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Q-Absorbance ratio and Chromatographic Method for the Analysis of Aspirin and Salicylic acid

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ABSTRACT

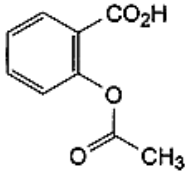
Two simple, specific, precise and accurate methods were developed for the analysis of Aspirin (ASA) in bulk and formulation forms in presence of its degradation product, salicylic acid (SA). The first method was based on determining Q-Absorbance ratios for aspirin and salicylic acid at two wavelengths; 251.4 nm (iso–absorptive point, λ_1) and 275.6 nm (λ_{max} of ASA, λ_2) and obtaining their absorptivity values which form the basis for their concentrations calculations. Beer's law was obeyed in the concentration range 50-450µg/ml and 10-90µg/ml for ASA and SA, respectively, with correlation coefficient not less than 0.999. The second method was based on development of stability-indicating chromatographic method (HPLC), reliable for the assay of aspirin in presence of salicylic acid. The stability-indicating property of the developed method was achieved on a C_{18} column (250*4.6mm) using a mixture of methanol and 1% (v/v) acetic acid aqueous solution (60:40 v/v) as mobile phase at 1ml/min flow rate and a UV detector set at 298nm. The constructed calibration curves were linear in the concentration range 1.0-5.0mg/ml and 30-270µg/ml for ASA and SA, respectively. The developed methods were optimized and validated in accordance to ICH guidelines. The validated methods were applied for the assay of aspirin in five brands low dose formulations as well as to identify and quantify any salicylic acid present using standard reference materials.

Introduction

Aspirin, acetylsalicylic acid (ASA; Fig. 1), (2-acetyloxy-benzoic acid) [1], was introduced in the late 1890s [2, 3]. Aspirin belongs to a group of medicines known as salicylates which have been recognized for more than 250 years [4].

Aspirin is used as analgesic and non–steroidal anti–inflammatory agent. It is also acting as anti–pyretic with anti–platelet action [5].

Aspirin is one of the most widely used medications in the world, with an estimated 40,000 tons of it being consumed each year [6]. Aspirin is classified as "Generally Regarded as Safe" and is widely used throughout the over–the–counter medicines [7, 8].It is on the WHO Model List of Essential Medicines [9].



Molecular formula: $C_9H_8O_4$

Molecular weight: 180.2

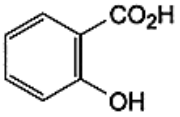
CAS no.: 50-78-2

Figure 1. Aspirin structure.

The structure of Acetylsalicylic acid shows an ester which is liable to undergo hydronium or hydroxide ion catalyzed hydrolysis, however the rate of OH^- catalyzed hydrolysis of esters is known to proceed faster than that of H_3O^+ ion.

Aspirin is stable in dry air, but readily hydrolyzes to acetic acid and salicylic acid when exposed to water or moist air [5]. High temperatures will speed the rate of hydrolysis. Low dose aspirin tablets can easily lose their efficacy if stored in unsuitable condition, even if that happened for short period of time.

Salicylic acid (SA; Fig. 2) is usually present in aspirin tablets (as hydrolysis product and/or synthetic precursor for aspirin). Salicylic acid is not as therapeutically effective as aspirin [5].



Molecular formula: $C_7H_6O_3$

Molecular weight: 138.1

CAS no.: 69-72-7

Figure 2. Salicylic acid structure.

The BP described acid–base residual titration method for the analysis of ASA raw material and its conventional tablets and HPLC method for enteric coated tablets [10], while the USP recommended acid–base residual titration method for raw material and HPLC method for both conventional and enteric coated tablets [11].

Several spectrophotometric and electrochemical [12-24], and chromatographic methods [25-35] were reported for analysis of ASA in the presence of SA acid in bulk, pharmaceutical dosage form and biological fluids.

Although literature survey revealed various methods for the determination of aspirin and salicylic acid, it is deemed useful to develop simpler, more accurate and less expensive methods for their routine analysis, especially in the developing countries.

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Materials and Methods

Reagents

Aspirin CDH (Central drug house (P) ltd., 99%w/w), India; Salicylic acid CDH (Central drug house (P) ltd., 99%w/w), India; Aspirin tablets (five brands) were collected from the local market; Methanol PRE, CARLO ERBA, S.A.S.; Methanol RS HPLC, CARLO ERBA, S.A.S.; Glacial acetic acid CDH (Central drug house ltd.), made in India.

Instrumentation and Analytical conditions

UV–VIS spectrophotometer instrument (UV–1800 240V, Shimadzu, Japan). HPLC chromatograph, LC-20AD Solvent delivery unit, DGU-20A3 Degasser, Rheodyne 7725i Sample injector, VP-ODS Column C18 250*4.6mm, SPD-20A UV/VIS Detector, Shimadzu, Japan).

Preparation of Sample and Standard solutions

Q-Absorbance ratio method

Standard stock solutions

Accurate quantities of ASA and SA standards were weighed, dissolved separately in methanol to obtain (solution A; 500µg/ml ASA, solution B; 2500µg/ml ASA, solution C; 500µg/ml SA).

Sample solution

Twenty tablets from each brand were accurately weighed and then powdered. To a quantity of the powder containing 0.3g of aspirin, 60 ml of methanol were added. The solution was shaken for 10 minutes, completed to 100 ml, mixed and filtered. 5 ml of the filtrate were diluted to 50 ml with methanol (solution D; 300µg/ml).

HPLC method

Standard stock solutions

Accurate quantities of ASA and SA standards were weighed, dissolved separately in a mixture of 60 volumes of methanol and 40 volumes of 1% (v/v) acetic acid aqueous solution to obtain (solution E; 25000µg/ml ASA, solution F; 1500µg/ml SA).

Sample solution

Twenty tablets were accurately weighed and then powdered. To a quantity of the powder containing 0.3 g of aspirin, 60 ml of methanol were added. The solution was shaken for 10 minutes, completed with 1% acetic acid aqueous solution to 100 ml, mixed and filtered through 0.45µm membrane filter (solution G; 3000µg/ml).

Procedures

Methods development

Q–Absorbance ratio method

Determination of iso–absorptive point and wavelength of maximum absorbance

In order to specify the iso–absorptive point and wavelength of maximum absorbance (λ_{max}) of ASA, six solutions of ASA and SA standards were prepared from standard stock solutions (A&C) as follows: ASA 100 µg/ml, (ASA 80µg/ml + SA 20µg/ml), (ASA 60µg/ml + SA 40µg/ml), (ASA 40µg/ml + SA 60µg/ml), (ASA 20µg/ml + SA 80µg/ml) and SA 100 µg/ml. All spectra were then recorded and their overlay graph was examined to determine the iso–absorptive points.

HPLC method

System suitability

In order to obtain a column/mobile phase system that would be suitable for the quantification of ASA, different mobile phase systems with different compositions and polarities were examined. Mobile phase system of 60 volumes of methanol to 40 volumes of 1% acetic acid aqueous solution was selected to get the best chromatographic resolution of ASA and SA in an isocratic elution. The detector was set at wavelength of 298 nm after examining the overlay spectra of ASA and SA, as it gives suitable ASA response and maximizes SA response in mixture of different concentration of both. Flow rate was of 1 ml/min and the injection volume was 20µL. System suitability tests as tailing factor, retention factor, separation factor, number of theoretical plates and resolution were then studied.

Methods validation

Calibration curves and linearity data

Q–Absorbance ratio method

Series of concentrations of standard ASA (50–450µg/ml) and SA (10–90µg/ml) were prepared from standard stock solutions (B&C) and spectra were recorded between 400 to 200 nm.

Calibration curves of ASA and SA were then constructed by plotting the absorbance at selected wavelengths (251.4nm &275.6nm) versus concentration in order to determine the absorptivity values and the validation parameters.

HPLC method

Serial concentrations of ASA (1.0–5.0mg/ml) and SA (30–270µg/ml) were prepared from standard stock solutions (E&F). Solutions were injected into the column at room temperature and their peak areas were measured. Calibration curves were then constructed, by plotting the peak areas versus corresponding concentrations, and validation parameters were determined.

Precision and Accuracy

The precision of the developed methods was evaluated by determining the within-day and between-day data were for three concentrations within the linearity range. The relative standard deviation (RSD) values were then calculated.

The accuracy of the methods was assessed by comparing the results obtained with the official method and calculating the t-value at 95% confidence level [36].

Assay of ASA tablet formulations

Q–Absorbance ratio method

The absorbance values of solution D were measured at 275.6 nm (λ_{max} of ASA) and 251.4 nm (iso–absorptive point) where absorptivity values were previously determined under calibration curve.

The concentrations of ASA and SA in sample solution were then calculated using the Q–Absorbance ratio method formula [37]:

$$\mathbf{C_X} = \frac{(\mathbf{Q_M} - \mathbf{Q_Y})}{(\mathbf{Q_X} - \mathbf{Q_Y})} \times \frac{\mathbf{A_1}}{\mathbf{a_{X1}}} \;\&\; \mathbf{C_Y} = \frac{(\mathbf{Q_M} - \mathbf{Q_X})}{(\mathbf{Q_Y} - \mathbf{Q_X})} \times \frac{\mathbf{A_1}}{\mathbf{a_{Y1}}}$$

Where the absorptivities of X at λ_1 & λ_2 are $\boldsymbol{a_{X1}}$ & $\boldsymbol{a_{X2}}$, the absorptivities of Y at λ_1 & λ_2 are $\boldsymbol{a_{Y1}}$ & $\boldsymbol{a_{Y2}}$, the absorbance of a sample at λ_1 & λ_2 are $\mathbf{A_1}$ & $\mathbf{A_2}$ and the concentration of X & Y are $\mathbf{C_X}$ & $\mathbf{C_Y}$, respectively.

HPLC method

Twenty µl volumes from solutions G were injected into the column at room temperature and their peak areas were measured.

Results

Q–Absorbance ratio method

Determination of maximum wavelength and the iso-absorptive point

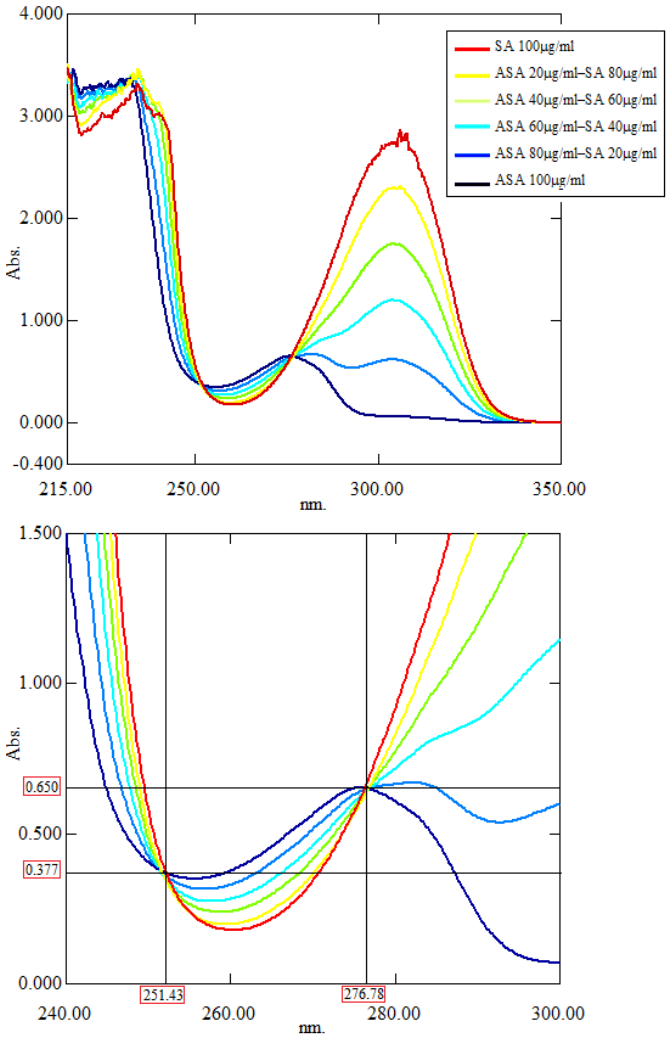
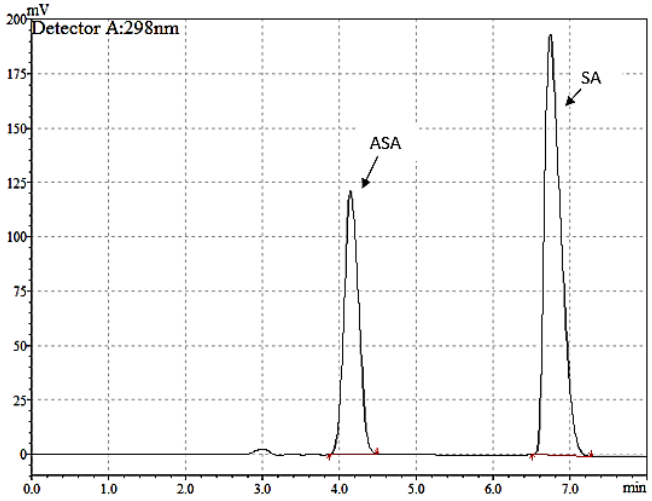


Figure 3. Overlay spectra of ASA, SA and mixtures of them.

HPLC method



Name	Conc. (µg/ml)	Ret. Time	Area	Height	Peak Start	Peak End	Area%
ASA	3000	4.138	1578598	120847	3.867	4.483	35.6103
SA	90	6.733	2854379	193126	6.508	7.267	64.3897

Figure 4. HPLC chromatogram of synthetic mixture of ASA 3000 & SA 90 (µg/ml) at λ 298 nm using mobile phase mixture of: 40% of acetic acid 1% (v/v) aqueous solution and 60% methanol.

Table 1. HPLC system suitability parameters.

	ASA	SA
RT	4.1	6.7
N	722	1259
K	0.4	1.2
α	3	
R	3.77	
T_f	1.05	1.22

RT: Retention time (min.), N: Number of theoretical plates, K: Retention factor, α: Separation factor, R: Resolution and T_f: Tailing factor

Linearity data for Q–Absorbance ratio method

Table 2. Summary for regression analysis data of ASA and SA.

	ASA		SA	
	275.6 nm	**251.4nm**	**275.6 nm**	**251.4nm**
Regression Equation*	A_{ASA}= 63.97C+0.0067	A_{ASA}= 37.096C+0.0009	A_{SA}= 57.018C+0.003	A_{SA}= 38.945C-0.0031
Slope	63.97	37.096	57.018	38.945
Intercept	0.0067	0.0009	0.003	0.0031
Correlation coefficient (r^2)	0.9999	0.9999	0.9996	0.9997
Linearity Range	50 – 450 µg/ml	50 – 450 µg/ml	10 – 90 µg/ml	10 – 90 µg/ml
Accuracy and Precision	100.131% ± 0.379	100.074% ± 0.667	100.367% ± 1.65	99.329%± 1.95
SE of intercept	0.0038	0.0036	0.0020	0.0060
SD of intercept	0.0113	0.0107	0.0012	0.0037
LOD	5.818 mg/ml	9.509 mg/ml	3.473 µg/ml	3.163 µg/ml
LOQ	17.629 mg/ml	28.815 mg/ml	10.523µg/ml	9.585 µg/ml

*C in g/100ml

Table 3. Summary of HPLC linearity data of ASA and SA.

	ASA	SA
Regression Equation	$PA_{ASA} = 491853 C_{ASA} + 126730$	$PA_{SA} = 29975\ C_{SA} + 94329$
Slope	491853	29975
Intercept	126730	94329
Correlation coefficient (r^2)	0.9991	0.9996
Linearity Range	1.0 – 5.0 mg/ml	30 – 270 μg/ml
Accuracy and Precision	99.713 % ± 0.846	99.922% ± 1.072
SE of intercept	18197.34	37966.66
SD of intercept	54592.02	113900
LOD	0.3663 mg/ml	12.539 μg/ml
LOQ	1.1099 mg/ml	37.998 μg/ml

Precision and Accuracy

Table 4. Precision and Accuracy results for the developed methods.

Parameter	Q-Absorbance ratio	HPLC
Within day precision, RSD%; n=3	0.31	0.28
Between-days precision, RSD%; n=3	0.49	0.55
t Stat*	2.453	2.630
P(t ≥T)	0.07	0.09
t Critical	2.776	2.776
F	5.985	3.09
P (f ≥F)	0.144	0.109
F Critical	19	19

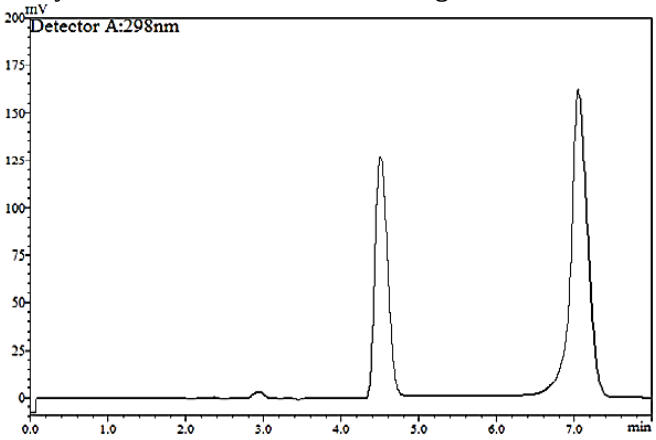
*Calculated at 95% confidence limit for 4 degrees of freedom

Assay of ASA tablet formulations using Q-Absorbance ratio method

Table 5. Q-Absorbance ratio method application for assay of ASA tablet formulations collected from Khartoum and Port Sudan (Five brands).

		C_{ASA}	C_{SA}	$C\%_{ASA}$	$C\%_{SA}$
Khartoum	**I**	297.94	4.05	99.314	1.349
	II	283.70	8.63	94.566	2.875
	III	279.71	13.46	93.235	4.485
	IV	249.07	24.15	83.025	8.048
	V	241.73	41.16	80.575	13.720
Port Sudan	**I**	281.05	21.42	93.682	7.141
	II	266.62	28.49	88.873	9.496
	III	268.76	26.96	89.588	8.986
	IV	247.10	48.11	82.366	16.037
	V	231.07	49.25	77.025	16.417

Assay of ASA tablet formulations using HPLC



Peak #	Name	Conc. (μg)	Ret. Time	Area	Height
1	ASA	3000	4.498	1484415	127047
2	SA	–	7.046	2340127	160918

Figure 5. HPLC chromatogram of a sample solution collected from Khartoum.

Table 6. Results of HPLC determination of ASA tablet formulations ($\bar{C}$%±SD, n=3) collected from Khartoum and Port Sudan (Five brands)

		$\bar{C}$%±SD	
		ASA	**SA**
Khartoum	**I**	98.026±0.328	1.193±0.013
	II	94.104±0.327	1.518±0.015
	III	93.148±0.267	2.548±0.026
	IV	84.030±0.439	4.562±0.018
	V	84.656±0.163	4.817±0.027
Port Sudan	**I**	97.030±0.624	1.520±0.022
	II	92.398±0.422	3.200±0.025
	III	93.200±0.555	2.897±0.014
	IV	87.751±0.649	7.702±0.036
	V	77.221±0.330	12.77±0.025

Discussion

The acceptability of analytical method corresponds directly to the performance characteristics that indicate its suitability and reliability for the intended analytical applications. From the commencement of official pharmaceutical analysis, analytical assay methods were included in the compendial monographs with the aim to characterize the quality of bulk drug materials by setting limits of their active ingredient content. In recent years, the assay methods in the monographs include titrimetry, spectrometry, chromatography, and capillary electrophoresis; also the electro analytical methods can be seen in the literature [38].

Simply accepting the analytical result could lead to rejection or acceptance of a product on the basis of a faulty analysis. There are various criteria which are used to judge the quality of an analysis [39].

The requirements for control of the quality of methods of analysis (validation) have been addressed by different organizations. The International Conference on Harmonization of technical requirements for registration of Pharmaceuticals for human use (ICH), The United States Food and Drug Administration (US FDA) and The United

States Pharmacopoeia (USP) have published specific guidelines for method validation [40- 42].

In our present study, two simple, specific and accurate methods were developed for the analysis of ASA and SA.

Q–Absorbance ratio method

Absorbance ratio method uses measurements at two wavelengths; the iso–absorptive wavelength ($\boldsymbol{\lambda_1}$) and the λ_{max} of one of the two components ($\boldsymbol{\lambda_2}$).

Iso–absorptive wavelength for two compounds is a wavelength in which the absorptivities of the two compounds are equal and hence they absorb UV–light in same manner, therefore measuring the absorbance of a mixture of the two compounds at that wavelength depends on the total concentration regardless the mixture composition ratio.

Overlay spectra of different mixtures of ASA and SA with total concentrations sum of 100μg/ml show that ASA and SA have two iso–absorptive wavelengths 276.8 nm and 251.4 nm (Figure 3).

Since there are two iso–absorptive points, it is possible to apply the Q–Absorbance ratio method for the determination of ASA and SA in different mixtures of them by two ways using different wavelength set; 1st iso–absorptive wavelength (276.8 nm) with λ_{max} of ASA (275.6 nm) and, 2nd iso–absorptive wavelength (251.4 nm)with λ_{max} of ASA.

However, error in Q–absorbance ratio method may be referred to practical difficulties in measurement specifically at the iso–absorptive wavelength. Therefore, in our present study we chose the 2nd iso–absorptive wavelength in the analysis in order to obtain reliable and accurate determination (251.4 nm and 275.6 nm).

HPLC method development

Results of HPLC showed that a good chromatographic separation of ASA and SA can be obtained in C_{18} column by the use of 1%(v/v) acetic acid aqueous solution and methanol in the ratio 40:60% (v/v) as a mobile phase and with flow rate of 1ml/min at 298 nm. Using these chromatographic conditions, the retention time of ASA is 4.1min and that of SA is 6.7min (Figures 4& 5).

Optimization and system suitability

Examining the retention time results, SA has much higher retention time than ASA, this cannot be explained in term of polarity since the polarity of SA is not lower than that of ASA. The delay of elution of SA can be attributed to that SA can form intramolecular H–bonding, and so it has less polar interaction with the mobile phase and subsequently higher retention time. Moreover, the acidic nature of the mobile phase hinders the ionization of the acidic phenolic and carboxylic groups of SA and so increases its retention in the column (pKa of ASA and SA are 3.50 & 2.97, respectively), this in turn enables good chromatographic separation between SA and ASA.

Specifying the detection wavelength that gives good response for ASA and SA; wavelength 280 nm gives very high response for ASA and very weak response for SA and as changing the wavelength toward higher values the ASA response decrease and that of SA increase; wavelength 295nm gives approximately similar responses for both compounds while wavelength 298 nm maximize the response of SA and give suitable ASA response.

Specifying the detection wavelength would be aided by UV overlay spectra of both compounds prepared with concentrations that match allowed content ratio in tablets (limit of SA in ASA: 0.3%w/w).

Different parameters of system suitability were checked and results obtained were summarized in Table 1.

Methods validation

The developed methods were validated to establish linearity, precision and accuracy according to ICH guidelines.

Linearity

Constructed calibration curves showed that developed methods are of good linearity and sensitivity for the determination of ASA in concentration range of 50-450μg/ml and for SA in concentration range of 10-90μg/ml; 1.0–5.0mg/ml for ASA and 30–270μg/ml for SA using Q–absorbance ration and HPLC methods, respectively. Regression analysis data are summarized in Tables 2&3.

Precision and accuracy

The methods repeatability (within the day) and the intermediate precision (between the days) were proved by the low obtained RSD% values (Table 4).

The accuracy of developed methods was assessed by comparison of the statistical results obtained with those of the official method. As the calculated *t*-values at 95% confidence limit were less than tabulated one, the result of developed methods can be considered as accurate as the official method (Table 4). Again probability values P ($|t| \geq T$) are greater than 0.05, the methods do not differ significantly.

Assay of tablets

The validated methods were applied for the assay of five different low dose ASA tablet formulations collected from two different provinces in Sudan. The response of the tablet dosage forms was measured and compared with respective to dry and humid weather (Khartoum and Port Sudan (Tables 5&6)

Comparison of results obtained between different brands of ASA tablet formulations collected from Khartoum and that collected from Port Sudan can be based on SA content. It was clear that all ASA tablets collected from Port Sudan have higher SA content than their corresponding ones that collected from Khartoum. The climate of Port Sudan is known to be of high temperatures and of high moisture contents. These conditions can accelerate the decomposition of ASA to SA and Acetic acid. Formulations I & III are not much affected unlike formulations II, IV &V. Formulations I & II are uncoated while formulation III, IV & V are coated. Packing materials, formulation excipients, coating materials and the storage time; all these can be controlling factors in the decomposition of ASA tablet formulations.

Conclusion

Q–Absorbance ratio and HPLC methods were developed and validated for the determination of five brands of ASA collected from Khartoum and Port Sudan. The developed methods proved to be simple, precise and accurate and can be applied for the routine analysis of ASA in the presence of SA.

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