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Field amplified sample injection–capillary electrophoresis–tandem mass spectrometry for the analysis of acrylamide in foodstuffs

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Abstract

This paper shows the applicability of capillary electrophoresis (CE) coupled to mass spectrometry (MS) for the analysis of acrylamide (AA) in foodstuffs. In order to obtain an ionisable compound amenable to be analysed by CE, acrylamide was derivatised with 2-mercaptobenzoic acid. Spectra in positive and negative modes were studied in order to select the best ionisation mode and multistep tandem mass spectrometry was used to obtain structural information. Maximum signal was observed when negative mode was used and MS/MS and MS^3 were selected for quantitation and confirmation, respectively. For the separation, a fused-silica capillary of 80 cm and 50 μm I.D. and 35 mM ammonium formate/ammonia solution at pH 10 as running electrolyte were used. The applicability of field amplified sample injection (FASI) in reversed polarity was evaluated in order to decrease detection limits. The developed FASI–CE–MS/MS method provided a detection limit of 8 ng g^{-1} and good linearity ($r = 0.999$) and precision (day-to-day lower than 15%). The method has been applied to the analysis of different representative food products and the results were compared with those obtained by LC–MS/MS.

Keywords: FASI; CE–MS/MS; CE–MS^n; Acrylamide; Food

1. Introduction

Acrylamide (AA) is a low molecular weight hydrophilic compound known mostly for its use as a monomer in the production of polyacrylamide which in turn is used in paper and textile industries, as flocculant in the treatment of wastewater, as soil conditioner, in ore processing and in cosmetics. The monomeric form of acrylamide has been shown to have neurotoxic properties in both animals and humans [1]. Moreover, it has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) on the basis of sufficient evidence for carcinogenicity in experimental animals and mechanistic considerations [2]. In addition, it has been regulated in EU countries by the EU 98/83 directive in drinking water [3] with a minimum quality requirement of 0.1 μg L^{-1} for water intended for human consumption. In this context, the discovery that cooking carbohydrate-rich foodstuffs at elevated temperatures results in the formation of acrylamide has caused considerable alarm [4,5] since levels as high as mg kg^{-1} have been reported [6–8]. However, how high the risk of contracting cancer is in humans after the intake of acrylamide-containing foods cannot be reliably estimated at present. For all these reasons, a worldwide monitoring of acrylamide in foodstuffs has started since 2002. Nowadays, although the underlying of the mechanisms of formation is not yet known in detail, the Maillard reaction between amino acids (asparagine) and reducing sugars is proposed as the main source of acrylamide [9,10] although in model experiments, other pathways have been proposed and several precursors of this compound such as acrolein, acrylic acid and other carbonyls [9,10] have also been identified.

To determine acrylamide in foodstuffs, sensitive and selective analytical techniques are needed. Nowadays, the most common analytical methods include liquid chromatography with UV detection [11–13] or coupled to tandem mass spectrometry (LC–MS/MS) [14–17] although gas chromatography mass spectrometry (GC–MS) with or without derivatisation of the analyte has also been applied [14–17]. Capillary electrophoresis using UV detection [18–20] has also been applied for the analysis of acrylamide in foodstuffs. Microemulsion electrokinetic chromatography (MEEKC) was firstly proposed in order to analyse acrylamide without derivatisation [18]. However, this method provided low selectivity and high detection limits. So, lately, in order to improve both, selectivity and sensitivity, a capillary zone electrophoresis (CZE) method was proposed [19]. In this

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method, a derivatisation procedure using 2-mercaptobenzoic acid, previously proposed for acrylamide analysis by LC–MS [21] was used. To further improve detection limits and to spread the applicability of the method over a wide range of samples, field amplified sample injection (FASI) was proposed [20]. With this approach limits of detection of low ng g^{-1} were obtained.

The aim of this work was to develop and optimise a CE–MS method for the analysis of acrylamide after its derivatisation with 2-mercaptobenzoic acid. In order to improve detection limits, the use of field amplified sample injection (FASI–CE–MS) in reversed polarity was evaluated. Quality parameters were established and then the method was applied to the analysis of acrylamide in different representative foodstuffs.

2. Experimental

2.1. Chemicals and consumables

Acrylamide (C_3H_5NO, 71 Da) (>99%) was purchased from Fluka (Buchs SG, Switzerland) and 2,3,3-D_3-acrylamide (98%) from Cambridge Isotope Laboratories (Andover, MA, USA). All the reagents used were of analytical grade. Methanol, hexane, formic acid (98–100%), acetic acid (100%), ammonium acetate, dichloromethane, ammonia solution (25%), hydrochloric acid (25%) and isopropanol were obtained from Merck (Darmstadt, Germany), and ammonium formate was provided from Fluka. The derivatising reagent was 2-mercaptobenzoic acid purchased from Sigma–Aldrich (Steinheim, Germany). Stock solutions of acrylamide (1 mg mL^{-1}) and D_3-acrylamide (1 mg mL^{-1}) were prepared in Milli-Q water, and stored at 4 °C for a maximum of 4 weeks.

Solid phase extraction (SPE) cartridges Strata-X-C (200 mg, 6 mL) and Isolute ENV+ (200 mg, 3 mL) were provided by Phenomenex (Torrance, USA) and IST (Hengoed, MId-Glamorgan, UK), respectively. Syringe filters 0.45 μm of nylon and nitrocellulose were purchased from Teknokroma (Barcelona, Spain). Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

The experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis instrument (Fullerton, CA, USA) coupled to a Classic LCQ mass spectrometer (Finnigan, San Jose, CA, USA) equipped with a tricoaxial pneumatically assisted electrospray ionisation (ESI) source and with an ion trap as mass analyser. The electrophoretic separations were carried out using uncoated fused-silica capillaries of 80 cm × 50 μm I.D., 35 mM ammonium formate-ammonia buffer (pH 10) as carrier electrolyte and +25 kV as capillary voltage. Samples were analysed applying capillary zone electrophoresis and hydrodynamic injection and FASI (−10 kV) were used. An injection time of 5 s for hydrodynamic injection was applied. For FASI, 35 s (3.5 kPa) of water plug, 35 s (−10 kV) of sample and 6 s (3.5 kPa) of additional water plug were injected. The CE instrument was controlled using a Beckman 32 Karat software version 5.0.

A standard solution of 20 μg mL^{-1} of acrylamide, previously derivatised (see Section 2.5) was used to optimise CE–MS coupling parameters. This solution was infused into the ESI source applying simultaneously an electrophoretic voltage of +25 kV and an overimposed pressure of 3.5 kPa on the CE inlet vial and −3 kV as electrospray needle voltage. The optimised conditions were: sheath gas (nitrogen) at a flow-rate of 6 arbitrary units (a.u.); spray voltage −3 kV; heated capillary temperature 175 °C; capillary protruding 0.18 mm; and distance to the heated capillary 15 mm. Moreover, 5–10 mm of the polyamide coating was removed from the fused-silica capillary outlet to improve the stability of the spray.

CE–MS data acquisition was carried out in negative full scan mode from *m/z* 100 to 300 in centroid mode using a maximum injection time of 200 ms and performing 3 μscans. Product ion scan mode with a maximum injection time of 200 ms and 3 μscans was used for CE–MS/MS data acquisition. To isolate the precursor ion, an isolation width of *m/z* 1.5 was applied, the trapping ratio frequency voltage (AQ) was set at 0.400 and the activation time (AT) was 30 ms. Precursor ions, product ion scan ranges, diagnostic product ions and the normalised collision energies (NCE %) used in the MS/MS and MS^3 experiments for both acrylamide and D_3-acrylamide are indicated in Table 1. Mass spectrometry data were processed with a Xcalibur 1.4 software version.

2.3. Capillary conditioning

New capillaries were pre-treated using 0.1 M hydrochloric acid for 30 min, water for 30 min, 1 M sodium hydroxide for 30 min, and finally were washed with water for 30 min. At the beginning of each session, the capillary was rinsed with sodium hydroxide for 15 min, water for 15 min, and with the carrier electrolyte during 30 min. The capillary was washed with carrier electrolyte for 5 min between runs and stored after rinsing with water at the end of each session.

2.4. Sample preparation and clean-up procedure

Five different representative food samples (potato crisps, biscuits, crisp bread, breakfast cereals and coffee) were collected from supermarkets in Barcelona (Spain). In order to extract acrylamide from these samples, a previously published purification method [22] was used. Briefly, prior to extraction, the foodstuffs were ground and homogenized using a supermixer blender system (Moulinex, Lyon, France) and an Ultraturrax T25 basic (IKA-Werke, Staufen, Germany), respectively. Sub-samples of 2 g (1 g for potatoes samples) were weighed into 15 mL centrifuge tubes, and 40 μL of 2,3,3-D_3-acrylamide (10 μg mL^{-1}) and 10 mL water were added. Each tube was shaken for 1 h on a rotating shaker (Rotatory Mixer 34526; Breda Scientific, Breda, The Netherlands). Then, the tubes were centrifuged at 4000 rpm for 30 min with a Selecta Centronic centrifuge (Selecta, Barcelona, Spain). A 5 mL aliquot of aqueous solution was filtered through a syringe filter of nitrocellulose (0.45 μm) and de-fatting was carried out by adding 2 mL of hexane and removing the organic phase. For clean-up, Strata-X-C SPE car-