Novel Spectrophotometric Methods for Determination ofSalicylamide and Ascorbic acid in their binary mixture

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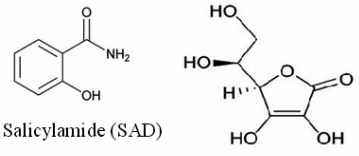
Summary: Simple, selective and precise four spectrophotometric methods were developed and validated for quantitative determination of Salicylamide (SAD) and Ascorbic acid (ASC, Vitamin C) in their binary mixture. Method A is Area under curve spectrophotometry, in which the area under curve in the wavelength ranges 225-245 nm and 265-285 nm were selected for determination of SAD and ASC. Method B is based on dual wavelength spectrophotometry, where ASC can be determined by difference in absorbance at 249.8 and 285.8 nm. On the same way; SAD is measured by difference in absorbance at 240.4 and 286.4 nm. Method C utilizes isoabsorptive point spectrophotometry where total concentration of SAD and ASC was calculated at their isoabsorptive points at 246.4 and 287 nm, while SAD concentration alone can be determined by first derivative spectrophotometry (^{1}D) at 315.4 nm, then ASC concentration can be determined by subtraction. Method D is ratio subtraction spectrophotometry, where ASC can be determined by dividing the spectrum of the mixture by the spectrum of the SAD (as a divisor) followed by subtracting the constant absorbance value of the plateau region, then finally multiplying the obtained spectrum by the spectrum of the divisor. The developed methods have been successfully applied for determination of the studied drugs in different laboratory prepared mixtures and in their pharmaceutical formulation. Statistical comparison between the results obtained by applying the proposed methods and the reported HPLC method was done, and it was found that there was no significant difference between them regarding both accuracy and precision.

Keywords: Salicylamide; Ascorbic acid; Area under curve; Dual wavelength; Isoabsorptive point.

Introduction

Ascorbic acid (ASC, vitamin C) is chemically known as 2, 3-Didehydro-L-threo-hexono-1, 4-lactone [1], Fig. 1. It is a water-soluble anti-oxidant vitamin that is essential for the synthesis of collagen and intercellular material and used for treatment and prevention of vitamin C deficiency (scurvy) [2]. Different titrimetric methods for determination of ASC in raw material or in tablets have been stated in the United States Pharmacopeia (USP) [3] and the British Pharmacopeia (BP) [4]. Also, several analytical methods including spectrophotometry [5–7], spectrofluorimetry [8], voltammetry [9-11], HPLC [12-15], and capillary electrophoresis [16] have been described for determination of ASC.

Salicylamide (SAD) is chemically known as 2-hydroxybenzamide [1], Fig. 1. It has analgesic, anti-inflammatory and antipyretic actions. It is used for pain, fever and inflammatory disorders such as osteoarthritis and rheumatoid arthritis [2]. USP [3] stated a non-aqueous titration method for the determination of SAD in raw material. Several analytical methods were described for determination of SAD, whether in dosage forms or in biological fluids. Such methods include; spectrophotometry [17], spectrofluorimetry [18, 19], HPTLC [20], HPLC [21,22] and capillary electrophoresis [23].



Salicylamide (SAD) Asorbic acid (ASC)

Fig. 1: Chemical structure of Salicylamide and Ascorbic acid.

Salicylamide and Ascorbic acid are co-formulated together in Cidal C® tablets for treatment of common cold associated with fever and muscular pain. Only one HPLC method has been cited in the literature for determination of SAD and ASC in their binary mixture [24]. The scientific novelty of the

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present work is that the proposed methods are simple, rapid, selective, less expensive and less time consuming than the reported HPLC method. The aim of this study is to develop and validate different spectrophotometric methods that can quantitate SAD and ASC in their binary mixture, the proposed methods was validated as per ICH guidelines [25].

Spectral Characteristics and Construction of Calibration Curves of Salicylamide and Ascorbic acid

Into two separate sets of 10 ml volumetric flasks, different aliquots containing 20–200 µg of each SAD and ASC solutions, were accurately transferred from their working solutions; then the volume was completed with double distilled water. The absorption spectra of 10 µg ml^{-1} of each of SAD and ASC were recorded using double distilled water as a blank in the range of 200-400 nm**.**

Area under Curve Spectrophotometric Method (AUC)

Area under the curves for the wavelength ranges 225-245 nm (λ_1-λ_2) and 265-285 nm (λ_3-λ_4) for determination of SAD and ASC were measured, then the absorptivity 'Y' value of each drug was determined at the selected wavelength ranges.

The absorptivity 'Y' values were determined as, Y= area under curve of component (from 225 to 245 nm or 265 to 285 nm)/concentration of the component (in µg ml^{-1}).

Mixtures of SAD and ASC were prepared and their areas under curves were measured at the selected wavelength ranges. Then the concentration of the two drugs was calculated by applying Cramer's rule using the corresponding equations.

Dual Wavelength Spectrophotometric Method

The absorbance values at 240.4 and 286.4 nm for SAD in the range of 2-20 µg ml^{-1} and at 249.8 and 285.8 for ASC in the range of 2-20 µg ml^{-1} were measured. SAD was determined by plotting the difference in absorbance at 240.4 and 286.4 nm (zero difference for ASC) against its corresponding concentration. Similarly for determination of ASC, the difference in absorbance at 249.8 and 285.8 nm (zero difference for SAD) was plotted against the corresponding concentrations. The calibration curve of each drug was constructed, then regression equations were calculated.

Isoabsorptive Point Spectrophotometric Method

Zero order absorbance spectra was recorded and the absorbance at 246.4 ($Aiso_1$) and 287 nm ($Aiso_2$) for ASC was measured. The first derivative curves (^1D) for SAD were obtained and the peak amplitude was measured at 315.4 nm using $\Delta\lambda$ = 4 and scaling factor = 10 (corresponding to zero crossing of ASC). The calibration curves relating the absorbance of ASC and peak amplitude of SAD at the selected wavelength were constructed against the corresponding concentration of each drug and regression equations were calculated.

Analysis of Laboratory Prepared Mixtures of SAD and ASC

The absorbance of mixtures containing different ratios of SAD and ASC were measured at 315.4 nm corresponding to the peak amplitude utilizing the first derivative spectrophotometry method for the concentration of SAD alone; and at 246.4 ($Aiso_1$) and 287 nm ($Aiso_2$) corresponding to total concentration of SAD and ASC in the mixture. Both the total concentration of SAD and ASC in the mixture and the concentration SAD alone were calculated using respective regression equations. Then, ASC concentration in the mixture was calculated by subtracting the SAD concentration from the total concentration.

Ratio Subtraction Spectrophotometric Method

The absorbance of different laboratory prepared mixtures of SAD and ASC was measured in the range of 200-400 nm, and were devided by the spectrum of 10 µg ml^{-1} of SAD (as a divisor). The absorbance in the plateau region at λ above 304 nm was subtracted; then the obtained spectra were multiplied by the spectrum of the divisor to obtain the spectra of ASC. ASC concentration was determined at its λ max 265.4 nm using its corresponding regression equation.

Analysis of Laboratory Prepared Mixtures of Salicylamide and Ascorbic acid

Into 10 ml volumetric flasks, different aliquots of SAD 50-200 µg and of ASC 20-100 µg were transferred from their respective working solution (100 µg ml^{-1}) in double distilled water into to prepare mixtures of different ratios of SAD and ASC, then concentration of each drug was obtained by applying the procedures mentioned under each proposed method.

Application to Pharmaceutical Formulation (Cidal C® tablets)

Powder and mix well ten Cidal C® tablets, transfer an accurately weighed portion of the powdered tablet equivalent to 100 mg of SAD and 10 mg of ASC was transferred into 100-ml volumetric

flask; add 75 ml methanol and sonicate for 30 min, filter, and then complete to volume with methanol. This solution is used as working solution for ASC (100 µg ml^{-1}), while a part of the solution is diluted to obtain working solution for SAD (100 µg ml^{-1}) using double distilled water as solvent. SAD and ASC were analyzed by applying the procedures mentioned under each proposed method, then the concentration of each drug was calculated from the corresponding regression equations.

Results and Discussion

Spectrophotometric methods are the most commonly used and popular techniques. The common availability of the instrumentation, the simplicity of the procedures, speed, precision and accuracy of the technique still make spectrophotometric methods attractive. Spectrophotometric methods are more economic and simpler, compared to methods such as chromatography and electrophoresis. While there is no literature reveals determination of Salicylamide and Ascorbic acid in their binary mixture and pharmaceutical formulation and as shown in Fig.2 there is severe overlapping between their spectra, so the objective is to establish different spectrophotometric methods for rapid quantification of Salicylamide and Ascorbic acid using the Area under curve, dual wavelength, isoabsorptive point and ratio subtraction spectrophotometric methods which are less expensive, less time consuming and required less sophisticated equipment rather then the reported HPLC method.

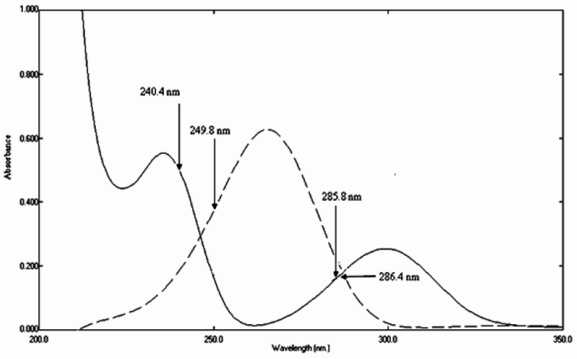

Fig. 2: Zero order absorption spectrum of 10 µg mL^{-1} of each of Salicylamide (___) and Ascorbic acid (----) using double distilled water as a solvent showing the selected wavelengths for dual wavelength method.

Area under Curve Spectrophotometric Method

In the simultaneous equations using AUC method, the absorptivity (Y) values of each of the two drugs were determined at the selected wavelength ranges, 225-245 nm (λ_1-λ_2) and 265-285 nm (λ_3-λ_4), as shown in Fig. 3. The 'Y' values were determined as, Y= area under curve of component (from 225 to 245 nm or 265 to 285 nm)/concentration of the component in µg ml^{-1}. The 'Y' values reported are the mean of ten independent determinations. By applying Cramer's rule in equations (1) and (2), concentrations of SAD and ASC can be obtained. Concentrations of the two drugs in pure form and in mixtures were calculated using the corresponding equations (1) and (2).

A_1=0.989 C_{SAD}+0.258 C_{ASC} (1) at 225-245 nm (λ_1-λ_2)
A_2=0.160 C_{SAD}+0.921 C_{ASC} (2) at 265-285 nm (λ_3-λ_4)

where, A_1 and A_2 are the area under curve of sample solutions at the wavelength range (λ_1-λ_2) and (λ_3-λ_4), respectively.

C_{SAD} and C_{ASC} are the concentrations of SAD and ASC in µg ml^{-1}, respectively.

0.989 and 0.160 are the absorptivity (Y value) of SAD at (λ_1-λ_2) and (λ_3-λ_4), respectively.

0.258 and 0.921 are absorptivity (Y value) of ASC at (λ_1-λ_2) and (λ_3-λ_4), respectively.

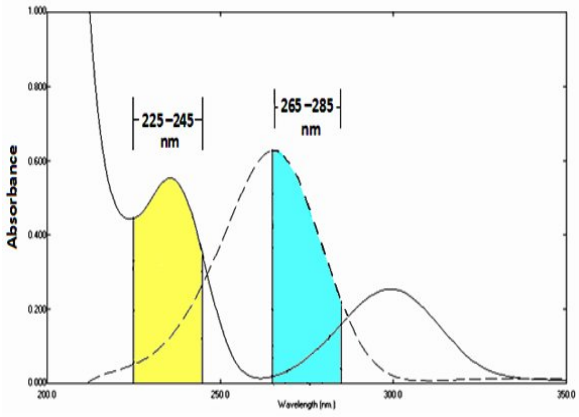

Fig. 3: Zero order absorption spectra of 10 µg mL^{-1} of Salicylamide (—) and 10 µg mL^{-1} of Ascorbic acid (-----) showing wavelength ranges for AUC method using double distilled water as a blank

Dual Wavelength Spectrophotometric Method

The principle of dual wavelength method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component. It can be utilized to a great extent without much

complication to calculate the unknown concentration of the component of interest in a mixture. The requirement for dual wavelength method is the selection of two wavelengths where the interfering component shows the same absorbance while the component of interest shows significant difference in absorbance with concentration.

Fig. 2 shows that the absorbance values of ASC are the same at 240.4 and 286.4 nm therefore these two wavelengths were selected for determination of SAD. The same for the two wavelengths 249.8 and 285.8 nm, the absorbance values of SAD are the same, hence those two wavelengths were selected for determination of ASC.

Difference in absorbances of SAD at 240.4 and 286.4 nm were plotted against its concentration in the range of 2-20 µg ml^{-1}, also for ASC, difference in absorbances at 249.8 and 285.8 nm were plotted against its concentration in the range of 2-20 µg $ml^{-1.}$ The concentration of SAD and ASC can be calculated from the following regression equations:

$A_1 = 0.0324\ C_{SAD} - 0.0030$, r = 0.9997
$A_2 = 0.0144\ C_{ASC} + 0.0209$, r = 0.9998

where, A_1 is the absorbance difference at 240.2 and 273.2 nm, A_2 is the absorbance difference at 230.8 and 259.2 nm. C_{SAD} and C_{ASC} are the concentration of SAD and ASC in µg ml^{-1}, respectively and r is the correlation coefficient.

Isoabsorptive Point Spectrophotometric Method

In this work, the so-called isoabsorptive point spectrophotometry developed by *Erram* and *Tipnis* [26 –28] is applied for determination of SAD and ASC in their binary mixture. The theory of this method could be confirmed experimentally by recording the absorbance spectra of 12 µg ml^{-1} of each SAD and ASC separately, and that of a mixture containing equal concentration of SAD and ASC (6 µg ml^{-1} of each of SAD and ASC), as shown in Fig. 4, the mixture and the pure drugs have different absorbance spectra; meanwhile they possess the same absorbance at their isoabsorptive point. Thus, by measuring the absorbance value at the chosen isoabsorptive point, the total concentration of the mixture could be calculated. By applying the suggested procedure the absorbance at 246.4 nm ($Aiso_1$) and 287 nm ($Aiso_2$) for ASC was obtained over different concentrations, while the concentration of SAD in a mixture of SAD and ASC could be calculated by first derivative spectrophotometry (^1D) and measuring the peak amplitude of SAD at 315.4 nm Fig. 5, corresponding to concentration of SAD only where no interference from ASC is observed. Then the concentration of ASC could be calculated by subtraction of SAD concentration from total mixture concentration.

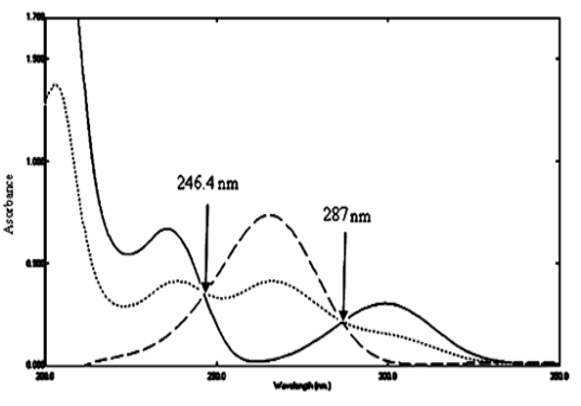

Fig. 4: Zero order absorption spectrum of 12 µg mL^{-1} Salicylamide (___) and 12 µg mL^{-1} Ascorbic acid (----) and mixture (salicylamide + Ascorbic acid 6 µg ml^{-1} of each) using double distilled water as a solvent.

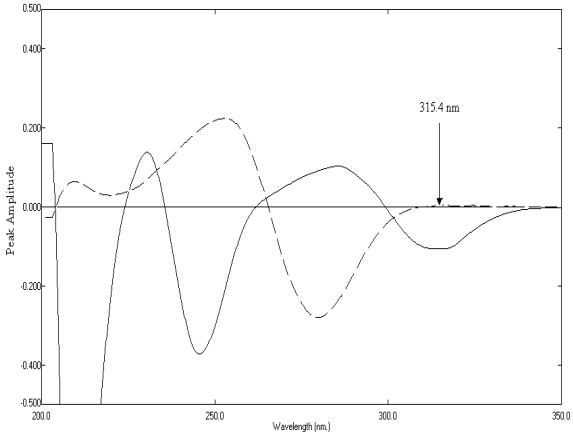

Fig. 5: First derivative absorption spectra of 10 µg mL^{-1} of Salicylamide (—) and 10 µg mL^{-1} of Ascorbic acid (----) using double distilled water as a solvent.

Linear correlations were obtained between absorbance at 246.4 and 287nm for ASC and its concentration in the range of 2–20 µg ml^{-1} and peak amplitude at 315.4 nm for SAD and its concentration in the range of 2–20 µg ml^{-1} from which the regression equations were calculated and found to be:-

$A = 0.0153 \quad C_{SAD} + 0.0225, \quad r = 0.9998$
$Aiso_1 = 0.0105 \quad C_{ASC} + 0.0014, \quad r = 0.9998$
$Aiso_2 = 0.0255 \quad C_{ASC} + 0.0418, \quad r = 0.9999$

where A is the peak amplitude of SAD, while $Aiso_1$ and $Aiso_2$ are the absorbance of ASC, C_{SAD} and C_{ASC} are the concentration in µg ml^{-1}, and r is the correlation coefficients.

Ratio Subtraction Spectrophotometric Method

Based on the theory of Ratio subtraction spectrophotometric method [29], ASC could be selectively determined in the presence SAD, where SAD can be determined by ^1D method. First, the linearity of ASC was determined in the concentration range 2–20 µg ml^{-1} at its λ_{max}= 265.4 nm in the zero order spectra. Second, the spectra of mixtures containing different concentration of SAD and ASC were divided by the spectrum of the SAD as a divisor where different divisor concentrations (5, 10, 15 and 20 µg ml^{-1}) were tried. The divisor of concentration 10 µg ml^{-1} of SAD was found to be the best regarding accuracy and precision when the method was used for calculation of ASC concentration in its laboratory prepared mixtures. The amplitude value in the plateau region at λ above 304 nm was subtracted from the spectra of the divided mixtures; the obtained spectra were then multiplied by the spectrum of the divisor as shown in Fig. 6**.** Finally, ASC concentrations in laboratory prepared mixtures were measured from the last spectra obtained at its λ_{max}= 265.4 nm. Linear correlation was obtained between absorbance of ASC at 265.4 nm and its concentration in the range of 2–20 µg ml^{-1} from which the regression equation was calculated and found to be:

$A = 0.0541C_{ASC} + 0.0854, \quad r = 0.9999$

where A is the absorbance, C_{ASC} is the concentration of ASC in µg ml^{-1} and r is the correlation coefficient.

The ability of the proposed spectrophotometric methods for determination of SAD and ASC in their binary mixtures was checked by analysis of different laboratory prepared mixtures containing different ratios of them where good results were obtained Table-1.

The usefulness of the proposed methods for assay of SAD and ASC in their pharmaceutical formulation was studied by analysis their tablet formulation (Cidal C® tablets). Furthermore, the validity of the proposed method was assessed by applying the standard addition technique, which showed accurate results and there was no interference from tablet excipients as shown in Table-2.

Method validation was performed according to USP guidelines [3] for all the proposed methods. Table-3 shows results of accuracy, repeatability and intermediate precision of the methods. Other regression equation parameters are shown in **Table 3**, which shows good linear relationship for the suggested methods as revealed by the correlation coefficients. Tables 4 and 5 show statistical comparison of the results obtained by the proposed methods and those obtained by the reported HPLC method. The calculated *t*- and *F*-values are less than the theoretical ones indicating that there is no significant difference between them with respect to accuracy and precision.

Table-1: Determination of Salicylamide and Ascorbic acid in laboratory prepared mixtures by the proposed spectrophotometric methods.

Mixture No.	Concentration (µg mL^{-1})		Recovery *%							
			AUC method		Dual wavelength method		^1D method	Isoabsorptive point method		Ratio-sub traction method
			SAD	ASC	SAD	ASC	SAD	ASC		ASC
	SAD	ASC	225-245 nm and 265-285nm		difference at 240.4 and 286.4 nm	difference at 249.8 and 285.8 nm	at 315.4 nm	at 246.4 nm	at 287 nm	at 265.4 nm
1	20	2	101.10	102.00	98.10	97.50	100.75	98.50	98.50	99.50
2	20	4	99.70	101.50	100.60	99.00	100.30	100.75	100.50	101.50
3	20	5	98.00	99.60	99.70	101.60	101.70	100.80	101.00	99.00
4	18	4	101.89	97.75	98.22	102.50	101.28	98.25	102.25	98.25
5	10	10	101.70	101.90	102.20	98.70	101.50	98.20	101.40	101.90
6	5	10	101.00	101.80	100.25	101.40	102.00	101.60	100.30	101.60
7	9	6	101.00	98.83	100.78	100.83	99.00	100.67	102.50	102.17
Mean ± SD			101.00 ±1.354	101.50 ± 1.735	100.25 ± 1.457	100.83 ± 1.829	100.28 ± 1.028	100.67 ± 1.446	101.00 ± 1.349	101.50 ± 1.594

*Average of 3 determinations.

Table-2: Determination of Salicylamide and Ascorbic acid in their pharmaceutical formulation by the proposed methods and application of standard addition technique.

Pharmaceutical formulation	The proposed methods	component	Taken ($\mu g\ mL^{-1}$)	Found* % ± SD	Standard addition technique **(Mean ± SD)
Cidal C® tablets claimed to contain 500mg SAD and 50 mg ASC/tablet (Batch No 121135W)	Area under curve method	SAD	8	101.25 ± 1.684	101.15 ± 1.648
		ASC	2	104.50 ± 1.368	100.27 ± 1.092
	Dual wavelength method	SAD	8	103.00 ± 1.169	100.97 ± 1.686
		ASC	2	105.00 ± 1.331	100.90 ± 1.364
	^{1}D method	SAD	8	101.87 ± 1.771	101.42 ±1.022
	Isoabsorptive point method	ASC ($Aiso_1$)	2	104.50 ± 1.273	100.06 ± 1.055
		ASC ($Aiso_2$)	2	106.00 ± 1.389	100.44 ± 1.750
	Ratio-subtraction method	ASC	2	105.00 ± 1.229	100.78 ± 1.256

*Average of 6 determinations.
** Average of 3 determinations.

Table-3: Results of assay validation parameters of the proposed methods for determination of Salicylamide and Ascorbic acid.

Parameters	Area under curve method		Dual wavelength method d		^{1}D method	Isoabsorptive point method		Ratio-subtraction method
	SAD	ASC	SAD	ASC	SAD	ASC ($Aiso_1$)	ASC ($Aiso_2$)	ASC
Range					2-20 ($\mu g\ mL^{-1}$)			
Slope	-	-	0.0324	0.0144	0.0105	0.0255	0.0153	0.0541
Intercept	-	-	-0.0030	0.0209	0.0014	0.0418	0.0225	0.0854
Correlation coefficient	-	-	0.9997	0.9998	0.9998	0.9999	0.9998	0.9999
Accuracy (mean ± SD)	100.08 ± 1.476	100.01 ± 1.150	99.85 ± 1.183	100.02 ± 1.322	100.75 ± 1.225	99.94 ± 0.982	99.89 ± 1.218	100.11 ± 0.946
Selectivity (mean ± SD)	101.00 ± 1.354	101.50 ± 1.735	100.25 ± 1.457	100.83 ± 1.829	101.28 ± 1.028	100.67 ± 1.446	101.00 ± 1.349	101.50 ± 1.594
Precision (%RSD)								
Repeatability*	1.32	1.15	1.30	1.24	1.23	1.13	1.17	1.15
Intermediate precision*	1.45	1.32	1.65	1.55	1.36	1.44	1.26	1.57
LOD** ($\mu g\ mL^{-1}$)	0.61	0.59	0.57	0.62	0.44	0.46	0.65	0.63
LOQ** ($\mu g\ mL^{-1}$)	1.82	1.76	1.72	1.86	1.33	1.38	1.96	1.89

* The intraday precision (n=3), average of three different concentrations repeated three times within day. The interday precision (n=3), average of three different concentrations repeated three times in three successive days.
** Limit of detection and quantitation are determined via calculations LOD = (SD of the response/slope) × 3.3; LOQ = (SD of the response/slope) × 10.

Table-4: Statistical comparison of the results obtained by the proposed methods and the reported method for the determination of pure Salicylamide and Ascorbic acid.

Items	Area under curve method		Dual wavelength method		^{1}D method	Isoabsorptive point method		Ratio-subtraction method	Reported method [24]*	
	SAD	ASC	SAD	ASC	SAD	ASC		ASC	SAD	ASC
						($Aiso_1$)	($Aiso_2$)			
Mean	100.08	100.01	99.85	100.02	100.75	99.94	99.89	100.11	100.46	99.41
SD	1.476	1.150	1.183	1.322	1.225	0.982	1.218	0.946	1.288	1.475
%RSD	1.475	1.149	1.185	1.322	1.216	0.982	1.219	0.945	1.282	1.484
N	10	10	10	10	10	10	10	10	6	6
Variance	2.178	1.322	1.404	1.748	1.501	0.964	1.483	0895	1.658	2.176
Student's t-test (1.761)**	0.144	0.051	0.544	0.279	0.249	0.205	0.548	0.127	-	-
F-test (3.482)**	1.314	1.645	1.186	1.244	1.106	2.257	1.465	2.431	-	-

* HPLC method using C_8 column using methanol: 0.03 M phosphate buffer mixture (55 :45 v/v) as a mobile phase with UV detection at 255 nm and a flow rate of 1 mL min^{-1}.
** Figures between parenthesis represents the corresponding tabulated values of t and F at P= 0.05.

Table-5: Statistical comparison of the results obtained by the proposed methods and the reported method for the determination of dosage form (Cidal C® tablets).

Items	Area under curve method		Dual wavelength method		^{1}D method	Isoabsorptive point method		Ratio-subtraction method	Reported method [24]*	
	SAD	ASC	SAD	ASC	SAD	ASC ($Aiso_1$)	ASC ($Aiso_2$)	ASC	SAD	ASC
Mean	101.25	104.50	103.00	105.00	101.87	104.50	106.00	105.00	102.50	104.50
SD	1.684	1.368	1.169	1.331	1.771	1.273	1.389	1.229	1.472	1.366
% RSD	1.663	1.309	1.641	1.268	1.738	1.218	1.310	1.170	1.436	1.307
N	6	6	6	6	6	6	6	6	6	6
Variance	2.836	1.871	1.366	1.771	3.136	1.620	1.716	1.510	2.167	1.866
Student's t-test (1.812)**	0.637	0.006	1.303	0.648	0.197	0.151	1.483	0.651	-	-
F-test (5.050) **	1.309	1.002	1.585	1.053	1.448	1.151	1.034	1.236	-	-

* HPLC method using C_8 column using methanol: 0.03 M phosphate buffer mixture (55 :45 v/v) as a mobile phase with UV detection at 255 nm and a flow rate of 1 mL min^{-1}.

** Figures between parenthesis represents the corresponding tabulated values of t and F at P= 0.05

Experimental

Instruments

A double beam UV-visible spectrophotometer (SHIMADZU,Japan) model UV-1601 PC with quartz cell of 1 cm pathlength, connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7.The spectral band width was 2 nm and wavelength-scanning speed 2800 nm/min.

Materials

(a) Pure standard

Salicylamide and Ascorbic acid were kindly supplied by Chemical Industries Development (CID) Company, Egypt. Their purity was found to be 100.46±1.288 and 99.41±1.475, respectively according to the reported HPLC method [24].

(b) Pharmaceutical Formulation

Cidal C® tablets (Batch No. 121135W) labeled to contain 500 mg of SAD and 50 mg of ASC manufactured by Chemical Industries Development (CID) Company, Giza, Egypt.

(c) Chemicals and reagents

All reagents and chemicals used throughout this work were of analytical grade and were used without further purification.

- Double distilled water (Otsoka Pharmaceuticals, Egypt)
- Methanol; HPLC grade (CHROMASOLV ®, Sigma-Aldrich Chemie Gmbh, Germany).

Standard Solutions

- Stock standard solutions of SAD and ASC (1 mg ml^{-1} in methanol): 0.1 gm of SAD and ASC were accurately weighed into two separate 100 ml volumetric flasks, 50 ml of methanol was added to each flask, shaken to dissolve then the volume was completed to the mark with methanol.
- Working standard solutions of SAD and ASC (100 µg ml^{-1}): 10 ml from stock solution of each of SAD and ASC were transferred into two separate volumetric flasks and the volume was completed to the mark with double distilled water.

Laboratory Prepared Mixtures

Mixtures containing different ratios of SAD and ASC were prepared using their respective working solutions in double distilled water including their ratio in marketed formulation.

Conclusion

This work presents simple, rapid, accurate and precise spectrophotometric methods for determination of Salicylamide and Ascorbic acid in their binary mixture.

Isoabsorptive point method allows the determination of ASC as well as SAD without any requirement of data manipulation. The ratio subtraction method is very simple, accurate, precise and does not require any sophisticated apparatus or computer programs. Its main advantage is the direct measurement of the drug at its characteristic λ_{max}, hence there is a potential for greater sensitivity and accuracy. Area under curve and dual wavelength spectrophotometric methods could be applied to the simultaneous determination of both drugs either in their pure powder form or in their combined preparati on.

These spectrophotometric methods can be regarded as a useful alternative to chromatographic

techniques in the routine quality control of pharmaceutical formulations.

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