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Determination of Amineptine and Amprolium Hydrochlorides through Ion Associates with Cobalt (II) Thiocyanate

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Abstract

Two new methods for the determination of amineptine (AMN) and amprolium (AMP) have been developed. The methods consist of extracting the ion - pairs between the drug and the inorganic complex $[Co(SCN)_4]^{-2}$. The optimal experimental conditions of both methods including pH, concentration of Co (II) and thiocyanate ions, and the organic solvents were studied. The optimum pH was found to be 3.9, nitrobenzene proved to be the most suitable solvent, giving quantitative extraction for the two drugs. The two drugs can be determined in the organic phase spectrophotometrically at 625 nm showing Sandell sensitivities of 0.19 and 0.12 μg cm^{-2} with relative standard deviation of 0.46 and 0.87 % for amineptine and amprolium, respectively.

The indirect method was also applied to measure cobalt in the organic phase by atomic absorption spectrometry at 240.7 nm, and the relative standard deviation of the method is approximately 0.35 and 0.29 % for amineptine and amprolium, respectively. The proposed methods were found to be suitable for the accurate, simple and rapid analysis of amineptine and amprolium hydrochlorides in the bulk drugs and in pharmaceutical forms.

Key words: Amineptine hydrochloride, amprolium hydrochloride, Co (II) thiocyanate, Ion- pairs, Spectrophotometry, AAS.

Introduction

Amineptine hydrochloride (AMN) 7-[(10, 11-dihydrodibenzo [a,d] cyclohepten-5-yl)amino] heptanoic acid hydrochloride, is a tricyclic antidepressant. As the hydrochloride it is formulated in tablets. Amineptine has been determined by gas chromatographic-mass fragmentographic [1], HPLC [2, 3], gas chromatographic- mass spectrometric [4], spectrophotometric [5] and ion-selective electrode [6] methods.

Amprolium hydrochloride (AMP) 1-(4-amino-2-propylpyrimidin-5-ylmethyl)-2methylpyridinium chloride hydrochloride, is an antiprotozoal used in veterinary practice, alone or in conjunction with other drugs such as ethopabate, for the control of coccidiosis, it is formulated in oral

solution for veterinary use only. Pharmacopoeial methods [7, 8] for its determination in bulk and its dosage forms involve non-aqueous titration and chromatography. Only one extraction- spectrophotometric method [9] was applied.

Spectrophotometric techniques offer significant economic advantages over gas chromatographic and HPLC and are more sensitive than titration methods. The present work aimed at finding the most suitable and highly sensitive direct and indirect spectrometric methods for the determination of amineptine and amprolium hydrochlorides in bulk and in pharmaceutical preparations

Complexes of SCN^- with Cr (III), Zn (II), Fe (III) and Co (II) were used to determine positively charged organic analytes [10, 11]. However, there are no literature data about their usage to form ion pairs with the studied drugs.

This paper describes the study of direct spectrophotometric determination of amineptine and amprolium via the formation of ion pairs with $[Co(SCN)_4]^{-2}$, extraction of the ionic complex into nitrobenzene, and subjected to spectrophotometric analysis. This study also aims to use the combination of extraction and AAS techniques, giving an increase in selectivity to the indirect determination of AMN and AMP in pure forms and in the pharmaceutical preparations by measuring the metal content of the organic phase. The quantitative reactions between $[Co(SCN)_4]^{-2}$ and AMN or AMP form the basis for the determination of these two drugs.

Experimental

Materials and reagents

Amineptine (ABI / Servier) and amprolium hydrochloride bulk drug was obtained from Lona Co. for Industrial Investments (S.A.E.) Cairo- Egypt and used without any purification. Amineptine tablets and a veterinary oral powder (Amprolium, 20 %) were obtained from local manufactures, Cobalt(II) chloride, ammonium thiocyanate, citric acid and sodium hydroxide were of analytical grade. Doubly- distilled water was used in all experiments.

Amineptine hydrochloride was used as 10^{-2} M and 2 mg ml^{-1} methanolic solutions.

10^{-2} M and 1mg ml^{-1} aqueous solutions of amprolium hydrochloride were used as standard solutions.

Apparatus

A Shimadzu UV-1601 recording spectrophotometer with quartz cells of 1 cm optical path length was used.
A Perkin-Elmer A Analyst 100 atomic absorption spectrometer was used with a hallow cathode lamp of cobalt at 240.7 nm, lamp current= 30mA and slit width= 0.2 mm with an air/ acetylene flame.

Recommended procedures

Determination of Amineptine and Amprolium hydrochlorides

a) Spectrophotometric method
Standard solutions of amineptine and amprolium 0.05-1.0 & 0.03-0.6 mg respectively, placed in a 50-ml capacity separating funnel each, 1.5 ml of cobalt (II) chloride (0.5M), 4 ml of ammonium thiocyanate (1M), 0.5 ml of 2 M potassium chloride and 5 ml of citrate buffer(20 mM) (pH=3.9) solutions were added, followed by 5 ml of nitrobenzene. After shaking the reaction mixture for 1 min the green-coloured layer is separated and filtered throw a Whatman No.41 filter paper. The absorbance is measured at 625 nm, against a reagent blank.

b) Atomic absorption spectrometric method (AAS)
The atomic absorption measurements were applied by direct nebulization of the organic extracts for the two drugs into the flame. The concentration of cobalt in the organic layer was measured at 240.7 nm. The calibration graphs were prepared with ten standard solutions ranging from 0.01- 0.2 and 0.01- 0.16 mg ml^{-1} for AMN and AMP, respectively.

Analysis of Servector (amineptine hydrochloride) tablets

An accurately weighed amount of the powdered tablets equivalent to 200 mg of drug was transferred to a beaker and extracted with methanol, filtrated through a filter paper and washed with methanol. The filtrates and washings were collected in a 100- ml standard flask and diluted to volume with methanol to obtain 2000 µg ml^{-1} of AMN, which was subjected to analysis by the recommended procedures.

Analysis of Veterinary oral powder (Amprolium, 20 %)

An accurately weighed amount of the veterinary oral powder equivalent to 100 mg of amprolium hydrochloride was dissolved in water with shaking for 5 min. The solution was filtered through a filter paper,

washed with water. The filtrate and washings were collected in a 100- ml standard flask and then diluted to volume with water, and then subjected to analysis by the recommended procedures.

Results and Discussion

On mixing aqueous solutions of tetrathiocyanatocobaltate (II) AMN or AMP in acidic medium, green ion-pairs are formed via the protonated nitrogen atom of both drugs as previously shown in literature [12].The extraction of these ion-pairs with different solvents such as chloroform, 1, 2- dichloroethane, dichloromethane, ethylacetate, *o*- nitrotoluene and nitrobenzene were studied. As with the ion-pairs formed with $[Co(SCN)_4]^{2-}$, ethylacetate extracts the ion pair but also extracts the $[Co(SCN)_4]^{2-}$ binary complex, while chloroform, 1,2-dichloroethane and dichloromethane fading the green colour of the ion-pairs, but the absorbance readings of *o*-nitrotoluene and nitrobenzene extracts are the maximum one also nitrobenzene is more suitable for AAS measurements than *o*- nitrotoluene. Therefore, nitrobenzene was chosen as the extraction solvent.

The effect of ammonium thiocyanate on the ion-pair formations and their extraction in nitrobenzene is shown in Fig.1a. The data show that 3.5- 4.0 ml of 1 M ammonium thiocyanate is required for maximum absorbance in a final volume of 10 ml aqueous solution and in presence of 0.5 ml (2 mg/ml) and 0.5 ml (1mg/ml) for AMN and AMP, respectively, after this, the absorbance was nearly constant. Also, it was found that 2.0 and 5.0 ml of 0.5 M Co (II) chloride in the final solution of 10 ml gave the maximum effect on the absorbance in the determination of AMN and AMP, respectively (Fig. 1b).The absorbance maximum of the associated species, is measured at 625 nm.

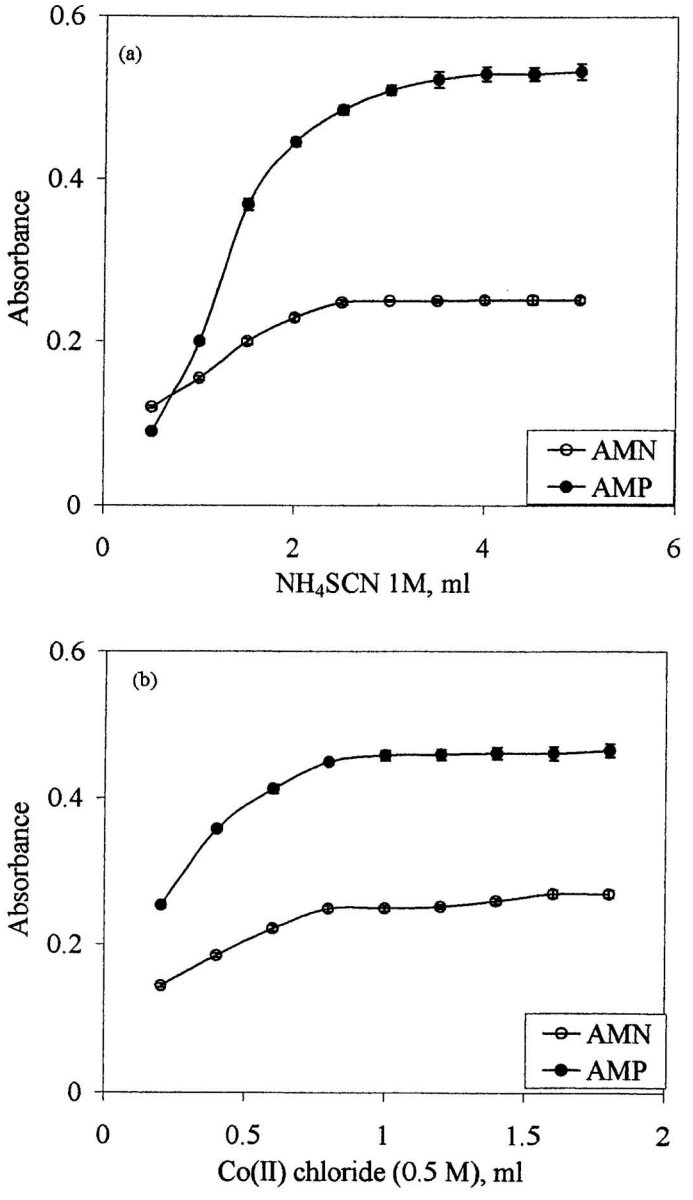

Fig.1. Effect of (a) NH_4 SCN and (b) Co (II) chloride concentrations on the reaction products of the ion- pair formations.

Effect of pH and ionic strength on ion-pair formation

The results were obtained by varying the pH of the aqueous phase within the range 2.15- 4.51 and using 100 μg ml^{-1} of drug. A maximum was clearly detected between 3.04 and 4.03 pH values at which AMN or AMP was found to be protonated thus helping ion- pair formation. Therefore a citrate buffer solution of pH 3.9 was chosen for further investigation.

As the shape of the absorption spectrum and position of the absorption maximum do not vary with pH, it is assumed that in this pH range only one type of complex is extractable.

The ionic strength also plays a significant role by its influence on the stability and intensity of recorded peaks. The effect of ionic strength and complex formation was followed in the range of 0.04- 0.4 M. At an ionic strength of 0.1M, spectra with optimum shape were recorded; therefore, 0.1 M KCl was chosen to carry out the procedure.

Shaking time and stability

The influence of the shaking time of the phases on the absorbance values was not found to be significant. Shaking time ranging from 30 sec to 3 min was studied, with the conclusion that 1 min is sufficient to obtain constant absorbance values. Consequently, the yield of a single extraction with 5.0 ml of nitrobenzene in optimal conditions with an organic: aqueous phase of 1: 2 is practically 100%.

The stability of the extracts obtained was studied by preparing a stock solution of each extract and comparing its absorbance over a period of time with that of a freshly prepared extract under the same conditions. It was found that the formation of the ion- pairs was rapid and the absorbance readings of the extracts of the associates were stable at a temperature range of 20 and 40°C; then room temperature, 25 $\pm$ 0.5° C, was used.

Stoichiometry of the ion-pair

The composition of the cobalt (II) and each drug in presence of excess amount of ammonium thiocyanate was determined by applying Job's method of continuous variation. The results indicate that 1:1 (metal: drug) ion-pairs are formed through the electrostatic attraction between positive protonated AMN. H^+ or AMP. H^+ and negative complex cobalt (II) thiocyanate. On the basis of the results obtained, it can be presumed that

the composition of the extracted ion- pair complex is [drug.H][Co(SCN)$_4$].

Relative stability constants of the complexes

The relative stability constants of the complexes were determined by applying the method of Sommer et al.[13] on the basis of results obtained by Job's method for the composition of the complexes and also by application of Job's method of non-equimolar solutions [14] (Table 1). By Job's method of non-equimolar solutions the curves for a five-and ten-fold excess of reagent were obtained (Fig.2).
The conditional stability constants were then calculated in the following way:

$$K' = \frac{(p-1)(1-2X_{max})}{(C_{drug})[(1+p)X_{max}-1]^2}$$

Where p = 5 or 10, X_{max} is projection of the peak maximum divided by the total volume of the organic solvent used for extraction in each case (5 ml). The values obtained by the two different methods are in good agreement. The stability constants values which are presented in Table 1 indicate the high stability of the ion-pairs.

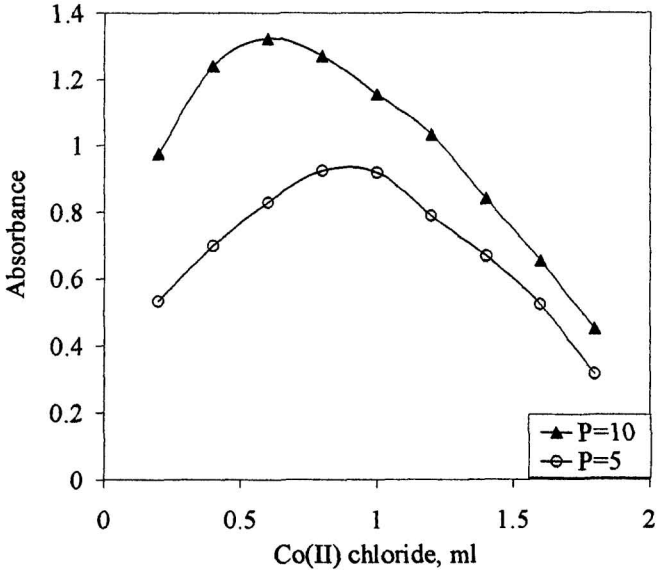

Fig.2. Job's curve of non-equimolar solutions for Co (II) - amprolium ion - pairs

Table 1
Conditional stability constants of the Co (II) – amprolium ion pair

Sommer's method[a]				
Log K'	log K'_{min}	log K'_{max}	SD*	RSD (%)
4.100	3.996	4.203	0.085	2.07

Job's method of non-equimolar solutions[a]

[Co(II)]	P	X_{max}	log K'	
$5x10^{-2}$	5	0.188	4.18	
$1x10^{-1}$	10	0.113	4.10	
			Mean:	4.14

*n = 5
[a] Conditions: pH= 3.9; μ =0.1and temperature = 25 ± 0.5° C.

Interferences

Interferents are mainly basic compounds such as metoclopramide, oxybuprocaine, avacan....etc that contain heteronitrogen aromatic nuclei. However, such compounds are not usually present with the examined drugs in pharmaceutical preparations and hence are not likely to cause analytical problems. Also cations such as Cr (111), Zn (11), Mo (V1)...etc which react with thiocyanate ions to form coloured compounds are not present. On the other hand, tablet fillers such as lactose, starch and stearic acid do not interfere in the proposed method

Analytical data

Under the experimental conditions described above, calibration graphs were constructed for both methods from six data points over the concentration ranges cited in Table 2. Regression analysis indicated a linear relationship between absorbance and concentrations (Table 2).

The molar absorptivity, Sandell sensitivity and the linear regression equation for each method are listed in Table 2. The correlation coefficients were between 0.9993 – 1.0002 indicating good linearity. The slopes of the calibration graphs reflect the sensitivity of the procedures.

The highest slope is that for amprolium, the drug of greatest expected basicity, due to the presence of a tertiary amine group. The detection limits for the two drugs are calculated as shown in Table 2.

Six replicate determinations at different concentration levels were carried out to test the precision of the methods, with standard deviations (RSD) less than 1%.

The accuracy and repeatability of the proposed methods were checked by the standard addition method. The good percentage recoveries and the values of standard deviation (Table 3) indicate the good accuracy and repeatability of the proposed methods.

Table 2
Linear range, sensitivity, precision and detection limit of the developed methods

Parameter	Spectrophotometric method		AAS method	
	AMN	AMP	AMN	AMP
λ_{max}(nm)	625	625	240.7	240.7
Beer's law limits ($\mu g\ ml^{-1}$)	10- 190	5- 120	10- 200	10- 160
Slope (m)	0.0052	0.0085	0.0026	0.0030
Intercept (b)	-0.0032	-0.0040	-0.0060	-0.0019
Correlation coefficient (r)	0.9999	1.0002	0.9998	0.9993
Relative standard deviation (%, n=6)	0.46	0.87	0.35	0.29
Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	1870	2239		
Sandell sensitivity ($\mu g\ cm^{-2}$)	0.19	0.12	0.39	0.34
Detection limit ($\mu g\ ml^{-1}$)	1.77	2.05	2.69	1.93

Analysis of commercial pharmaceutical dosage forms

The proposed methods for the determination of amineptine and amprolium hydrochlorides were applied to their pharmaceutical formulations (Servector tablets, and veterinary oral powder, Amprolium, 20 %). It was carried out on the same batch of samples together with the USP [7] and reference [5] methods. The results were compared statistically by the Student's t- test and Variance ratio F- test (Table 3). The experimental values did not exceed the theoretical ones indicating the absence of any significant difference between the methods compared.

Table 3
Determination of amineptine and amprolium hydrochlorides in their pharmaceutical preparations

Drug	Mean recovery ± SD (%)[a]		
	Proposed methods		BP [7] and Reference method [5]
	Spectrophotometric	AAS	
Amineptine hydrochloride (pure drug)	99.86 ± 0.41 t = 0.39 F = 4.50	99.74 ± 0.55 t = 0.28 F = 1.24	99.69 ± 0.87[5] (2.306)[d] (6.390)[d]
Amineptine hydrochloride (servector 100 mg)[b]	100.59 ± 0.58 t = 0.97 F = 1.29	99.58 ± 0.64 t = 0.44 F = 1.06	99.76 ± 0.66[5]
Amprolium (pure drug)	100.09 ± 0.31 t = 0.60 F = 3.59	99.29 ± 0.53 t = 1.75 F = 1.24	99.91 ± 0.59[7]
Amprolium (oral powder)[c]	99.88± 0.57 t= 0.24 F= 1.18	99.69± 0.31 t= 0.32 F= 4.00	99.79± 0.62[7]

[a] Mean recovery ± standard deviation of five determinations.
[b] Servector tablets (Servier Egypt Industries Limited, under license of Les Laboratories Servier-France).
[c] Veterinary oral powder (Adco Co., Egypt)
[d] Critical values of t =2.306 and F= 6.390 at $p = 0.05$.

Conclusion

The proposed methods are based on the extraction of the drugs as their cobalt –thiocyanate ion pairs from acidic solution into nitrobenzene and measurements of its green colour spectrophotometrically and also atomic absorption signal of cobalt. Hence the absorbance due to cobalt is related to the drug concentration. As the direct determination of the two drugs by AAS was not possible, an indirect atomic absorption spectrometric technique was adopted. The methods described here are highly sensitive and rapid, and are free from interferences from a large number of ions. The drugs concentration in pharmaceutical preparations samples can be determined without a masking agent, which is a distinct advantage of the methods. Interferences from common cations were virtually eliminated by means of a solvent extraction step. Use of a solvent extraction allows pre-concentration of the drug which improves precision, sensitivity and detection limit. The proposed methods have been successfully applied to the separation and determination of microgram amounts of the drug in their formulations. Hence the procedures are suitable for the routine laboratory determination of the drugs.

REFRENCES

1. Sbarra, C., Negrini, P., Fanelli, R.: J. of Chromatography, 1979; 162, 31- 38
2. Nicot, G., Lachatre, G., Gonnet, C., Valette, J.P., Bromet, N.: J. of Chromatography, 1984; 306 , 279- 290
3. Rop, P. P., Spinazzola, J., Bresson, M., Conquy, T., Viala, A.: J. Chromatogr. Biomed. Appl., 1990; 532, 351- 361.
4. Tsaconas, C., Padieu, P., D'. Athis, P., Mocaer, E., Bromet, N.: J. Chromatogr., 1989; 487, 313- 329
5. Abdel-Gawad, F. M.: J. Pharm. Biomed. Anal., 1998; 16, 793-799
6. Issa, Y. M., Abdel-Ghani, N. T., Shoukry, A. F., Ahmed, H. M.: Microchim. Acta, 1999; 132, 83- 88
7. British Pharmacopoeia (Veterinary) London: The Stationery Office, 2001; p. 33-34
8. United States Pharmacopoeia 23 NF 18, US pharmacopoeial Convention, Inc. 1995; 113- 114
9. Shoukry, A. F., Risk, M. S., Issa, Y. M., Atia, E. M.: Microchim. Acta, 1997; 127 , 269- 272
10. Nerin, C. Garnica, A., Cacho, J.: Anal. Chem. 1985; 57, 34
11. Nerin, C., Garnica, A., Cacho, J.: Anal. Chem. 1986; 58, 2617
12. Abdel- Gawad, F. M., El- Guindi, N. M.: Anal. Lett. 1995; 28, 1437-1447

13. Sommer, L. , Kuban, J., Havel, L. 'Spectrophotometric Studies of Complexation in Solution' Tomus XI Chemia 7, Opus 1, 1970, p. 25-27
14. Vosburg, W.C., Cooper, G.R.: J. Am. Chem. Soc., 1941; 63, 437

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