

Methodology for the Determination of Triterpenic Compounds in Apple Extracts

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Abstract : Apples are among the most commonly consumed fruits in the world. Based on data from the year 2014, approximately 84.63 million tons of apples are grown per annum. Apples are widely used in food industry to produce various products and drinks (juice, wine, and cider); they are also used unprocessed. Apples in human diet are an important source of different groups of biological active compounds that can positively contribute to the prevention of various diseases. They are a source of various biologically active substances – especially vitamins, organic acids, micro- and macro-elements, pectins, and phenolic, triterpenic, and other compounds. Triterpenic compounds, which are characterized by versatile biological activity, are the biologically active compounds found in apples that are among the most promising and most significant for human health. A specific analytical procedure including sample preparation and High Performance Liquid Chromatography (HPLC) analysis was developed, optimized, and validated for the detection of triterpenic compounds in the samples of different apples, their peels, and flesh from widespread apple cultivars 'Aldas', 'Auksis', 'Connel Red', 'Ligol', 'Lodel', and 'Rajka' grown in Lithuanian climatic conditions. The conditions for triterpenic compound extraction were optimized: the solvent of the extraction was 100% (v/v) acetone, and the extraction was performed in an ultrasound bath for 10 min. Isocratic elution (the eluents ratio being 88% (solvent A) and 12% (solvent B)) for a rapid separation of triterpenic compounds was performed. The validation of the methodology was performed on the basis of the ICH recommendations. The following characteristics of validation were evaluated: the selectivity of the method (specificity), precision, the detection and quantitation limits of the analytes, and linearity. The obtained parameters values confirm suitability of methodology to perform analysis of triterpenic compounds. Using the optimised and validated HPLC technique, four triterpenic compounds were separated and identified, and their specificity was confirmed. These compounds were corosolic acid, betulinic acid, oleanolic acid, and ursolic acid. Ursolic acid was the dominant compound in all the tested apple samples. The detected amount of betulinic acid was the lowest of all the identified triterpenic compounds. The greatest amounts of triterpenic compounds were detected in whole apple and apple peel samples of the 'Lodel' cultivar, and thus apples and apple extracts of this cultivar are potentially valuable for use in medical practice, for the prevention of various diseases, for adjunct therapy, for the isolation of individual compounds with a specific biological effect, and for the development and production of dietary supplements and functional food enriched in biologically active compounds. Acknowledgements. This work was supported by a grant from the Research Council of Lithuania, project No. MIP-17-8.

Keywords : apples, HPLC, triterpenic compounds, validation

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