Determination of three triterpenic acids in dried rosemary by HPLC-fluorescence detection with DIB-Cl

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Introduction

Rosemary has been widely used as a functional food owing to its antioxidative and anti-inflammatory activities. Recently, ursolic acid (UA) and betulinic acid (BA) which are triterpenic acids in rosemary take notice of their skin whitening activities. Therefore the determination of the acids in rosemary is important for the quality evaluation rosemary products. In this study, an HPLC-fluorescence (FL) detection method with 4-(4,5-diphenyl-1*H*-imidazole-2-yl)benzoyl chloride (DIB-Cl) as a derivatization reagent was developed for UA, BA and oreanolic acid (OA). Moreover, the method was applied to determine these acids in commercialy avairable dried rosemary.

Materials and Methods

Extraction: 50 mg of dried rosemary grinded with a finger masher were added to 3 mL of EtOH. The mixture was sonicated for 40 min and then filtered with a membrane filter. Derivatization: 100 μL of sample were added to the mixture of 100 μL of 1% trimethylamine in CH_3CN and 100 μL of 1mM DIB-Cl in CH_3CN suspension. The resultant was stood for 5 min at room temperature and applied to HPLC analysis. HPLC: The triterpenic acids were separated on a Wakopak Handy ODS (250×4.6 mm) with 25 mM acetate buffer (pH 4.5)/MeOH/CH_3CN (=8:10:82 v/v/v%). The fluorescence intensity (FI) of the eluent at 365 (λ_{ex}) and 490 nm (λ_{em}) was monitored.

Results and Discussion

After optimization of separation and derivatization conditions, the triterpenic acids were well separated without interfering peaks from rosemary extract with 26 min (BA), 29 min (UA) and 30 min (OA) of retention time (Fig. 1). The calibration curves using rosemary extract with standards indicated good linearities ($r \geqq 0.997$) in the range of 2.5-100 ng/mL. The detection limits at a 3σ of internal peak in rosemary extract for BA, UA and OA were 0.2, 0.4 and 0.5 ng/mL, respectively. Accuracy (ranging from 80.5 to 106.8%), precisions of intra-day (less than 5.6%) and inter-day assays (less than 6.8%) were acceptable (n=5).

The concentration ranges of BA, UA and OA in commercially available dried rosemary (n=7) were 8.0-13.7 mg/g, 10.0-13.3 mg/g and 20.9-31.9 mg/g, respectively.

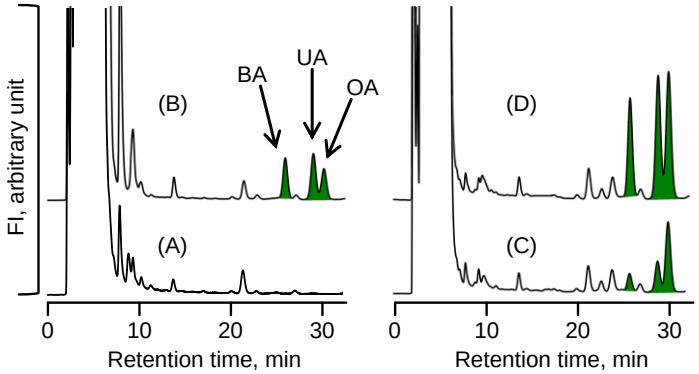

Figure 1: Chromatograms of the proposed method. Sample: blank (A), standards with 10 ng/mL (B), rosemary extract (C) and that spiked with 20 ng/mL of standards (D).

Conclusion

The proposed method was enough sensitive to determine the triterpenic acids in dried rosemary and could be successfully applied to analyse the acid compounds in practical samples. Therefore the method might be a powerful tool for the quality evaluation of rosemary products on the basis of three triterpenic acids amounts.