



DEVELOPMENT OF FOUR UV-SPECTROMETRIC TECHNIQUES FOR CONCURRENT ESTIMATION OF ASPIRIN AND SILDENAFIL CITRATE IN THEIR BINARY MIXTURE AND PHARMACEUTICAL FORMULATIONS

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The combination of aspirin (ASP) and sildenafil citrate (SIL) into single dosage form has been of interest for a considerable period; because it has a synergistic effect for the handling of many diseases. Therefore, UV-spectrometric techniques were desperately required for the concurrent estimation of ASP and SIL in their binary mixture and pharmaceutical formulations. In this paper, four simple, low-cost and precise UV-spectrometric techniques were evolved for the concurrent estimation of ASP and SIL. The advanced methods include treatment of the zero-order spectrum by ratio subtraction (RS) along with spectrum subtraction method (SS), absorption correction method (ACM), area under the curve correction method (AUCCM) and induced dual wavelength method (IDWM). Moreover, UV-spectrometric purity analysis via spectral ratio factor (SRF) was developed; which considered the first UV-spectrometric purity testing to verify the purity of ASP in pharmaceuticals formulations.

All approaches were linear; in the range of 25.0-100.0 µg/mL for ASP, and 10.0-50.0 µg/mL for SIL. The mean percentage recoveries for ASP were 100.92, 100.33, 100.79 and 100.18% respectively, and for SIL were 99.27, 99.56, 99.83 and 99.44% respectively. The SRF values were approximating unity and showed high purity of the resolved peaks. The results were statistically compared with the HPLC reported method and no significant variance was discerned.

Keywords: Aspirin, Concurrent estimation, Sildenafil, UV-Spectrometric techniques.

INTRODUCTION

The combination of multiple drugs into single dosage form has become a common strategy in drug development. It improves patient docility and reduces production costs.

ASP can be added to the therapy of erectile dysfunction, revealing a feasible therapeutic action, especially in patients at higher risk of cardiovascular diseases¹. Moreover, the utilization of low-dose aspirin and SIL may be useful in the handling of thin endometrium²⁻⁴. In addition, sildenafil citrate can be added to low dose aspirin and low molecular weight heparin to improve fetal growth parameters in endangered pregnant

women in uteroplacental blood flow in cases of high-risk pregnancy⁵. The combining of ASP and SIL has demonstrated no pharmacokinetic interaction between them. Also, SIL has no effects on prothrombin or bleeding times in patients taking SIL alone or concurrently with ASP⁶. Therefore, oral dispersible tablets of ASP and SIL were evolved by the direct compression method⁷. ASP is chemically 2-(Acetyloxy)benzoic acid, has also been named as acetylsalicylic acid, Figure 1⁸. Aspirin is widely used in low doses in preventing antiplatelet aggregations in patients at dicey of occlusive vascular events⁹. Many analytical approaches have been announced for the definition of ASP like spectrophotometry¹⁰, HPLC¹¹, RP-HPLC¹², UPLC¹³, TLC¹⁴, capillary

zone electrophoresis¹⁵ and raman spectroscopy¹⁶. SIL is chemically 5-[2-Ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate, which belongs to phosphodiesterase inhibitors PDEs, specific type 5, Figure 1⁸. It was initially synthesized to treat angina pectoris and hypertension. Then, a concomitant as penile erection was discovered and now it is long-established in the handling of erectile dysfunction¹⁷. Also, SIL has FDA approval for the handling of pulmonary arterial hypertension¹⁸⁻²². Various techniques were applied for SIL definition like spectrophotometric methods^{23&24}, HPLC²⁵, RP-HPLC²⁶, GC^{27&28}, capillary zone electrophoresis²⁹, X-ray fluorescence spectrometry³⁰.

Literature review showed one UV-spectrometric technique for the concurrent estimation of ASP and SIL based on zero crossing first-derivative UV-spectrometry³¹. Also, one HPLC method was announced for the concurrent determination of ASP and SIL¹. However, these previous studies have a low sensitivity in quantification, and have an important constraint on all results discussed in this area.

The target of this study is to evolve easy, rapid, and accurate approaches for the concurrent estimation of aspirin and sildenafil citrate. These spectral techniques are: ratio subtraction (RS) along with spectrum subtraction method (SS), absorption correction method (ACM), area under the curve correction method (AUCCM) and induced dual wavelength method (IDWM). Also, to ensure that ASP is free from its degradation product and to check the purity and similarity of SIL, the spectral ratio factor (SRF) was estimated. The advanced methods were verified in concurrence with International Conference on Harmonisation (ICH) criteria³².

MATERIALS AND METHODS

Devices and software

The analysis was conducted on JASCO V-650, employing a pair of 1.0 cm quartz cells. Scans were executed at 200.0 to 400.0 nm. To homogenize the solutions an Ultrasonic bath model power sonic 405 (Korea) was employed. A SARTORIUS balance model 2474 (Germany) was adopted; with accuracy ± 0.1

mg. Calibrated volumetric flasks, whatman filter paper grade 91 and micropipettes were used.

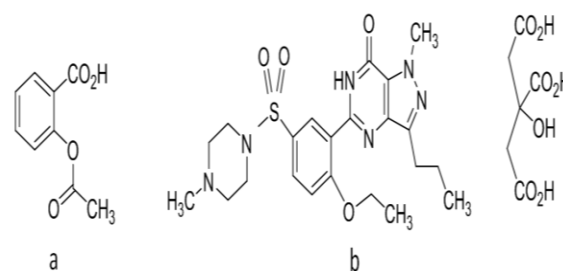


Fig. 1: Structural formula of (a) ASP and (b) SIL.

Chemicals

Standard API of ASP and SIL were supplied by JQC (Huayin); China, Xuhuang; China, certified to have a purity of 99.8%, 99.5% respectively. Ethanol of analytical grade (Scharlau Chemie. S.A, Spain) was utilized. Aspirin® Bayer enteric-coated tablets; any tablet claimed to consist of 100 mg ASP and Vega® film-coated tablets; any tablet claimed to consist of SIL amounting to 50 mg base (Asia pharmaceutical industries, Syria) were purchased from a pharmacy. Sodium Starch Glycolate (Hzhou Zhewang, China), Cross Povidone (Shaanxi greenyo, China), Aerosil (Fengchen, China), Magnesium stearate (Peter Greven Asia SDN.BHD, Malaysia), Talc (euro Minerals, Austria) and Lactose (Shreeji pharma international, India) were used to prepare oral dispersible tablets⁷.

Preparation of standard solutions

Stock solutions of 1000.0 $\mu\text{g/mL}$ of ASP and SIL were prepared independently by dissolving 25.0 mg in 22.0 mL ethanol and sonicated for 8 min, then diluted the volume to 25.0 mL utilizing the same solvent. Working solutions were diluted from the stock solutions to get ASP and SIL at a concentration of 50.0 $\mu\text{g/mL}$ for each.

Laboratory synthetic mixtures preparation

Five combinations including expected ratios of ASP and SIL were equipped into a set of 5 mL volumetric flasks from stock standard solutions using ethanol as a solvent. Moreover, pharmaceutical drug of ASP was mixed with specific concentration of SIL, and vice versa. This process was undertaken to demonstrate

that the excipients of either of these drugs did not interfere with each other.

Preparation of pharmaceutical products

10 tablets of ASP (100 mg/enteric-coated tablet) and 10 tablets of SIL (50 mg base/film coated tablet) were weighed and their average weight was calculated. Amounts equivalent to 25.0 mg ASP and 12.5 mg SIL were weighed, conveyed to 25.0 mL volumetric flask and dissolved in 22.0 mL ethanol, then sonicated for 8 min and diluted the volume with ethanol. A suitable amount filtered then 0.5 mL was withdrawn from the solution and conveyed into 5.0 mL volumetric flask, the volume was diluted with ethanol to get a final concentration of ASP and SIL equal to 100.0 $\mu\text{g}/\text{mL}$ and 50.0 $\mu\text{g}/\text{mL}$, respectively.

Oral dispersible tablet having label claim for ASP 150.0 mg and SIL 50.0 mg was designed in the laboratory⁷. In the same way, a final concentration of (90.0 $\mu\text{g}/\text{mL}$ ASP and 30.00 $\mu\text{g}/\text{mL}$ SIL) were acquired.

RESULTS AND DISCUSSION

The absorption spectra of ASP and SIL in ethanol show partial overlap. The ratio of the two ingredients is (3:1) or (2:1) for ASP and SIL, respectively.

In their absorption spectra, it was observed that ASP shows two maximum absorption at 226 nm and 276 nm. Using λ_{max} 276 nm enables us to determine both ingredients in the whole range of linearity. SIL has λ_{max} at 294 nm. Many UV-spectrometric techniques were

advanced over many years. The aim of this diversity in these methods is to get simple methods that achieve high sensitivity, accuracy and precision for assessing the ingredients in their pharmaceutical tablets. Zero order Spectral characteristics are disclosed in Figure 2.

Ratio subtraction method (RS)

This method³³ is carried out when one ingredient is more prolonged than the other. It can determine the less continuous ingredient (ASP) in the binary combinations. This method is applied through three steps as follows:

- **Step 1:** (ASP+SIL) admixture is divided by a specific divisor SIL' to get a new spectrum representing (ASP/SIL' + constant).
- **Step 2:** This constant is canceled through subtraction.
- **Step 3:** The D_0 spectra of ASP is gained by multiplying SIL' by the spectrum ASP/SIL' which have got from the second step.

The constant was acquired from the spectrum [(ASP+SIL)/SIL'] in the region where the spectrum of SIL is more extended (300.0-320.0 nm) as disclosed in Figure 3. The ratio spectra of admixture is disclosed in Figure 4. To select the leading concentration divisor of SIL, average absolute difference study (AAD) was done. 40 $\mu\text{g}/\text{mL}$ gave the slightest variance between recorded and postulated constants as disclosed in Table 1.

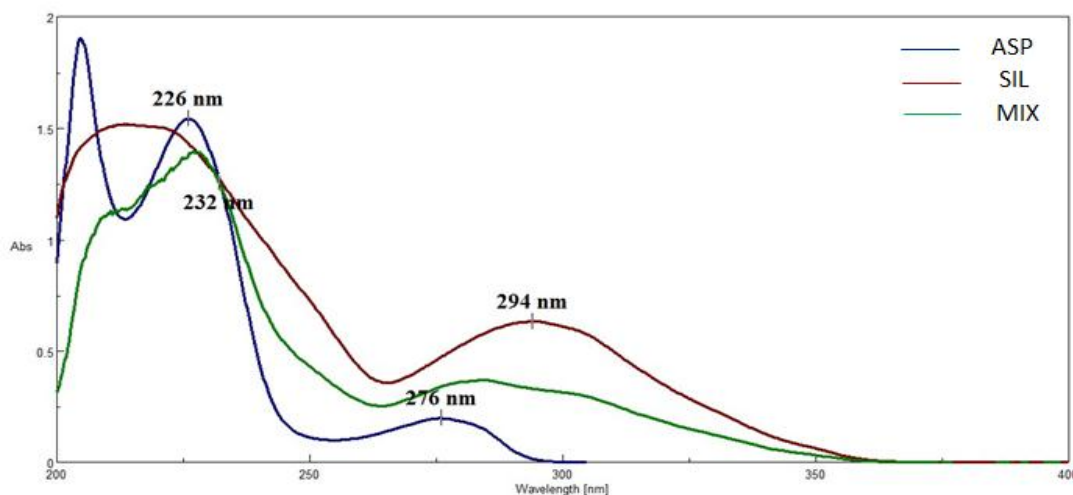


Fig. 2: D_0 spectra of ASP (30.0 $\mu\text{g}/\text{mL}$), SIL (30.0 $\mu\text{g}/\text{mL}$) and an admixture consisting of (15.0 $\mu\text{g}/\text{mL}$) of ASP and (15.0 $\mu\text{g}/\text{mL}$) of SIL.

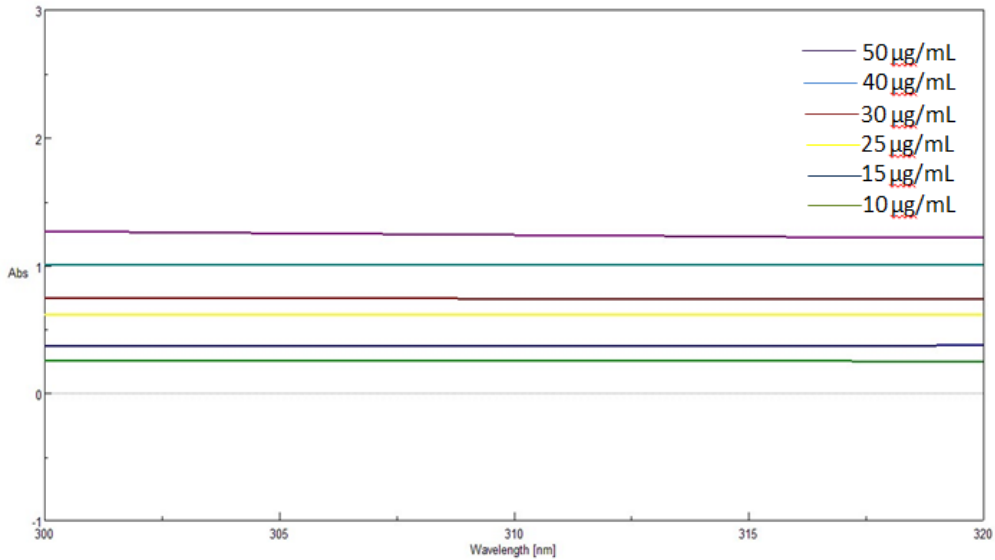


Fig. 3 : The constant value obtained by division the D_0 spectra of SIL (10.0-50.0 $\mu\text{g/mL}$) by the divisor spectra (40.0 $\mu\text{g/mL}$ SIL).

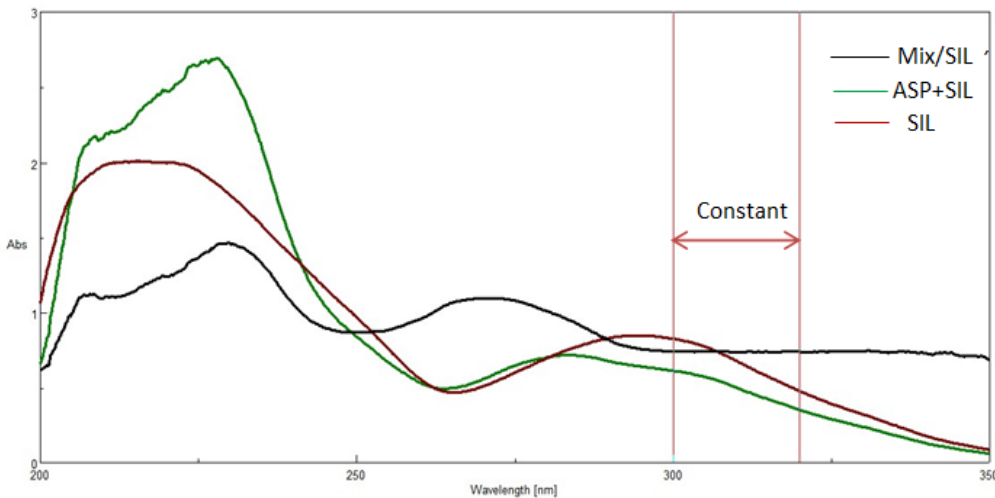


Fig. 4: The ratio spectra of admixture gained by dividing this admixture (30.0 $\mu\text{g/mL}$ ASP + 30.0 $\mu\text{g/mL}$ SIL) by SIL (40.0 $\mu\text{g/mL}$) as a divisor.

Table 1: Selection the appropriate concentration divisor of SIL in RS method.

Mix no.	(SIL) divisors					
	15 $\mu\text{g/mL}$		25 $\mu\text{g/mL}$		40 $\mu\text{g/mL}$	
	Post ^a	Rec ^b	Post ^a	Rec ^b	Post ^a	Rec ^b
1	2.096	2.212	1.22	1.233	0.737	0.747
2	2.840	2.779	1.651	1.615	0.999	0.978
3	1.028	1.055	0.597	0.613	0.362	0.371
4	1.733	1.818	1.007	1.056	0.610	0.640
5	0.707	0.669	0.411	0.389	0.249	0.235
AAD ^c	0.065		0.028		0.017	

The values represent the constant at the plateau region (300.0-320.0 nm) for SIL.

^apostulated value of constant.

^brecorded value of constant.

^caverage absolute difference between recorded and postulated constant values.

Spectrum subtraction method (SS)

This method³³ is based on direct subtraction of the D₀ spectra of ASP gained by RS method from the D₀ spectra of the binary combinations.

Spectral ratio factor (SRF)

This method³⁴ is advanced to verify the purity and uniformity between two spectra of the same ingredient. It relies on measuring the absorbance at three wavelengths; two of them is λ₁ and λ₂ at both sides of λ_{max}. Then, the absorbance ratio A₁=(A_{λ_{max}}/A_{λ₁}) and A₂=(A_{λ_{max}}/A_{λ₂}) is calculated. SRF is assured by dividing (A₁/A₂) of the sample by (A₁/A₂) of the standard ingredient. For ASP, the absorbance was recorded at λ_{max} (276.0 nm), λ₁ (272.0 nm) and λ₂ (280 nm). For SIL, the absorbance was recorded at λ_{max} (294.0 nm), λ₁ (285.0 nm) and λ₂ (304.4 nm). The SRF values showed a high purity of the resolved peaks. It was predestined that there were no distinction in ASP spectrum, which ticked that there was no trace of salicylic acid; which has a spectral range of 295-350 nm³⁵. The variances appeared at the ends of the spectrum; which demonstrates the good results attained despite the percent error acquired by the variances in

absorbance at the ends of the spectrum as disclosed in Figure 5 (a) and (b).

Absorption correction method (ACM)

This method^{36&37} was evolved to determine ASP and SIL concurrently. It was observed that SIL has an extensive peak with maximum absorption λ_{max} at 294 nm with no absorbance for ASP. but at this wavelength, interference was observed between ASP and SIL. So, wavelength at 305 nm was chosen for estimation of SIL which gives the best results in quantification without any absorbance and interference with ASP spectrum. SIL interferes with ASP at its λ_{max} 276 nm; So, the absorption factor was estimated for unadulterated SIL. The absorption of ASP estimation was done using the absorption factor of unadulterated SIL (A at 276/A at 305 nm=0.823) concurring to the Eq. (1):

$$A_{ASP,276\text{ nm}} = A_{\text{mix},276\text{ nm}} - \left(\frac{A_1}{A_2}\right) \times A_{\text{mix},305\text{ nm}} \quad (1)$$

Where; A₁/A₂ is the absorption factor of unadulterated SIL (A_{SIL, 276 nm}/A_{SIL, 305 nm}). Standard curves were constructed for ASP and SIL between absorbances of D₀ spectra (ASP at 276 nm, SIL at 305 nm) and their concentrations in (µg/mL).

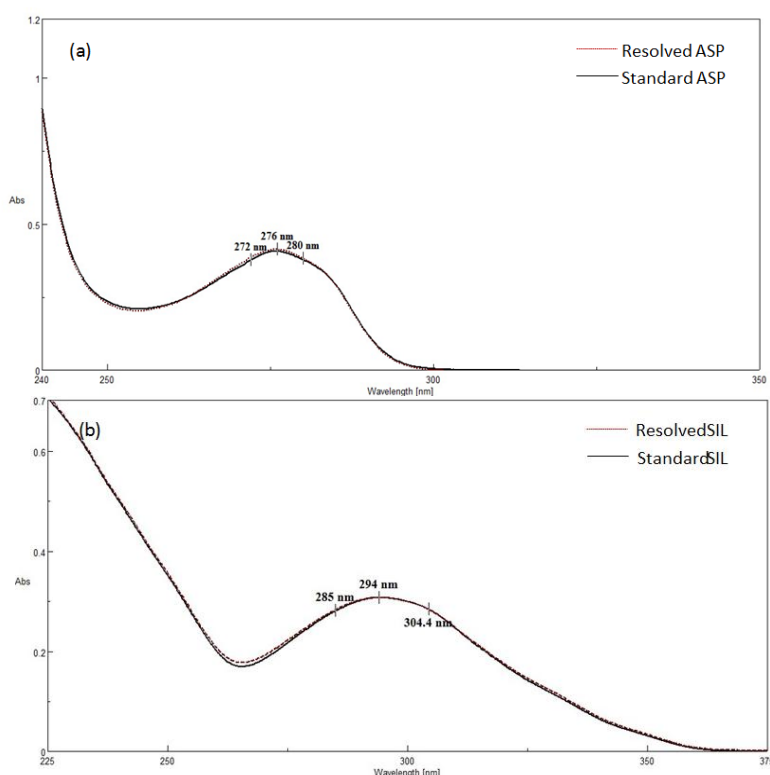


Fig. 5: (a) The variances between the standard and resolved spectrum for ASP (60.0µg/mL), (b) The variances between the standard and resolved spectrum for SIL (15.0 µg/mL).

Area under the curve correction method (AUCCM)

This method^{38,39} depends on AUC of its D₀ spectra. To raise the sensibility of ACM; absorbance has been replaced by AUC. SIL shows some interference at the region 270-285 nm ($\lambda_1-\lambda_2$), while ASP does not show any interference at the region 304-310 nm ($\lambda_3-\lambda_4$). So SIL can be determined directly at 304-310 nm. AUC of ASP estimation was done using the AUC factor of unadulterated SIL ($F_{AUC}=2.245$) concurring to the Eq.(2):

$$AUC_{ASP(270-285\text{ nm})} = AUC_{mix(270-285\text{ nm})} - \left(\frac{AUC_{(1-2)}}{AUC_{(3-4)}} \right) \times AUC_{mix(304-310\text{ nm})} \quad (2)$$

Where; $AUC_{(1-2)}/AUC_{(3-4)}$ is area under curve factor of unadulterated SIL [$AUC_{SIL(270-285\text{ nm})} / AUC_{SIL(304-310\text{ nm})}$].

Standard curves were constructed for ASP and SIL by plotting AUC values for ASP at 270-285 nm and SIL at 304-310 nm versus their concentrations in ($\mu\text{g/mL}$).

Induced dual wavelength method (IDWM)

This method⁴⁰ has been advanced over dual wavelength method. Various wavelengths were tested and it was observed that (280, 293 nm) and (294, 278 nm) are the best wavelengths in selectivity and sensitivity. To oust the vestige of SIL on ASP spectrum, the

equality factor of unadulterated SIL was reckoned; $F_{eqSIL}=(A_{SIL\text{ at }280\text{ nm}}/A_{SIL\text{ at }293\text{ nm}})=0.833$. In the same way, SIL can be estimated by reckoning the absorbance variances at 294 nm and 278 nm, $F_{eqASP}=(A_{ASP\text{ at }294\text{ nm}}/A_{ASP\text{ at }278\text{ nm}})=0.095$.

Standard curve of ASP was constructed between the absorbance variance values of ASP at D₀ spectra after multiplying by F_{eqSIL} $\Delta A=A_{280\text{ nm}} - (A_{293\text{ nm}}*0.833)$ and their corresponding concentrations. Similarly, the standard curve of SIL was constructed between the absorbance variance values of SIL at D₀ spectra after multiplying by F_{eqASP} $\Delta A=A_{294\text{ nm}} - (A_{278\text{ nm}}*0.095)$ and their corresponding concentrations.

Methods validation and statistical analysis

The advanced approaches were verified in concurrence with ICH criteria³².

Linearity of ASP and SIL was implemented by measuring six concentrations of ASP and SIL separately. Range of linearity for ASP and SIL is within 25-100 $\mu\text{g/mL}$ and 10-50 $\mu\text{g/mL}$, respectively. All advanced approaches showed that correlation coefficient values are closer to 1 which gives good linearity as disclosed in table 2.

Limit of detection (LOD), limit of quantification (LOQ) were exposed as disclosed in Table 2.

Table 2: Validation parameters for ASP and SIL.

Parameters	ASP				SIL			
	RS	ACM	AUCCM	IDW	SS	ACM	AUCCM	IDW
Wavelength (nm)	276	276	270 and 285	280 and 293	294	305	304 and 310	294 and 278
N	6	6	6	6	6	6	6	6
Linearity ($\mu\text{g/mL}$)	25-100	25-100	25-100	25-100	10-50	10-50	10-50	10-50
Intercept	-0.0137	-0.0137	-0.198	-0.0061	-0.0157	-0.0101	-0.0488	-0.0138
Slope	0.007	0.007	0.0967	0.0057	0.0217	0.0195	0.1109	0.02
Regression equation	$Y=0.007x-0.0137$	$Y=0.007x-0.0137$	$Y=0.0967x-0.198$	$Y=0.0057x-0.0061$	$Y=0.0217x-0.0157$	$Y=0.0195x-0.0101$	$Y=0.1109x-0.0488$	$Y=0.02x-0.0138$
Correlation coefficient	0.9999	0.9999	0.9999	0.9998	0.9996	0.9999	0.9999	0.9996
LOD ($\mu\text{g/mL}$)	2.1433	2.1433	1.6838	1.6438	0.7801	0.8544	0.9224	0.8162
LOQ ($\mu\text{g/mL}$)	6.4948	6.4948	5.1026	4.9811	2.3640	2.5890	2.7951	2.4733
System Suitability Testing(RSD%) ^a	0.31	0.13	0.11	0.11	0.09	0.30	0.25	0.09
Accuracy ^{b,c}	100.92±1.05	100.33±1.24	100.79±1.00	100.18±1.65	99.27±0.85	99.56±0.80	99.83±0.78	99.44±0.83
Repeatability ^{b,d}	0.88	1.05	0.92	0.97	1.24	1.15	1.24	1.20
Intermediate precision ^{b,d}	1.37	1.66	1.41	1.49	1.16	1.12	1.18	1.13

^aAverage of six replicates of 80 $\mu\text{g/mL}$ for ASP, and 40 $\mu\text{g/mL}$ for SIL.

^bAverage of three experiments.

^cMean of the concentrations (30, 50, 70 $\mu\text{g/mL}$) for ASP, and (15, 25, 35 $\mu\text{g/mL}$) for SIL \pm SD.

^dRSD% of the concentrations (40, 60, 80 $\mu\text{g/mL}$) for ASP, and (20, 30, 40 $\mu\text{g/mL}$) for SIL.

$$LOD = 3.3 \frac{SD}{Slope}$$

$$LOQ = 10 \frac{SD}{Slope}$$

Accuracy of the advanced approaches was computed and expressed as the percentage recoveries and standard deviation (SD) of unadulterated ASP and SIL. Moreover, the approach of standard addition was implemented by adding known amounts of ASP and SIL at three various concentrations (80-100-120%). Results are disclosed in Table 3.

The precision of the advanced approaches was appreciated by analyzing three samples of ASP and SIL in triplicates on the same day

(Intra-day or repeatability) and on three various successive days (Inter-days or intermediate precision). Acceptable and satisfying results RSD% were disclosed in Table 2.

System suitability testing was implemented by calculating RSD% for six replicated samples of ASP and SIL as disclosed in Table 2.

Specificity was executed by testing laboratory equipped admixtures consisting of diverse concentrations of ASP and SIL. The results were disclosed as mean recovery of all laboratory equipped admixtures (Recovery % ± SD) as displayed in Table 4.

Table 3: Standard addition method.

Method	Level	Bayer®+Vega®		ODT	
		ASP	SIL	ASP	SIL
		Drug recovery%± SD	Drug recovery%± SD	Drug recovery%± SD	Drug recovery%± SD
ACM	80%	101.45±1.16	98.69±0.52	99.08±1.21	96.07±0.00
	100%	101.30±0.53	99.37±0.20	101.94±1.96	96.87±0.71
	120%	101.47±0.94	97.44±1.78	99.69±1.36	100.28±0.71
AUCCM	80%	102.93±1.40	99.51±0.51	98.54±1.50	96.12±0.16
	100%	102.52±0.31	99.71±0.25	101.29±1.46	97.71±0.94
	120%	102.55±1.02	97.79±1.61	99.77±1.27	100.99±0.42
IDWM	80%	102.57±0.22	97.86±0.75	99.87±2.10	94.55±0.28
	100%	101.40±0.65	98.66±0.58	101.45±0.81	96.93±1.22
	120%	101.43±0.93	97.26±2.59	99.55±1.56	98.65±1.21

Table 4: Estimation of ASP and SIL in laboratory equipped combinations and tablet formulations.

Mix ratio	ASP:SIL ^a (µg/mL)	ASP				SIL			
		Recovery %				Recovery %			
		RS	ACM	AUCCM	IDW	SS	ACM	AUCCM	IDW
1:1	30:30	103.03	97.70	97.80	96.29	99.24	99.73	99.93	99.55
2:1	80:40	102.39	101.24	102.19	99.76	97.70	97.06	96.80	98.00
3:1	45:15	100.86	99.52	99.52	99.33	99.65	98.95	99.04	98.85
4:1	100:25	101.43	100.88	100.81	101.53	102.25	101.08	101.81	101.28
6:1	90:15	101.28	100.85	100.67	101.05	100.78	99.41	99.28	99.54
Mean ^b	--	101.80 ±1.43	100.04 ±1.79	100.20 ±1.92	99.59 ±2.21	99.92 ±1.91	99.25 ±1.76	99.37 ±1.99	99.45 ±1.60
SRF		0.9890	--	--	--	1.0055	--	--	--
Pharmaceutical Forms									
3:1 ^b	90:30	101.75	99.82	100.09	99.82	100.01	99.57	100.23	99.57
3:1 ^c	90:30	101.43	99.61	100.39	99.61	99.95	99.21	99.40	99.21
Mean ^d	--	101.59 ±1.56	99.71 ±2.05	100.24 ±1.77	99.71 ±2.05	99.98 ±1.75	99.39 ±1.87	99.82 ±2.11	99.39 ±1.87
SRF		0.9770	--	--	--	1.0159	--	--	--

^aAverage of three experiments.

^bAspirin Bayer® tablets and standard SIL.

^cVega® tablets and standard ASP.

^dAverage of the recovery of all laboratory equipped admixtures ± SD.

All advanced UV-spectrometric techniques were statistically compared with announced HPLC method¹. t and F values were reckoned to evaluate the differences in data for statistical significance. The results were judged to be less than the critical ones as disclosed in Table 5. Table 6 shows One-way ANOVA results gained by the advanced approaches and the one announced by HPLC. These results demonstrate that there was no considerable variance between the advanced methods and the announced one.

Comparative study between all advanced UV-spectrometric techniques

A comparative study was done between the advanced UV-spectrometric techniques in term of sensitivity, simplicity, and cost. Sensitivity was expressed as the Limit of detection (LOD). The simplicity of the advanced methods was explained by the advantages and limitations of these methods. All advanced methods do not require a specific software and do not demand a mixed solvent system, which reduces resulting costs. All results were disclosed in Table 7.

Table 5: Statistical analysis of the advanced UV-spectrometric techniques and the announced HPLC method.

Bayer®+ Vega®	Parameters	ASP					SIL				
		RS	ACM	AUCCM	IDW	RM ^b	SS	ACM	AUCCM	IDW	RM ^b
	Mean ^a	102.50	100.71	101.29	99.84	102.04	100.23	100.17	100.34	99.98	100.83
	SD	1.87	1.54	1.96	2.78	1.71	1.69	1.24	1.20	1.61	1.12
	Variance	3.50	2.38	3.84	7.71	2.91	2.86	1.55	1.43	2.60	1.26
	N	5	5	5	5	5	5	5	5	5	5
	t-test ^c	0.41	1.29	0.64	1.51	--	0.66	0.88	0.67	0.97	--
	F-test ^d	1.20	1.22	1.32	2.65	--	2.27	1.23	1.14	2.06	--
	SRF	0.9788	--	--	--	--	1.0112	--	--	--	--
ODT	Parameters										
	Mean ^a	101.41	100.41	100.49	100.03	99.47	99.71	99.13	99.88	99.89	99.66
	SD	1.42	1.59	1.54	1.97	1.82	0.79	1.24	1.11	0.75	1.70
	Variance	2.02	2.53	2.38	3.87	3.32	0.62	1.54	1.24	0.56	2.88
	N	5	5	5	5	5	5	5	5	5	5
	t-test ^c	1.88	0.86	0.95	0.46	--	0.06	0.56	0.25	0.28	--
	F-test ^d	1.64	1.31	1.40	1.17	--	4.65	1.87	2.33	5.18	--
	SRF	0.9900	--	--	--	--	1.0156	--	--	--	--

^aAverage of five experiments.

^bReported method is HPLC method.

^cThe tabulated value of Student's t-test is 2.776 at p = 0.05.

^dThe tabulated value of F value is equal to 6.39 at p = 0.05.

Table 6: Results of one-way ANOVA.

	Source of variation	Degree of freedom	Sum of squares	Mean Square	P value ^a	F value ^a	F critical ^a
ASP	Between columns	9	42.949	4.772	0.228	1.384	2.12
	Within columns	40	137.950	3.449	--	--	--
	Total	49	180.900	--			
SIL	Between columns	9	9.412	1.046	0.763	0.632	2.12
	Within columns	40	66.198	1.655	--	--	--
	Total	49	75.611	--			

^aThere was no significance variance among these methods using one-way ANOVA at p < 0.05.

Table 7: A comparative study between the advanced UV-spectrometric techniques used for simultaneous determination of ASP and SIL.

Method	Advantages	Limitations	LOD ($\mu\text{g/mL}$)
Ratio subtraction method (RS)	Measured at zero order spectra Ensured high accuracy and precision	only the less extended component can be determined Require three steps to obtain the recovered spectrum	2.1433for ASP
Spectrum subtraction method (SS)	Simple, uncomplicated method. It has only one step to get the zero order spectra	Applied as a supplementary method	0,7801for SIL
Absorption correction method (ACM)	Measured at zero order spectra	Only applied when one of the two components has interference at λ_{max} with the second component, while the second component has no interference with the first one at λ_2 Require for calculate absorption factor	2.1433for ASP 0.8544 for SIL
Area under the curve correction method (AUCCM)	Measured at zero order spectra The sensitivity of this method is improved, compared to ACM, because the values obtained are always higher than the values obtained from the absorbances	One of the components of the binary mixture must have a free region Require for calculate the AUC factor	1.6838 for ASP 0.9224 for SIL
Induced dual wavelength method (IDWM)	Measured at zero order spectra	Require for calculate equality factor. Any aberration in these two selected wavelengths leads to artificial results	1.6438 for ASP 0.8162 for SIL

Conclusion

This work permits the concurrent estimation of ASP and SIL in binary synthetic admixtures and pharmaceutical formulations. All UV-spectrometric techniques used are uncomplicated, ecofriendly and economic. These approaches do not necessitate any sophisticated techniques, do not waste time and are easy to apply.

All proposed approaches showed similar accuracy, precision and sensitivity. In addition, RS and SS methods enable us to ascertain the purity of the resolved spectrum through the SRF technique; which is considered much simpler than the other methods have been used.

These results demonstrate the potential superiority of all proposed approaches over the UV announced one. Moreover, the solvent used in this technique (ethanol) is more environmentally friendly and more preferable than solvents used in literature review.

The evaluation of these methods included comparison between results of these studies and published HPLC work and no significant variance was discerned.

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نشرة العلوم الصيدلانية جامعة أسيوط



تطوير أربع تقنيات طيفية للأشعة فوق البنفسجية للتحديد المتزامن للأسبرين وسيترات السيلدينافيل في مزيجهما الثنائي وفي التركيبات الصيدلانية

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اعتبر الجمع بين الأسبرين وسيترات السيلدينافيل ضمن جرعة وحيدة موضع اهتمام لفترة طويلة؛ وذلك بسبب التأثير التآزري لعلاج العديد من الأمراض. لذلك، هناك حاجة ماسة لاستخدام تقنيات التحليل الطيفي في المجال فوق البنفسجي للتحديد المتزامن للأسبرين وسيترات السيلدينافيل في مزيجهما الثنائي وفي المستحضرات الصيدلانية. في هذه الورقة البحثية تم تطوير أربع تقنيات طيفية بسيطة ومنخفضة التكلفة ودقيقة من أجل التحديد المتزامن للأسبرين وسيترات السيلدينافيل. تشمل الطرائق المطورة معالجة طيوف المرتبة الصفريّة وذلك باستخدام طريقة الطرح النسبي جنبًا إلى جنب مع طريقة طرح الطيف، وطريقة تصحيح الامتصاصية، وطريقة المساحة تحت المنحني المصححة، وطريقة طول الموجة المزدوج المستحث. علاوة على ذلك، تم تطوير طريقة طيفية لتحديد نقاوة وذلك باستخدام معامل النسبة الطيفية؛ الذي يعتبر أول اختبار طيفي تم استخدامه للتحقق من نقاوة الأسبرين في الأشكال الصيدلانية. تراوحت خطية جميع التقنيات ضمن المجال ٢٥.٠-١٠٠.٠ ميكروجرام/مل للأسبرين، و ١٠.٠-٥٠.٠ ميكروجرام/مل لسيترات السيلدينافيل. وكان متوسط النسبة المئوية لاسترداد للأسبرين ٩٢.٩٢ و ١٠٠.٣٣ و ١٠٠.٧٩ و ١٠٠.١٨٪ على التوالي، و ٩٩.٢٧ و ٩٩.٥٦ و ٩٩.٨٣ و ٩٩.٤٤٪ لسيترات السيلدينافيل على التوالي. كانت قيم معامل النسبة الطيفية قريبة من الواحد وأظهرت درجة نقاوة عالية للقمم التي تم استردادها. تمت مقارنة النتائج إحصائياً مع طريقة الكروماتوجرافيا السائلة عالية الأداء المذكورة ولم يلاحظ أي فرق معنوي بين النتائج.