Atomic Absorption and Spectrophotometeric Determinations of Salicylhydroxamic Acid in Its Pure and Pharmaceutical Dosage Forms

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A new method for the indirect determination of salicylhydroxamic acid (SHAM) using atomic absorption spectrometry (AAS) was proposed. The method is based on precipitating the ion associate complex of SHAM with $[Cu(NH_3)_4]^{2+}$. The excess, unreacted, Cu^{2+} ions were determined using AAS.

Another spectrophotometric method based on measuring the absorbance of the formed $[Cu(NH_3)_4]$-SHAM complex in dioxane was proposed. The green color of the complex formed was measured at 330 nm.

The two methods were successfully used for determining SHAM in its pure and pharmaceutical dosage forms. Using AAS, the drug was determined over a concentration range of 3.06 to 30.63 μg/mL with an average relative standard deviation of 1.6 to 2.2% and a recovery of 97.52 to 102.4%. The spectrophotometric method gave a linear concentration range of 1.53 to 18.38 μg/mL with an average relative standard deviation of 1.2 to 1.3% and recovery of 96.73 to 101.31%.

The results obtained showed that the methods developed are precise and accurate. They are shown to be suitable for routine SHAM quality control in its pure and pharmaceutical dosage forms.

Key Words: Atomic absorption spectrometry (AAS), spectrophotometry, SHAM, salicylhydroxamic acid, drug analysis, Cu complexes.

Introduction

Salicylhydroxamic acid (SHAM) has been reported to prevent the formation of mucopolysaccharides sulfate and consequently prevents the formation of calcium mucopolysaccharides sulfate which upon aggregation with Ca^{++} forms calcium oxalate stones[1]. SHAM also prevents the formation of phosphate stones by

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inhibiting urease enzyme activity. The splitting of urea to ammonia and carbon dioxide is then catalyzed by the urease enzyme in cases of urinary tract infection. By inhibiting urease activity, SHAM reduces ammonia formation and keeps urea acidic. It also reduces serum uric acid and reduces the incidence of ureate and uric stones[1].

SHAM has a potent antispasmodic action on urethras and prevents renal colic. Its analgesic effect is 1.65 times more potent than that of acetylsalicylic acid, whereas its antipyretic effect is weaker than that of acetylsalicylic acid[1].

SHAM has a salicylate and hydroxamic acid moieties (Form 1). It is a potent chelating agent for Ni^{++}, Ca^{++}, Fe^{++}, Co^{++} etc. with stability constants decreasing in the order Ni > Ca > Fe.

Talipova et al. have spectrophotometrically studied the reaction of rhenium(VII) with salicylhydroxamic acid in hydrochloric acid solution[2]. Ti(IV) extracted from mineral samples and aluminum alloys was determined photometrically as a Ti(IV)-salicylhydroxamic acid complex[3]. Hydroxamic acid was used as a chelatometric indicator in determining iron(III)[4]. Traces of molybdenum and tungsten were determined by extraction followed by the polarographic measurement of their salicylhydroxamates[5].

Salicylhydroxamic acid was used for the liquid-liquid extraction separation of iridium in the presence of rhodium and ruthenium using iso-butanol[6]. It was also used for determining vanadium in its steel alloy and vanadium ores[7]. Vanadium traces were determined using 5-bromo-salicylhydroxamic acid by solid phase spectrometry[8]. The vibrational spectra and thermodynamic functions of salicylhydroxamic acid have also been reported[9]. Synthesis and vibrational studies of molybdenum di-nitrosyl complexes with hydroxamic acids were reported[10]. Khadikar et al. have studied crystalline bis-salicylhydroxamato chelates of bivalent metal ions by low-frequency IR spectroscopy[11].

Salicylhydroxamic acid was determined using reversed phase high performance liquid chromatography[12]. It was also determined by measuring the absorbance of its V(V) complex at 620 nm. A detection limit at a concentration as low as 50 μM was reported[13]. A potentimetric determination of salicylhydroxamic acid based on the inhibition of urease activity was also reported[14]. A linear calibration graph in the range of 0.5 to 7 μg/mL with a detection limit of 0.1 μg/mL was obtained. Shetty et al. have used HPLC for determining salicylhydroxamic acid and its metabolites in rat urine[15]. Cu(II) chelates of some biologically active ligands such as salicylhydroxamic, salicylamide and gallic acid were studied by ESR[16].

Capitan et al. have reported the use of salicylhydroxamic acid in spectrophotometric extractive determination of Ti(IV) in mineral samples and aluminum alloys. Ti(IV)-salicylhydroxamic acid complex and its mixed ligand, Ti(IV) - salicylhydroxamic acid –thiocyanate complex, were formed and measured[17].

Atomic absorption spectrometry (AAS) occurs in the forefront of the most sensitive and widely used analytical techniques. The reason is attributed to its low detection limit, simplicity, reproducibility and low running costs compared to other analytical tools. Spectrophotometry is well known for its simplicity, availability and fast use for large numbers of analytical determinations.

The aim of this investigation was to develop new methods for SHAM determination using AAS and spectrophotometry. The AAS method is based on precipitating $[Cu(NH_3)_4]^{2+}$-SHAM ion associate complex by adding excess $[Cu(NH_3)_4]^{2+}$ to SHAM solution. Excess, unreacted, Cu^{2+} ions in the supernatant solutions will be determined using AAS. The spectrophotometric method is based on measuring the green color developed upon adding $[Cu(NH_3)_4]^{2+}$ to SHAM solution in 50% dioxane-water. Both methods will be compared with each other and with published ones.

Experimental

Apparatus

pH values were measured using an Orion Research digital pH meter, Model 601A, with an accuracy of $\pm$ 0.05 pH units.

AAS measurements were carried out using a Shimadzu Atomic Absorption/Flame Emission spectrometer, type AA-625-01 (Kyoto, Japan).

A Perkin Elmer Lambda 3B double beam UV - VIS and a computerized Shimadzu 1201 UV - VIS single beam spectrophotometer with 1 cm quartz or matched silica cells were used for absorbance measurements.

Conductometric measurements were carried out using a YSI model 32 M conductivity meter with cell constant K = 1 (TOA Electronic Ltd., Japan).

Materials

Double distilled water from glassware and twice distilled absolute ethyl alcohol were used. Dioxane, methanol and copper sulfate were supplied by Aldrich. All reagents were of analytical grade.

Pharmaceutical preparations

A SHAM sample of 99.9% purity was provided by the El Nasr Company for pharmaceutical and chemical industries, Cairo, Egypt.

SHAM capsules containing 300 or 600 mg per capsule produced by the El Nasr Company for pharmaceutical and chemical industries, Cairo, Egypt, were purchased locally.

Reagents

A 10^{-3} M SHAM solution was prepared by dissolving the accurate weight of the drug into dilute methanolic ammonium hydroxide or ethanol. Similarly, 10^{-3} M solutions of [Cu (NH$_3$)$_4$] SO$_4$ were prepared by dissolving the required amounts of CuSO$_4$ into distilled water and adding ammonium hydroxide until a permanent blue color was achived[18]. A 0.1 M NaCl solution, adjusted to the required pH, was used as a filling solution to adjust the pH and ionic strength.

Working solutions of lower concentrations were prepared by dilution from the stock standard solutions.

Standard solution for AAS

A standard solution (1000 μg/mL) of Cu(II) was prepared by transferring 2.528 g of anhydrous pure copper sulfate into a 1000 mL measuring flask; 50 mL of concentrated nitric acid was added. The solution was well shaken and made up to the mark with distilled water. The resulting solution, stored in a plastic bottle, is stable for approximately 1 year.

Analytical procedures

Atomic absorption

Measurement parameters

Cu(II) was measured by AAS in the absorption mode at 324.8 nm using air/acetylene. A slit width of 1.9 Å was used. The relative noise was 1.0 with an approximate absorption sensitivity of 0.15 μg/mL. The lamp current was 10 mA with a burner height of 4 cm and gas flow of 2.3 mL/s.

Calibration graph for AAS

A calibration graph was constructed using the standard copper solution previously prepared. Solutions having concentrations of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 μg/mL Cu^{2+} were measured. Each measurement was performed at least four times to check the reproducibility.

Conductometric titration

The stoichiometric composition of copper associate complex was identified by conductometric titration of 50 mL (4.0×10^{-5} M) SHAM in ethyl alcohol with a 10^{-3} M solution of [Cu (NH$_3$)$_4$] SO$_4$. A 1:1 associate complex ([Cu(NH$_3$)$_4$]:SHAM) was obtained. This ratio was confirmed by elemental analysis, molar ratio and continuous variation methods (Table 1).

Table 1. Composition, molar ratio, color and elemental analysis of Cu(NH$_3$)$_4$]$^{2+}$-SHAM ion associate complexe.

Ion Assoc. Composition	M.R.	Color	Calculated (Found)			
			C%	H%	N%	M%
[Cu (NH$_3$)$_4$](C$_7$H$_5$N$_1$O$_3$)	1:1	Green	30.27	6.13	25.22	22.90
			(29.00)	(6.20)	(25.00)	(23.00)

Preparation of ion associate complex

Copper ion associate complex was prepared by mixing 0.002 mol of SHAM with 0.002 mol of [Cu (NH$_3$)$_4$] SO$_4$ in methanol. The precipitated complex was filtered, washed and dried. Confirmation of the composition was performed using C, H, N and metal content elemental analysis (Table 1).

Effect of pH and ionic strength

The effects of pH and ionic strength on the solubility of prepared SHAM-ion associate complex were examined by measuring dissociated Cu^{2+} ion concentration at different pH and ionic strength values. The lowest solubility was recorded at an ionic strength of 0.1 and pH of 7.0 (Figure 1).

Determination procedures

Atomic absorption

Authentic sample (general procedure)

Into 50 mL measuring flasks were transferred successive aliquots of 1.0 to 10.0 mL of 10^{-3} M ammoniacal SHAM solutions. To each flask was added 1.0 mL of 10^{-2} M [Cu (NH$_3$)$_4$] SO$_4$. Flasks were made up to the mark with optimum pH and ionic strength solutions. The resulting solutions were shaken well and left to stand for 5 h. The precipitates were filtered through a Whatman P/S filter paper (12.5 cm).

The 2.0 mL aliquots of concentrated HNO_3 were added to each filtrate and its Cu^{2+} content was measured by AAS using the previously mentioned measuring parameters. The metal ions consumed in complex formation were calculated by subtraction. This value is equivalent to the SHAM concentration in the sample. Thus, SHAM was indirectly determined.
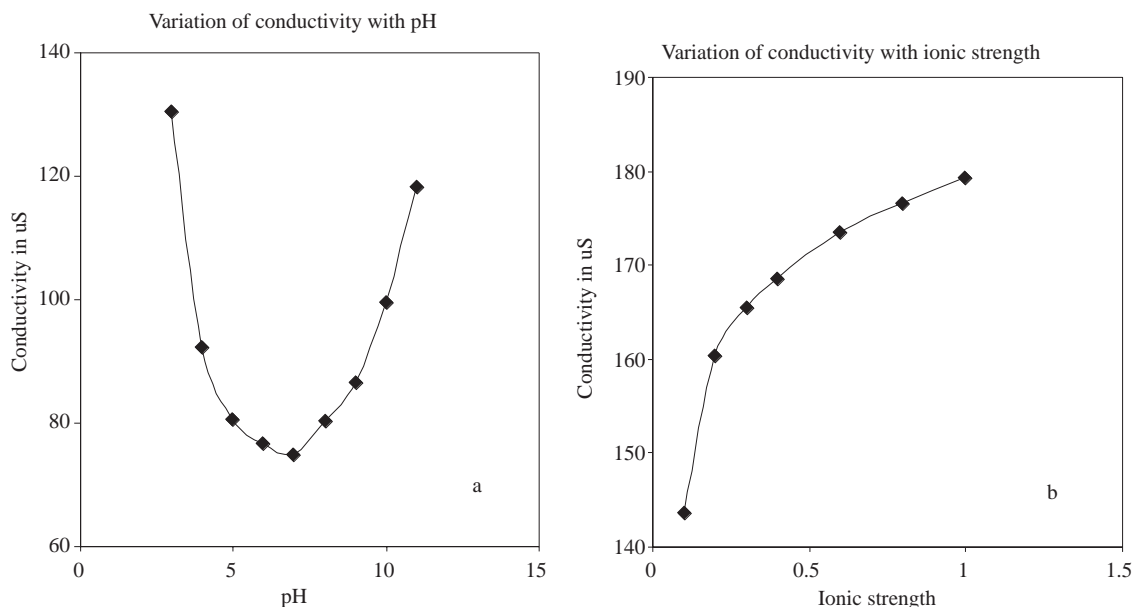

Figure 1. Effects of pH and ionic strength on conductivity of $Cu(NH_3)_4$-SHAM ion associate complex.

Pharmaceutical preparations

To determine SHAM in pharmaceutical preparations, 10 capsules were carefully evacuated and ground to a fine powder. Powder aliquots containing 0.1-1.6 mg of SHAM were transferred to 50 mL measuring flasks. The analysis was performed as in the general procedure.

Spectrophotometry

Authentic sample (general procedure)

Into 10 mL measuring flasks were transferred successive aliquots of 0.1-1.2 mL of 10^{-3}M SHAM containing 1.53 to 18.38 μg/mL. For each flask, 2 mL aliquots of 10^{-3} M Cu $(NH_3)_4SO_4$ and 5 mL of 1,4-dioxane were added. Solutions were made up to 10 mL with distilled water. The solutions were well shaken and absorbance was measured at 330 nm against 1,4 dioxane as a reagent blank. A calibration graph was plotted as absorbance versus concentration.

Pharmaceutical preparations

An accurate weight of finely ground capsules', powder containing 15.314 mg of SHAM was dissolved into dilute methanolic ammonia solution and transferred into a 100 mL measuring flask. The solution was made up to the mark by the same solution. Into 10 mL measuring flasks were transferred successive aliquots of 0.1-1.2 mL of this solution. Cu $(NH_3)_4SO_4$ and 1,4-dioxane were added and measurements were performed as in the general procedure.

Results and Discussion

A-Atomic Absorption

The elemental analysis given in Table 1 shows that SHAM forms a 1:1 (reagent:drug) complex with [Cu $(NH_3)_4$]$^{2+}$. After comparing stability at different pH and ionic strength values, we found that the formed complex is stable (pK$_{sp}$ = 10.89) at pH = 7.0 and ionic strength = 0.1.

Table 2 shows optimum pH and ionic strength for the least solubility of the prepared ion associate complex. Solubility of the ion associates was determined by putting a certain amount of the complex in the solution of required pH and ionic strength for several days. The amount of Cu^{2+} ions released was measured by AAS. A solubility value of $10^{-5.45}$ M and a solubility product of $10^{-10.98}$ were obtained, indicating a relatively high stability complex.

Table 2. Optimum pH and ionic strength for least solubility (pS) and solubility product (pK$_{sp}$) of Cu(NH$_3$)$_4$]$^{2+}$-SHAM ion associate complex at 25 °C.

| Ion Associate	pH	I	pS	PK$_{sp}$
[Cu $(NH_3)_4$]$(C_7H_5N_1O_3)$	7.0	0.1	5.45	10.98

pS = -log (solubility), (S).

pK$_{sp}$ = - log (solubility product), (K$_{sp}$).

Conductometric titration

The stoichiometric composition of the copper associate complex was further confirmed by conductometric titration. A 1:1 (reagent:drug) ratio was obtained.

Reaction mechanism

The formation mechanism of copper associate complex in alkaline medium is represented in Form 1. SHAM is converted into doubly charged negative species in alkaline solution by hydrogen ionization. In the presence of divalent metal ion complex, an associate complex is precipitated with the formula given in Table 2.

Form 1
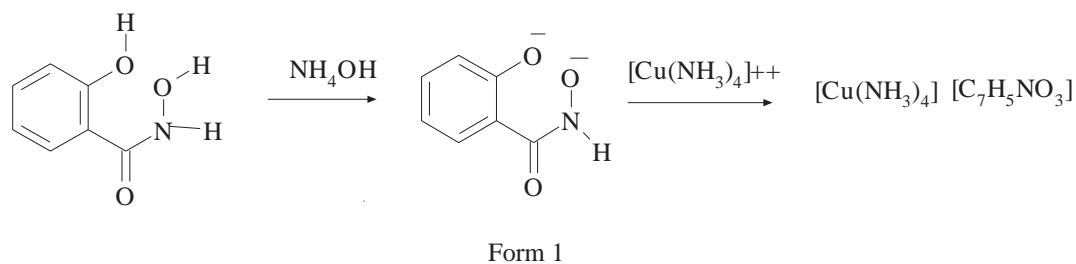
Calibration graph

AAS measurements for three series of standard solutions of Cu^{2+} ions were measured. Straight line calibration graphs over a concentration range of 2-7 μg/mL Cu^{2+} were obtained. The corresponding regression equation gave Y= 0.0352 X, where Y is the absorbance and X is the concentration in μg/mL. The calibration curve shows a correlation coefficient of 0.944 (Figure 2).
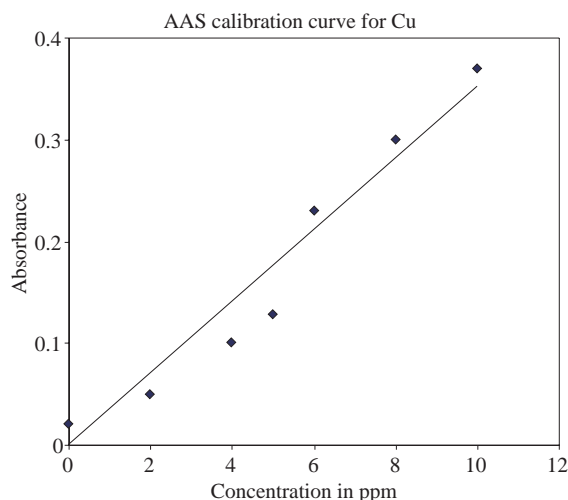
Figure 2. Atomic absorption calibration curve for Cu(II) ions.

Determination of SHAM in authentic samples

SHAM in pure solution was determined precisely and accurately over the concentration range 3.06-30.63 μg/mL using the proposed method under the optimum pH and ionic strength conditions given in Table 2.

The results revealed SHAM recoveries in the range of 99.50 to 102.31%, indicating good accuracy (Table 3). The low values of relative standard deviation reflect the precision of the method. The 3.06-30.63 μg/mL linear dynamic range of the proposed method indicates a relatively wide concentration range. Student's t-test at the 95% confidence limit gave t-values < 2.0, indicating a statistically insignificant difference between the true (taken) and found (measured) values.

Table 3. Determination of SHAM in authentic samples and in pharmaceutical preparations using AAS and spectrophotometry.

Sample	Taken (μg.mL^{-1})	Recovery%	Mean RSD%	t-test t	F-test F^1	F^2
Atomic Absorption						
Authentic	3.06-30.63	99.50-102.31	1.60	0.63-2.89	1.51	3.52
300 mg capsules [a]	3.06-30.63	98.00-102.40	2.16	1.85-2.22	3.24	1.93
600 mg capsules [a]	3.06-30.63	97.52-101.31	2.20	1.19-2.26	3.36	1.86
Spectrophotometry						
Authentic	1.53-18.38	96.73-101.31	1.30	2.02-5.03	1.51	5.33
300 mg capsules [a]	1.53-18.38	96.73-101.31	1.20	2.18-5.45	3.24	6.25
600 mg capsules [a]	1.53-18.38	96.73-101.31	1.20	2.18-5.45	3.36	6.25

RSD: Relative standard deviation (four determinations).

[a] El Nasr Co for Pharm. and Chem. Ind., Cairo, Egypt.

F^1 based on comparing standard deviations of the proposed methods.

F^2 Based on comparing standard deviations of the proposed methods with the method in reference [14].

Determination of SHAM in pharmaceutical preparations

SHAM was determined in pharmaceutical preparations using the proposed method at the recommended pH and ionic strength (Table 3). Recoveries obtained are in the range of 97.52 to 102.40%, showing the fair accuracy of the method. Relative standard deviation% ≤ 2.2 reflects its high precision. SHAM capsules was determined over a concentration range of 3.06 to 30.62 μg/mL. Student's t-test at the 95% confidence limit gave t-values < 0.98 indicating a statistically insignificant difference between the true and measured values.

Selectivity

Since the formation of ion associate requires the existence of negatively charged species in the presence of $[\text{Cu (NH}_3)_4]^{2+}$, this species could only be produced by the hydrolysis of SHAM. Other expedients and fillers added to the pharmaceutical preparation will not interfere. Thus, no extraction was needed to separate SHAM from the drug matrix.

B-Spectrophotometry

Stoichiometry

The molar ratio of copper ion associate complex was again confirmed by Job's method of continuous variation and by the molar ratio method. The results indicated the formation of a 1:1 (reagent:drug) complex (Figure 3).
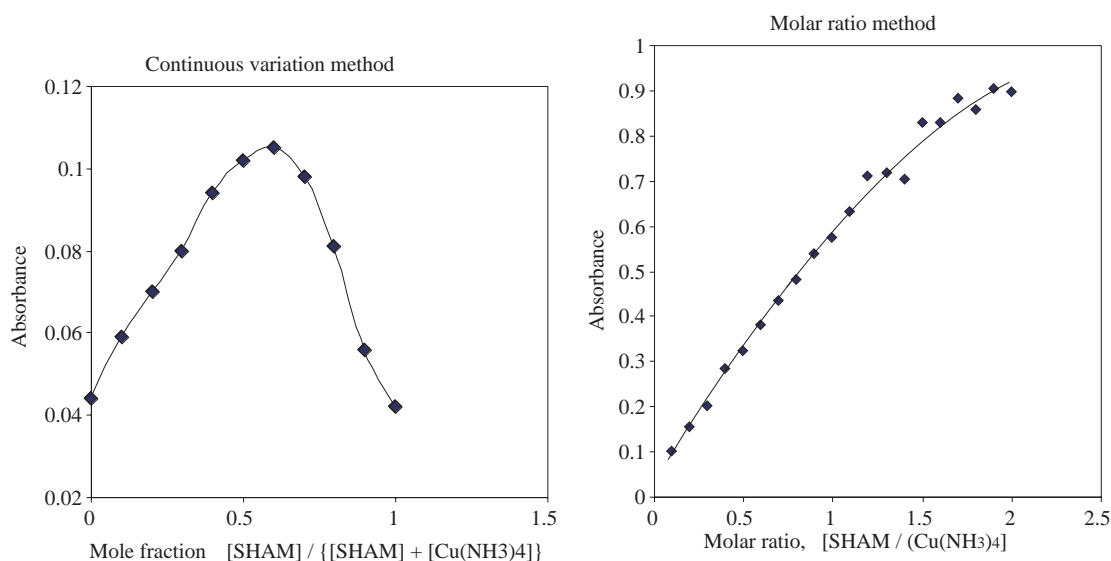

Figure 3. Molar ratio and continuous variation plots for $\text{Cu(NH}_3)_4$-SHAM ion associate complex.

Calibration graph (Beer's law)

Four series of standard solutions were measured by adding successive increments (0.1 mL) of 10^{-3}M SHAM to 1.2 mL of 10^{-3} M, $[\text{Cu (NH}_3)_4]^{2+}$ as mentioned in the general procedure. A linear calibration graph was obtained in the concentration range of 1.53-18.37 μg/mL SHAM (Figure 4). A more accurate range was calculated using a Ringbom calibration graph based on plotting T% versus log concentration in μg/mL. A concentration range of 2.0 to 18.0 μg/mL was obtained.

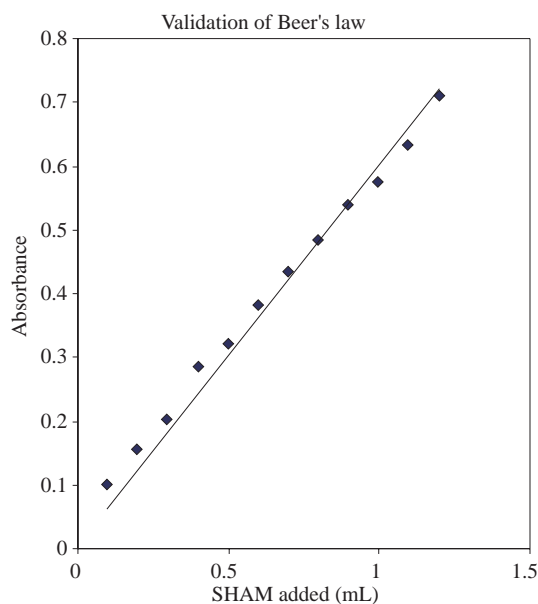**Figure 4**. Spectrophotometric Calibration curve for $Cu(NH_3)_4$-SHAM ion associate complex.

Precision values as given by the relative standard deviations (RSD%) are depicted in Table 3. Slope, intercept, correlation coefficient, molar absorptivity and Sandell sensitivity index were calculated and given in Table 4.

Table 4. Spectral characteristics of SHAM–$[Cu(NH_3)_4]^{2+}$ complex calibration curve.

Parameter	Value
Range	1.53-18.37 μg/mL
Slope	0.60
Intercept	0.01
Correlation coefficient[a]	0.99
Molar absorptivity	4.8×10^3 L.mol^{-1}.cm^{-1}
Specific absorptivity	0.031 L.g^{-1}.cm^{-1}
Sandell sensitivity	0.032 μg/mL

[a] Twelve points, each of which was determined three times at least covering a range of 1.5-18.37 μg/mL SHAM.

Applying the least-square's method to four calibration graphs gave confidence limits for the slope and intercept using a significant test at the 0.05 probability level. The regression line is represented by

$$\mathbf{Y = (0.5999 \pm 0.02)\ X}$$

where X is the average reference assay and Y is the average absorbance. This equation can be utilized for determining SHAM in pure samples, SHAM capsules or any synthetic laboratory mixture. A low quantification limit (LOQ) of 1.53 μg/mL was detected.

Pharmaceutical preparation

The validity of the developed methods was checked by determining SHAM in pharmaceutical preparations. The results obtained are given in Table 3.

Applying the least-squares method to four determinations gave a regression line equation (taken vs. found) represented by

$$Y = 0.9822\ X \pm 0.02$$

where X is the average reference assay (taken) and Y is the average of the measured values (found). The relative average errors were found to be in the range 1.31-1.96%. An average recovery of 96.73-101.31% and relative standard deviation of 0.05-0.08 were obtained. Variation coefficients ranged from 1.07 to 5.08 at 0.18-0.54 μg/mL of SHAM. These values indicate the accuracy and precision of the developed method.

Precision

The mean relative standard deviations for the four determinations are ≤ 2.2 for the atomic absorption method and ≤ 1.30 for the spectrophotometric method. This level of precision is quite suitable for the quality control analysis of pharmaceutical preparations and pure compounds.

Applying Student's t-tests for four determinations on pure SHAM gave t-values of 0.63-2.89 and 2.02-5.45 using AAS and spectrophotometry, respectively. The corresponding t-values for 300 and 600 mg capsules are 1.85-2.22 and 1.19-2.26, respectively using AAS and 2.18-5.45 for both capsules using spectrophotometry. These values indicate a statistically insignificant difference between the mean of measured concentration and real content at the 95% confidence level.

Comparing developed AAS and spectrophotometric methods using the F-test gave average F-values of 1.51, 3.24 and 3.26 for pure, 300 and 600 mg capsules, respectively.

Comparing each of the developed methods with the potentiometric method[14] gave F-values of 1.86-3.52 for AAS and 5.33-6.25 for spectrophotometry. These values indicate an insignificant difference in precision between each of the two proposed methods and the potentiometric one at the 95% confidence level (Table 3).

This indicates that the proposed methods are accurate and precise and can be used simply for routine work in drug control laboratories.

Conclusion

This paper reports a new example of ion associate complex application in drug analysis. AAS has the advantages of being fast and simple compared to other analytical techniques. The AAS method showed wide dynamic range, high sensitivity, low quantification limit and no interference.

The spectrophotometric method, based on measuring the absorbance of formed complex in 50% 1,4-dioxane at 330 nm, was successfully utilized for determining SHAM in its pure form as well as in pharmaceutical preparations. A quantification limit of 1.53 μg/mL and a smaller dynamic range of 1.53-18.38 were obtained.

The proposed methods proved to be sensitive, accurate and precise, as well as simple to handle with higher tolerance limits relative to previously published methods.

Student's t-values indicate the absence of systematic errors. F-values indicate the absence of significant differences in precision between both proposed methods and between each of the proposed and potentiometric methods[14].

Therefore, these two methods can be safely used for the quality control of SHAM in its pure form and in pharmaceutical formulations. The methods can be carried out without the pretreatment of pharmaceutical samples.

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