Clinica Chimica Acta, 195 (1990) 47–56
Elsevier

CCA 04898

Atomic absorption and UV-VIS absorption spectrophotometric determination of oxalate in urine by ligand exchange extraction

J.A. Muñoz Leyva, M.P. Hernández Artiga, M.M. Aragón Méndez and J.J. Quintana Pérez

Department of Analytical Chemistry, Faculty of Sciences, University of Cádiz, Cádiz (Spain)

(Received 2 October 1989; revision received 1 October 1990; accepted 5 October 1990)

Key words: Oxalate; Urine; Copper oxinate; Ligand exchange extraction; UV-VIS absorption spectrophotometry; Atomic absorption spectrophotometry

Summary

A new method for oxalate determination in urine is described. It neither requires precipitation nor previous extraction of oxalate and is free from the interferences often found with analyes of urine samples. The method is not time consuming and is suited to routine work.

Introduction

The determination of trace anion using ligand exchange extraction is an established technique [1]. The extraction occurs when an organic solution of a metallic complex is shaken with an aqueous solution containing the anion. Some anions such as oxalate, citrate, cianide, sulphide and carbonate have been determined previously by ligand exchange extraction followed by UV-VIS absorption spectrophotometry [2–6].

In this paper a new method for the determination of oxalate is proposed. Briefly copper, as oxinate in organic solution, is extracted by oxalate in aqueous solution with which the copper forms a compound which is soluble in the aqueous layer.

The indirect determination of oxalate is possible since copper(II) extraction to the aqueous phase provokes organic layer decolorisation. The signal decrease produced

Correspondence to: J.A. Muñoz Leyva, Department of Analytical Chemistry, Faculty of Sciences, University of Cádiz, Apdo. 40, Puerto Real, 11500 Cádiz, Spain.

in the organic layer is measured by UV-VIS absorption spectrophotometry (UVAS) and by atomic absorption spectrophotometry (AAS).

We now report the behaviour of Cu(II) oxinate in several organic solvents, the variables which influence the extraction process in order to choose the best conditions to determine oxalate and the application of the method to urine samples.

Materials and methods

Instrumentation

A Pye-Unicam SP-9 atomic absorption spectrometer with flame, a Perkin-Elmer 575 spectrophotometer and a Metrhom E-516 pH meter with a combined glass-calomel electrode, The instrumental conditions for AAS and UVAS are described in Table I.

Reagents

Copper(II) oxinate ($CuOx_2$) in the solid state was prepared according to the gravimetric method [7]. Solutions of this product were prepared in methyl isobutyl ketone, *n*-butyl acetate or isoamyl alcohol (in the latter case the spectrophotometric absorbance necessarily has to be between 0.600 and 0.750).

All chemicals used were of analytical reagent grade.

Procedures

1. In order to study the behaviour of aqueous solutions of oxalate when they are shaken with $CuOx_2$ in isoamyl alcohol, the following procedure was used. A portion

TABLE I

Instrumental conditions for AAS and UVAS

	AAS
Lamp	Cu hollow catnode lamp
Lamp current	4 mA
Analytical line	324.8 nm
Spectral bandwidth	0.5 nm
Integration time	2 s
Flame	stoichiometric air/acetylene flame
Zero set	I.A.
	UVAS
Wavelength	395 nm
Cells	1.0 cm glass cell

of a 5.68×10^{-4} mol/l oxalate solution (1–30 ml) was added to 2 ml of 1 mol/l acetic acid-sodium acetate buffer solution (pH 4.55), diluted to 50 ml with distilled water and shaken for 3 min in a separatory funnel with 10 ml of copper(II) oxinate (concentration is about 9.1×10^{-5} mol/l in isoamyl alcohol). After 20 min, to allow phase separation, the aqueous layer was discarded, the organic phase dried with anhydrous sodium sulfate and transferred into stoppered glass tubes. A blank was prepared in the same way, but without oxalate. The absorbances of the organic layers were measured by UVAS and by AAS with the instrumental setting described in Table 1.

2. To determine oxalate amount in real urine, urine samples were collected over 24 h, diluted with distilled water ten times and their pH values adjusted to approximately 4.5 with $HClO_4$ 10^{-2} mol/l. To five portions, each of 40 ml, of this solution were added 1, 2, 3, 4 and 5 ml respectively of 5.68×10^{-4} mol/l oxalate solution, 2 ml of buffer solution (1 M acetic acid–acetate, pH 4.55) and diluted to 50 ml with distilled water. Continue as described in procedure (1) allowing longer times for complete phase separation (about 30 min) due to the formation of froth. Plot the absorbance differences versus oxalate concentrations added and calculate the origin ordinate (Or.Or.). The oxalate concentration in the urine sample is obtained by the expressions:

$$c(\text{molar}) = \frac{\text{Or.Or.}-0.022}{0.124} \times 1.14 \times 10^{-4} \text{ for UVAS}$$

$$c(\text{molar}) = \frac{\text{Or.Or.}-0.026}{0.125} \times 1.14 \times 10^{-4} \text{ for AAS.}$$

Results

Behaviour of Cu(II) oxinate in organic solvents

Cu(II) oxinate spectra showed a maximum at 415 nm in butyl acetate (BA) and in methylisobutylketone (MIBK) and at 395 nm in isoamyl alcohol (IA) when they were recorded immediately after being prepared. $CuOx_2$ is not stable in these solvents. Its absorbance measured by UVAS or by A.A.S. is slowly increasing. The solutions in BA and in IA were stabilized heating them in a hot water bath for a few minutes, remaining stable at least for a week when they were kept in the darkness.

The behaviour of $CuOx_2$ in the three solvents when it is extracted into aqueous solution at different pH is shown in Fig. 1. $CuOx_2$ in BA is extracted at $pH < 5$, in MIBK at $pH < 7$ and in IA at $pH < 4.5$.

Oxalate determination by UVAS and A.A.S.

The samples were prepared as follows: 10 ml of $CuOx_2$ in I.A. 9.1×10^{-5} mol/l are manually shaken for 3 minutes with the aqueous solution containing oxalate. After phase separation, the organic layer is dried with Na_2SO_4 anhydrous and measured by UVAS and A.A.S.

The influence of the oxalate aqueous solution pH was determined adjusting it with

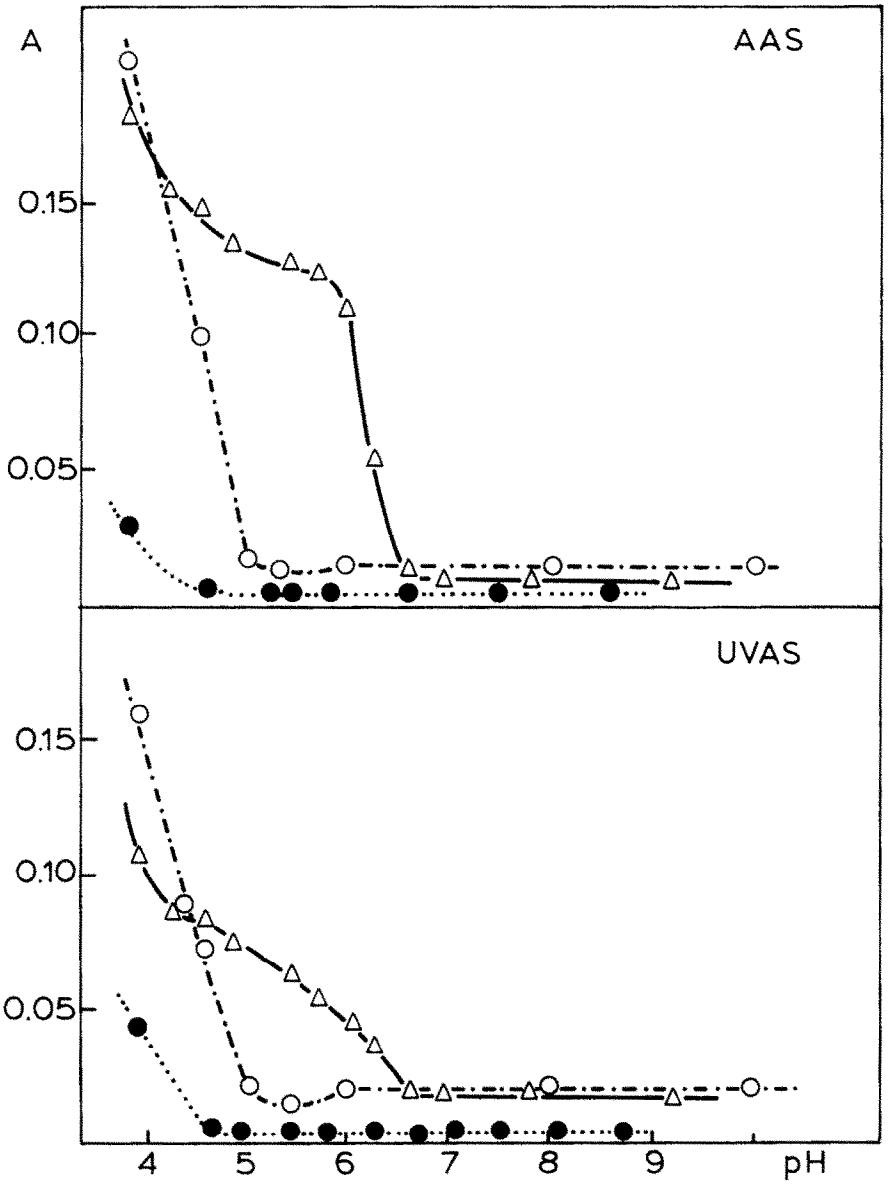

Fig. 1. Influence of the aqueous phase pH on the extraction. (△) MIBK, (●) IA and (○) BA. A: difference of absorbance between the $CuOx_2$ (blank) and the samples.

$HClO_4$ or NaOH to avoid possible interferences from other ions. The results are shown in Fig. 2.

Several shaking times, carried out manually, and several periods for phase separation, were tested. The results are found in Table II. Also, in this table is shown the effect of the volume ratio V_{aq}/V_{org} and the influence of the $CuOx_2$ concentration in I.A. used for extraction.

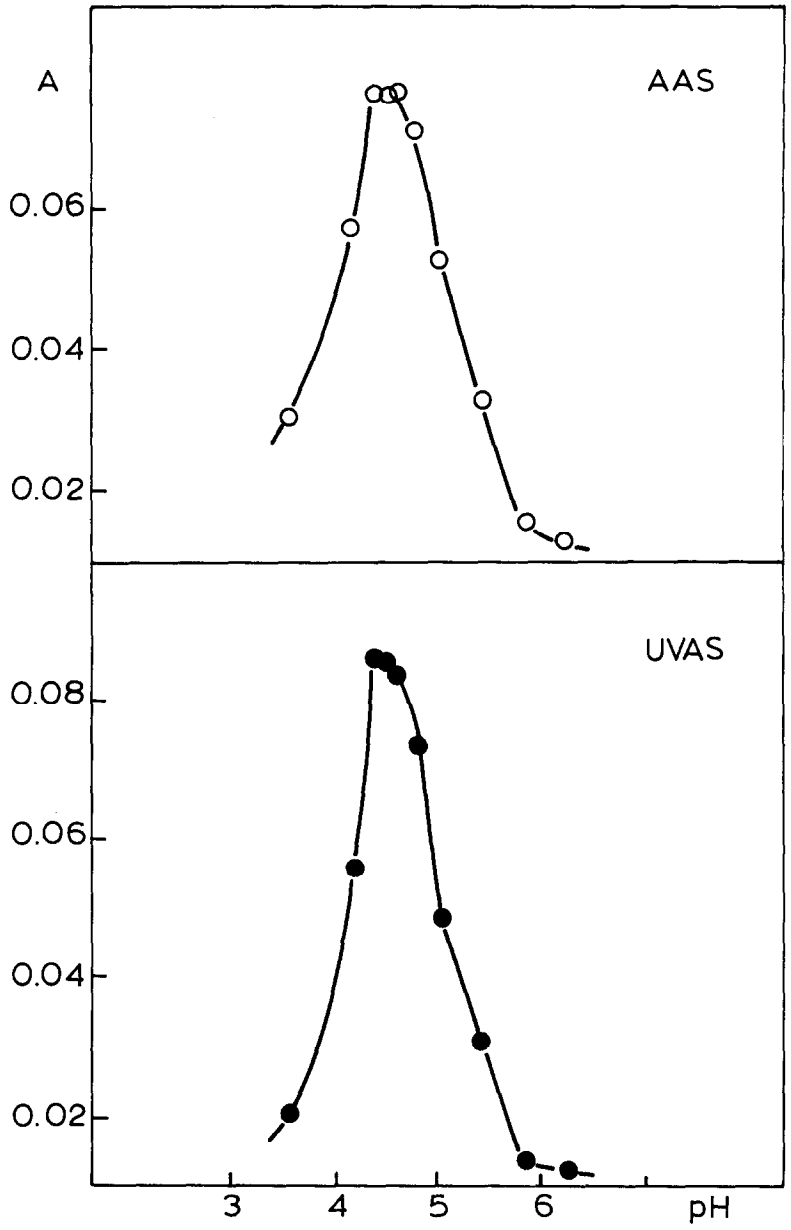

Fig. 2. Influence of the pH in the extraction with oxalate aqueous solutions from Cu(Ox)2 in IA. A. difference of absorbance between the $CuOx_2$ (blank) and the samples.

Calibration graphs and reproducibility

The samples were prepared as described in the procedure with oxalate concentrations from 0.64 ppm (7.3×10^{-6} mol/l) to 30,7 ppm (3.5×10^{-4} mol/l). The organic phase was measured by UVAS at 395 nm and by A.A.S. with Cu lamp. The results

TABLE II

Influence of several variables in oxalate determination* at pH 4.55

Shaking time[a]			Period for phase separation[b]	
Min		*A*	Time (min)	*A*
1		0.078	1	0.065
2		0.080	3	0.069
3		0.080	5	0.071
4		0.081	15	0.098
5		0.082	20	0.098

V_{aq}/V_0	A	mol/l	A0	A
20:1	Not enough volume	9.11×10^{-5}	0.592	0.080
	left	1.14×10^{-4}	0.740	0.079
10:1	0.068	1.32×10^{-4}	0.860	0.010
5:1	0.037	2.53×10^{-4}	1.645	–0.059

(*). A = Difference between blank absorbance (A_0) and sample absorbance. Other experimental conditions were:

(a) $[Ox_2Cu]_0 = 9.1 \times 10^{-5}$ mol/l; $[C_2O_4]_{aq} = 4.4 \times 10^{-5}$ mol/l; period for phase separation = 15 min; $V_{aq}/V_0 = 5:1$.

(b) $[Ox_2Cu]_0 = 6.7 \times 10^{-5}$ mol/l; $[C_2O_4]_{aq} = 8.8 \times 10^{-5}$ mol/l; $V_{aq}/V_0 = 5:1$; shaking time = 3 min.

(c) $[Ox_2Cu]_0 = 6.7 \times 10^{-5}$ mol/l; $[C_2O_4]_{aq} = 2.6 \times 10^{-5}$ mol/l; shaking time = 3 min.; period for phase separation = 15 min.

(d) $[C_2O_4]_{aq} = 4.4 \times 10^{-5}$ mol/l shaking time = 3 min; $V_{aq}/V_0 = 5:1$; period for phase separation = 15 min.

are shown in Fig. 3. The calibration graphs show that there are two different slopes, one from 1.13×10^{-5} mol/l to 1.13×10^{-4} mol/l and another from 1.36×10^{-4} mol/l to 3.39×10^{-4} mol/l. The least-squares equations which fit the calibration curves are:

$A = 1408c + 0.026$ for UVAS

$A = 1320c + 0.024$ for AAS

for the first concentration range, and

$A = 616c + 0.132$ for UVAS

$A = 528c + 0.136$ for AAS

for the second concentration range. The molar absorptivity was 1410 for the first concentration range and 640 $l \cdot mol^{-1} \cdot cm^{-1}$ for the other one. The sensitivity of the A.A.S. measures was 3.4×10^{-6} mol/l and 7.9×10^{-6} mol/l, respectively.

The reproducibility of the method was studied for 11 determinations with 4.4×10^{-5} mol/l of oxalate; the samples were prepared as described in the procedure. The statistical values setting the confidence level at 95% are: standard deviation 5.4×10^{-4} and 7.2×10^{-4}, and relative error (%) 1.1 and 1.6 for AAS and for UVAS, respectively.

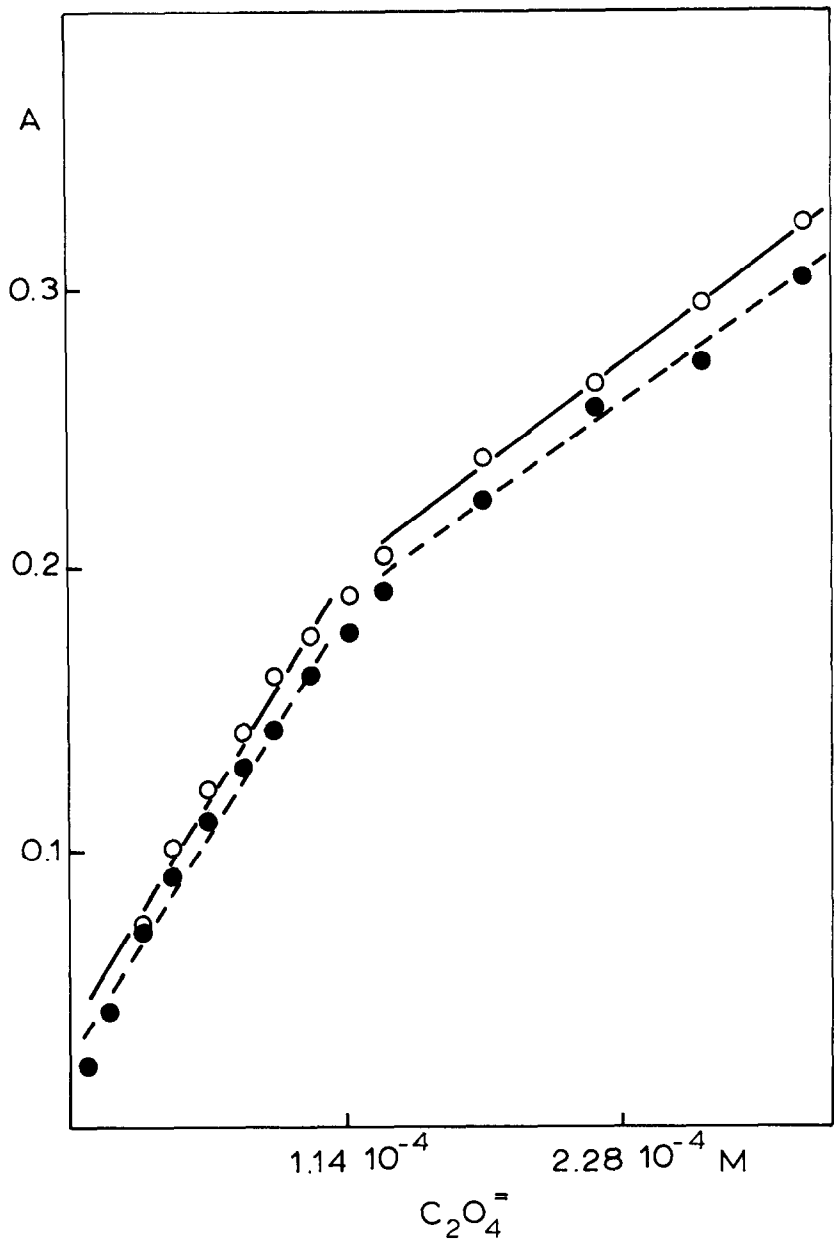

Fig. 3. Calibration curves for oxalate determination. (○) UVAS, (●) AAS.

Interferences

The samples were prepared as described in the procedure, with the interferent species five time diluted respect to the level that they usually have in urine (8), and 5.0 ppm of oxalate (5.7×10^{-5} mol/l) in the aqueous phase. The results are shown in Table III.

TABLE III

Influence of the species usually found in urine for 5 ppm of oxalate (5.7×10^{-5} mol/l).

Interferent	Conc.(ppm)*	error(%) MS	AA S
Organic compounds			
Albumin	10	+2.3	+0.9
DNA	2	−1.8	+ 1.8
Urea	4000	+3.5	−0.9
Uric acid	140	+0.9	+3.6
Creatinine	240	+7.9	+8.0
Creatine	16	+4.4	+6.3
Glucose	200	+3.5	+1.8
Bilirubin	0.5	+1.8	+5.8
β-Phenylpyruvic acid	0.5	0.0	−2.6
α-Ketobutyric acid	3	+1.9	−1.6
Histidine	2	+2.8	−1.6
Acetone	0.5	0.0	−4.1
β-Hydroxybutyric acid	3	0.0	+3.3
Nor-adrenalin	0.1	−3.7	−1.6
Dopamine	0.1	−1.9	−3.3
Acetylcholine	12	−3.7	+1.6
Serotonin	12	−1.9	−1.6
Imidazole-4-acetic acid	12	+2.8	+3.3
Glycine	0.5	+3.6	+8.9
Adrenalin	0.1	+7.2	−8.9
Inorganic Compounds			
Ammonium	140	+0.9	+5.4
Chloride	1300	0.0	+1.6
Potassium	500	−1.8	+3.4
Nitrate	60	+0.9	+1.7
Nitrite	20	−7.9	+3.4
Carbonate	60	−0.9	+2.6
Sulphate	20	+0.9	+4.3
Calcium	40	−6.2	−0.9
Cobalt(II)	1	−6.2	−11.2
Lead(II)	0.02	+1.8	−7.8
Iron(III)	1	−10.6	+28.4
Iodide	1	0.0	−8.6
Phosphate	200	−7.9	−14.6
Zinc	0.2	−2.6	−8.6
Magnesium	30	−28.3	−23.3

*The concentrations are five times smaller as usually found in urine because the urine samples are diluted ten times.

Oxalate determination in urine

With the aim of determining this species in real urine samples, as synthetic urine which contained all the components shown in Table III at the same concentrations was prepared. In order to determine oxalate in synthetic urine and afterwards in real samples, we obtained several calibration curves in synthetic urine. In one case this urine was diluted five times and in the other one ten times. The oxalate concentrations in both series are 2.26×10^{-5} mol/l, 4.52×10^{-5} mol/l, 6.78×10^{-5} mol/l, 9.04×10^{-5} mol/l and 1.13×10^{-4} mol/l.

The above mentioned series were repeated several times at 1:10 dilution. The results were quite similar and we have obtained two mean calibration curves which are:

$A = 1091c + 0.022$ for UVAS (a)

$A = 1100c + 0.026$ for AAS. (b)

The oxalate determination in real urine was carried out applying the multiple-addition method as described in the procedure using the calibration curves (a) and (b).

Discussion

Before carring out the extraction with oxalate some properties of the Cu(II) oxinate in organic solvents were studied, such as: absorption spectra of the metallic complex, stability and pH zone of the aqueous phase in which the complex remains in the organic phase after extraction. The organic solvents studied were: BA, MIBK and IA, all of them suitable to be used in flame atomic absorption. After this preliminary study IA was choosen, because reextraction by water occurs at lower pH, the metallic complex is unstable in MIBK and it shows low solubility in BA.

To carry out the oxalate determination based on the extraction of CuOx2 in isoamyl alcohol, several variables such as influence of the pH of the aqueous solution, phase volumen ratio, agitation time and phase separation time were established. As we can observe in the Fig. 2, the maximum amount of Cu(II) extracted takes place between pH 4.4 and 4.6. Therefore the buffer solution choosen was 1M acetic acid/sodium acetate at pH 4.55. Other optimal experimental conditions are shown in Table II.

The results show that the determination of oxalate presents little interferences. Therefore, it is confirmed the possibility to determine oxalate in real urine samples by this method. The least-squares equations which fit the two different slopes of the calibration graphs show the convenience of working with the first straight line in order to get more sensitivity.

The oxalate concentration in real urine samples applying the multiple-addition method can be calculated from the equations obtained with synthetic urine samples due to the similar slopes of the calibration graphs in both cases. The oxalate concentration was calculated from the equations (a) and (b) as follows:

$0.114 = 1091c + 0.022$; $c = 8.1 \times 10^{-5}$ mol/l

$0.098 = 1100c + 0.026$; $c = 6.5 \times 10^{-5}$ mol/l

since the samples were diluted ten times the oxalate concentration in this urine sam-

ple is 8.1×10^{-4} mol/l measured by UVAS and 6.5×10^{-4} mol/l measured by AAS.

Comparing the results obtained in the oxalate determination in natural urine by UVAS with the ones obtained by AAS, led us to the conclusion to propose the AAS as a more reliable technique since it is more selective than UVAS (it can be deduced in this case from the calibration curves for natural urine) and the results are closer to the normal values described in the literature.

Acknowledgement

The authors are grateful to CAICYT (Project PA85/264) for financial support.

References

1 Valcarcel M. y Silva M. Teoria práctica de la extracción líquido–líquido, Madrid: Alhambra, 1984; 223–225.

2 Roman Ceba M, Muñoz Leyva JA, Vinagre Jara F. Spectrophotometric determination of anions by reextraction through the exchange of ligands: the determination of traces of oxalate using Ferric indol-2-carboxylate in isoamyl alcohol. Microchem J 1980;25:443–457.

3 Ibid, Determinación espectrofotométrica de aniones mediante reextracción por intercambio de ligandos. II. Determinación de trazas de citrato usando indol-2-carboxilato férrico en alcohol isoamílico. An Quim 1981;77:94–97.

4 Ibid, Spectrophotometric determination of sulphide in water by reextraction and ligand exchange using chloroform solutions of copper quinolin-8-olate, Analyst 1982;107:781–786.

5 Ibid, Preliminary study of nickel, ferric, lead and copper oxinates in reactions of reextraction through the exchange of ligand: The use of chloroform solutions of the copper oxinate in the spectrophotometric determination of cyanide, Microchem J 1982;27:265–275.

6 Galindo Riaño MD, Garcia Vargas M, Muñoz Leyva JA. Simple spectrophotometric determination of total carbonate by reextraction via ligand exchange using chloroform solutions on uranyl quinolin-8-olate, Anal Lett 1988;21:641–652.

7 Erdey L. Gravimetric analysis, Part 2, First ed. Oxford: Pergamon Press, 1965.

8 Urianálisis moderno. Miles Lab. S.A.E. 4ª ed. Madrid, 1981.