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ASKORBİK ASİT VE RUTİN'İN BİRİNCİ TÜREV UV – SPEKTROFOTOMETRİSİ İLE BİRARADA MİKTAR TAYİNİ

THE SIMULTANEOUS DETERMINATION OF ASCORBIC ACID AND RUTIN BY FIRST - DERIVATIVE UV - SPECTROPHOTOMETRY

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ÖZET

Askorbik asit ve Rutin içeren tabletlerde ilaç etken maddeleri türev spektrofotometrisi ile hiçbir belirteç kullanılmadan ve herhangi bir ayırma işlemi uygulanmadan tayin edildi.

Askorbik asit ve Rutin içeren Rutinal C tabletlerde Askorbik asit ve Rutin metanol ile ekstraksiyon, süzme, uygun seyreltme ve sırasıyla 258.8 ve 337.4 nm 'deki birinci türev absorbans değerlerinin ölçülmesiyle tayin edildi.

Türev spektrofotometrisi ile bulunan sonuçların kıyaslanması amacıyla aynı tabletler, USP XXI'de Askorbik asit tabletler için önerilen yöntemle analiz edildi. Rutin miktar tayini için farmakopelerde bir yöntem önerilmediğinden Rutin miktarı 358,4 nm'deki absorbans değeri ölçülerek spektrofotometrik olarak saptandı.

Elde edilen bulgular doğruluk ve presizyon yönünden t ve F testleri yardımıyla % 95 güvenirlik düzeyinde istatistiksel olarak karşılaştırıldı. Ortalamalar ve presizyonlar arasında anlamlı fark olmadığı görüldü.

Anahtar kelimeler : Askorbik asit, rutin, türev spektrofotometrik yöntem.

SUMMARY

The active ingredients in ascorbic acid and rutin containing pharmaceutical tablets have been determined by derivative spectrophometry without requiring any reagents or preliminary separations.

Ascorbic acid and rutin have been extracted with methanol from Rutinal C tablets, filtered off, diluted to appropriate volume, and quantitatively estimated by measuring their first derivative absorbance values, $dA/d\lambda$ at 258.8 and 337.4 nm, respectively.

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With the purpose of comparison of the results obtained by derivative spectrophotometry, the same tablets were analyzed for ascorbic acid by the method recommended in USP XXI. Since a suitable method for rutin was not available in the pharmacopoeias, rutin was estimated by direct UV - spectrophotometry utilizing the absorbance at 358.4 nm.

The obtained results were statistically compared in respect to accuracy and precision by the aid of t and F tests on the basis of 95 % confidence level. There were no significant differences between the corresponding means and precisions.

Key words : Ascorbic acid, rutin, derivative spectrophotometry

INTRODUCTION

Rutin and ascorbic acid are together used in some commercial preparations because rutin has been synergistic with ascorbic acid. Determination of the drugs in these preparations is not possible by simultaneous UV spectrophotometry due to the spectral overlap. Some time consuming methods have been used such as orthogonal function spectrophotometric (1). dual wavelength spectrophotometric (2), and extractive separation followed by dual wavelength spectrophotometric (3) methods have been employed. Dual wavelength spectrophotometry has also been used in the analysis of ascorbic acid - rutin - procain HC1 containing suppositories (4).

Compared with conventional spectrophotometric determinations, derivative spectrophotometry (5-7) has proved to be a great value in eliminating the interference from excipients and coformulated drugs (8-10). The derivative spectrophotometry represents an elegant approach to the problem of resolving spectral overlap in pharmaceutical analysis. It has been succesfully used for the analysis of pharmaceutical dosage forms containing several drugs alone or in mixtures (11-15).

In this work aiming to apply derivative spectrohotometry to the analysis of binary pharmaceutical mixtures, a quantitative method for the individual determination of ascorbic acid and rutin in mixtures has been developed and applied to pharmaceutical tablets in the country.

EXPERIMENTAL

Equipment

A Philips 8700 UV -Vis spectrophotometer equipped with Hellma, 100 -QS, quarts cuvettes of dimensions 10.10.45 nm has been used in the photometric measurements.

Chemicals

Pharmaceutical purity grade ascorbic acid and rutin were supplied from Dilmen Laboratories.

Distilled technical methanol was used in the preparation of solutions.

Stock solutions : Methanolic solutions of ascorbic acid and rutin were prepared in 1 mg. ml^{-1} concentration.

Standart solution : 40 μg ml^{-1} solutions were prepared by appropriate dilution with methanol from the stock solutions.

3% w/V metaphosphoric acid - 8% V/V acetic acid solution (HPO_3 - AcOH).

2,6 - dichlorophenol - indophenol solution (2,6 DCPIP)

0.25 g. 1^{-1} of 2,6 -DCPIP (sodium salt) and 0.21 g. 1^{-1} sodium hydrogen carbonate.

Standard ascorbic acid solution : 1mg ml^{-1} in HPO_3 - AcOH solution for indophenol titration.

Establishing the calibration curve : The first - derivative absorption spectra of ascorbic acid and rutin standard solutions within the 4.0 - 20.0 μg. ml^{-1} concentration range in the wavelength interval of 190 - 425 nm using 2 nm bandwidths at a scanning speed of 500 nm. min-1were recorded. The first derivative absorbance values (^1D) at 258.8 nm for ascorbic acid, and at 337.4 nm for rutin were correlated to the corresponding substance concentrations via calibration curves. The linear equations of the calibration curves were computed by the leastsquares approximation.

Recommended procedure for analysing pharmaceutical tablets

Twenty pharmaceutical tablets were accurately weighed and ground together. An aliquot corresponding to 50 mg ascorbic acid of the fine grained material was transferred to a 50 ml flask. 30 ml CH_3OH was added, agitated for 10 min, and diluted to volume with methanol. The well - agitated mixture was filtered off, and the first 15 ml of the filtrate was discarded. The next 5 ml was taken and diluted with methanol to

volume in a 100 ml volumetric flask. A suitable aliquot of this final solution was diluted to a concentration range between 4.0 - 20.0 μg. ml^{-1} of ascorbic acid and rutin, and the first derivative absorbance values (^1D) against CH_3OH were recorded at 258.8 nm for ascorbic acid, and at 337.4 nm for rutin. The active ingradient contents of the tablets were determined from the ^1D - values using the corresponding calibration curves.

Determination of ascorbic acid using USP XXI method (Titration with 2,6 - DCPIP solution)

The 2,6 -DCPIP solution was standardised by titration with 2.0 ml standard ascorbic acid solution and 5.0 ml HPO_3-AcOH solution to the end - point (a persistent rosy pink colour). The consumption of the blank was determined by titrating 2,6 -DCPIP solution with 7.0 ml of HPO_3-AcOH solution plus a given amount of water equivalent to the volume of 2,6 -DCPIP solution used in the previous standardisation titration.

For the sample titration, a volume of the filtrate equivalent to about 2 mg of ascorbic acid, was transferred to a 50 ml conical flask and was then titrated with 2,6 - DCPIP solution using the same procedure as described above including the titration of the blank.

RESULTS AND DISCUSSION

As can be seen from the absorption spectra of ascorbic acid and rutin in Fig. 1, rutin can be individually determined spectrophotometrically in the presence of ascorbic acid by the use of its 358.4 nm absorption peak without any interference of the latter. On the other hand, ascorbic acid may not be estimated in the same mixture by a similar procedure due to the interferent absorption peak of rutin.

From the simultaneous first - derivative spectra of the two substances shown in Fig. 2, rutin's first - derivative absorbance at 258.8 nm may be observed to be zore. Thus ascorbic acid content of the mixture was determined by measuring the first - derivative absorbance at this wavelenght, i. e., $^1D_{258.8}$ without any effect of rutin. On the other hand the value at 337.4 nm of the same spectrum, i. e., $^1D_{337.4}$, was utilized for rutin assay.

The standard calibration curve for ascorbic acid is drawn as $^1D_{258.8}$ values vs. ascorbic acid concentration in the range 4.0 - 20.0 μg. ml^{-1} and the regression equation of the curve are given in Fig. 3. Likewise, the cal

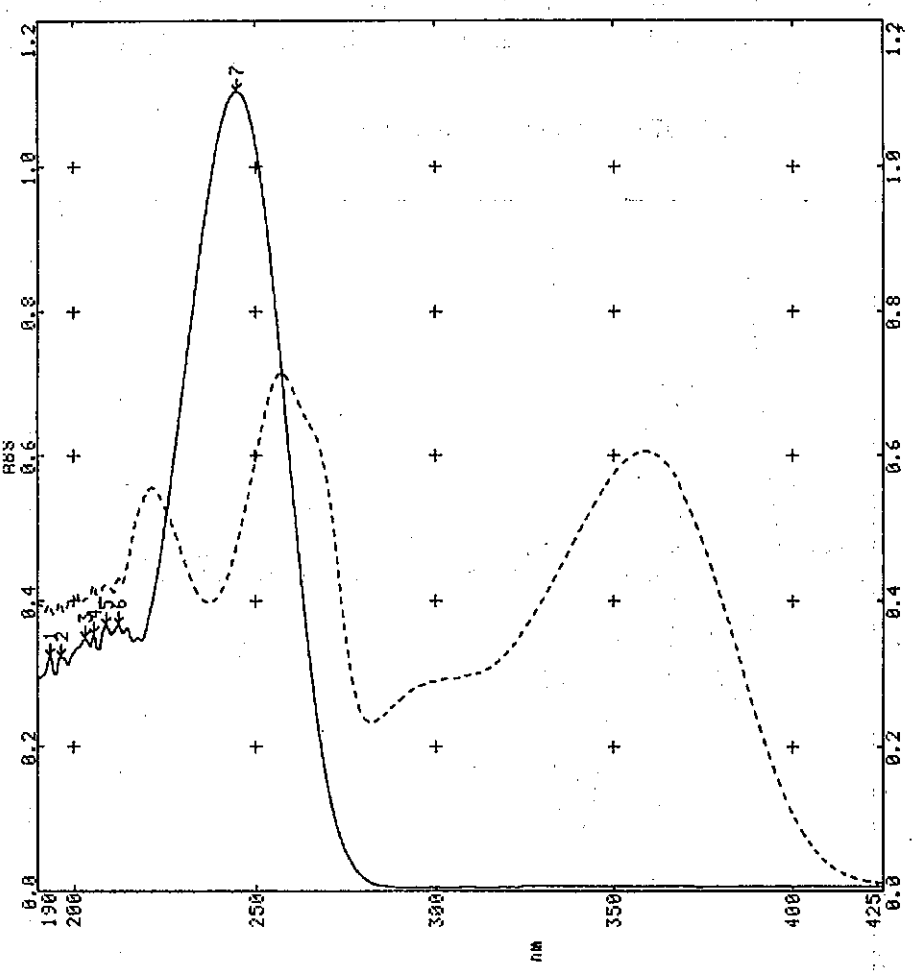


Fig. 1 : The absorption spectra of ascorbic acid (————) and rutin (-------) in μg. ml $^{-1}$ methanolic solutions.

ibration curve and the regression equation for rutin as $^1D_{337.4}$ vs. rutin concentration in the range of 4.0 - 20.0 μg. ml^{-1} are shown in Fig. 4.

For statistically comparing the derivative - spectrophotometric assay results of pharmaceutical tablets containing the above ingredients, the same tablets were assayed by USP XXI recommended procedure (16) for ascorbic acid. Due to the absence of a suitable procedure for rutin assay in pharmacopoeias, BP , USP the rutin content of the tablets were estimted by measuring the absorbance at 358.4 nm, and correlating this

value to the calibration curve between A358.4 and rutin concentration (C) in the range of 4.0 - 20.0 μg. ml^{-1}, which is expressed by thel inear equation;

$$A_{358.4} = 0.0307\ C + 0.0006\ (r = 0.9999)$$

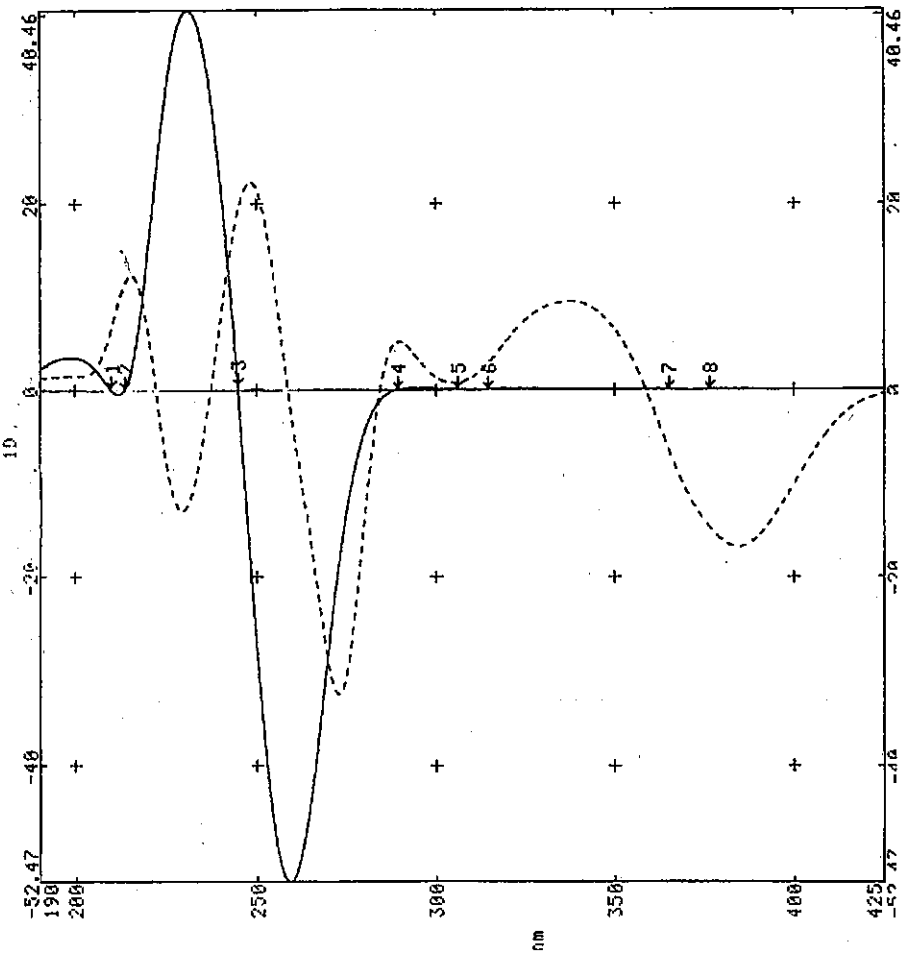
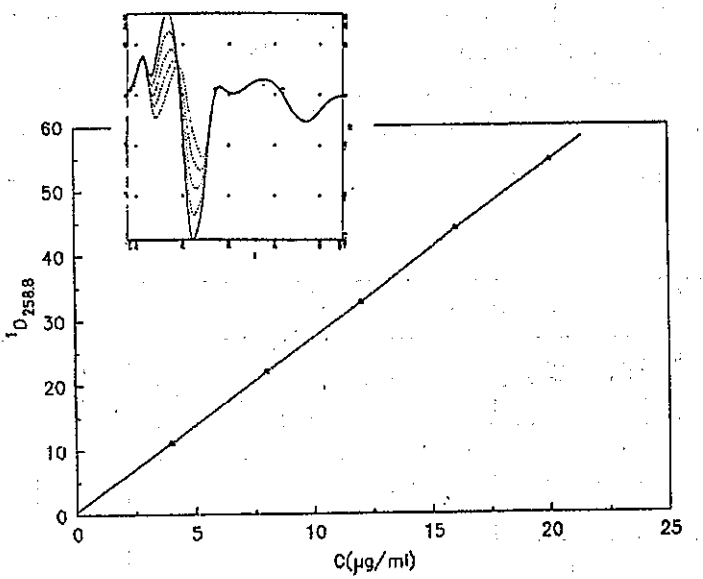
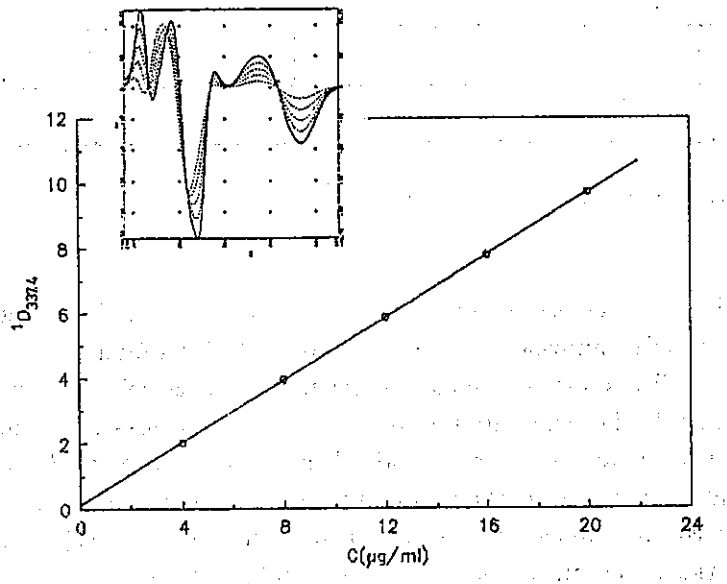


Fig. 2 : The first derivative spectra of ascorbic acid (———) and rutin (------) in 20 μg. ml^{-1} methanolic solutions.



$[^1D_{258.8} = 2.578C - 0.072 \ (r=0.9999)]$

Fig. 3 : The standard calibration curve for ascorbic acid assay and the regression equation of 1D258.8 as a function of ascorbic acid concentration, C.



$[^1D_{337.4} = 0.481C + 0.106 \ (r=0.9999)]$

Fig. 4 : The standard calibration curve for rutin assay and the regression equation of 1D337.4 as a function of rutin concentration, C.

These statistical comparisons are depicted in Table 1. Table 1 contains information of the mean (X), standard deviartion (S), relative standard deviation (RSD, %) and the result range on the basis of 95 % confidence level ($X \pm t * s / \sqrt{n}$), derived from 5 replicate determinations.

Table 1 : The assay results of Rutinal – C [*]

Statistical parameters	ASCORBIC ACID				RUTIN			
	Derivative spectrophotometric method		USP method		Derivative spectrophotometric method		Absorption spectrophotometric method	
	mg/tablet	%	mg/tablet	%	mg/tablet	%	mg/tablet	%
$\bar{X}$	97.4	97.4	97.7	97.7	102.1	102.1	101.1	101.1
S	0.85		1.48		0.94		1.14	
% S	0.87		1.52		0.92		1.13	
$\bar{X}+t.s/\sqrt{n}$	96.4-98.4		96.0-99.4		101.0-103.2		99.8-102.4	
t test	0.35				1.35			
F test	3.03				1.47			
$n_1=n_2=5$ P=0.05	t_{table}: 2.31				F_{table}: 6.39			

[*] Each tablet contains 100 mg ascorbic acid and 100 mg rutin .

The results obtained by the developed method and by the standard procedures of comparison were compared on 95% confidence level by the aid of t and F tests for means and precisions, respectively. As can be seen from Table 1, the calculated t and F values were less than the corresponding ones obtanied from standard tables for the selected confidence level and population number. Thus there were no significant differences between the developed and standard methods for the assay of ascorbic acid - rutin tablets in respect to accuracy and precision.

The developed derivative - spectrophotometric method may be recommended for routine laboratory analyses in view of its speed. simplicity, sensitivity and reproducibility.

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