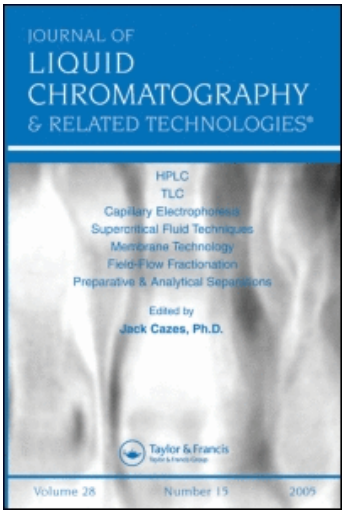

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Liquid Chromatographic Determination of Rutin and Ascorbic Acid-Binary Mixture in Pharmaceutical Preparations

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LIQUID CHROMATOGRAPHIC DETERMINATION OF RUTIN AND ASCORBIC ACID-BINARY MIXTURE IN PHARMACEUTICAL PREPARATIONS

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Abstract:

A liquid chromatographic procedure for the separation and determination of rutin and ascorbic acid in oral dosage forms is described. The dosage content of tablets and soft gelatin capsules is diluted and chromatographed on a Lichrosorb (C_{18}) column with a mobile phase of water-methanol (55-45 V/V) and detection at 250 nm. Phenacetin was used as internal standard. The calibration is linear with correlation coefficient of 0.9999 for each component. Recoveries of spicked excipients averaged 99.5% and 99.2% for rutin and 100.2% and 99.8% for ascorbic acid in tablets and soft gelatin capsules, respectively. The method met USP XXII requirements for system suitability with proper resolution between two adjacent peaks. The relative standard deviation (RSD) of peak response of each component (obtained by chromatographing five replicates of standard solution) is less than 2% and tailing factor of each component is not greater than 1.5. The method can be used for composite and content uniformity assay of rutin and ascorbic acid in tablets and soft gelatin capsules.

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Introduction

Rutin and ascorbic acid combination dosage forms are available as tablets and soft gelatin capsules. These dosage forms are used for treatment of disease states characterized by capillary bleeding associated with increased capillary fragility. The binary mixture is not a compendial one. Different spectrophotometric techniques were applied for the analysis of such mixture. Orthogonal function [1] and dual-wavelength techniques [2] were used. There was no HPLC method reported for the simultaneous determination for rutin and ascorbic acid in their dosage forms.

This paper describes a method for simultaneous quantitation of rutin and ascorbic acid in oral dosage forms. the method is used for the composite and content uniformity assay of these products. The method was validated according to the USP XXII system suitability criteria [3]. The HPLC method was simple, rapid and accurate than the spectrophotometric methods.

Experimental

Chemicals and Reagents:

Methanol and water were HPLC grade. Rutin, ascorbic acid and phenacetin were kindly supplied by Pharco Pharm. Co. (Alexandria, Egypt) and used without further purification, their purity was certified to be 99.8%, 99.5% and 99.5%,respectively.

Apparatus :

Hewlett-Packard high performance liquid chromatograph model HP 1090 equipped with UV diode-array detector, binary DR 5 solvent delivery system and interfaced with HP/85-B personal computer. The peak areas integrations were performed using HP 3392A integrator. The samples were injected automatically using an auto-in-

jection system. A 250 X 4.6 mm ID, reverse phase column (Lichrosorb RP-18, E.Merk, Darmstadt, Germany), packed with 10 um particles, was used.

Chromatographic Conditions :

The mobile phase was prepared by mixing methanol and water (pH ca 3.0) in ratio of 55:45, respectively. The ratio of the solvent in mobile phase may be adjusted to achieve optimum resolution between major peaks. The mobile phase was vacuum filtered and deaerated by ultrasonication before use.

With all components of the system in place, the mobile phase was passed through the column at a flow of 1 ml min^{-1}. The detector was set at 250 nm with bandwidth of 5 nm. The temperature was ambient. The integrator (HP 3392A) parameters were set to a chart speed of 0.3 cm min^{-1}, peak width of 0.04, threshold of 3 and an attenuation of 2 ^ 3.

Standard Solutions

Accurately weighed quantities of standard rutin, ascorbic acid and phenacetin were dissolved in ethanol to obtain a solution having known concentrations of 0.45, 0.05 and 0.25 mg ml^{-1}, respectively. The synthetic mixtures were prepared by transferring different aliquots from rutin and ascorbic acid standard solutions with constant 2 ml of the internal standard to 50 ml volumetric flasks.

Sample Solutions

For composite and content uniformity assays, an amount equivalent to one average tablet or soft gelatin capsule, or a single unit, was transferred to a 100 ml volumetric flask, diluted with 50 ml ethanol, sonicated for 10 minutes to dissolve and made up to volume with

ethanol. A 1 ml portion of this solution was further diluted to 10 ml in a volumetric flask.

Calculations

$$\text{Quantities in mg} = \frac{\text{Peak ratio response from sample solution}}{\text{Peak ratio response from standard solution}} \times \text{Conc.of Standard(mg/ml)} \times \frac{\text{Average weight (g) (tablet or capsule)}}{\text{Sample weight (g)}}$$

Method Validation

System Suitability

With all system components in place, the column was equilibrated with mobile phase at flow rate of 1 ml min^{-1} for at least 30 min or untill a steady baseline was obtained. Five replicates, 5 µl each of standard solution were chromatographed. The peak responses of rutin and ascorbic acid were recorded. The resolution factor, relative retention times, relative standard deviation (RSD) and tailing factors for each peak were calculated according to criteria described in USP XXII.

Linearity

Five concentrations of rutin and ascorbic acid in the range of 0.5-2.5 and 1-5 µg ml^{-1}, respectively, with 50 µg ml^{-1} constant concentration from phenacetin were prepared in ethanol and chromatographed. The linearity of each component was established by linear regression analysis of peak area ratio responses versus concentration.

Accuracy

An amount of rutin, ascorbic acid and excipient mixture equal to one tablet or capsule weight was di-

ssolved in ethanol and the resulting solutions assayed in triplicates. The results obtained were compared with label claim. A solution containing only excipients was also prepared in ethanol, filtered and chromatographed to establish any interference that may occur due to excipients.

Recovery

Solutions of excipients were spiked with each active component from 50 - 150% of the label claim and assayed. The recovery of each component was calculated and compared with the amount added.

RESULTS AND DISCUSSION

The assay and content uniformity results for tablet and capsule dosage forms are summarized in Tables 1 and 2. The results indicate that rutin and ascorbic acid

Table 1
Assay Results of Rutin and Ascorbic Acid in Commercial Products

Compound	Tablet[a]	S.G.Capsule[b]
Rutin	59.50 mg (99.17%)[c]	94.80 mg (99.60%)
Ascorbic Acid	161.50 mg (100.94%)	99.50 mg (99.50%)

a- Ruta-C tablets (El Kahira Pharm. Co., Cairo, Egypt), with 60 and 160 mg of rutin and ascorbic cid, respectively.
b- Rutin-C soft gelatin capsules (Pharco Pharm. Co., Alexandria, Egypt) with 50 and 100 mg of rutin and ascorbic acid, respectively.
c- Mean of three determinations; assay as percentage label claim.

Table 2

Contents Uniformity of Results for Rutin and Ascorbic Acid in Tablets and Soft Gelatin Capsules

	FOUND							
	RUTIN				ASCORBIC ACID			
	TABLET		CAPSULE		TABLET		CAPSULE	
	mg	%	mg	%	mg	%	mg	%
1.	60.30	100.5	49.70	99.4	160.47	100.3	99.20	99.2
2.	60.70	101.3	50.20	100.4	160.80	100.5	100.50	100.5
3.	59.10	98.5	48.95	97.9	160.16	100.1	98.80	98.8
4.	59.70	99.5	49.45	98.7	159.68	99.8	99.30	99.3
5.	60.48	100.8	49.75	99.5	161.76	101.1	98.90	98.8
6.	60.60	101.0	49.10	98.2	159.68	99.8	99.30	99.3
7.	59.28	98.8	50.65	101.3	160.16	100.1	98.90	98.9
M.	60.02	100.1	49.68	99.3	160.39	100.2	99.27	99.3
S.	0.608	1.034	0.552	1.120	0.673	0.420	0.536	0.536
R.	1.013	1.033	1.111	1.127	0.420	0.420	0.540	0.540

M = mean,; S = population standard deviation and
R = relative standard deviation.

can be quantified in pharmaceutical dosage forms using the proposed HPlC method. Figure 1 shows proper resolution between any two peaks. The reproducibilty of the method is indicated by the (RSD) of less than 2% for five replicate injections of standard solution as evident from Table 3.

The relative retention times are 2.09, 3.88 and 5.09 minutes for ascorbic acid, rutin and (IS), respectively. A typical chromatographic run is completed in about 6 minutes. The tailing factor for each peak is calculated to be not greater than 1.5.

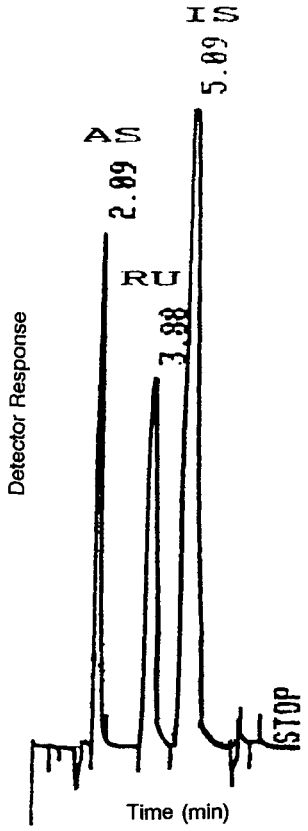

Fig. 1: *A typical chromatogram of 5 ul injection of synthetic mixture of Ascorbic acid (AS); Rutin (RU) and Phenacetin (IS).*

A linear response are obtained for rutin and ascorbic acid in concentration ranges of 0.5 - 2.5 µg ml^{-1} and 1 - 5 µg ml^{-1}, respectively. Regression analysis on the calibration curves relating peak area ratio (Y) and concentration C (µg ml^{-1}), with correlation coefficient (r), gave the following equations :

Y (Rutin) = -8E-4 + 0.172 C (r= 0.9999)

Y (Ascorbic acid) = -4E-4 + 0.045 C (r= 0.9999)

Table 3

Reproducibility of Standard Solution

Injection Number	Peak Area Responses Ascorbic Acid	Rutin	IS
1	176150	742310	1667500
2	178230	743500	1666600
3	177300	745630	1670800
4	178090	747480	1670400
5	176400	750000	1666400
Mean	1777234	745784	1668340
± SD	848.5	2755	1886
RSD	0.479	0.369	0.113

SD=population standard deviation; RSD=reltive standard deviation.

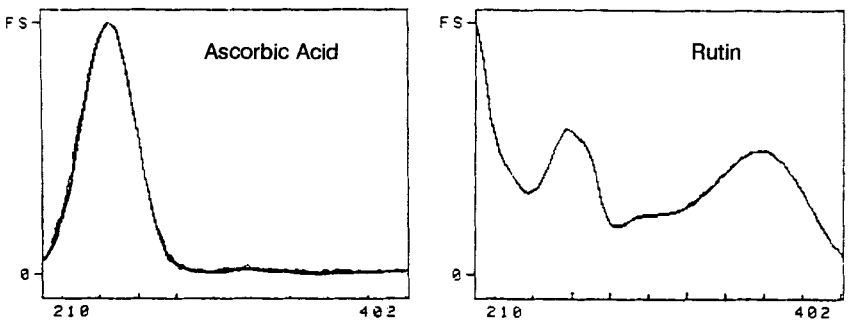

Fig. 2: *Peak purity check of the eluted drugs by overlapping spectra.*

The accuracy of the method is established by achieving reproducible results (Table 1). The excipients solution showed no peaks. The recovery of active components from a spicked excipient solution afforded excellant results. The percentage recoveries were summarized as follows: for rutin 99.5% and 99.2%, for ascorbic acid 100.2% and 99.8% for tablet and soft gelatin capsule excipients, respectively.

Peak purity control and identification are important in the method development and in routine quality control applicaions. The Diode-array detector system enables us to positively confirm peak purity and peak idendity [4]. Figure 2 showed an overlayed UV spectra taken, for every peak, during a peak's elution. It is very clear from the shown spectra that they were superimposed which confirms the purity of every peak in the chromatogram. The structure of the eluted peaks was achieved by comparing them with known standards.

The proposed HPLC method is simple, accurate and reproducible. In the absence of any compendial method for the simultaneous determination of rutin and ascorbic acid, the proposed method can be used for routine quality control and stability evaluations of these products.

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REFERENCES

1- D.Feng and W.Peng, *Yaowu Fenxi Zazhi*, 9(4),237 (1989) through Anal. Abstr. 52; 6E2 (1990).

2- J.Ge, G.Yang, R.Yu and Q.Yang, *Nanjing Yaoxueyuan Xuebao*, 16(1), 42 (1985) through Anal. Abstr. 48; 10E24 (1986).

3- The United States Pharmacopeia, 22nd Rev., U.S. Pharmacopeial Convention, Rockville, MD,1990,p.1566.

4- L.Huber, Application of Diode-Array Detection in HPLC, Hewlett-Packard Publication Number 12-5953-2330.

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