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Evaluation of volatile ion-pair reagents for the liquid chromatography–mass spectrometry analysis of polar compounds and its application to the determination of methadone in human plasma

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Abstract

A liquid chromatography method using volatile ion-pairing reagents and tandem mass spectrometry was developed to obviate observed matrix effect for ionizable polar compounds. The present study investigated the addition of volatile ion-pair reagents to the reconstitution solution instead of the mobile phase to enhance the efficiency of chromatographic separation and minimize the sensitivity loss due to the formation of ion-pairs. The volatile ion-pair reagents used were perfluorinated carboxylic acids with *n*-alkyl chains: heptafluorobutanoic acid (HFBA), nonafluoropentanoic acid (NFPA), tridecafluoroheptanoic acid (TDFHA) and pentadecafluorooctanoic acid (PDFOA). The model analytes evaluated were *N*-methylnicotinamide (MNA) chloride, *N*-methyl 2-pyridone 5-carboxamide (2PY) and phenylephrine. The effects of alkyl chain length and the concentrations of the ion-pair reagents on the retention of analytes were studied, as well as the effect of pH on the retention of phenylephrine. The volatile ion-pair reagents in the reconstitution solution showed significant effect on the retention of the ionizable polar compounds, and the sensitivity of detection was improved for plasma samples through decreasing the matrix effect. This methodology was successfully applied to establish a quantitative assay for the polar drug substance methadone in human plasma with a concentration range from 0.1 to 50 ng/mL. Ion-pair reagents not only shifted the retention time but also reduced the carry-over peak for methadone.

Keywords: LC–MS; Polar compounds; Sensitivity enhancement; Volatile ion-pairing reagents

1. Introduction

Small organic molecules are commonly separated by reverse-phase liquid chromatography (RP-LC). However, conventional reverse-phase liquid chromatography often lacks the ability to adequately retain small polar molecules. The drawbacks of inadequate retention when using high performance liquid chromatography coupled with mass spectrometry (HPLC/MS) are significant: not only can polar interference peaks co-elute with the analyte; but unresolved endogenous species from the sample matrix can lead to ion suppression and unreliable quantitation [1]. In order to increase the retention of polar molecules, the compounds may require time-consuming derivatization procedures or the use of hydrophilic interaction chromatography (HILIC). HILIC, which employs polar stationary phases (such as silica, cyano, diol or amino column) and aqueous-organic mobile phases; although HILIC is frequently used in bioanalytical applications recently [2], poor retention and peak shape sometimes can be observed. Another alternative approach

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to effectively increase the retention of ionizable polar compounds is ion-pair chromatography. Traditional ion-pair chromatography uses either alkyl sulfonates for increased retention of protonated bases and other cations, or tetra alkyl ammonium salts for the increased retention of ionized acids and other anions [3–6]. However, these ion-paring reagents are not volatile and are therefore not compatible with mass spectrometry (MS). Extensive use of HPLC/MS for a wide variety of pharmaceutical separations suggests the importance of using volatile ion-pairing reagents for the separation of small ionizable polar molecules. Triethylamine, dibutylamine, tetrabutylammonium acetate and other agents [7–9] have been used as ion-pairing reagents for native or chemically modified oligonucleotides and other anions, such as sodium borocaptate, in LC/MS in the negative ion-detection mode. Perfluorinated carboxylic acids are ion-pairing reagents for cations and have been applied to the analysis of underivatized small peptides [10–12]. Due to the formation of ion pairs between the analyte and ion-pairing reagent, ionization efficiency can be impaired and poor detection sensitivity might be observed. A well-known instance is that severe ion suppression is noticed when using trifluoroacetic acid (TFA) to improve the separation of amino acids and some small peptides. Post-column infusion of isopropanol and propionic acid has been used to address this problem, which is referred to as the 'TFA Fix' [13]. Ion suppression was also observed for other alkyl chain perfluorinated carboxylic acids [14]. Kwon and Moini used atmospheric chemical ionization MS, which relies on gas-phase reactions rather than solution ionization, to overcome this problem when using NFPA as a mobile-phase additive [15]. Keever et al. employed a post-column infusion of propionic acid to minimize the electrospray signal suppression of HFBA for the quantitative determination of ceftiofur in milk [16].

Most of the applications involving volatile ion-pairing reagents with LC/MS have been limited to biomolecules such as oligonucleotides and amino acids. Applications to polar pharmaceuticals have not been extensively investigated, although it is necessary to develop approaches to achieve appropriate retention that allows sensitive quantification in biological matrices. In the present study, various perfluorinated carboxylic acids, HFBA, NFPA, TDFHA and PDFOA, were evaluated as ion-pairing reagents. *N*-Methylnicotinamide (MNA) chloride, *N*-methyl 2-pyridone 5-carboxamide (2PY) and phenylephrine (Fig. 1) were selected as the model analytes and analyzed in biometric. MNA, a metabolite of niacin [17], is a quaternary ammonium that is ionized in the mobile phase at pH 1–13; by contrast, its methabolite 2PY is a weak acid that is not ionized in the normal pH range. It is necessary to detect MNA and 2PY simultaneously to understand the pharmacokinetic profile of niacin. Phenylephrine, which is used for the temporary relief of sinus congestion caused by allergies or colds, is an amphoteric compound with dissociation constants of 8.9 (–OH) and 10.1 (–NH). In acidic conditions, the secondary amino group can be ionized. When using the typical RP separation, the retention times of these polar compounds were less than 1.2 min and ion suppression matrix effect was observed. Although reverse-phase ion-pair HPLC analysis of MNA and phenylephrine has been reported [18–20], these separation methods

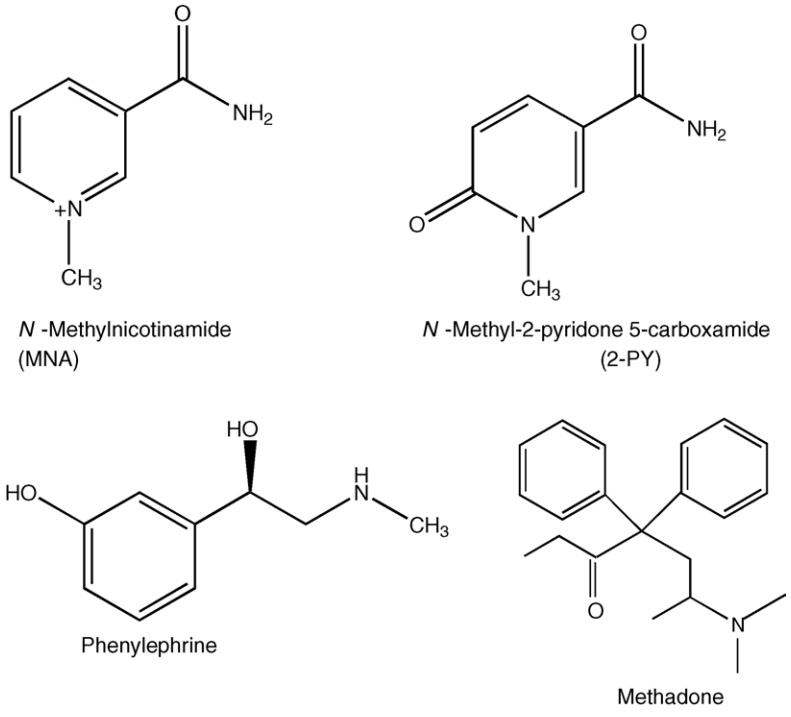

Fig. 1. Chemical structure of the three model compounds and methadone.