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Validated spectrophotometric methods for simultaneous determination of troxerutin and carbazochrome in dosage form

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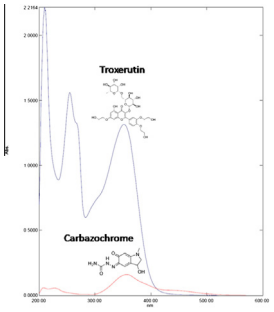
HIGHLIGHTS

- Troxerutin (TXN) is co-formulated with Carbazochrome (CZM).
- No method was reported for determination of TXN and CZM in their mixture form.
- Four spectrophotometric methods were developed for their simultaneous determination.
- The methods were validated according to the ICH guidelines.
- The results were statistically compared to the manufacturer's method.

GRAPHICAL ABSTRACT



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ABSTRACT

Four simple, accurate, sensitive and precise spectrophotometric methods were developed and validated for simultaneous determination of Troxerutin (TXN) and Carbazochrome (CZM) in their bulk powders, laboratory prepared mixtures and pharmaceutical dosage forms. Method A is first derivative spectrophotometry (D^1) where TXN and CZM were determined at 294 and 483.5 nm, respectively. Method B is first derivative of ratio spectra (DD^1) where the peak amplitude at 248 for TXN and 439 nm for CZM were used for their determination. Method C is ratio subtraction (RS); in which TXN was determined at its λ_{max} (352 nm) in the presence of CZM which was determined by D^1 at 483.5 nm. While, method *D* is mean centering of the ratio spectra (MCR) in which the mean centered values at 300 nm and 340.0 nm were used for the two drugs in a respective order. The two compounds were simultaneously determined in the concentration ranges of 5.00–50.00 μg mL^{-1} and 0.5–10.0 μg mL^{-1} for TXN and CZM, respectively. The methods were validated according to the ICH guidelines and the results were statistically compared to the manufacturer's method.

Introduction

Troxerutin (TXN) is chemically designated as 2-[3,4-bis(2-hydroxyethoxy)phenyl]-5-hydroxy-7-(2-hydroxyethoxy)-4-oxo-4H-chromen-3-yl6-o-(6-deoxy-β-D-mannopyranosyl)-β-D-glucopyranoside, it is a flavonol and known as vitamin P4 [1]. TXN has a considerable broad pharmacological activities; it improves capillary function, reduces capillary fragility and abnormal leakage. It is also used for reducing the occurrence of night cramps, treatment of varicose veins and hemorrhoids [2]. Carbazochrome (CZM) is chemically designated as [(3-Hydroxy-1-methyl-6-oxo-2,3-dihydroindol-5-ylidene)amino]urea and it is an oxidation product

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of adrenaline and used as anti-hemorrhagic agent [3]. Both drugs are co-formulated (TXN 150 mg and CZM 1.5 mg) and the dosage form was shown to have a good efficacy and safety profile in non-surgical patients with acute uncomplicated hemorrhoids. Their chemical structures are shown in Fig. 1.

Several methods were reported on each drug individually. For TXN, it was determined by spectrophotometry [4–6], capillary electrophoresis [7] and high-performance liquid chromatography (HPLC) [8–10], electrochemistry [11] and capillary electrochromatography [12]. While for CZM, it was determined by spectrophotometry [13], high-performance liquid chromatography (HPLC) [14,15] and Chemiluminescence [16].

No method was reported for determination of TXN and CZM in their mixture form. So, our aim was to develop and validate simple spectrophotometric methods for simultaneous determination of both drugs in their pure form, laboratory prepared binary mixture and pharmaceutical formulation.

Experimental

Instruments

A double beam UV–visible spectrophotometer (SHIMADZU, Kyoto, Japan) model UV-1650, pc with quartz cell of 1 cm path length, connected to IBM compatible computer operated with UV-probe personal spectroscopy software version 2.21. The spectral band width is 2 nm and wavelength scanning speed is 2800 nm/min. Mean centering computations were done using Matlab® 6.5 with PLS-Toolbox.

Samples

Pure samples of TXN and CZM were kindly supplied by Minapharm for Pharmaceuticals and Chemical Industries, Cairo, Egypt. Both were certified to contain 99.90% w/w according to the manufacturer's method. Fleboton® ampoules, labeled to contain 150 mg of TXN and 1.5 mg of CZM were manufactured by Minapharm Pharmaceuticals and Chemical Industries, Egypt (batch No. 9DE0219) and were obtained from local market.

Chemicals and solutions

Methanol spectroscopic grade was used. Stock standard solutions of TXN and CZM (1.0 mg mL^{-1}) were prepared in methanol. Working standard solutions of TXN and CZM (0.1 mg mL^{-1}) were prepared by an additional dilution of their stock standard solutions with methanol. A set of laboratory prepared mixtures containing different ratios of TXN (10.0–50.0 μg mL^{-1}) and CZM (0.5–7.5 μg mL^{-1}) was prepared.

Procedures

Construction of calibration curves

Aliquots of TXN working standard solution (0.1 mg mL^{-1}) equivalent to 50.0–500.0 μg mL^{-1} and of CZM working standard solution (0.1 mg mL^{-1}) equivalent to 5.0–100.0 μg mL^{-1} were accurately transferred into a series of 10 mL volumetric flasks; the volume was completed to the mark with methanol. The zero order spectra of the prepared solutions were recorded using methanol as a blank in the range of 200–600 nm.

For D^1 method

The D^1 curves of the scanned spectra were recorded using $\Delta\lambda = 4$ and scaling factor = 10. Calibration curves were then constructed by plotting the values of the peak amplitude of D^1 curves at 294 nm for TXN (corresponding to zero crossing of CZM) and 483 nm for CZM (corresponding to zero absorbance of TXN) versus the corresponding concentrations and the regression parameters were computed.

For DD^1 method

The scanned spectra of TXN were divided by a standard spectrum of 10.0 μg mL^{-1} CZM while the spectra of CZM were divided by a standard spectrum of 40.0 μg mL^{-1} TXN and the first derivative of the ratio curves (DD^1) for each compound were then obtained with $\Delta\lambda = 4$ and scaling factor = 10. Calibration curves were constructed by plotting the peak amplitude at 248 and 439 nm of the DD^1 curves versus the corresponding concentrations of TXN and CZM, respectively and the regression parameters were computed.

For ratio subtraction method (RS)

For the determination of TXN, a calibration curve was constructed relating the absorbance of zero order spectra of TXN at 352 nm to the corresponding concentrations and the regression

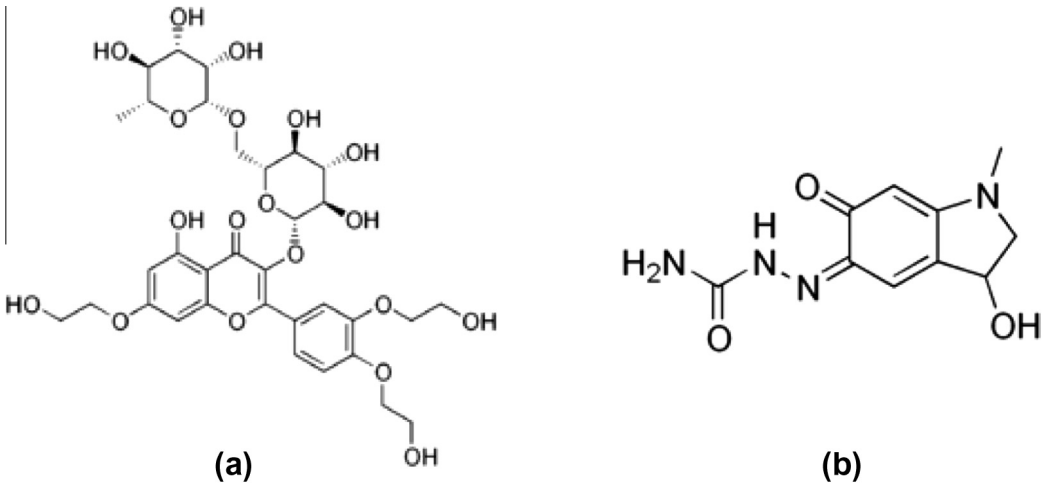

Fig. 1. Chemical structure of (a) truxerutin and (b) carbazochrome.