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Analytical Methods

Simultaneous determination of individual isothiocyanates in plant samples by HPLC-DAD-MS following SPE and derivatization with *N*-acetyl-L-cysteine

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ABSTRACT

The procedure for the isothiocyanates (ITCs) determination that involves derivatization with *N*-acetyl-L-cysteine (NAC) and separation by HPLC was developed. Prior to derivatization, plant ITCs were isolated and purified using solid-phase extraction (SPE). The optimum conditions of derivatization are: 500 μL of isopropanolic eluate obtained by SPE combined with 500 μL of derivatizing reagent (0.2 M NAC and 0.2 M $NaHCO_3$ in water) and reaction time of 1 h at 50 °C. The formed dithiocarbamates are directly analyzed by HPLC coupled with diode array detector and mass spectrometer if required. The method was validated for nine common natural ITCs. Calibration curves were linear ($R^2 \geqslant 0.991$) within a wide range of concentrations and limits of detection were below 4.9 nmol/mL. The recoveries were in the range of 83.3–103.7%, with relative standard deviations <5.4%. The developed method has been successfully applied to determine ITCs in broccoli, white cabbage, garden cress, radish, horseradish and papaya.

1. Introduction

Isothiocyanates (ITCs) are regarded as the most biologically active breakdown products of glucosinolates – secondary metabolites of plants, mainly from *Brassicaceae* family. Upon plant tissue damage induced by pest attack or other disruption events, e.g. cutting or chewing, glucosinolates (GLs) undergo hydrolysis catalyzed by the enzyme myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1) to the unstable intermediate which rearranges further to ITCs and related products, such as nitriles, epithionitriles, indoles, thiocyanates or oxazolidine-2-thiones (Mithen, Armah, & Traka, 2011). The formation of specific products depends on the variety of factors, including metal ions, pH, protein cofactors and side chain structure (Wittstock & Burow, 2007). Among GL hydrolysis products, ITCs ensure the most effective barrier against plant pathogenic microorganisms (Aires et al., 2009). This group of compounds triggers also several biological activities that discourage herbivore attacks and thus represents environment friendly biopesticides that can be used in biofumigation process (Kusznierewicz et al., 2012; Pilipczuk, Piekarska, Kusznierewicz, Bartoszek, & Namieśnik, 2013). However, the feature of ITCs that warranted them the position of the most investigated phytochemical family is their ability to reduce the incidence and progression of human cancers and to prevent inflammation (Mithen et al., 2011). The chemopreventive effect of ITCs involves multiple mechanisms that include inhibition of phase I cytochrome P450 enzymes, stimulation of phase II detoxification enzymes, ceasing inflammatory reactions, inhibition of angiogenesis, induction of cell cycle arrest and apoptosis of tumor cells (Navarro, Li, & Lampe, 2011). Importantly, numerous epidemiological studies have demonstrated the high potency of ITCs in prevention of lung, prostate, breast, bladder, colorectal, pancreatic and other less frequent human cancers (Higdon, Delage, Williams, & Dashwood, 2009).

Equally important are synthetic ITCs, which are widely applied as valuable starting materials for a wide range of chemical reactions. For instance, they are used for synthesis of glycolipids with thiourea- or urea-linkers (Mathiselvam, Loganathan, & Varghese, 2013), 1,3,5-triazine derivatives needed in organic chemistry and medicinal research (Li, Tu, Jiang, Wang, & Tu, 2013) or other compounds with important functional groups such as thiosemicarbazides (Pandurangan, Kitchen, McCabe, & Gunnlaugsson, 2013),

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