



Volatility Evaluation of Mobile-Phase/Electrolyte Additives for Mass Spectrometry

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In this study evaporative light scattering detection is used to evaluate the volatility of several volatile acids, bases and corresponding salts. Depending on their volatility the acids, bases and their mixtures are classified in four categories: non-volatile, low volatility (volatile up to 5 mM), medium volatility (volatile up to 25 mM) and high volatility (volatile at least up to 100 mM).

Introduction

Until the mid-1980s the only valid reason for using a volatile mobile phase in high performance liquid chromatography (HPLC) was for purification purposes as the collected mobile phase could easily be removed by freeze drying.^{1,2} However, the commercial introduction of powerful new detectors based on electrospray mass spectrometry (ESI-MS), evaporative light-scattering detection (ELSD) and chemiluminescent nitrogen detection (CLND), which all require volatile mobile phases, changed the analytical approach to mobile-phase selection.

In particular, the couplings of capillary electrophoresis (CE) or LC with ESI-MS are now the methods of choice for polar, low-volatility and/or thermolabile compounds. One of the major parameters to consider with the above hyphenations is the volatility of the mobile phase/electrolyte used. Non-volatile mobile phases/electrolytes lead to

- decreased signal intensities and signal-to-noise ratios
- pollution of mass spectrometers resulting in source blockages, decreasing the sensitivity of the system during this period and seriously affecting the accuracy of quantitative results.³⁻⁵

Two major methods have been presented to overcome these problems. The first uses on-line membrane suppressors^{3,6-9} or different valve switching techniques^{10,11} to remove/replace

non-volatile counter-ions after separation and prior to detection. These techniques have been successfully used for the removal of non-volatile salts/ion-pairing reagents (usually strong electrolytes), such as phosphate anion, alkanesulfonate anion, sodium cation and tetraalkylammonium cation.^{3,6-11} However, these techniques present several drawbacks: they usually result in loss of chromatographic resolution because of adsorption or retention by the suppressor or columns used, and they may decrease analyte signal, complicate instrumentation and make automation difficult.⁹

The second involves the substitution of non-volatile additives with volatile ones. The only problem here is that the additive nature and concentration may change chromatographic retention and resolution even if the pH of the mobile phase/electrolyte is held constant. It must be pointed out here that advances in chromatographic supports using high-purity silicas and new “endcapping” methods allow symmetrical, high theoretical plate peaks to be retained after the substitution of “phosphate-like” additives with volatile ones.

Early evaluations of volatile mobile-phase additives used formate, acetate, carbonate and bicarbonate ammonium salts for coupling LC with thermospray mass spectrometry.¹²⁻¹⁵ However, electrolyte volatility was estimated only through the boiling point of the mobile-phase additives, the monitoring of

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mass spectrometry pollution and long-term signal stability of test solutes. Furthermore, several papers have been dedicated to the evaluation of HPLC and CE volatile eluents, and electrolytes compatible with ESI–MS, in relation to the separation efficiency of biological compounds, such as proteins, drugs etc.^{16–18}

Recently, perfluorinated carboxylic acids were evaluated as volatile anionic ion-pairing reagents for the chromatographic separation of underivatized amino acids and small peptides, with silica- and carbon-based stationary phases.^{19–24} Several aliphatic amines were also evaluated as volatile cationic ion-pairing reagents for the chromatographic separation of inorganic anions, organophosphoric acids, sulfobutyl ether- β -cyclodextrins and amino acids.^{25–27} All the above compounds contain no/weak UV chromophores, so ELSD was used for their detection. Eluent volatility was confirmed by ELSD as this

detection technique requires a volatile mobile phase to avoid high background noise. Indeed, the diverse capabilities of ELSD and its chromatographic requirements, identical to those of MS, make it an inexpensive way to develop LC methods directly transposable to LC–ESI–MS.^{20,28,29}

In this study the inherent ability of ELSD to respond to the presence of semi-/non-volatile compounds is used for the volatility evaluation of 10 acids, 10 bases and their corresponding salts.

Experimental

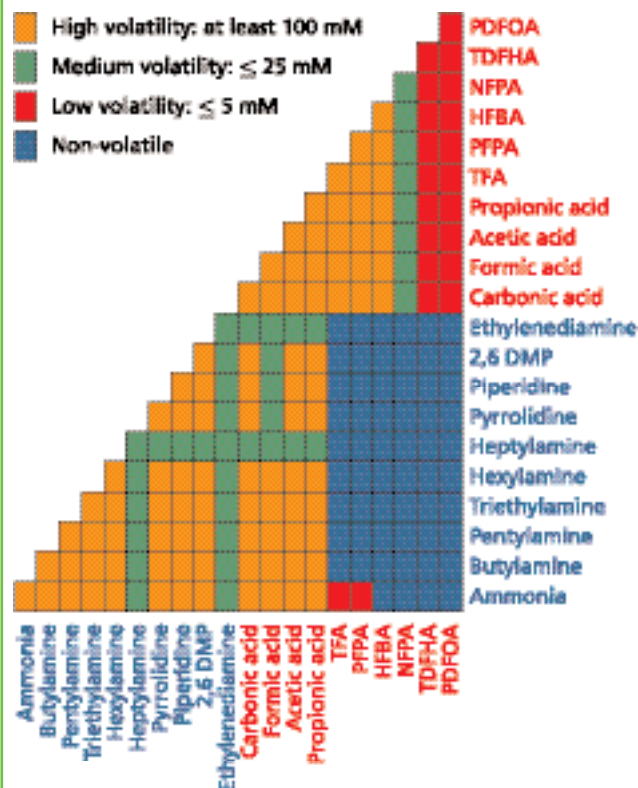
Reagents: All reagents were purchased from Sigma (St. Quentin, Fallavier, France), Aldrich (St. Quentin, Fallavier, France) or Interchim (Montluçon, France), and were of analytical grade if available, or of the highest available purity (at least 96%) if not. The 10 acids tested were carbonic, formic, acetic, propionic, trifluoroacetic (TFA), pentafluoropropionic (PFPA), heptafluorobutyric (HFBA), nonafluoropentanoic (NFPA), tridecafluoroheptanoic (TDFHA) and pentadecafluorooctanoic (PDFOA). The 10 bases tested were ammonia, butylamine, pentylamine, triethylamine, hexylamine, heptylamine, pyrrolidine, piperidine, 2,6 dimethyl piperidine (2,6 DMP) and ethylenediamine. All salts were aqueous equimolar mixtures of acids and bases except for the formate, acetate and carbonate ammonium, which were commercially available salts. All the other carbonate salts were obtained by gaseous CO₂ saturation in the base solution thermostated at 0 °C in a water bath. All the possible combinations, acid, base, salts, as well as acid–acid and base–base were tested. The volatility of all the acids, bases and salts were tested up to 100 mM as this concentration is considered as the upper level used with mass spectrometry. PDFOA is not soluble at 25 mM in water. The solubilization of 5 mM of TDFHA and PDFOA in water was aided by sonication. These were the only reagents of which mixtures with other acids and bases were tested up to 5 mM. 18 M Ω deionized water was delivered by an Elgastat UHQ II system (Elga, Antony, France) and was used as HPLC-grade eluent and for the preparation of the different mobile phases.

Apparatus: An ELSD model Sedex 55 was used for the study set as follows; gain = 7, gas pressure = 2.5 bar, evaporative tube temperature = 60 °C. A Beckman (Fullerton, California, USA) model 128 system gold binary pump was used for the mixture of mobile phase A (100% water) and mobile phase B (additive at 100 mM in water). Once an additive had been tested, the system was cleaned for approximately 15 min with water. No chromatographic column was used. However, adequate tubing (fused silica) was used to increase background pressure to 50 bar and, consequently, to regulate the mobile-phase flow-rate to eliminate pulsation. The flow-rate was 1 mL/min.

Discussion

All the acids and bases tested in this study were at least volatile at 2 mM; the additives most frequently used with MS are also included. Some more acids and bases were also tested but were eliminated either because they were non-volatile or because of solubility problems with water (especially the organic bases). The volatilities of these additives (and their combinations) were tested at 5, 25 and 100 mM. The ELSD background noise induced by the additives was measured in triplicate and if it exceeded 1.5 mV it was considered non-volatile.

Figure 1: Volatility evaluation of potential mobile-phase/electrolyte additives.



TFA = trifluoroacetic acid, PFPA = pentafluoropropionic acid, HFBA = heptafluorobutyric acid, NFPA = nonafluoropentanoic acid, TDFHA = tridecafluoroheptanoic acid, PDFOA = pentadecafluorooctanoic acid, 2,6 DMP = 2,6 dimethyl piperidine.

Figure 1 summarizes the results obtained. Non-volatile mixtures of additives are shown in blue, low volatility (≤ 5 mM) in red, medium volatility (≤ 25 mM) in green and high volatility (~ 100 mM) in orange. As can be seen, almost all combinations of perfluorinated carboxylic acids with bases resulted in non-volatile salts. The highest homologues of perfluorinated carboxylic acid, TDFHA and PDFOA, cannot be used in concentrations higher than 5 mM. It must be pointed out here, however, that low concentrations (0.5 mM of PDFOA or 1 mM of TDFHA) were enough to provide retention and high liquid chromatographic selectivities among very polar underivatized amino acids.^{19,20} Perfluorinated carboxylic acids can be used as anionic volatile ion-pairing reagents in the place of the non-volatile alkylsulfonates.

Heptylamine and ethylenediamine were the least volatile bases, although their mixtures with carbonic, formic, acetic and propionic acids are volatile, allowing their buffering. Though aliphatic amines are volatile and can be used instead of tetrabutylammonium, they are not strong bases. As a result, the pH of the mobile phase should be controlled in such a way as to allow the protonation of these amines in order to obtain chromatographic retention of negatively charged compounds.

In general, higher homologues of compounds of the same family are less volatile with the exception of formic and acetic acid. Indeed, acetate salts are more volatile than formate salts. Finally, most of the acids/bases/salts in orange were still volatile at 100 mM.

It is important to note that the nebulizer characteristics (droplet size distribution), the filter of big droplets (glass chamber) and the % of aerosol directed towards the drift tube influence the vaporization process at low temperatures. Changes in the flow-rate of the mobile phase as well as the drift tube characteristics (length, speed of aerosol) may slightly modify the present results. Nevertheless, several of the mobile phases presented here have been tested with LC-MS and found to be volatile (no salts deposit in the curtain plate after several hours of pumping), confirming the accuracy of the presented results.

Conclusions

This is the first study in which a quantitative approach is applied to the volatility of mobile-phase/electrolytes additives. Acids, bases and their corresponding salts are divided into four categories depending on their volatility. It is demonstrated that perfluorinated carboxylic acids cannot be buffered with any base as they form non-volatile salts. Strong anionic non-volatile ion-pairing reagents such as alkylsulfonates can be replaced by perfluorinated carboxylic acids, while the cationic ion-pairing reagent tetralkylammonium cation can be replaced by aliphatic amines. The list of additives tested in this study is not an exhaustive one from a volatility point of view and organic synthesis could provide us with new volatile ionic/ionizable acids or bases. As a result, this article could be updated in the future.

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