ISSN (1230-0322)
2 0 1 1 , Vol. 61, Suppl. 1

EuroFoodChem XVI

Translating food chemistry into health benefits

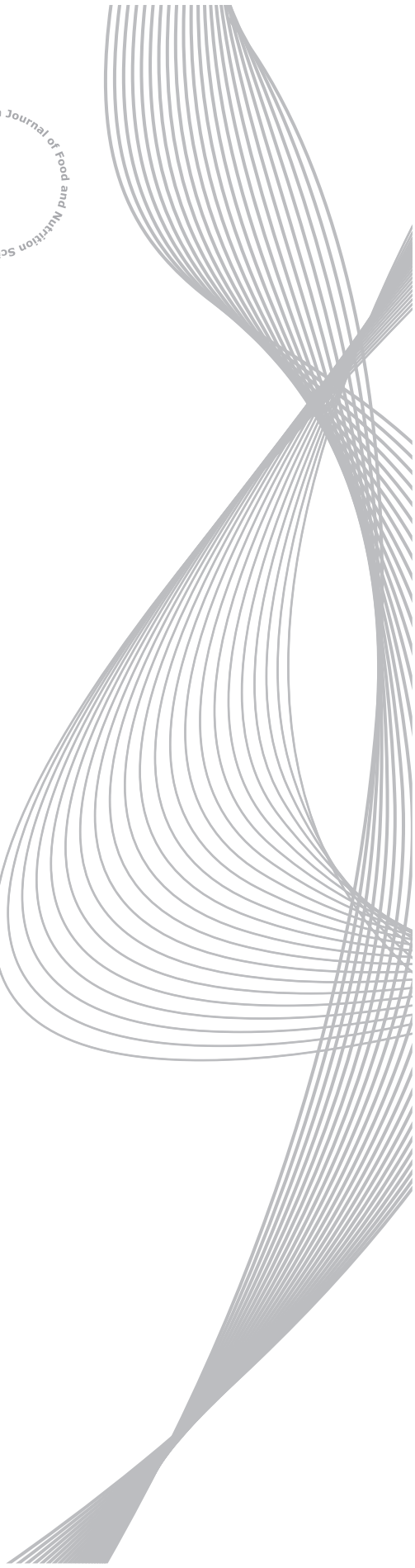
Conference Proceedings

Gdansk 6th-8th July, 2011

Published

by the Division of Food Sciences, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn

Polish Journal of Food and Nutrition Sciences
formerly Acta Altimentaria Polonica



Food analyses and bioanalyses

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Application of High Performance Liquid Chromatography with UV and Tandem MS Detection for Analysis of β-Carotene in Food Supplement Based on Conifer Needle Extract

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Key words: dietary supplements, β-carotene, liquid-liquid extraction, SPE, liquid chromatography, tandem masspectrometry

Homeopathic drugs and non-synthetic food supplements are getting wider attention recently due to the widespread opinion that synthetically developed drugs are more harmful than those of plant origin. On the other hand, drugs produced according to GMP and tested according to GLP standards have definite and unambiguous composition with clinically confirmed mode of action and possible side effects contrary to food supplements of plant origin. Therefore it is important to develop analytical procedures to establish the composition and concentration of compounds present in dietary supplements.

A reliable and selective analytical method for determination of β-carotene in conifer needle extract using high performance liquid chromatography with UV and tandem MS detection was developed. The HPLC-UV method involves extraction with organic solvent, polar compound removal, clean-up with SPE columns, extract evaporation and dissolution in mobile phase. Application of tandem masspectrometry improves selectivity of elaborated assay and allow to avoid a SPE purification step.

In order to choose the most suitable extraction solvent n-hexane, n-heptane, petroleum ether, cyclohexane, isooctane, MTBE, toluene, dichloromethane, and ethyl acetate were evaluated. For further purification of conifer needle extract pre-packed solid phase clean-up cartridges containing alumina, silica and amino modified silica were tested.

Single factor dispersion analysis was used to ascertain influence of the extraction procedure and instrumental analysis on the obtained results.

The chromatographic separation was achieved on a RP C-18 column using acetonitrile-methanol-dichloromethane (75/15/10 v/v/v) as a mobile phase in isocratic mode. During this study main parameters of the method were checked. The calibration curves were linear in the interval from 5-1000 μg/g with correlation coefficients higher than 0.9997. The recoveries were in range from 92%-107% for 100, 200 and 250 μg/g spiking levels. The precision calculated from interday repeatability was from 1.5% to 3.3% for the same spiking levels.

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