ORIGINAL ARTICLE

THE ELABORATION OF NOVEL ANALYTICAL METHODS FOR CLEMASTINE AND TIMOLOL

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Manuscript received: June 2016

Abstract

Based on the reactions between clemastine/timolol and the silicotungstic acid, $H_4[SiW_{12}O_{40}]$, combinations by ionic association, hardly soluble in water, having the following formula: $[CL]_4[SiW_{12}O_{40}]\cdot 2H_2O$ and $[TM]_4[SiW_{12}O_{40}]\cdot 2H_2O$ are obtained. The confirmation of the structures of the new complexes and their setting conditions for processing were performed in previous work. The obtained data were used for the development of new methods for the assay of the two studied drugs. The established methods are accurate and reproducible.

Rezumat

Având la bază reacția clemastinei și timololului cu acidul silicowolframic, $H_4[SiW_{12}O_{40}]$, s-au obținut combinații prin asociere ionică, greu solubile în apă, având următoarea formulă: $[CL]_4[SiW_{12}O_{40}]\cdot 2H_2O$ and $[TM]_4[SiW_{12}O_{40}]\cdot 2H_2O$. Confirmarea structurii complecșilor formați și condițiile lor de prelucrare s-au stabilit într-un studiu anterior. Datele obținute au fost utilizate în elaborarea unor noi metode analitice, pentru dozarea celor două substanțe studiate. Metodele elaborate sunt exacte și reproductibile.

Keywords: clemastine, timolol, silicotungstic acid, congo red, ion-association combination

Introduction

Clemastine,(2*R*)-2-[2-[I-1-(4-chlorophenyl)-1-phenylethoxy] ethyl-1-methyl pyrrolidine (Figure 1), it is an antihistaminic drug used to reduce the effects produced by activating the H1-receptor by histamine, and timolol, 1-[1,1-dimethyl ethyl-(amino)]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl] oxy] 2-propanol is a drug with antihypertensive effect used in ocular hypertension and glaucoma.
The spectrophotometric methods used for the clemastine fumarate and timolol maleate determination have known lately a special attention due to their multiple advantages (simplicity, accuracy, low cost) [3, 5-7, 13, 15, 19]. Also, a series of chromatographic methods were developed, for the assay of the two studied substances [1, 2, 4, 8-11, 14, 16-18, 20-22]. In the presence of the silicotungstic acid, clemastine and timolol form, in a range of pH between 1 and 5, a yellow, respectively, pink microcrystalline precipitates. The resulting compounds, $[CL]_4[SiW_{12}O_{40}]\cdot 2H_2O$ and $[TM]_4[SiW_{12}O_{40}]\cdot 2H_2O$, are 4:1 ion association complexes. To describe the obtained complexes, we used spectral analysis (IR and UV-Vis), thermal analysis (TG, DTG, DSC), and then we assayed the molecular weight and the water solubility at 25°C [12]. Based on the precipitation reactions, we intended to establish some simple and sensitive methods for the assay of clemastine and timolol in bulk. The silicotungstic acid (abbreviated SiWo), due to its voluminous anion, used for the assay of a small number of inorganic and organic compounds, forms with these organic bases ion pairs complexes, characterised by a low solubility in water, correlated with its high molecular weight.

Materials and Methods

Reagent. All chemical reagents and solvents were provided from Merck (Germany): silicowolframic acid (SiWo), acetonitril, dioxan, dimethylformamide (DMF), ethanol, methanol, methylethylketon (MEC) which were of analytical grade. The water used in all reactions was distilled water. Clemastine fumarate (CLF) was provided by Promedic (Romania) and timolol maleate (TMM) by Merck (Germany).
The $2.5\cdot 10^{-3}$ M silicotungstic acid standard solution in MEC (the titrimetric factor was determined by titration with a $2.5\cdot 10^{-3}$ M sodium methoxide standard solution, prepared in benzene and methanol, using a 0.2% red of Congo indicator solution, prepared a solvents mixture MEC:methanol, in a ratio 1:1).
The $2.5\cdot 10^{-3}$ M sodium methoxide standard solution in benzene and methanol was prepared by dilution,

using a 0.1 M solution, whose factor was determined by titration with benzoic acid in the presence of thymol blue indicator.

The 0.1 M sodium methoxide in benzene and methanol standard solution was prepared by dissolving a quantity of 2.5 g sodium in 100 mL methanol and adding benzene to 1000 mL.

The 0.1% (w/v) thymol blue solution was prepared by dissolving the accurate weighed amount of 0.10 g in 100 mL DMF.

The 3% (w/v) SiWo solution was prepared by dissolving the accurate weighed amount of 3.00 g SiWo in 100 mL distilled water.

The 0.2% Congo Red solution prepared in mixed MEC:methanol, using a ratio 1:1 (v:v), was prepared by dissolving the accurate weighed amount of 0.20 g Congo Red in 100 mL solvents mixture.

The 2 M hydrochloric acid solution was prepared by diluting a 38% HCl Merck (Germany) solution with distilled water.

Acetonitril, dioxan, etanol, MEC and methanol were of spectroscopic grade

Equipment. The spectral measurements were recorded with a UV-Vis Spectrophotometer Lambda 2 Perkin Elmer, in a 1 cm thick quartz cells. An analytical balance (MettlerTolledo AT 261 Delta Range) and an ultrasonic bath Elma 9331-1 (Barstead, Lab-Line) were also used.

Methods

Direct titrimetric methods. Appropriate amounts (0.05 - 0.08 g) of drugs were accurately weighed and dissolved in 25 - 30 mL MEC:metanol (1:1). After addition of 7 - 8 drops of red of Congo solution in mixed MEC:metanol (1:1), the solutions were titrated with $2.5 \cdot 10^{-3}$ M silicotungstic acid standard solution in MEC until the indicator turns from red - orange to blue - grey.

Indirect titrimetric methods. Samples of about 0.10 g were weighed. CLF was dissolved in 20 - 30 mL ethanol, while TMM was dissolved in 50 - 60 mL distilled water. Then, 5 - 10 mL 2M hydrochloric acid solution were added and the samples were heated using a water bath, at 50 - 60°C. The precipitation reagent was added under continuous stirring, until complete precipitation and then a small volume of reagent was added in excess. The precipitates were left to stand for 30 - 40 minutes at 50 - 60°C, then were filtered through a G4 filter (previously washed with DMF) and were purified by washing with a saturated solution of precipitate and then with distilled water, until the washings waters no longer gave an acid reaction. The precipitates obtained were dissolved in 30 - 40 mL DMF, 3 - 4 drops of thymol blue were added and then the solutions were titrated with 0.1 M sodium methoxide standard solution, until the change of colour, from yellow to blue. It is recommended to carry out the titration slowly.

Spectrophotometric assay. Appropriate amounts (accurate weighed) of CLF (0.10 g), respectively TMM (0.05 g) were quantitatively precipitated with SiWo solution (according to the procedure described in the titrimetric method). The precipitates obtained in both cases were separated by a crucible G4, purified and quantitatively dissolved in acetonitrile (for CLF), in methanol (for TMM) and then filled with the same solvent to 100 mL in a volumetric flask (Solution A). Using the A solutions, B solutions were prepared, by diluting 2 mL (for CLF), respectively 10 mL (for TMM) with the same solvents to 50 mL in a volumetric flask. The B solutions were used for plotting the calibration curves as follows: volumes of 1.0; 2.0; till 10.0 mL B solutions were diluted to 10 mL, using acetonitrile for CLF and methanol for TMM. The maximum absorbances of the solutions were registered at 268.9 nm (for CLF) against acetonitrile, respectively at 266.4nm (for TMM) against methanol, in 10 mm quartz cell.

Results and Discussion

Direct titrimetric methods

Silicotungstic acid is a strong acid in non-aqueous solvents (MEC, MEC:methanol, dioxane). It forms with CLF and TMM compounds insoluble in water. CLF forms with SiWo a yellow ionic association complex, $[C_{21}H_{26}ClNOH]^{+}_4[SiW_{12}O_{40}]^{4-} \cdot 2H_2O$,($(CL)_4[SiWo] \cdot 2H_2O$). From the thermogravimetric analysis it can be seen that the precipitate is stable up to 96°C [12]. The precipitate can then be processed through the drying at about 50 - 60°C.

In acidic medium (pH ~ 1), SiWo reacts with TMM, also forms a pink microcrystalline precipitate, $[C_{13}H_{25}N_4O_3S]^{+}_4[SiW_{12}O_{40}]^{4-} \cdot 2H_2O$, ($(TM)_4[SiWo] \cdot 2H_2O$), easy to separate. From the thermogravimetric analysis it can be seen that the precipitate is stable in the temperature range of 95 - 150°C [12]. The precipitate can then be processed through the drying at about 100 - 110°C.

The general reaction between the anion and the protonated cation of basic drugs is presented below:

$$[SiW_{12}O_{40}]^{4-} + 4BH^{+} + 2H_2O = [BH]^{+}_4[SiW_{12}O_{40}]^{4-} \cdot 2H_2O.$$

The organic solvents chosen for the assay ensure a homogenous system throughout the titration. They have a dissolving effect on both the reactants and the reaction products. MEC is a neutral solvent from the ketones class. It serves for widening the scale of the pH during titration and for decreasing the effect of dissociation of methanol. The equivalence point has been highlighted with the

Congo Red indicator. Congo Red indicator has basic properties and participates, in the experimental conditions, in the protonated form. Before the end point, the precipitate surface of $[BH]_4[SiW_{12}O_{40}]$ will have a primary layer consisting of base protonated ions. Thus, the solution has the red colour of the indicator cationic form, which is not adsorbed by the precipitate of $[BH]_4[SiW_{12}O_{40}]$. After the equivalence, in the presence of the silicotungstic ions, the electric charge of the precipitate surface will change and the protonated ions of the indicator will act as counter ions, being adsorbed at the precipitate surface, which acquires a blue - grey colour for the solution. The method encountered the determination of small amounts of drugs and it is easy to apply. Errors are situated within the allowed range for volumetric method so the assay is characterized by precision and accuracy.

The statistical processing of results is presented in Table I.

Table I

Analytical results of direct titrimetric assay

Drug	M_r	S	S_x	Confidence range (at 95% confidence level)	RSD%
CLF	100.12	0.0527	0.0160	100.12 ± 0.03	0.0526
TMM	99.76	0.0105	0.0033	99.76 ± 0.01	0.0105

Where: S = Standard Deviation; S_x = Standard error of mean; N = 10

Indirect titrimetric methods

Using the drugs precipitation, followed by dissolution, the new developed ion pairs complexes were determined following two new indirect acid-base methods. The reagents, the solvents and the titrants have been selected in order to achieve the quantitative precipitation reactions and to ensure a homogeneous system throughout the titration. The purpose of the precipitation reactions was to separate the drugs from complex matrices. Then, drugs were titrated as ionic association complexes with very low solubility in water, but easy soluble in anhydrous solvent. These methods assure the quantitative precipitation of CLF, respectively TMM, using as reagent the silicowolframic acid. The obtained ionic association compounds were separated and dissolved in dimethylformamide (DMF) and then were titrated with sodium methoxide solution, using thymol blue to indicate the end point. DMF acts as a protophilic solvent; it decreases the drug's basic functions and increases the heteropolyacid function to a strong acid function, so it can be titrated with a strong base solution.

The indirect titrimetric methods use the following reactions:

$$[SiW_{12}O_{40}]^{4-} + 4BH^+ + 2H_2O = [BH^+]_4[SiW_{12}O_{40}]^{4-}\cdot 2H_2O$$

$$[BH^+]_4[SiW_{12}O_{40}]^{4-}\cdot 2H_2O + 4(CH_3)_2N\text{-}CHO = 4B +$$

$$[(CH_3)_2N^+H\text{-}CHO]_4[SiW_{12}O_{40}]^{4-} + 2H_2O$$

$$[(CH_3)_2N^+H\text{-}CHO]_4[SiW_{12}O_{40}]^{4-} + 4CH_3ONa = 4(CH_3)_2N\text{-}CHO + Na_4[SiW_{12}O_{40}] + 4CH_3OH$$

The statistical processing of results is presented in Table II.

Table II

Analytical results of indirect titrimetric assay

Drug	M	S	S_x	Confidence range (at 95% confidence level)	RSD%
CLF	99.75	0.044	0.0140	99.75 ± 0.03	0.043
TMM	99.77	0.012	0.0038	99.77 ± 0.01	0.012

Where: S = Standard Deviation ; Sx = Standard error of mean; N = 10

Spectrophotometric assay

The obtained ionic association complexes were analysed using spectrophotometric methods, in order to establish the amount of CLF and TMM. The ionic association compounds of CLF and TMM are soluble in DMF, dimethylsulfoxide, acetonitrile, methanol, sparingly soluble in acetone. For the spectrophotometric determinations, the solution of $[CL]_4[SiWo]\cdot 2H_2O$ in acetonitrile and the solution of $[TM]_4[SiWo]\cdot 2H_2O$ in methanol, were used.

The UV absorption spectrum of $[CL]_4[SiWo]\cdot 2H_2O$, prepared in acetonitrile, was scanned and showed a maximum absorption at 268.9 nm, stable for at least two hours. Also, the SiWo solution in acetonitrile had a maximum absorption at 271.9 nm. The UV absorption spectrum of $[TM]_4[SiWo]\cdot 2H_2O$, prepared in methanol, was scanned and showed a maximum absorption at 266.4 nm, stable for at least two hours, while SiWo in methanol showed a maximum absorbance at 248.1 nm.

The absorbance linearity increases with the concentration of the ionic association complexes (Figures 1 and 2).

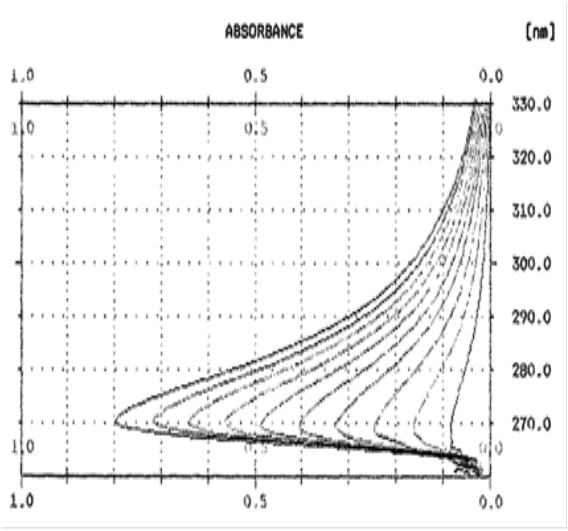

Figure 1.
Variation of $[CL]_4[SiWo]\cdot 2H_2O$ absorbance *versus* concentration

The validation of the spectrophotometric method

A linear relationship between concentration and absorbance has been noticed in the studied range of concentrations. The statistical parameters for calibration curve were given in the regression equation calculated from the calibration curve.

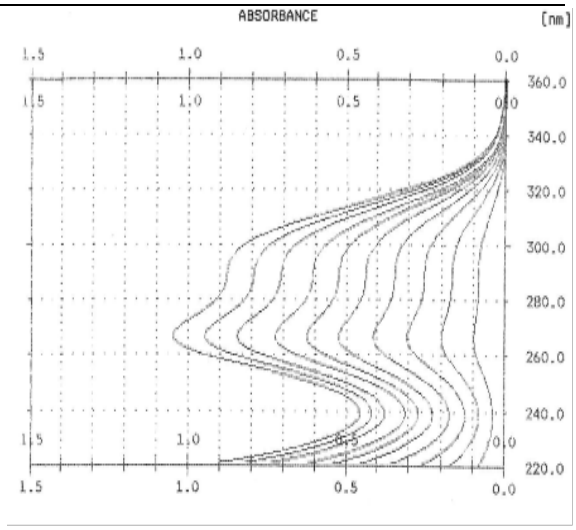

Figure 2.
Variation of $[TM]_4[SiWo]\cdot 2H_2O$ absorbance *versus* concentration

The LOD and LOQ of clemastine and timolol by the proposed methods were determined using the standard deviation of the blank. An appropriate number of blank samples (n = 6) where analysed and the standard deviation of the responses was calculated. LOD and LOQ were calculated as $3.3\cdot\sigma/S$ and $10\cdot\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of the blank.

The statistical processing of the results obtained by the spectrophotometric assay is presented in Table III.

Table III
Summary of the validation parameters

Parameter	Drug	
	CLF	TMM
λmax	269 ± 2 nm	266 ± 2 nm
Beer`s law limits (mg/mL)	$0.39\cdot10^{-2}$ - $3.90\cdot10^{-2}$	$0.95\cdot10^{-2}$ - $9.5\cdot10^{-2}$
Linear regression equation	Y = 20.3X + 0.009	Y = 11.1X - 0.008
Square of correlation coefficient (R^2)	0.9998	0.9999
Molar absorptivity ($L\cdot moli^{-1}\cdot cm^{-1}$)	9630	4754
Specific absorbance	208	108
Limit of detection (LOD, mg/mL)	$2.15\cdot10^{-3}$	$3.17\cdot10^{-3}$
Limit of quantification (LOQ, mg/mL)	$6.5\cdot10^{-3}$	$9.6\cdot10^{-3}$

Accuracy was studied taking samples of clemastine and timolol in order to obtain concentrations between 80 - 100% of the amount of interest. The procedures described for the calibration curves plotting were used for samples preparation. For each concentration level, three solutions were prepared. The absorbance of UV spectra was registered. The results show that in the range of the concentration from $1.6\cdot10^{-2}$ to $3\cdot10^{-2}$ mg CLF/mL and $2.5\cdot10^{-2}$ to $7.4\cdot10^{-2}$ mg TMM/mL measurements are accurate (Table IV).

A summary of validation data obtained for timolol and clemastine assay is presented in Table IV. The intermediate precision was studied by determinations with freshly prepared solutions using the same procedure for repeatability. Three solutions of CLF and TMM were prepared and, for each of them, the UV absorbance was three times recorded. CLF and TMM concentrations were calculated using the regression equation. The data were processed statistically and are shown in Table IV.

Table IV
Validation data

Validation criterion		Drug	Results	
			Confidence range	RSD%
Accuracy		CLF	99.69 ± 0.320	0.387
		TMM	99.87 ± 0.010	0.014
Precision	Repeatability	CLF	99.72 ± 0.170	0.241
		TMM	99.88 ± 0.010	0.012
	Intermediate precision	CLF	99.54 ± 0.260	0.432
		TMM	99.90 ± 0.010	0.012

Conclusions

Six methods have been developed for the determination of clemastine fumarate and timolol maleat in bulk drug. Clemastine fumarate and timolol maleate form with the silicotungstic acid ion associations complexes, insoluble in water, with the formula $[CL]_4[SiW_{12}O_{40}]\cdot 2H_2O$ and $[TM]_4[SiW_{12}O_{40}]\cdot 2H_2O$. The precipitation reactions were used to elaborate new titrimetric, spectrophotometric methods, respectively. The present visual titrimetric methods are simply and economically, compared with the reported chromatographic methods. The spectrophotometric methods are characterized by simplicity, since they do not involve any critical experimental variables. The accuracy, reproducibility, simplicity and cost-effectiveness of the proposed methods suggest their application in the quality control laboratories, where the modern and expensive instruments are not available.

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