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Systematic comparison of sensitivity between hydrophilic interaction liquid chromatography and reversed phase liquid chromatography coupled with mass spectrometry☆

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ABSTRACT

Hydrophilic interaction liquid chromatography (HILIC) appears as a promising strategy to increase sensitivity with electrospray ionization source (ESI/MS). In the present study, peak heights, background noises and signal-to-noise ratios (S/N) obtained with HILIC–MS/MS and RPLC–MS/MS conditions were systematically compared using a dataset of 56 basic drugs possessing diverse physico-chemical properties. Various mobile phase conditions were investigated, including different pH (3 and 6 in HILIC; 3, 6 and 9 in RPLC) and flow rates (300, 600 and 1000 μL/min). The average gain in sensitivity obtained between HILIC and RPLC was equal to 7 and 10 at pH 3 and 6, respectively. However, this value was not reliable, since it was altered by a few compounds possessing an "extreme" behaviour (gain in sensitivity from 100-fold to >8000-fold better). Then, the median gain in sensitivity, equal to 4 in our case, whatever the pH, should be considered. For about 90% of the tested compounds and analytical conditions, the best S/N was systematically attained under HILIC mode. Thanks to PCA representation, it was shown that the basic compounds with pKa between 6 and 8 generally had the best sensitivity in HILIC at pH 6, while the best sensitivity for basic analytes possessing pKa higher than 8 was usually obtained in HILIC at pH 3. As previously reported, the sensitivity gain in HILIC vs. RPLC was explained by the difference in acetonitrile concentration at elution (in average 29% ACN in RPLC and 82% ACN in HILIC at pH 6) leading to better analytes' desolvation. However, it seems that this high proportion of solvent also favourably influenced the ionization by modifying pH and pKa. Indeed the weakest bases of our training set of compounds (pKa between 2 and 5) showed an unexpectedly strong gain in sensitivity, between 20 and 100-fold in comparison to RPLC. These results prove that the ionic character of analytes in solution (i.e., pKa and pH) and the ionization mechanism (i.e., proton transfer) also play an important role for explaining the sensitivity enhancement in HILIC.

1. Introduction

Liquid chromatography coupled to mass spectrometry (LC–MS) has become the technology of choice for drug discovery and bioanalysis, due to ease of method development, high specificity and sensitivity [1–3]. Nowadays, limits of quantitation (LOQ) in the range of 10–100 pg/mL are routinely obtained using LC coupled to adapted detection devices such as the well-known triple quadrupole devices (QqQ) [2]. However, in drug discovery many metabolites have to be determined at very low concentration and then, there is a need to further enhance sensitivity [4]. There are various available solutions to decrease LOQ in LC–MS: (i) enhance analyte ionization efficiency, through the selection of proper ESI parameters, including desolvation temperature, gas flow or capillary voltage settings, (ii) change mobile phase pH, flow rate and organic modifier nature and proportion to modify ionization efficiencies and chromatographic performance [2,5,6], (iii) increase chromatographic resolution to obtain sharper peaks and reduce co-elution with endogenous compounds from the matrix [7,8], (iv) use a more sensitive MS device, although this solution remains expensive [9].

Hydrophilic interaction liquid chromatography (HILIC) appears as an alternative approach to reversed-phase liquid chromatography (RPLC) for improving sensitivity with MS detection. HILIC refers to the combination of polar stationary phase and an aqueous-polar organic solvent mobile phase containing an important proportion (>60%) of organic modifier (usually acetonitrile). The retention mechanism is principally based on the partition of analyte between a water-rich layer formed at the surface of the stationary phase and

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the bulk mobile phase [10,11]. Hydrogen bonding, dipole–dipole interaction and ion exchange are also involved in the interaction mechanism. HILIC can be applied for the analysis of hydrophilic analytes, but also for a wide range of charged ionizable compounds [12–16]. Conversely, non-polar neutral compounds have generally a limited retention due to the lack of ionic interactions. The highly volatile organic mobile phase in HILIC condition provides, not only low column backpressure, but also higher spraying and desolvation efficiency, leading to a sensitivity increase in ESI-MS [11,17,18], but also with other detectors based on nebulization/evaporation processes, such as evaporative light scattering detector (ELSD) and corona charged aerosol detector (CAD) [19,20]. Naidong et al. [21] compared LC–MS/MS sensitivity improvement for seven polar compounds and reported a gain in sensitivity ranging from 5 to 8-fold for the four basic analytes and up to 20-fold for the three acidic compounds. Other groups also demonstrate sensitivity enhancement under HILIC condition for most of the tested polar compounds [19,22–25]. However, in these studies, only a limited number of model compounds were selected.

In the present work, the peak heights, background noise and signal-to-noise ratio (S/N) obtained under HILIC and RPLC conditions were systematically compared using a large set of 56 representative basic drugs. These compounds cover a broad range of lipophilicity and ionization constants. Various chromatographic parameters were investigated, including mobile phase pH (3 and 6 in HILIC; 3, 6 and 9 in RPLC) and flow rates (300, 600 and 1000 μL/min). Only columns packed with sub-2 μm particles (UHPLC column type) were employed, since this state-of-the-art technology gives access to superior resolution, as well as lower analysis time and solvent consumption than conventional HPLC [26]. Columns packed with sub-2 μm particles were particularly relevant in HILIC due to the reduced pressure drop observed with highly organic mobile phase (low viscosity), leading to a better compatibility with LC instruments. In addition, frictional heating effects which may be detrimental in RPLC with sub-2 μm particles might be significantly reduced in HILIC, since the observed pressure drops were in average 2–3 times less than in RPLC. As example, using columns of 50 mm × 2.1 mm, 1.7 μm, the pressure drop was only equal to 150–350 bar under HILIC conditions at 500 μL/min.

2. Experimental

2.1. Chemical and reagents

Water was obtained from a Milli-Q Water Purification System from Millipore (Bedford, MA, USA). Acetonitrile (ACN), methanol (MeOH), formic acid and acetic acid were of ULC–MS grade and purchased from Biosolve (Valkenswaald, Netherlands). Ammonium hydroxide was from Sigma–Fluka (Buchs, Switzerland).

Formate buffer 10 mM (pH 3) was prepared with an adapted volume of formic acid and pH was adjusted to 3.0 with ammonium hydroxide 28%. Acetate buffer 10 mM (pH 6) was prepared with an adapted volume of acetic acid and pH was adjusted to 6.0 with ammonium hydroxide 28%. Ammonium buffer 10 mM (pH 9) was prepared with an adapted volume of ammonium hydroxide 28% and pH was adjusted to 9.0 with formic acid.

2.2. Pharmaceutical compounds dataset

The training set of 56 basic compounds, covering a broad spectrum of pKa (calculated basic pKa values vary from 6 to 11, with only rare exceptions in the 2–6 range) and log *P* (calculated log *P* values vary from −1.2 to 5.6), as shown in Fig. 1, included the following drugs: 6-monoacetylmorphine, acebutolol, adenosine, alprazolam, alprenolol, amphetamine, antipyrine, bisoprolol, buprenorphine, bupropion, buspirone, clonidine, cocaethylene, cocaine, codeine, dextromethorphan, dibucaine, dihydrocodeine, diltiazem, doxepin, fentanyl, flurazepam, heroin, hydroxyzine, imipramine, ketamine, lidocaine, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethamphetamine (MDEA), methadone, methamphetamine, methylephedrine, naloxone, naltrexone, norcocaine, norephedrine, nortriptyline, noscapine, papaverine, perphenazine, pethidine, pindolol, prilocaine, propranolol, pseudoephedrine, pyrilamine, salbutamol, sulpiride, terfenadine, tetracaine, thebaine, tolazoline, tramadol, triazolam and triprolidine. These compounds were purchased from Sigma–Aldrich (Steinheim, Germany) or Lipomed (Arlesheim, Switzerland).

Stock solutions of each individual sample were prepared at 1 mg/mL in pure MeOH and the final concentrations were selected to have similar signal to noise ratios for all compounds at pH 3 in RPLC. Because of the additional selectivity from MS device, two mixtures of compounds were prepared and 2 μL of each mixture was injected on our UHPLC–MS/MS platform. The first mixture contained 28 drugs including adenosine, alprenolol, bupropion, heroin, norephedrine, perphenazine, propranolol, terfenadine each at 1 μg/mL; alprazolam, amphetamine, dihydrocodeine, thebaine each at 500 ng/mL; dextromethorphan, methylephedrine, pseudoephedrine each at 200 ng/mL; dibucaine, doxepin, fentanyl each at 100 ng/mL; buspirone, MBDB, norcocaine, pethidine, prilocaine, sulpiride, tramadol each at 50 ng/mL and naloxone, diltiazem and lidocaine at 400, 30 and 10 ng/mL, respectively. The second one contained 28 drugs including clonidine, naltrexone, triazolam and triprolidine each at 500 ng/mL; 6-monoacetylmorphine, antipyrine, MDA, tolazoline each at 400 ng/mL; MDEA, methamphetamine, pindolol, pyrilamine each at 200 ng/mL; cocaethylene, flurazepam, hydroxyzine, ketamine, methadone, noscapine, salbutamol each at 100 ng/mL; cocaine, papaverine, tetracaine each at 50 ng/mL; imipramine, acebutolol each at 25 ng/mL; and nortriptyline, buprenorphine, codeine and bisoprolol at 2, 1.5, 1 μg/mL and 5 ng/mL, respectively. Both samples were analyzed in the positive ESI mode, using SRM experiments. These mixtures were prepared using at least 95% ACN or water within the sample diluents for HILIC and RPLC conditions, respectively, to attain reasonable peak shape [27]. No solubility issue occurred, because of the low drugs concentrations within the sample mixture.

2.3. Instrumentation

The chromatographic experiments were performed using an Acquity Ultra Performance Liquid Chromatography (UPLC™) system from Waters (Milford, MA, USA). This instrument was equipped with a binary solvent manager with a maximum delivery flow rate of 2 mL/min, an autosampler with a 2 μL loop operating in the full loop injection mode, and a column manager composed of a column oven, set at 40 °C. This UHPLC system was hyphenated with a Waters TQD triple quadrupole mass spectrometer fitted with a Z-spray electrospray ionization (ESI) source. The ESCi® ionization source operated in the ESI positive mode and Selected Reaction Monitoring (SRM) was performed. Nitrogen was used as drying gas.

In a first instance, the ionization source parameters were optimized in HILIC and RPLC, using a mixture of 4 model compounds (i.e. norcocaine, clonidine, adenosine and doxepine). The source temperature, cone gas flow and source extractor voltage were identical in both modes (120 °C, 20 L/h and +3 V, respectively). The capillary voltages in HILIC and in RPLC were set at +3 kV and +2 kV, respectively. Due to the higher proportion of organic modifier in the HILIC mobile phase, the desolvation gas temperature in HILIC and RPLC modes were set at 350 and 450 °C, respectively, while the gas flows were set at 600 and 800 L/h, respectively.