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The control of nitrilotriacetic acid in edetic acid and its salts by liquid chromatography

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Abstract

Two liquid chromatographic methods have been developed and validated for the determination of nitrilotriacetic acid (NTA) in edetic acid (EDTA) and its salts. In one method NTA and EDTA are separated on a polymer column without pretreatment, and are detected amperometrically using a glassy-carbon electrode. In the other method NTA and EDTA are complexed with ferric ions and the complexes separated on a porous graphite carbon stationary phase with ultraviolet detection at 215 nm. Both methods were sufficiently selected and sensitive to allow the control of NTA (0.1% m/m) in sample of EDTA and its salts.

Keywords: Nitrilotriacetic acid; EDTA; Complexation; Reversed-phase chromatography; Porous graphite carbon amperometry

1. Introduction

Nitrilotriacetic acid (NTA) which is considered to be a potential human carcinogen [1] is a synthetic impurity of ethylenediaminotetraacetic acid (EDTA) and its salts. Thus, NTA is specifically limited to 0.1% m/m in the relevant monographs of the European Pharmacopoeia [2] and the US Pharmacopoeia [3–5] based on a liquid chromatographic separation and of the complexes of EDTA and NTA with copper with UV detection [6]. However, different laboratories have reported that there were some disadvantages with this method. There are a number of other reports on the separation of the complexes of aminopolycarboxylic acids with metal ions usually copper [7–9] and ferric ions [8,10–15] ions with UV detection. These methods vary with respect to the pH of the mobile phase, the presence or absence of a counter-ion and the type of stationary phase employed. Apart from reversed phase materials [7–13] other phases which have been used to separate the Fe complexes of NTA and EDTA include graphite carbon [16] and anion exchange resins [14,15].

Ion-exchange chromatographic methods for the determination of NTA in water samples, using

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both amperometric and potentiometric detection [17] and the separation of NTA and EDTA on an ionPac AS7 column with UV detection after post-column derivatisation [18] have been described. Aminopolycarboxylic acids have also been analysed by gas-chromatography [5,19] and capillary electrophoresis [20–22].

These acids can be chromatographed without pre-treatment and detected by amperometry using carbon-paste, glassy carbon [23] or platinum electrodes [24].

This report describes chromatographic methods for the separation of aminopolycarboxylic acids with Fe (III) complexation and without complexation using UV and amperometric detection, respectively.

2. Materials and methods

2.1. Chemicals and reagents

Trichloroacetic acid, sodium hydroxide, copper (II) nitrate trihydrate, ethylene glycol and sodium acetate trihydrate were all obtained from Merck (Germany); methanol and glacial acetic acid were obtained from Riedel de Haën (Germany); tetrabutylammonium dihydrogenphosphate and iron (III) sulphate pentahydrate were obtained from Sigma-Aldrich (Germany) and NTA from Fluka (Switzerland). Samples of edetic acid, sodium calcium edetate and disodium edetate were obtained from Sigma-Aldrich and the European Pharmacopoeia. De-ionised water was obtained using an Elgastat UHQPS, PHO203 system.

2.2. Instrumentation

The liquid chromatographic system comprised a model P 1000 XR isocratic pump, an AS 100 XR automatic injector and a Spectra Series UV 150 variable wavelength detector (Thermoseparation Products, Orsay, France). The electrochemical detector ED 40 with a platinum electrode was from Dionex Corporation (USA) and all acquired data were stored and processed using MILLENNIUM Software (Version 3.1) from Waters (USA).

2.3. Stationary phases

Stainless steel columns (250 × 4.6 mm) packed with octadecylsilyl silica gel (Hypersil-ODS, 5 μm) and styrene-divinylbenzene co-polymer (PLRP-S, 8 μm) were supplied by Thermo Hypersil Keystone Ltd (UK) and Polymer Laboratories Ltd. (UK), respectively. A stainless steel column packed with porous graphitic carbon (Hypercarb, 100 × 4.6 mm, 5 μm) was obtained from Thermo Hypersil Keystone Ltd.

2.4. Mobile phases

Mobile Phase A: Tetrabutylammonium dihydrogen phosphate solution (5 g/l) adjusted to pH 7.7 with dilute sodium hydroxide solution: methanol: water (850:115:35; v/v/v). Mobile Phase B: Ferric sulphate pentahydrate (50.0 mg) dissolved 0.1 M sulphuric acid (100 ml) and de-ionised water (700 ml), was adjusted to pH 2.5 with dilute sodium hydroxide solution and ethylene glycol (20 ml) added before making up to 1000 ml with de-ionised water. Mobile Phase C was a 0.1% m/v solution of trichloroacetic acid in water.

2.5. Preparation of solutions

Copper (II) nitrate (2.5 g) was dissolved in de-ionised water and made up to 250 ml with de-ionised water (cupric reagent). Ferric sulphate pentahydrate (10 g) was dissolved in de-ionised water and made up to 1000 ml with de-ionised water (ferric reagent).

2.6. System suitability test solutions

Equal amounts of EDTA and NTA were dissolved in de-ionised water for the direct amperometric method, or in the reagent solution for methods employing UV detection of the complex. Weights and volumes are those specified for the corresponding test solutions.

2.6.1. Test solution 1

A quantity of the sample (0.100 g) was dissolved (initially in 1 ml 1 M sodium hydroxide if

necessary) and diluted to 10.0 ml with either the cupric or ferric reagent solution.

2.6.2. Reference solution 1

A quantity of NTA (50.0 mg) was dissolved in either the cupric or ferric reagent solution and diluted to 50.0 ml with the same reagent solution. A portion (1.0 ml) of this solution was diluted to 100.0 ml with the same reagent solution.

2.6.3. Test solution 2

A quantity of the sample (0.100 g) was dissolved initially in 1 ml 1 M sodium hydroxide if necessary and diluted to 25.0 ml with ferric reagent solution.

2.6.4. Reference solution 2

A quantity of NTA (40.0 mg) was dissolved in ferric reagent solution and diluted to 100.0 ml with the same reagent solution. A portion (1.0 ml) of this solution was diluted to 100.0 ml with ferric reagent solution.

2.6.5. Test solution 3

A quantity of the sample (50.0 mg) was dissolved in water, if necessary in 1 M sodium hydroxide, and diluted to 50.0 ml with de-ionised water.

2.6.6. Reference solution 3

A quantity of NTA (50.0 mg) was dissolved in de-ionised water and diluted to 50.0 ml with de-ionised water. A portion (1.0 ml) of this solution was diluted to 1000.0 ml with de-ionised water.

2.7. Calibration solutions

Two series of five solutions of NTA were prepared at the following concentrations 25, 50, 75, 100 and 125% of the limiting concentration of NTA in EDTA (0.1% NTA in EDTA) in the presence and absence of EDTA. Spiked solutions of the samples were prepared as for the test solutions but to which were added an amount of NTA corresponding to the limiting concentration of NTA in EDTA (0.1% m/m).

The limit of quantification was determined at the concentration of a solution of NTA which gave a peak whose height was ten times greater than the base-line noise.

2.7.1. Chromatographic conditions

2.7.1.1. System A. Test solutions 1 and reference solutions 1 were injected on to either the Hypersil-ODS column or the PLRP-S column. The flow rate of mobile phase A was 1 ml/min and the detection wavelength was 270 nm.

2.7.1.2. System B. Test solutions 2 and reference solutions 2 were injected on to the Hypercarb column which was eluted with Mobile Phase C at 1.0 ml/min. The detection wavelength was 215 nm.

2.7.1.3. System C. Test solutions 3 and reference solutions 3 were injected onto the PLRP-S column eluted with Mobile Phase 3 at 1.0 ml/min. Amperometric detection was with a glassy carbon electrode with an applied voltage of 1.3 V.

3. Results and discussion

The currently used Cu (II) complexation methods [2–5] for measuring small amounts of NTA in EDTA and its salts suffers from a number of disadvantages including the presence of system peaks due to the reagent eluting very near to NTA. Moreover, the performance of the stationary phase can also degenerate rapidly due to the mobile phase. Consequently it is considered that these methods are insufficiently robust to be employed for the routine control of NTA in EDTA and its salts.

To overcome the problem of the degradation of the octodecylsilyl silica caused by the mobile phase, a co-polymer stationary phase which is stable over a larger range of pH (1–12) was examined. However, the chromatographic profile was similar to that obtained with the silica based column in that a small system peak interfered with the peak due to NTA. Whilst the peak shape for NTA (t_R 4.8 min) was good, EDTA eluted too close (t_R 6.5 min) to it. The effect of altering the pH of the mobile phase in the range of 6.7–9.5 was examined but with no improvement in the separa-

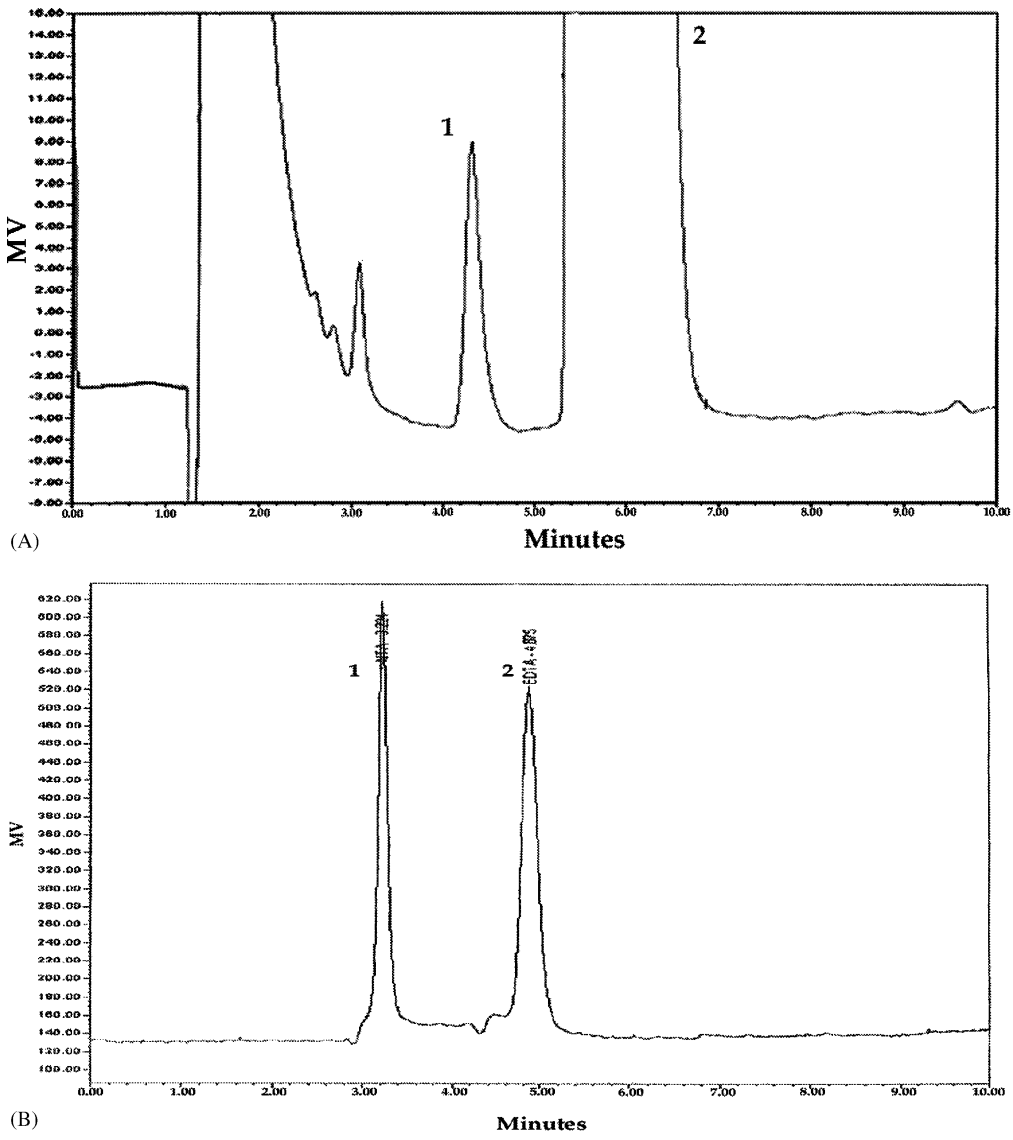

Fig. 1. (A) Liquid chromatogram of a solution of EDTA (4 mg/ml) spiked with the limiting concentration of NTA (0.004 mg/ml). Column, Hypercarb 5 μm (4.6 × 100 mm); Mobile phase, 0.1 mM Fe (III) sulphate in 0.1 M H_2SO_4, pH 1.5 solution containing 2% ethylene glycol; UV detection at 215 nm, Peak 1, NTA, Peak 2, EDTA. (B) Liquid chromatogram of an equimolar solution of EDTA and NTA. Column, PLRP-S 8 μm (4.6 × 250 mm), Mobile phase: 0.1% w/v trichloroacetic acid, pH 1.5, Detector, electrochemical, voltage 1.2 V, Peak 1, NTA, Peak 2, EDTA.

tion. It had been reported that the optimum complexation of Cu (II) and Fe (III) with EDTA occurs at pH 7.7 (6) and pH 4.5 (16). Ferric ion was then examined for its suitability for complexation with EDTA and NTA with an acidic mobile phases. Although with these conditions there was adequate separation of NTA and EDTA there was a system peak near to the peak due to NTA in the pH range of 1.5–4.5.

In an effort to reduce or eliminate "ghost peaks" and peak splitting the metal ion had been added to the mobile phase [16]. With the co-

polymer phase, varying the pH in the range of 1.5–4.5 did not produce a satisfactory separation of NTA from EDTA. However, with porous graphitic carbon as stationary phase as originally described (16) there was excellent separation of NTA and EDTA with no interference from the reagent peak (Fig. 1A). The pH of the mobile phase was altered and the effects on resolution, peak symmetry and peak area were examined. There was good separation ($R_s \sim 6$) and symmetry of the peaks in the pH range 1.5–2.5. As there was an apparent increase in the response at pH 2.5, this pH was chosen for further examination. Since a requirement of the method developed was to measure small amounts of NTA in a large excess of EDTA for maximum sensitivity a detection wavelength of 215 nm was chosen. The limit of quantification of NTA was 1 μg/ml for a 0.4% w/v solution of EDTA. Linear relationships for NTA concentration versus detector response were obtained both in the absence ($y = 25\,363x - 4605.6$, $R^2 = 0.989$) and in the presence of EDTA ($y = 36\,968x + 5070$, $R^2 = 0.989$) around the limiting concentration of NTA. Precision of replicated injections at these various concentrations was also shown to be acceptable (0.68–5.90% in the range 1–5 μg ml). Three samples were analysed for their content of NTA (Table 1). No NTA was detected in a sample of disodium edetate (Sigma-Aldrich) nor in a sample of sodium calcium edetate (Ph. Eur) whereas edetic acid contained approximately 10 ppm of NTA. The samples were also 'spiked' with NTA at the limiting concentration: the recovery of NTA was calculated to be around 100% (Table 1).

Subsequently a modification of a published method (24) demonstrated that NTA and EDTA were separated on a co-polymer column and detected amperometrically with a glassy carbon electrode. NTA and EDTA were separated with either 0.1% w/v trichloroacetic acid (TCA) or 0.1% w/v trifluoroacetic acid (TFA) as mobile phases. Since, when an injection of the TFA mobile phase was made there was a small peak eluting at the same retention time as NTA, the TCA mobile phase was chosen (Fig. 1B). An optimum response was achieved with a mobile phase pH of 1.5 with a detector voltage of 1.3 V. Thus with these condi-

Table 1
Comparision of the concentrations of NTA test and spiked test solutions of edetic acid and edetate salts

Sample	Concentration	Hypercarb column/complexation			Polymer column/amperometric		
		NTA (μg/ml) n = 6	NTA in EDTA (% w/v)	Recovery (%)	NTA (μg/ml)	NTA in EDTA (%)	Recovery (%)
Sodium calcium edetate	(1 mg/ml)	NQ	NQ	NQ	NQ	NQ	NQ
	(4 mg/ml)	NQ	NQ	NQ	NQ	NQ	NQ
	(1 mg/ml+1 μg/ml NTA)				0.94±0.02	0.094	94
	(4 mg/ml+4 μg/ml NTA)	3.89±0.02	0.103	103			
Disodium edetate	(1 mg/ml)	NQ	NQ	NQ	NQ	NQ	NQ
	(4 mg/ml)	NQ	NQ	NQ	NQ	NQ	NQ
	(1 mg/ml+1 μg/ml NTA)				1.06±0.09	0.106	106
	(4 mg/ml+4 μg/ml NTA)	3.99±0.04	0.100	100			
Edetic acid	(1 mg/ml)	NQ	NQ	NQ	NQ	NQ	NQ
	(4 mg/ml)	0.040±0.03	0.001				
	(1 mg/ml+1 μg/ml NTA)				0.97±0.02	0.097	97
	(4 mg/ml+4 μg/ml NTA)	3.99±0.07	0.100	100			

NQ, non-quantifiable; ±, standard deviation.

tions the limit of quantification of NTA was 0.5 μg/ml for a test concentration of 1.0% m/m EDTA. A linear relationship of detector response against concentration of NTA was obtained both in the presence ($y = 1E+0.6x-61\,550$, $R^2 = 0.996$) and absence of EDTA ($66\,9566x-6037$, $R^2 = 0.991$), and the repeatability of injection at each of these concentrations around the limiting concentration was acceptable (R.S.D. 1.9–5.3 in the range 0.3–1.5 mg/ml). The same samples which were previously examined were re-analysed for their content of NTA using this method (Table 1).

In summary, two LC methods have been developed for the determination of NTA in EDTA and its salts. One method involves separation of the ferric complexes of the aminopolycarboxylic acid and UV detection and the other the separation of the aminopolycarboxylic acids on a polymeric column with amperometric detection. Although either method can be employed, the reverse-phase chromatographic separation of the ion-complexes with UV detection is preferred since there is a better separation of NTA from EDTA and although the method is not so sensitive as the method using amperometric detection, it is more precise. Both methods, unlike the presently described compendial methods are suitable for routine use since the column performances are stable with use.

References

[1] Guidelines for drinking water quality, second ed., vol. 1, Recommendations in World Health Organisation, Geneva, 1993, pp. 74–75.
[2] Edetic Acid Monograph 1612 European Pharmacopoeia fourth ed., Council of Europe, Strasbourg, France, 2001.
[3] Edetate calcium disodium United States Pharmacopoeia 24, United States Pharmacopoeial Convention, Rockville, MD, USA, 2000, pp. 622.
[4] Edetate disodium p 622 Ibid.
[5] Edetic acid, National Formularly 19, United States Pharmacopoeial Convention, Rockville, MD, USA, 2000.
[6] D.G. Parkes, M.G. Caruso, J.E. Spradling, III, Anal. Chem. 53 (1981) 2154–2156.
[7] J. Bauer, D. Heathcote, S. Krogh, J. Chromatogr. 369 (1986) 422–425.
[8] M. Deacon, M.R. Smyth, L.G.M.T. Tuinstra, J. Chromatogr. A 659 (1994) 349–357.
[9] L. Hall, L. Takahashi, J. Pharm. Sci. 77 (1988) 247–250.
[10] R. Geschke, M. Zehringer, Fresenius J. Anal. Chem. 357 (1997) 773–776.
[11] E.L. Inman, R.L. Clemens, B.A. Olsen, J. Pharm. Biomed. Anal. 8 (1990) 513–520.
[12] D.E. Richardson, G.H. Ash, P.E. Harden, J. Chromatogr. 688 (1994) 47–53.
[13] D.L. Venezky, W.E. Rudzinski, Anal. Chem. 56 (1984) 315–317.
[14] J. Harmsen, A. Van Den Toorn, J. Chromatogr. 249 (1982) 379–384.
[15] C. Randt, R. Wittlinger, W. Merz, Fresenius, J. Anal. Chem. 346 (1993) 728–731.
[16] O. Stalberg, T. Arvidsson, J. Chromatogr. A 684 (1994) 213–219.
[17] W. Buchberger, P.R. Haddad, P.W. Alexander, J. Chromatogr. 546 (1991) 311–315.
[18] Dionex application note, http://www.dionex.com/app/tree.taf?asset_id = 9195.
[19] H.B. Lee, T.E. Peart, K.L.E. Kaiser, J. Chromatogr. A 738 (1996) 91–99.
[20] R.L. Sheppard, J. Henion, Anal. Chem. 69 (1997) 2901–2907.
[21] C.S. Burgisser, A.T. Stone, Environ. Sci. Technol. 31 (1997) 2656–2664.
[22] W. Buchberger, S. Mulleder, Mikrochim. Acta 119 (1995) 103–111.
[23] J. Dai, G.R. Helz, Anal. Chem. 60 (1988) 301–305.
[24] J.W. Johnson, H.W. Jiang, S.B. Hanna, W.J. James, J. Electrochem. Soc. 119 (1972) 574–580.