxy groups [1,19]. Keeping these materials free of surface hydroxy groups is difficult since most separations of tetracycline require pH values close to that at which the silica-based supports decompose. The analysis of tetracycline by LC would be facilitated if a selective detection method were available that did not respond to most compounds in the sample matrix.

We have already shown how europium(III) and terbium(III) can be used as

0378-4347/88/$03.50

selective luminescent chromophores for the LC detection of aromatic aldehydes, aromatic ketones [20], nucleotides, and nucleic acids [21]. Excited-state tetracycline is capable of transferring energy to Eu(III) by an intramolecular process [22]. Luminescence characteristic of the Eu(III) is then observed. In this report we describe the use of Eu(III) as a chromophore in LC for the detection of tetracycline.

EXPERIMENTAL

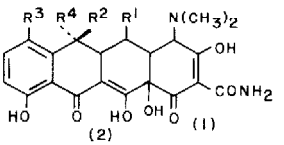
Reagents

Tetracycline hydrochloride, doxycycline hydrochloride, and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) were obtained from Sigma (St. Louis, MO, U.S.A.). Oxycycline, minocycline, demeclocycline, and chlortetracycline as their hydrochloride salts were a gift of Dr. Lars Christersson (State University of New York, Buffalo, NY, U.S.A.). The structures of the tetracyclines employed in this study are shown in Fig. 1. Europium oxide was purchased from Alfa Products (Danvers, MA, U.S.A.). Disodium ethylenediaminetetraacetic acid (Na_2EDTA) was obtained from Aldrich (Milwaukee, WI, U.S.A.). Ammonium chloride was obtained from Mallinckrodt (St. Louis, MO, U.S.A.). Concentrated aqueous ammonia was obtained from VWR Scientific (San Francisco, CA, U.S.A.). Mobile phases were prepared from HPLC-grade solvents.

Apparatus

The liquid chromatograph and post-column addition system have been described [20,21]. The same apparatus, without a column, was used for flow injection analysis (FIA). The columns used for the separations of tetracycline were C_8 silica (25 cm × 4.6 mm, 10 μm) from Alltech (Avondale, PA, U.S.A.), C_1 silica (125 mm × 4.6 mm, 5 μm) (Shandon phase) custom-packed by Alltech, and C_{18} polystyrene-divinylbenzene (ACT-1) from Interaction Chemicals (Mountain View, CA, U.S.A.). The flow-rates of the pre- and post-column pump were adjusted to 1 ml/min.

The fluorescence detector was a Perkin-Elmer LS-5 spectrofluorometer fitted



R1	R2	R3	R4	
H	OH	H	CH_3	TETRACYCLINE
H	OH	Cl	CH_3	CHLORTETRACYCLINE
OH	H	H	CH_3	DOXYCYCLINE
OH	OH	H	CH_3	OXYCYCLINE
H	H	H	H	MINOCYCLINE
H	OH	Cl	H	DEMECLOCYCLINE

Fig. 1 Structure of tetracyclines.

with either an LC flow cell or a cuvette holder. For LC detection, the following parameters were set: excitation monochromator, 392 nm; emission monochromator, 616 nm; excitation slit width, 5 nm; emission slit width, 5 nm; response setting, 4. For cuvette studies, the parameters were the same except the emission monochromator was scanned. For time-resolved measurements, which employ a delay time between the source pulse and the start of data acquisition, the instrument was switched to the phosphorescence mode and appropriate delay and gate times were set.

Preparation of mobile and post-column phases

All mobile phases were prepared as specified in the literature. Several post-column phases were employed.

Post-column phase A (PC-A) consisted of a solution of $Na[Eu(EDTA)]\cdot 5H_2O$ (10^{-4} *M*) and ammonium chloride (0.2 *M*) in water (pH 9 using concentrated aqueous ammonia). The Eu(III) complex with EDTA was prepared by a literature method [23]. The Eu(III) complex with EDTA can also be prepared in solution by using a 1:1 molar ratio of Eu(III) chloride or nitrate and Na_2EDTA.

Post-column phase B (PC-B) consisted of $Na[Eu(EDTA)]\cdot 5H_2O$ (10^{-4} *M*), ammonium chloride (0.2 *M*) (pH 9 or 10)–acetonitrile (90:10, v/v).

Post-column phase C (PC-C) consisted of $Na[Eu(EDTA)]\cdot 5H_2O$ (10^{-4} *M*), CAPS (0.2 *M*) (pH 9 or 10 using 4 *M* sodium hydroxide)–acetonitrile (90:10, v/v). Dimethylformamide was also employed as the organic modifier in PC-B and PC-C. Since the flow-rate of the post-column phase matched that of the liquid chromatograph, the concentration of Eu(III) at the detector was $5\cdot 10^{-5}$ *M*.

Preparation of tetracycline standards

Standard solutions of tetracycline were prepared by weighing the appropriate amount of tetracycline hydrochloride into a volumetric flask and diluting to the mark with the particular mobile phase in use.

Evaluation of mobile phases

Preliminary evaluations of mobile phases were carried out by cuvette study. A 50:50 (v/v) mixture of the mobile phase, which was 10^{-5} *M* in tetracycline, and PC-A was prepared. If no precipitate was observed, the emission spectrum between 570 and 670 nm was recorded at an excitation wavelength of 392 nm.

Analysis of gingival crevice fluid

Samples of gingival crevice fluid were obtained by placing a small strip of filter paper in the gingival crevice for 10 s. The volume of sample drawn onto the strip was measured using a gingival fluid meter (Periotron). The strips were kept in vials at −70 °C until ready for use. The vials were removed from the freezer and a 100-μl aliquot of the pre-injection phase (No. 1 in Table I) was added. The pre-injection phase was allowed to sit in contact with the strip for 15 min. The strip was then removed from the vial and the sample analyzed using FIA. Three replicate injections (20 μl) were made.

RESULTS AND DISCUSSION

Sensitized Eu(III) luminescence is not a viable detection method with every LC phase described for the separation of tetracycline. Most of the reversed-phase methods for tetracycline employ mobile phases of low pH. Detection based on sensitized Eu(III) luminescence, however, is best performed at basic pH [22].

The pH dependence of the energy transfer between tetracycline and Eu(III) reflects changes in the site of binding. The bonding of tetracycline with gadolinium(III), which is expected to be the same as that with Eu(III), has been studied using nuclear magnetic resonance (NMR) spectroscopy [24]. It was found that at pH 2.0 and 6.5, the NMR data were consistent with preferential binding of the lanthanide ion at position 1 (see Fig. 1). At a pH of 8.6 the hydroxy group near site 2 (see Fig. 1) is deprotonated. The NMR data were now consistent with chelate bonding of the lanthanide ion at site 2. Site 2 places the Eu(III) closer to the benzoyl moiety. The benzoyl moiety in tetracycline is probably responsible for the energy transfer to Eu(III).

For detection of tetracycline using Eu(III) luminescence to be viable, the pH of the LC effluent must be adjusted post-column. This requires the use of a buffer in the LC mobile phase that can be readily overcome via post-column adjustment. The need for a basic pH to optimize the sensitivity of the method also precludes the use of chloride and nitrate salts of Eu(III). These salts, which primarily exist as aquated lanthanide ions in aqueous solutions, form insoluble hydroxides or oxides at pH values above 7. Any insoluble materials would clog the post-column mixing tee and reaction coil.

The solubility problem was overcome by adding EDTA to the phases containing Eu(III). Complexes of lanthanide ions with EDTA are known to be water-soluble and stable at pH values as high as 12 [25]. In addition, the size of the lanthanide ions prevents the EDTA from fully encapsulating the metal [26]. As a result the lanthanide ion has available coordination sites at which donor compounds can bond without having to displace the EDTA ligand. The intensity of Eu(III) luminescence from solutions containing Eu(III) nitrate, tetracycline, and EDTA at pH 9 or 10 was larger than that of the sample without EDTA at pH 6. In the absence of EDTA, a pH of 6 is the highest value one could reasonably employ in LC without concern for possible precipitation or europium oxides or hydroxides.

The emission spectrum from 590 to 660 nm of a 50:50 (v/v) mixture of methanol–acetonitrile–0.01 *M* oxalic acid (pH 2) (1:1.5:5)–tetracycline (10^{-5} *M*) and PC-A is shown in Fig. 2b. The excitation wavelength was 392 nm and corresponds to absorption by tetracycline. This wavelength was found to maximize the emission from Eu(III). The bands at 592, 616, and 651 nm are characteristic of emission from Eu(III). The emission spectrum in Fig. 2a is that of PC-A with no tetracycline. The peaks in Fig. 2b are therefore the result of energy transfer from tetracycline to Eu(III). The emission profile in Fig. 2b suggests that a filter instrument would be sufficient for LC detection.

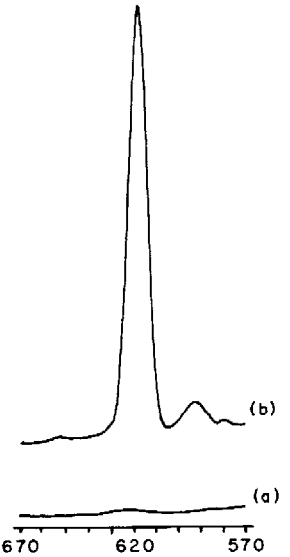


Fig. 2. Emission spectrum from 590 to 660 nm of (a) PC-A and (b) a 50:50 (v/v) mixture of methanol–acetonitrile–0.01 *M* oxalic acid (pH 2) (1:1.5·5)–tetracycline (10^{-5} *M*) and PC-A. Excitation wavelength, 392 nm.

Evaluation of mobile phases

Of the mobile phases considered [7,9–18,27,28] thirteen were found that were compatible with sensitized Eu(III) luminescence detection. These are listed in Table I. Several were eliminated on the basis that the mobile phase buffer was too concentrated to be adjusted to a pH of 9 using the post-column phase. Mobile

TABLE I

MOBILE PHASES COMPATIBLE WITH SENSITIZED EUROPIUM(III) LUMINESCENCE DETECTION

No.	Mobile phase	Reference
1	10^{-3} *M* Na_2EDTA, 0.12 *M* KNO_3–acetonitrile (90:10)	1
2	10^{-3} *M* Na_2EDTA, 0.12 *M* KNO_3–dimethylformamide (90:10)	1
3	Methanol–acetonitrile–0.01 *M* oxalic acid (pH 2) (1:1.5:5)	2
4	Glycine buffer (pH 2.1)–acetonitrile (72:28)	3
5	0.01 *M* NaH_2PO_4 (pH 2.4)–acetonitrile (75:25)	6
6	Methanol–water (40:60) containing 0.1% trifluoroacetic acid	11
7	Acetonitrile–water (27:73) containing 0.1% trifluoroacetic acid	11
8	Acetonitrile–water–0.1 *M* NaH_2PO_4 (pH 2.6) (20:70:10)	12
9	10^{-3} *M* EDTA (pH 6.6)–tetrahydrofuran (85:15)	14
10	10^{-3} *M* EDTA (pH 6.6)–methanol (82:18)	15
11	0.005 *M* EDTA–methanol (70:30)	16
12	0.05 *M* phosphate buffer (pH 2.5)–acetonitrile (90:10)	18
13	0.2 *M* oxalate–0.1 *M* Na_2EDTA–dimethylformamide (55.20:25)	5
14	10^{-4} *M* Na[Eu(EDTA)], 10^{-3} *M* EDTA, 0.2 *M* ammonium chloride (pH 9, ammonia)–methanol (60:40)	–

phases 12 and 13, because of weaker Eu(III) emission, were judged less suitable for use with sensitized Eu(III) luminescence. The ammonia–ammonium chloride buffer was chosen for the post-column phase because of its suitable pK_a value. Ammonia also exhibits weaker complexation with lanthanide ions than water [29]. The concentration of the buffer was selected as a reasonable upper limit to employ with LC pumps and mobile phases. Since enhanced Eu(III) luminescence was not observed for all mobile phases, certain buffers apparently interfered with the energy transfer.

A set of chromatograms for a test mixture of tetracyclines using the conditions described by Oka et al. [2] is shown in Fig. 3. The concentrations of each of the compounds in the mixture was 10^{-5} *M*. The chromatogram obtained using ultraviolet detection at 280 nm is shown in Fig. 3a. The chromatogram obtained using fluorescence detection with a post-column phase containing no Eu(III) is shown in Fig. 3b. Fig. 3c shows the chromatogram obtained using fluorescence detection with a post-column phase that contains Eu(III).

The signals for oxycycline and tetracycline using fluorescence detection were significantly enhanced when Eu(III) was added to the post-column phase. In the case of minocycline, no peak, indicative of no energy transfer, was noted with Eu(III). The other three compounds exhibited small peaks with Eu(III). For tetracycline, the detection limit, measured as that amount of sample that gave a

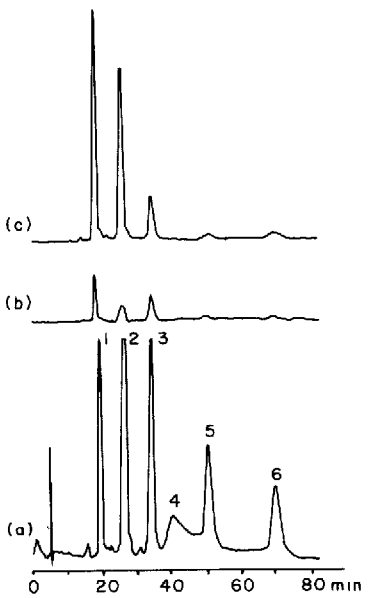


Fig. 3. Chromatograms of a test mixture of oxycycline (1), tetracycline (2), demeclocycline (3), minocycline (4), chlortetracycline (5), and doxycycline (6) obtained using: (a) ultraviolet detection at 280 nm, mobile phase 3 in Table I, flow-rate 1 ml/min; (b) fluorescence detection (excitation wavelength, 392 nm, emission wavelength, 616 nm), mobile phase 3 in Table I, flow-rate 1 ml/min; post-column phase: 0.2 *M* ammonium chloride (pH 9.0), flow-rate 1 ml/min; (c) fluorescence detection (excitation wavelength, 392 nm, emission wavelength, 616 nm), mobile phase 3 in Table I, flow-rate 1 ml/min, post-column phase; PC-A, flow-rate 1 ml/min. Column: C_8 (see Experimental section for description).

signal three times the peak-to-peak noise of the baseline, obtained using sensitized Eu(III) luminescence was $5 \cdot 10^{-7}$ *M* (4.8 ng of injected sample). The detection limits under these conditions, which are not optimized, are comparable to those with ultraviolet detection (4–5 ng) [2,6,8]. The response for tetracycline using sensitized Eu(III) luminescence was linear over the range from the detection limit to 10^{-5} *M*.

The retention times we observed for the tetracyclines are longer than those reported by Oka et al. [2]. In part, this can be attributed to the smaller particle diameter of our stationary phase (5 versus 10 μm). Another reason, however, is that our stationary phase probably had less surface coverage of hydroxysilane groups. During the course of our study the retention times of the tetracyclines gradually increased. We believe the mobile phase caused a gradual decomposition of the solid support leading to removal of the surface C_8 groups. Minocycline was particularly susceptible to the surface decomposition as its retention time changed from 20 to 34 min. This change was large enough to cause an alteration in the elution order of minocycline. While we have not compared commercial columns, we would recommend that high-quality analytical columns be purchased for tetracycline analyses. Were we able to duplicate the retention times of Oka et al. [2], we believe that the detection limit for tetracycline using sensitized Eu(III) luminescence would be lower than 4.8 ng.

Investigations of three other LC mobile phases were undertaken. The first (No. 4 in Table I) was used with a C_8 column. Under these conditions tetracycline eluted at approximately 6.6 min with severe tailing. The sensitivity with this mobile phase was not as great as that with the phase of Oka et al. [2]. The other two phases (Nos. 1 and 2 in Table I) were used with a C_1 column. Employing acetonitrile as the organic modifier and adjusting the pH of the mobile phase to 2.6 through the addition of nitric acid, tetracycline exhibited tailing. A detection limit of $5 \cdot 10^{-7}$ *M* (4.8 ng) was obtained with a post-column phase at pH 10. Employing dimethylformamide as the organic modifier, tetracycline exhibited a symmetrical peak. A detection limit of $1 \cdot 10^{-7}$ *M* (1 ng) was obtained with a post-column phase at pH 10.

The use of CAPS as the buffer for the post-column phase was also investigated. The higher pK_a value of the CAPS buffer (10.4) compared to the ammonia buffer (9.25) allows a more alkaline pH. The response obtained for tetracycline (10^{-5} *M*) using a 50:50 (v/v) mixture of a mobile phase (No. 1, Table I) with PC-C was essentially the same at pH 10 and 11. The sensitivity with CAPS buffer at pH 10 was better by a factor of 5, however, than that with ammonia buffer at pH 10. The use of CAPS buffer at pH 10 is therefore recommended for the detection of tetracycline by sensitized Eu(III) luminescence.

A C_{18} reversed-phase column utilizing a styrene-divinylbenzene copolymer has recently been developed. The styrene-divinylbenzene support is stable at all pH values. Retention of tetracyclines on this column was found to depend on the concentration of EDTA, concentration of Eu(III), pH, and percentage organic modifier in the mobile phase. At pH 9 or greater, however, the number of theoretical plates achieved for tetracycline was only about 200. The column was therefore judged unsuitable for our purposes.

Applications

Sensitized Eu(III) luminescence detection was applied to the LC separation of tetracycline in urine, blood, serum, and gingival crevice fluid. The chromatogram obtained for urine using ultraviolet detection at 280 nm is shown in Fig. 4a. The chromatogram obtained using fluorescence detection with post-column addition of Eu(III) is shown in Fig. 4b. The selectivity of detection based on sensitized Eu(III) luminescence results in considerably fewer peaks in the chromatogram. The likelihood that a coeluting peak would have the requirements for energy transfer to Eu(III) and interfere with the detection of tetracycline are reduced. Similar findings were noted with blood serum.

Tetracycline was the only compound detected in gingival crevice fluid using Eu(III) luminescence. Therefore, FIA coupled with sensitized Eu(III) luminescence detection was suitable for measuring tetracycline. Fig. 5a shows the response for a sample of gingival crevice fluid obtained from a person who had received tetracycline orally. Fig. 5b shows the response for a sample obtained from a dog that had not been given tetracycline. The instrument sensitivity in Fig. 5b is an order of magnitude higher than that in Fig. 5a. The essential absence

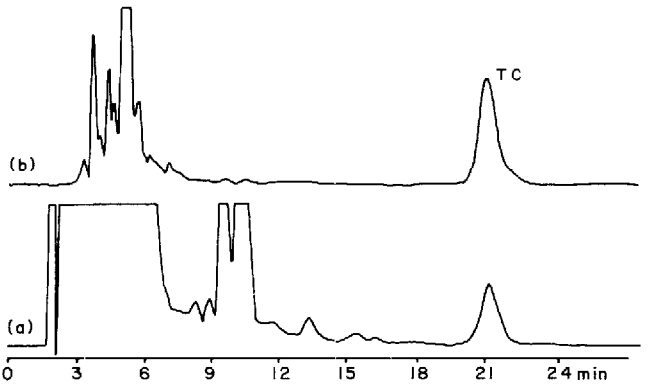


Fig. 4. Chromatogram of a sample of urine spiked with tetracycline (TC) obtained using: (a) ultraviolet detection at 280 nm, mobile phase 3 in Table I, flow-rate 1 ml/min; (b) fluorescence detection (excitation wavelength, 392 nm; emission wavelength, 616 nm), mobile phase 3 in Table I, flow-rate 1 ml/min; post-column phase: PC-A, flow-rate 1 ml/min Column: C_8 (see Experimental section for description).

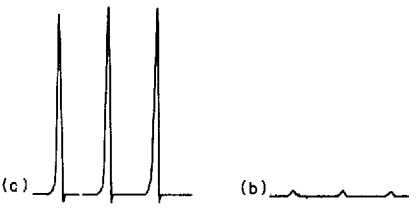


Fig. 5. Analysis of gingival crevice fluid using FIA. Pre-injection phase, No. 1 in Table I; post-injection phase, PC-B (pH 10); excitation wavelength, 392 nm, emission wavelength, 616 nm. (a) Subject given tetracycline orally; (b) subject not given tetracycline. Sensitivity in (b) 10×greater than in (a).

of signal in Fig. 5b indicates that there were no compounds in this sample that either fluoresced at the conditions employed or transferred energy to Eu(III). The relative standard deviation of the three injections shown in Fig. 5a was 2.1%. This is typical of the precision attainable with the method. The detection limit, assessed as that concentration of tetracycline that gave a signal-to-noise ratio of 3, was measured to be $5 \cdot 10^{-8}$ M (480 pg injected). The response was linear from $5 \cdot 10^{-8}$ to $1 \cdot 10^{-5}$ M.

Fluorescent impurities in samples will pose a problem with FIA. Any signal obtained using a post-injection phase without Eu(III) can be attributed to fluorescence from impurities. Eu(III) has a longer excited-state lifetime than most fluorescent organic compounds. Fluorescence from impurities can therefore be eliminated by time-resolved methods. In analyses of tetracycline in saliva, coffee was found to contain fluorescent impurities that interfered with the analysis. The fluorescence from these impurities was eliminated by incorporating a 30-μs delay time between the pulse of the excitation lamp and the acquisition of data. Considerable luminescence from Eu(III) was still observed at this delay time.

The detection limit measured for tetracycline using a delay time of 30 μs was $2.5 \cdot 10^{-7}$ M (2.4 ng of injected sample). The higher detection limit obtained in the time-resolved mode contrasts with previous observations of species with long excited-state lifetimes [21,30]. In these reports, it was found that the reduction in background scatter and fluorescence from impurities that occurred with the delay time more than offset the reduction in signal from the long-lived excited-state species [21,30]. Our results suggest that background scatter and fluorescence was not significant at the conditions employed.

ACKNOWLEDGEMENTS

We wish to thank Research Corporation, through a Leo H. Baekeland Grant, and the National Science Foundation (Biological Instrumentation Program, fluorescence spectrophotometer; College Science Instrumentation Program, liquid chromatograph) for supporting this work. We also thank Dr. Lars Christersson of the Periodontal Disease Clinical Research Center, State University of New York at Buffalo, for providing several of the tetracycline compounds and samples of gingival crevice fluids.

REFERENCES

1 J.H. Knox and J Jurand, J. Chromatogr., 186 (1979) 763.
2 H. Oka, K Uno, K.I. Harada, K. Yasaka and M. Suzuki, J. Chromatogr., 298 (1984) 435.
3 A P. De Leenheer and H.J.C.F. Nelis, J. Chromatogr , 140 (1977) 293.
4 H.J.C.F. Nelis and A.P. De Leenheer, J. Chromatogr., 195 (1980) 35.
5 W.N. Barnes, A. Ray and L.J. Bates, J. Chromatogr., 347 (1985) 173.
6 R. Bocker and C.J. Estler, Arzneim.-Forsch/Drug Res., 29 (1979) 1690.
7 B.G. Charles, J.J. Cole and P.J. Ravenscroft, J. Chromatogr., 222 (1981) 152.
8 G.D Mack and R.B. Ashworth, J. Chromatogr. Sci., 16 (1978) 93.
9 J. Hermansson, J. Chromatogr., 232 (1982) 385.
10 J.Y.C. Hon and L.R Marray, J. Liq. Chromatogr., 5 (1982) 1973.
11 T. Hasan and B.S. Cooperman, J. Chromatogr., 321 (1985) 462.

12 N.A. Botsoglou, V.N. Vassilopoulos and D.C. Kufidis, Chim. Chron., 13 (1984) 37.
13 S.L. Ali and T Strittmatter, Int. J. Pharm., 1 (1978) 185.
14 A.M. Depaolis, T.E. Britt, A.J. Holman, E.J. McGonigle, G. Kaplan and W.C. Davies, J. Pharm. Sci., 73 (1984) 1650.
15 A. Aszalos, C. Haneke, M.J. Hayden and J. Crawford, Chromatographia, 15 (1982) 367.
16 I. Nilsson-Ehle, T.T. Yoshikawa, M.C. Schotz and L.B. Guze, Antimicrob. Agents Chemother , 9 (1976) 754.
17 J. Torel, J. Cillard, P. Cillard and M. Vie, J. Chromatogr., 330 (1985) 425.
18 Y. Onji, M. Uno and K. Tanigawa, J. Assoc. Off. Anal. Chem., 67 (1984) 1135.
19 J.H. Knox and J. Jurand, J. Chromatogr., 110 (1975) 103.
20 E.E. DiBella, J.B. Weissman, M J. Joseph, J.R. Schultz and T.J. Wenzel, J. Chromatogr., 328 (1985) 101.
21 T.J Wenzel and L.M. Collette, J. Chromatogr., 436 (1988) 299.
22 L.M. Hirschy, E.V. Dose and J.D. Winefordner, Anal. Chim. Acta, 147 (1983) 311.
23 T.J. Wenzel, M.E. Ashley and R.E. Sievers, Anal. Chem., 54 (1982) 615
24 M. Celotti and G.V. Fazakerley, J. Chem. Soc. Perkin 2, (1977) 1319.
25 G.A. Elgavish and J. Reuben, J. Am. Chem. Soc., 99 (1977) 1762.
26 M.D. Lind, B. Lee and J.L. Hoard, J. Am. Chem. Soc., 87 (1965) 1611.
27 N. Muhammad and J.A. Bodnar, J. Pharm. Sci., 69 (1980) 928.
28 K. Dihuidi, M.J. Kucharski, E. Roets, J. Hoogmartens and H. Vanderhaeghe, J. Chromatogr., 325 (1985) 413
29 T. Moeller, in J.C. Bailar, Jr., H.J. Emeleus, R. Nyholm and A.F. Trotman-Dickenson (Editors) Comprehensive Inorganic Chemistry, Vol. 4, Pergamon Press, 1973, p. 28.
30 R.A. Baumann, C. Gooijer, N.H. Velthorst and R W. Frei, Anal. Chem., 57 (1987) 1815.