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Simultaneous determination of rutin and ascorbic acid mixture in their pure forms and combined dosage form

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ABSTRACT

A simple, rapid, sensitive and selective high performance liquid chromatographic (HPLC) method with ultraviolet detection has been developed for simultaneous determination of ascorbic acid and rutin in pure forms and pharmaceutical dosage form. HPLC separation was performed on Phenomenex C18 analytical column with 0.1% v/v acetic acid in water and acetonitrile (75:25, v/v), as mobile phase. The separation was done at ambient temperature with flow rate of 1 mL·min^{-1} in isocratic mode. HPLC measurements were carried out using ultraviolet detection wavelength at 257 nm. The average retention times were 2.72 and 7.00 min for ascorbic acid and rutin, respectively. The calibration plots were constructed over the concentration range of 5.0–30.0 for ascorbic acid and 10.0–60.0 μg·mL^{-1} for rutin. The limits of detection were 1.06 and 1.89 μg·mL^{-1} and limits of quantification were 3.54 and 6.31 μg·mL^{-1} for ascorbic acid and rutin, respectively. The proposed HPLC-UV method was successfully applied for determination of ascorbic acid in its tablets and for simultaneous determination of the studied drugs in their laboratory prepared mixtures and in pharmaceutical formulation. Statistical comparisons of the results with the reference method show an excellent agreement and indicate no significant difference in respect to accuracy and precision.

1. Introduction

Rutin is a well-known and widely used citrus flavonoid glycoside between flavonol quercetin and disaccharide rutinose [1]. It is found in many foods, such as buckwheat, onion, lemon, apple, orange, and grapefruit. Rutin has a lot of benefit pharmacological effects, such as anti-inflammatory, antimicrobial, antioxidant, and antihypertensive effects [2–5]. Furthermore, rutin has anti-carcinogenic effect at sub-toxic concentration [6] through inhibition of vascular endothelial growth factor. On the other hand, Ascorbic acid (Vitamin C) is an essential nutrient for humans. It is commonly present in vegetables and fresh fruits, especially citrus fruits. Ascorbic acid has a highly effective antioxidant activity by decreasing the oxidative stress. Vitamin C is not only used for preventing and treating scurvy but also for treatment of certain respiratory diseases such as allergic rhinitis [7]. The chemical structures of rutin and ascorbic acid are shown in Fig. 1.

The combination of rutin and ascorbic acid is very useful for maintenance of the normal conditions in the wall of blood vessels and capillaries. This combination is intended for oral administration to guard against different types of haemorrhage such as cerebral haemorrhage, other capillary haemorrhagic complications, haematuria, and increased capillary permeability as in allergic diseases [8].

Different methods have been developed for the individual determination of ascorbic acid [9–17]and rutin [18–22]. In addition, Several methods have been reported for simultaneous determination of mixture of these active ingredients like UV spectrophotometry [23,24], electrochemical method [25–27], chemiluminescence [28], capillary electrophoresis [29–32], high-performance liquid chromatography (HPLC) [33–36]and NIR FTIR [37].

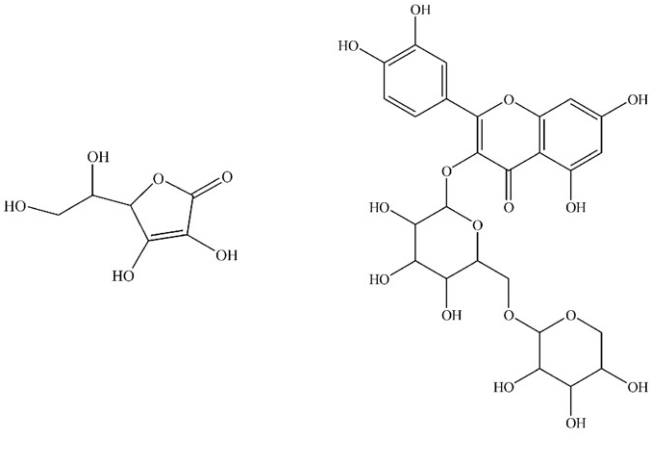

Fig. 1. The chemical structures of ascorbic acid and rutin.

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The objective of this study is to develop a simple, rapid, sensitive and selective method for determination of rutin and ascorbic acid mixture in their pure forms and pharmaceutical dosage forms. The proposed method is based on UV measurement of the investigated drugs after their physical separation through an HPLC system.

In comparison with other conventional spectroscopic methods, the proposed HPLC-UV method is more sensitive and selective. While in comparison with the previously reported HPLC methods, the proposed method is simpler as

a- It does not require any reagent or sample preparation.
b- The mobile phase does not contain any complicated buffers which cause column corrosion.
c- The mobile phase does not contain methanol which has several bad health effects and affect the chromatographic conditions.

So, the proposed HPLC-UV method is ideally suited for simultaneous determination of ascorbic acid and rutin in quality control laboratories.

2. Materials and methods

2.1. Apparatus

- Digital balance, Radwag Wagi Elektroniczne WTP 3000 (Bracka, Radom, Poland).
- Sonicator, Wise Clean WUC-A-10H, Dahan Scientific Co. Ltd. (Wonju-si, Gangwon-do, Korea).
- Milwaukee SM 101 pH meter, Portugal (Milwaukee, Portugal).
- Water distiller, BHANU BASIC/PH4, MK-I (Bangalore, India).
- The HPLC equipment used in the present study was the Knauer system (Knauer, Berlin, Germany). This included Smartline S-1000 quaternary pumps, coupled with Smart line S-2600 UV–VIS multiwavelength detector and Knauer dynamic mixing chamber. The separation was carried out on Phenomenex C18 (4.6 × 250 mm, 10 μm, Phenomenex, Bondo Ione, USA) reversed phase column at ambient temperature. The mobile phase is composed of 0.1% v/v glacial acetic acid in water and acetonitrile (HPLC grade), using isocratic elution system. The mobile phase was filtered under vacuum through a 0.45 μm membrane filter and degassed before use. Chromatograms were processed by using an Eurochrom for windows program (Basic edition V3.05, Advanced Scientific Instruments Wissenschaftliche Gerätebau, Berlin, Germany). The analysis was carried out at a flow rate of 1.0 $mL \cdot min^{-1}$ with the detection wavelength set at 257 nm, and the injection volume was set at 20 μL using 20 μL sample loop. The peak identification was based on the comparison of retention times and UV spectra with those of authentic compounds.

2.2. Reagents and solutions

Ascorbic acid (99.8% purity) and rutin (99.9% purity) reference standards were kindly supplied by Kahira Pharmaceutical and Chemical Industries Company (Cairo, Egypt) and were used without further purification. Acetonitrile, acetic acid and methanol (E-Merck, Darmstadt, Germany) were of HPLC grade.

The following available commercial preparations were analyzed;

- Ruta C 60® tablets [Kahira Pharmaceutical and Chemical Industrial Co., Cairo-Egypt] labeled to contain 60 mg of rutin and 160 mg of ascorbic acid per tablet.
- Cevarol® tab [Memphis Co. for Pharmaceutical and Chemical Industries (MEMECO)-Egypt] labeled to contain 500 mg of ascorbic acid per tablet.

2.3. Standard solution preparation for establishing calibration curve

1 $mg \cdot mL^{-1}$ stock solution of each standard drugs (rutin and ascorbic acid) was prepared by dissolving 100 mg of each drug in 5 mL of methanol (HPLC grade), and then diluted to 100 mL with the mobile phase [0.1% v/v acetic acid in water: acetonitrile (75:25, v/v/)]. Stock solution of ascorbic acid was prepared daily, protected from light and stored at −20 °C until use. On the other hand, the rutin stock solution was stable for seven days when kept in the refrigerator. The working standard solutions of 100 μg mL^{-1} were freshly prepared by further dilution with mobile phase solution.

2.4. General analytical procedures

Serial dilutions were carried to prepare different concentrations for establishing standard calibration curves. HPLC analysis was carried out by injection of 20 μL of each standard solution. The mobile phase [0.1% v/v acetic acid: acetonitrile (75:25, v/v/)] was prepared daily, filtered and sonicated before use. The mobile phase was isocratic eluted at a flow rate of 1.0 $mL \cdot min^{-1}$ on Phenomenex C18 reversed phase column kept at ambient temperature (~25 °C) with UV detection at 257 nm. The regression equations were computed and calculations were performed.

2.5. Assay of laboratory-prepared mixtures

Different ratios of rutin and ascorbic acid mixtures were prepared in laboratory and were analyzed using the proposed HPLC-UV method. The concentrations were calculated from the corresponding regression equations.

2.6. Procedures for pharmaceutical dosage form analysis (Ruta-C 60® tablets and Cevarol® tablets)

Twenty tablets of each pharmaceutical formulation were weighed accurately and were ground into fine powder. For Ruta-C 60® tablets, an accurately weighed portion of the powder equivalent to 60 mg of rutin and 160 mg of ascorbic acid, was extracted by sonication in methanol. For Cevarol® tablets, a quantity of the powder equivalent to 25 mg of ascorbic acid was weighed accurately and was dissolved by sonication in methanol. In both solutions, the extract was filtered. The filtrate was diluted with mobile phase several times to adjust the final concentrations of working standard solutions. Twenty micro liters from each working tablet solution samples was directly injected into the HPLC column, and the general procedure was followed. The nominal contents of pharmaceutical preparations were calculated using the corresponding regression equations.

3. Results and discussion

A simple HPLC-UV method has been developed for simultaneous determination of rutin and ascorbic acid in their pure forms and pharmaceutical formulations. The proposed HPLC-UV method is very simple and rapid as it does not require any reagent or sample preparation. Furthermore in the previously reported HPLC methods [33–36], the mobile phase consisted of either complicated buffers which cause column corrosion, or methanol which has several disadvantages such as forming a relatively viscous solution with water giving high pressures, lower elution strength than acetonitrile, and producing a high noise in UV detection region. In addition, acute or chronic exposure to methanol by inhalation or ingestion has several health hazard effects such as blurred vision, headache, dizziness, and nausea. However in the proposed HPLC-UV method, the mobile phase simply consisted of 0.1% v/v acetic acid in water and acetonitrile in the ratio of 75:25 (neither buffer nor methanol was used in the HPLC mobile phase system).