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A new method for rapid determination of indole-3-carbinol and its condensation products in nutraceuticals using core–shell column chromatography method

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ABSTRACT

Indole-3-carbinol is a natural glucosinolate known for prevention of human breast, prostate and other types of cancer and it started to be used in commercial preparations, as food supplements. However no analytical method has been proposed for quality control of nutraceuticals with this substance yet. In this paper a new high-performance liquid chromatography (HPLC) method using core–shell column for separation of indole-3-carbinol and its condensation/degradation products was developed and used for the quantitative determination of indole-3-carbinol in nutraceuticals. Separation of indole-3-carbinol, its condensation/degradation products and internal standard ethylparaben was performed on the core–shell column Kinetex 5 μ XB-C18 100A (100 × 4.6 mm), particle size 5.0 μm, with mobile phase acetonitrile/water according to the gradient program at a flow rate of 1.25 mL min^{-1} and at temperature 50 °C. The detection wavelength was set at 270 nm. Under the optimal chromatographic conditions good linearity of determination was achieved. Available commercial samples of nutraceuticals were extracted with 100% methanol using ultrasound bath. A 5-μL sample volume of the supernatant was directly injected into the HPLC system. The developed method provided rapid and accurate tool for quality control of nutraceuticals based on cruciferous vegetable extracts with indole-3-carbinol content. The presented study showed that the declared content of indole-3-carbinol significantly varied in the different nutraceuticals available on the market. Two analyzed preparations showed the presence of condensation/degradation products of indole-3-carbinol which were not officially declared by the manufacturer. Moreover, further two analyzed nutraceutical preparations showed absolutely no content of declared amount of indole-3-carbinol.

1. Introduction

Indole-3-carbinol (I3C) is the breakdown product of the naturally occurring glucosinolates, mainly in cruciferous vegetables such as broccoli, Brussels sprouts, cabbage, cauliflower and garden cress. I3C is released from its glucosinolate precursor glucobrassicin when brings into contact with myrosinase [1]. In an acidic solution like the human stomach or under acidic conditions in vitro (0.05 M hydrochloric acid, pH 1.5 for 60 min), I3C is rapidly converted into an array of acid condensation products and modified derivatives [2]. In vivo assessment of I3C and its products suggest that 3, 3′-diindolylmethane (DIM), an I3C acid-condensation product, is one of the major bioactive compounds responsible for the benefits associated with I3C, because DIM has distinct targets and greater bioactivity [3,4]. I3C together with its metabolic products has indicated anti-tumor activity. These indole derivatives have been shown to suppress the proliferation of various cancer cell lines, including those of breast, colon, prostate, and endometrium, by targeting a wide spectrum of signaling pathways governing apoptosis, cell-cycle progression, hormonal homeostasis, DNA repair, angiogenesis, and multiple-drug resistance. Moreover, indole-3-carbinol proves to be an effective chemoprotective agent against estrogen responsive cancer such as breast and cervical cancer, in part, because it functions as a negative regulator of estrogen by inhibiting ERα signaling and altering cytochrome P450-mediated estrogen metabolism [5].

The intrinsic instability of indole-3-carbinol in acidic milieu arises from the vinyl hemiaminal moiety of the indole ring. This unique structural feature underlies the high susceptibility of indole-3-carbinol to acid catalyzed dehydration and condensation

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to generate a complex series of oligomeric products in vivo, including DIM (3, 3′-diindoylmethane), ICZ (indolo[3,2b]-carbazole), LTr1 (a linear trimer), CTr (a cyclic trimer), and CTet (a cyclic tetramer) [1,4,5].

Prevention and protection against chemical carcinogens by phytochemicals presented in extensively consumed glucosinolate-containing cruciferous vegetables is of great interest. It provides a safe and cost effective means of cancer prevention [6]. Direct and indirect research evidences demonstrated the benefits of cruciferous vegetable in prevention of metabolic disorders, asthma and Alzheimer's disease, along with antimicrobial activity against the number of pathogens. A large number of plant derived compounds including indole-3-carbinol have been identified for prevention and treatment of cancer [7]. Cancer inhibition mechanisms by indole-3-carbinol were established for human breast cancer cells [8] and for human prostate cancer cells [9]. Other health protective effect were studied, e.g. long term treatment with 200 mg of indole-3carbinol twice a day has been demonstrated to be effective in chemoprevention of respiratory papillomatosis caused by human papilloma virus [10,11]. The above mentioned beneficial biological properties create a wide scope for preparation of food supplements containing indole-3-carbinol. Therefore indole-3-carbinol started to be used in multicomponent commercial preparations, food supplements and nutraceuticals. Despite the benefits of indole-3-carbinol in the auxiliary cancer treatment, no analytical method for quality control of this substance in nutraceutical products has been proposed.

Nutraceuticals and food supplements are known as dietary supplements that deliver a concentrated form of a presumed bioactive agent from food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods [12]. Food supplements are taken alone or in combination and marketed in dose forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles and powders designed to be taken in measured small unit quantities [13]. Nutraceutical products together with functional foods belong to the most rapidly growing sectors in the food and personal care product industry. The main problem associated with nutraceutical products is their legal classification. Being neither food nor pharmaceuticals, they often stay in a gray area between both, which makes the quality control and regulation very difficult [13,14]. No specific regulation exists in Europe to control nutraceuticals, although they are considered under the same laws that regulate medicine and drug. The only aim is to harmonize the legislation and to ensure that these products are safe and appropriately labeled so that consumers can make informed choices of appropriate supplement [15]. In the USA, the Food and Drug Administration regulates dietary supplements under a different set of guidelines than those covering conventional foods and drug products [12]. Because of consumers trust in the safety, beneficial biological activity and efficiency of these products, there is a need of efficient quality control measures [16]. From this point of view, new modern analytical methods covering the quality control of nutraceuticals and food supplements must be developed.

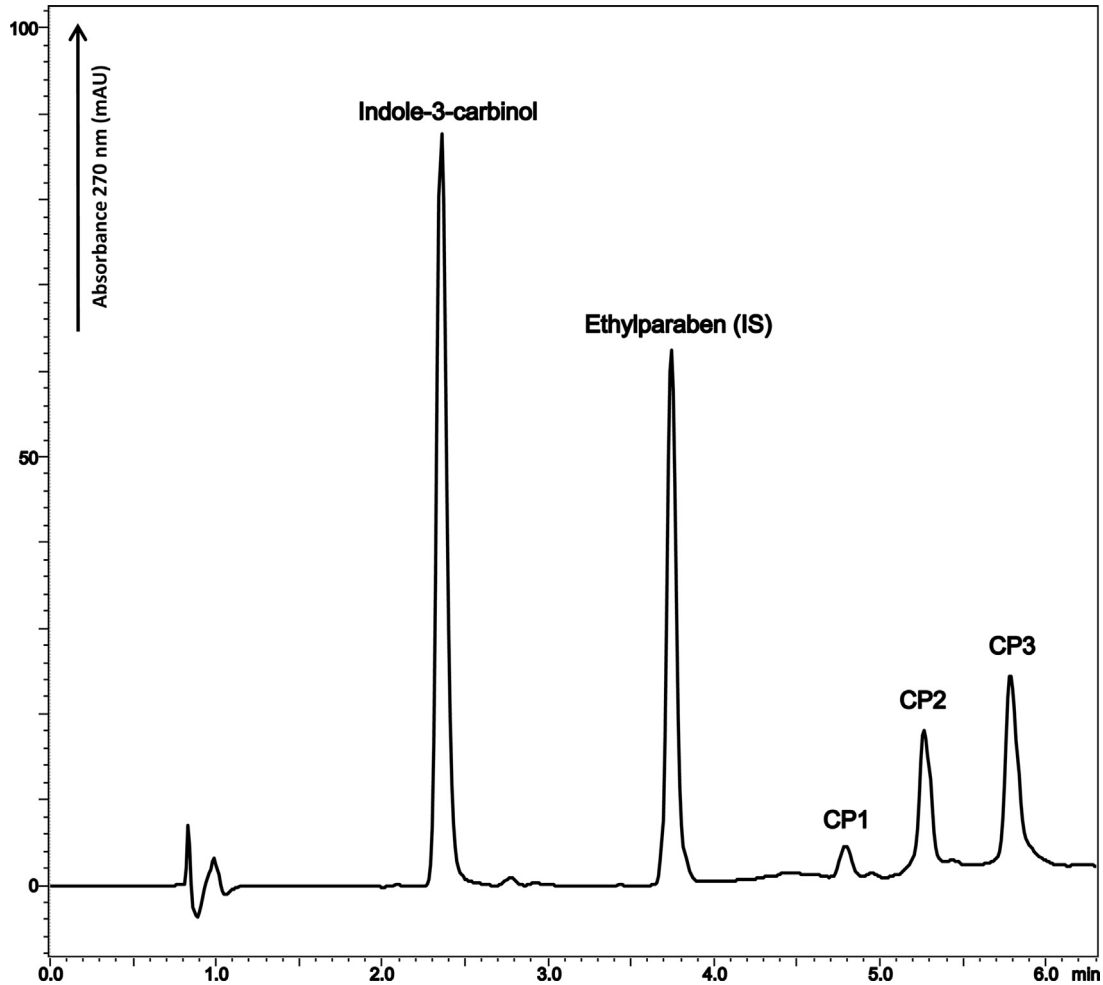

Fig. 1. Chromatogram of indole-3-carbinol, ethylparaben (IS) and condensation products CP1, CP2 and CP3 separation in standard solution performed on the core–shell column Kinetex 5 μ XB-C18 100A (100 × 4.6 mm), particle size 5.0 μm, with mobile phase acetonitrile/water according to the gradient program at temperature 50 °C.