

Spectrophotometric Methods for Simultaneous Determination of Two Hypouricemic Drugs in their Combined Dosage Form

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Abstract

Four simple, precise and accurate spectrophotometric methods have been developed for simultaneous determination of Allopurinol (ALP) and Benzbromarone (BENZ) in their bulk powder and pharmaceutical dosage form. Method (I) is dual wavelength analysis, method (II) is Q-analysis (graphical absorbance ratio) method, method (III) is the mean centering of ratio spectrophotometric (MCR) method while method (IV) is the extended ratio subtraction method (EXRSM).

In method (I) two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 238.2 and 261.2 nm ALP had equal absorbance values; therefore, these two wavelengths have been used to determine BENZ; on a similar basis 253 and 274.4 nm were selected to determine ALP. Method (II) involves the formation of Q-absorbance equation using the respective absorptivity values at 245.8 nm (isoabsorptive point) and 250 nm (λ_{\max} of ALP). In method (III) the absorption spectra of both ALP and BENZ with different concentrations were recorded over 210-280 and 210-275 nm, respectively, divided by the spectrum of suitable divisor of both ALP and BENZ and then they obtained ratio spectra were mean centered. Method (IV) starts with the normal ratio subtraction method (RSM) for determination of ALP at its λ_{\max} (250 nm), while an extension of the already developed method has been established as a new approach for BENZ determination at its λ_{\max} (238 nm).

Accuracy, precision and recovery studies of the developed methods have been carried out in order to confirm their accuracy. Specificity of the methods was also tested by their application for determination of different synthetic mixtures and they have been successfully used for drugs determination in their combined dosage form. Statistical comparison of the developed methods with the reported HPLC one showed no significant difference.

Keywords: Allopurinol; Benzbromarone; Dual wavelength analysis; Q-analysis; Spectrophotometry; Mean centering method; Extended ratio subtraction method

Introduction

Allopurinol (ALP), is (1, 5-Dihydro-4H-Pyrazolo [3, 4-d] pyrimidin-4-one) [1], Figure 1, It is an official drug in British (BP) & United states (USP) pharmacopoeias [1,2] which is used for treatment of gout and hyperuricaemia [3]. It is a xanthine oxidase inhibitor [4-7], which prevents the oxidation of hypoxanthine to xanthine & xanthine to uric acid [8]. Thus results in the reduction of urate and uric acid concentrations in plasma and urine.

Benzbromarone (BENZ), is (3,5-dibromo-4-hydroxyphenyl)-(2-ethyl-3-benzofuranyl)methanone [1], Figure 2, It is an official drug in BP pharmacopoeias [1] which is used as a hypouricemic drug. It increases the excretion of uric acid by blocking renal tubular reabsorption & thus reduces plasma concentrations and increases the excretion of uric acid [9,10].

Combination of ALP and BENZ has the advantages of greater therapeutic effect than with either drug alone [11]. This combination causes manifold reduction in uric acid concentrations in plasma and urine as compared to double dose of the individual drug when used alone [12]. Also, this combination helps to decrease the dose of each active ingredient, and as a result, decreases the side effects of each of component if given separately in high doses [13].

Reviewing the literature in hand, only one report has been published for determination of the studied mixture which depended on measuring BENZ using zero order spectra at its λ_{\max} =356 while

ALP was determined by using (²D) amplitudes at 281.4 nm or by measuring the amplitudes of the second derivative of the ratio spectra curves (2DD) at 282.4 nm after using a standard spectrum of 8 ugml⁻¹ BENZ as a divisor [11]. Also, the studied drugs have been analysed by TLC-Densitometric method using acetone: chloroform: NH₃ (5:4:0.01, by volume) as a developing system and by RP-HPLC method using phosphate buffer pH=4.0-acetonitrile-methanol (50:30:20,by volume) as a mobile phase [11].

Due to the pharmaceutical importance of this combination and from the previous literature review, it is important to develop simple, sensitive, time saving and cost effective methods for simultaneous analysis of the studied drugs which can be used for their quality control analysis. The developed work aimed to develop & validate two spectrophotometric methods, Dual wavelength analysis, Q-analysis method, mean centering of ratio spectrophotometric method (MCR) and extended ratio subtraction method (EXRSM) for simultaneous

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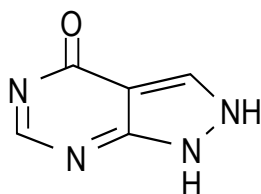


Figure 1: Chemical Structure of Allopurinol.

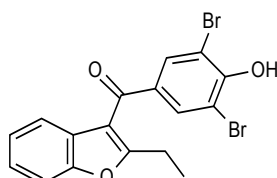


Figure 2: Chemical Structure of Benzbromarone.

determination of both ALP & BENZ. The developed methods have advantages over the published ²D method nor 2DD methods [11] on using zero order absorption spectra without derivatization steps and hence signal to noise ratio is enhanced. Also, it is time and cost effective than the reported chromatographic methods and they do not need sophisticated apparatus or sample pretreatment. The proposed methods have been optimized and validated as per the International Conference on Harmonization (ICH) guidelines ICH, and were found to comply with the acceptance criteria [14].

Experimental

Instruments

Double beam UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) model UV-1601 PC with 1cm quartz cells, connected to IBM compatible computer. The bundled software, UV-PC personal spectroscopy software version 3.7 was used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

Materials

Pure standards: Standard ALP and BENZ were kindly supplied by GLOBAL NAPI PHARMACEUTICALS, 2nd Industrial Zone, 6th of October City- Egypt. With claimed purity of 98.36% and 98.43% according to a reported HPLC method.

Pharmaceutical dosage form: Alloben[®] tablets (100/25) (B.N.100251) labeled to contain 100 mg Allopurinol+25 mg Benzbromarone and were manufactured by GLOBAL NAPI PHARMACEUTICALS, 2nd Industrial Zone, 6th of October City- Egypt.

Solvents: Methanol HPLC grade (CHROMASOLVE[®], Sigma-Aldrich chemie GmbH., Germany) was used for the two methods.

Standard solutions: Standard stock solutions each of ALP and BENZ containing 1000 µgml⁻¹ of ALP and BENZ were prepared separately in methanol.

Working solutions: Working standard solutions of ALP and BENZ (100 µgml⁻¹) were obtained by dilution of the respective stock solutions with methanol.

Procedure

Spectral characteristics and wavelength selection: The absorption

spectra of 20 µgml⁻¹ each of ALP, BENZ and their 1:1 mixture (containing 10 µgml⁻¹ each) in methanol were recorded over the range of 200-400 nm using methanol as a blank. The overlain spectra were observed for selection of the suitable wavelengths for each of the developed methods, Figure 3.

Linearity

Method I (Dual wavelength method): Standard solutions of both ALP and BENZ in the range of 5-60 and 5-20 µgml⁻¹, respectively were separately prepared by appropriate dilutions of their respective working standard solutions in methanol and then scanned in the range of 200-400 nm. Absorbance values at 253 and 274.4 nm (for ALP) and at 238.2 and 261.2 nm (for BENZ) were measured. ALP was determined by plotting the difference in absorbance at 253 and 274.4 nm (difference is zero for BENZ) against its corresponding concentration. Similarly for determination of BENZ, the difference in absorbance at 238.2 and 261.2 nm (difference is zero for ALP) was plotted against the corresponding concentration. The concentrations of the two drugs were calculated each from the corresponding calibration curve equation.

Method II (Q-analysis method): Standard solutions containing 5-60 µgml⁻¹ of ALP and 5-20 µgml⁻¹ of BENZ were prepared separately using methanol as a solvent. The absorption spectra of the prepared solutions were recorded in the range of 200-400 nm and the absorbance values at 245.8nm (λ_{iso}) and 250nm (λ_{max} of ALP) were measured from which the absorptivity values for both drugs at the selected wavelengths were calculated. The method employs Q values and the concentrations of the studied drugs in the prepared solutions were determined by using the following equations:

$$C_x = [Q_m - Q_y / Q_x - Q_y] \times A / A_x$$

$$C_y = [Q_m - Q_x / Q_y - Q_x] \times A / A_y$$

Where C_x and C_y are the concentrations of ALP and BENZ in µgml⁻¹, respectively; Q_m is the absorbance of the sample at $\lambda_{245.8}$ / absorbance of sample at λ_{250} ; Q_x is the absorptivity of ALP at λ_{250} / absorptivity of ALP at $\lambda_{245.8}$; Q_y is the absorptivity of BENZ at λ_{250} / absorptivity of BENZ at $\lambda_{245.8}$; A_x is the absorbance of ALP at 245.8; A_y is the absorbance of BENZ at $\lambda_{245.8}$; and A is the absorbance of the sample at $\lambda_{245.8}$.

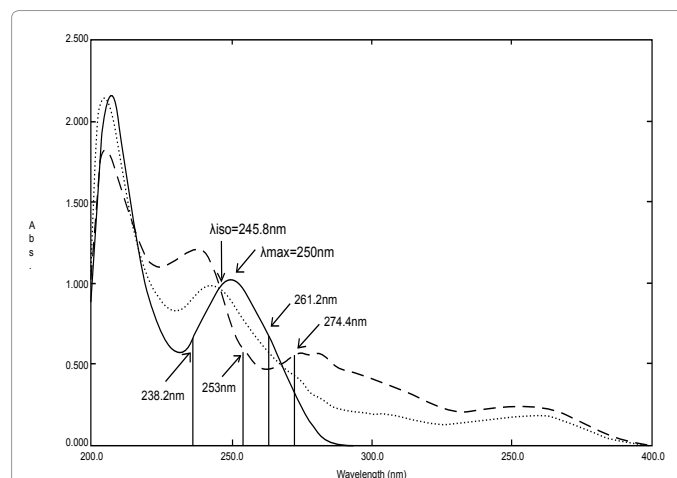


Figure 3: Zero-order absorption spectra of 20 µgml⁻¹ each of ALP (—), BENZ (---) and 1:1 mixture (.....) contains 10 µgml⁻¹ of each using MeOH as a solvent.

Method III (Mean centering of ratio spectra method): Standard solutions of ALP equivalent to 5-50 $\mu\text{g mL}^{-1}$ were accurately transferred from its standard working solution (100 $\mu\text{g mL}^{-1}$) into a set of 10 mL measuring flasks and the volume was adjusted using methanol. The absorption spectra of the prepared solutions were recorded in the range of 210-280 nm, divided by the standard spectrum of 20 $\mu\text{g mL}^{-1}$ of BENZ and then they obtained ratio spectra were mean centered. By the same way the spectra of different concentrations of standard solutions of BENZ in the range of 5-25 $\mu\text{g mL}^{-1}$ were recorded. The stored spectra were divided by the standard spectrum of 10 $\mu\text{g mL}^{-1}$ of ALP to obtain the ratio spectra which were then mean centered. Calibration curves for both ALP and BENZ were constructed by plotting the amplitude values of their respective mean centered ratio spectra from 233.4-257.4 and 228.4-252.8 nm (peak to peak) against their corresponding concentrations, respectively.

Method IV(Extended ratio subtraction method): Standard solutions containing 5-50 $\mu\text{g mL}^{-1}$ of ALP and 5-20 $\mu\text{g mL}^{-1}$ of BENZ were prepared separately by appropriate dilutions of their respective working standard solutions in methanol and then scanned in the range of 200-400 nm. As an extended ratio subtraction method (EXRSM) starts with the ratio subtraction method (RSM), where a mixture of ALP and BENZ showed overlapped spectra, ALP represents unextended spectrum and BENZ represents extended spectrum. ALP can be determined through usual ratio subtraction method (RSM) by dividing mixtures (ALP and BENZ) by a standard spectrum of BENZ' (20 $\mu\text{g mL}^{-1}$) as a divisor producing a new curve that represents ALP/BENZ' + BENZ/BENZ' (constant). Measuring the values of these constants in the plateau region (290-308 nm) followed by subtracting of these constant values, then multiplication the obtained curve after subtraction by standard spectrum of BENZ' (20 $\mu\text{g mL}^{-1}$) which is the same divisor used, therefore the obtained spectrum is the zero order absorption spectrum of ALP. The concentrations of ALP are calculated using the regression equation representing the linear relationship between the absorbance at its λ_{max} versus the corresponding concentration of ALP.

This can be summarized as the following:

$$\text{ALP+BENZ} / \text{BENZ}' = \text{ALP} / \text{BENZ}' + \text{BENZ} / \text{BENZ}' \text{ (constant)}$$

$$\text{ALP} / \text{BENZ}' + \text{constant} - \text{constant} = \text{ALP} / \text{BENZ}'$$

$$\text{ALP} / \text{BENZ}' * \text{BENZ}' = \text{ALP}$$

BENZ can be determined through an extension of the already developed method which has been established as a new approach, where BENZ can be determined by dividing the obtained zero order spectrum of ALP by a standard spectrum of ALP' (60 $\mu\text{g mL}^{-1}$) to get the value of the constant (ALP/ALP'). Dividing the spectrum of the mixtures (ALP and BENZ) by a standard spectrum of ALP' (60 $\mu\text{g mL}^{-1}$) which is the same divisor resulted in a new curve that represents BENZ/ALP' + ALP/ALP' (constant) where ALP/ALP' is the previously obtained constant followed by subtraction of this constant, then multiplication the obtained curve after subtraction by a standard spectrum of ALP' (60 $\mu\text{g mL}^{-1}$) which is the divisor and the obtained spectrum is the zero order spectrum of BENZ.

This can be summarized as the following:

$$\text{BENZ} / \text{ALP}' + \text{ALP} / \text{ALP}' - \text{ALP} / \text{ALP}' = \text{BENZ} / \text{ALP}' * \text{ALP}' = \text{BENZ}$$

Analysis of laboratory prepared mixtures: Different laboratories prepared mixtures containing different ratios of ALP and BENZ were

prepared from their respective working standard solutions (100 $\mu\text{g mL}^{-1}$). Zero order absorption spectra of these mixtures were recorded using methanol as a blank and then the differences in absorbance values at 253,274.4 & at 238.2, 261.2 nm (For method I) were measured, the absorbance values at 250 & 245.8 nm (For method II) were recorded, the absorbance values from 233.4 & 257.4 and 228.4 & 252.8 nm (peak to peak) were calculated (For method III) and the absorbance values at 250 & 238 nm were determined (For method IV). From the calculated regression equations, concentrations of ALP and BENZ in the prepared mixtures were calculated.

Analysis of the marketed formulation: Ten Alloben® tablets were weighed and crushed to obtain a fine powder. An accurately weighed amount equivalent to 100 mg ALP and 20 mg BENZ was transferred into 100-mL calibrated measuring flask and the volume was completed with methanol. The prepared solution was sonicated for 20 mins and the solution was then filtered. The filtrate was appropriately diluted with methanol to prepare a working solution equivalent to 0.1 mg mL^{-1} of ALP and 0.02 mg mL^{-1} of BENZ. Further dilutions have been made on the prepared working solution to prepare concentrations within the linearity range of each drug. The absorbance values at the selected wavelengths were determined and the methods given under analysis of laboratory prepared mixtures were then followed.

Recovery studies: To study the accuracy of the proposed methods, recovery studies were carried out by applying the standard addition technique at different levels (80%, 100% and 120%) where known amounts of the studied drugs were separately added to the pre-analyzed Alloben® tablets powder and the percentage recoveries were then calculated.

Results and Discussion

As shown in Figure 3, Zero order absorption spectra of ALP and BENZ show strong spectral overlap which interfere with direct spectrophotometric analysis of the studied drugs without derivatization. The suggested dual wavelength and Q-analysis methods provide simple, rapid, convenient and accurate way for simultaneous analysis of ALP and BENZ in their combined dosage form without derivatization steps.

Methods development and optimization

The main step in the development and validation of an analytical method of analysis is to improve the conditions and parameters which should be followed in the analysis [15,16]. Different solvents were studied (methanol, ethanol, acetonitrile, water, 0.1 N HCl and 0.1N NaOH). The criterion employed was the sensitivity of the method and availability of the solvent. From a solvent effect studies and spectral behaviors of ALP and BENZ, methanol was selected as a solvent for the two suggested methods.

Dual wavelength method: The developed dual wavelength method provides a simple method for selective determination of both ALP and BENZ using their zero order absorption spectra. The principle of this method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent on the interfering component [15,17]. The pre-requisite for this method is the selection of two wavelengths where the interfering component shows the same absorbance value while the component of interest shows significant difference in absorbance with concentration [15]. Different wavelengths were tried such as [271.4 & 284, 258.4 & 267.2 and 253 & 274.4 nm] for ALP and [240.2 & 259.2, 225.4 & 237.8 and 238.2 & 261.2 nm] for BENZ. Using the absorbance values at 253 and 274.4 nm

[where BENZ has the same absorbance] gave the best selectivity when used for determination of ALP. On the other hand, the absorbance values at 238.2 and 261.2 nm were chosen for determination of BENZ where the best results were obtained.

Calibration curves for ALP and BENZ were constructed by plotting the difference in absorbance values at the selected wavelengths for each drug against their corresponding concentrations. ALP and BENZ obeyed Beer Lambert's Law in the concentration ranges of 5-60 and 5-20 $\mu\text{g mL}^{-1}$, respectively with good correlation coefficients. Regression equation parameters are given in Table 1.

Q-analysis (graphical absorbance ratio) method: This method depends on the property that for the substance that obeys Beer's Lambert's law at all wavelengths, the ratio of absorptivity (or absorbance) values at any two wavelengths are constant, independent on the concentration or path length. This ratio is referred as Q-ratio [15]. One of the two selected wavelengths is an iso-absorptive point and the other is the wavelength of maximum absorption of one of the two components [15].

From the overlain spectra of the two drugs and their mixture, Figure 3, it is evident that ALP and BENZ show iso-absorptive points at 215.8, 245.8 and 267.6 nm; ALP has λ_{max} at 250 nm while BENZ has λ_{max} at 238 and 275 nm. Using the absorbance values at 245.8 nm (λ_{iso}) and 250 nm (λ_{max} for ALP) gave the best results regarding selectivity. The absorbance values at 245.8 and 250 nm for ALP in the range of 5-60 $\mu\text{g mL}^{-1}$ were obtained and similarly for BENZ absorbance values in the range of 5-20 $\mu\text{g mL}^{-1}$ were measured, absorptivity coefficients were determined for both drugs and the average values were taken. The absorptivity values and the absorbance ratio were used to develop the following sets of equations from which the concentration of each component in the sample can be calculated:

$$C_{\text{ALP}} = (Q_m - 0.8055/1.0639 - 0.8055) \times A/0.0484$$

$$C_{\text{BENZ}} = (Q_m - 1.0639/0.8055 - 1.0639) \times A/0.0457$$

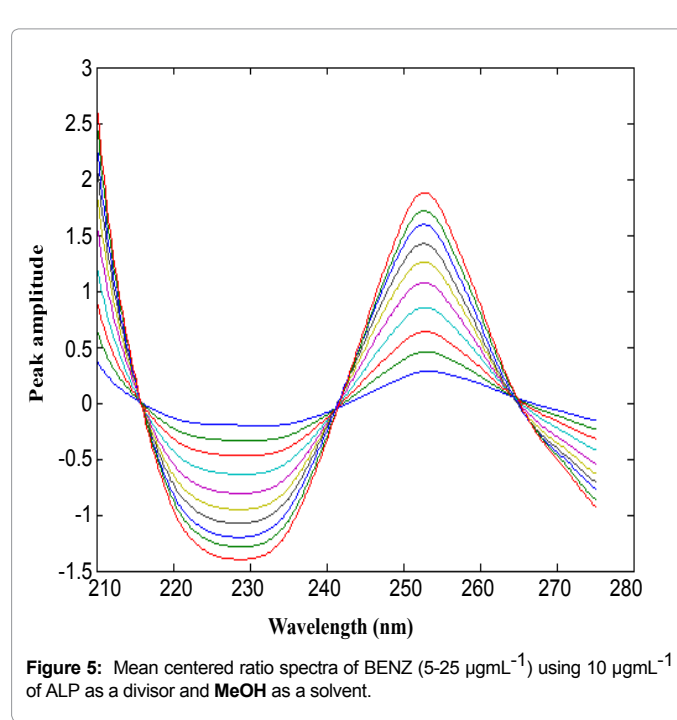
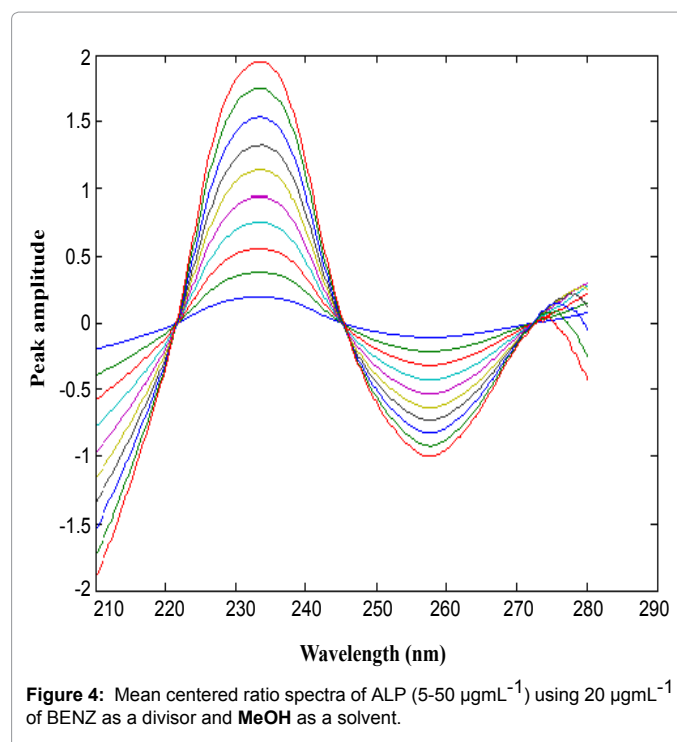
Where C_{ALP} is the concentrations of ALP in $\mu\text{g mL}^{-1}$; C_{BENZ} is the concentrations of BENZ in $\mu\text{g mL}^{-1}$; Q_m is the absorbance of sample at $\lambda_{245.8}$ /absorbance of sample at λ_{250} ; and A is the absorbance of the sample at $\lambda_{245.8}$.

Mean centering of ratio spectra spectrophotometric method (MCR): To optimize the developed MCR method [18-20], different parameters were tested. Since the wave length range taken has a great effect on the obtained mean centered ratio spectra, different wave length ranges were tested and the best results were obtained upon using wave length ranging from 210-280 and 210-275 nm for ALP and BENZ, respectively. The effect of divisor concentration on the selectivity was checked by testing several concentrations each of BENZ (5, 10, 35 and 50 $\mu\text{g mL}^{-1}$). The best results regarding sensitivity and selectivity were obtained upon using 20 $\mu\text{g mL}^{-1}$ of BENZ and 10 $\mu\text{g mL}^{-1}$ of ALP as divisors. To construct the calibration curves of the proposed method, the absorption spectra of the standard solutions of ALP with different concentrations were recorded in the wave length range from 210-280 nm and divided by the standard spectrum of BENZ (20 $\mu\text{g mL}^{-1}$). Then, mean centering of the resulted ratio spectra has been obtained and the concentrations of ALP were determined by measuring the amplitude values of the mean centered ratio spectra from 233.4 to 257.4 nm (peak to peak) as shown in Figure 4.

By the same way, different standard solutions of BENZ with different concentrations were recorded and divided by the standard spectrum of ALP (10 $\mu\text{g mL}^{-1}$) and the ratio spectra were obtained

which were then mean centered. The amplitude values from 228.4 to 252.8 nm (peak to peak) in the obtained mean centered ratio spectra were used for determination of BENZ as shown in Figure 5. The computed regression parameters for each of the studied drugs are given in Table 1.

Extended ratio subtraction method (EXRSM): Extended ratio subtraction method (EXRSM) starts after the application of the ratio subtraction method (RSM) [20,21]. The (RSM) method depends



on that, when mixtures of ALP and BENZ where the spectrum of BENZ is more extended, Figure 3, the determination of ALP in the mixtures can be done by scanning the zero order spectra of the laboratory prepared mixtures (ALP and BENZ), dividing them by a carefully chosen concentration of standard BENZ' ($20 \mu\text{g mL}^{-1}$) as a divisor producing new ratio spectra which represent ALP / BENZ' + constant as shown in Figure 6, then subtraction of the values of these constants BENZ / BENZ' in the plateau region (290-308nm) as shown in Figure 7, followed by multiplication of the obtained spectra by the divisor BENZ' ($20 \mu\text{g mL}^{-1}$) as shown in Figure 8. Finally, the original spectra of ALP can be obtained, Figure 8 which were used for direct estimation of ALP at 250 nm and calculation of the concentration from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of ALP at 250 nm against the corresponding concentrations). The determination of BENZ can be done by the extended ratio subtraction by dividing these obtained spectra of ALP by a carefully chosen concentration of standard ALP' ($60 \mu\text{g mL}^{-1}$) producing ratio spectra represent the constants ALP/ALP' in plateau (220-280 nm) as shown in Figure 9. The previously scanned zero order absorption of the laboratory prepared mixtures (ALP and BENZ) were divided by standard ALP' ($60 \mu\text{g mL}^{-1}$) as a divisor

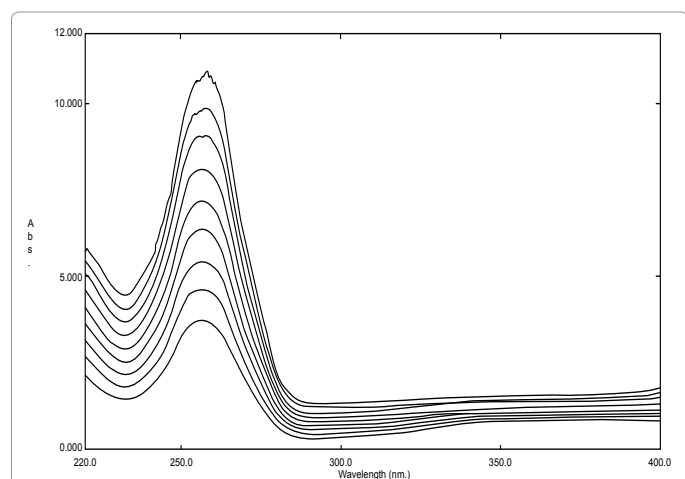


Figure 6: Ratio spectra of laboratory prepared mixtures of ALP and BENZ using $20 \mu\text{g mL}^{-1}$ of BENZ' as a divisor and MeOH as a solvent.

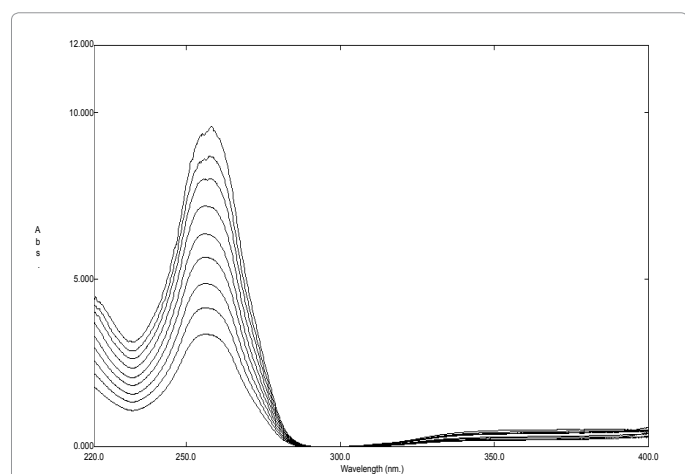


Figure 7: Ratio spectra of laboratory prepared mixtures of ALP and BENZ using $20 \mu\text{g mL}^{-1}$ of BENZ' as a divisor and MeOH as a solvent after subtraction of the constant.

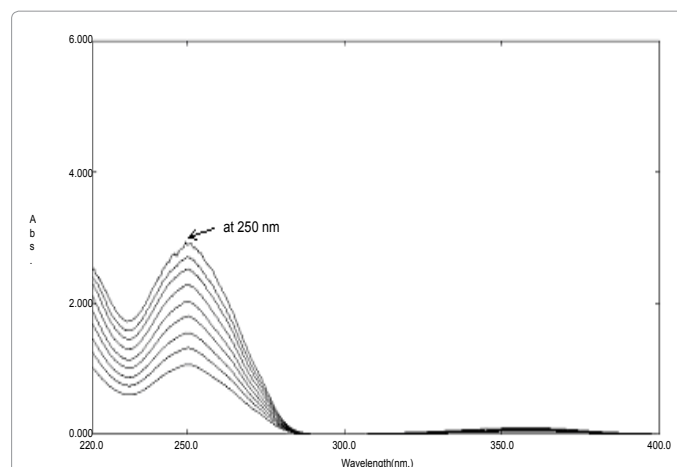


Figure 8: The zero order absorption spectra of ALP obtained by the proposed ratio subtraction method for the analysis of laboratory prepared mixtures after multiplication by the divisor BENZ'.

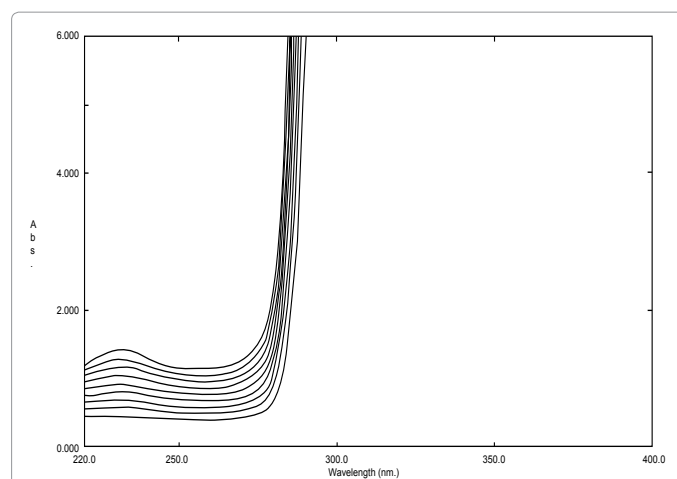
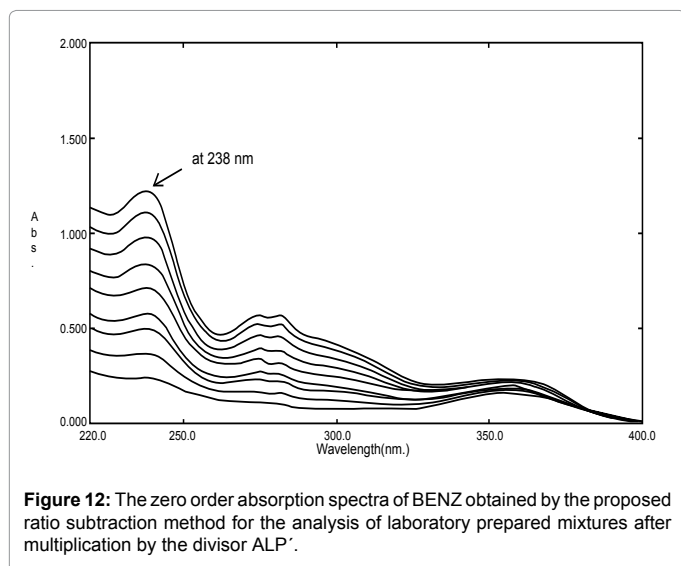
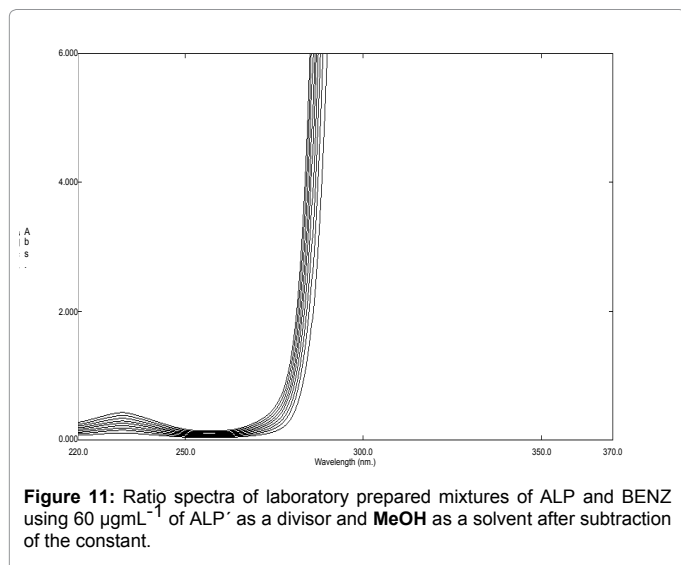
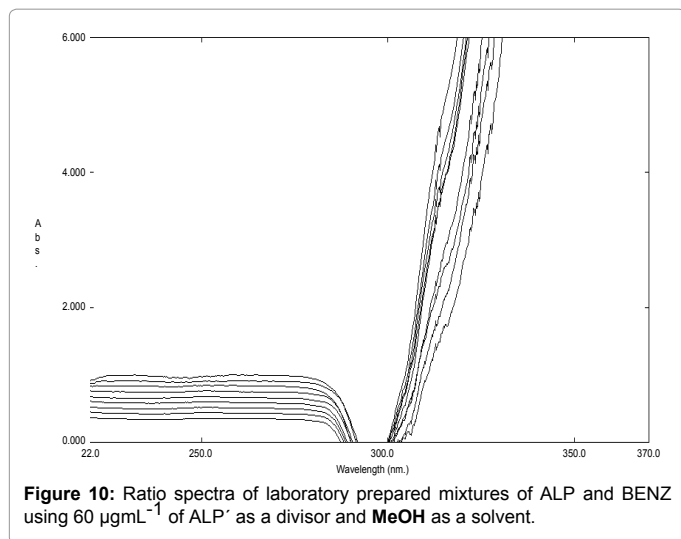


Figure 9: Ratio spectra of obtained spectra of ALP using $60 \mu\text{g mL}^{-1}$ of ALP' as a divisor and MeOH as a solvent.

producing new ratio spectra which represent BENZ / ALP' + constant as shown in Figure 10, then subtraction of these obtained spectra by the divisor ALP' ($60 \mu\text{g mL}^{-1}$) as shown in Figure 11. Finally, the original spectra of BENZ, Figure 12 can be obtained which are used for determination of BENZ at 238 nm and calculation of the concentration from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of BENZ at 238 nm against the corresponding concentrations).

The extended ratio subtraction method has advantage that the extended drug in the mixture can be determined at its λ_{max} which cannot be achieved by the previously established ratio subtraction method [21] which has been used for determination of unextended drug only. Therefore, the two methods are considered to be complementary to each other as the two components of interest in the mixture can be determined.

The proposed methods have been successfully applied for determination of the studied drugs in bulk powder as well as in their combined dosage form. The results obtained upon using the suggested methods for analysis of ALP and BENZ in Alloben[®] tablets, Table 2, showed good agreement between the amounts estimated and those claimed by the manufacturer. Moreover, results obtained by the



suggested methods showed no significant difference when compared with those obtained by applying the reported HPLC method [11] as confirmed from F and t values presented in Table 2. The developing methods have advantages on being simple, easy to be applied and they do not need sophisticated apparatus or programs.

Methods validation

Validation of the method has been carried out according to ICH guidelines [14].

Linearity and range: The calibration range for ALP and BENZ was established through considerations of the practical range necessary according to adherence to Beer-Lambert's law and the concentration of ALP and BENZ present in the pharmaceutical dosage form to give accurate, precise and linear results. Linearity ranges of both ALP and BENZ are shown in Table 1.

Accuracy: The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of ALP and BENZ and the concentrations were obtained from the corresponding regression equations. Good percentage recoveries were obtained and were presented in Table 1.

Accuracy of the methods was further assured by applying the standard addition technique where good results were obtained, confirming the accuracy of the proposed methods, Table 2.

Precision

Repeatability: Three concentrations of ALP and BENZ ($10, 14, 18 \mu\text{g mL}^{-1}$) were analyzed three times intra-daily using the proposed methods. acceptable RSD% values were obtained, confirming the repeatability of the methods, Table 1.

Intermediate precision: The previous procedures were repeated inter daily on three different days for the analysis of the three chosen concentrations and RSD% values were calculated Table 1.

Specificity: To test the selectivity of the developed dual wavelength and Q-analysis methods, they were applied for analysis of number of laboratory prepared mixtures containing ALP and BENZ in different ratios within their linearity ranges. The good percentage recoveries and low RSD% values shown in Table 3, confirming the high selectivity of the suggested methods.

Conclusion

Four simple, specific and accurate spectrophotometric methods have been developed and validated for simultaneous determination of ALP and BENZ in pure form, laboratory prepared mixtures and combined dosage form. The developed dual wavelength analysis and Q-method differ from the reported spectrophotometric ones in using zero order absorption spectra and no derivatization, so signal to noise ratio is enhanced. Also they are time consuming and cost-effective than the chromatographic methods that make them suitable for laboratories lacking the facilities for these methods. Moreover, the developed MCR method has advantages over the published methods in being more simple, rapid and data processing step is not time consuming as it does not need the application of complex algorithms. Also, it does not need derivative steps and so signal to noise ratio has enhanced. On the other hand, the developed extended ratio subtraction method has advantage that the extended drug in the mixture can be determined also because the traditional ratio subtraction method was used for determination of unextended drug only. Hence, both extended and unextended drug in the mixture can be determined in one step.

Parameters	Dual wavelength method		Q-analysis method		MCR method		EXRSM	
	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ
λ (nm)	Difference in absorbance between 253 & 274.4 nm	Difference in absorbance between 238.2 & 261.2 nm	24.8 nm(λ iso) and 250 (λ_{max})		Peak amplitudes (233.4 & 257.4 nm)		Peak amplitudes (228.4 & 252.6 nm)	
Beer's law range	5-60 μgml^{-1}	5-20 μgml^{-1}	5-60 μgml^{-1}	5-20 μgml^{-1}	5-50 μgml^{-1}	5-25 μgml^{-1}	5-50 μgml^{-1}	5-20 μgml^{-1}
Regression equation	$Y=0.0368x-0.0126$	$Y=0.0438x-0.1063$	$C=(Q_m-0.8055/1.0639-0.8055) \times A/0.0484$	$C=(Q_m-1.0639/0.8055-1.0639) \times A/0.0457$	$Y=0.0591x-0.0004$	$Y=0.1535x-0.2765$	$Y=0.0517x-0.0064$	$Y=0.0664x-0.0967$
Correlation coefficient	0.9999	0.9998	--	--	0.9999	0.9999	0.9998	0.9999
Accuracy	99.75	99.67	99.34	99.72	99.88	100.04	99.11	100.03
Precision								
Repeatability	1.293	1.286	1.463	0.924	1.317	0.727	0.753	0.598
Intermediate precision	0.528	1.05	1.092	1.199	0.433	0.551	0.513	0.286

Table 1: Linear regression and analytical parameters of the proposed methods for determination of Allopurinol and Benzbromarone.

Parameters	Dual wavelength method		Q-analysis method		MCR method		EXRSM method		Reported HPLC**	
	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ
Alloben® tablets ^a (B.N.100251)	100.85 ± 1.131	98.02 ± 0.973	99.06 ± 1.275	99.67 ± 1.305	99.33 ± 1.190	98.47 ± 1.475	101.04 ± 1.261	98.72 ± 1.012	97.47 ± 1.250	97.70 ± 0.924
Standard addition ^b	99.45 ± 1.050	99.97 ± 1.286	98.05 ± 1.283	99.27 ± 1.137	98.53 ± 1.366	99.83 ± 0.927	99.11 ± 1.015	99.39 ± 0.795	98.36 ± 0.847	98.43 ± 1.281
F-test ^c	1.072 (4.387)	1.846 (4.875)	1.553 (4.235)	1.795 (4.228)	1.824 (4.357)	1.254 (4.208)	1.054 (4.148)	2.234 (4.526)		
Student's t-test ^c	0.593 (2.160)	0.735 (2.252)	0.813 (2.228)	0.487 (2.200)	1.147 (2.146)	0.955 (2.261)	1.342 (2.323)	0.953 (2.252)		

^a Average of six determinations.

^b Average of 3 determinations.

^c The values in the parenthesis are the corresponding theoretical values at $p=0.05$.

**Reported RP-HPLC method using phosphate buffer PH: 4.0-acetonitrile-methanol (50:30:20v/v/v) as a mobile phase with UV detection at 250nm

Table 2: Determination of the studied drugs in the pharmaceutical preparations by the proposed methods and statistical comparison with reported HPLC method.

Mix no	Ratio ALP: BENZ	Taken amount		Found recovery%**							
		ALP	BENZ	Dual wave length		Q-analysis		MCR		EXRSM	
				ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ
1	5:1*	25	5	100.37	99.77	97.64	99.67	99.68	99.73	100.81	97.27
2	3:1	30	10	99.47	99.14	99.3	100.82	97.25	99.11	97.87	98.37
3	2:1	24	12	100.23	98.36	100.54	98.24	100.48	97.12	98.24	98.42
4	1:1	15	15	100.39	100.35	98.75	97.30	98.96	97.34	100.12	97.93
5	1:2	10	20	99.73	99.11	99.79	99.07	99.38	99.61	97.96	99.44
6	3:2	30	20	99.47	98.11	99.76	98.64	98.43	98.41	98.90	100.80
7	5:1*	40	8	98.63	100.80	99.55	100.14	97.34	97.75	98.63	97.53
8	5:2	30	12	98.64	99.38	98.58	98.96	98.43	97.79	98.58	98.67
Mean±RSD%				99.62 ± 0.711	99.38 ± 0.922	99.24 ± 0.900	99.11 ± 1.116	98.75 ± 1.118	98.37 ± 1.008	98.89 ± 1.049	98.55 ± 1.133

* Corresponding to their ratio in pharmaceutical formulation

** Average of 3 determinations

Table 3: Determination of the studied drugs in the laboratory prepared mixtures by the proposed spectrophotometric methods.

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