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Development and Validation of UV Spectrophotometric Methods for Simultaneous Quantitative Estimation of Two Hypouricemic Drugs in Their Bulk Powder and Combined Solid Dosage Form

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ABSTRACT

Five Simple, accurate, sensitive and low cost spectrophotometric methods have been developed for simultaneous determination of allopurinol (ALP) and benzbromarone (BENZ) without preliminary separation. Method (I) is bivariate method, method (II) is simultaneous equation method, method (III) is area under the curve method (AUC) while method (IV) and method (V) are based on an extension area and presence of isoabsorptive point, called absorbance subtraction and amplitude modulation methods, respectively. All the proposed methods were validated and successfully applied for simultaneous determination of allopurinol (ALP) and benzbromarone (BENZ) in their bulk powder and pharmaceutical solid dosage form] Alloben® 100/25 tablets[. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95 % confidence level and no significant difference was observed regarding accuracy and precision.

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) and United States (USP) Pharmacopoeias [1,2] which is used for treatment of gout and hyperuricemia[3]. It is a xanthine oxidase inhibitor [4-7], which prevents the oxidation of hypoxanthine to xanthine & xanthine to uric acid [8]. This 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 hypouricaemic 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, a report has been published for determination of the studied mixture which depended on measuring BENZ using zero order spectra at its λ_{max}= 356 nm 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 analyzed by TLC-Densitometric method using acetone: chloroform: NH_3 (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]. Also, the studied drugs have been analyses by four spectrophotometric methods: dual wavelength method in which 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, Q-analysis method using the respective absorptivity values at 245.8 nm (isoabsorptive point) and 250 nm ($\lambda_{max}$of ALP), Mean centering method in which 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, Extended ratio subtraction method which 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) [12]. Also, the studied drugs have been analyzed by TLC-Densitometric method using chloroform: methanol (9.2:0.8, v/v) as a developing system and by RP-HPLC method using sodium acetate buffer (pH=4.5, adjusted with acetic acid): acetonitrile: triethylamine (50:50:0.5, by volume) as a mobile phase [13].

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 and validate five UV spectrophotometric methods, bivariate, simultaneous equation, area under the curve, absorbance subtraction and amplitude modulation methods for simultaneous determination of both ALP and BENZ. The developed methods are 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].

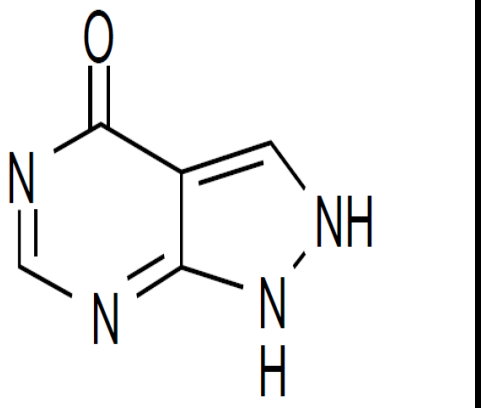

Figure 1: Chemical Structure of Allopurinol.

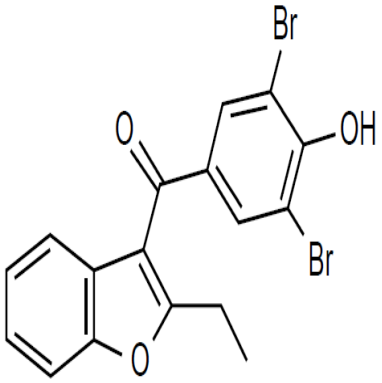

Figure 2: Chemical Structure of Benzbromarone.

MATERIALS AND METHODS

Instruments:

- Shimadzu UV-Visible 1800 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells. Scans were carried out in the range from 200 to 400 nm at 0.1 nm intervals.

- Sonicator (Q sonica, LLC, 53 Church Hill Road Newtown, CT. U.S.A).

Chemicals and solvents:

Methanol, analytical grade (El-Nasr Company, Egypt).

Pure and market samples:

– Pure samples: standard ALP and BENZ were kindly supplied by GLOBAL NAPI PHARMACEUTICALS, 2nd Industrial Zone, 6th of October City- Egypt. The percentage purity was found to be 99.99± 1.340 and 100.68 ± 1.570 for ALP and BENZ, respectively according to the reported dual wavelength method.

– Pharmaceutical solid dosage form (market sample): Alloben® tablets (100/25) (B.N. B 10601) labeled to contain 100 mg Allopurinol+25 mg Benzbromarone and were manufactured by GLOBAL NAPI PHARMACEUTICALS, 2nd Industrial Zone, 6th of October City- Egypt.

Stock and working standard solutions:

– Stock standard solutions: Stock standard solutions each of ALP and BENZ containing 1000 μgml^{-1} of ALP and BENZ were prepared separately in methanol.

– Working standard solutions: Working standard solutions of ALP and BENZ (100 μgml^{-1}) were obtained by dilution of the respective stock solutions with methanol.

Procedures:

Spectral characteristics and wavelength selection: The absorption spectra of 16 μgml^{-1} each of ALP, BENZ and their 1:1 mixture (containing 8 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**.

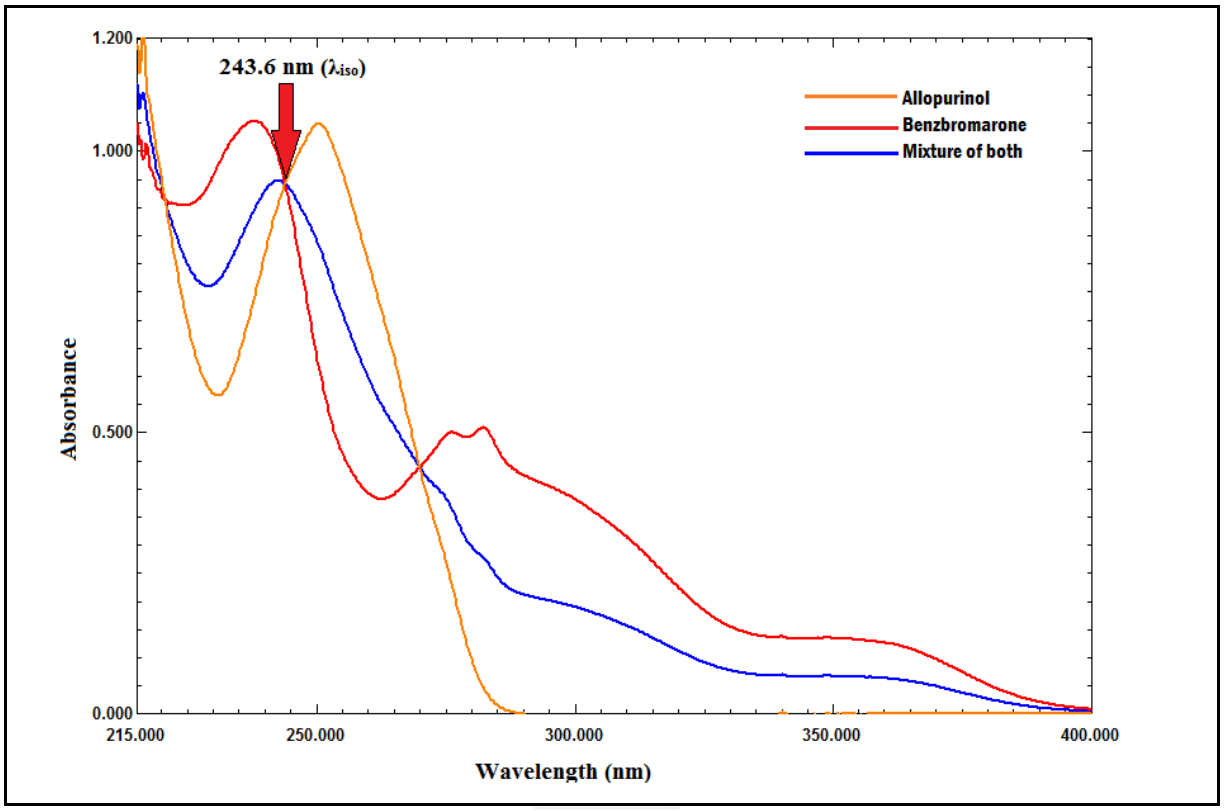

Figure 3: Zero-order absorption spectra of 16 µgml^{-1} each of ALP, BENZ and their 1:1 mixture contains 8 µgml^{-1} of each using methanol as a solvent

Linearity: (construction of the calibration graph):

Method I (Bivariate method):[15]

The calibration graphs were constructed over the concentration ranges from (2 to 16 µgml^{-1}) of ALP and (1 to 16 µgml^{-1}) of BENZ by transferring different aliquots of their standard solutions (100 µgml^{-1}) containing (20 - 160 µg) of ALP and (10 - 160 µg) of BENZ into two separate series of 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank. The absorbance was measured at 233 and 253 nm and then the corresponding regression equations were computed at the selected wavelengths for both ALP and BENZ. The obtained slope and intercept values were used for calculating the concentrations of both ALP and BENZ using the following equations:

$$C_A = (A_{AB1} - e_{AB1} - m_{B1}C_B) / m_{A1} \quad (1)$$

$$C_B = [m_{A2} (A_{AB1} - e_{AB1}) + m_{A1} (e_{AB2} - A_{AB2})] / m_{A2}m_{B1} - m_{A1}m_{B2} \quad (2)$$

Where:

- C_A, C_B are the concentrations of ALP, BENZ, respectively.

- m_{A1}, m_{A2} are the slope values of ALP at λ_1, λ_2.

- m_{B1}, m_{B2} are the slope values of BENZ at λ_1, λ_2.

- A_{AB1}, A_{AB2} are the absorbance values of the binary mixture at λ_1, λ_2.

- e_{AB1}, e_{AB2} are the sum of the intercepts of ALP, BENZ at λ_1, λ_2.

According to Kaiser method,[16] the slope values of the linear regression equations for both ALP and BENZ at different wavelengths were used to calculate the sensitivity matrices (K**)** to find out the optimum pair of wavelengths (highest matrix value) at which the binary mixture was determined.

$$K = \begin{vmatrix} m_{A1} & m_{B1} \\ m_{A2} & m_{B2} \end{vmatrix}$$

The measured absorbance values at 233 and 253 nm for both ALP and BENZ versus the final concentrations in $\mu g ml^{-1}$were plotted to get the calibration graphs for both ALP (2-16$\mu g ml^{-1}$) and BENZ (1-16 $\mu g ml^{-1}$), the regression equations were derived.

Method II (Simultaneous equation method):[17,18]

The calibration graphs were constructed over the concentration ranges from (2 to 16 $\mu g ml^{-1}$) of ALP and (1 to 16 $\mu g ml^{-1}$) of BENZ by transferring different aliquots of their standard solutions (100 $\mu g ml^{-1}$) containing (20 - 160 µg) of ALP and (10 - 160 µg) of BENZ into two separate series of 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank. The absorbance values at 250 and 238 nm were recorded. Absorbance and

absorptivity values were used for calculating the concentrations of both ALP and BENZ using the following equations:

$$C_X = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \quad (3)$$

$$C_Y = \frac{A_1 a_{X2} - A_2 a_{X1}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \quad (4)$$

Where C_x is the concentration of ALP and C_y is the concentration of BENZ

a_{X1} and a_{X2} are the absorptivities of ***ALP*** at ***250*** and ***238nm***, respectively.

a_{Y1} and a_{Y2} are the absorptivities of ***BENZ*** at ***250*** and ***238nm***, respectively.

A_1 and A_2 are the absorbances of samples at ***250*** and ***238nm***, respectively.

The measured absorbance values at 250 and 282 nm for ALP and BENZ versus the final concentrations in µgml^{-1} were plotted to get the calibration graphs and the regression equations were derived.

Method III (Area under the curve method):[19,20]

The calibration graphs were constructed over the concentration ranges from (2 to 16 µgml^{-1}) of ALP and (1 to 16 µgml^{-1}) of BENZ by transferring different aliquots of their standard solutions (100 µgml^{-1}) containing (20 - 160 µg) of ALP and (10 - 160 µg) of BENZ into two separate series of 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank. Areas under curve at 226-243 nm and 245-257 nm were recorded. Areas under the curve and area absorptivity values were used for calculating the concentrations of both ALP and BENZ using the following equations:

$$C_x = (A_{m1}\, a_{y2} - A_{m2} a_{y1}) / (a_{x1}\, a_{y2} - a_{x2}\, a_{y1}) \quad (5)$$

$$C_y = (A_{m2} - a_{x2} C_x) / a_{y2} \quad (6)$$

Where C_x is the concentration of ALPand $C_y$is the concentration of BENZ

A_{m1} and A_{m2} is the area under the curve of the mixture at the wavelength range (226-243 nm)

and (245-257 nm) respectively.

a_{X1} and a_{X2} are the absorptivities of ***ALP*** at the wavelength range (226-243 nm) and (245-257 nm) respectively.

a_{Y1} and a_{Y2} are the absorptivities of ***BENZ*** at the wavelength range (226-243 nm) and (245-257 nm) respectively.

The obtained area under curve values at 226 – 243 nm and 245 – 257 nm for both ALP and BENZ versus the final concentrations in μgml^{-1} were plotted to get the calibration graphs and the regression equations were derived.

Method IV (Absorbance subtraction method):[21,22]

The calibration graphs were constructed over the concentration ranges from (2 to 16 μgml^{-1}) of ALP and (1 to 16 μgml^{-1}) of BENZ by transferring different aliquots of working standard ALP and BENZ solutions (100 µg/ml) containing 20 - 160 µg and 10 - 160 µg, respectively into a series of 10-ml volumetric flasks and completed to the mark with methanol. The zero order absorbance of each set was scanned in the range of 200 – 400 nm. The absorbance of ALP and BENZ was measured at 243.6 nm (λ_{iso}) and the calibration graph was constructed. The absorbance factor for BENZ at 243.6 nm and 289 nm ($A_{243.6}$/ A_{289}) was calculated, and used for calculating the concentrations of ALP and BENZ at 243.6 nm in the mixture.

The absorbance values at 243.6 nm (iso-absorptive point) versus the drug concentrations in μgml^{-1} were plotted to get the calibration graphs and the regression equations were derived.

Method V (Amplitude modulation method):[23]

The calibration graphs were constructed for both ALP and BENZ in the ranges of 1-16 μgml^{-1} by transferring different aliquots of working standard ALP and BENZ solutions (100 μgml^{-1}) containing 10 - 160 µg into a series of 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank and then divided by the normalized absorption spectrum of BENZ solution (1 µg/ml). The amplitude of ratio spectra of BENZ at 243.6 nm (iso-absorptive point) was recorded. Also, The amplitude of ratio spectra of ALP at 243.6 nm (iso-absorptive point) was recorded after subtraction of the constant value at 289 nm (amplitudes in plateau

region) and the concentrations of both ALP and BENZ were calculated from the regression equation at 243.6 nm.

The measured amplitudes of ratio spectra for both ALP and BENZ at iso-absorptive point 243.6 nm versus the final concentrations in μgml^{-1} were plotted to get the calibration graphs and the regression equations were derived.

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 μgml^{-1}). Zero order absorption spectra of these mixtures were recorded using methanol as a blank.**For method I**, the absorbance of each mixture was recorded at 233 and 253 nm, ALP and BENZ concentrations were calculated using equations 1 & 2, respectively. **For method II**, the absorbance of each mixture was recorded at 250 and 238 nm, ALP and BENZ concentrations were calculated using equations 3 & 4, respectively. **For method III**, areas under curve at 226-243 nm and 245-257 nm were recorded; ALP and BENZ concentrations were calculated using equations 5 & 6, respectively. **For method IV**, the absorbance of BENZ in the mixtures at iso-absorptive point was determined by using absorbance factor then subtracted from the recorded absorbance at 243.6 nm (λ_{iso}) to get the absorbance of pure ALP, the concentrations of both ALP and BENZ in the mixture were calculated from the corresponding regression equation at 243.6 nm (λ_{iso}). **For method V**, the absorbance of each mixture was recorded at 289 nm after division by a normalized absorption spectrum of BENZ then the concentrations of BENZ were calculated from the corresponding regression equation at 243.6 nm (λ_{iso}) after that the ratio spectra were subtracted at 289 nm then the absorbance values at 243.6 nm were recorded thus the concentrations of ALP were calculated from the corresponding regression equation at 243.6 nm (λ_{iso}).

Extraction and application of the pharmaceutical formulation (solid dosage form):

Pharmaceutical sample stock solution ($1000/250 \mu gml^{-1}$): Ten **Alloben®** tablets were accurately weighed and finely powdered, then a quantity equivalent to 100 mg of ALP and 25 mg of BENZ was extracted three times with 25 ml of methanol by mixing well for 10 minutes by vigorous shaking then the prepared solution was sonicated for 20 minutes, finally the prepared solution has been filtered through whatman filter paper No. 41 into 100 ml

volumetric flasks. Filter paper was washed with methanol, adding washings to the volumetric flask and the volume was made up to the mark with methanol.

Pharmaceutical sample working solution (100/25 µgml^{-1}): It was freshly prepared by suitable dilution from its stock solution using methanol as diluent in another 100 ml-volumetric flasks to prepare concentrations within the linearity range of each drug. Determination of both ALP and BENZ content of the tablets were carried out following the methods are given under analysis of laboratory prepared mixtures.

RESULTS AND DISCUSSION:

Five different spectrophotometric methods were applied for the simultaneous determination ALP and BENZ in binary mixture, whose spectra showed high degree of interference **figure 3** which hinder the direct UV determination of the two drugs in their mixture form. Sensitivity and selectivity of the applied methods were assessed and compared.

Methods development and optimization of experimental conditions:

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 [24,25].

Method I (Bivariate method):

In order to apply the bivariate method for simultaneous determination of ALP and BENZ in binary mixture, the absorbance of the two components individually at eight different selected wavelengths was recorded in the region of overlapping; 225, 233, 237, 241, 253, 261, 269 and 277 nm. The calibration curve equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that; there was a linear relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths for both ALP and BENZ showed a satisfactory linear regression coefficient (r^2> 0.9992).

According to Kaiser method, the slope values of the linear regression equations for both drugs at the selected wavelengths were used to calculate the sensitivity matrices (K) to find out the optimum pair of wavelengths at which the binary mixture was recorded. It was found that; the slopes at 233 and 253 nm gave the maximum value of K (**table 4)** and thus chosen for the analysis.

Method II (Simultaneous equation method):

In this method, simultaneous quantitative estimation of both ALP and BENZ in binary mixture was carried out by simultaneous equation method. The absorbance values were measured at 250 nm and 238 nm for both ALP and BENZ. The absorptivity coefficients of each component at both wavelengths were determined. The concentrations of both ALP and BENZ in laboratory prepared mixture and pharmaceutical formulation was determined by substituting the absorbance and absorptivity coefficient in the previous equations 3 & 4.

Method III (Area under the curve method):

In this method, the area under the curve for both ALP and BENZ were recorded over the ranges of at (226 - 243 nm) and (245 - 257 nm) as shown in **figure 4** for ALP and **figure 5** for BENZ. The absorptivity values of both ALP and BENZ were determined at each wavelength range. The concentrations of both ALP and BENZ were obtained by using the previous equations 5 & 6.

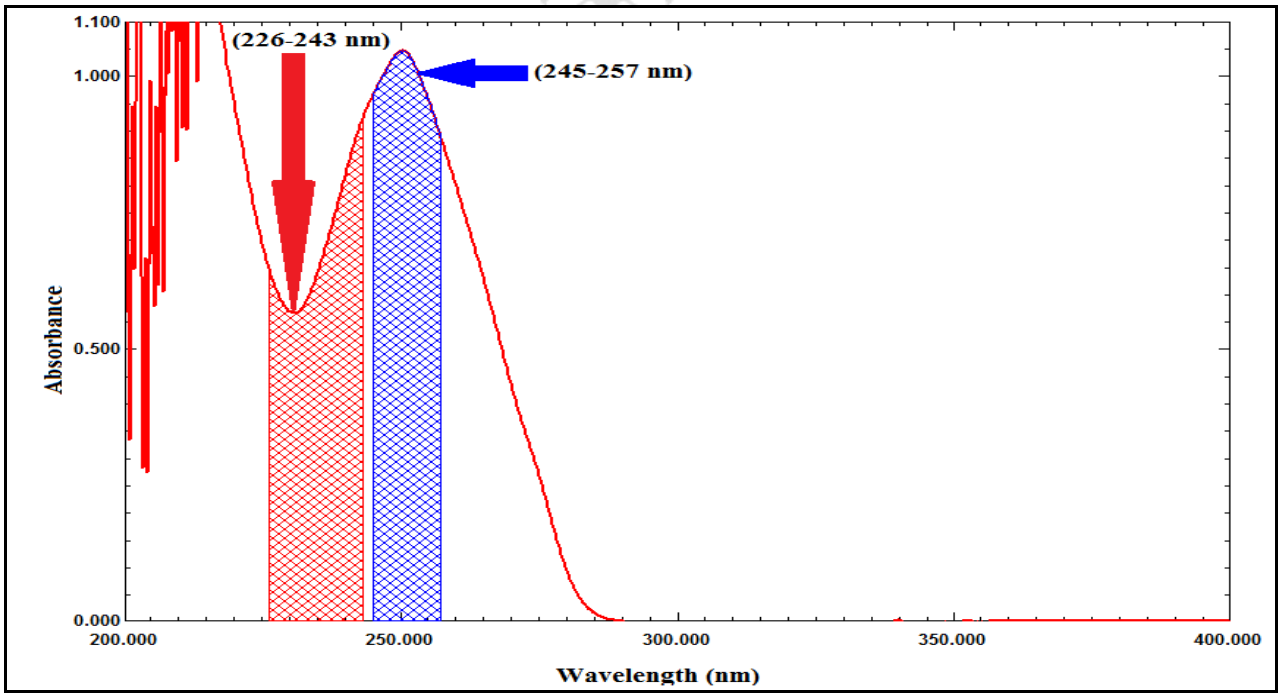

Figure 4: Zero-order absorption spectrum of ALP (16 µgml^{-1}) showing area under the curve over the ranges of (226 – 243 nm) and (245 – 257 nm)

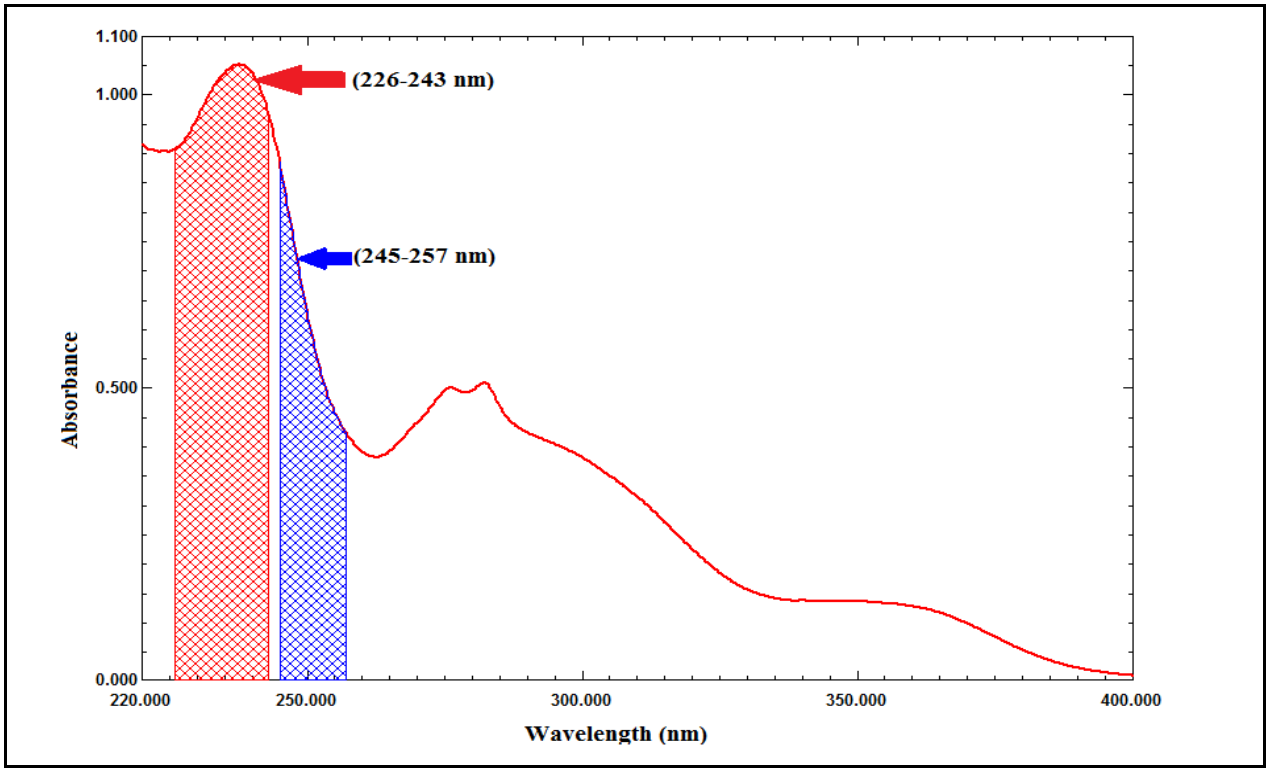

Figure 5: Zero-order absorption spectrum of BENZ (16 µgml^{-1}) showing area under the curve over the ranges of (226 – 243 nm) and (245 – 257 nm)

Method IV (Absorbance subtraction method):

The zero-order absorption spectra of both ALP and BENZ, show severe overlap, with isosbestic point at 243.6 nm and the spectrum of BENZ is more extended in plateau region from 289 – 400 nm in which the spectrum of ALP show no absorbance as shown in **figure 3.**

In this method**;** for the simultaneous determination of ALP and BENZ in binary mixture, we will utilize their iso-absorptive point at 243.6 nm. The absorbance corresponding to ALP or BENZ, separately, at iso-absorptive point can be calculated using absorbance factor ($A_{243.6}/A_{289}$) which is the average of the absorbance of different concentrations of BENZ at iso-absorptive point (243.6 nm) to that at 289 nm. The absorbance of BENZ alone in the mixture at iso-absorptive point calculated by multiplication the absorbance of the mixture at 243.6 nm to absorbance factor and then the absorbance of ALP can be obtained by subtraction absorbance of BENZ obtained from the absorbance of the mixture at iso-absorptive point.

Absorbance of BENZ in the mixture at ($\lambda_{243.6}$) = ($A_{243.6}/A_{289}$) x $A_{289(mix)}$.

Absorbance of ALP in the mixture at ($\lambda_{243.6}$) = $A_{(mix\ at\ 243.6nm)}$- $A_{(BENZ\ at\ 243.6nm)}$

Concentrations of ALPand BENZ can be determined by substitution the obtained absorbance values corresponding to ALP and BENZ in iso-absorptive point (243.6 nm) regression equation.

Method V (Amplitude modulation method):

The zero-order absorption spectra of ALP and BENZ, show sever overlap and iso-absorptive point at 243.6 nm as shown in **figure 3**.

This method is based on two facts; the first that the iso-absorptive point whenever present in an absorption spectrum will be retained at the same point even after division by a one component as a divisor in the ratio spectrum as shown in **figure 6**, while the second that the results of manipulating ratio spectra techniques are greatly affected by the choice of the divisor. So to eliminate the effect of the divisor, we will use the normalized spectrum of BENZ (1 μgml^{-1}) (normalized spectrum is prepared mathematically by using sum of different spectra of one drug in the binary mixture (BENZ) divided by the total concentrations). Since the two components, exhibiting this point have equal absorptivities, by dividing the spectrum of the binary mixture by the normalized BENZ divisor spectrum, we obtain the ratio spectra. At the iso-absorptive point of ratio spectra, the amplitude value was modulated to concentration. The amplitude value of the constant can be determined at the plateau region at 289 nm, which is equal to the amplitude constant value of BENZ along the whole spectrum. At the iso-absorptive point (243.6 nm), the amplitude of the ratio spectra at this point will be equal to the sum of the amplitudes of ALP and BENZ. After the subtracting recorded amplitude at 289 nm (constant) from the amplitude value at 243.6 nm (iso-absorptive), we get the corresponding recorded amplitude of ALP as shown in **figure 7**, which is equivalent to recorded concentration of ALP in the mixture ($C_{Recorded}$ of ALP), while the recorded amplitude of constant value will be directly equal to the recorded concentration of BENZ in the mixture ($C_{Recorded}$ of BENZ), to eliminate any error due to signal to noise ratio, the actual concentrations of ALP and BENZ could be calculated by using their corresponding unified regression equation at isosbestic point 243.6 nm.

$$C_{Recorded} = 1.0266\ C - 0.006.$$

Where, $C_{Recorded}$ is the recorded amplitude of ratio spectrum at 243.6 nm and C is the corresponding concentration of ALP or BENZ.

The two main requirements of this method are the existence of isoabsorptive point of both components at zero points and consequently in the ratio spectra, and the extension of the spectra of one component. The advantage of amplitude modulation method over other mathematical techniques utilizing the constant is the reduced manipulation steps and only one divisor is needed in order to determine both components in the mixture. By using the normalized divisor, the results are not affected by the choice of divisor. This method has an advantage over the isoabsorptive point at zero order that it measures the concentration of both components with no need for other complementary method to measure one of the components in the mixture. In addition, this method has advantages over the newly developed absorbance subtraction method is that by using the normalized divisor, the obtained amplitude at the ratio spectrum will directly represent the concentration of each component and the risk of error upon the determination of absorbance factor of lower absorbance as well as the manipulation steps will be reduced by elimination of the absorbance factor calculation step.

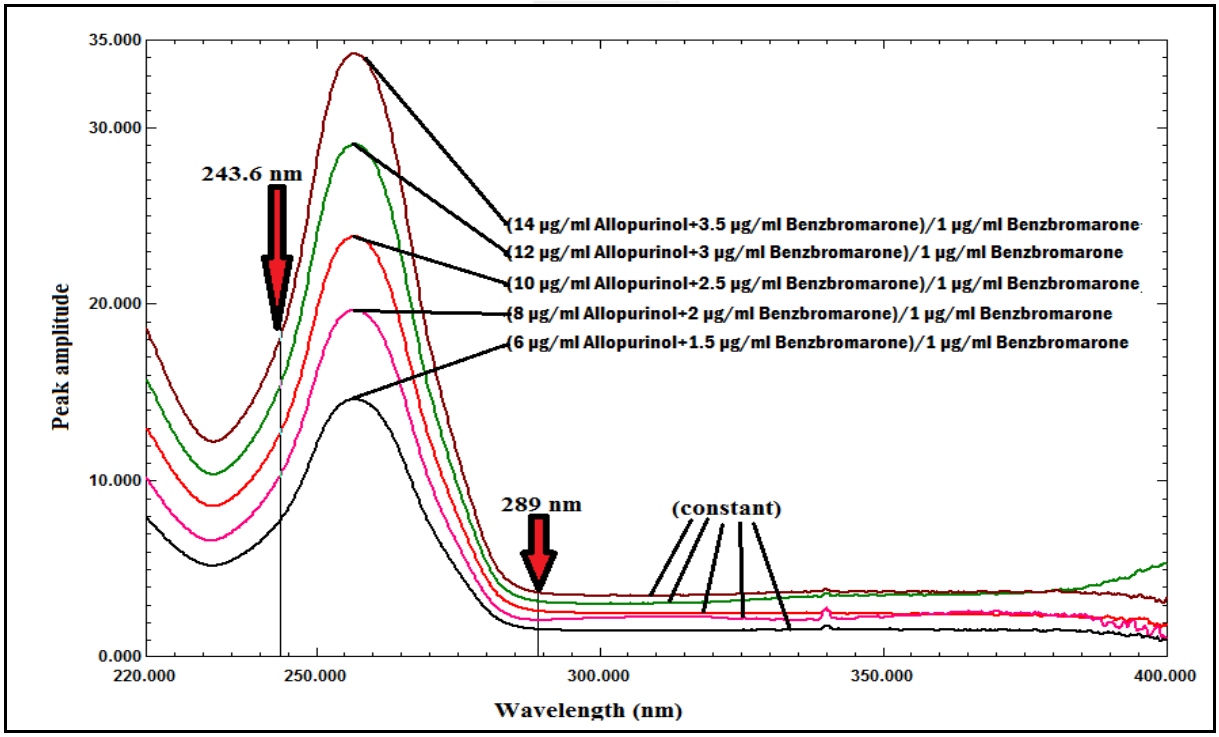

Figure 6: Ratio spectra of laboratory prepared mixtures of ALP (6 – 14 µgml^{-1}) and BENZ (1.5 – 3.5 µgml^{-1}) using 1 µgml^{-1} of BENZ as a divisor

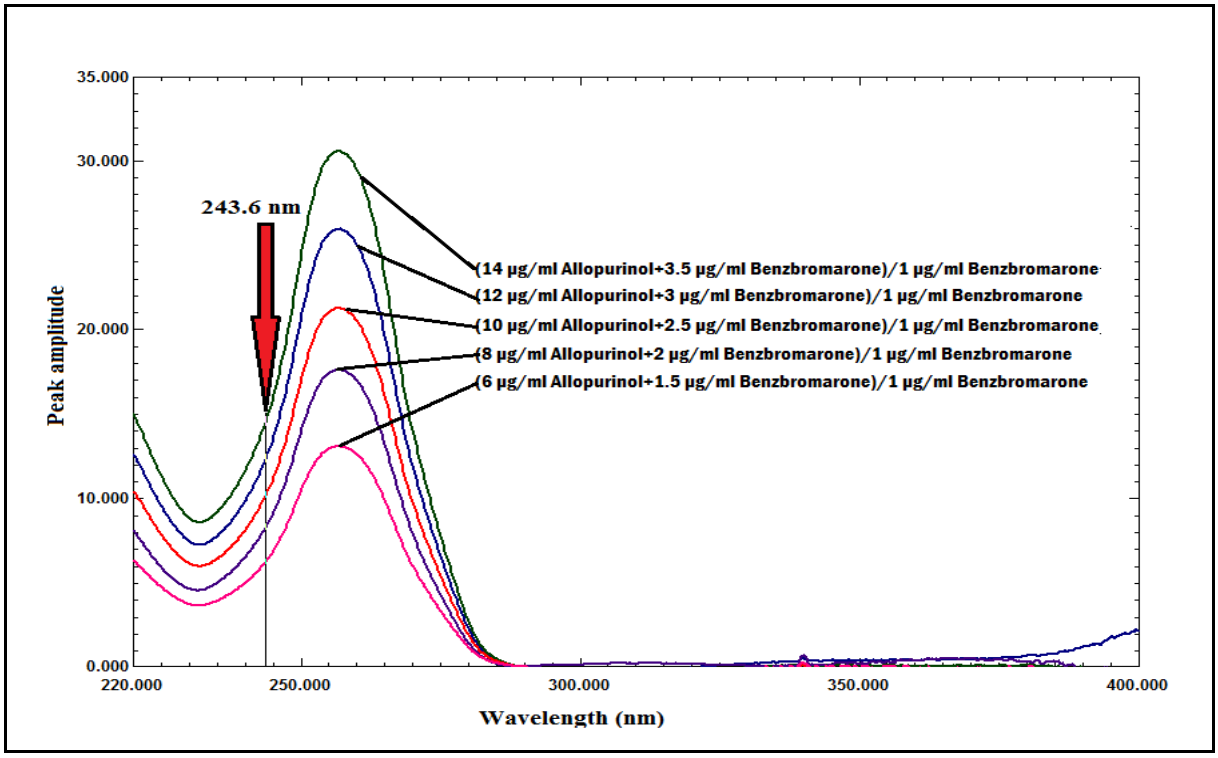

Figure 7: Ratio spectra of laboratory prepared mixtures of ALP (6 – 14 µgml^{-1}) and BENZ (1.5 – 3.5 µgml^{-1}) using 1 µgml^{-1} of BENZ as a divisor after subtraction of the constant

Method validation:

Validation of the methods 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 (2, 8, 14 μgml^{-1}) were chosen in the five methods for both ALP and BENZ except in bivariate method, the concentrations of (4, 8, 14 μgml^{-1}) for BENZ were chosen and in simultaneous equation method, the concentrations of (4, 8, 14 μgml^{-1}) for ALP were chosen. These concentrations 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 five methods, they were applied for analysis of number of laboratory prepared mixtures containing ALP and BENZ in the same ratios as the pharmaceutical preparation and within their linearity ranges. The good percentage recoveries and low RSD% values are shown in **table 3**, confirming the high selectivity of the suggested methods.

CONCLUSION

The mentioned UV spectrophotometric methods were validated and successfully applied for simultaneous determination of ALP and BENZ in binary mixtures and in their available dosage form. The methods were validated and statistically compared, and no significant differences were found among them. The proposed procedures are simple and do not require sophisticated techniques or instruments. They are also sensitive, selective and can be used for the routine analysis of ALP and BENZ in their available dosage forms.

Table 1: Linear regression and analytical parameters of the proposed methods for determination of allopurinol and benzbromarone

Parameters	Bivariate method		Simultaneous equation method		AUC method		Absorbance subtraction method		Amplitude modulation method	
	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ
λ(nm)	233 & 253 nm		250 & 238 nm		(226-243) & (245-257) nm		(λ_{iso}) at 243.6 nm			
Beer's law range	2-16 μgml^{-1}	1-16 μgml^{-1}	2-16 μgml^{-1}	1-16 μgml^{-1}	2-16 μgml^{-1}	1-16 μgml^{-1}	2-16 μgml^{-1}	1-16μgml^{-1}	1-16μgml^{-1}	1-16μgml^{-1}
LOD (μgml^{-1})	0.339 & 0.308	0.371 & 0.327	0.231 & 0.377	0.266 & 0.378	0.246 &0.251	0.306 & 0.304	0.233	0.392	0.343	0.371
LOQ (μgml^{-1})	1.027 & 0.934	1.124 & 0.991	0.699 & 1.142	0.807 & 1.146	0.746 & 0.761	0.926 & 0.920	0.706	1.188	1.040	1.124
Regression equation	**Y=0.0392x -0.0316 Y=0.063x -0.006**	**Y=0.066x -0.031 Y=0.032x -0.016**	**Y=0.066x -0.011 Y=0.049x -0.028**	**Y=0.068x -0.031 Y=0.040x -0.021**	**Y=0.7638x -0.5105 Y=0.7578x -0.1288**	**Y=1.0994x -0.5116 Y=0.4723x -0.2299**	**Y=0.0604x -0.0223**	**Y=0.0612x -0.0278**	**Y=1.026x -0.006**	**Y=1.0708x -0.4854**
Correlation coefficient	0.9996 0.9997	0.9996 0.9997	0.9998 0.9995	0.9996 0.9998	0.9998 0.9998	0.9997 0.9997	0.9998	0.9997	0.9996	0.9997
Accuracy	99.36	100.54	99.28	101.12	99.59	100.55	99.94	101.43	99.73	100.26
Precision										
Repeatability	0.538	0.837	0.628	0.712	1.249	1.116	0.713	0.528	1.151	0.599
Intermediate precision	1.425	0.920	1.487	0.606	1.789	1.006	1.120	0.823	1.715	0.810

Table 2: Determination of the studied drug in the pharmaceutical preparation by the proposed methods and statistical comparison with reported dual wavelength method

Parameters	Bivariate method		Simultaneous equation method		AUC method		Absorbance subtraction method		Amplitude modulation method		Reported dual wavelength method**	
	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ
Alloben® tablets[a] (B. N. B 10601)	100.47 ± 1.285	100.76 ± 1.128	99.79 ± 1.417	101.84 ± 0.805	100.13 ± 1.551	101.39 ± 0.624	100.32 ± 1.255	99.41 ± 1.696	100.14 ± 1.157	101.04 ± 0.830	99.99 ± 1.340	100.68 ± 1.570
Standard addition[b]	100.32 ± 0.953	100.60 ± 1.134	100.71 ± 0.428	100.15 ± 0.747	100.61 ± 0.788	101.14 ± 0.683	98.60 ± 0.637	100.29 ± 0.805	100.98 ± 0.734	100.85 ± 1.345		
F-test [c]	0.928 (6.388)	0.517 (6.388)	1.113 (6.388)	0.269 (6.388)	1.343 (6.388)	0.160 (6.388)	0.882 (6.388)	1.139 (6.388)	0.747 (6.388)	0.281 (6.388)		
Student's t-test [c]	0.575 (2.306)	0.093 (2.306)	0.232 (2.306)	1.462 (2.306)	0.156 (2.306)	0.939 (2.306)	0.398 (2.306)	1.226 (2.306)	0.187 (2.306)	0.450 (2.306)		

a Average of five determinations.

b Average of four determinations.

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

** Reported dual wavelength method using the absorbance difference (253.4-274.4 nm) for determination of ALP while the absorbance difference for BENZ is zero and using the absorbance difference (230-266.8 nm) for determination of BENZ while the absorbance difference for ALP is zero.

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

Taken amount µgml-1		Found recovery %									
		Bivariate method		Simultaneous equation method		AUC method		Absorbance subtraction method		Amplitude modulation method	
ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ	ALP	BENZ
4	1	101.08	99.55	98.39	100.24	99.18	101.54	100.78	101.19	———	———
6	1.5	99.77	100.54	98.85	101.71	99.70	101.95	98.19	101.28	100.59	100.40
8	2	98.97	100.62	98.53	100.68	99.28	101.49	101.04	99.64	100.04	100.62
10	2.5	101.01	101.97	99.36	101.75	100.28	101.43	99.94	98.65	98.25	100.37
12	3	100.46	101.34	100.53	101.75	101.34	101.88	101.69	97.99	99.49	101.18
14	3.5	———	———	———	———	———	———	———	———	100.65	100.92
Mean ± RSD%		100.26 ± 0.888	99.13 ± 0.860	100.80 ± 0.905	101.23 ± 0.706	99.96 ± 0.889	101.66 ± 0.232	100.33 ± 1.345	99.75 ± 1.479	99.80 ± 0.992	100.70 ± 0.344

Table 4: Values of the sensitivity matrix determinants calculated according to Kaiser's Method[16]

(K X 10^6) for the mixture of allopurinol and benzbromarone by the bivariate method.

λ/ λ	WL225	WL233	WL237	WL241	WL253	WL261	WL269	WL277
WL225	0	662.56	328.44	-300.68	-2270.72	-1717.84	-504.8	695.96
WL233		0	-398.24	-1077.24	-2898.64	-2190.48	-871.68	418.88
WL237			0	-714.73	-2801.74	-2118.38	-735.52	625.96
WL241				0	-2376.46	-1798.92	-425.16	941.45
WL253					0	-8.2	778.96	1609.18
WL261						0	591.12	1214.86
WL269							0	596.48
WL277								0

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