ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 97–105

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of lignocaine and amprolium in pharmaceutical formulations using AAS

W.F. El-Hawary

Chemistry Department, Faculty of Science, Cairo University Giza, Egypt

Received 30 March 2001; received in revised form 16 May 2001; accepted 29 May 2001

Abstract

The ion-associate complexes of lignocaine hydrochloride (Lig.Cl) with ammonium reineckate (Rk) or sodium cobaltithiocyanate, and that of amprolium hydrochloride (Amp.Cl) with ammonium reineckate, have been prepared. The precipitated ion-associates were subjected to elemental analyses, infrared and nuclear magnetic resonance spectroscopy and determination of the metal content for elucidation of their structures. The solubilities of the solid ion-associate complexes have been studied and their solubility products were determined at different temperatures at the optimum pH for their quantitative precipitation. The thermodynamic parameters ΔH, ΔG and ΔS for the dissolution of the ion-associate complexes were calculated. These ion-associate complexes have been used for the quantitative determination of the above mentioned drugs by precipitating them with an excess of the inorganic metal complex ions and determining the excess metal complex ions using atomic absorption spectrometry. The method was applied for the determination of the above drugs in pure solution and pharmaceutical preparations. 0.135–135.4 and 0.158–157.6 mg of lignocaine and amprolium, respectively, can be determined with mean relative standard deviations (R.S.D.) 0.92–1.20% and recovery values of 99.18 ± 0.48 to 100.12 ± 0.34% indicating high precision and accuracy.

Keywords: Ion-associate complexes; Lignocaine; Amprolium; Atomic absorption spectrometry; Pharmaceutical analysis

1. Introduction

The investigated drugs are important pharmaceutical compounds. Lignocaine hydrochloride (Lig.Cl) is a local anaesthetic of the amide type and is widely used by injection and for local applications to mucous membranes [1]. Amprolium hydrochloride ($Amp.Cl_2$) is an antiprotozoal agent used in veterinary practice for the control of coccidiosis in poultry [1].

The pharmacopoeial methods for determination of both lignocaine and amprolium depend on high performance liquid chromatography (HPLC) technique [2]. Several methods, also, have been reported for determination of lignocaine; they include titrimetry [3,4], spectrophotometry [5–7], HPLC [8–10], TLC [11], GC [12–15] and potentiometry using ion selective electrodes [16–18]. Various techniques have been, also, used for determination of amprolium; including gravimetry [19], spectrophotometry [20–22], HPLC [23–25], GC [26], TLC [27]and potentiometry using ion selective electrode [28].

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00514-3

Although some atomic absorption spectrometric methods (AAS) have been reported for determination of lignocaine [29–31], yet, most of them need extraction prior to measurement. Also, this technique has not been applied for the determination of amprolium. The present work presents a new atomic absorption method for the determination of lignocaine and amprolium. Atomic spectrometry is characterized by high sensitivity and accuracy enabling determination of the drugs with simple and rapid method. The method is free from interference by excipients present in the drug formulations. The determination is based on the precipitation of the ion-associates of the drugs with an excess of reineckate (Rk) or cobaltithiocyanate complexes, then measuring the equilibrium concentration of the excess metal ion complexes in the supernatant solution using AAS. This method of analysis depends on the formation of sparingly soluble ion-associates containing the studied drugs and the inorganic metal complex ion. So, it is worthy to study and determine the solubility of such solid ion-associates, as the accuracy of the determination depends mainly on their low solubility.

2. Experimental

2.1. Materials and reagents

All reagents used were of analytical grade. Lignocaine hydrochloride ($C_{14}H_{23}N_2OCl$, MW = 270.8) and amprolium hydrochloride ($C_{14}H_{20}N_4Cl_2$, MW = 315.2) were supplied by ADWIC (Egypt). Ammonium reineckate, cobalt sulphate, ammonium thiocyanate, sodium chloride, methyl parapen, propyl parapen, sucrose, glucose and lactose were Aldrich products. The pharmaceutical preparations assayed were purchased from local market. They include Xylocaine 2% injection solution (containing 2% (w/v) lignocaine hydrochloride, sodium chloride, methyl parapen, propyl parapen and sterilized water) produced by Astra (Sweden); Lignocaine cream (containing 5% (w/w) lignocaine hydrochloride in water washable base) produced by NILE Co. for Pharm. and Chem. Industries (Egypt); and amprolium 20% soluble powder (containing 20% (w/w) amprolium hydrochloride in sucrose, glucose or lactose) for veterinary use produced by El-Nasr Pharm. Chem. Co. (Egypt). The water was always twice distilled from all glass equipments.

2.2. Preparation of the ion-associate complexes

Lignocaine–cobaltithiocyante ion-associate (2:1) was prepared by mixing an aqueous solution containing 0.1 mole of Co(II) ion with an equal volume of a solution containing 0.4 mole of ammonium thiocyanate, then adding the formed cobaltithiocyanate complex to a solution containing 0.2 mole of lignocaine hydrochloride. Lignocaine–reineckate ion-pair (1:1) and amprolium–reineckate ion-associate (1:2) were prepared by mixing one volume of a solution containing 0.1 mole reineckate with an equal volume of a solution containing 0.1 mole lignocaine hydrochloride, or 0.05 mole amprolium hydrochloride, respectively. The formed precipitates were filtered, washed with distilled water till free from the soluble ions that may be leached (free from chloride ion), and dried at room temperature. To elucidate the formation and structure of the ion-associate complexes, the products were subjected to elemental analysis at the Microanalytical Center of Cairo University, infrared and NMR spectroscopy and determination of the metal ion content.

2.3. Effect of pH on the solubility of ion-associates

The suitable pH values at which the ion-associates exhibit the lowest solubility were determined by saturating a series of solutions of different pH values, ranging from 1 to 10 (adjusted with HCl or NaOH), with the solid ion-associates. The solutions were left to stand for a week, with occasional shaking, to attain a stable equilibrium. Thereafter, the saturated solutions were filtered in dry beakers, and the equilibrium concentrations of the metal ions, present in the form of soluble inorganic complex ions, were measured using AAS. The solubility and solubility product of the ion-associates were calculated, from the concentration of the metal ions.

2.4. Effect of temperature

The effect of temperature on the solubility of the ion-associates was studied by saturating a series of solutions of different temperature, at the optimum pH, with the solid ion-associates. The metal ion content, present in the form of soluble complex ion, was measured using AAS, and hence the solubility and solubility product were calculated at different temperatures.

2.5. Preparation of standard solutions

Standard solutions containing Co or Cr ions were prepared by weighing 1.000 g of highly pure Cr-metal or Co-powder (Aldrich), dissolving in concentrated HNO_3 and completing to 1 l with distilled water. The 1000 μg ml^{-1} solutions were stored in plastic bottles, which have been pre-soaked in diluted HNO_3. The solutions are stable for approximately 1 year.

Aqueous solutions containing 0.1 M ammonium reineckate or 0.1 M cobaltithiocyanate complexes were always freshly prepared and standardized using AAS. 0.1 M solutions of the investigated drugs were prepared and standardized [17,28]. The solutions were stored in dark bottles. Dilute solutions were prepared by accurate dilution.

2.6. Apparatus

The IR-absorption spectra were obtained by applying the KBr-disc technique using Shimadzu FTIR-8201 PC Fourier Transform Infrared Spectrophotometer. The proton nuclear magnetic resonance spectra (^1H NMR) were scanned using Varian Gemini 200, 200 MHz (H-NMR) spectrometer, the solvent used was DMSO, using TMS as internal standard. The pH values of the solutions were measured using CHEMITRIX type-62 digital pH-meter. The determination of metal ion content was carried out using Perkin–Elmer 2380-AAS. Air–acetylene was used as a fuel under the recommended conditions [32] (Co and Cr were measured at 240.7 and 357.9 nm with slit width of 0.2 and 0.7 nm, respectively). Calibration graphs were constructed using standard ammonium cobaltithiocyanate or ammonium reineckate complexes, by performing triplicate measurements using solutions containing 0, 10, 20, 30 and 50 μg ml^{-1} of the respective inorganic complex ion. The calibration graphs are straight lines passing by the origin.

2.7. Procedures for determination of the drugs

Aliquots (0.1–10 ml) of 0.01 M solution of the drug were transferred to a 25 ml measuring flask. To each flask, a known excess (1–5 ml) of 0.1 M solution of the inorganic complex ion (ammonium reineckate or cobaltithiocyanate) was added, then completed to the mark with distilled water. The solutions were shaken well, left to stand for 30 min, to assure complete precipitation, and filtered. 1 ml of the filtrate was diluted to 10 or 25 ml with distilled water, then the metal ion concentration was measured using AAS. The excess metal complex ion was determined; from which the consumed metal complex in the formation of the ion-associate was calculated and the drug concentration was, thus, determined indirectly.

For analysis of the drugs in the pharmaceutical formulations, aliquots equivalent to (0.20–100.0 mg) of lignocaine hydrochloride or amprolium hydrochloride were transferred quantitatively and dissolved in 100-ml measuring flask and treated as previously described.

3. Results and discussion

3.1. Preparation and characterization of the ion-associates

The ion-associates were prepared as described in the experimental part and subjected to elemental analysis, infrared and nuclear magnetic resonance spectroscopy, for elucidation of their structure.

The metal content of the ion-associates was determined by AAS after their digestion with concentrated nitric acid. A calibration curve was constructed for each metal ion using standard solution of the metal ion. The results of elemental analysis for C, H, N, S and metal content are in

good agreement with those required by the suggested formulae, i.e. formation of 1:1 Lig–Rk ion-pair, 1:2 Amp–Rk and 2:1 Lig–$Co(SCN)_4$ ion-associates.

Table 1 lists the important IR-bands of the drugs under investigation, together with their assignment. Beside these bands, a new strong band was observed in the spectra of the ion-associates at 2073–2083 cm^{-1} region. The appearance of this band, which is assigned to ν_{NCS} of the ion-associate, can be taken as an evidence for the formation of the ion-associates.

A substantial support for the structure of the ion-associates is gained by considering the NMR-spectra of some ion-associates. The NMR spectrum of Lig–$Co(SCN)_4$ ion associate shows a triplet at 1.09–1.30 ppm assigned to the aliphatic CH_3 group (Table 1). The integrated area of this signal is equivalent to 12 protons. The signal of the aromatic CH_3 is shown at 2.12 ppm, with integrated area equivalent to, also, 12 protons. The aliphatic CH_2 attached to methyl group shows a quartet at 3.09–3.16 ppm, with integrated area equivalent to eight protons. Also, the CH_2 attached to both nitrogen and carbonyl groups shows a signal at 4.1 ppm equivalent to four protons. The integrated curve shows six CH-aromatic protons at 7.1 ppm and two NH protons at 9.4 ppm. The results obtained from the NMR-spectrum indicate the presence of two Lig^+ ions within the structure of the ion-associate, and confirm the formation of 2 Lig:1 $Co(SCN)_4$ ion-associate, which is consistent with the results obtained by elemental analysis.

3.2. Solubility of the ion-associates

The choice of a suitable pH value at which the ion-associate exhibits the lowest solubility is of prime importance in the use of such compounds in quantitative analysis. To determine this pH value, the solubility and solubility product of the compounds were determined at 25 °C in solutions of varying pH values (pH 1–10). It was found that, for all the studied ion-associates, by increasing the pH value of the medium, the solubility of the ion-associates decreased slightly until pH 4–5, then it remained constant from pH 5 to 9. Above pH 9, the solubility of the ion-associates containing reineckate anion increases again, while that of the Lig–$Co(SCN)_4$ decreases. This can be explained by considering the solubility equilibrium of the ion-associate.

$$Lig\text{–}Rk \rightleftharpoons Lig^+ + Rk^-$$

$$Lig_2[Co(SCN)_4] \rightleftharpoons 2Lig^+ + [Co(SCN)_4]^{2-}$$

Table 1
Assignment of the most important IR-bands of the investigated drugs and NMR-signals of lignocaine–cobaltithiocyanate ion-associate

IR-bands (cm^{-1})			NMR of Lig–$Co(SCN)_4$		
Lig.Cl	$Amp.Cl_2$	Band-assignment	Number of protons	Chemical shift (ppm)	Assignment
3386	–	ν_{NH}	12	1.09–1.30	Aliph. CH_3
2976	2980	ν_{CH} asym.	12	2.12	Arom. CH_3
2922	2920	ν_{CH} symm.	8	3.09–3.16	Aliph. CH_2
2618	2617	$\nu_{NH_2^+}$	4	4.10	CH_2 group
–	2362	ν_{NH^+}	6	7.10	CH-aromatic
1672	–	$\nu_{C=O}$	2	9.40	NH group
1655	1645	$\nu_{C=C}$			
1545	1529	$\nu_{C=C}$			
1477	1450	$\nu_{C=C}$			
–	1494	$\nu_{C=N}$			
–	1373	δ_{NH_2}			
1271	1305	ν_{C-N} arom.			
1197	–	ν_{C-N} aliph.			

Table 2
Effect of temperature on the solubility of lignocaine hydrochloride and amprolium hydrochloride ion-associates

T (K)	Ammonium reineckate					Sodium cobaltithiocyanate				
	pS	pK_{SP}	ΔG	ΔS	ΔH	pS	pK_{SP}	ΔG	$-\Delta S$	ΔH
Lignocaine hydrochloride										
293	3.50	7.00	39.26	13.04	43.081	3.07	8.62	48.35	90.34	21.88
298	3.45	6.90	39.36	12.48		3.05	8.56	48.83	90.44	
303	3.38	6.76	39.21	12.77		3.03	8.49	49.25	90.33	
308	3.33	6.66	39.27	12.37		3.02	8.45	49.82	90.71	
313	3.26	6.51	39.01	13.00		2.99	8.38	50.21	90.51	
318	3.19	6.38	38.84	13.33		2.97	8.31	50.59	90.28	
323	3.15	6.30	38.96	12.76		2.96	8.28	51.20	90.77	
328	3.10	6.20	38.93	12.65		2.94	8.22	51.61	90.64	
333	3.04	6.07	38.70	13.15		2.92	8.17	52.08	90.69	
338	3.00	6.00	38.82	12.60		2.90	8.10	52.41	90.33	
Amprolium hydrochloride										
293	3.93	11.19	62.77	111.53	95.74					
298	3.85	10.95	62.47	111.64						
303	3.75	10.65	61.78	112.08						
308	3.67	10.41	61.38	111.56						
313	3.56	10.08	60.40	112.91						
318	3.46	9.78	59.54	113.84						
323	3.42	9.65	59.67	111.67						
328	3.33	9.39	58.96	112.13						
333	3.25	9.15	58.33	112.34						
338	3.18	8.94	57.85	112.10						

pS, $-$log of solubility; pK_{SP}, $-$log of solubility product; ΔG (kJ mole^{-1}); ΔS (J K^{-1} mole^{-1}); ΔH (kJ mole^{-1}).

In acid medium, the hydrogen ions may react with the complex anion to dissociate it, while in basic medium, the hydroxyl ions may react with the drug ion. Thus, the equilibrium is shifted toward the direction of increasing solubility. In case of ion-associates containing $[Co(SCN)_4]^{2-}$, the decrease in solubility above pH 9 may be attributed to the hydrolysis of $[Co(SCN)_4]^{2-}$. However, it is to be mentioned that the effect of pH is rather weak, and the present study can be applied safely over a wide range of pH values.

The solubility and solubility product of the ion-associates were determined at different temperatures (25–65 °C). The results (Table 2) show that by increasing the temperature, the solubility increases indicating that the process of dissolution of the ion-associates is endothermic because the lattice energy is usually greater than the solvation energy. The heat of solution of the ion-associates was calculated by applying the Van't Hoff isochore relation, thus

$$pK_{SP} = \frac{\Delta H}{2.303RT} + \text{constant}$$

where, K_{SP} is the solubility product of the ion-associate; ΔH is heat of solution (kJ mole^{-1}); R is universal gas constant (8.31 J mole^{-1} K^{-1}); and T is absolute temperature.

Thus, a plot of pK_{SP} against $1/T$ is a straight line with a slope equals to ($\Delta H/2.303R$), from which ΔH can be calculated.

Also, the Gibb's free energy change (ΔG) and the entropy change (ΔS) of the dissolution were calculated at different temperatures using the following equations.

$$\Delta G = -RT \ln K_{SP}$$

$$\Delta G = \Delta H - T\Delta S$$

The values of the solubility (S), solubility product (K_{SP}), ΔG and ΔS at different temperatures, and ΔH have been calculated and listed in Table 2. The free energy change (ΔG) increases

with decreasing the solubility of the Lig–Rk and Amp–Rk ion-associates, while it increases with increasing the solubility of the Lig–$Co(SCN)_4$ ion–associate. In the latter case the entropy changes (ΔS) have negative values.

3.3. Determination of the drugs in pure solutions and pharmaceutical preparations

Lignocaine and amprolium were determined precisely and accurately in pure solutions by applying the proposed method. Calibration curves were constructed using standard ammonium cobaltithiocyanate or ammonium reineckate solutions by plotting their concentrations (in $\mu g\ ml^{-1}$) versus absorbance. The calibration curves are straight lines passing by the origin having slopes of 0.025 and 0.045, respectively. From these curves, the concentration of the excess metal complex ion was determined; from which the drug concentration was determined indirectly. The results given in Table 3 reveal that the recoveries are 100 ± 0.34 and $99.65 \pm 0.32\%$ in case of lignocaine using reineckate and cobaltithiocyanate, respectively; and the recovery for determination of amprolium using reineckate is $99.74 \pm 0.34\%$. In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression of the observed drug concentration against the theoretically taken values was calculated. The slope of the regression lines amounts to 0.994–0.997 and the intercepts are in the range -5.71×10^{-3} to 3.62×10^{-3}. The correlation coefficients are always 0.999 indicating that there is no systematic difference between the determined and true concentrations over a wide range. Also, the Student's t-test was applied. The calculated t-values range from 2.06 to 3.64, which are lower than the tabulated value at 99.9% confidence level and eight degrees of freedom (5.04). All the above results reflect the high accuracy of the proposed method.

The reproducibility of the method is shown by calculating the relative standard deviation (R.S.D.). The very low values of R.S.D., ranging from 0.92 to 0.98%, indicate the precision of the present method.

The F-test was applied to indicate whether there is a significant difference between the proposed method and the official ones [2,7,22]. The F-values were found to be in the range 2.88–3.55, which are lower than the tabulated value at 95% confidence level and four degrees of freedom in the numerator and denominator (6.39). This means that there is no significant difference between the proposed method and the official ones.

The validity of the method for application for real substances was tested by using it for determination of the cited drugs in their pharmaceutical preparations. Preliminary experiments show that the excipients, which are present in the pharmaceutical preparations (sodium chloride, methyl parapen, propyl parapen, sucrose, glucose and lactose) do not interfere with the determination of the drugs, as there is no interaction between them and ammonium reineckate or ammonium cobaltithiocyanate.

The results of determination of lignocaine in Xylocaine 2% injection solution and Lignocaine cream 5% and determination of amprolium in 20% amprolium powder for veterinary use, are given in Table 3. The results indicate good recoveries (99.18 ± 0.48 to $99.61 \pm 0.36\%$), reflecting the high accuracy of the determination, in addition to the precision indicated by the very low value of R.S.D. (0.88–1.2%). The excipients of the formulations did not show any effect on the determination, which reflect the high selectivity of the method. Also, the sensitivity of the method can be revealed by the very low concentration, which can be determined by this method (as low as 0.2 mg ml^{-1} of the drug can be determined with fair accuracy).

4. Conclusion

Finally, it can be concluded that the proposed method can be applied satisfactorily for the determination of lignocaine and amprolium in pure solutions and in their pharmaceutical preparations. Amprolium can be determined with fair accuracy after precipitation as $Amp(Rk)_2$. Lignocaine can be determined accurately after precipitation either as Lig–Rk or $Lig_2[Co(SCN)_4]$.

Table 3
Determination of the investigated drugs in pure solutions and pharmaceutical preparations and statistical treatment of the results

Sample	Taken (mg)	Recovery[a] (%)	R.S.D. (%)	Slope of regression line[b]	Intercept of regression line	Correlation coefficient of regression line	t-Value (5.04)[c]	F-value (6.39)[d]
Lignocaine–reineckate ion-pair								
Pure sample	0.135–135.4	100.12 ± 0.34	0.98	0.997	3.62×10^{-3}	0.999	2.06	3.25
Xylocaine injection	0.200–100.0	99.21 ± 0.36	1.12					
Lig. Cream	0.500–100.0	99.4 ± 0.52	1.20					
Lignocaine–cobaltithiocyanate ion-associate								
Pure Sample	1.354–135.4	99.65 ± 0.32	0.92	0.994	-5.71×10^{-3}	0.999	3.64	3.55
Xylocaine injection	2.00–100.0	99.61 ± 0.36	0.98					
Lig. cream	5.00–100.0	99.58 ± 0.28	0.88					
Amprolium–reineckate ion-associate								
Pure sample	0.158–157.6	99.74 ± 0.34	0.96	0.996	-2.26×10^{-3}	0.999	2.90	2.88
Amp. powder	0.200–100.0	99.18 ± 0.48	1.18					

[a] Mean recovery of five determinations.
[b] Determined mg vs. theoretically taken mg.
[c] t-Tabulated at 99.9% confidence level and eight degrees of freedom.
[d] F-tabulated at 95% confidence level and four degrees of freedom in the numerator and denominator.

However, the precipitation as Lig–Rk is superior over that as $Lig_2[Co(SCN)_4]$, as the former has a much lower solubility and, so, a lower concentration can be determined.

The presented methods for determination of lignocaine and amprolium are simpler than the official methods [2], which depends on use of liquid chromatography. The liquid chromatography is non-available technique in all chemical analysis laboratories. The official methods involve usage of many reagents and several preparations. Also, a pure drug samples should be available for preparation of standards. As low as 2.0 and 0.5 mg ml^{-1} of lignocaine and amprolium, respectively, can be determined with the official methods; while the presented methods can be used for determination of as low as 0.2 mg ml^{-1} of both drugs. The R.S.D. of the presented methods are comparable with that of the official ones (1.5 and 1.0% in case of lignocaine and amprolium, respectively).

It is convenient to compare the presented methods with the published spectral methods. Most of the published spectrophotometric methods for determination of lignocaine [6,7] use non-aqueous solvents and need extraction prior to the determination. Also, the produced colored compounds are non-stable over 3 h, or need long time for developing their maximum color intensity [7]. The narrow concentration range, which can be determined by these methods (up to 0.40 mg ml^{-1} [6] and 0.9–10.0 mg [7], compared with the presented method 0.2–135.4 mg) is another disadvantage. The AAS method used by El-Ries [29] for determination of lignocaine is very sensitive (1–10 μg ml^{-1} can be determined), but it is subjected to many interfernces. The AAS methods of Lei [30] and Nerin [31] need extraction with non-aqueous solvents and measuring the absorbance in the organic phases, which need special precautions.

All the spectrophotometric methods used for determination of amprolium [20,22] need extraction with organic solvents. The method of Severijnen [22] involves long time pretreatment, and narrow concentration range can be determined (0.5–2.0 mg of amprolium). Although the method developed by Shoukry [20] is sensitive, but the stability of the colored products is low (about 6 h.), as well as it requires extraction of the products with chloroform. There are no AAS-methods found in literature for determination of amprolium.

Comparing these methods with the presented ones reveals that, although the presented methods are more time consuming than some other methods, yet they exhibit fair sensitivity and accuracy, and they are very simple methods for determination of both of lignocaine and amprolium. Moreover, they have no interferences from the excipients present in the drug formulations, thus no extraction is needed, and the reproducibility of the results is superior to that obtained from other methods.

References

[1] J.E.F. Reynolds (Ed.), Martindale. The Extra Pharmacopoeia, The Pharmaceutical Press, London, 1993, pp. 510, 1010.
[2] United State Pharmacopoea, XXIII, US Pharmacopeial Convention, Inc., MD, 1995, pp. 113, 887.
[3] G.X. Li, R.Q. Zheng, W.X. Shi, C.M. Dong, Yaowu Fenxi Zazhi 14 (1994) 59 [Anal. Abstr. 56 (1994) 10G115].
[4] A.A. Abou-Ouf, M.I. Walash, M. Rizk, F. Belal, J. Drug Res. 11 (1981) 81.
[5] M.I. Arufe-Martinez, J.L. Romero-Palanco, J. Gamero-Lucas, M.A. Vizcaya-Rojas, J. Anal. Toxicol. 13 (1989) 337.
[6] J. Lemli, I. Knockaert, Pharm. Weekbl. Sci. Ed. 5 (1983) 124.
[7] M.S. Rizk, Y.M. Issa, A.F. Shoukry, E.M. Atia, Anal. Lett. 30 (15) (1997) 2743.
[8] Y. Kohda, Y. Kakiuchi, M. Miyabe, S. Sato, H. Toyooka, E. Sagara, J. Appl. Ther. Res. 2 (1) (1998) 33.
[9] M. Parissi-Poulou, I. Panderi, J. Liq. Chromatogr. Relat. Technol. 22 (7) (1999) 1055.
[10] L. Kang, H.W. Jun, J.W. McCall, J. Pharm. Biomed. Anal. 19 (5) (1999) 737.
[11] L. Zivanovic, D. Zivanov-Stakic, D. Radulovic, J. Pharm. Biomed. Anal. 6 (1988) 809.
[12] B. Vetter, R. Benecke, D. Olthoff, Pharmazie 43 (1988) 553.
[13] M. Gulea, N. Palibroda, Z. Moldovan, A.D. Abraham, Chromatographia 28 (1989) 24.
[14] E.H.M. Koster, N.S.K. Hofman, G.J. De Jong, Chromatographia 47 (11/12) (1998) 678.
[15] Y. Shen, L. Mathiasson, J.A. Jonsson, J. Microcolumn Sep. 10 (1) (1998) 107.
[16] N. Guecer, J. Ebel, R. Groening, Pharm. Acta Helv. 68 (1993) 129.

[17] Y.M. Issa, A.F. Shoukry, M.S. Rizk, E.M. Atia, J. Chem. Technol. Biotechnol. 64 (1995) 379.
[18] L. Campanella, L. Aiello, C. Colapicchioni, M.D. Sammartino, M. Tomassetti, Artif. Nat. Percept. Proc. Ital. Conf. Sens. Microsyst. 2 (1997) 121.
[19] H. Krepska, R. Pakula, Chem. Anal. (Warsaw) 12 (1967) 293.
[20] A.F. Shoukry, M.S. Rizk, Y.M. Issa, E.M. Atia, Mikrochim. Acta 127 (1997) 269.
[21] J. Hrdlicka, J. Stolejda, Biol. Chem. Vet. 14 (1978) 233.
[22] M. Severijnen, A.M.G. Buyzen-Satijn, F.G. Buizer, Analyst 100 (1975) 328.
[23] E.J. Kentzer, L.S. Cottingham, R.L. Smallidge, J. Assoc. Off. Anal. Chem. 71 (1988) 251.
[24] G.B. Cox, K. Sugden, Analyst 101 (1976) 738.
[25] Y. Kao, C. Cheng, C. Liu, Zhongguo Nongye Huaxue Huizhi 36 (2) (1998) 163 [Chem. Abstr. 129 (1998) 135337t].
[26] M. Petz, H.P. Their, H. Vogt, Z. Lebensm.-Unters. Forsch. 170 (1980) 329.
[27] P.W. Hammond, R.E. Weston, Analyst 94 (1969) 921.
[28] Y.M. Issa, M.S. Rizk, A.F. Shoukry, E.M. Atia, Mikrochim. Acta 129 (1998) 195.
[29] M.A. El-Ries, F.M. Abou Attia, F.M. Abdel-Gawad, S.M. Abu El-Wafa, J. Pharm. Biomed. Anal. 12 (1994) 1209.
[30] G. Lei, S. Lin, X. Chi, Guangpuxue Yu Guangpu fenxi 11 (1991) 57 [Anal. Abstr. 55 (1993) 5G111].
[31] C. Nerin, A. Garnica, J. Cacho, Anal. Lett. 24 (1991) 1347.
[32] Perkin–Elmer, Analytical Methods for Atomic Absorption Spectrophotometry, 1982.