I6 - HPLC/DAD Method for Determination of Flavonoids Rutin and Quercetin in Herbal Supplements

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Flavonoids are a large group of polyphenolic components possessing benzo-γ-pyronic structure and are widely distributed in plants. The chemical nature and the biological activity of flavonoids depends on the structural class to which they belong, the degree of hydroxylation, the degree of polymerization, and the presence of other substituents and bonds. *Ginkgo biloba* is one of the top-selling botanicals in the world. It has supported efficacy for the treatment of cerebrovascular disease and dementia. *Ginkgo biloba* leaf extract is said to contain more than 20 kinds of flavonoids. We have analyzed quercetin and rutin as representative flavonoids in the commercially available herbal supplements containing *Ginkgo biloba* leaf extract. HPLC analyses were performed using a Schimadzu LC-2010 chromatographic system (Schimadzu, Kyoto, Japan) consisting of a LC-20AT Prominence liquid chromatograph pump with a SPD-M20A Prominence Diode Array Detector. Chromatographic separation was performed on a Purospher® STAR RP-18e reversed-phase column (250 X 4.0 mm I.D.; particle size 5 µm) in a gradient mode with a mobile phase constituted of: acetonitrile: 3% phosphoric acid (85% phosphoric acid was used). The elution was carried out at a flow rate of 1.50 ml /min. All analyses were performed at room temperature (24 +/- 2°C). Rutin was monitored at 255 nm, while quercetin at 375 nm. Data analyses were done using Class VP 7.3 Software. The proposed method was validated according to the guidelines set by the International Conference on Harmonization for validation of analytical procedures. The identification of flavonoids was done by comparison of retention times of the analyzed components, their UV spectra and by standard addition method. Calibration curves were obtained using standard solutions of rutin and quercetin with concentrations ranged from 0.01 – 0.08 mg/ ml. Correlation coefficients were 1.0 and 0.9998 for rutin and quercetin, respectively. The precision of the method was confirmed by assessment of repeatability and reproducibility. Relative standard deviations obtained in the investigation of repeatability were: 0.52 % and 0.05 % for rutin and quercetin, respectively. Relative standard deviations obtained in the investigation of reproducibility were: 0.82 % and 0.95 % for rutin and quercetin, respectively. The average recovery for samples containing rutin and quercetin were 99.2 % and 101.2 % for rutin and quercetin, respectively. The limits of detection for rutin and quercetin were 0.95 ng/ml and 1.25 ng/ml, respectively, which indicates an excellent sensitivity of the proposed method. From the results presented, it can be concluded that this method is simple, easy to perform and specific for routine determination of flavonoids rutin and quercetin in herbal preparations containing *Ginkgo biloba* extract.

Key words: flavonoids, rutin, quercetin, *Ginkgo biloba*, HPLC, validation