Colorimetric and Atomic Absorption Spectrometric methods for the determination of Hyoscine N-butylbromide in pharmaceutical formulations using Cobalt (II) and Chromium (III) thiocyanate complexes

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Abstract

Two simple and sensitive colorimetric and atomic absorption spectrometric procedures have been established for determination of hyoscine N-butylbromide (Hyo.Br) in pure form and in pharmaceutical formulation. The methods are based on formation of an insoluble coloured ion-associate between the examined drug and tetrathiocyanatocobaltate (CoTC) or hexathiocyanatochromate (CrTC). The first method involves extraction of Hyo-CoTC and Hyo-CrTC ion-associates with methylene chloride and isobutyl alcohol, followed by measuring the absorbance at 630 and 557 nm, respectively. Optimization of the extraction conditions is investigated. Beer's law is obeyed in the concentration ranges 144.20-865.20 and 72.00-640.00 μg/ml using CoTC and CrTC, respectively. The molar absorptivities, Ringbom ranges, Sandell sensitivities and quantification and detection limits are also calculated. The second method is based on measuring the absorbance of the excess cobalt or chromium in the aqueous solution, after precipitation of the drug, at 240.7 and 357.9 nm using atomic absorption spectrometer, respectively. Linear application ranges, characteristic concentrations and detection limits of Hyo.Br are 0.404-1.617 mg/ml, 16.11 and 10.78 μg/ml in the case of CoTC, while 0.040-0.283 mg/ml, 29.50 and 2.70 μg/ml in the case of CrTC. The present methods have been successfully applied for the determination of the drug in commercial dosage

form. The data obtained by the developed methods are compared with the official one.

Keywords

Hyoscine N-butylbromide (scopolamine N-butylbromide), Ion-associates, Colorimetry, Atomic absorption spectrophotometry, Thiocyanate complexes

Introduction

Hyoscine N-butylbromide (scopolamine N-butyl bromide) [149-64-4] is an antispasmodic agent. It is used to treat the painful spasm of the stomach and intestines (including spasm associated with irritable bowel syndrome), reproductive organs and urinary system. Several methods have been reported for the quantitative determination of Hyo.Br including spectrophotometry [1-3], first [1,4] and second [5,6] derivative spectrophotometry, densitometry [7], HPLC [8-17], TLC [18-20], GC [21-24] and electrophoresis [25,26]. The British pharmacopoeia [27] recommended a titrimetric method with potentiometric detection of the end point for determination of Hyo.Br. Other methods include conductimetric titration [28] and ion-selective electrodes based on different types of ion pairs [29-32] have been reported.

Reaction of the investigated drug with the thiocyanate complexes of cobalt and chromium has not been examined before and also no atomic absorption method for the quantification of this drug has been published. Although densitometric [7], HPLC [8-17] and GC [21-24] methods were reported for the determination of the cited drug yet the proposed colorimetric and atomic absorption methods are less expensive, without loss of the accuracy and hence more suitable for application in quality control laboratories in developing countries. Therefore, the aim of the present work is to develop simple, sensitive and validated visible spectrophotometric and atomic absorption spectrometric procedures for the determination of Hyo.Br based on formation of coloured ion-associates. The optimum experimental conditions are thoroughly studied and under these

conditions, the procedures provide highly selective and sensitive methods for determination of the drug in commercial dosage form.

Experimental

Reagents and solutions

All chemicals and reagents were of analytical grade and water was always bi-distilled. Ammonium thiocyanate, sodium chloride, chromium chloride, sodium hydroxide and hydrochloric acid are Merck products, while cobalt chloride, methylene chloride and isobutyl alcohol are Aldrich products. Hyo.Br and buscopan injection (each 1 ml contains 20 mg Hyo.Br), was obtained from local manufacturer, Chemical Industries Development Company, CID, Egypt.

Solid hexathiocyanatochromate, $[Cr(SCN)_6]^{3-}$ (CrTC), was prepared by using Cruser and Miller method [33]. 10^{-2} M solution was prepared by dissolving the required amount of the pure solid in the appropriate amount of bi-distilled water. A standard solution, 10^{-2} M, of tetrathiocyanatocobaltate, $[Co(SCN)_4]^{2-}$ (CoTC), was freshly prepared by dissolving 0.01 mole of cobalt, as $CoCl_2.6H_2O$ and the required amount of ammonium thiocyanate (0.04 mole), in 100 ml bi-distilled water. 10^{-2} M aqueous solution of Hyo.Br was used as a standard solution. Sodium chloride (0.1-1.0 mol/l) was used to control the ionic strength of the medium, while HCl and NaOH (0.001-1.00 mol/l) were used to adjust the pH.

Apparatus

A Perkin-Elmer UV-Vis spectrometer model Lamda 1 with quartz cells of 1 cm optical path length was used. A Perkin-Elmer 2380 atomic absorption spectrometer was used with a hallow cathode lamp for cobalt at 240.7 nm, slit width 0.2 nm and current lamp 30 mA and a hallow cathode lamp for chromium at 357.9 nm, slit width 0.7 nm and current 25 mA. Air-acetylene flame was used for all measurements. All pH measurements were made with Metrohm titroprocessor model 682.

Preparation of the solid ion-associates

The solid ion-associates were prepared by mixing 0.01 mole of the metal reagents with the calculated amount of the drug to prepare 1:1 and 1:2 reagent:drug in case of CoTC and 1:1, 1:2 and 1:3 in case of CrTC. The precipitates were filtered, thoroughly washed with bi-distilled water, dried at the room temperature and subjected to elemental analysis (C, H and N) at the Microanalytical Center, Faculty of Science, Cairo University. The metal content was determined by using atomic absorption measurement. The stoichiometry of the formed ion-associates was determined by mole ratio and continuous variation methods [34,35].

General Procedure
Spectrophotometric method

Into a 50 ml separating funnel, a volume containing 1.80-21.63 mg of Hyo.Br was transferred, 3 ml of 10^{-2} M CoTC or CrTC were added and the total volume was completed to 25 ml with bi-distilled water. The formed Hyo-CoTC ion-associate was extracted with 10 ml methylene chloride by shaking for 6.0 min, while Hyo-CrTC was extracted with 7.5 ml isobutyl alcohol by shaking for 4.0 min. The extraction process is repeated twice with the same amount of solvents to be sure of complete recovery of the ion-associates. The reaction mixture was allowed to separate into two phases then the organic layer was collected, dried over anhydrous sodium sulphate and completed to 25 ml with the same solvent. The absorbances of the extracts were measured at the recommended λ_{max} against a reagent blank prepared in the same manner.

Atomic absorption method

To different aliquots of 10^{-2} M drug solution (0.40-2.83 mg) in 10 ml measuring flask, 3.5 ml 10^{-2} M standard CrTC were added and then the total volume of the solution was completed to the mark with 1.0 M NaCl adjusted to pH 5.0 using 0.001 M HCl. In case of CoTC, the concentration of the drug and reagent were 10^{-1} M (4.04-16.17 mg Hyo.Br and 3.5 ml 10^{-1} M CoTC, respectively) and then

the total volume was completed to 10 ml with 0.3 M NaCl solution adjusted to pH 6.0. The solution was shaken well and filtered through Whatman filter paper (No. 42). 2.0 ml portion of the filtrate was acidified with 1 ml concentrated nitric acid and completed to 10 ml with bi-distilled water. The absorbance of the unreacted metal ion was measured at the recommended wavelengths, subtracted from the absorbance of the blank solution and hence the resulting absorbance value was used in determination of the drug concentration from calibration graphs prepared by the same method.

Assay of buscopan ampoules

For analysis Hyo.Br in pharmaceutical formulation, ten ampoules of buscopan (20 mg/ml) product were mixed and 10 mg transferred in 25 ml calibrated flask then analyzed in the same way as the pure solution using the general procedures.

Results and discussion

Spectrophotomertic method

Careful investigations were carried out to establish the most favorable conditions to achieve maximum colour intensity in quantitative determination of Hyo.Br. The absorption spectra, under the optimum conditions, are shown in Fig. 1, which revealed that the ion-associates with CoTC and CrTC absorbed maximally at 630 and 557 nm, respectively. The reagent blanks prepared under similar conditions showed no absorption.

Nature of the medium

The reaction between Hyo.Br and the cited reagents was performed in different media followed by extraction of the formed products and measuring their absorbance values at the corresponding λ_{max}. The results showed that, presence of different buffer (universal, acetate and borate) of different pH values (2-10) and different volumes of HCl and NaOH (0.1-1.0 M) did not stimulate the formation of the

ion-associates and their extraction, so formation of Hyo-CoTC and Hyo-CrTC is proceed favorably in aqueous media.

Selecting of the extracting solvent and optimization of the extraction procedure

Several organic solvents such as toluene, chloroform, and isobutyl alcohol, benzene and carbon tetrachloride were tested for extraction of the ion-associates in order to provide an applicable extraction procedure. Methylene chloride and isobutyl
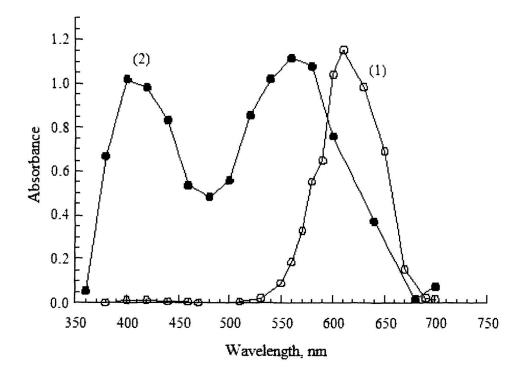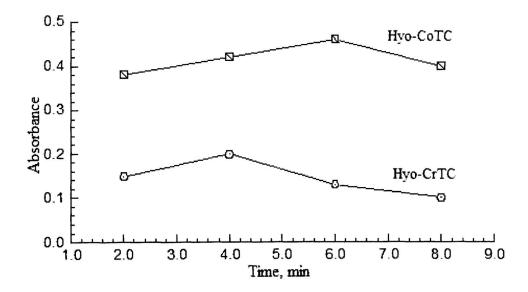

Fig. 1. Absorption spectra of Hyo-CoTC (1) and Hyo-CrTC (2) ion-associates.

alcohol were preferred for selective extraction of Hyo-CoTC and Hyo-CrTC from the aqueous phase using 10 and 7.5 ml (for efficient extraction), respectively. Double extractions were adequate to achieve quantitative recovery of the coloured product,

with shaking time 6.0 and 4.0 min (Fig. 2) for Hyo-CoTC and Hyo-CrTC ion-associates, respectively. The coloured extracts were stable for more than 24 h in both examined systems.

Stoichiometry and formation constants of the ion-associates

The composition of the ion-associates was studied by Job's method of continuous variation and the mole ratio method. The results obtained reveal the formation of 1:2 and 1:3 CoTC:Hyo.Br and CrTC:Hyo.Br, respectively, Fig. 3. These results were confirmed by elemental analysis data of the solid ion-associates. The calculated values of C, H, N and Co% for $Hyo_2[Co(SCN)_4]$ ion-associate were 54.50, 5.90, 8.30 and 12.60, while the values found were 54.80, 5.40, 8.40 and 12.50, respectively. In case of $Hyo_3[Cr(SCN)_6]$ ion-associate, the calculated values of C, H, N and Cr% were 53.30, 5.80, 8.11 and 12.35 and the found values were 53.00, 5.70, 8.00 and 12.35%, respectively.

Fig. 2. Effect of shaking time on the extraction of the reaction products of Hyo.Br with CoTC and CrTC.

The stability constants of the formed ion-associate complexes were calculated according to the equation [36]:

$$K_f = (A/A_m) / [(1- A)/A_m]^{n+1} \, C^n \, n^n$$

where A is maximum absorbance obtained from Job's continuous variation curve, A_m is the absorbance corresponding to the intersection of the two tangents of the curve in Figure 3, C is the concentration corresponding to maximum absorbance, n is the amount of the drug in the reaction product. Using this equation, K_f was found to be 6.42×10^6 and 1.51×10^3 for Hyo-CoTC and Hyo-CrTC, respectively.

The Gibbs free energy change of the reaction was also calculated adopting the following equation:

$$\Delta G = -2.303 \, RT \log K_f$$

where ΔG is Gibbs free energy of the reaction, R is the universal gas constant, T is the absolute temperature and K_f is the formation constant of the reaction.

The values of ΔG were found to be -9.34 and -4.36 Kcal/mol for Hyo-CoTC and Hyo-CrTC, respectively. The negative sign of ΔG points out the spontaneous nature of the reactions.

Atomic absorption method

The method is based on precipitation of the drug with a slightly excess of CoTC or CrTC followed by measuring the absorbance of the unreacted metal ion in the filtrate. Accordingly, the quantitative determination of the drug could be performed.

The optimum pH and ionic strength values, at which the ion-associates exhibit the lowest solubility, have been investigated. The method based on addition of solid ion-associates to a series of solutions (each of 25 ml) of NaCl-HCl and NaCl-NaOH of different pH and ionic strength values. The solution-solid mixtures
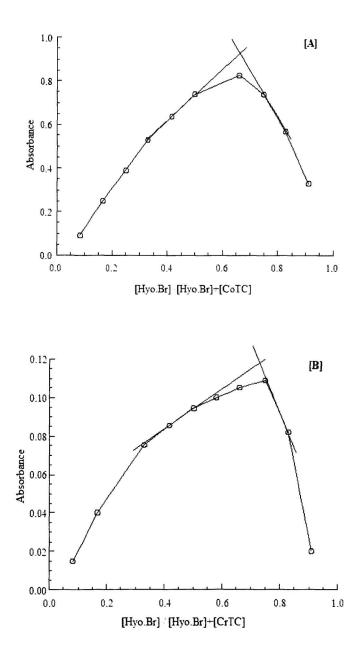
Fig. 3. The continuous variation plots for the stoichiometry of the reaction of Hyo.Br with CoTC [A] and CrTC [B].

were shaken for 4-6 hours and left to stand for at least one day, to attain the equilibrium, and then the metal ion content was measured using atomic absorption spectrometer. The results showed that the optimum pH and ionic strength values were 6.0 and 0.3 for Hyo-CoTC while 5.0 and 1.0 for Hyo-CrTC ion-associate.

Validation of methods
Spectrophotometric method

Under the optimized experimental conditions, there was a linear correlation between the absorbance and the drug concentration over the ranges 144.20-865.20 and 72.00-640.00 $\mu g/ml$ with molar absorptivities 3.56×10^2 and 0.90×10^2 l mol^{-1} cm^{-1} and sandell's sensitivities 1.24 and 4.90 $\mu g/cm^2$ for CoTC and CrTC, respectively, Table 1. The linear regression analysis using the method of least squares was applied to evaluate the slope, intercept and correlation coefficient values. The results, Table 1, showed good linearity of the calibration graphs ($r \geq 0.9977$) and the intercepts are close to zero. Linearity was also evaluated from the standard deviation of the slope (S_b) and found to be $\leq 8.09 \times 10^{-4}$. Optimization of the linear application ranges (Ringbom) was evaluated by plotting the percentage of transmittance vs. the logarithmic value of the concentration in $\mu g/ml$. The confidence interval of intercepts at 95% level were calculated and found to be 3.80×10^{-2} and 7.33×10^{-3} for CoTC and CrTC, respectively, which means that the calculated intercept is not significantly different from zero. Thus, the present method is free from constant errors independent of the concentration of Hyo.Br.

The sensitivity of the present method was checked by calculating the limit of detection and the limit of quantification which were found to be 5.56 and 14.03 $\mu g/ml$ for CoTC and 18.53 and 46.80 $\mu g/ml$ for CrTC, Table 1.

Precision as percentage of relative standard deviation (R.S.D.%) and accuracy as percentage relative error (R.E.%) of the suggested method were estimated by measuring different concentrations within the linear range. The results

showed good accuracy and high precision of the proposed extractive spectrophotometric procedure, Table 1.

The performance of the proposed method was compared with that of the official method (based on potentiometric titration of the drug with 0.1 N $AgNO_3$) [27] by applying t- and F-tests [37], Table 1. The results showed that the two methods are in comparable precision and there is no significant difference between the mean values obtained by the two methods.

Atomic absorption method

The ion-associates of a standard series of Hyo.Br were precipitated in 0.3 or 1.0 M NaCl solutions, adjusted to pH 6.0 or 5.0 with 0.001 M HCl, using an excess of CoTC and CrTC, respectively. The absorbance values of the reacted metal anion complex, equivalent to the reactant drug, were deduced from the difference between the absorbance of the blank solution (total metal anion complex) and those of the unreacted metal anion complexes. These values were plotted against the drug concentration, where linear calibration relations were obtained.

The present method is as good as the method reported in the British pharmacopoeia [27], and being applicable over relatively wider concentration ranges of Hyo.Br 0.404-1.617 and 0.040-0.283 mg/ml using CoTC and CrTC, respectively. Also, the proposed method has the advantage of high sensitivity for determination of Hyo.Br, as indicated by the characteristic concentration values (16.11 and 29.50 µg/ml for CoTC and CrTC, respectively). Low concentration of the drug can be determined by the present method as revealed from the values of the detection limits (10.78 and 2.70 µg/ml).

In order to determine the accuracy and the precision of the method, solutions containing three different concentrations of the drug were prepared and analyzed in quadruplicate. The standard errors and relative standard deviations values shown in Table 2 can be considered satisfactory, at least for the concentration levels examined.

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Parameter	CoTC	CrTC
Optimum medium	Aqueous	Aqueous
Extracting Solvent	Methylene chloride	Isobutyl alcohol
λ_{max} (nm)	630	557
Beer's law ($\mu g\ ml^{-1}$)	144.20-865.20	72.00-640.00
Ringbom range ($\mu g\ ml^{-1}$)	170.20-763.80	110.49-597.42
Molar absorptivity (L $mol^{-1}\ cm^{-1}$)	3.56×10^2	0.90×10^2
Sandell's sensitivity ($\mu g\ cm^{-2}$)	1.24	4.90
LOD ($\mu g\ ml^{-1}$)	5.56	14.03
LOQ ($\mu g\ ml^{-1}$)	18.53	46.80
Regression equation _Slope (a)_ _S.D. of slope (S_a)_ _tS_a^1_ _Intercept (b)_ _S.D. of intercept (S_b)_ _tS_b^1_ _Correlation coefficient (r)_	8.09×10^{-4} 2.71×10^{-5} 7.52×10^{-5} -1.50×10^{-2} 1.37×10^{-2} 3.80×10^{-2} 0.9977	2.04×10^{-4} 2.06×10^{-5} 5.72×10^{-6} 6.39×10^{-4} 7.33×10^{-3} 2.03×10^{-3} 0.9998
Recovery[2] ± S.D.%	99.66±0.63	99.37±0.17
R.E.%	-0.90	1.9
R.S.D.%	1.06	1.44
_t_-value _(3.18)[3]_	0.28	0.44
_F_-value _(9.28)[4]_	2.17	1.15

LOD: limit of detection   LOQ: limit of quantification   R.E.: Relative error
Y= a X + b where X: is the concentration in $\mu g/ml$, Y: absorbance, a: slope and b: intercept.
[1]: Confidence interval of intercept and slope at 95% confidence level
[2]: Mean value for four replicate analyses within the linear limits.
[3]: Tabulated t-value at three degrees of freedom and 95% confidence level.
[4]: Tabulated F-value at three degrees of freedom and 95% confidence level.

Tab. 1. Spectrophotometric characteristics and statistical data of the regression equations

In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression of observed drug concentration against

the theoretical values obtained using the official method was calculated. The Student t-test (at 95% confidence level) and F-test were applied [37]. The calculated t and F-values were found be lower than the tabulated values at 95% confidence limit. This means that there is no systematic difference between the determined and true concentrations; thus, the proposed method is of the same accuracy as the official method [27]. The results of statistical treatment of data are presented in Table 2.

Parameter	CoTC	CrTC
pH	6.00	5.00
Ionic strength (μ)	0.30	1.00
Linear range (mg ml^{-1})	0.404-1.617	0.040-0.283
Characteristic concentration (μg ml^{-1})	16.11	29.50
Detection limit (μg ml^{-1})	10.78	2.70
Regression equation *Slope (a)* *Intercept (b)* *Correlation coefficient (r)*	0.9962 -4.80x10^{-2} 0.9999	0.9985 1.65x10^{-3} 0.9999
Mean Recovery ± S.D.%	99.61±0.75	99.67±0.56
R.E.%	-0.39	-0.33
R.S.D.[1]%	1.47	0.98
t-value *(3.18)*[2]	0.28	0.26
F-value *(9.28)*[3]	1.22	2.77

a: slope and b: intercept of regression equation for the theoretical and the observed mg of Hyo.Br
[1]: For four replicate analyses within the linear limits.
[2]: Tabulated t-value at three degrees of freedom and 95% confidence level.
[3]: Tabulated F-value at three degrees of freedom and 95% confidence level.

Tab. 2. Analytical characteristics of the atomic absorption method and statistical data of regression analysis

Interferences

No interference was observed in the determination of Hyo.Br with CoTC and CrTC from the presence of camphor, menthol, glucose, lactose, sucrose, starch,

and magnesium stearte (which may be present in its pharmaceutical preparation) applying spectrophotometric and atomic absorption spectrometric procedures. The results indicate that up to 50 fold excess do not interfere (absorbance changes by ±3.0% in the absorbance of is non-interference) which may be present in its pharmaceutical preparation.

Analytical Applications

Determination of Hyo.Br in its dosage form checks the validity of the proposed methods. Buscupan injection (20 mg Hyo.Br/ml) was analyzed spectrophotometrically and by atomic absorption using the mentioned reagents. The results were recorded in Table 3, and compared statistically with the official method [27], reveal that recoveries were in the range 99.65±1.50 to 101.60±0.18 reflecting a high accuracy, in addition to the high precision indicated by very low values of relative standard deviations (≤ 1.84).

The validity of the proposed methods was evaluated by statistical analysis between the results obtained and that of the official method [27]. Regarding the calculated student's t-test and variance ratio F-test (Table 3), there is no significant difference between the proposed and the official method regarding the accuracy and precision.

Comparison with other methods

The performance of the proposed method was compared with those of other existing spectrophotometric methods (Table 4) that reveals that the proposed methods are of comparable sensitivity, precision and accuracy, moreover the present methods are of better dynamic range. Therefore, following the recommended procedures, one can easily assay Hyo.Br within short time (5-15 min) of mixing the reagents in an aqueous (atomic absorption procedure) and non-aqueous (spectrophotometric procedure) media.

Conclusion

The proposed methods are simple and rapid. They do not involve tedious procedural steps or expertise like HPLC and other methods. In addition, the reagents and equipment used in the present study are inexpensive and common in most of the quality control laboratories.

Hyo.Br reacts with CoTC and CrTC complexes forming coloured ion-associate compounds with molar ratio 2:1 and 3:1, respectively. The suggested spectrophotometric methods enable the determination of Hyo.Br in the ranges 144.20-865.20 and 72.00-640 µg/ml using Hyo-CoTC and Hyo-CrTC systems, respectively. The CoTC method is more sensitive as indicated by the molar absorptivity ($\varepsilon = 3.56 \times 10^2$ L mol^{-1} cm^{-1}) and LOD (5.56 µg/ml) values, Table 1. In case of the atomic absorption method, although CoTC is applied for a wider concentration range (0.404-1.617 mg/ml) of Hyo.Br, CrTC is more sensitive as it determines 0.040-0.283 mg/ml and showed a lower detection limit (2.70 µg/ml).

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	Claimed amount (mg/ml)	Found in sample (mg/ml)[a]	Recovery % ± S.E.	R.S.D. %	t-value	F-value
Buscopan injection (CID, Company, Egypt)	20					
Spectrophotometric procedure						
CoTC		20.22	101.10±0.13	1.79	1.45	2.07
CrTC		20.12	100.61±0.23	1.08	1.08	1.88
Atomic absorption procedure						
CoTC		20.32	101.60±0.18	0.96	1.23	3.54
CrTC		19.93	99.65±1.50	1.84	0.36	1.76
Official method [27]		19.99	99.95±0.93	1.62	(3.18)[1]	(9.28)[2]

a: Average value of four determinations.
S.E.: Standard error
[1]: Tabulated t-value at three degrees of freedom and 95% confidence level.
[2]: Tabulated F-value at three degrees of freedom and 95% confidence level.

Tab. 3. Determination of Hyo.Br in commercial injection solution using the proposed procedures compared statistically with official method

Reagent	Medium	λ_{max} (nm)	Range[a]	ε[b]	Reco-very%	R.S. D.%	Ref.
Direct spectrophotometry							
Reineckate	Acetone	306.2	0.20-4.50	nr	99.30	0.76	[1]
Extractive spectrophotometry							
Bromocresol purple	Chloroform	408	200-600	nr	97.0-101.0	nr	[2]
Bromocresol green	Methylene chloride	400	4.00-35.00	nr	nr	nr	[3]
Methyl orange	Methylene chloride	440	4.00-44.00	nr	nr	nr	[38]
Tetrathiocyanato-cobaltate	Methylene chloride	630	144.20-865.20	3.56x 10^2	99.66 ±0.63	1.06	Present method
Hexathiocyanato-chromate	Isobutyl alcohol	557	72.00-640.00	0.90x 10^2	99.37 ±0.17	1.44	Present method
First derivative spectrophotometry							
No reagent	Aqueous	219.8	4.00-27.80	nr	99.80	0.08	[1]
Picric acid	Chloroform	300-500	5.00-20.00	nr	99.50-100.6	nr	[4]
Second derivative spectrophotometry							
0.1 M HCl		212.5	3.50-20.00	nr	99.15 ±1.48	0.08	[5]
Sodium tetraphenylborate	1,2-Dichloroeth ane	UV	1.20 mg	nr	nr	nr	[6]
Indirect spectrometric methods							
Tetrathiocyanato-cobaltate	Aqueous	240.7	0.404-1.617 mg/ml	nr	99.61 ±0.75	1.47	Present method
Hexathiocyanato-chromate	Aqueous	357.9	0.040-0.283 mg/ml	nr	99.67 ±0.56	0.98	Present method

[a]: All the application ranges mention in the table are expressed in µg/ml except when it was mentioned; [b]: *L.mol⁻¹.cm⁻¹*, *nr*: not reported

Tab. 4. Comparison of reagents of previously reported spectrophotometric methods with that of the proposed method for Hyo.Br determination

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