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Investigation of the separation of heterocyclic aromatic amines by reversed phase ion-pair liquid chromatography coupled with tandem mass spectrometry: The role of ion pair reagents on LC–MS/MS sensitivity

F. Bianchi, M. Careri *, C. Corradini, L. Elviri, A. Mangia, I. Zagnoni

Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43100 Parma, Italy

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Abstract

Reversed phase ion-pair chromatography (RP-IPC) of seven heterocyclic aromatic amines encompassing quinoline (IQ, MeIQ), quinoxaline (MeIQx), pyridine (PhIP) and carboline derivatives (AαC, Harman, Norharman) was carried out with formate as counter ion in an aqueous eluent with acetonitrile as organic modifier. TSKgel ODS-80TS was used as the stationary phase. With the aim of acquiring a better insight into the mutual influence of ion-pair reagent and the organic modifier upon solute retention, the study was performed by using an experimental design approach able to evidencing the effect of the simultaneous variation of the two factors. A model for the chromatographic behavior of the amines is proposed that includes classical ion-pair mechanism involving formate in the case of MeIQx, PhIP, Harman and Norharman. A competitive ion-exchange mechanism was hypothesized to govern retention of quinoline compounds, whereas electrostatic interactions and hydrogen bond formation with the silanols of the stationary phase were judged to be responsible for the retention of AαC. Further, the chromatographic behavior of the analytes using the formic acid-ammonium formate buffer in the mobile phase was compared with that observed using acetic acid-ammonium acetate buffer. The method based on the use of RP IPC with tandem mass spectrometry when the eluent contained formate buffer at pH 2.8 exhibited higher detectability with respect to that achieved using the acetate buffer.

Keywords: Heterocyclic aromatic amines; Liquid chromatography–mass spectrometry; Ion-pair reagents

1. Introduction

Heterocyclic aromatic amines (HAAs) are biologically active substances identified in a wide variety of food [1]. These compounds are known to possess a high mutagenic activity in the Ames test [2] and are recognized as possible human carcinogens. The major pathway for the metabolic activation of HAAs starts with the hydroxylation of the hexocyclic amino group, catalyzed mainly by cytochrome P4501A2, followed by acetylation or sulphation to form direct-acting reactive mutagens that alter DNA and genome [3].

As illustrated in a review devoted to the determination of HAAs in foods [4], high-performance liquid chromatography (HPLC) and gas chromatography (GC) are the techniques predominantly used for analysis of these substances, GC being less convenient than HPLC because of the need of chemical derivatization. As regards to HPLC, separation of HAAs is commonly performed on a reversed-phase (RP) C_{18} column [5–15]. However, a drawback of ordinary RPLC is that organic solute ions exhibit poor peak shapes and inadequate retention. In this context, ion pair RPLC represents a more effective technique for retention

* Corresponding author. Tel.: +39 0521 905477; fax: +39 0521 905557.
E-mail address: careri@unipr.it (M. Careri).

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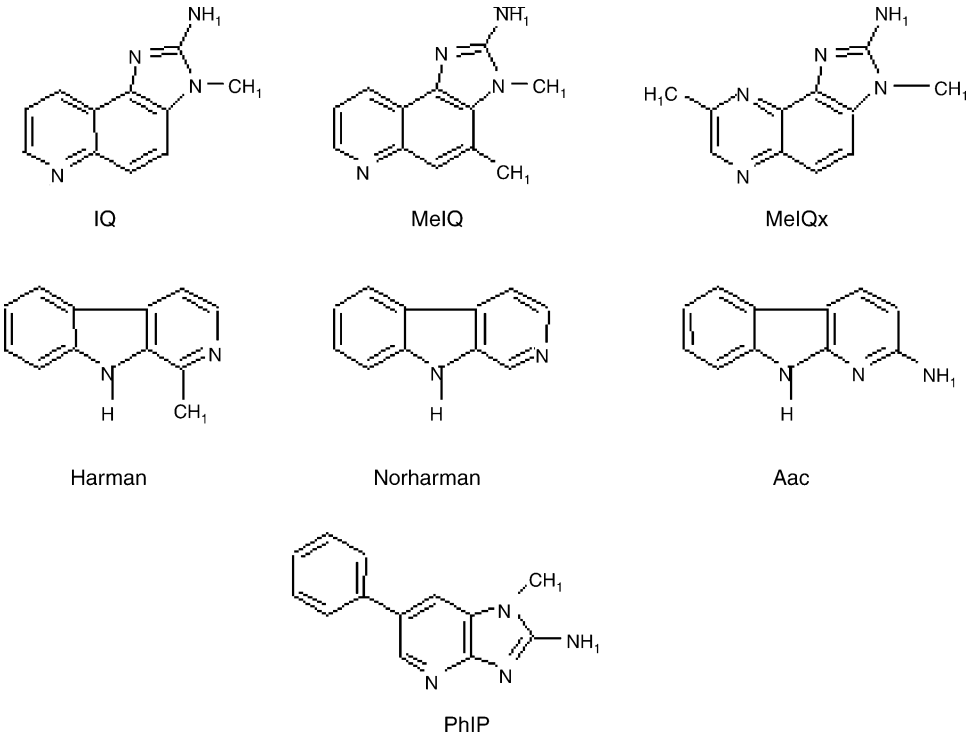

Fig. 1. Chemical structures of heterocyclic amines investigated.

of basic compounds because of the improvement in selectivity and peak shape of ionizable solutes. In spite of this, to our knowledge this mechanism has been scarcely investigated for separation of HAAs [16]. In that paper the development of a method for determination of some HAAs by ion-pair chromatography on a new phenyl-hexyl stationary phase with coulometric electrode array detection is described [16].

In the past few years many papers dealing with the analysis of these mutagenic compounds by LC coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) have been published [5,7,8,10,12–17]. In most of these studies, LC has been performed under RP conditions using buffered mobile phases, salts used as buffers likely acting as ion-pair reagents. However, taking into account that in LC–MS systems the performance of electrospray (ESI) and atmospheric pressure chemical ionization (APCI) is known to be affected by the solvent composition, scarce attention has been paid to the evaluation of the ion pair reagent capable of providing the highest sensitivity when MS and MS/MS detection is used.

In the present investigation we studied the influence of the concentration of a common volatile ion pairing reagent suitable for LC–MS separation and the percentage of the organic modifier on the retention of seven HAAs encompassing quinoline (IQ, MeIQ), quinoxaline (MeIQx), pyridine (PhIP) and carboline derivatives (AαC, Harman, Norharman) (Fig. 1). With the aim of acquiring a better insight into the mutual influence of these chromatographic factors upon solute retention, the study was performed by using an experimental design approach able to evidencing the effect of the simultaneous variation of the two factors. Further, the chromatographic behavior of the analytes using the formic acid-ammonium formate buffer was compared with that observed using acetic acid-ammonium acetate buffer in the mobile phase. LC–MS/MS method based on the use of formate-based eluent exhibited higher detectability with respect to that achieved using the acetate buffer.

2. Experimental

2.1. Chemicals

Harman (2-methyl-β-carboline) and norharman (9H-pyrido[3,4-*b*] indole) were from Sigma–Aldrich (Germany). IQ (2-amino-3-methyl-3H-imidazo[4,5-*f*]quinoline), MeIQ (2-amino-3,4-dimethyl-3H-imidazo[4,5-*f*]quinoline), MeIQx (2-amino-3,8-dimethyl-3H-imidazo[4,5-*f*]quinoxaline), PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), AαC (2-amino-9H-pyrido[2,3-*b*]indole) were from Toronto Research Chemicals Inc. (Canada).

Stock standard solutions of HAAs at concentrations of 0.5 mg/ml were prepared in methanol and stored in the dark at 4 °C. Working standard solutions were prepared daily by diluting stock solutions with HPLC-grade water. Ammonium formate, formic acid, ammonium acetate, acetic acid, HPLC-grade acetonitrile and HPLC-grade water were from Carlo Erba (Milan, Italy).