Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 417 - 418 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.201
Short communication

Quantification of rutin in *Calendula flos* using a chemometric approach based on ATR-FTIR spectra

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Introduction

Calendula officinalis L. is a plant of great medicinal importance, which is widely used in the pharmaceutical, cosmetic and phytotherapeutic industries due to its rich phytochemical content. (Shahane et al., 2023). As one of its bioactive compounds, rutin is a flavonoid glycoside, known for its pharmacological properties, such as antioxidant, antiviral, and anti-inflammatory effects (Al-Huqail et al., 2023).

ATR-FTIR spectroscopy is a rapid, non-destructive analytical method that requires minimal or no sample preparation. Combined with chemometric analysis, it is used for product validation and quality control (Agatonovic-Kustrin et al., 2020).

The aim of the research was to assess the potential of vibrational spectroscopic techniques for rapid quantification of rutin in *Calendula officinalis* L. dry flower material, which represents one of the major flavonoid constituents and an important quality parameter of Calendula flos. In addition, ATR-FTIR analysis of powdered Calendula flos samples originating from different cultivation sites from Kosovo was performed, and a multivariate chemometric approach was applied for development and optimization of calibration models. Additionally, standard addition of rutin was employed in order to improve the robustness and predictive performance of the developed model against the reference analytical technique (HPLC).

Materials and methods

Plant material

Flower samples of *Calendula officinalis L.*, Asteraceae were collected from 11 different geographical locations in Kosovo in three consecutive years of cultivation. The samples were dried and mechanically grounded to obtain homogeneous material and subjected to spectroscopic analysis and extraction.

HPLC method for quantification of rutin and quercetin

The extraction was performed using 0.5 g of dried and crushed plant material with 10 mL of 50% (v/v) ethanol. The mixture was sonicated for 15 minutes at room temperature and subsequently filtered through filter paper.HPLC analysis was performed on an Agilent 1200 Series system equipped with a DAD detector using an XBridge C18 column (250 × 4.6 mm, 5 µm) at 25 °C with a flow rate of 1 mL/min and a 10 µL injection volume. Compounds were separated

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using a gradient of acetonitrile and 1% orthophosphoric acid and identified at 360 nm by comparing retention times and UV spectra with authentic standards.

ATR-FTIR analysis of Calendula flos samples

ATR-FTIR spectra were recorded using a Cary 630 FTIR spectrometer equipped with an attenuated total reflection (ATR) module (Agilent Technologies, USA). The spectra were acquired directly from the powdered samples by placing the material onto the ATR crystal under constant pressure.

Spectra were recorded in the spectral range of 4000 - 650 cm^{-1} with a spectral resolution of 4 cm^{-1} using 32 accumulated scans per spectrum.

Development of PLS calibration models for rutin quantification

PLS calibration models for rutin quantification were developed using ATR-FTIR spectra of *Calendula flos* as independent X variables and HPLC-determined rutin concentrations as dependent Y variables.

Results and discussion

The obtained concentrations of quercetin were found within a narrow concentration range (40.4 - 45.2 mg/g dry weight), whereas rutin concentrations varied substantially between the investigated samples (43.0–294.4 mg/g dry weight). Such variability indicates significant influence of environmental factors, cultivation conditions, harvesting period, and genetic variability on flavonoid biosynthesis in Calendula flos. In contrast, quercetin concentrations exhibited only minor variability, making this compound unsuitable for reliable ATR-FTIR chemometric quantification. Therefore, subsequent ATR-FTIR chemometric analysis focused exclusively on rutin quantification.

ATR-FTIR spectra of powdered *Calendula flos* samples showed characteristic absorption bands associated with flavonoid structures, particularly in regions corresponding to O-H stretching vibrations, aromatic C=C stretching vibrations, and C-O stretching vibrations of glycosidic moieties. Differences in intensity and shape of these absorption bands correlated with rutin concentrations determined by the reference HPLC method.

A preliminary PLS model was first constructed using the ATR-FTIR spectra of all investigated samples and the corresponding HPLC-determined rutin concentrations. This initial model showed satisfactory correlation between spectral data and rutin content; however, its predictive performance was limited since the rutin concentrations of the available samples were not evenly distributed across the calibration range but were predominantly located at the concentration extremes.

To improve the predictive ability of the model and reduce the error of prediction, additional calibration samples were prepared by standard addition of rutin to ground *Calendula flos*, followed by acquisition of new ATR-FTIR spectra. The resulting PLS model showed improved agreement between predicted and determined rutin concentrations, as reflected by lower RMSEC and RMSECV values, indicating better calibration accuracy, and predictive stability.

Conclusion

The obtained results demonstrate that ATR-FTIR spectroscopy combined with PLS regression analysis represents a rapid, non-destructive, and environmentally friendly analytical approach for rutin quantification in *Calendula flos*. Compared with conventional chromatographic methods, the proposed method requires minimal sample preparation, reduced solvent consumption, and significantly shorter analysis time, making it highly suitable for routine quality control and rapid screening of herbal raw materials and its products.

References

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