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# An HPLC method for the evaluation of the ACE inhibitory activity of plant proteins hydrolysates

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**Abstract**
Recently in vitro and in vivo studies have demonstrated that peptides obtained from hydrolysis of plant and animal proteins have a direct action on the Angiotensin Converting Enzyme (ACE), the key enzyme involved in blood pressure regulation in humans, competing with angiotensin I for the active site. Food ACE-inhibitory peptides from milk and dairy products, egg, wheat and legumes may be of interest in lowering human pressure maintaining health (Fujita et al., 2000; Vermeirssen et al., 2004). The evaluation of the ACE-inhibitory activity is mainly performed by spectrophotometric methods, based on the use of the model tripeptide hippuryl-histidyl-leucine, HHL (Cushman and Cheung, 1971). However, these techniques show low specificity and sensitivity when applied to complex peptide mixtures obtained from food, containing several interferences. It was thus necessary to develop an innovative strategy based on more accurate methods. For this reason a method based on HPLC coupled with a DAD detector was developed. The variables of the assay had to be adapted at the new analytical conditions, in particular the substrate and enzyme concentrations, the buffer and time/temperature of the enzymatic reaction. The optimized method was tested on three drugs (captopril, enalapril, and lisinopril) commonly used in the treatment of hypertension and two tripeptides (IPP and VPP) responsible of ACE inhibitory activity in milk hydrolyzates. Then the method was applied to enzymatic mixtures obtained from digestion of proteins from the main legumes: soybean, pea, lupin, bean, lentil, chickpea.

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# In-house validation of chromatographic speciation methods for arsenic in fishery products

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**Abstract**
The relatively high concentrations of arsenic found in shellfish in recent years have contributed to raise the threshold of attention of European Union, in terms of food security. Among the various factors that influence the toxicity of arsenic, the chemical form is of particular significance, given the high toxicity of the inorganic form in respect of the organic that frequently contaminates fishery products. The Expert Committee of FAO / WHO defined a provisional tolerable weekly intake (PTWI) of 15 mg/kg b.w. only for the inorganic form and, in view of definition of residual limits for arsenic in fish, it becomes necessary to have analytical methods able to differentiate inorganic form from the organic ones (e.g. monomethyl- and dimethylarsenic acid, arsenobetaine, arsenocholine). The purpose of this study was to optimize and validate an analytical method for the speciation of arsenic in fishery products, that could identify and quantify the organic forms of arsenic. Screening of organic forms of arsenic was carried out using HPLC (high performance liquid chromatography) coupled to a tandem mass spectrometry detector, while determination of total arsenic was carried out using the atomic absorption spectroscopy. The validation procedure was conducted according to the requirements of the European Community to allow the use of the present method by the Official Control laboratories. The matrices considered for method optimization and validation have been fish, molluscs and crustaceans from the coasts of southern Italy. Obtained results allowed the method to enter within the routinely activities of the laboratory and require method accreditation.