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MICRO-DETERMINATION OF ITOPRIDE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS AND URINE SAMPLES USING ICP-ATOMIC EMISSION SPECTROMETRY

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ABSTRACT

Ion-associate complexes of Itopride hydrochloride with zinc(II) thiocyanate, sodium cobaltinitrite and ammonium reineckate are precipitated. The solubility of the ion associates at the optimum pH and ionic strength values have been examined. Saturated solutions were prepared at different temperatures (25 – 60^{o}C) under the optimum precipitation conditions and the metal ion contents (zinc, chromium and cobalt) in the supernatant were determined. The solubility products were thus calculated at different temperatures and the thermodynamic parameters ΔH, ΔG and ΔS were calculated. A new accurate and precise method based on inductively coupled plasma atomic emission spectrometry for the micro-determination of Itopride hydrochloride (0.63-71.08 µg/ml) in pure solutions, pharmaceutical formulations and urine samples is given.

KEWORDS: **Pharmaceutical analysis, ion-associate complexes, ICP-atomic emission spectrometry.**

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INTRODUCTION

Itopride hydrochloride; Ip HCl is chemically hydrochloride salt of N-{[4-(2- dimethyl amino ethoxy) phenyl] methyl}-3, 4-dimethoxy-benzamide.is an anti-emetic drug and used in the management of gastrointestinal symptoms like nausea, vomiting, nonulcer dyspepsia, emesis and chronic gastritis[1]. Itopride has anticholinesterase (AchE) activity[2] as well as dopamine D2 receptor antagonistic activity and is being used for the symptomatic treatment of various gastrointestinal motility disorders. On oral administration, Itopride is rapidly and extensively absorbed and peak serum concentrations are achieved within 35 minutes after oral dosing[3]. Food does not affect its absorption[4]. Itopride is metabolized in the liver by N-oxidation to inactive metabolites by the enzyme flavin-containing monooxygenase (FMO).The half life of Itopride is about 6 hours. It is excreted mainly by the kidneys as metabolites and unchanged drug[5]. Because of the pharmaceutical properties of Ip HCl we found it important to prepare new ion associates containing Itopride and to study and elucidate their chemical structures to be applied to the analysis of Ip HCl. Various reports have been described for the determination of Ip HCl, those are spectrophotometric [6-10] , HPLC[11-15], LC-MS[16], HPTLC[17,18] and RP-HPLC[19,20]. Though these methods are sensitive, they require expensive instruments, careful control of conditions, suffer from lack of selectivity, time consuming and trained personnel. Despite the availability of sophisticated and sensitive instruments, for routine quantitative analysis. Although inductively coupled plasma atomic emission Spectrometry (ICP-AES) is a rapid method and has very low detection limits which can not be reached by most of the above mentioned methods, it has not been applied yet to the determination of Ip HCl. The present work includes a new ICP-AES method for the micro-determination of Ip HCl. The method is based on precipitation of the ion associates formed from the reaction of Ip HCl with $[Zn(SCN)_4]^{2-}$, ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$, or $[Co(NO_2)_6]^{3-}$. The metal ion content present in saturated solutions of these ion associates is determined employing ICP-AES and is used to calculate the concentration of Ip HCl. ICP-AES is well suited for this type of determination because of its accuracy, precision, sensitivity, and freedom from interference.

MATERIALS AND METHODS

Double-distilled water and analytical grade reagents were used to prepare all solutions. Itopride hydrochloride (Kahira Pharm. & Chem. Ind., Egypt), ammonium reineckate, sodium cobaltinitrite and zinc acetate were Aldrich products, Ganaton tablets, containing 50 mg Ip HCl per tablet were obtained from (Kahira Pharm. & Chem. Ind., Egypt), Ampty tablets, containing 150 mg Ip HCl per tablet and Itokine tablets, containing 50 mg Ip HCl per tablet were obtained from (Taj Pharmaceuticals limited, India).

APPARATUS

The pH of the solutions was measured using an Orion Research Model 701A digital pH-meter. Direct coupled plasma atomic emission measurements were carried out using ICPE-9000 Shimadzu plasma atomic emission spectrometer and atomic absorption measurements were made on AA-6650 Shimadzu atomic absorption spectro-photometer. Conductimetric measurements were carried out using conductivity measuring bridge type M.C.3 model EBB/10 (K_{cell} = 1); [Chertsey, Surry, England]. The IR absorption spectra were obtained by applying the KBr disk technique using a PYE UNICAM SP – 300 infrared spectrometer.

PREPARATION OF THE ION-ASSOCIATES

The ion associates were prepared by mixing solutions containing 1 x 10^{-3} mol of Zn(II) with a solution containing 4 x 10^{-3} mol of potassium thiocyanate and the requisite amount of Ip HCl. Sodium cobaltinitrite and ammonium reineckate 1 x 10^{-3} mol of the solution was mixed with the calculated amount of Ip HCl. The precipitates obtained were filtered, thoroughly washed with distilled water, and dried at room temperature. They were subjected to elemental microanalysis, infrared spectroscopy, nuclear magnetic resonance and

determination of the metal content.

EFFECT OF pH ON THE SOLUBILITY OF ION-ASSOCIATES

The choice of a suitable pH value at which the ion associates exhibit the lowest solubilities and the effect of pH on the degree of completeness of ion-associate formation were studied as follows: the solid ion associates were added to form saturated solutions in a series of solutions of different pH values ranging from 1 to 10; the pH value was adjusted with 0.1 *M* HC1 or 0.1 *M* NaOH. The solutions were shaken for 4-6 hrs and left to stand for a week to attain a stable equilibrium. Then the saturated solution is filtered in a dry beaker (rejecting the first few milliliters of filtrate). One milliliter of the filtrate is transferred into a 100-ml measuring flask containing 1 ml of concentrated $HN0_3$ and the volume is filled to the mark with distilled water. The equilibrium concentration of the metal ion present in the form of soluble inorganic complex ion is measured using ICP-AES, and hence the solubility of the precipitate is evaluated, from which the solubility products of the ion associates were calculated.

EFFECT OF IONIC STRENGTH ON THE SOLUBILITY OF ION-ASSOCIATES

A series of saturated solutions of the ion associate adjusted to the optimum pH value and having different ionic strength (0.1-1.0) was prepared using NaCl as the electrolyte. The same procedures as those used in the determination of the effect of pH have been followed to determine the optimum ionic strength values at which ion associates have the lowest solubilities.

EFFECT OF TEMPERATURE

The effect of temperature on the solubility of ion associates and the heat of the solution of ion associates were studied by preparing a suspension of the ion associate in solutions at the optimum pH and ionic strength values at different temperatures (25, 35, 45, and 60°C). The metal ion content present in the form of soluble complex ion is measured using ICP-AES and the heat of the solution of the ion associates was determined applying the Van't Hoff isochore relation; thus:

$$\log S = - \Delta H / 2.303RT + \text{constant}, \quad (1)$$

where H is the heat of solution KJ mol^{-1} ; R is the universal gas constant, 8.3 J mol^{-1} K^{-1} ; and T is the absolute temperature in K. Thus, a plot of log S against $1/T$ is a straight line with a slope equal to ($- \Delta H / 2.303 R$) from which ΔH is calculated.

Gibb's free energy change (ΔG) and the entropy change (ΔS) are calculated using Equations. (2) and (3), respectively,

$$\Delta G = -RT \ln K_{sp}, \quad (2)$$

where K_{sp} is the solubility product of ion associate and

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

PREPARATION OF STANDARD SOLUTIONS

Standard solutions of cobalt (II), chromium and zinc are prepared by weighing 1.0 g of a high-purity sample (cobalt powder, chromium shot and zinc metal, respectively), transferring it to a 1-liter measuring flask and then adding 50 ml of concentrated HNO_3. After dissolution the solution is diluted to 1 liter with deionized water. The 1000-ppm solution is stored in a plastic bottle which has been presoaked in dilute $HN0_3$. The solution is stable for approximately one year.

CALIBRATION OF ICP-AES

Under the recommended conditions, calibration graphs were constructed of aqueous standards of cobalt(II), chromium(III) and zinc(II) in 1 M

$HN0_3$ by performing triplicate measurements using solutions containing 0, 10, 20, and 50 ppm analyte concentrations as previously reported [21, 22]. The calibration graphs are straight lines passing through the origin.The different parameters used for the measurement of cobalt (II), chromium(III) and zinc(II) are listed in Table 1.

TABLE 1

Analytical Parameters for the Measurement of Co, Cr and Zn Using ICP- AES

Element	Wavelength (nm)	Plasma Order	Plasma position	DL (mg/L)	LDR (mg/L)	BEC (mg)	RSD x BEC (%)
Co	236.37	95	0	0.02	0.2-1000	0.8	1 x 1.7
Cr	267.71	84	0	0.01	0.1-1000	0.4	7 x 0.7
Zn	206.20	109	0	0.01	0.1-1000	0.3	10 x 0.9

Note. DL, detection limit; LDR, linear dynamic range; BEC, background equivalent concentration; RSD, relative standard deviation. For all elements: state, ion; entrance slits, 50 x 300 µm; exit slits, 100 x 300 µm.

CONDUCTIMETRIC MEASUREMENTS

The stoichiometry of the ion associates was elucidated by conductimetric titration of Ip HCl with the metal complex solutions.

ANALYTICAL DETERMINATION OF Ip HCL IN AQUEOUS SOLUTIONS

Aliquots (0.04 - 4.5 ml) of 0.001 *M* Ip HCl solution are quantitatively transferred into 25-ml measuring flasks. To each flask 1.0 ml of 0.01 *M* standard solution of Zn(II) thiocyanate, cobaltinitrite, or ammonium reineckate is added and the flask is filled to the mark with the recommended buffer solution of the optimum pH and ionic strength values. The solutions are shaken well and left to stand for 15 min and then filtered through Whatman P/S paper (12.5 cm), and the equilibrium metal ion concentration in the filtrate is determined using ICP-AES. The metal ion consumed in the formation of ion associates is calculated and the drug concentration is determined indirectly.

ANALYTICAL DETERMINATION OF Ip HCl IN PHARMACEUTICAL FORMULATIONS AND URINE SAMPLES

The itopride-containing pharmaceutical preparations (Ganaton, Ampty and Itokine tablets) were successfully assayed using the present method. Sampling were made by grinding (10, 8 and 12 tablets) then taking 1.50-68.15, 2.25-70.25 and 2.50-71.00 µg / ml of the Ganaton, Ampty and Itokine tablets, respectively. A certain amount of the cited drug within the applied concentration range (5.50 – 65.25 µg / ml) was spiked into urine of a healthy person. At the optimum conditions the tablets and urine samples were analyzed applying the above mentioned procedure.

RESULTS AND DISCUSSION

COMPOSITION AND STRUCTURE OF ION-ASSOCIATES

The results of elemental analysis (Table 2) of the produced solid ion associates reveal that two itopridinium cations form ion associates with one $[Zn(SCN)_4]^{2-}$ and three $[Co(NO_2)_6]^{3-}$ while only one Ip combines with $[Cr(NH_3)_2(SCN)_4]^-$ to form a 1:1 ion associate. These results are comparable to the previously reported results.[21-23] Conductimetric titrations of the investigated inorganic complexes with Ip HCl were performed to give insight into the stoichiometric compositions of the ion associates formed in solutions. For all ion associates, the characteristic curves break at a molecular ratio ([Ip] / $[x]^{n-}$) of about 2, confirming the formation of 2:1 (Ip : x^{2-}) ion associates except in the case of the reineckate anion where the curve exhibits a sharp break at the 1:1 molecular ratio and in case of cobaltinitrite anion the curve exhibits a sharp break at the 3 :1 molecular ratio . The results obtained coincide with the elemental analysis of the precipitated ion associate.

TABLE 2
Elemental Analysis, Composition, and Some Physical Properties of Itopride hydrochloride Ion - Associates.

Ion-associate composition	m. p. °C	Molar ratio	Color	% found (calculated)				
				C	H	N	S	Metal
$(C_{20}H_{26}N_2O_4)_2[Zn(SCN)_4]$	320	2:1	white	52.06 (52.10)	5.08 (5.13)	11.00 (11.05)	12.56 (12.63)	6.38 (6.44) Zn
$(C_{20}H_{26}N_2O_4)[Cr(NH_3)_2(SCN)_4]$	267	1:1	violet	42.54 (42.60)	4.68 (4.73)	16.52 (16.56)	18.85 (18.93)	7.59 (7.69) Cr
$(C_{20}H_{26}N_2O_4)_3[Co(NO_2)_6]$	342	3:1	pink	51.12 (51.17)	5.49 (5.54)	11.89 (11.94)	------	4.41 (4.05) Co

EFFECT OF pH ON THE FORMATION OF ION-ASSOCIATES

The choice of a suitable pH value at which the ion associate exhibits the lowest solubility (Table 3) is of prime importance in the use of such compounds in quantitative analysis. To determine this pH value, the solubility and the solubility products of the compounds are determined at 25°C in solutions of varying pH values. From the obtained results, it was observed that increasing the pH value of the medium decreases the solubility of the ion associate, although only slightly, until a certain pH value (Table 3), when it then increases again. This can be explained by considering the solubility equilibrium of the ion associate, e.g.,

$$(Ip)_2[Zn(SCN)_4] \rightleftharpoons 2\,Ip^{+} + [Zn(SCN)4]^{2-}.$$

In acid medium, the hydrogen ion may react with the complex anion, $[Zn(SCN)_4]^{2-}$, while in basic medium the hydroxyl ions may react with the itopridinium ion or the zinc thiocyanate complex. However, it is of note that the effect of pH is rather weak and the present method can be applied safely over a wide range of pH values.

EFFECT OF IONIC STRENGTH ON THE SOLUBILITY OF ION-ASSOCIATES

The choice of a suitable μ value at which the ion associates exhibit the lowest solubility is also of prime importance in the use of such ion associates in quantitative analysis. The solubility and the solubility product values of ion associates at different μ values (0.1-1.0) have been investigated at the optimum pH values. It was found that increasing the μ value of the medium decreases the solubility of the ion associates, probably due to the salting out effect, until the optimum μ value is reached (Table 3). It then increases again due to complexation reactions between the base cations and the concentrated NaCl in the medium that form the drug precipitate, and hence the concentration of the metal ion increases, leading to an increase in the calculated solubility values. The values of the solubility and solubility product at the optimum conditions of pH and ionic strength (μ) are given in Table (3). The results indicate that the present ion associates are so sparingly soluble that Ip HCl can be determined accurately and precisely by the indirect method through precipitation of its ion associates with Zn(II) thiocyanate, sodium cobaltinitrite and ammonium reineckate complexes.

TABLE 3
Solubility and Solubility Product Values at 25°C of Ip HCl Ion Associates at Their Optimum pH and Ionic Strength (μ) Values.

Ion Associate	pH	μ	pS	pK_{sp}
$(Ip)_2[Zn(SCN)_4]$	3.0	0.3	2.35	6.45
$(Ip)_3[Co(NO_2)_6]$	4.0	0.4	2.04	6.72
$(Ip)[Cr(NH_3)_2(SCN)_4]$	5.0	0.5	3.45	6.90

Note. pS, -log solubility.
pk_{sp}, -log solubility product.

EFFECT OF TEMPERATURE ON THE SOLUBILITY OF ION-ASSOCIATES

The solubility of ion associates was investigated at different temperatures (25, 35, 45, and 60°C) and the heat of solution (ΔH), Gibb's free energy change (ΔG), and the entropy change (ΔS) have been calculated (Table 4). The results show that Ip HCl is better determined at 25°C than at higher temperatures, providing the optimum conditions of pH and ionic strength. This is because increasing temperature increases the solubility where the process of dissolution of the precipitates is endothermic because the lattice energy is usually greater than the solvation energy and hence the stability of ion associates decreases. Gibb's free energy ΔG increases when the solubility of ion associates is decreased.

TABLE 4
Solubility (S), Solubility Product (K_{sp}), and Some Thermodynamic Functions of Itopride Ion Associate at Different Temperatures

parameter	$(Ip)_2[Zn(SCN)_4]$				$(Ip)[Cr(NH_3)_2(SCN)_4]$				$(Ip)_3[Co(NO_2)_6]$			
t °C	25	35	45	60	25	35	45	60	25	35	45	60
S (g mol/liter)	5.62×10^{-4}	4.23×10^{-4}	5.12×10^{-4}	6.23×10^{-4}	5.51×10^{-5}	6.33×10^{-5}	7.19×10^{-5}	8.33×10^{-5}	1.42×10^{-5}	2.83×10^{-5}	3.95×10^{-5}	4.33×10^{-5}
K_{sp}	7.04×10^{-10}	2.95×10^{-10}	5.33×10^{-10}	2.20×10^{-9}	3.04×10^{-9}	3.98×10^{-9}	5.14×10^{-9}	6.92×10^{-9}	1.96×10^{-10}	1.84×10^{-9}	6.61×10^{-9}	9.42×10^{-9}
ΔG (kJ mol^{-1})	52.12	19.89	56.38	55.10	48.53	24.72	50.37	51.94	51.52	51.43	49.71	51.09
ΔS (kJ mol^{-1})	8.90	8.72	8.32	7.95	6.69	6.54	6.27	5.96	15.27	14.76	14.35	13.64
ΔH (kJ mol^{-1})	2.6×10^3				4.5×10^3				2.0×10^3			

ANALYTICAL DETERMINATION OF Ip HCl IN AQUEOUS SOLUTIONS, TABLETS AND URINE SAMPLES

Ip HCl was determined precisely and accurately in aqueous solutions, pharmaceutical formulations (Ganaton, Ampty and Itokine tablets) and urine using the present method. The results given in (Table 5) reveal that for ammonium reineckate the recovery is 100.13 %, reflecting a high accuracy which in addition to the high precision indicated by very low values of relative standard deviations. For zinc thiocyanate and cobaltinitrite the recovery range is between 98.56 and 98.89 % -less accurate than that for ammonium reineckate.

CONCLUSION

The present method is applicable over a wider concentration range; (0.63 - 71.32 μg/ml) than that of Gupta et al[8], Choudhary et al [9] and Zate et al [10] where 5-50, 10-50 and 5-100

µg/ml solution of Ip HCl can be determined, respectively. In pharmaceutical analysis it is important to test the selectivity toward the excipiences and the fillers added to the pharmaceutical preparations. Fortunately, such materials mostly do not interfere. This is clear from the results obtained for the pharmaceutical preparations (Table 5) that these excipiences do not interfere. Although the present method is more time consuming (20 min) in comparison to other methods such as (15 min for HPLC), it exhibits the advantages of simplicity, precision, higher sensitivity, accuracy and convenience. Moreover, the reproducibility of the results are superior to those obtained from other methods such as chromatography. [15, 17, 18] Therefore, the method should be useful for routine analytical and quality control assay of the investigated drug in dosage forms. In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression[24] of observed drug concentration against the theoritical values (five points) was calculated. Student' s *t*-test [25] (at 95% confidence level) was applied to slope of the regression line (Table 6) and showed that it didn' t differ significantly from the ideal value of unity. Hence, it can be concluded that there are no systematic differences between the determination and true concentration over a wide range. The standard deviations (S.D.) can be considered satisfactory at least for the level of concentrations examined. Although the present method is more time consuming than some other methods, it exhibits fair sensitivity and accuracy. Moreover, the reproducibility of the results is superior to that obtained from other methods.

TABLE 5

Analytical Determination of Itopride hydrochloride in Aqueous Solution , Tablets and Urine Samples by ICP-AES

Sample	Amount taken (µg)	Mean recovery (%)	Mean RSD (%)
$[Zn(SCN)_4]^{2-}$			
Pure Ip HCl solution	0.63 - 71.08	98.89	1.05
Ganaton Tablets[(a)]	1.50 - 68.15	98.88	1.08
Ampty Tablets[(b)]	2.25 - 70.25	98.86	1.08
Itokine Tablets[(b)]	2.50 -71.00	98.88	1.06
Urine	5.50- 65.25	98.87	1.05
$[Cr(NH_3)_2(SCN)_4]^{1-}$			
Pure Ip HCl solution	0.63 - 71.08	100.13	0.75
Ganaton Tablets[(a)]	1.50 - 68.15	100.08	0.85
Ampty Tablets[(b)]	2.25 - 70.25	100.09	0.85
Itokine Tablets[(b)]	2.50 -71.00	100.08	0.78
Urine	5.50- 65.25	100.09	0.77
$[Co(NO_2)_6]3-$			
Pure Ip HCl solution	0.63 - 71.08	98.75	1.08
Ganaton Tablets[(a)]	1.50 - 68.15	98.56	1.06
Ampty Tablets[(b)]	2.25 - 70.25	98.74	1.07
Itokine Tablets[(b)]	2.50 -71.00	98.75	1.06
Urine	5.50- 65.25	98.76	1.07

Note. RSD, relative standard deviation (five determinations).

(a) Kahira Pharm. & Chem. Ind. Co. , Egypt.

(b) Taj Pharmaceuticals Limited, India

TABLE 6

Linear regression analysis for Itopride hydrochloride using zinc thiocyanate, ammonium reineckate and sodium cobaltinitrite.

Parameter	Zinc thiocyanate	Ammonium reineckate	Sodium cobaltinitrite
Optimum concentration range (µg / ml)	0.63 – 71.08	0.63 – 71.08	0.63 – 71.08
Shift or intercept of the regression line [a]	0.029	0.035	0.031
Slope of regression line	1.0048	1.0032	1.0056
Student's / (2.31)[b]	2.11	2.05	2.04
Range of error (%)	100 + 1.1	100 + 1.3	100 + 1.2

(a) Observed versus theoretical.

(b) Tabulated 95% confidence limit (for slope).

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