Analytical Chemistry Insights

ORIGINAL RESEARCH

A Validated Reverse Phase HPLC Method for the Determination of Disodium EDTA in Meropenem Drug Substance with UV-Detection using Precolumn Derivatization Technique

Bhavil Narola, A.S. Singh, M. Mitra, P.R. Santhakumar and T.G. Chandrashekhar

Ranbaxy Research Laboratories, Plot No 20, Sector-18, Udyog Vihar Industrial Complex, Gurgaon-122001, Haryana, India. Corresponding author email: bhavil.narola@ranbaxy.com

Abstract: This paper deals with development and validation of a high performance liquid chromatographic method for the quantitative determination of disodium EDTA (Ethylenediaminetetraacetic acid) in Meropenem active pharmaceutical ingredient (API). EDTA was derivatized with Ferric chloride solution by heating at 70 °C in water bath for about 20 minutes and the chromatographic separation achieved by injecting 100 µL of the derivatized mixture into a Waters HPLC system with photodiode array detector using a Phenomenex Luna C18(2) column (250 × 4.6 mm), 5 µ. The mobile phase consisting of 5% methanol and 95% of 0.7 g/L solution of Tetra butyl ammonium bromide and 4.6 g/L solution of sodium acetate trihydrate in water (pH adjusted to 4.0 with the help of acetic acid glacial) and a flow rate of 1 milliliter/minute. EDTA eluted at approximately 6 minutes. The method was suitably validated with respect to specificity, linearity of response, precision, accuracy, ruggedness, stability in analytical solution, limit of quantitation and detection and robustness for its intended use.

Keywords: EDTA, HPLC, precolumn derivatization, Meropenem, ferric chloride

Analytical Chemistry Insights 2011:6 7–14

doi: 10.4137/ACI.S5953

This article is available from http://www.la-press.com.

Introduction

Ethylenediaminetetraacetic acid (EDTA) (Fig. 1) is a powerful chelating agent, forming stable complexes with most metal ions. Due to its ability to sequester metal ions, EDTA is widely used in medicine, chemical industry, food technology, agriculture and pharmaceutical technology. EDTA in its disodium salt or calcium disodium salt form is frequently used in pharmaceuticals because of its stability, compatibility and low toxicity. In the field of analytical chemistry, besides its use in complexometric titrations, EDTA has been reported to be very useful ligands for the complexation of metals, which enables their chromatographic separations.[1]

Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections, including meningitis and pneumonia. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. EDTA is being used in the synthesis of Meropenem. Therefore the quantification of the residual EDTA is essential as per regulatory requirement.

Various analytical methods have been proposed for the determination of EDTA in a wide variety of sample matrices.[2] They include titrimetry,[3] spectrophotometry,[4] electrochemistry eg, polarography,[5] differential pulse polorography,[6] catalytic potential tirtrimetry,[7] differential pulse anodic stripping voltammetry,[8] amperometry,[9] capillary electrophoresis,[10] and chromatography. Among them, gas chromatography and HPLC (reverse phase ion-pair or ion exchange retention mechanism) appear to be the prevailing techniques, despite the fact that EDTA lacks volatility and exhibits low UV/visible absorptivity. The gas chromatographic methods always include a time consuming derivatization steps, in which EDTA is converted into methyl, ethyl, propyl and butyl esters to obtained volatility.[11,12]

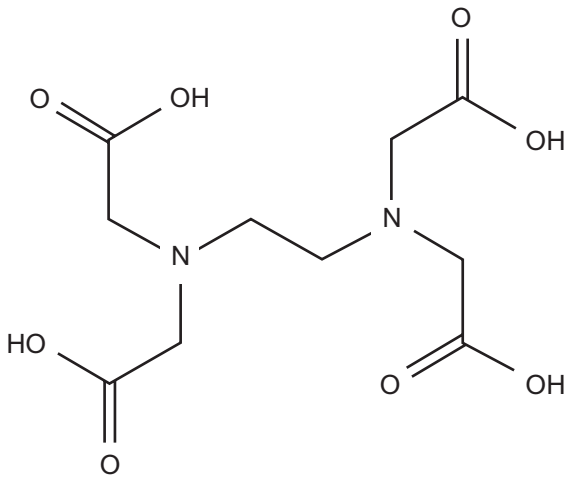

Figure 1. Structure of EDTA.

This paper describes development and validation of derivatized method with direct UV-detection for the quantitative determination of disodium EDTA in Meropenem drug substance. This method also has advantages over some techniques as mentioned in above references,[3–12] like here EDTA response is measured by direct UV detection with enhanced sensitivity and method is simpler, highly reproducible, specific and accurate, compare to using complex techniques like titrimetry, spectrophotometric, capillary electrophoresis or GC technique. As EDTA does not contain any chromophoric group, it is very difficult to determine EDTA by direct UV detection. Hence a method has been optimized and developed by derivatizing disodium EDTA with ferric chloride solution. The method has been optimized with respect to reaction time, derivatization temperature and derivatization reagent volume and suitably validate for its intended use.

Experimental

Reagents and chemicals

Di-sodium salt of Ethylenediaminetetra-acetic acid dehydrate was purchased form Qualigens, Sodium acetate trihydrate was purchased from Qualigens (AR grade), Tetra butyl ammonium bromide was purchased from Spectrochem (AR grade), Methanol (HPLC grade), Acetic acid glacial was purchased from Rankem (AR grade), Ferric chloride anhydrous was purchased from Qualigens (AR grade) and Meropenem was obtained from Ranbaxy Labs. Ltd. (India). All the above materials were used without any further purification. Water (HPLC grade) was used for the preparation of solutions.

Chromatography

The analytical separations were carried out on a Waters HPLC system, equipped with a 2695 separation module and 2996 photodiode array detector. The analytical column was a Phenomenex Luna C18 (2) column

(250 × 4.6 mm), 5 µ. The mobile phase consisted of premixed and degassed solution of buffer and methanol in the ratio of [95:5] [v/v]. Mobile phase was prepared by dissolving 4.1 gm of sodium acetate trihydrate and 0.64 gm Tertrabutyl ammonium bromide in 950 mL of water and than added 50 mL of methanol, mixed and pH adjusted to 4.0 with acetic acid glacial. The mobile phase was filtered through a 0.45 µm membrane filter. Water used as a diluent for the preparation of solutions. The flow rate was 1 mL/min and runtime was 10 minute for EDTA standard solution and 60 minutes for Meropenem sample solutions. Column temperature was maintained at 30 °C. UV detection was measured at 254 nm and the volume of sample injected was 100 µL. The control of the HPLC system and data collection was by Empower software.

Derivatization process of standard and sample solution

Standard solution preparation

A standard stock solution at 1 milligram per milliliter (mg/mL) was prepared by dissolving disodium salt of EDTA in diluent (Accurately weighed and disolved disodium salt of EDTA dihydrate 63.5 mg equivalent to 50 mg of EDTA in 50 mL of diluent). The derivatization process was carried out by transferring 10 mL of standard stock solution into 100 mL volumetric flask. Add 5 mL of Ferric chloride solution (About 0.64 gm of Ferric chloride anhydrous transfer to 200 mL volumetric flask and dissolve with 5 Ml 1 N solution of hydrochloric acid and make up the volume to 200 mL with water). Shake well and keep in water bath at 70 °C for 20 minutes, finally make-up the volume with diluent after it attains room temperature. Dilute the solution with diluent to achieve the final concentration 0.0002 mg/mL.

Sample solution preparation

A sample solution was prepared by dissolving 500 mg of Meropenem sample in diluent, in 25 mL volumetric flask. The derivatization process was carried out by adding 2 mL of Ferric chloride solution. Shake well and keep in water bath at 70 °C for 20 minutes, finally make-up the volume with diluent after it attains room temperature. The final concentration is 20 mg/mL.

Results and Discussion

Method validation

Specificity

Blank, sample solution and sample solution spiked with EDTA (at 0.001% of sample concentration) along with other known related substances of Meropenem were chromatographed individually as per the method to examine interference, if any, with EDTA peak.

No peak from the blank was observed at the retention time of EDTA peak. The peak purity plot of EDTA shows that the peak is pure and has no co eluting peaks, indicating specificity of the method (Figs. 2–4).

Linearity of response

The linearity of response for EDTA was determined in the range as given in Table 1. Data shown in Table 1 indicates that the response is linear over

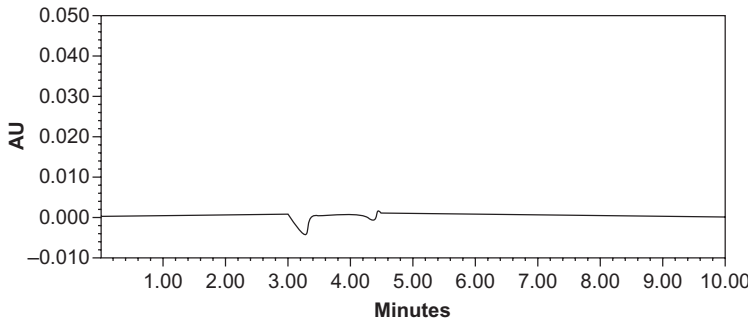

Figure 2. Chromatogram of blank.

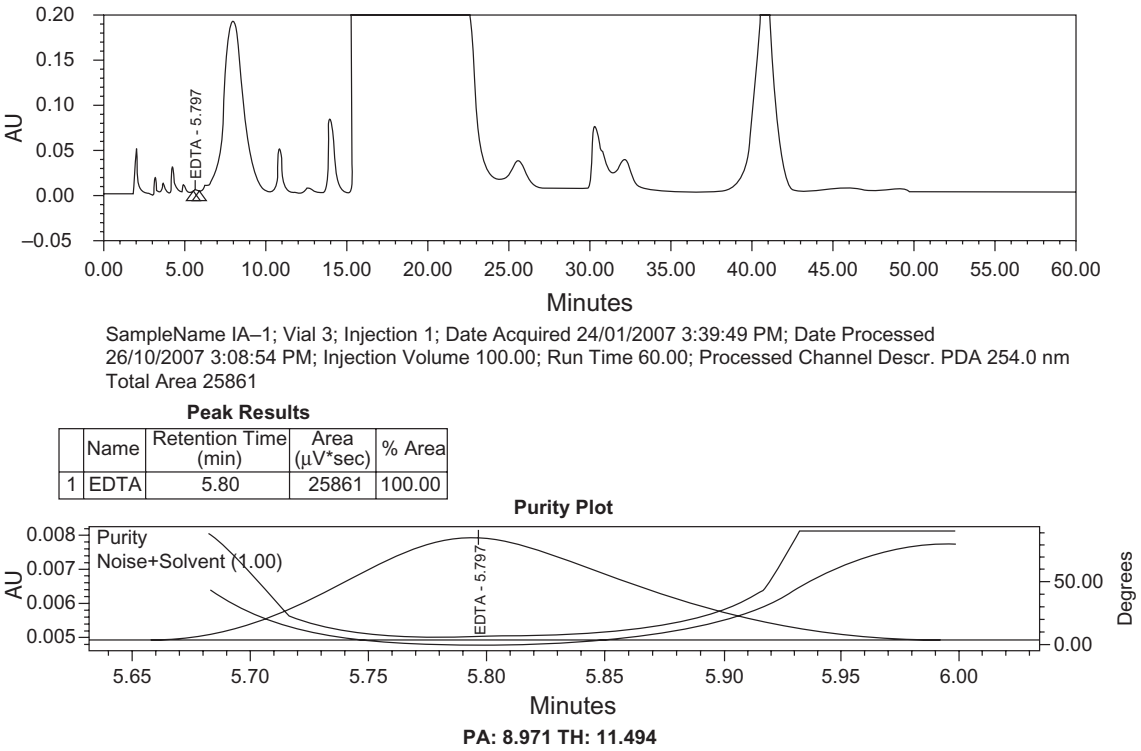

Figure 3. Chromatogram and Peak purity plot of EDTA in sample.
Abbreviations: PA, Purity angle; TH, Purity threshold.

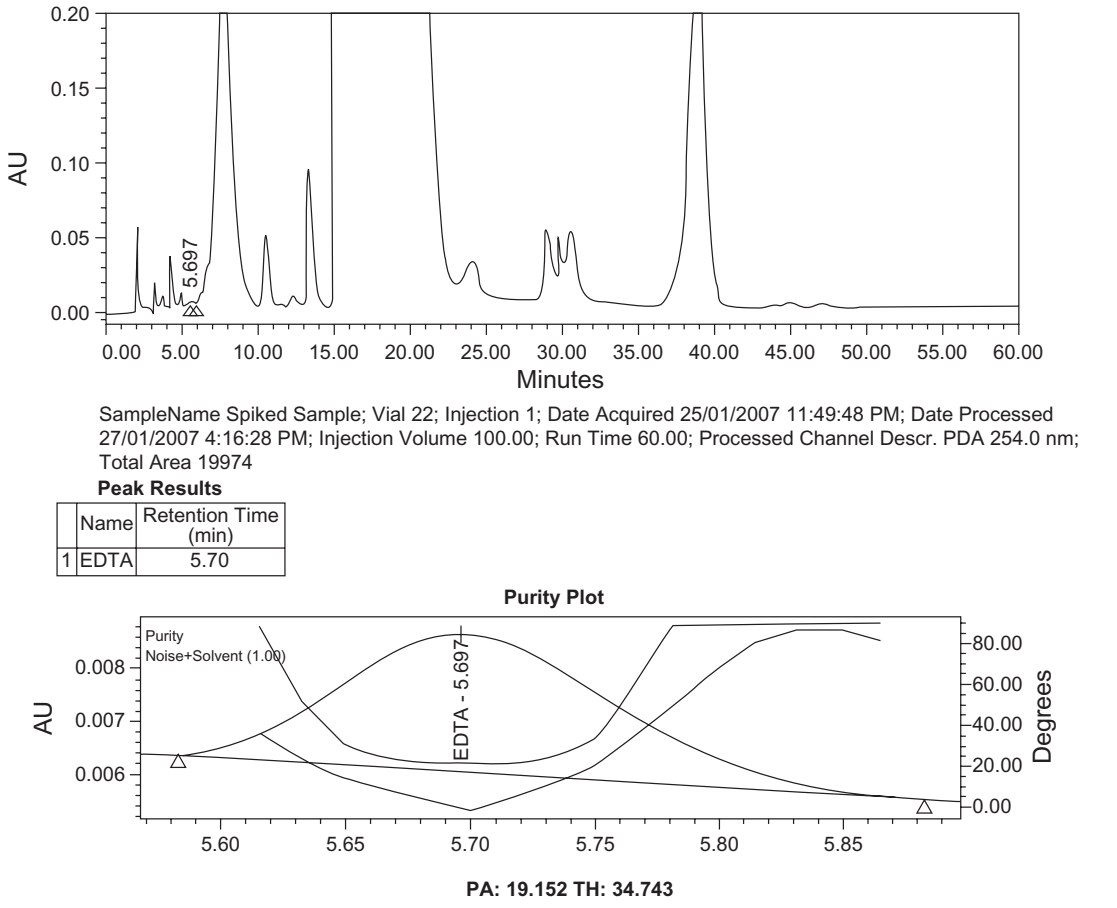

Figure 4. Chromatogram and Peak purity plot of EDTA in Sample spiked with known related substances of Meropenem.

Table 1. Linearity of response.

Conc. (µg/mL)	Area counts (µV*sec.)
0.035	6072
0.058	10390
0.116	18963
0.231	36709
0.347	55300
0.579	92690
1.157	186442
2.315	359162
3.472	549143
Slope	157156
Intercept	989
CC	0.99991

Abbreviation: CC, Correlation coefficient.

the specified range. (Acceptance criteria: Correlation coefficient should not be less than 0.98).

Precision

System precision

Standard solution was injected six times into the HPLC system. Data shown in Table 2 indicate an acceptable level of precision for the analytical system. (Acceptance criteria: RSD should not be more than 5.0%).

Method precision

Six samples of a single batch of Meropenem API were prepared and analysed by the proposed method. Data is shown in Table 3. The % RSD value indicates that the method has an acceptable level of precision (Acceptance criteria: RSD should not be more than 10%).

Accuracy

Known amount of sample was taken separately into nine different flasks and spiked with known quantities of EDTA at three different levels, each in triplicate. The samples were analysed by the proposed method and the amount of EDTA recovered after making corrections for the amount already present were calculated. Data shown in Table 4 indicate that the method has an acceptable level of accuracy. (Acceptance criteria: Recovery should be in the range of 80%–120%).

Table 2. System precision.

Injection No.	Area counts (µV*sec.)
1	36737
2	36288
3	36640
4	36710
5	36548
6	36699
Mean	36604
SD	169
RSD (%)	0.46

Table 3. Method precision.

Sample No.	EDTA (ppm)
1	8.078
2	7.908
3	7.791
4	7.456
5	7.276
6	7.108
Mean	7.603
SD	0.3815
RSD (%)	5.02

Ruggedness

Ruggedness of the method was verified by analysing samples of a single batch of Meropenem API by two different analysts using two different instruments and columns on different days. The mean, standard deviation and % RSD for EDTA is shown in Table 5. Ruggedness of the method is shown by the overall RSD value of 4.87% between the two sets of data. (Acceptance criteria: Overall RSD should not be more than 10%).

Table 4. Accuracy.

Recovery level	EDTA		
	Amount added (ppm)	Amount recovered (ppm)	% Recovery
Level-1, Rec 1	58.009	53.783	92.71
Level-1, Rec 2	57.840	53.656	92.77
Level-1, Rec 3	57.817	52.790	91.31
Level-2, Rec 1	115.372	108.048	93.65
Level-2, Rec 2	115.609	107.659	93.12
Level-2, Rec 3	113.896	104.715	91.94
Level-3, Rec 1	232.650	217.232	93.37
Level-3, Rec 2	231.172	215.982	93.43
Level-3, Rec 3	232.197	218.217	93.98
Mean			92.92
SD			0.850
RSD (%)			0.91

Table 5. Ruggedness.

Sample No.	Disodium EDTA (ppm)	
	Set I	Set II
1	8.078	8.466
2	7.908	8.027
3	7.791	7.859
4	7.456	7.926
5	7.276	7.963
6	7.108	7.513
Mean	7.603	7.959
SD	0.3815	0.3071
RSD (%)	5.02	3.86
Overall Mean	7.781	
Overall SD	0.3790	
Overall RSD (%)	4.87	
Set	I	II
Analyst	1	2
Instrument No.	A	B
Column No.	X	Y

Stability in analytical solution

A sample solution of Meropenem API was prepared and kept at 10 °C. The sample solution was analysed initially and at various time intervals. Data is shown in Table 6. As the cumulative % RSD up to 975 min. for EDTA meets the acceptance criteria, it is concluded that sample is stable in analytical solution at 10 °C for about 16 h. (Acceptance criteria: Cumulative RSD should not be more than 10%).

Limit of quantitation and detection

The limit of quantitation and detection for EDTA were determined from linearity data using the formulae* and verifying the predicted LOQ and LOD values by showing precision by making six replicate injections of lower concentration solutions of EDTA. Data is shown in Table 7a and summarized in Table 7b. (Acceptance criteria: RSD should not be more than 10% for LOQ and should not be more than 33% for LOD).

Table 6. Stability in analytical solution (At 10 °C).

Time (min.)	Area counts (µV*sec.)	Cumulative RSD (%)
Initial	23892	–
61	23629	0.78
123	23455	0.93
184	23145	1.33
246	23756	1.23
307	23244	1.24
369	22678	1.77
490	21361	3.53
611	21230	4.34
732	20521	5.33
854	19465	6.72
975	18146	8.56

Table 7a. Limit of quantitation and detection.

Conc.	LOQ	LOD
µg/mL	0.034	0.023
ppm	1.700	1.150
Injection	Area counts (µV*sec.)	
1	5901	4415
2	5849	4793
3	6034	3695
4	5930	5378
5	5578	4291
6	5649	4813
Mean	5824	4564
SD	175	571
RSD (%)	3.00	12.51

Robustness

Robustness of the method was investigated by varying the instrumental conditions such as flow rate (±10%), column oven temperature (35 °C), organic content in mobile phase (±2%), wavelength of detection (±5 nm) and pH of mobile phase (±0.2). Sample solution was analysed under each condition and EDTA content was calculated. The mean, standard deviation and % RSD for each set of data are shown in Table 8. Robustness of the method is indicated by the overall % RSD between the data of Set I and data at each variable condition. However, under the condition of Temperature (35 °C), change in organic content in mobile phase (±2%) and change in pH of mobile phase (±0.2%), the %

Table 7b. Summary of LOQ and LOD values.

S.no.	Component	LOQ		LOD	
		µg/mL	ppm	µg/mL	ppm
1	EDTA	0.034	1.700	0.023	1.150

$$* \text{ LOQ } (\mu g/mL) = \frac{10 \times \text{Residual standard deviation}}{\text{Slope}}$$

$$* \text{ LOD } (\mu g/mL) = \frac{3.3 \times \text{Residual standard deviation}}{\text{Slope}}$$

Table 8. Robustness.

S.no.	EDTA (ppm)									
	Set I	**Set II**	**Set III**	**Set IV**	**Set V**	**Set VI**	**Set VII**	**Set VIII**	**Set IX**	**Set X**
1	8.078	7.948	8.201	8.274	8.037	9.505	17.146	22.761	22.067	12.021
2	7.908	7.889	8.135	7.765	7.688	10.520	18.165	23.884	24.582	14.773
3	7.791	7.753	8.119	8.206	7.511	7.561	17.459	20.609	22.260	11.461
4	7.456	–	–	–	–	–	–	–	–	–
5	7.276	–	–	–	–	–	–	–	–	–
6	7.108	–	–	–	–	–	–	–	–	–
Mean	7.603	7.863	8.152	8.082	7.745	9.195	17.590	22.418	22.970	12.752
SD	0.3815	0.1000	0.0435	0.2763	0.2676	1.5036	0.5220	1.6642	1.3997	1.7728
RSD (%)	5.02	1.27	0.53	3.42	3.46	16.35	2.97	7.42	6.09	13.90
Overall Mean		7.690	7.786	7.762	7.650	8.134	10.932	12.541	12.725	9.319
Overall SD		0.3323	0.4083	0.4091	0.3375	1.1359	5.0095	7.4603	7.7211	2.7394
Overall RSD (%)		4.32	5.24	5.27	4.41	13.96	45.82	59.49	60.68	29.40

Notes: Set I—Control (Proposed method); Set II—Variation in wavelength (UV = 249 nm); Set III—Variation in wavelength (UV = 259 nm); Set IV— Variation in flow rate (–10%); Set V—Variation in flow rate (+10%); Set VI—Column oven temperature (35 °C); Set VII—Variation in organic content in mobile phase (–2%); Set VIII—Variation in organic content in mobile phase (+2%); Set IX—Variation in pH of mobile phase (–0.2); Set X—Variation in pH of mobile phase (+0.2).

RSD is above the acceptance criterion. Hence it is recommended to adhere to the method. (Acceptance criteria: Overall RSD should not be more than 10%).

Conclusion

A simple isocratic reverse phase method for the determination of EDTA was developed and validated using direct UV detection. The method is selective, precise and accurate and was successfully applied to the analysis of commercially available Meropenem drug substances. EDTA is not easily detected by HPLC using UV detection because of absence of a chromophoric group. Derivatization with Ferric chloride solution is a simple and very effective means of enhancing the chromatographic detection of the compound.

Acknowledgment

Authors are thankful to Ranbaxy Research Laboratories for providing necessary facilities and for permission to publish this article.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Buchbergr W, Haddad PR, Alexander PW. Separation of metal complexes of ethylenediaminetetraacetic acid in environmental water samples. *J chromatogr*. 1991;558:181–6.
2. Sillanpaa M, Sihvonen ML. Analysis of EDTA and DTPA. *Talanta*. 1997;44: 1487–97.
3. Clinkemaille GG. Determination of nitrilotriacetic acid and ethylenediaminetetraacetic acid in granular detergent formulations. *Anal Chim Acta*. 1968;43:520–2.
4. Hamano T, Mitsuhashi Y, Kojima N, et al. Sensitive spectrophotometric method for the determination of ethylenediaminetetraacetic acid. *Analyst*. 1993;118:909–12.
5. Nomura T, Nakagava G. Tensammetric determination of microgram amounts of EDTA. *J Electroanal Chem*. 1980;111:319–24.
6. Stolzberg RJ. Determination of ethylenediaminetetraacetate and nitrilotriacetate by differential pulse polorography. *Anal Chim Acta*. 1977;92:139–48.
7. Hadjiioannou TP, Koupparis MA, Efstathiou CE. Semiautomatic indirect titration of alkaline earth-ions with catalytic end point indication. *Anal Chim Acta*. 1977;88:281–7.
8. Voulgaropoulos A, Tzivanakis N. Use of ion exchangers for the voltammetric determination of NTA and EDTA in natural waters. *Electroanalysis*. 1992;4:647–51.
9. Fogg AG, Fernandez-Arciniega MA, Alonso RM. Amperometric flow injection determination of ethylenediaminetetraacetic acid (EDTA) at an electrochemically pre-treated glassy carbon electrode. *Analyst*. 1985;110:1201–4.
10. Pozdniakova S, Ragauskas R, Dikcius A, Padarauskas A. Determination of EDTA in used fixing solutions by capillary electrophoresis. *Fres J Anal Chem*. 1999;363:124–5.
11. Sorvari J, Sillanpaa M, Sihvonen ML. Development of a gas chromatographic method for the simultaneous determination of trace amounts of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid in natural waters. *Analyst*. 1996;121:1335–9.
12. Nishikawa Y, Okumura T. Determination of nitrilotriacetic acid and ethylenediaminetetraacetic acid in environmental samples as their methyl ester derivatives by gas chromatography-mass spectrometry. *J Chromatog A*. 1995;690:109–18.