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Analysis of heterocyclic amines in chicken by liquid chromatography with electrochemical detection

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Abstract

The present paper describes a method based on liquid chromatography with electrochemical detection (LC–ECD) for the determination of heterocyclic amines (HAs) in food samples. In order to obtain extracts clean enough to quantify heterocyclic amines by liquid chromatography with electrochemical detection, an additional purification step based on a cationic exchanger (CBA) as sorbent was included in a clean-up performed by several solid-phase extraction steps. Two different mobile phases, acetonitrile–50 mM ammonium acetate (10:90) at pH 5.25, and acetonitrile–50 mM ammonium acetate (30:70) at pH 6.0, were used for the LC separation of polar and less polar amines. Optimum working potentials were +1000 and +950 mV for polar and less polar HAs, respectively. The obtained figures of merit showed detection limits of 5–79 pg for standards, 2–30 ng g^{-1} for beef extract and 0.3–2.9 ng g^{-1} for fried chicken sample, and day-to-day precisions of 1.2–7.6% for standards and 2.8–10.4% for fried chicken sample. The applicability of the LC–ECD method has been demonstrated by the determination of the amines in griddled and fried chicken.

Keywords: Heterocyclic amines; Food; LC–ECD

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1. Introduction

Several epidemiological studies have shown diet to be an important factor in the global variation of human cancer rates, and heterocyclic amines (HAs), potent mutagens present at ppb levels in cooked foods, appear to play a role in the aetiology of human cancer [1]. HAs belong to a large group of substances present in grilled and fried meat, poultry, and fish [2,3]. The types and amounts of HAs formed can be attributed to parameters such as time, temperature, and cooking procedures [4,5]. These amines belong to two main classes of compounds: aminoimidazoazaarenes and aminocarbolines (Fig. 1), the first type being generated from precursors such as glucose, creatine/creatinine and free amino acids at ordinary cooking temperatures [6]. In contrast, aminocarbolines, called pyrolitic HAs, are formed at temperatures above 300 °C through a pyrolitic reaction [7].

To date, more than 20 different mutagenic and/or carcinogenic heterocyclic amines have been isolated and identified in cooked food samples and several model systems [8–10]. In order to estimate intake and risk to human health, it is important to quantify these compounds in a variety of cooked foods. However, the high complexity of the sample matrix along with the low level of concentration of HAs requires the use of sensitive and selective analytical methodologies. Usually, laborious clean-up procedures that include extraction, purification and pre-concentration steps [11], followed by a separation technique [12] mainly liquid chromatography (LC) and gas chromatography (GC) are used. The detection systems commonly used are UV, electrochemical, fluorescence and MS.

Because of the frequent appearance of co-extracted compounds from the food sample matrix, the use of highly selective techniques like mass spectrometry [13–18] is cur-

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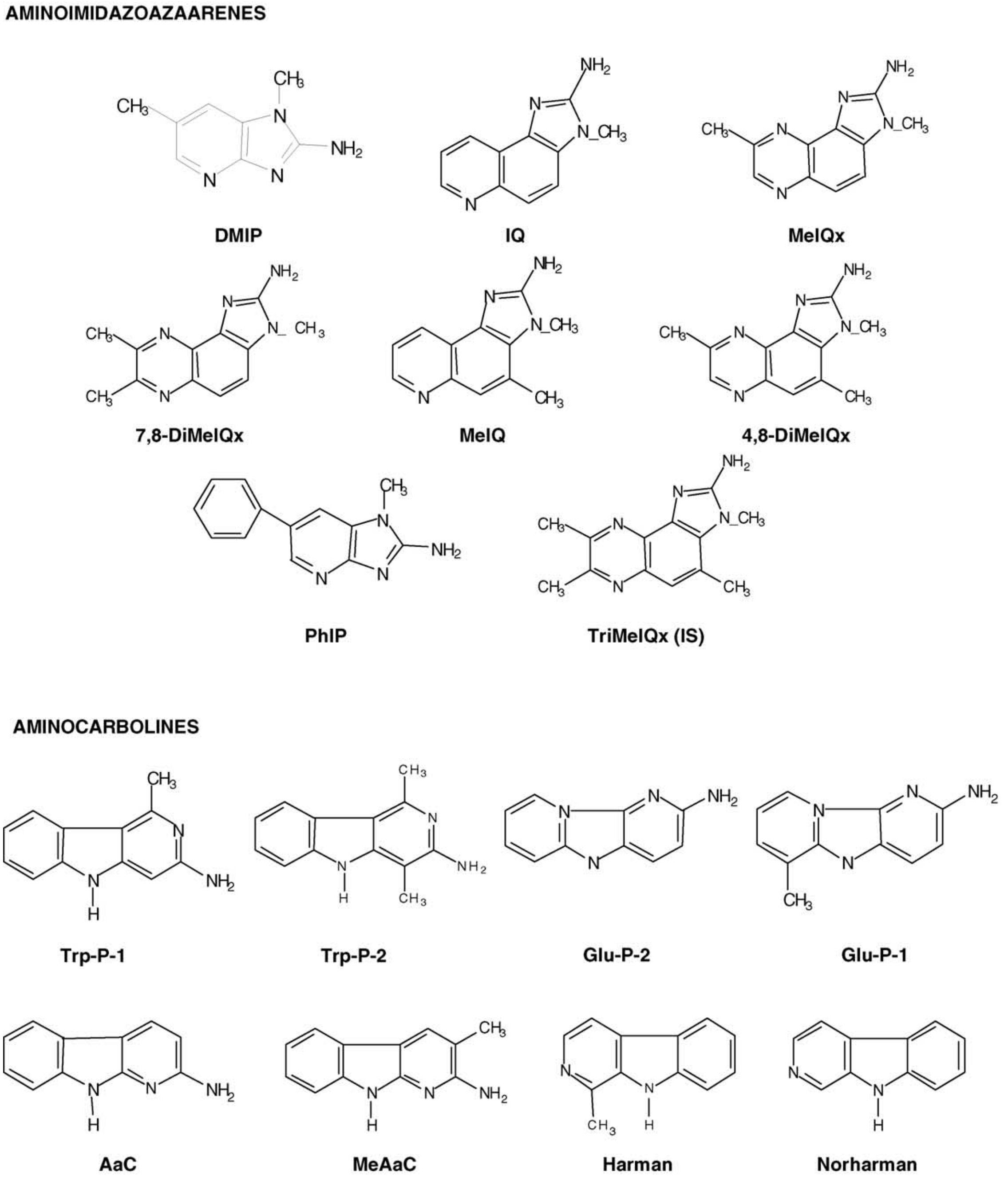

Fig. 1. Chemical structure of studied heterocyclic amines.

rently preferred. However, due to the relatively high cost of mass spectrometry instrumentation, the application of this approach might be beyond the possibilities of some laboratories. In those cases, the use of electrochemical or fluorescence detection can be a good alternative. Fluorescence detection [11,19] offers high sensitivity and selectivity in comparison with UV detection but, unfortunately, several HAs are not fluorescent. On the other hand, electrochemical detection (ECD) [20–30] presents good sensitivity and, moreover, all HAs, without exception, give good analytical signals because all of them can be easily oxidised. To date, several works on the analysis of HAs using LC–ECD have been published. The first studies using this detection system for the analysis of these compounds were published by Grivas and Nyhammer [20] and Takahashi et al. [21,22]. These works were focused on the determination of some aminoimidazoazaarenes, those known as IQ-type compounds, in model systems [20] and beef extracts [21,22]. The development of methodologies based on electrochemical detection for the analysis of this type of compounds were the objective of the works of several authors such as Van Dyck et al. [23], Murkovic et al. [24,25] and Ohe [26]. Billedeau et al. [27] and Galceran et al.