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Zero-Order Spectrophotometry and First-Order Derivative Spectrophotometry for the Quantitative Estimation of Allopurinol in a Pharmaceutical Formulation

Saadet Dermiş*°, Erdal Dinç*, Mehmet Emin Kuşçu*, Merve Biliktü*

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Abstract

In this study, two different spectrophotometric methods, direct absorbance measurement method and first derivative spectrophotometry were proposed for the quantitative analysis of allopurinol in tablets. In the application of direct absorbance measurement to the analysis of allopurinol, calibration curve was obtained by measuring the absorbance values at 245.0 nm in the zero-order spectra. In first derivative method, calibration equation was computed by using the $dA/d\lambda$ values at 257.6 nm. Linearity range for both methods were found between 4.0-36.0 µg/mL for the analysis of allopurinol. The proposed methods were checked by analyzing external test samples containing allopurinol and using standard addition samples. Analysis results showed that the applied methods were precise, accurate and reliable for the quantitation of the related drug.

Keywords: Spectrophotometry, Quantitative analysis, Allopurinol, Tablets

* Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100 Tandoğan-Ankara, Turkey
° Corresponding author: dermis@pharmacy.ankara.edu.tr

Özet

Farmasötik Formülasyonlarda Allopurinol'ün Sıfırıncı Dereceden ve Birinci Dereceden Türev Spektrofotometrisi Yöntemleriyle Kantitatif Analizi

Bu çalışmada tabletlerde Allopurinol'ün kantitatif analizi için doğrudan absorbans ölçümü ve birinci türev spektrofotometrisi yöntemlerini kapsayan iki farklı spektrofotometrik yöntem geliştirildi. Allopurinol'ün analizi için 245.0 nm dalga boyunda doğrudan absorbans ölçümleri yapılarak kalibrasyon eğrisi elde edildi. Birinci dereceden türev spektrofotometrisi yönteminde 257.6 nm de dalga boyunda dA/dλ değerleri kullanılarak kalibrasyon denklemi hesaplandı. Allopurinol'ün miktar tayini için her iki yöntemin uygulanmasında doğrusal çalışma aralığı olarak 4.0-36.0 µg/mL bulunmuştur. Hazırlanan test numunelerinin analizi ve standart ekleme tekniği kullanılarak önerilen analitik yöntemler valide edildi. Analizler, ilgili ilacın miktar tayini için kullanılan yöntemlerin kesin, doğru ve güvenilir olduğunu gösterdi.

Anahtar Kelimeler: Spektrofotometri, Kantitatif Analiz, Allopurinol, Tablet

1. Introduction

Alloprinol is known chemically as 1,5-dihydro-4 H-pyrazolo (3,4-d) pyrimidin-4-one. Figure 1 shows chemical structure of allopurinol. Alloprinol is potent inhibitors of xanthine oxidase, commonly used in the treatment of chronic gout or of hyperuricaemia associated with leukaemia, radiotheraphy, antineoplastic agents and treatment with diuretics conditions (1). In treatments, allopurinol is administered orally. Allopurinol is a structural analogue of the natural purine base, hypoxanthine. As mentioned above, allopurinol is an inhibitor of xanthine oxidase, the enzyme responsible for the conversion of hypoxanthine to xanthine and of xanthine to uric acid, the end product of purine metabolism in man. Alloprinol is metabolized to the corresponding xanthine analogue, oxypurinol (alloxanthine), which also is an inhibitor of xanthine oxidase.

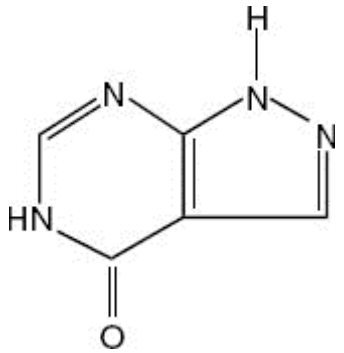

Figure1. Chemical formula of Allopurinol

Several analytical techniques including spectrophotometric method [2-5], spectrofluorimetric method [6,7], HPLC [8-11], LC-MS [12-14], capillary electrophoresis [15,16] and electrochemical analysis [17,18] were used for the determination of allopurinol in samples.

The quantitative estimation and quality control of pharmaceutical preparation are very important requirements for the drug industry and human health. These preparations contain active compounds with a constant amount of excipient, which may give overlapping spectral bands with main active compound. In such case, the analysis of pharmaceutical formulation is a very difficult problem. In order to eliminate this, derivative spectrophotometry is very useful technique to get precise, accurate and reliable results.

In our study, zero-order spectrophotometry and first derivative spectrophotometry, based on the use of the measurements of absorbances at 245.0 nm and $dA/d\lambda$ value at 257.6 nm, were applied to the quantification of allopurinol in a commercial formulation. The performance of the proposed two approaches was tested by analyzing external test samples of the related drug. Satisfactory analysis results were obtained by applying both zero-order spectrophotometry and first derivative spectrophotometry to the analysis of tablets containing allopurinol.

2. Material and Methods

2.1. Apparatus and Software

A Shimadzu 1601 double beam spectrophotometer with fixed slit width (2 nm) connected to a desktop computer loaded with Shimadzu UVPC program was used to record the zero-order spectra of calibration set external test and tablet samples. Data treatments were performed by using Microsoft Excel Software. Spectra were plotted by using Matlab Software.

2.2. Pharmaceutical Preparation

A commercial tablet preparation (Urikoliz® tablets produced by Sandoz Pharm. Ind., Istanbul, Turkey) containing 300mg allopurinol per tablet was analyzed.

2.3. Standard, calibration and validation solutions

Standard stock solution (10 mg/100 mL) was prepared by dissolving 10 mg of allopurinol in 100 mL calibrated flask in methanol. Calibration solutions of allopurinol in the concentration range of 4.0-36.0 µg/mL were prepared by using the standard stock solution. For the validation of the method, external test samples in the working concentration of 4.0-36.0 µg/mL and standard addition samples in three different concentration levels 4.0, 8.0 and 12.0 µg/mL were prepared from stock solution of allopurinol.

2.4. Analysis procedure of Commercial Tablet

In the analysis of commercial preparation containing 300 mg allopurinol per tablet, 20 tablets were accurately weighed and powdered in mortar. An accurately weighed quantity of tablet powder, equivalent to one tablet was transferred to 100 ml volumetric flask and dissolved by sonication with sufficient quantity of methanol and volume was made to the mark with methanol. Sample solution was then filtered by 0.42 µm syringe filter. The filtrate was diluted by using methanol into working concentration range for the spectral analysis.

3. Results and Discussions

In this study, the UV zero-order spectra of allopurinol in the concentration range of 4.0-36.0 µg/mL were recorded between 200-300 nm. Figure 2a shows the absorption spectra of the analyzed drug in the mentioned concentration range. In first application of the analysis of the related drug zero-order spectrophotometry also named direct absorbance measurement method was proposed for the quantification of allopurinol in tablets. In the application of this zero-order spectrophotometry, linear regression equation for the drug was computed by applying least squares regression analysis to concentration set and absorbance at 245 nm in the zero-order spectra. The first method gave a linear response to allopurinol from 4.0 to 36.0 µg/mL. Regression analysis and statistical results were given in Table 1. Calibration equation was used for the estimation of allopurinol in samples.

In this study, another method is first derivative method for the quantitation of allopurinol in samples. In the application of this method, first derivative of the zero-order UV spectra of allopurinol and its samples was calculated by using the interval of $\Delta\lambda$=8 nm in the spectral range of 200-300 nm. Their first derivative spectra were smoothed by using the smoothing function of $\Delta\lambda$=8 nm. In Figure 2b, first derivative spectra of allopurinol in the calibration range of 4.0-36.0 µg/mL were shown in Figure 2b. It was concluded that the concentration of allopurinol was proportional to the $dA/d\lambda$ values at 257.6 nm. Using the relationship between concentration and $dA/d\lambda$ values, linear regression analysis and its statistical results were listed in Table 1. Allopurinol in samples was determined by using the linear regression equation.

3.1. Validation of the method

The method to analyze allopurinol tablets was validated. Detection limits, linearity, accuracy and precision, and sample recovery were estimated. A good linearity with high correlation co-

Table 1. Linear regression analysis and statistical results

Parameter	Zero-order spectrophotometry	First order spectrophotometry
λ (nm)	249.0	257.6
m	$8.34x10^{-3}$	$2.34x10^{-3}$
n	$-3.96x10^{-3}$	$-3.40x10^{-4}$
r	0.9996	0.9998
SE(r)	$2.4x10^{-3}$	$5.53x10^{-4}$
SE(m)	$9.25x10^{-5}$	$2.13x10^{-5}$
SE(n)	$1.87x10^{-3}$	$4.31x10^{-4}$
LOD	0.63	0.52
LOQ	2.24	1.84

m= Slope of regression equation
n= Intercept of regression equation
r= Correlation coefficient of regression equation
SE(m) = Standard error of the slope
SE(n) =Standard error of the intercept
SE(r) = Standard error of correlation coefficient
LOD = Limit of detection (µg/mL)
LOQ = Limit of quantitation (µg/mL)

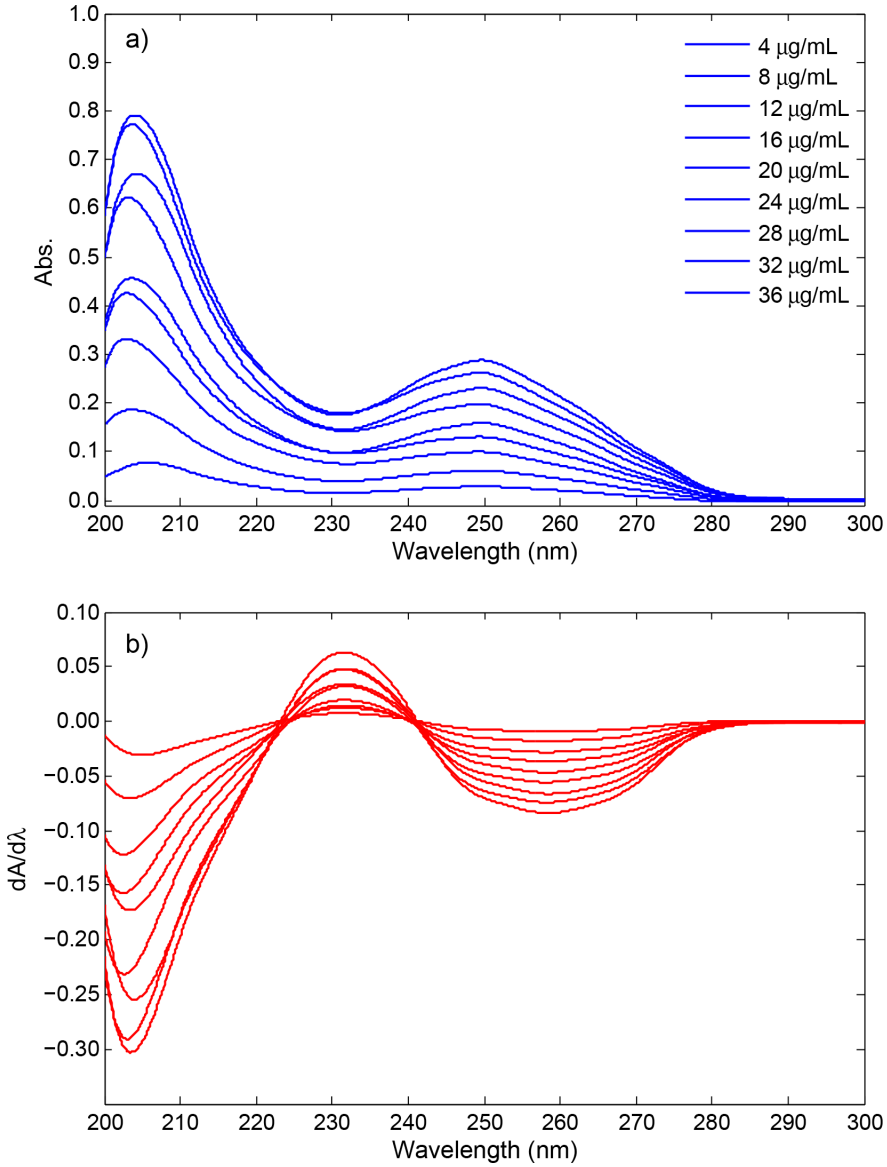

Figure 2. Zero-order spectra **(a)** and their first-order spectra **(b)** of allopurinol in the concentration range of 4.0-36.0 µg/mL

efficients was observed for the application of zero-order spectrophotometry and first derivative method to the analysis of allopurinol in the investigation concentration range of 4.0-36.0 µg/mL (see Table 1). The correlation coefficients (r) were 0.9996 and 0.9998 indicating good linearity, respectively. For each method, limit of detection (LOD) and limit of quantitation (LOQ) were computed by using the standard deviation of y-intercept of the calibration curve. The LOD and LOQ values were listed in Table 1. In order to estimate the accuracy and precision of the proposed zero-order spectra and first derivative methods, an independent sample set containing allopurinol was prepared and analyzed by using two proposed methods. From the analysis results, recovery values, standard deviation and relative standard deviation were calculated for the estimation accuracy and precision for zero-order spectrophotometric and first derivative methods. Their results were presented in Table 2. For both methods, relative standard devia-

Table 2. Recovery results of the test samples by the proposed methods

Sample		*Zero-order spectra*		*First derivative*	
Run	*Added*	*Found*	*Recovery*	*Found*	*Recovery*
1	4	3.91	97.8	4.10	102.4
2	12	12.43	103.6	12.18	101.5
3	20	19.53	97.6	20.07	100.3
4	28	28.09	100.3	28.38	101.4
5	36	35.01	97.2	35.84	99.6
		Mean	99.3		101.0
		SD	2.67		1.10
		RSD	2.68		1.09

SD = Standard deviation
RSD= relative standard deviation

Table 3. Recovery results of allopurinol in standard addition samples by zero-order spectrophotometry and first order spectrophotometry

		Zero-order spectrophotometry		*First order spectrophotometry*	
Sample no.	*Added (µg/mL)*	*Found (µg/mL)*	*Recover (%)*	*Found (µg/mL)*	*Recover (%)*
1	4	4.01	100.2	4.07	101.7
2	8	8.11	101.3	8.17	102.1
3	12	12.51	104.2	12.1	100.8
		Mean	101.9		101.6
		SD	2.1		0.69
		RSD	2.06		0.67

SD = Standard deviation
RSD= relative standard deviation

tions between five different samples were 2.68 % and 1.09 %, indicating method repeatability or precision, respectively.

In order to check the effect of tablet excipients on the determination of allopurinol, standard addition samples were analyzed. Added recovery, standard deviation and relative standard deviation were calculated. Numerical values of the analysis results were shown in Table 3. According the analysis results of standard addition samples, no interference of tablet excipients was reported. Stability problem of the samples and standards was not observed during analysis.

3.2. Method Application to Tablet Analysis

As described above, direct absorbance measurement and derivative methods were used for the quantitative analysis of allopurinol in tablets. Determination results of allopurinol in tablets were presented in Table 4. From this table, a good agreement was reported for the implementation of zero-order spectrophotometry and first derivative method in the determination of allopurinol in commercial tablets.

Table 4. Tablet results for allopurinol in tablets by zero-order spectrophotometry and first order spectrophotometry

	mg/tablet	
Sample number	***Zero-order spectrophotometry***	***First order spectrophotometry***
1	295.9	301.8
2	302.2	301.0
3	311.7	296.5
4	295.8	303.9
5	305.0	302.0
Mean	302.1	301.0
SD	6.68	2.75
RSD	2.21	0.91

SD = Standard deviation
RSD = relative standard deviation

4. Conclusions

In analyses, comparable results were generally obtained by applying both zero-order spectrophotometry and first derivative method to the analysis of commercial tablets. However, as can be seen from Table 4 for the application of the methods to the analysis of commercial tablets, first derivative approach was more precise and accurate than the zero-order spectrophotometric procedure. The proposed methods are simple, rapid and reliable for the quantification of allopurinol in tablets compared to previously reported methods. No interference was observed from the tablets excipients which indicate the specificity and selectivity of methods. It was concluded that the analytical methods were specific, precise, linear, and accurate for the analysis of active compound. It was observed that the proposed methods can be used for the quantitative estimation and reliable routine analysis of pharmaceuticals containing allopurinol drug.

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