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(Research Article)

Green UV Spectrophotometric Methods for Simultaneous Determination of Aspirin and Esomeprazole in Laboratory Prepared Capsules

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Abstract: Simple, green and accurate UV spectrophotometric methods were established aiming for simultaneous estimation of aspirin and esomeprazole without prior separation. These methods are zero order absorption, ratio subtraction, mean centering and absorbance subtraction. In zero order method esomeprazole was measured at 302.0 nm at which aspirin has no absorbance while aspirin was determined by a ratio subtraction at 225.0 nm after deduction of overlapping caused by esomeprazole. In mean centering, the mean centered values were estimated at 228.0 nm and 262.0 nm for aspirin and esomeprazole respectively. In absorbance subtraction method the two drugs intersect at isoabsorptive point at 237.0 nm and esomeprazole spectrum is further prolonged where aspirin has no absorbance. The isoabsorptive point can be used for their determination using calculated factor called absorbance factor. These methods are effective for estimation of aspirin and esomeprazole in dosage form. Moreover, the greenness of the suggested methods was evaluated demonstrating minimum hazardous effect on the environment.

Keywords: Aspirin, Esomeprazole, Zero order, Ratio subtraction, Mean centering, Absorbance subtraction.

1. INTRODUCTION

Aspirin (ASP) is 2-Acetyloxybenzoic acid. It is a colorless or white crystals. It is soluble in water, ethanol, chloroform and ether; it is also soluble in acetates and citrates solutions, with decomposition, in alkali hydroxides and carbonates solutions [1]. It is a member of NSAID with analgesic, anti-inflammatory and antipyretic properties. It hinders the enzyme cyclooxygenase causing direct inhibition of the biosynthesis of prostaglandins and thromboxane from arachidonic acid. It is also used in the initial treatment of angina pectoris and myocardial infarction as well as in the avoidance of cardiovascular events at risk patients [2]. Numerous methods have been established for the analysis of ASP especially in mixture with other drugs. The more recent articles were spectrophotometric [3-8], spectrofluorometric [9-12], electrochemical [13], densitometric [14-16] and HPLC methods [17-23]. Esomeprazole (ESZ) is 5-Methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl2-pyridyl) methyl] sulfinyl] benzimidazole. It is soluble in organic solvents such as ethanol, DMSO and dimethyl formamide (DMF). It is very slightly soluble in water [24]. Esomeprazole inhibits the H+/K+-adenosine triphosphatase enzyme leading to reduction of gastric acid secretion [25, 26]. It is used in the management of peptic ulcer and NSAID-associated ulcer, in gastro-esophageal reflux and the Zollinger-Ellison syndrome [2]. Numerous analytical methods for determining ESZ have been developed either alone, or in mixture with other

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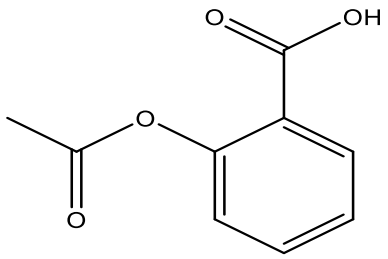
drugs in pharmaceuticals and in biological fluids [27, 28]. Limited methods were published for the simultaneous estimation of ASP and ESZ as spectrophotometric [29], HPTLC [30], UPLC [31] and HPLC methods [32].

Green analytical chemistry was announced to minimize the hazardous of procedures on workers and the environment. It has gained an excessive interest between chemists focused on making their procedures environmentally friendly [33]. Green chemistry is used as it confirms consumption of safe, less toxic solvents and the use of small quantities of reagents [33]. Therefore, this study was concentrated on the establishment of eco-friendly, simple, fast, economic and delicate methods for analysis of aspirin and esomeprazole in dosage form. The greenness of the established methods as well as the reported one was evaluated to demonstrate the effect of the established methods on the environment.

A



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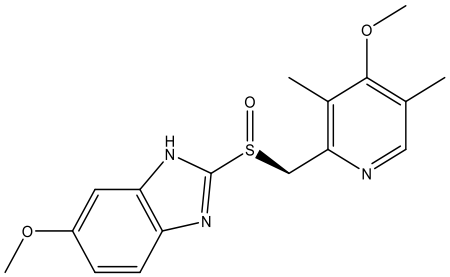


Figure1. Chemical structure of aspirin (A) and esomeprazole (B).

2. METHODS

2.1. Instruments

Shimadzu UV-Visible 1650 Spectrophotometer, (Tokyo, Japan), linked to UV probe program with two matched 1 cm path-length quartz cell.

2.2. Pure samples

Pure ASP as gift samples (B.N.ER00003) was obtained from Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. Its purity was 99.83% by application of the reported method [29].

Pure ESZ sodium (B.N. 202510110001) was friendly provided from Chemipharm Pharmaceutical Industries, Giza; Egypt. The purity was 100.14%by using the reported method [29].

2.3. Laboratory prepared capsules

Laboratory prepared Co-formulated capsule Axanum[34], were prepared in the laboratory as follows: ASP and ESZ were finely ground separately and an quantity of the fine powder equal to 81.0 mg of ASP and 20.0 mg of ESZ were mixed together with sucrose (13.7 mg), magnesium stearate (1.0mg) and hydroxypropylcellulose (3.3mg) in hard gelatin capsule.

2.4. Chemicals and reagents

Ethanol (Sigma-Aldrich).

2.5. Standard solutions

Stock solutions of ASP and ESZ (1mg/mL) were made in ethanol by dissolving 100 mg of ASP or ESZ in 25 mL ethanol and completed to 100 mL. Working standard solutions of ASP or ESZ (100µg/ mL) were prepared by dilution with ethanol.

2.6. Procedures

2.6.1. Spectral characteristics

The spectra of ASP and ESZ exhibit one way of overlapping with isoabsorptive point at 237.0 nm, this overlapping interferes with direct estimation of ASP in combination with ESZ while the spectrum of ESZ is more extended and can be determined at 302.0 nm at which ASP has no absorbance. The isoabsorptive point can be used for their determination using calculated factor called absorbance factor **Figure (2)**.

2.6.2. Construction of calibration curves

Volumes of standard ASP or ESZ solution (100 µg /mL) equivalent to definite range were transferred accurately into two distinct sequences of 10-mL volumetric flasks and completed with ethanol. The UV absorption spectra of the prepared solutions were scanned from 200.0-400.0 nm using ethanol as a blank and saved.

-Zero-order absorption

The spectra of ESZ were assessed at 302.0 nm. A linear calibration curve was produced between the absorbance at 302.0 nm and the equivalent concentration of ESZ then the regression equation was computed.

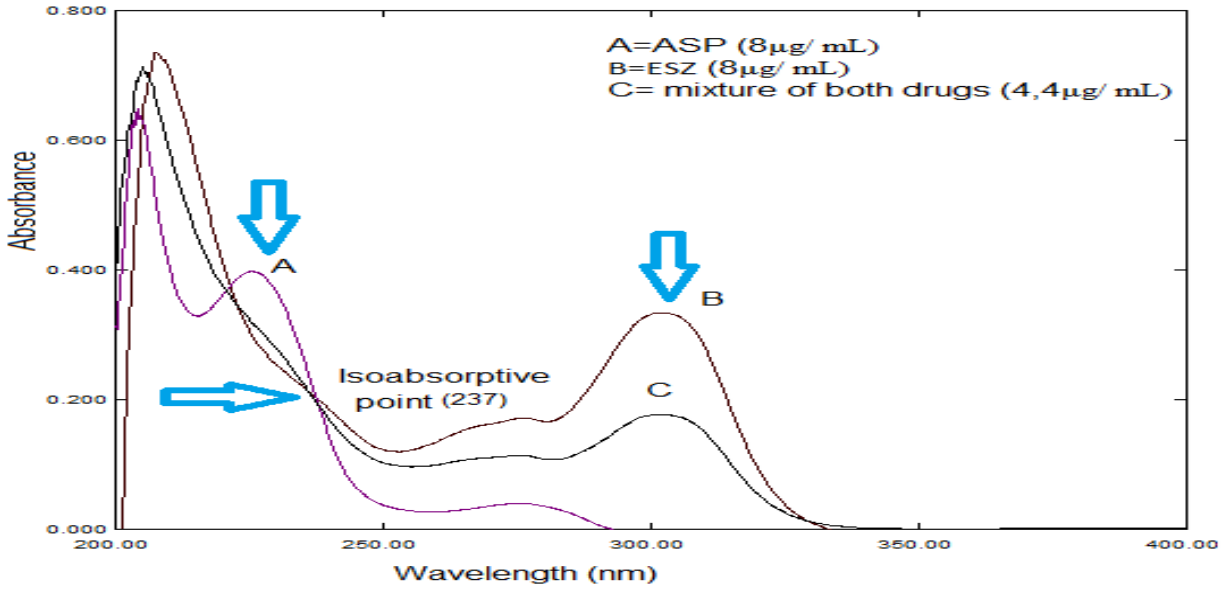


Figure 2. Zero order spectra of (A): Aspirin (8 µg/ mL), (B): Esomeprazole (8 µg/ mL) and (C): Mixture of Aspirin and Esomeprazole 4 µg/ mL for each in ethanol.

-Ratio subtraction

The laboratory prepared spectra of mixture of ASP and ESZ were divided by 20 µg/mL of ESZ (extended drug). The response value at 302.0 nm in the ratio spectra was subtracted from each ratio spectrum, after that the obtained spectra were multiplied by 20 µg/mL of ESZ spectrum. The response measured at 225.0 nm was corresponding to ASP.

-Mean centering method

The absorption spectra of each drug were divided by that of (20µg/mL) of the other drug to obtain the ratio spectra, the ratio spectra of ASP (from 210.0 to 280.0 nm) or ESZ (from 200.0 to 280.0 nm) were mean centered then ASP was measured at 228.0 nm while ESZ at 262.0 nm.

Absorbance subtraction method

The absorbance of ASP at 237.0 nm (λ_{iso}) was recorded and a calibration graph of the absorbance versus the drug concentration was created. The absorbance values of ESZ at 237.0 nm and 302.0 nm were recorded and the absorbance factor was calculated [$A_{237.0}$/ $A_{302.0}$]. The absorbance of ESZ at 237.0 nm in mixture with ASP was obtained by multiplying the absorbance of the mixture at 302.0 nm by the absorbance factor. Finally the absorbance of ASP at 237.0 nm was obtained by subtraction of ESZ absorbance at this wavelength from the absorbance of the mixture and the concentration ofach drug was determined from the regression equation at 237.0 nm.

-Analysis of laboratory prepared mixture

Into a set of 10- mL volumetric flaks, different volumes of ASP and ESZ within linearity range were transferred from standard solutions (100 µg /mL), then analyzed by the proposed spectrophotometric method as previously mentioned. The concentration of each drug was obtained from the resultant regression equation.

-Application to laboratory prepared capsules

An exact amount of ingredients of ten of laboratory prepared capsules equivalent to 40.5mg of ASP and 10 mg of ESZ were dissolved in 30 mL ethanol, stirred for 30 min. and completed to 100 mL with ethanol then filtered to get concentration of 405µg/mL for ASP or 100 µg/mL for ESZ respectively then the proposed methods as previously mentioned were followed. The concentrations of ASP and ESZ were obtained from the regression equation. The standard addition technique was applied to confirm the validity of the proposed methods where fixed volume of the prepared capsule solution equivalent to (8.1) µg/mL of ASP or (2) µg/mL of ESZ were mixed with different volumes of the pure of each drug then the concentrations of ASP and ESZ were obtained from the regression equation.

3. RESULTS

The purpose of this study is to develop new validated green UV- spectroscopic methods for analysis of ASP and ESZ mixture in pure form and in laboratory prepared capsules.

3.1. Method validation

The suggested spectrophotometric methods were validated in consistent with the ICH guidelines [35].

3.1.1. Linearity

Linear relationships were attained by the suggested methods using different concentrations of drugs. The obtained response was plotted against concentration. Beer's law was obeyed in the range of 2–24 µg/mL for ASP in all studied methods. ESZ linearity range was found to be 2-24 for zero order and ratio subtraction methods, 2-12 for mean centering and 2-20 for absorbance subtraction method. The regression data were in **Table1**.

3.1.2. Limits of detection and quantitation

LOD and LOQ were estimated using these equations:

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve. The values were summarized in **Table 1**. The minor values of LOD and LOQ display respectable sensitivity.

3.1.3. Accuracy and precision

Accuracy and precision were calculated using three different concentrations of ASP or ESZ in pure form (8.1, 12.15 and 16.2 µg/mL) or (2, 3 and 4 µg/mL), each in triplicate, in one day (intra-day) and in three successive days (inter-day), then the accuracy (R%) and precision (RSD%) were calculated. The good %R, recorded in **Table 1**, confirm excellent accuracy.

3.1.4. Robustness

It was checked by repeating each method with minor variations in the wavelength at which the analysis was done (±0.2 nm). The response stayed relatively unchanged, approving robustness of the procedure. RSD % did not exceed 1.34, 1.56 and 1.02 for ASP for ratio subtraction, mean centering and absorbance subtraction methods respectively and1.24, 1.78 and 1.12 for ESZ for zero order absorption, mean centering and absorbance subtraction methods respectively confirming robustness of the method.

3.1.5. Selectivity

The established methods were applied to different mixtures of ASP and ESZ where good recoveries were obtained **Table 2**.

3.1.6. Analysis of laboratory prepared capsule

The developed procedures were used for estimation of ASP and ESZ in laboratory prepared capsule .The obtained results were in good agreement, indicating no interfering from other ingredients. **Table3**.

Moreover, the standard addition technique was applied to approve the validity of the proposed methods. **Table4**. The obtained results were statistically compared with a reported method [29]. It was appeared that no essential differences by applying t-test and F-test at 95% confidence level [36]. Finally the established methods showed better sensitivity than the reported spectrophotometric method [29].It could measure concentrations 2 µg/mL for ASP or ESZ.

4. DISCUSSION

4.1. Zero order absorption [37]

The zero-order absorption spectrum of ESZ at 302.0 nm showed no interference from ASP, which allowed for direct estimation of ESZ at this wavelength.

4.2 Ratio Spectra methods

The selection of the divisor was of extreme significance, so various concentrations of divisor were used (4, 8, 10, 14, 18 and 20 µg/mL); the most appropriate one was (20 µg/mL), as it showed excellent results consistent with selectivity and sensitivity; **Figure 3, 4**.

4.2.1. Ratio subtraction [38]

ASP and ESZ mixtures absorption spectra were divided by ESZ (20 µg/mL) spectrum to develop the ratio spectra; **Figure 5** the response in plateau region at 302 nm were subtracted; **Figure 6**, the obtained spectra were multiplied by ESZ spectrum (20 µg/mL); **Figure 7** to obtain the final spectra. The response at 225.0 nm in the obtained spectra is relative to ASP concentration lacking overlapping from ESZ.

4.2.2. Mean centering method [39]

Stored ratio spectra of the ASP and ESZ were mean centered. The obtained results at 228.0 nm are relative to the concentrations of the ASP lacking overlapping from ESZ (divisor); **Figure 8** whereas values at 262.0 nm were used for ESZ analysis; **Figure 9**.

Table 1. Assay parameters for the determination of ASP and ESZ by the proposed UV spectrophotometric methods

Parameters	Zero order	Ratio subtraction	Mean centering		Absorbance subtraction	
	ESZ	ASP	ASP	ESZ	ASP	ESZ
Wavelength (nm)	302.0	225.0	228.0	262.0	237.0	
Linearity range (µg / mL)	2-24	2-24	2-24	2-12	2-24	2-20
Regression parameters **- Slope± (S_Y)** **- Intercept ±(S_X)** **- SD of residual (S_{YX})** **-Correlation coefficient (r)**	0.0459±0.0002 -0.0178±0.003 0.004 0.9999	0.0536± 0.001 -0.0081± 0.008 0.010 0.9994	0.0284± 0.0003 0.0003± 0.004 0.005 0.9994	0.1182± 0.001 -0.1525± 0.008 0.008 0.9997	0.0344±0.0004 -0.0302±0.006 0.006 0.9993	0.0353±0.0003 -0.0376±0.003 0.004 0.9997
LOD (µg / mL)	0.29	0.62	0.58	0.22	0.58	0.37
LOQ (µg / mL)	0.87	1.87	1.76	0.68	1.74	1.13
Accuracy (% recovery)	98.34	99.14	100.13	100.54	99.51	100.67
Precision (%RSD) **- Repeatability*** **- Intermediate precision****	0.79 1.22	0.62 0.86	0.97 1.41	0.85 1.52	1.13 1.92	1.03 1.84

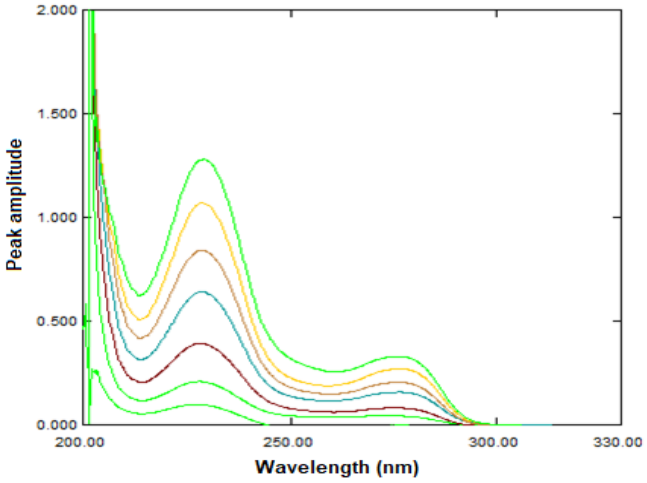


Figure 3. Ratio spectra of ASP at various concentrations (2, 4, 8, 12, 16, 20, 24 µg/mL) using (20 µg/mL) of ESZ as divisor and ethanol as blank.

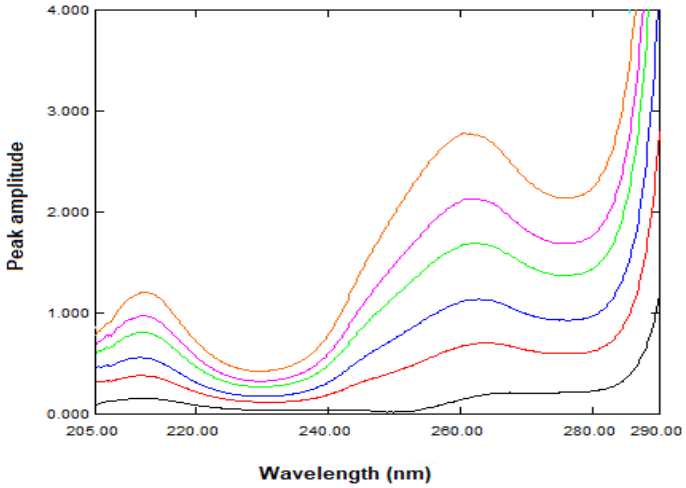


Figure 4. Ratio spectra of ESZ at various concentrations (2, 4, 6, 8,10, 12 µg/mL) using (20 µg/mL) of ASP as divisor and ethanol as blank.

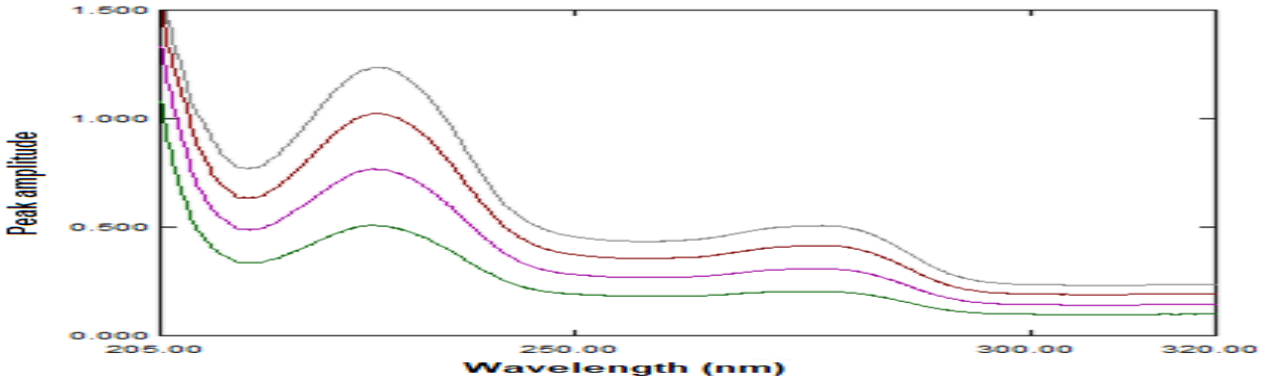


Figure 5. Ratio spectra of laboratory prepared mixture of ASP and ESZ using (20 µg/mL) of ESZ as divisor and ethanol as blank.

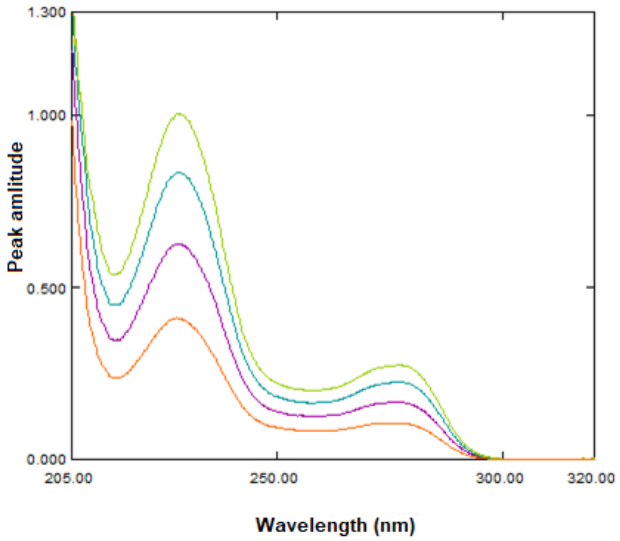


Figure 6. Ratio spectra of laboratory prepared mixture of ASP and ESZ after subtraction of ESZ at 302.0 nm.

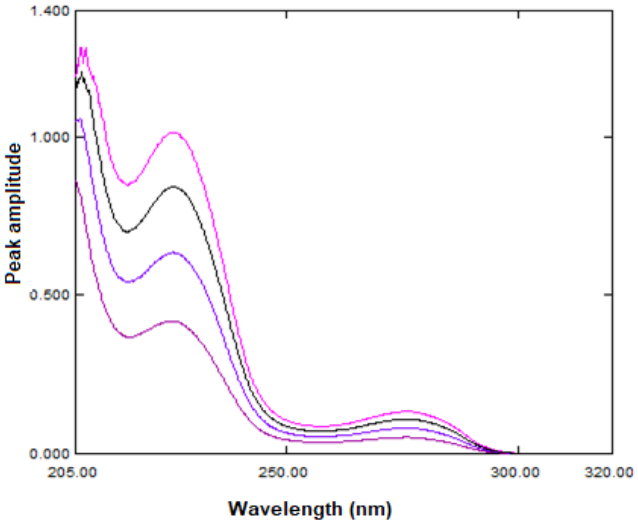


Figure 7. Final spectra of laboratory prepared mixture of ASP and ESZ after multiplication by the divisor spectrum.

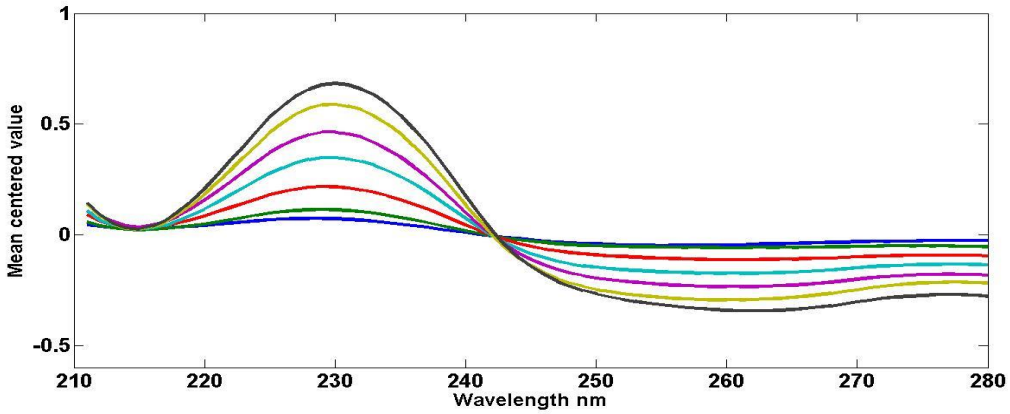


Figure 8. Mean centering of the ratio spectra of ASP at various concentrations (2, 4, 8, 12, 16, 20 and 24 µg/mL) using 20 µg/mL of ESZ as a divisor.

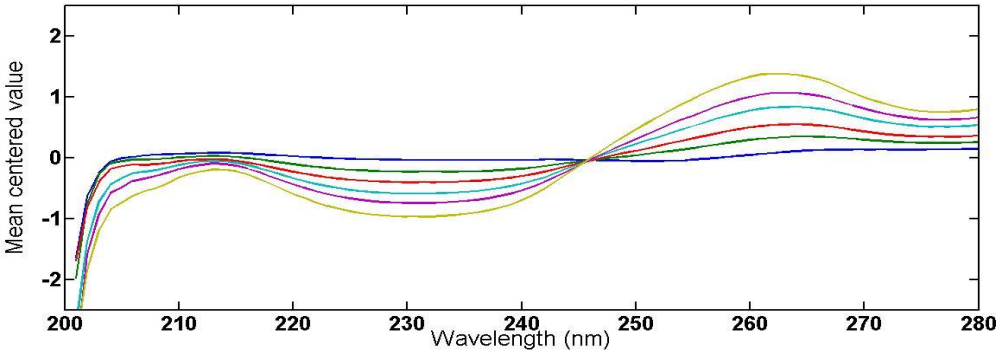


Figure 9. Mean centering of the ratio spectra of ESZ at various concentrations (2, 4, 6, 8, 10 and 12 µg/mL) using 20 µg/mL of ASP as a divisor.

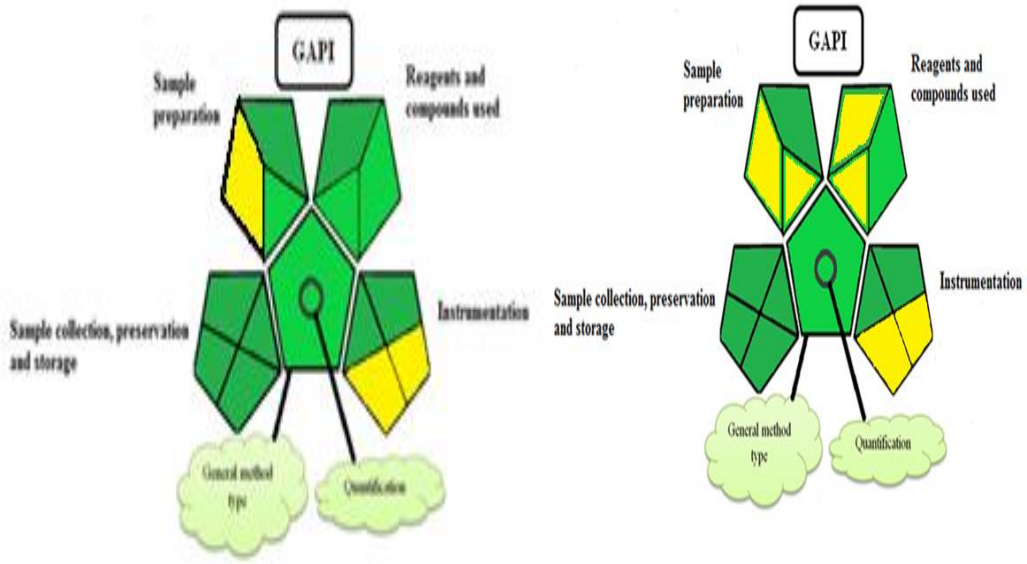
4.2.3. Absorbance subtraction method [40]

The absorbance factor ($A_{237.0}/A_{302.0}$) for ESZ was calculated by measuring different concentrations and taking the average value. The absorbance of ESZ at 237.0 nm in mixture with ASP can be obtained by multiplying its absorbance at 302.0 nm by the absorbance factor. The absorbance of ASP at 237.0 nm can be obtained by subtraction of the absorbance of ESZ at 237.0 nm from that of the mixture at the same wavelength; **Figure 2.** The regression equations at isoabsorptive point 237.0 nm can be used for estimation of the drugs concentration.

4.3. Evaluation of greenness of the methods

Green Analytical Procedure Index (GABI) is a novel tool for the assessment of the greenness of the whole method.

GAPI assessment characterizes each step of the procedure[33]. The developed methods were evaluated for greenness together with a reported method, applying GAPI tool, **Figure 10**, which showed the excellent green of the suggested methods. Furthermore, the analytical Eco-scale was applied. The score obtained by deducting penalty points from the base of 100 points is the result of the analytical Eco-scale[41]. After calculation, the proposed methods score is 95 as presented in **Table 5** proving that the proposed method is an excellent green method of analysis. It is established from these evaluation tools that the established methods are greener than the reported method demonstrating minimum hazardous effect on the environment.



Proposed methods.

Reported method [29].

Figure 10. The green assessment profile for the proposed methods in comparison with the reported method, using the GAPI tool.

Table 2. Determination of ASP and ESZ in laboratory prepared mixtures by the proposed UV- spectrophotometric methods

Conc. of ASP (µg/mL)	Conc. of ESZ (µg/mL	Zero order		Ratio subtraction		Mean centering				Absorbance subtraction			
		Found conc. of ESZ(µg/mL)	Recovery % of ESZ	Found conc. of ASP (µg/mL)	Recovery % of ASP	Found conc. of ASP (µg/mL)	Recovery % of ASP	Found conc. of ESZ(µg/mL)	Recovery % of ESZ	Found conc. of ASP (µg/mL)	Recovery % of ASP	Found conc. of ESZ (µg/mL)	Recovery % of ESZ
8.1	**2**	2.03	101.52	8.01	100.18	7.98	98.54	1.97	98.66	8.05	99.42	2.05	102.51
12.15	3	3.04	101.37	12.24	100.74	12.08	99.47	3.01	100.45	12.17	100.19	3.04	101.46
16.2	4	4.07	101.83	15.98	98.64	15.96	98.56	4.05	101.30	16.11	99.47	3.91	97.82
20.25	5	4.94	98.82	20.21	99.82	20.31	100.30	4.96	99.26	20.23	99.91	4.94	98.85
4	4	4.02	100.50	4.01	100.25	3.97	99.26	4.04	101.02	4.02	100.59	3.99	99.81
Mean% ± SD		100.81±1.22			99.93±0.79		99.23±0.73		100.14 ±1.38		99.92± 0.49		100.09± 1.90

Table 3. Results obtained by the proposed methods compared with a reported method for determination of ASP and ESZ in pharmaceutical preparations.

Parameters	Zero order method	Ratio subtraction method	Mean centering method		Absorbance subtraction method		Reported method [29]	
	ESZ	ASP	ASP	ESZ	ASP	ESZ	ASP	ESZ
Linearity range (µg/mL)	2– 24	2– 24	2– 24	2-12	2-24	2-20	20-50	5-30
n	4	4	4	4	4	4	4	4
Mean %	99.91	100.64	100.34	99.84	99.34	100.42	99.83	100.14
SD	1.35	1.94	1.36	1.88	1.37	1.63	1.88	1.55
Variance	1.82	3.76	1.85	3.54	1.88	2.65	3.55	2.39
Student's t-test	0.224 (2.447)	0.599 (2.447)	0.443 (2.447)	0.244 (2.447)	0.421 (2.447)	0.249 (2.447)	——	——
F -value	1.316 (9.277)	1.064 (9.277)	1.917 (9.277)	1.478 (9.277)	1.885 (9.277)	1.105 (9.277)	——	——

Figures in parenthesis are the theoretical t- and F- values at p = 0.05.

- *Reported method involved the use of first derivative of the ratio spectra by measurements of the amplitudes at 221.0 nm for ASP and 291.0 nm for ESZ, respectively using methanol as solvent.*

Table 4: Application of standard addition technique for the simultaneous determination of ASP and ESZ by the proposed UV spectrophotometric methods

Preparation	ASP		ESZ		Zero order	Ratio subtraction	Mean centering		Absorbance subtraction	
					ESZ	ASP	ASP	ESZ	ASP	ESZ
Laboratory prepared capsules	Claimed taken (µg/mL)	Added conc. (µg/mL)	Claimed taken (µg/mL)	Added conc. (µg/mL)	Recovery % of added	Recovery % of added	Recovery % of added	Recovery % of added	Recovery % of added	Recovery % of added
	8.1 8.1 8.1	2 4 6	2 2 2	2 4 6	99.90 101.23 100.34	99.45 100.68 99.91	101.49 102.11 99.79	99.56 98.75 101.98	98.79 102.07 100.06	100.21 99.19 102.03
Mean%± SD					100.49± 0.68	100.01 ± 0.62	101.13±1.20	100.10±1.68	100.31±1.65	100.48±1.44

Table 5: Eco-scale penalty points [40] for comparing between the proposed methods and the reported method

Proposed methods		**Reported method ([29])**	
Reagents	**PPs**	**Reagents**	**PPs**
Ethanol	0	Water	0
		Methanol	6
Instrument	0		0
Occupational hazards	0		0
Waste	5		5
Total PPs	Σ5		Σ11
Eco-Scale	95		89
	Excellent green analysis		

5. CONCLUSIONS

It can be concluded that the proposed spectrophotometric methods for the simultaneous determination of ASP and ESZ in bulk or in their combination are green, simple, accurate, fast, economic and do not require initial pretreatment steps. The greenness evaluation of the methods was applied, demonstrating the highest greenness of the developed methods. Consequently, these methods were efficiently performed for assessment and regular quality control analysis of ASP and ESZ with minimum sample preparation.

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Conflict of interest: The authors declare that there are no conflicts of interest.

Ethics statement: N A

Author contribution: Authors MF and NR are the supervision of the work designed the study and the protocol. Author AH finished the experimental study and statistical analysis, wrote the first draft and managed literature searches. All authors agreed the final manuscript.

Acknowledgments: NA

List of abbreviations: ASP: Aspirin, **ESZ**: Esomeprazole, **GAPI**: Green Analytical Procedure Index, **ICH**: International Conference on Harmonization, **LOD**: Limit of Detection, **LOQ**: Limit of Quantification, **RSD**: Relative Standard Deviation, **SD:** Standard Deviation, **UV**: Ultraviolet

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