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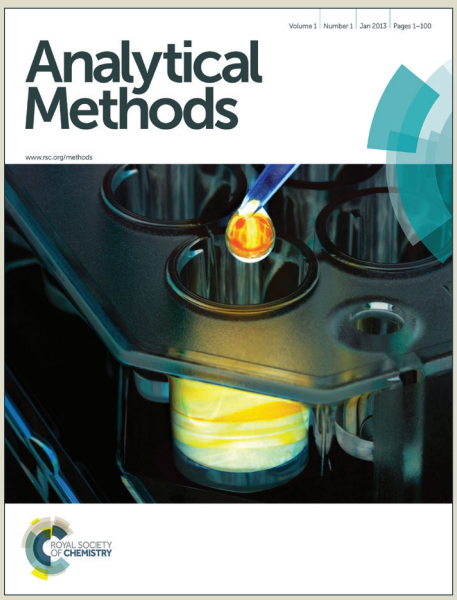

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Simultaneous Determination of Methocarbamol and Aspirin Binary Mixture in their Combined Tablets by Derivative and Ratio Derivative Spectrophotometry

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Abstract

Two simple and rapid spectrophotometric methods were developed for simultaneous determination of methocarbamol (MET) and aspirin (ASP) in their combined dosage form. The first method, zero-crossing first derivative spectrophotometry, depends on measuring the first derivative amplitude values at 279.2 nm and 246.6 nm for the simultaneous determination of MET and ASP, respectively. The second method, ratio first derivative spectrophotometry, depends on measuring the peak amplitudes of the first derivative of the ratio spectra at 284 nm for MET and at 238.2and 246 nm for ASP determination. The calibration graphs were linear over the range of 2.0-30.0 μg/mL for MET and ASP by the two proposed methods. The detection limits of the first derivative method were found to be 0.2998 and 0.3227 μg/mL and the quantitation limits were 0.9084 and 0.9780 μg/mL for MET and ASP, respectively. For the ratio first derivative method, the limits of detection and quantitation were 0.2795 and 0.8470 μg/mL for MET, respectively while, ASP limits **of** detection and quantitation were 0.2485 and 0.7530 μg/mL at 238.2 nm and 0.4173 and 1.2647 μg/mL at 246 nm. The proposed methods were applied successfully to the assay of the studied **drugs** in their combined tablets. The results were favorably compared to those obtained by the **reference** methods.

Key words: methocarbamol; aspirin; first derivative, ratio first derivative; spectrophotometry; tablets.

I. Introduction:

Methocarbamol (MET), (±)-3-(*o*-Methoxyphenoxy)-1,2-propanediol 1-carbamate (1) (Fig 1), is a centrally acting skeletal muscle relaxant whose action is due to general depressant effects on the central nervous system (2). The United States Pharmacopoeia (USP) (1) **recommended** direct measurement of the absorbance in methanol at 274 nm for the determination of MET raw material and HPLC method with UV detection for its tablet and injection dosage forms. Spectrophotometric (3), spectrofluorometric (4), electrochemical (5), HPLC (6), and supercritical fluid chromatographic methods (7) were also reported for the determination of MET **either in pharmaceutical formulations and/or in biological fluids**.

Aspirin (ASP), 2- (Acetyloxy) benzoic acid (1) (Fig 1), is a salicylate NSAID having analgesic, anti-inflammatory, and antipyretic properties. Its action depends on the inhibition of the cyclo-oxygenase (COX) enzyme by irreversible acetylation of the enzyme, which results in direct inhibition of the biosynthesis of prostaglandins and thromboxanes from arachidonic acid (2).

Regarding ASP, the USP (1) and the British Pharmacopoeia (BP) (8) recommended back titration methods for the analysis of ASP raw material, while the USP described an HPLC method for the analysis of ASP tablets (1).

Several analytical methods **were** reported for the estimation of ASP like, spectrophotometry (9), spectrofluorometry (10-12), capillary electrophoresis (13, 14), HPTLC (15) and HPLC (16, 17) either in dosage forms or in biological fluids.

Combination of MET and ASP is used to relieve the pain associated with the muscle spasm. MET and ASP were determined in their binary mixture *via* HPLC (18), GC (19) and supercritical fluid chromatography (20).

The objective of the present work was to develop two simple, rapid and accurate derivative spectrophotometric methods for the simultaneous determination of MET and ASP binary mixture based on using first derivative method adopting the zero-crossing technique and ratio first derivative spectrophotometric method.. The proposed methods could be applied successfully for the determination of these compounds in their dosage forms.

II. Experimental

II.A. Instrumentation

- Spectrophotometric analyses were carried out on a Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer with 1-cm path length quartz matched cuvettes. Absorption spectra of both drugs

were recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto.

The first derivative spectra of both drugs were derived in the wavelength range (200 - 350) nm using $\Delta\lambda = 8$ nm and scaling factor = 100.

The ratio derivative spectra for MET were derived in the wavelength range (200-300) nm using $\Delta\lambda = 2$ nm and scaling factor = 1 for smoothing of ratio spectra and $\Delta\lambda = 4$ nm for the first derivative of ratio spectra with scaling factor = 10. Meanwhile, the ratio derivative spectra for ASP were derived in the wavelength range (200-270) nm using $\Delta\lambda = 2$ nm and scaling factor = 1 for smoothing of ratio spectra and $\Delta\lambda = 4$ nm for the first derivative of ratio spectra with scaling factor = 10.

II.B. Reagents and chemicals

All the chemicals used were of Analytical Reagent grade and the solvents were of spectroscopic grade.

- Methocarbamol, pure sample 99.50% (batch # TR080111) was kindly provided by Sigma Pharmaceutical Company, Cairo, Egypt, and was used as received.
- Aspirin, pure sample, 99.84% was kindly provided by EL Nasr Pharmaceutical Company, Cairo, Egypt, and was used as received.
- Laboratory prepared tablets (400mg methocarbamol, 325mg aspirin, 20mg talc powder, 15mg maize starch, 15mg lactose and 7mg magnesium stearate per tablet).
- Methanol (Sigma-Aldrich, Germany).

II.C. Preparation of standard solutions

Stock standard solutions of MET and ASP (100 µg / mL each) were prepared by accurately weighing 10.0 mg of each and transferred separately to 100 mL measuring flasks, then complete to the mark with methanol. The stock solutions were further diluted to obtain the working concentration ranges. The standard solutions were found to be stable for at least one week without alteration when kept in the refrigerator.

II.D. Construction of the calibration curves

Aliquots of the working solutions covering the final concentration range of 2.0-30.0 µg/mL for MET and ASP were transferred into a series of 10 mL volumetric flasks, diluted with methanol to the mark and mixed well, and the zero-order absorption spectra were recorded against methanol as a blank.

For the determination by first derivative spectrophotometry, the absolute values of the first order derivatives were measured at 279.2 nm for MET and 246.6 nm for ASP. The derivative amplitudes were then plotted against the final concentrations to get the calibration graphs. Alternatively, the corresponding regression equations were derived.

For the determination by ratio derivative spectrophotometry, the first derivative of the ratio spectra (the spectra of MET divided by the spectrum of 10.0 µg/mL ASP solution and the spectra of ASP divided by the spectrum of 10.0 µg/mL MET solution) were recorded. The amplitudes at 284 nm for MET and at 238.2and 246 nm for ASP were measured. The derivative amplitudes were then plotted against the final concentrations to get the calibration graphs. Alternatively, the corresponding regression equations were derived.

II.E. Procedure for the laboratory prepared mixture

Accurately measured aliquots of the working standard solutions of both drugs were transferred into a series of 10 mL volumetric flasks to prepare different laboratory-prepared mixtures of ASP and MET in the ratio of nearly 1:1.25(their pharmaceutical ratio), respectively. The solutions were then diluted with methanol to volume, mixed, and analyzed as described under "*construction of the calibration curves"*. The concentration of each drug was determined using, either the calibration curve or the corresponding regression equation.

II.F. Procedure for laboratory prepared tablets

A quantity of the powder of the laboratory prepared tablets equivalent to 325 mg of ASP and 400 mg MET (in their pharmaceutical ratio nearly 1:1.25) was transferred into a small conical flask and extracted with 3 x 30 mL of methanol and the solution was sonicated for 10 min then filtered into 100 mL volumetric flask. The conical flask was washed with few milliliters of methanol; the washings were passed into the same volumetric flask which was then completed to the mark with methanol to give the working solution. Aliquots over the concentration range (2.0-30.0 µg/mL) were transferred into 10.0 mL volumetric flasks. The general procedure was then applied as under "*construction of calibration curves*", and the nominal content of tablets was determined either from the previously plotted calibration graphs or using the corresponding regression equations.

III. Results and discussion

The UV spectrum of MET solution in methanol showed absorption maxima at 206, 225 and 275 nm, while that of ASP showed absorption maxima at 210, 227, 278 and 305nm (Fig. 2). MET can't be determined in the presence of ASP by conventional UV spectrophotometry because

of the obvious overlap between MET spectrum and that of ASP throughout the maxima of MET , while ASP can be determined but with very low sensitivity at 305 nm as MET does not show any significant absorption in the vicinity of this fourth maximum of ASP. Thus, derivative and ratio derivative spectrophotometry were applied for the quantification of both drugs in the presence of each other.

III.A. First Derivative Spectrophotometry

Because the derivative spectrophotometric technique enhances the detectability of the minor features of the UV absorption spectrum, the first derivative spectra of both MET and ASP (Fig. 3) displays features which may permit more specific and selective determination of each drug in the presence of the other. The zero-crossing method is the most common procedure for conducting analytical calibration in derivative spectrophotometry. The first derivative amplitudes at 279.2nm (zero-crossing of ASP) and at 246.6 nm (zero-crossing of MET) were chosen for the simultaneous determination of MET and ASP, respectively, in their binary mixtures.

III.B. Ratio Derivative Spectrophotometry

Fig. 4 shows the ratio spectra of different concentrations of MET standards (spectra divided by the spectrum of a solution containing 10.0 μg/mL of ASP) while Fig. 5 shows their first derivative spectra. In this figure, the amplitude at 284 nm ($^1DD_{284}$) in the ratio derivative spectra corresponds to MET present in the solution, so it can be used for its quantitative determination.

Likewise, Fig. 6 and 7 show the ratio spectra of different concentrations of ASP standards (spectra divided by the spectrum of 10.0 μg/mL MET solution) as well as their corresponding first derivative

spectra, on the basis of which, ASP can be quantified by measuring the amplitude at 238.2 and 246 nm ($^1DD_{238.2, 246}$).

The influence of Δλ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; Δλ = 4 nm was considered as suitable for both drugs. For selecting the standard solution as divisor, different concentrations were tested and different calibration curves were obtained.

The best results in terms of signal-to-noise ratio, sensitivity and repeatability were obtained by using the spectra of 10.0 µg/mL of MET and ASP solutions as divisors in the determination of ASP and MET, respectively.

IV. Validation

To assess the validity of the proposed method, it was tested for linearity, range, limits of quantification, limits of detection, accuracy, precision, specificity and solution stability.

IV.A. Linearity and Range

The calibration graphs for the determination of MET and ASP by the proposed methods were constructed by plotting the derivative amplitudes *versus* the concentrations as shown in Figures (8 and 9).

Linear regression analysis of the data by first derivative method gave the following equations:

DA = 0.0002 +0.05 C (r =0.9999) for MET at 279.2 nm ($^1D_{279.2}$)

DA = -0.1431+0.10C(r =0.9999) for ASP at 246.6 nm ($^1D_{246.6}$)

and by the ratio first derivative method gave the following equations:

DA = -0.027+ 0.16C (r = 0.9999) for MET at 284.0 nm ($^1DD_{284.0}$)

DA = -0.831+0.72C (r = 0.9999) for ASP at 238.2 nm ($^1DD_{238.2}$)

DA = -0.863+0.66 C (r = 0.9999) for ASP at 246.0 nm ($^1DD_{246.0}$)

where DA is derivative amplitude, C is the concentration of the drug (μg/mL) and r is correlation coefficient.

Statistical analysis (21) of the data gave high values of correlation coefficients (*r*) of the regression equations, small values of the standard deviations of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small values of percentage relative standard deviation and percentage relative error (Table 1, 2). These data proved the linearity of the calibration graphs and the agreement of the results with Beer's law.

IV.B. Limits of quantification (LOQ) and limits of detection (LOD)

The limits of quantification (LOQ) and limits of detection (LOD) were determined according to ICH Q2 (R1) recommendations (22). The results are summarized in Table 1and 2.

LOQ and LOD were calculated according to the following equations:

$LOQ = 10S_a/b$

$LOD = 3.3S_a/b$

Where, S_a is the standard deviation of the intercept of regression line and b is the slope of the regression line.

IV.C. Accuracy

To prove the accuracy of the proposed method, the results of the assay of the studied drugs were compared with those of the official USP methods(1) for MET raw material and tablets as well as ASP tablets but in case of ASP raw material the results were compared with the HPLC comparison method described by *Kees et al* (17). Statistical analysis of the results using Student's *t*-test and variance ratio *F*-test (21) revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Tables 3 and 4).

IV.D. Precision

IV.D.1. Intra-day precision

To assess the intra-day precision of the proposed methods, it was applied to the determination of three concentrations of both MET and ASP in pure form on three successive times. The high accuracy and precision of the proposed derivative spectrophotometric methods were established by the small values of percentage error and percentage RSD (Table 5, 6).

IV.D.2. Inter-day precision

Evaluation of the inter-day precision of the developed spectrophotometric methods was performed by repeated analysis of three concentrations of MET and ASP in pure form for a period of three successive days. Small values of percentage error and percentage RSD indicate the high accuracy and precision of the proposed derivative spectrophotometric methods. The results are also summarized in Table 5 and 6.

IV.E. Specificity

The specificity of each of the proposed methods was investigated by observing any interference encountered from common dosage form excipients. It was shown that these compounds did not interfere with the results of the proposed methods (Tables 9 and 10).

IV.F. Solution stability

The stability of the stock solutions was determined by quantitation of MET and ASP compared with freshly prepared standard solutions. No significant change was observed in standard solution response, relative to freshly prepared standard. The results obtained in both cases proved that the sample solution and mobile phase used during the assay were stable up to 7 and 3 days, respectively.

V. Applications

V.A. Analysis of MET and ASP in the Laboratory Prepared Mixtures

The proposed methods were applied to the simultaneous determination of ASP with MET in laboratory prepared mixtures containing different concentrations of both drugs in 1:1.25 ratios. The amplitude of first derivative and ratio derivative techniques were measured for both drugs. The concentrations of both drugs in the laboratory-prepared mixtures were calculated according to the linear regression equations of the calibration graphs. The values of %RSD and %Er were calculated and the results indicate high accuracy of the proposed method as shown in Tables (7 and 8).

V.B. Analysis of MET and ASP in laboratory prepared tablets

The proposed methods were applied to the determination of the studied drugs in laboratory-prepared co-formulated tablets. The specificity of the method was investigated by observing any interference encountered from the common tablet excipients. The excipients didn't interfere with the proposed method. The results of the proposed methods were compared with those obtained using the official USP methods (1). Statistical analysis (21) of the results obtained using student's *t*-test and variance ratio *F*-test revealed no significant difference between the performance of the compared methods regarding the accuracy and precision, respectively (Tables 9 and 10).

Conclusion

Two new simple, sensitive and time saving methods were developed for the simultaneous determination of MET and ASP in their binary mixtures. The proposed methods could be utilized for the assay of the combination of MET and ASP in pharmaceutical formulations. The developed methods could be regarded as useful alternatives to the reported sophisticated techniques in the routine quality control of pharmaceuticals with a relatively inexpensive instrumentation.

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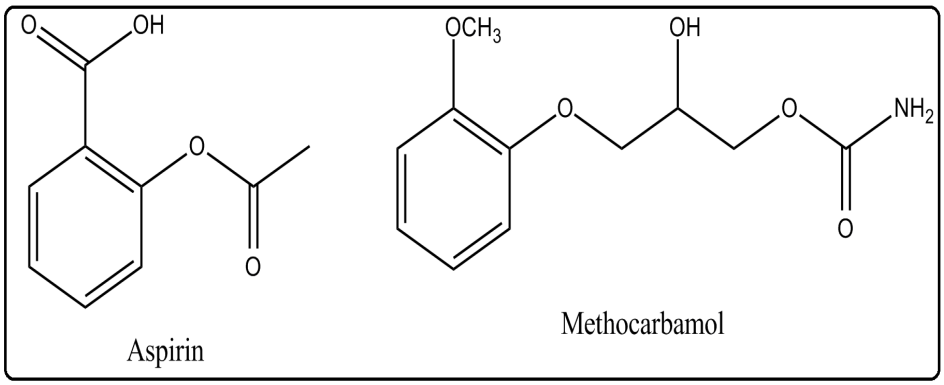

Figure (1): structural formulae of the studied drugs.

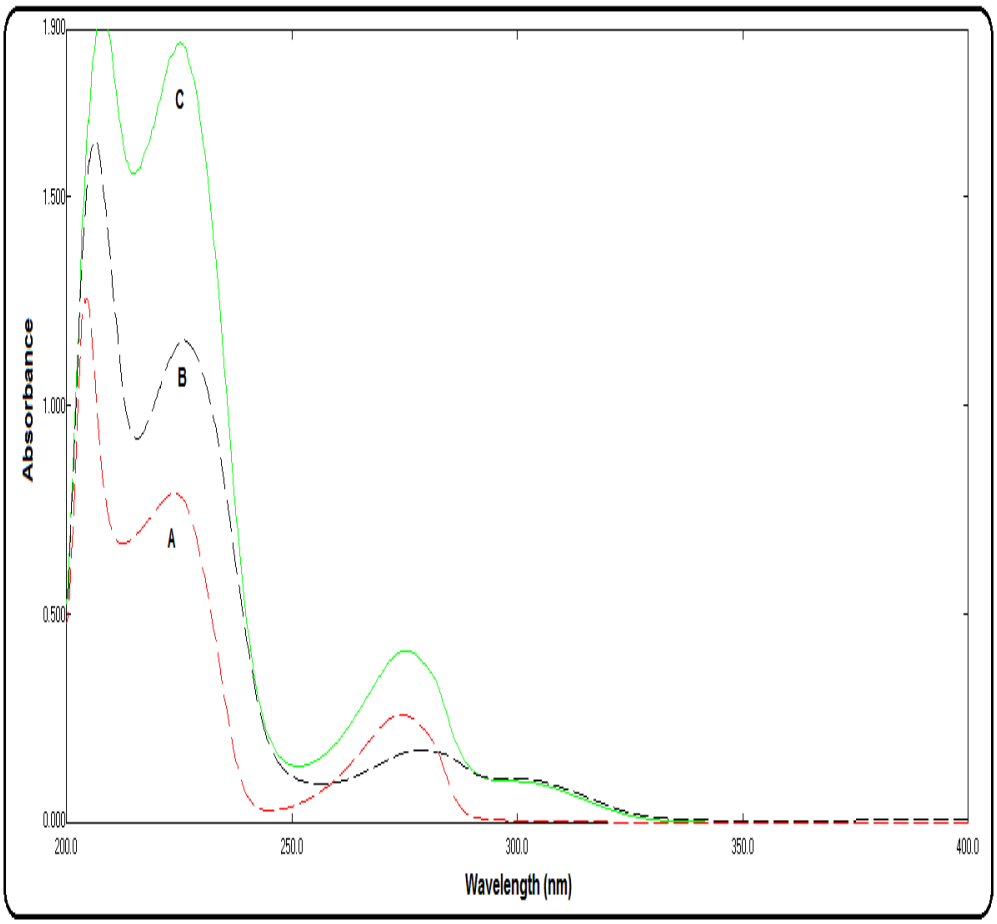

Figure (2): Zero order absorption spectra of MET (A; 25.0 µg/mL), ASP (B; 25.0 µg/mL) and a mixture of 25.0 µg/mL of both MET and ASP (C) in methanol.

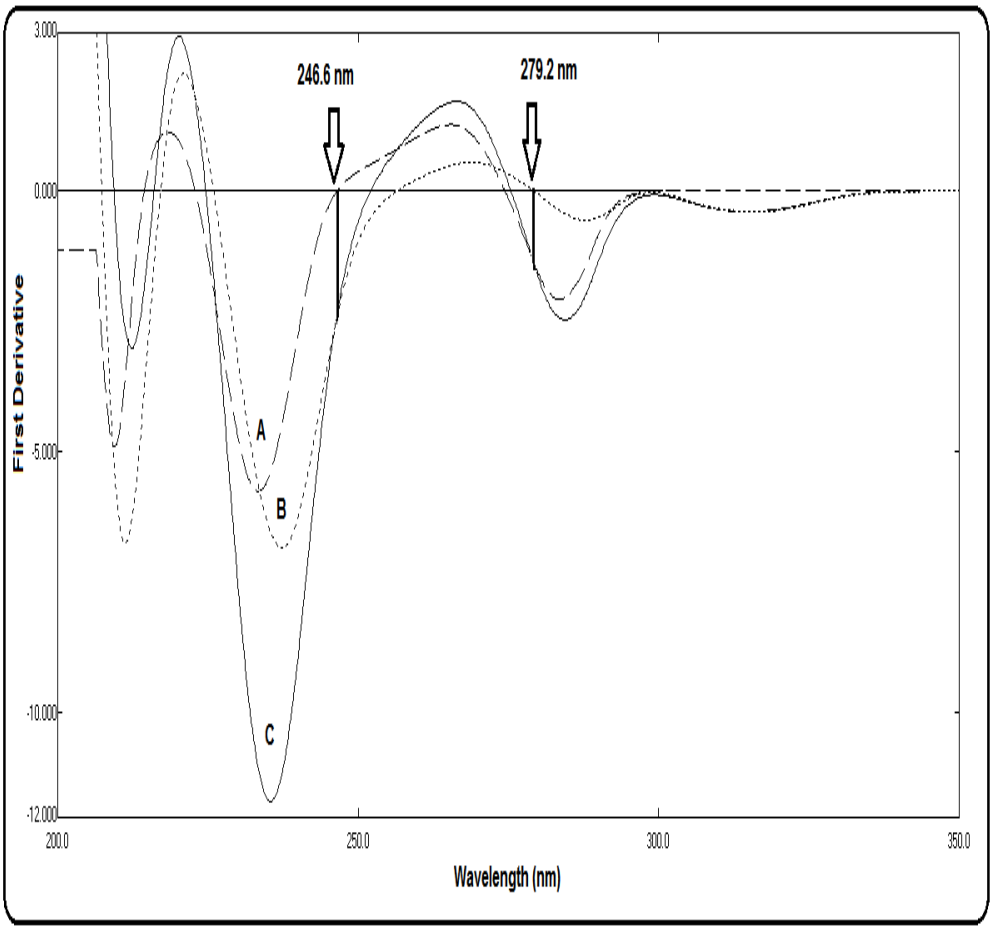

Figure (3): First order derivative spectra of MET (A; 25.0 µg/mL), ASP (B; 25.0 µg/mL) and a mixture of 25.0 µg/mL of both MET and ASP (C) in methanol.

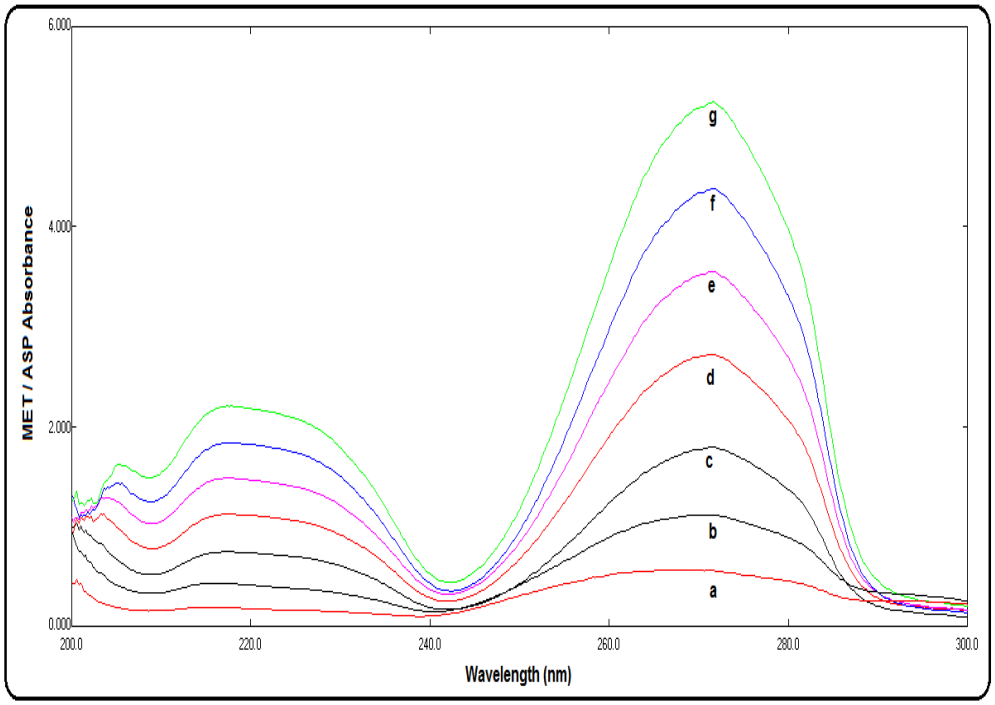

Figure (4): Ratio spectra of MET (2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/mL) when 10.0 µg/mL ASP was used as divisor.

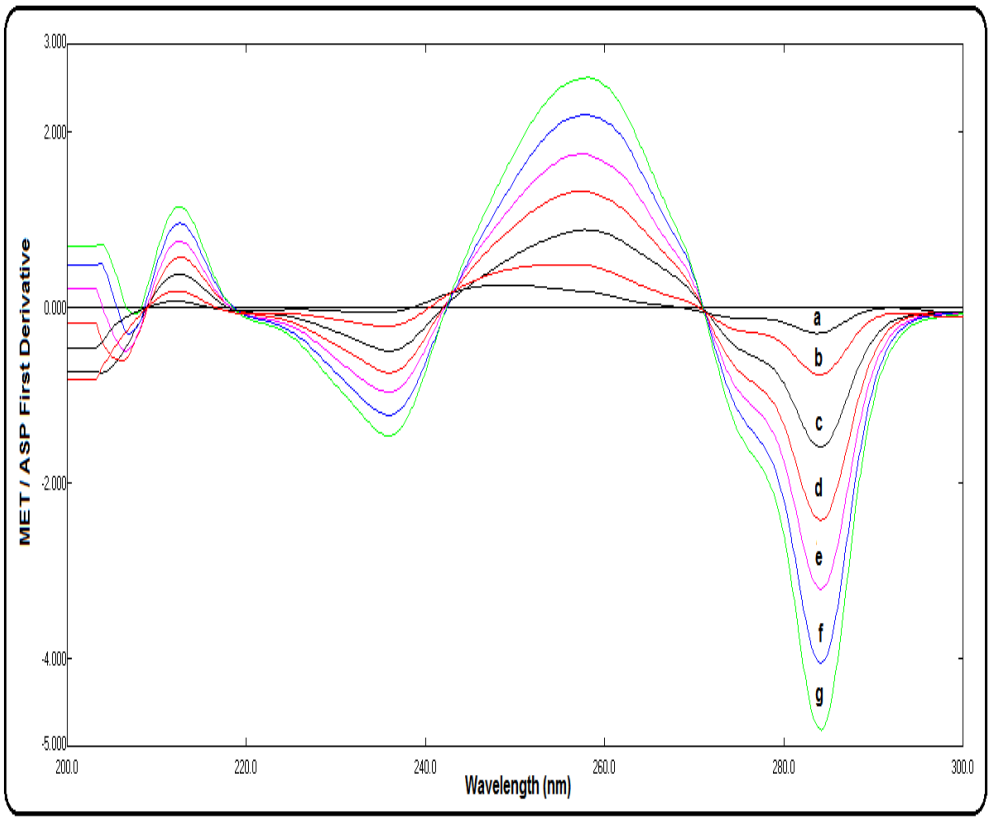

Figure (5): First derivative of the ratio spectra of MET (2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/mL) when 10.0 µg/mL ASP was used as divisor.

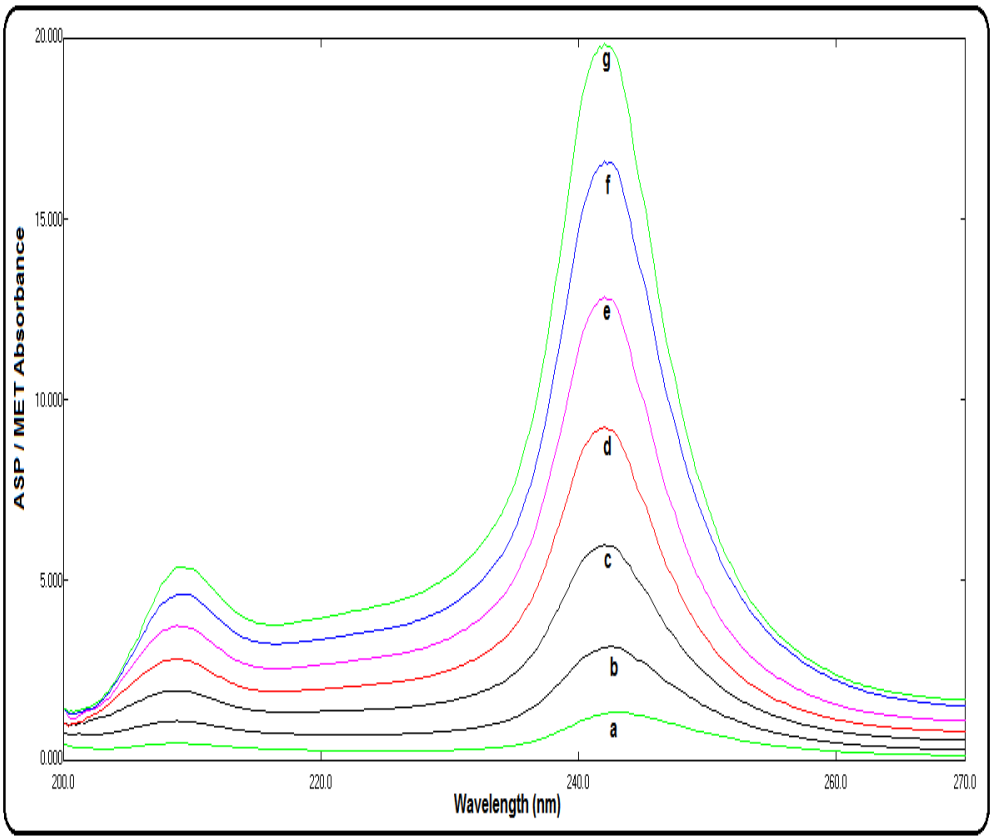

Figure (6): Ratio spectra of ASP (2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/mL) when 10.0 µg/mL MET was used as divisor.

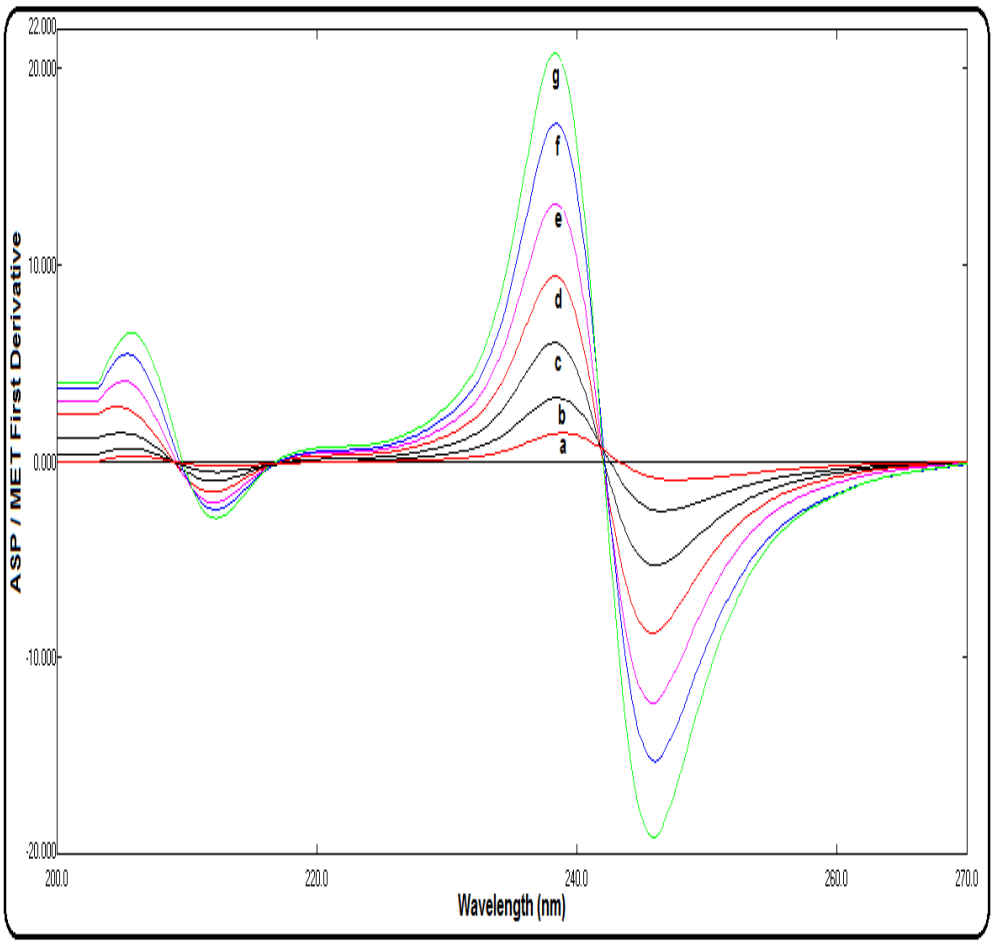

Figure (7): First derivative of the ratio spectra of ASP (2.0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/mL) when 10.0 µg/mL MET was used as divisor.

Table (1): **Analytical performance data for the determination of the studied drugs by first derivative spectrophotometric method.**

Parameter	MET	ASP
Concentration range (µg/mL)	2.00-30.00	2.00-30.00
Correlation coefficient	0.9999	0.9999
Slope	0.0531	0.1014
Intercept	0.0002	-0.1431
Limit of detection (LOD) (µg/mL)	0.2998	0.3227
Limit of Quantification (LOQ) (µg/mL)	0.9084	0.9780
Standard deviation of the residuals ($S_{y/x}$)	0.0068	0.0139
Standard deviation of the intercept (S_a)	0.0048	0.0099
Standard deviation of the slope (S_b)	0.0003	0.0005
% RSD (SD x 100 / X)	0.9410	0.7870
% Error (% RSD / √n)	0.3560	0.2970

Table (2): **Analytical performance data for the determination of the studied drugs by ratio first derivative spectrophotometric method.**

Parameter	MET	ASP	
		λ= 238.2 nm	λ= 246.0 nm
Concentration range (µg/mL)	2.00 – 30.00		
Correlation coefficient	0.9999	0.9999	0.9999
Slope	0.1621	0.7148	0.6560
Intercept	-0.0271	-0.8316	-0.8631
Limit of detection (LOD) (µg/mL)	0.2795	0.2485	0.4173
Limit of Quantification (LOQ) (µg/mL)	0.8470	0.7530	1.2647
Standard deviation of the residuals ($S_{y/x}$)	0.0193	0.0757	0.1166
Standard deviation of the intercept (S_a)	0.0137	0.0538	0.0830
Standard deviation of the slope (S_b)	0.0008	0.0030	0.0046
% RSD (SD x 100 / X)	0.8491	0.8960	1.0852
% Error (% RSD / √n)	0.3200	0.339	0.4112

Table (3): **Application of the first derivative spectrophotometric method to the determination of MET and ASP in pure form.**

Parameters	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found	Comparison methods [1,17]
MET	2.00	2.0160	80 100	98.56
	5.00	4.9303	98.61	100.22
	10.00	9.9209	99.21	99.82
	15.00	15.1940	101.29	
	20.00	19.9962	99.98	
	25.00	25.1375	100.55	
	30.00	29.8832	99.61	
X			**100.01**	**99.53**
SD			**±0.94**	**± 0.88**
t		**0.744(2.306)***		
F		**1.180(19.33)***		
ASP	2.00	1.9734	98.67	99.38
	5.00	5.0108	100.22	98.80
	10.00	10.0404	100.40	100.78
	15.00	14.9122	99.41	
	20.00	20.1686	100.84	
	25.00	24.7742	99.10	
	30.00	30.0700	100.23	
X			**99.84**	**99.66**
SD			**±0.79**	**±1.02**
t		**0.316 (2.305)***		
F		**1.675(5.143)***		

*Figures between parenthesis are the tabulated *t* and *F* values, respectively at *p*=0.05 (21)**.**

Table (4): **Application of the ratio first derivative spectrophotometric method to the determination of MET and ASP in pure form.**

Parameters	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found[a]	Comparison methods [1,17]
MET	2.00	1.9870	99.35	98.56
	5.00	4.9173	98.35	100.22
	10.00	10.0068	100.07	99.82
	15.00	15.1333	100.89	
	20.00	19.9636	99.82	
	25.00	25.1518	100.61	
	30.00	29.8526	99.51	
X			**99.80**	**99.53**
SD			**±0.85**	**± 0.88**
t		**0.454(2.306)***		
F		**1.046(5.143)***		
ASP (at λ=238.2nm)	2.00	2.0148	100.74	99.38
	5.00	5.0806	101.61	98.80
	10.00	9.9771	99.77	100.78
	15.00	14.8036	98.69	
	20.00	20.0498	100.25	
	25.00	25.0862	100.34	
	30.00	29.9827	99.94	
X			**100.19**	**99.66**
SD			**±0.90**	**±1.02**
t		**0.839 (2.306)***		
F		**1.286(5.143)***		
ASP (at λ=246 nm)	2.00	2.0184	100.92	99.38
	5.00	5.0794	101.59	98.80
	10.00	9.9742	99.74	100.78
	15.00	14.7913	98.61	
	20.00	20.2181	101.09	
	25.00	24.7913	99.17	
	30.00	30.1267	100.42	
X			**100.22**	**99.66**
SD			**±1.09**	**±1.02**
t		0.767 (2.306)*		
F		1.140(19.33)*		

*Figures between parenthesis are the tabulated *t* and *F* values, respectively at *p*=0.05 (21).

[a]Each result is the average of three separate determinations.

Table (5): **Precision data for determination of the two studied drugs by the first derivative method.**

Concentration added (µg/ml)	% Found ± SD	% RSD	% Error
MET			
Intra-day			
5.00	99.65± 0.95	0.95	0.55
15.00	100.34± 0.72	0.72	0.41
25.00	99.35± 0.91	0.91	0.53
Inter-day			
5.00	99.22±1.00	1.00	0.58
15.00	100.08± 0.68	0.67	0.39
25.00	100.23± 0.88	0.88	0.51
ASP			
Intra-day			
5.00	100.13 ± 0.86	0.86	0.50
10.00	100.18± 0.94	0.94	0.54
20.00	99.73 ± 0.61	0.61	0.35
Inter-day			
5.00	99.33± 1.07	1.08	0.62
10.00	99.71± 0.83	0.83	0.48
20.00	100.44 ± 0.68	0.68	0.39

N.B. Each result is the average of three separate determinations.

Table (6): **Precision data for determination of the two studied drugs by the ratio first derivative method.**

Concentration added (µg/ml)	% Found ± SD	% RSD	% Error
MET			
Intra-day			
5.00	100.22± 0.70	0.70	0.40
15.00	99.55± 0.91	0.85	0.49
25.00	99.56± 0.18	0.92	0.53
Inter-day			
5.00	99.92±1.30	1.31	0.75
15.00	99.27± 1.07	1.07	0.62
25.00	100.42± 0.85	0.85	0.49
ASP (λ=238.2nm)			
Intra-day			
8.00	99.24 ± 1.16	1.17	0.68
16.00	100.15± 0.73	0.73	0.42
24.00	100.38± 0.91	0.91	0.52
Inter-day			
8.00	99.63± 0.78	0.79	0.45
16.00	100.55 ± 0.75	0.74	0.43
24.00	99.92 ± 0.87	0.87	0.50
ASP (λ=246 nm)			
Intra-day			
8.00	99.55 ± 0.81	0.81	0.47
16.00	100.50± 1.03	1.03	0.59
24.00	99.30 ± 0.88	0.88	0.51
Inter-day			
8.00	100.13 ± 0.88	0.87	0.51
16.00	99.53 ± 0.96	0.96	0.56
24.00	99.94 ± 0.66	0.66	0.38

N.B. Each result is the average of three separate determinations.

Table (7): **Assay results for the determination of MET and ASP in laboratory-prepared mixtures by first order derivative method**.

Sample	Conc. taken (µg/mL)		Conc. found (µg/mL)		% Found[a]	
	MET	**ASP**	**MET**	**ASP**	**MET**	**ASP**
MET and ASP mixture	10.00	8.125	9.849	8.061	98.49	99.21
	20.00	16.250	20.046	16.001	100.23	98.47
	30.00	24.375	29.748	24.626	99.16	101.03
X					**99.29**	**99.57**
± SD					**±0.88**	**±1.32**
% RSD					**0.88**	**1.32**
% Error					**0.51**	**0.76**

[a]Each result is the average of three separate determinations.

Table (8): **Assay results for the determination of MET and ASP in laboratory-prepared mixtures by ratio first order derivative method**.

Sample in the laboratory prepared mixture	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found[a]
MET	10.00	9.873	98.73
	20.00	19.884	99.42
	30.00	29.676	98.92
X			**99.02**
± SD			**±0.36**
% RSD			**0.36**
% Error			**0.21**
ASP (λ=238.2nm)	8.125	8.102	99.72
	16.250	16.349	100.61
	24.375	24.585	100.86
X			**100.40**
± SD			**±0.60**
% RSD			**0.60**
% Error			**0.35**
ASP (λ=246nm)	8.125	8.1169	99.90
	16.250	15.9461	98.13
	24.375	24.2994	99.69
X			**99.24**
± SD			**±0.97**
% RSD			**0.97**
% Error			**0.56**

[a]Each result is the average of three separate determinations.

***Table (9)*: Application of the first derivative spectrophotometric method for determination of the studied drugs in their laboratory-prepared tablets.**

Preparation	Conc. taken (µg/mL)		Conc. found (µg/mL)		% Found[a]			
					Proposed method		Reference method [(1)]	
	MET	ASP	MET	ASP	MET	ASP	MET	ASP
Prepared tablets (MET 400 mg + ASP 325 mg/ tablet)	10.00	8.125	10.140	8.184	101.40	100.73	99.11	98.80
	20.00	16.250	19.910	16.114	99.55	99.16	101.18	100.67
	30.00	24.375	30.261	24.702	100.87	101.34	99.71	99.78
x					**100.61**	**100.41**	**100.00**	**99.75**
± SD					**±0.95**	**±1.13**	**± 1.07**	**± 0.94**
% RSD					**0.95**	**1.12**		
% Error					**0.55**	**0.65**		
t					**0.735(2.776)***	**0.781(2.776)***		
F					**1.250(19.00)***	**1.446(19.00)***		

[a]Each result is the average of three separate determinations.

*Figures between parenthesis are the tabulated *t* and *F* values, respectively at p=0.05 (21).

***Table (10)*: Application of the ratio first derivative spectrophotometric method for determination of the studied drugs in their laboratory-prepared tablets.**

Determined drug in the laboratory prepared tablets	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found[a]	
			Proposed method	Reference method [(1)]
MET	10.00	10.172	101.72	99.11
	20.00	20.164	100.82	101.18
	30.00	29.850	99.50	99.71
x			**100.68**	**100.00**
± SD			**± 1.12**	**± 1.07**
% RSD			**1.11**	
% Error			**0.64**	
T			**0.763(2.776)***	
F			**1.099(19.00)***	
ASP (λ=238.2 nm)	8.125		8.179	100.66
	16.250		15.985	98.37
	24.375		24.660	101.17
X			**100.07**	**99.75**
± SD			**± 1.49**	**± 0.94**
% RSD			**1.49**	
% Error			**0.86**	
T			**0.312(2.776)***	
F			**2.542 (19.00)***	
ASP (λ=246nm)	8.125		8.243	101.45
	16.250		16.196	99.67
	24.375		24.509	100.55
X			**100.56**	**99.75**
± SD			**± 0.89**	**± 0.94**
% RSD			**0.89**	
% Error			**0.51**	
T			**1.082 (2.776)***	
F			**1.104 (19.00)***	

[a]Each result is the average of three separate determinations.

*Figures between parenthesis are the tabulated *t* and *F* values, respectively at *p*=0.05 (21).