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## Full Length Article

# New, simple, sensitive and validated spectrophotometric method for the determination of salicylhydroxamic acid in capsules and raw material according to the ICH guidelines



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## ABSTRACT

This work was aimed to develop a rapid, simple, selective, and precise spectrophotometric method for the estimation of salicylhydroxamic acid found in both capsules and raw materials. Spectrophotometric detection was conducted at maximum absorption of 294 nm with the aids of methanol: water (1:99, v/v) as solvent. The figures of merit of the newly developed method were validated for linearity, specificity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ). The detector response for the salicylhydroxamic acid was linear over the concentration range studied 0.1–50  $\mu\text{g mL}^{-1}$  with a correlation coefficient ( $R^2$ ) of 0.9999. Accuracy was between 99.0% and 101.7% with a mean value of 100.07%. The intra- and inter-day precisions, expressed as relative standard deviation (R.S.D.), were less than 0.032% and 0.421%, respectively. LOD and LOQ were 0.031 and 0.098  $\mu\text{g mL}^{-1}$ , respectively. Results confirmed that the excipients in the commercial capsules did not interfere with the method and can be employed for routine quality control analysis of salicylhydroxamic acid whether in capsules or raw materials.

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## 1. Introduction

Salicylhydroxamic acid (SHAM) ( $\text{C}_7\text{H}_7\text{NO}_3$ ), which is a phenolic compound [1–3], is considered an intense and irreversible inhibitor used against bacteria. Additionally plant urease generally utilized for urinary tract diseases and known for its pharmaceutical applications (Fig. 1). This structure of SHAM is similar to urea, however, is not hydrolyzable by the urease [3–8]. It prevents the development/formation of calcium oxalate stones in kidneys [9,10]. At the point when administered orally, it is metabolized to salicylamide which applies pain relieving, antipyretic and anti-inflammatory effects. SHAM is additionally viewed as a typical ligand that utilized as a part of the combination of metal-crowns [4]. The mechanism of action of SHAM to stop the development of phosphate stones is by back off urease catalyst action [9]. The change procedure of urea to carbon dioxide and ammonia is then catalyzed by the impact of urease enzyme in the case of occurrence of urinary tract disease. When urease action is restrained, SHAM suppress ammonia formation and holds urea acidic [9]. Moreover,

it diminishes serum uric acid and the rate of uric stones and ureate [11].

Literature search uncover that few methods for the estimation of SHAM have been accounted for whether the kinetics of the acid and base hydrolysis of SHAM and O-acetyl-salicylhydroxamic acid (OAc-SHAM) at various parameters, for example, time, temperature and pH, utilizing reversed phase performance liquid chromatography with UV detector (RP-HPLC/UV) [9], or for assessing SHAM in entire blood utilizing HPLC/UV [13].

SHAM was additionally evaluated by measuring the absorbance of its V(V) complex at 620 nm and limit of detection was 50  $\mu\text{M}$  [14]. Moreover, potentiometric method for the estimation of SHAM in light of the inhibition of urease action was likewise conducted [15]. A linear calibration curve between 0.5 and 7  $\mu\text{g mL}^{-1}$  with a limit of detection of 0.1  $\mu\text{g mL}^{-1}$  was achieved. Shetty et al. utilized HPLC for measuring SHAM and its metabolites in the urine of rat [16]. Cu(II) can chelate some active ligands, for example, salicylamide, salicylhydroxamic and gallic acid which were likewise analyzed by electron spin resonance (ESR) [17]. Besides, Capitan et al. have reported the utilization of SHAM in spectrophotometric methods for determination of Ti(IV) in aluminum alloys and mineral specimens as well. The complex Ti(IV)-SHAM and its blended ligand namely, Ti(IV)-SHAM – thiocyanate complex, were

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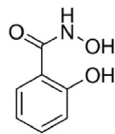


Fig. 1. The structure of salicylhydroxamic acid ( $pK_a$  7.40 and 9.70) [12].

estimated [18]. On the other hand, Salem created a few methods for the estimation of SHAM utilizing atomic absorption spectrophotometry (AAS) and spectrophotometry methods. The AAS technique depends on precipitating the  $[Cu(NH_3)_4]^{2+}$ -SHAM complex with the aid of excess of  $[Cu(NH_3)_4]^{2+}$  to SHAM solution. The  $Cu^{2+}$  ion in the supernatant layer was determined by AAS. The spectrophotometric technique is relies on upon measuring the green color produced by adding  $[Cu(NH_3)_4]^{2+}$  to SHAM solution consisted of 50% dioxane: water solution [19].

Spectrophotometry is notable for its ease of use, minimal effort, low cost, and availability in all research centers. In addition, the pretreatment steps prior sample preparation is at times essential prior the application of such technique in order to overcome sample interferences and to preconcentrate the analyte before subjected to analysis [19–21].

The target of the present work is to create simple, fast, specific and accurate UV spectrophotometric method for the estimation of SHAM in raw materials and capsules. This method was additionally validated for the accompanying parameters, for example, linearity, accuracy, precision, sensitivity, ruggedness, and robustness. The limits of detection (LOD), as well as quantification (LOQ), were also determined. Also, the experimental parameters for the developed method were validated in accordance with the International Conference on Harmonization (ICH) rules Q2 (R1) (ICH, 2005) [22]. The created method is consequently recommended for the normal routine analysis in the quality control unit.

## 2. Experimental

### 2.1. Materials and chemicals

Salicylhydroxamic acid working standard (>99%) and SHAM (300 mg capsules) were a kind gift from El Nasr Pharmaceutical Chemicals Co. "ADWIC". Salicylhydroxamic acid raw materials were purchased from Haoyuan Chemexpress Co. Ltd (MOLBASE, Shanghai, China) and Shangrao New Future Environment Protection Technology Co., Ltd. (Shangrao, China). Methanol used in this work was of analytical grade which purchased from Sigma-Aldrich (St Louis, USA). All the other chemicals and reagents used were of analytical grade. Double distilled water was used for the preparation of all solutions.

### 2.2. Method development

#### 2.2.1. Instrumentation

Spectroscopic analysis was carried out using Double beam Shimadzu recording UV–Visible Spectrophotometer (Kyoto, Japan) model 1800 with 10 mm path length quartz cells. The solutions were made fresh on mass basis using a Mettler Toledo balance (Switzerland) model JB1603-C/FACT with a precision of  $\pm 0.01$  mg. Double distilled water was produced in our laboratory using GFL-2008 water (Burgwedel, Germany).

#### 2.2.2. Preparation of standard solutions

A stock solution of salicylhydroxamic acid working standard containing  $1000 \mu\text{g mL}^{-1}$  was prepared in distilled water by transferring the required amount of salicylhydroxamic acid in 500 mL

with the aid of 5 mL methanol. It was made up to mark using distilled water. Then, a series of 100 mL volumetric flasks with varying fractions were topped up to mark with distilled water in order to prepare different standard differing in concentration in the range  $0.1\text{--}50 \mu\text{g mL}^{-1}$ . All other solutions were stored refrigerated in the dark when not in use.

#### 2.2.3. Preparation of sample (capsules and raw materials)

Twenty capsules of SHAM (300 mg) were weighed. Equivalent to 100 mg salicylhydroxamic acid was quantitatively transferred into 500 mL volumetric flasks. Then it was dissolved using 5 mL of methanol. After that it was shaken for 5 min, 20 mL distilled water was added, shaken again for another 2 min, and finally topped up to the mark with distilled water to attain a final concentration of  $200 \mu\text{g mL}^{-1}$ . The solution was filtered using Whatman filter paper. The filtrate was diluted to obtain the desired concentration within the linearity range studied. The absorbance of sample solutions was measured and the amount of salicylhydroxamic acid was determined using the calibration curve. In the same way, the raw materials were also prepared by transferring 100 mg each and transferred to 500 mL volumetric flask. After that, the same procedure in the preparation of capsules was followed.

### 2.3. Method optimization

#### 2.3.1. Selection of $\lambda_{\text{max}}$ wavelength

The wavelength at which the maximum absorption (294 nm, Fig. 2) occurs is selected for further analysis. A definite concentration of salicylhydroxamic acid solution was scanned in UV range of 200–800 nm. Methanol: water (1:99, v/v) was used as a blank. The absorbance of solutions was measured at 294 nm against blank and calibration curve of salicylhydroxamic acid was built up accordingly.

### 2.4. Method validation

The assay of salicylhydroxamic acid was validated taking into consideration linearity, LOD and LOQ, precision, accuracy, robustness, ruggedness and specificity. Validation of the parameters was carried in the light of the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005) [22]. Below are the parameters that have been investigated.

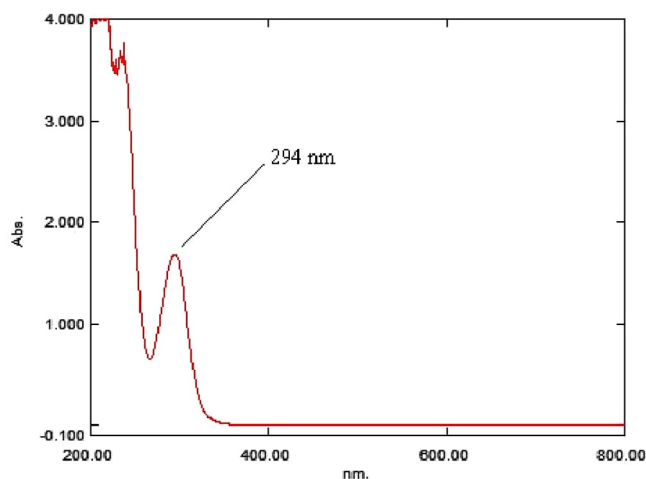


Fig. 2. UV spectra of salicylhydroxamic acid between 200 and 800 nm.

#### 2.4.1. Sensitivity

The sensitivity of the developed method was investigated by calculating the LOD, and LOQ. This was carried out by preparing a series of concentrations of the drug solutions. LOD and LOQ were carried out by a proper diluting known concentration of salicylhydroxamic acid till the average responses attained were 3 or 10 times the standard deviation of the responses for six measurements [23,24]. LOD and LOQ values were 0.031, and 0.098  $\mu\text{g mL}^{-1}$ , respectively.

#### 2.4.2. Specificity and selectivity

SHAM capsules of label claim 300 mg containing salicylhydroxamic acid of concentration 2  $\mu\text{g mL}^{-1}$  was prepared in methanol: water (1:99, v/v). On the other hand 2  $\mu\text{g mL}^{-1}$  of standard salicylhydroxamic acid, in addition, to sample solutions prepared were analyzed using the developed method. The expected amount of capsules was compared with that of the pure salicylhydroxamic acid solution of the same concentration.

#### 2.4.3. Linearity and range

Standard solutions containing salicylhydroxamic acid were prepared in a mixture of methanol: water (1:99, v/v) from a fresh stock solution (1000  $\mu\text{g mL}^{-1}$ ) to construct the calibration curve. The least square regression analysis was carried out for the obtained data. Calibration curve consisted of ten different concentrations in the range 0.1–50  $\mu\text{g mL}^{-1}$  for salicylhydroxamic acid. Each concentration level was performed thrice. The equation of the calibration curve attained was  $y = 0.02x + 0.004$ . It was obtained by plotting the absorbance (y) as a function of analyte concentration (x) in  $\mu\text{g mL}^{-1}$ .

#### 2.4.4. Accuracy

An appropriate amount of SHAM capsules powder was weighed and then spiked with known amount of the standard compound. After that, each sample was analyzed thrice. In brief, three different concentration levels of SHAM capsules solution using methanol: water (1:99, v/v) as solvent were prepared namely; 1, 5 & 10  $\mu\text{g mL}^{-1}$  and spiked with three different concentrations of salicylhydroxamic acid standard solution which prepared using methanol: water (1:99, v/v) (2, 15 & 30  $\mu\text{g mL}^{-1}$ ). Then the concentrations (x) of the resulting solutions were calculated using the calibration curve. The accuracy was reported as % recovery  $\pm$  standard deviation. Accuracy values obtained were in the range of 99.00–101.70% as indicated in Table 1. The good accuracy results obtained reveal the potential of the developed method for the quantification of the analyte in capsules pharmaceutical formulation.

#### 2.4.5. Precision

Intra- and inter-day precision were used in order to investigate the precision of the developed method. It was done by analyzing three different concentration levels namely; 0.5, 10 and 25  $\mu\text{g mL}^{-1}$  of standard solutions. The intra-day (repeatability) was estimated by analyzing the nine replicates on the same day. On the other hand, inter-day variation (intermediate precision) was carried out over six consecutive days. Intra-day precision, expressed as the percentage relative standard deviation, RSD, was 0.121–

**Table 2**

Intra and inter-day precision for the determination of salicylhydroxamic acid.

Amount ( $\mu\text{g mL}^{-1}$ )	RSD (%)
<i>Intra-day precision (n = 9)</i>	
0.50	0.322
10	0.121
25	0.143
<i>Inter-day precision (n = 27)</i>	
0.50	0.421
10	0.332
25	0.114

**Table 3**

Robustness results of salicylhydroxamic acid in capsules and raw materials upon changing  $\lambda_{\text{max}}$  294 nm i.e.,  $\pm 1.0$  nm.

Trade name	(%) $\pm$ SD (293 nm)	(%) $\pm$ SD (295 nm)
SHAM	91.10 $\pm$ 0.21	91.50 $\pm$ 0.29
Raw material 1	52.80 $\pm$ 0.31	52.40 $\pm$ 0.46
Raw material 2	102.40 $\pm$ 0.17	101.90 $\pm$ 0.18

Average of three determinations.

0.322% (Table 2), while inter-day precision was 0.114–0.421%, indicating the good precision of the developed method. Reproducibility was also determined by analyzing three different concentrations of salicylhydroxamic acid namely; 0.5, 10 and 25  $\mu\text{g mL}^{-1}$  on different Shimadzu UV spectrophotometers. The RSD values were less than 0.110%.

#### 2.4.6. Robustness

Robustness of the developed method was also done by slight altering the  $\lambda_{\text{max}}$  used in the analysis ( $\lambda_{\text{max}}$  294 nm) i.e.,  $\pm 1.0$  nm (Table 3).

#### 2.4.7. Ruggedness

The ruggedness of the developed method was achieved by analyzing salicylhydroxamic acid by different analysts using similar conditions. The %RSD value was found less than 1.5%.

#### 2.4.8. Analysis of capsules and raw materials using the current method

The content of salicylhydroxamic acid in capsules with label claims 300 mg per capsule and in raw materials were quantified using the developed method. The content of twenty capsules was weighed and the average content per capsule was calculated. Then, equivalent to 100 mg of salicylhydroxamic acid was weighed. The same procedure under Section 2.2.3 was followed for both capsules and raw materials. The prepared solutions were assayed using the proposed method. The % assay results were then reported.

### 3. Results and discussion

The maximum absorption of salicylhydroxamic acid was detected at 294 nm and overlay spectra of salicylhydroxamic acid

**Table 1**

Accuracy results for the determination salicylhydroxamic acid spiked in SHAM capsules.

Amount of sample taken (A) ( $\mu\text{g mL}^{-1}$ )	Amount of standard added (B) ( $\mu\text{g mL}^{-1}$ )	Total amount (A + B) ( $\mu\text{g mL}^{-1}$ )	Total amount found ( $\mu\text{g mL}^{-1}$ )	% Recovery* (Mean $\pm$ SD)
1	2	3	3.05	101.7 $\pm$ 0.0039
5	15	20	19.8	99.00 $\pm$ 0.0041
10	30	40	39.8	99.50 $\pm$ 0.0029

\*Indicates mean of six determinations (n = 6); SD: Standard deviation.

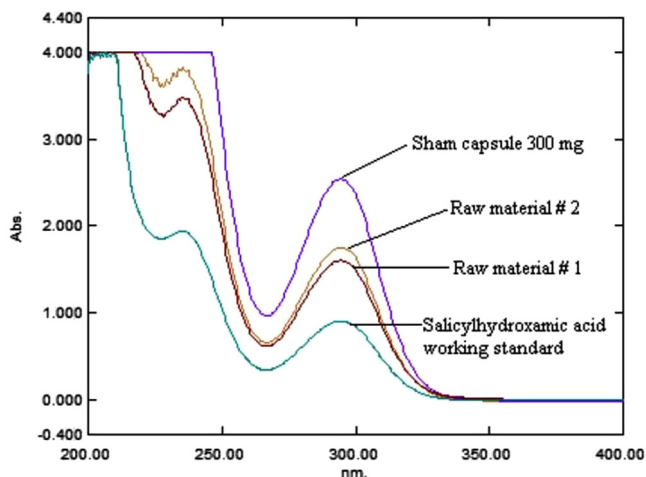


Fig. 3. Overlay spectrum of salicylhydroxamic acid working standard, raw materials, and capsule 300 mg.

working standard, raw materials and capsule were recorded (Fig. 3).

The current method was found to be simple, sensitive, accurate, precise, economical and rapid for the routine analysis of salicylhydroxamic acid not only in capsules but also in raw materials.

### 3.1. Analytical method validation parameters

The developed method was validated in accordance with the ICH guidelines (Q2) (R1) (ICH, 2005) [22].

#### 3.1.1. Linearity and range

The linearity of an analytical method is the figure of merits in method validation step. It is defined as the ability to get results that comply with Beer's law [25]. The characteristic parameters of our newly method are slope 0.02, intercept 0.004, and the correlation coefficient of 0.9999 which show a good linearity of the calibration curve (Table 3). Working solutions which contain the standard were prepared as prescribed earlier to draw the calibration curve. Calibration curve contained ten different concentra-

tions ( $0.1\text{--}50\text{ }\mu\text{g mL}^{-1}$ ) for salicylhydroxamic acid and each concentration level was performed in a trice. Calibration curve with regression equation was  $y = 0.02x + 0.004$  with good correlation coefficient (0.9999) between the standard concentration (x) and mean absorbance ( $n = 3$ ) show a good linearity of standard curve (Table 4).

#### 3.1.2. Precision

The precision of an analytical method reflects the degree of scattering occurred between a series of measurements obtained under particular conditions [26]. Intra- and inter-day tests were used to prove the precision of the developed method. The later was conducted by analyzing three concentration levels namely;  $0.5$ ,  $10$  and  $25\text{ }\mu\text{g mL}^{-1}$  of standard solutions. Specifically, intraday precision (repeatability) can be defined as the use of analytical procedure within a laboratory under a short period time through analyzing nine replicates on the same day by the same analyst using the same equipment. On the other hand, inter-day precision (intermediate precision) implies the evaluation of variations in the analysis when a method is used within a laboratory on different days (conducted over six consecutive days), by different analysts [24]. The %RSD for the intra-assay precision and intermediate precision for all the three concentration levels were below  $0.322$ , and  $0.421\%$ , respectively (Table 2) indicating the good precision of the developed method.

#### 3.1.3. Accuracy

The accuracy is defined as the closeness of results to accepted true value. It was determined by conducting recovery tests [27]. An appropriate amount of SHAM capsules powder was weighed and spiked with known amount of the standard compound, and each sample was analyzed in a trice. The results obtained were between  $99.00\%$  and  $101.70\%$  (Table 1). The obtained results support the accuracy of the developed method.

#### 3.1.4. Specificity

The developed method was found selective and specific as there is no interferences occurred as reflected by the accuracy results.

#### 3.1.5. LOD/LOQ

Standard solutions showed good linearity ( $r^2 > 0.9999$ ) over the concentration range tested. The sensitivity of the current method is higher compared with the reported spectrophotometric one (Salem, 2003; [11] the LOQ was  $1.53\text{ }\mu\text{g mL}^{-1}$ ) or even the potentiometric one reported by Hassan et al., 1997 (the LOD was  $0.1\text{ }\mu\text{g mL}^{-1}$ ) [15]. The LOD for SHAM was  $0.031\text{ }\mu\text{g mL}^{-1}$ , while the LOQ was  $0.098\text{ }\mu\text{g mL}^{-1}$ . LOD and LOQ were calculated using the following formulas ( $\text{LOD} = 3.3\text{ }\sigma/S$ ), and ( $\text{LOQ} = 10\text{ }\sigma/S$ ), respectively.

#### 3.1.6. Robustness

The slight variation in the  $\lambda_{\text{max}}$  ( $\pm 1.0\text{ nm}$ ) gave assay results as indicated in Table 3, indicating the robustness of the current method.

Table 4

Results of validation parameters obtained by the newly developed method.

$\lambda_{\text{max}}$	294 nm
Beer's law range ( $\mu\text{g mL}^{-1}$ )	0.1–50
Slope	0.02
Intercept	0.004
Correlation coefficient	0.9999
Accuracy	99.0–101.7%
Precision (%RSD)	<0.421%
Robustness	<0.46%
Ruggedness	<1.5%
LOD ( $\mu\text{g mL}^{-1}$ )	0.031
LOQ ( $\mu\text{g mL}^{-1}$ )	0.098

Table 5

Assay results of salicylhydroxamic acid in capsules and raw materials.

Trade name	Manufacturer	Generic Name	Label claim (mg)	(%) <sup>a</sup> $\pm$ SD
SHAM	ADWIC	Salicylhydroxamic acid	300	90.88 $\pm$ 0.17
Raw material 1	MOLBASE	Salicylhydroxamic acid	NA	51.00 $\pm$ 1.06
Raw material 2	SHANGRAO	Salicylhydroxamic acid	NA	100.13 $\pm$ 0.18

<sup>a</sup>Average of three determinations.



### 3.1.7. Application of the newly UV-Spectrophotometer method

The developed method has been successfully applied for the determination of SHAM capsules and raw materials (two different suppliers). In agreement with ICH guidelines the assay values for all formulations studied i.e., capsules and raw materials (1 & 2) were found 90.88, 51.00, and 100.13%, respectively (Table 5). Results indicate good agreement between the current method and the manufacturer's claimed values were found (Table 5).

## 4. Conclusion

A simple, reliable, accurate and reproducible spectrophotometric method for the determination of salicylhydroxamic acid (SHAM) in capsules and raw materials was successfully developed as per the ICH guidelines. The good analytical performance with regards to validation parameters was achieved. All the validated data attained are in agreement with the ICH guidelines Q2 (R1) (ICH, 2005) [22]. Once compared with the reported spectrophotometric method (Salem, 2003) [11], the developed method exhibits higher sensitivity. The LOD and LOQ were  $0.031 \mu\text{g mL}^{-1}$  and  $0.098 \mu\text{g mL}^{-1}$ , respectively. Good recoveries of SHAM were obtained in the range of 99.00–101.70% (Table 1) in different samples confirming the accuracy of developed method. The developed method is thus recommended to be implemented as a quality control protocol in pharmaceutical industries.

## Conflict of interest statement

Khalidun Mohammad Al Azzam declares that he has no conflict of interest.

Wafaa El Kassed declares that she has no conflict of interest.

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