

New and sensitive spectrophotometric method for determination of xipamide in pure and dosage forms by complexation with Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II) ions

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A new, rapid, sensitive and selective method for the spectrophotometric determination of xipamide in pure and dosage forms is proposed. The method is based on the reaction of xipamide with Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II) ions to form stable colored complexes. The formed complexes have maximum absorbance at 500, 390, 335, 445, 325 and 333 nm for Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II), respectively. Optimum conditions for determination, stoichiometry and stability of the formed complexes have been determined. Beer's law is obeyed up to concentration range 54.64-1995.75 $\mu\text{g ml}^{-1}$ of xipamide with apparent molar absorptivities ranging from 0.43×10^3 - $7.20 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$. The proposed method has been applied successfully for the determination of xipamide in pure form and representative pharmaceutical formulations with high precession and good accuracy.

Keywords: Xipamide; Spectrophotometric determination; Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II); complexation.

INTRODUCTION

Xipamide (4-chloro-5-sulfamoylsalicyloyl-2⁶-dimethyl-anilide) figure 1 is one of the sulfamoyl diuretics (carbonic anhydrase inhibitors) (Fischer and Lenhartz., 1970). Carbonic anhydrase is an enzyme responsible for the catalytic reversible hydration of carbon dioxide and dehydration of carbonic acid. Since the publication of a clinical study with xipamide, more information about this compound has been available (Knauf and Mutschler, 1984). Pharmacological investigations (Strandhoy JW, Gomoll, 1979) proved that the compound differed from the thiazides in its site of action in the renal tubule. Reference has been made to an action in the ascending limb of the loop of henle (Piyasena et al., 1975). Several techniques such as fluorescence spectrometry (Sobel and Mutschler, 1980), voltammetric (Legorburu et al., 1993) and high performance liquid chromatographic (Dadgar and Kelly, 1988; Bodenan et al., 1990; Kim et al., 2004) methods have been used for determination of xipamide in different samples.

However, most of these methods are sometimes rather complicated because of need to separate interfering compounds and hazard solvents should be used.

The present investigation propose a selective and sensitive method for determination of xipamide using UV-Vis spectrophotometry technique due to the resulting experimental rapidity, simplicity and the wide range of application of this method. The method based on the formation of some stable Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II) complexes with xipamide. The optimum reaction conditions and other analytical parameters are evaluated. The statistical analysis of the obtained results indicates that the method is precise and accurate. No interferences were observed from excipients and the validity of the method was tested against reference methods.

Experimental

All reagents and solvents used in the present investigation were of analytical or pharmacopoeialgrade purity from Aldrich, BDH, Riedel de Haen or Fluka,

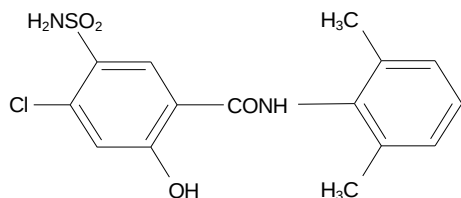


Figure 1. Structure of xipamide.

Bidistilled, deionized water was used throughout.

Solutions and reagents

Stock solution (1×10^{-3} M) of xipamide (Asta) was prepared by dissolving an accurately weighed amount of xipamide in the required volume of methanol. Metal stock solution (1×10^{-3} M) was prepared by dissolving the appropriate amount of ferric chloride anhydrous (Fluka), copper(II) acetate monohydrate (Merck), lanthanum(III) nitrate hexahydrate (BDH), uranyl(II) acetate dihydrate (Riedel de Haen), thorium(IV) nitrate tetrahydrate (Fluka) and zirconyl(II) chloride octahydrate (Fluka) in double distilled water and was standardized by the recommended method (Vogel, 1986). Working solutions were prepared by suitable dilutions with deionized water or methanol. The solutions used for investigation of the effect of diverse ions were prepared from sulfate or nitrate of the tested cations and sodium or potassium salts of the tested anions. The universal, acetate, borate and phosphate buffer solutions of varying pH values were prepared as described by Britton (Britton, 1952).

Apparatus

A Shimadzu model UV/Vis-1601PC spectrophotometer with 1 cm quartz cells was used to measure the absorbance. The pH measurements were made with Q Metrohm digital model 713 pH-meter with a calomel glass electrode of sensitivity ± 0.001 pH units. Hewlett Packard model 1100 high performance liquid chromatography with a variable wavelength detector was used. All measurements were performed at room temperature ($25 \pm 0.01^\circ\text{C}$).

Recommended procedures

A series of solutions containing up to 5.0 ml (1×10^{-2} M) of the metal ions and 0.2-2.8 ml (1×10^{-2} M) of xipamide was mixed in 10 ml measuring flask and then diluted up to the mark with methanol. The mixture was allowed to stand for 10 min. The absorbance at the maximum wavelength (λ_{max}) was measured against a blank solution contains the same concentration of the metal

ion in methanol. The calibration graphs were prepared by using the same procedure (at least ten concentration points) and were linear passing through the origin.

General procedure for the determination of xipamide in tablets

Since xipamide is readily soluble in diethyl ether, this solvent has been used for extraction of xipamide from tablets. Also, many of the excipient formulation components are insoluble in diethyl ether and are separated with a simple filtration procedure. To minimize tablet composition variation, 20 tablets were finely ground. A portion of powder was placed in 100 ml glass stopper flask and dissolved in diethyl ether. After vigorous stirring for 15 min with magnetic stirrer, the solution was sonicated for 15 min and was filtered off. The ether extract is evaporated to dryness on a water bath at 40°C and cooled. The assay was completed as described previously. To check the applicability of the developed procedure for the analysis of xipamide in tablets, the results were compared with those of a reference method (HPLC) (Dadgar and Kelly, 1988).

RESULTS AND DISCUSSION

Optimization

Investigations were carried out to establish the most favorable conditions for the complexation reaction of xipamide with the metal ions under study, to achieve the optimum conditions for maximum color development in the determination of xipamide. Investigation of the effect of media on the development of colored complexes showed that methanol is the best media. In acetate, borate, phosphate and universal buffers, the color of the complexes fades by time probably due to dissociation of the formed complexes by the effect of buffer constituents (Issa and Maghrabi, 1974).

Electronic spectra and selection of the suitable wavelength

As shown in figure 2, the absorption spectra of the xipamide-metal complexes were shifted to longer wavelength compared with that of xipamide it self. This shift of the absorption maxima can be attributed to increase delocalization of the π -electrons on complexation leading to decrease in the energy gap between the excited and ground states (Babko and Philipenko, 1971). The absorption spectra of the xipamide complexes in the UV-Vis region exhibits maximum absorption at 500, 390, 335, 445, 325 and 333 nm for Fe(III), Cu(II), La(III), $\text{UO}_2(\text{II})$, Th(IV) and ZrO(II), respectively, using the same amount of xipamide as a blank. At the maximum wavelengths the

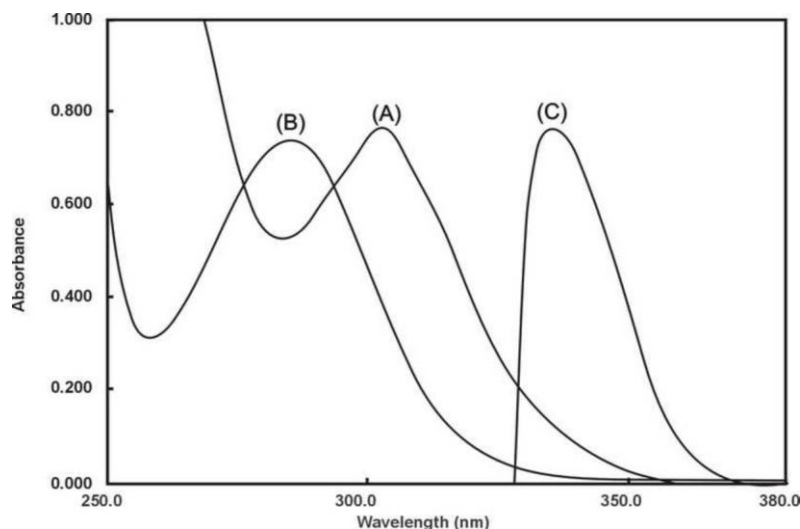


Figure 2. The electronic absorption spectra of xipamide, La(III) and La(III)-xipamide complex in methanol. **(A)** Xipamide against methanol as a reference, **(B)** La(III) against methanol as a reference and **(C)** La(III)-complex against xipamide and methanol as a reference.

absorption of both xipamide and metal solution were negligible, so, these longer wavelength peaks have been used in all subsequent measurements of the absorbance.

Effect of time and sequence of addition

The influence of time on complex formation was studied by measuring the absorbance of xipamide-metal complexes at increasing time intervals. The obtained results showed that all the complexes are formed instantaneously except for ZrO(II) complex which is formed after 15 min and the absorbance remain constant for more than 24 h. The obtained results for the effect of two different sequences of addition (metal to ligand and ligand to metal) to select the most suitable one for developing the colored complexes showed that the two sequences have the same effect on the formation of all xipamide complexes with metal ions under investigation except for ZrO(II) where the sequence ligand-metal is the best one.

Effect of metal ion concentration

The effect of metal ion concentration on the absorbance of xipamide complexes was investigated by varying the metal ion concentration while other variables were held constant. A constant and maximal absorbance was obtained when the metal ion concentration exceeded three times the reagent concentration. So, the metal ion concentration should be used in large excess during xipamide determination.

Effect of foreign ions

The interference from the presence of additives and excipients less than 2.5% is considered to be tolerable. The additives that are usually present in pharmaceutical formulations such as starch, lactose, microcrystalline cellulose, povidone, colloidal silicone dioxide and polyethylene glycol has no effect on the determination of xipamide through complex formation with studied metal ions. This conclusion is based on the fact that both additives and excipients have no effect on the band position characterized for complex formation between the metal ions and xipamide. The reaction of xipamide with Cu(II) in presence of a variety of species show the following:

- a- Turbidity of the solution occurs in case of carbonate, bicarbonate, mono, di and triphosphate, tetraborate as well as molybdate.
- b- The absorbance decreases at the same wavelength in case of sulphite, acetate, bromate, chloride, bisulphate, fluoride, nitrate, sulphate and perchlorate which indicates the removal or replacement of the xipamide by these ions.
- c- The absorbance increases in case of thiocyanate and iodide due to formation of ternary complexes.

Stoichiometry, stability constants and free energy changes

The continuous variation (Issa et al., 1975) and mole ratio (Zayan et al 1973), methods are used to investigate the stoichiometric composition of xipamide complexes with Fe(III), Cu(II), La (III), UO₂(II), Th(IV) and ZrO(II) ions.

Table 1. Spectrophotometric analytical characteristics of xipamide complexes with Fe(III), Cu(II), La(III), UO₂(II) Th(IV) and ZrO(II) ions

Metal ion ($\lambda_{\max}$)	M/L ratio	$\log \beta_n$	$-\Delta G$	$\varepsilon \times 10^{-3}$ (S.S.)	U.L. Beer (R.R.)	C.C. (S.D.)	L.O.Q. $\times 10^4$ (L.O.D. $\times 10^5$)	R.S.D.* (% E)	t-test* (F-ratio)
Fe ³⁺ (500)	1:1 1:2	4.44 8.84	0.25 0.50	2.78 (0.02)	149.00 (31.93-149.00)	0.9982 (0.0032)	0.60 (1.80)	0.655 (± 0.618)	1.13 (1.55)
Cu ²⁺ (390)	1:1 1:2	3.17 7.39	0.18 0.42	0.48 (1.66)	1995.75 (443.50-1773.78)	0.9985 (0.0020)	0.60 (1.80)	0.315 (± 1.115)	1.19 (1.43)
La ³⁺ (335)	1:1 1:2	3.92 6.70	0.22 0.38	0.43 (0.83)	212.81 (53.21-212.81)	0.9975 (0.0011)	0.60 (1.80)	0.908 (± 1.162)	1.26 (1.54)
UO ₂ ²⁺ (445)	1:1 1:2	3.41 5.46	0.19 0.31	0.54 (1.43)	1561.12 (283.86-1277.30)	0.9995 (0.0075)	0.60 (1.80)	0.195 (± 1.039)	1.15 (1.40)
Th ⁴⁺ (325)	1:1 1:2	4.09 8.88	0.23 0.50	7.19 (0.06)	54.64 (14.90-54.64)	0.9997 (0.0008)	0.60 (1.80)	0.169 (± 1.502)	1.10 (1.05)
ZrO ²⁺ (333)	1:1	4.63	0.26	7.20 (0.07)	56.77 (14.19-56.77)	0.9984 (0.0075)	0.60 (1.80)	0.115 (± 1.297)	1.23 (1.48)

$\lambda_{\max}$ – a suitable wavelength in nm; $\log \beta_n$ – logarithm of stability constant; (ΔG^*) – free energy change (KJ mol⁻¹); ε – molar extinction coefficient (L mol⁻¹ cm⁻¹). C.C. – correlation coefficient; (S.S.) – Sandell's sensitivity; U.L. Beer – upper limit in Beer's law plot ($\mu\text{g mL}^{-1}$); (R.R.) – Ringbom range ($\mu\text{g mL}^{-1}$); (S.D.) – standard deviation; L.O.Q. - Limit of quantification (mol L⁻¹); (L.O.D) - Limit of detection (mol L⁻¹); *Average of six determinations; R.S.D. – relative standard deviation; (%E) – relative error; t- and F-values for 5 degree of freedom and 95% confidence level.

The obtained results revealed the formation of 1:1 and 1:2 (M:L) complexes (Table 1). The logarithmic stability constants ($\log \beta_n$) and the free energy changes (ΔG^*) of the formed complexes were calculated from the data of continuous variation and mole ratio methods applying equations 1 and 2 (Harvey A, Manning, 1950).

$$\beta_n = \frac{\frac{A}{A_m}}{\left[1 - \frac{A}{A_m}\right]^{n+1} C_l^n n^2} \rightarrow (1)$$

$$\Delta G^* = -2.303 RT \log \beta_n \rightarrow (2)$$

where β_n is the stability constant of the metal chelate, A is the absorbance at ligand concentration C_L , A_m is the absorbance at full color developed, n is the order of the complex formed, T is the absolute temperature and R is the gas constant. The values obtained (Table 1) for 1:2 (M:L) complexes are nearly double those of 1:1 complexes which revealed that the stability of the complexes increases with increasing the number xipamide molecules attached to the central metal ion.

Adherence to Beer's law, sensitivity and reproducibility

After optimization of the conditions for the spectrophotometric determination of xipamide by chealation with Fe(III), Cu(II), La (III), UO₂(II), Th(IV) and ZrO(II) ions, obedience to Beer's law was tested. On plotting the absorbance as a function of xipamide concentration linear calibration graphs were obtained up

54.64-1995.75 $\mu\text{g mL}^{-1}$ concentration range of xipamide depends on the used metal ion (figure 3). The straight lines are passing through the origin for Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II) complexes. The high values of correlation coefficients and small values of standard deviations indicate the good linearity of all calibration graphs and the confirmatory of Beer's law to absorbance measurements. The apparent molar absorptivities ranged from 0.43×10^3 - 7.20×10^3 L mol⁻¹ cm⁻¹ and the Sandell's sensitivities (Sandell, 1959), lied in 0.06-1.66 $\mu\text{g mL}^{-1}$ range.

The optimum ranges for the determination of xipamide were determined from Ringbom plots and the results are summarized with the other analytical parameters in Table 1. The reproducibility of the investigated method was checked by analyzing solution containing 54.64 ppm of pure xipamide. The variation coefficients were found to be 0.42%, 0.54%, 0.66%, 0.57%, 0.46% and 0.63% whereas the relative errors were found to be 0.61%, 54%, 43%, 74%, 55% by complexation with Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II), respectively.

Applications of the proposed method

To confirm the usefulness of the proposed method, it was extended for the determination of xipamide in tablets manufactured by the local company. The concentration of xipamide in the dosage form was calculated from the appropriate calibration graphs. The results are compared with those obtained by applying official method (High Performance Liquid

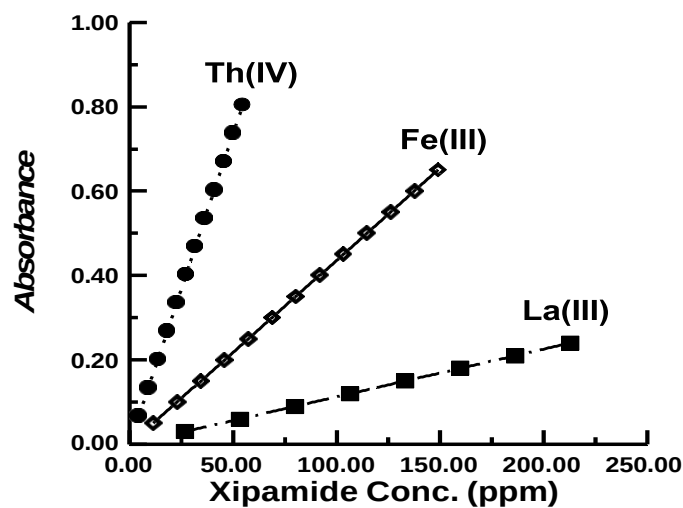


Figure 3. Absorbance vs concentration plots for Fe(III)-xipamide ($\lambda = 500$ nm), La(III)-xipamide ($\lambda = 335$ nm), and Th(IV)-xipamide ($\lambda = 325$ nm) complexes.

Table 2. Spectrophotometric determination of xipamide in pharmaceutical formulation by the proposed procedure and the official method.

Proposed procedure						Official procedure				
Metal used	Certified conc. (mg)	Found conc. (mg)	% Recovery	t-value	F-ratio	Taken conc. (mg)	Found conc. (mg)	% Recovery	R.S.D.	Ref.
Fe ³⁺	90	89.92	99.91	1.89	1.20	500	499.74	99.95	0.54	[16]
Cu ²⁺	500	503.78	100.76	1.01	1.32				0.97-1.50	[10]
La ³⁺	100	97.94	97.94	1.33	1.62				5.80	[6]
UO ₂ ²⁺	500	500.29	100.06	1.13	1.51					
Th ⁴⁺	30	30.06	100.19	0.70	1.23					
ZrO ²⁺	40	39.94	99.84	0.69	1.09					

Chromatography) (Dadgar and Kelly,1988). Comparison between the experimental mean values obtained by the investigated method and the values obtained by HPLC was carried out by calculating F- and t-values. From Table 2, it is found that for the investigated method F-values = 1.09-1.62 and t-values = 0.69-1.89 for five degree of freedom (P=0.05) and six replicates (n=6) at 95 % confidence level. The tabulated values did not exceed the theoretical F-values (5.05) and t-values (2.57) (Miller, (1986), which means that all samples are not subject to systematic error (accurate), i.e. no significant difference between accuracy of the proposed and the official procedures. Also, the values of standard deviations (S.D.), relative standard deviations (R.S.D.) and percentage error are very small indicating high precision (Hinchey, 1969) of the investigated method in case of Cu(II), UO₂(II), Th(IV) and ZrO(II) xipamide complexes. The obtained values are better than those calculated for HPLC procedure which is used as a reference procedure (Table 2). The relative standard deviation of the Cu(II), UO₂(II), Th(IV) and

ZrO(II) complexes are better than the values corresponding to the complex formation between xipamide and other metal ions.

CONCLUSIONS

In conclusion, this study has demonstrated the feasibility of the use of UV-Vis spectroscopy and complexation reaction for determination of xipamide. The determination process based on the ability of xipamide to form stable 1:1 and 1:2 (M:L) chelates with Fe(III), Cu(II), La(III), UO₂(II), Th(IV) and ZrO(II) ions. The proposed method is applied for determination of xipamide in pure and dosage forms with high precession and good accuracy.

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