

Second and Fourth Derivative Spectrophotometric Determination of Terbutaline Sulfate and Glyceryl Guaiacolate in Expectorant Syrup

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Ekspektoran Şurupta Terbutalin Sülfat ve Gliseril Gayakolatın, İkinci ve Dördüncü Türev Spektrofotometrik Tayini

Özet: Bu çalışmada ekspektoran bir şurupta terbutalin sülfat ve gliseril gayakolatın tayini için türev spektroskopisi (D_2 ve D_4) yöntemi kullanılmıştır. Sonuçlar, bu iki maddenin sıfırıncı türev spektroskopik değerleri ile karşılaştırılmıştır. Bu yöntemler arasındaki fark, uygulanan student's t testi ile çok anlamlı bulunmuştur ($p < 0.05$). Uygun dalga boyunda, D_2 ve D_4 türev absorbans değerleri, terbutalin sülfat için 5.0-60.0 $\mu\text{g/ml}$ ve gliseril gayakolat için 5.0-120.0 $\mu\text{g/ml}$ aralıklarında, konsantrasyonla lineer bir ilişki göstermiştir. Terbutalin sülfat asidik ortamda kloroformla ekstraksiyondan sonra 0.1 N HCl çözeltisinde, gliseril gayakolat ise direkt olarak 0.1N NaOH çözeltisinde tayin edilmiştir. Ticari şurupta terbutalin sülfatın 2. ve 4. türev spektroskopik yöntemlerle tayininde varyasyon katsayıları sırası ile % 2 ve % 2, gliseril gayakolat için bu değerler % 1, % 1 bulunmuştur.

Anahtar Sözcükler : Terbutalin sülfat, Gliseril gayakolat, Sıfırıncı, İkinci ve Dördüncü Türev Spektroskopisi, Şurupta miktar tayini.

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Summary: Second (D_2) and fourth (D_4) derivative spectroscopic methods were used for the determination of terbutaline sulfate and glyceryl guaiacolate in expectorant syrup and the results were compared with their zero derivative spectroscopic values. The significance test for these methods was made with student's t test. The values obtained gave significantly different results ($p < 0.05$). The D_2 and D_4 derivative absorbance values were linearly correlated with concentration in the tested ranges, 5.0-60.0 $\mu\text{g/ml}$ for terbutaline sulfate and 5.0-120.0 $\mu\text{g/ml}$ for glyceryl guaiacolate at appropriate wavelengths. Terbutaline sulfate was determined in 0.1N HCl solution after extracting in acidic medium with chloroform, while glyceryl guaiacolate was directly determined in 0.1N sodium hydroxide solution. Coefficient of variations for the assay of terbutaline sulfate in commercial syrup by second and fourth derivative spectroscopic methods were 2 % and 2 % respectively. The corresponding values for glyceryl guaiacolate were 1 %, and 1 %.

Keywords : Terbutaline sulfate, Glyceryl guaiacolate, Zero, Second, and Fourth Derivative Spectroscopy, Determination in syrup.

Introduction

Terbutaline sulphate I (1[3,5-dihydroxyphenyl]-2-tert-butyl-aminoethanol sulfate) (Fig: 1) is extensively used for the treatment of bronchial asthma and chronic obstructive diseases for its receptor effects. It can be administered individually by injection, inhalation or orally as tablet. Guaiphenesin

II (glycerylguaiacolate), [1,2-Propanediol, 3-(2-methoxy)] (Fig: 1) has expectorant effect^{1,2} and is used in combination with I in an expectorant syrup formulation.

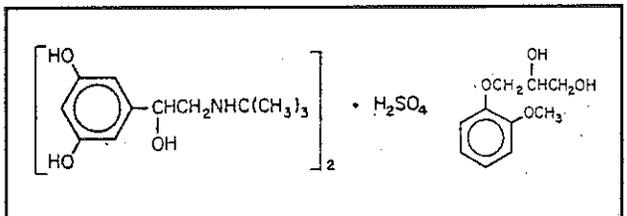


Fig: 1 - Chemical structure of terbutaline sulfate and glyceryl guaiacolate.

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There are very few methods reported for determination of these drugs in a mixture³. Several methods were proposed for the assay of I and II individually in pharmaceutical preparations, such as spectrophotometry⁴⁻¹⁷, thin layer chromatography¹⁸, high performance liquid chromatography (HPLC)¹⁹ for I and gas chromatography^{20,21} HPLC²²⁻²⁵, spectrophotometry²⁵⁻²⁸ for II.

Conventional ultraviolet spectrophotometric methods for the assay of drugs in pharmaceutical formulations are subject to background irrelevant absorption due to formulation excipients in high ratios.

The technique of derivative spectrophotometry has been reported to be useful in eliminating matrix interference in the assay of many active principals in pharmaceutical dosage forms.

This paper describes a sensitive, accurate and simple derivative spectrometric method for the determination of terbutalin sulfate and glyceryl guaiacolate in a syrup, based on the measurement of second and fourth derivative absorbances of samples prepared by dilution with 0.1N NaOH or by a simple solvent extraction procedure.

Experimental

Materials

Standart terbutaline sulfate and glyceryl guaiacolate were obtained from Refik Saydam Central Institute of Hygiene, Ankara. Commercial syrup preparations having 30 mg terbutaline sulfate, 1330 mg glyceryl guaiacolate, sodyum benzoate as a preservative agent and the other basic syrup ingredients per 100 ml were purchased from the Turkish market.

Instrument

Zero-order (D_0) and corresponding second (D_2) and fourth (D_4) derivative spectra were recorded in 1 cm quartz cells in the wavelength range 350-200 nm using a Shimadzu UV-160 A spectrophotometer with 2 nm spectral slit width. The D_2 values of terbutaline sulfate and glyceryl guaiacolate were measured at 275 nm and 273 nm respectively and their D_2 ($d^2 A/d\lambda^2$) and D_4 ($d^4 A/d\lambda^4$) values were measured

at 281.4 nm, 277.4 nm ($\Delta\lambda$: 4.2 nm, $\Delta\lambda$: 3.6 nm, N: 6) and 281 nm, 277 nm ($\Delta\lambda$: 4.2 nm, $\Delta\lambda$: 3.6, N: 6) respectively.

Solutions

Stock terbutaline sulfate standart solution: Dissolve 100 mg. terbutaline sulfate in 0.1N HCl solution and dilute to 100 ml with 0.1N HCl.

Stock glyceryl guaiacolate standard solution: dissolve 100 mg glyceryl guaiacolate in 0.1N sodium hydroxide solution and dilute to 100 ml with the same solution.

Sample preparation:

Sample solution for terbutaline sulfate determination: Transfer accurately 5 ml aliquot of syrup to a separatory funnel. Acidify with 0.5 ml 1N HCl and extract successively with five 10 ml portions of chloroform. Reject the chloroform extracts. Transfer the acidic aqueous phase into the 50 ml volumetric flask and complete to volume with 0.1N HCl.

Sample solution for glyceryl guaiacolate: Transfer 2.5 ml aliquot of syrup into 50 ml volumetric flask and complete to volume with 0.1N NaOH. From this solution pipette 5 ml into a 100 ml volumetric flask, and complete to volume with 0.1N NaOH.

Methods

Calibration curve of terbutaline sulfate standard solution: Six dilutions were prepared from freshly prepared terbutaline sulfate stock solution in the concentration range 5.0-60.0 $\mu\text{g/ml}$ and second and fourth derivative absorbances of each solution were measured at 281.4, 277.4 nm respectively.

Accuracy of the method for terbutaline sulfate: Nine replicates of sample solution prepared as described above were spiked with 0.75, 1.0, 1.5 ml of a standart terbutaline sulfate solution at 1 mg/ml concentration and zero-order and D_2 , D_4 values were measured.

Precision of the method: The zero-order, D_2 and D_4 values of fourteen replicates of sample solutions prepared from five separate batches of commercial syrup were measured.

Accuracy for glyceryl guaiacolate: Nine replicates of sample solution prepared as described above were spiked with 1 ml, 2 ml, and 3 ml of a standard glycreyl guaiacolate solution at 1.66 mg/ml concentration and D_2 and D_4 values were measured.

Precision of the method: The zero, D_2 and D_4 values of twelve replicates of the sample solutions prepared from four separate batches of commercial syrup were measured.

Synthetic mixtures: Mixture of both drugs as in ratio in commercial syrup were prepared and the methods for determination of the active substances in syrup were applied.

Results and Discussion

The zero order absorption spectra of the solution of terbutaline sulfate and glyceryl guaiacolate in 0.1N HCl give maxima at 276 and 273 nm respectively (Fig. 2) and interfere significantly. Although application of derivative technique may prove to be useful in simultaneous determination of such drugs, since glyceryl guaiacolate was present in more than 40- fold excess over terbutaline sulfate in the syrup, direct derivative spectroscopic determination of the latter in acidic solution was not possible. On the other hand, the UV spectrum of terbutaline sulfate shows a bathochromic shift in alkaline media to 290 nm due to its phenolic structure and degrades in 2 hours to give zero absorbance. The degradation of terbutaline in alkaline solution is due to rapid oxidation by dissolved oxygen. Thus

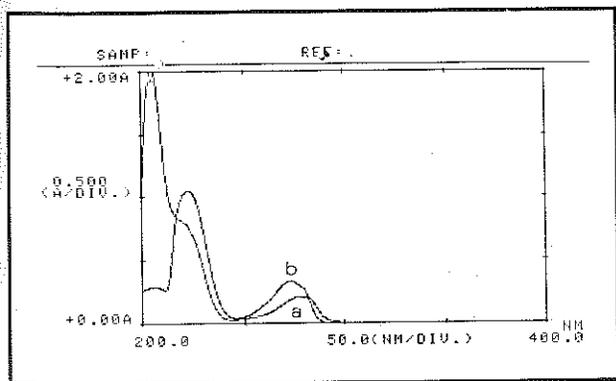


Fig. 2 - Zero-order UV spectra of terbutaline sulfate solution in 0.1N HCl 10 µg/ml (a), glyceryl guaiacolate solution in 0.1N HCl 10 µg/ml (b).

terbutaline sulfate was assayed after the syrup had been extracted with chloroform in acidic media. Chloroform rather than ether has been used or extraction of glyceryl guaiacolate from syrup, due to the superior solubility of this solute in chloroform. For this purpose, the sample solution obtained was subjected to zero-order, second and fourth derivative spectrophotometric analysis. Comparison of the results in Table-1 shows that better recoveries are obtained by second and fourth derivative techniques, which reveals the elimination of non-specific absorbances (Fig.: 3A) by derivation. On the other hand glyceryl guaiacolate was assayed in the same manner directly in alkaline media (0.1N NaOH) where interference from terbutaline sulfate could be eliminated easily due to bathochromic shift observed in its UV spectra and its rapid degradation. The assay results (Table-1) supported the above mentioned claim.

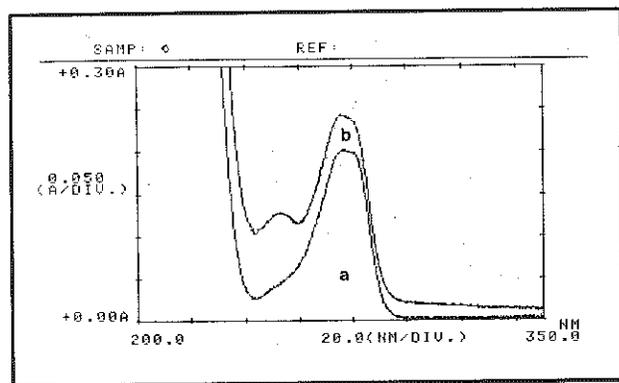


Fig. 3 - Zero-order UV spectra of terbutaline sulfate standard solution in 0.1N HCl 30 µg/ml (a), sample solution prepared from commercial syrup for terbutaline sulfate in 0.1N HCl, 30 µg/ml (b).

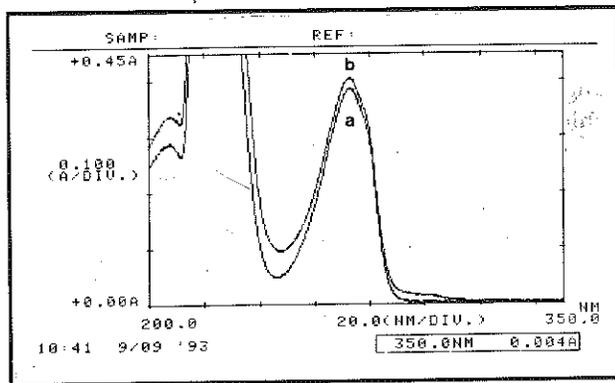


Fig. 4 - Zero-order UV spectra of glyceryl guaiacolate standard solution in 0.1N NaOH 33.25 µg/ml (a), sample solution prepared from commercial syrup for glyceryl guaiacolate in 0.1N NaOH 33.25 µg/ml (b).

Table - I: Assay results of terbutaline sulfate and glyceryl guaiacolate in syrup

Syrups	Recovery % (mean \pm C.V.*%)							
	Assays (n)	Terbutaline sulfate			Assays (n)	Glyceryl guaiacolate		
		D ₀	D ₂	D ₄		D ₀	D ₂	D ₄
Batch No: 1	3	116 \pm 1	99 \pm 0.3	99 \pm 1	2	106 \pm 0.4	100 \pm 1	100 \pm 1
Batch No: 2	2	112 \pm 2	95 \pm 0.4	94 \pm 0.4	4	105 \pm 0.5	100 \pm 0.6	100 \pm 0.5
Batch No: 3	3	112 \pm 3	100 \pm 1	99 \pm 2	4	106 \pm 1	101 \pm 1	101 \pm 1
Batch No: 4	3	114 \pm 2	100 \pm 1	99 \pm 2	2	107 \pm 0.2	101 \pm 0.4	101 \pm 1
Batch No: 5	3	112 \pm 2	97 \pm 2	99 \pm 0.5	-	-	-	-
Total Mean:	-	113 \pm 2	98 \pm 2	98 \pm 2	-	106 \pm 1	101 \pm 1	101 \pm 1
t (Do-D2):	18.3	** (DF: 26)	p<0.05		16.0	(DF: 22)		p<0.05
t Do-D4):	18.0	(DF: 26)	p<0.05		14.0	(DF: 22)		p<0.05

* C.V.: Coefficient variations

** DF: Degree of freedom

The derivative absorbance values of both compounds were measured at wavelengths corresponding to their max. values, 231.4 nm (D₂) and 277.4 nm (D₄) for terbutalin sulfate and 231 nm (D₂) and 277 nm (D₄) for glyceryl guaiacolate (Fig. 5, 6).

Linearity: The second and fourth derivative absorbance values of both compounds were linearly related with concentration in the concentration ranges stated in the experimental part, with correlation coefficients 0.9999 for terbutaline sulfate (D₂, D₄) and 1.000 and 0.9993 for glyceryl guaiacolate (D₂, D₄) (Table-2).

Accuracy, precision and specificity: The accuracy of the method was tested by standard addition principle and assaying of the synthetic mixtures of two drugs. The recoveries for added drugs to syrup sample (in quantities presented in Table-3) were in the range 99.0%-100.0% for both D₂ and D₄ of two drugs, which indicates satisfactory accuracy, also showing the elimination of interference from the syrup excipients which specifies the method. The results for the synthetic mixtures were in the range 100.0%-101.0% for D₂ and D₄ of the terbutaline sulfate and in the range 99.0%-101.0% for D₂ and D₄ of glyceryl guaiacolate (Table-4).

Quantitation based on zero-order absorbances of sample solutions prepared from synthetic mixtures yielded high results. The mean was as high as 109

% (Table-4). It was thought that the high result could arise from interaction between two active sub-

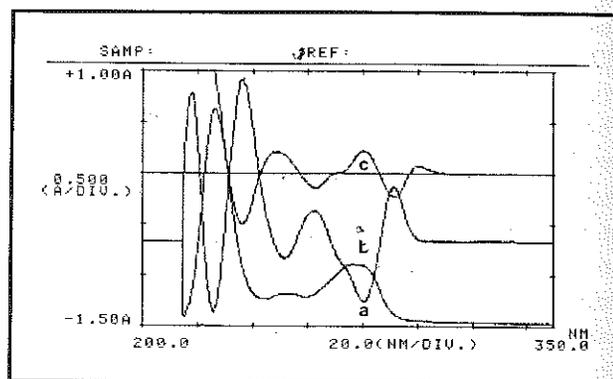


Fig: 5 - Zero-order (a), second-order (b), fourth-order (c) derivative UV spectra of sample solution prepared from commercial syrup for terbutaline sulfate in 0.1N HCl, 30 µg/ml.

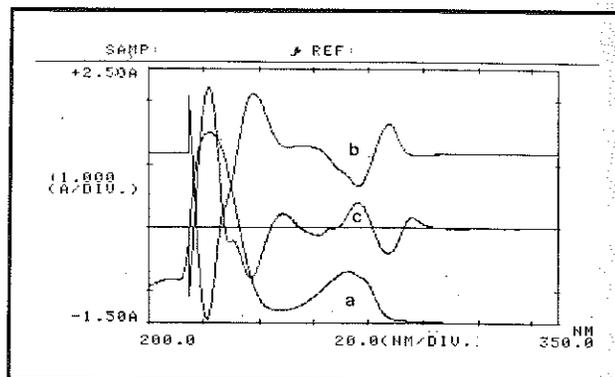


Fig: 6 - Zero-order (a), second-order (b), fourth-order (c) derivative UV spectra of sample solution prepared from commercial syrup for glyceryl guaiacolate in 0.1N NaOH, 33.25 µg/ml.

Table - 2: Regression analysis of calibration curves

	Terbutaline sulfate		Glyceryl guaiacolate	
	D ₂	D ₄	D ₂	D ₄
Regression equation	$Y = 6,95.10^{-3}X + 2,75.10^{-3}$	$Y = 7,3.10^{-3}X + 3,46.10^{-3}$	$Y = 1,07.10^{-2}X + 8,58.10^{-3}$	$Y = 1,13.10^{-2}X + 1,25.10^{-2}$
Correlation Coefficient	0.9999	0.9999	1.000	0.9998

Where X: Concentration (µg/ml) and Y: Derivative absorbance, n: 6 for terbutaline sulfate, n: 8 for glyceryl guaiacolate, level of significance $p < 0.05$

Table - 3 : Recovery of terbutaline sulfate and glyceryl guaiacolate added to commercial syrup

Recovery % (mean±C.V.*%)											
Terbutaline sulfate						Glyceryl guaiacolate					
		D ₂		D ₄				D ₂		D ₄	
Added (mg)	Assays (n)	Found	Recovery	Found	Recovery	Added (mg)	Assays (n)	Found	Recovery	Found	Recovery
0.750	3	0.748	100±3	0.720	96±1	1.66	3	1.657	100±2	1.653	100±1
1.000	3	1.007	101±1	1.018	102±0.6	3.32	3	3.315	100±0.6	3.279	99±1
1.500	3	1.437	99.1±0.1	1.465	98±2	4.98	3	4.971	100±0.3	4.910	99±0.6
total mean		100±1		99±1				100±1		99±0.9	
± C.V.% :											

*C.V = Coefficient variations

Table-4 : Recovery results of terbutaline sulfate and glyceryl guaiacolate in synthetic mixtures

% (mean ± CV*%)									
	Assays (n)	Terbutaline sulfate			Assays (n)	Glyceryl guaiacolate			
		D ₀	D ₂	D ₄		D ₀	D ₂	D ₄	
Synthetic mixtures	9	108±2	100±2	101±2	7	100±0.4	99±0.8	101±1	
t(D ₀ -D ₂)	:	7.3,	** (DF:7),	p<0.05					
t(D ₀ -D ₄)	:	7.0,	(DF:7),	p<0.05					

* CV : Coefficient variations

** DF : Degree of freedom

stances during extraction by chloroform. Since, at the end of the extraction, thin layer chromatography was applied on the aqueous phase to check whether glyceryl guaiacolate had been exhausted by chloroform. But, this drug could not be detected. Additionally, a solution containing only glyceryl guaiacolate was studied by the proposed method and did not give glyceryl guaiacolate specific peaks in U.V. spectrophotometry.

Zero-order mean value of sample solutions prepared from syrup was also as high as 113%. It was thought that this, even higher mean result value compared to synthetic mixtures demonstrate interferences of the syrup excipients.

Zero-order values of the sample solutions prepared from synthetic mixtures for glyceryl guaiacolate were not high as seen in Table-4. Therefore the

zero order values, as high as 6% of the sample solutions prepared from syrup for glyceryl guaiacolate, also indicate the presence of interference of absorbing syrup excipients (Table-1).

The precision test was performed at 30.00 µg/ml concentration for terbutaline sulphate and at 33.25 µg/ml for glyceryl guaiacolate, the coefficient variations being 2% (D₂), 2% (D₄) and 1% (D₂), 1% (D₄), respectively.

The assay results obtained by applying second and fourth derivative technique to commercial syrup, gave satisfactory recoveries (Table-1). The significance test for zero-order and derivative (D₂ and D₄) methods was made with student's t test (Table-1A). The low probability values (p<0.05), showed that these methods give significantly different results, which support use of derivative technique as method of choice.

Conclusion

According to our experimental results, the second and fourth derivative spectroscopic methods are satisfactory with respect to precision and accuracy. Both are applicable to the determination of terbutaline sulfate and glyceryl guaiacolate in syrup with coefficient variation (CV) 2% (D₂), 2% (D₄) and 1% (D₂), 1% (D₄), respectively.

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