



# Performance of amines as silanol suppressors in reversed-phase liquid chromatography



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## ABSTRACT

In reversed-phase liquid chromatography, cationic basic compounds yield broad and asymmetrical peaks, as a result of their ionic interaction with the anionic free silanol groups present in the silica-based stationary phases (commonly derivatised with C18 groups). A simple way to improve the peak shape is the addition to the hydro-organic mobile phase of a reagent (usually called additive) with cationic character. This associates with the stationary phase to prevent the access of analytes to the free silanol groups. Cationic additives may interact electrostatically with the anionic silanols. The hydrophobic region of the additive may also associate with the alkyl chains bound to the stationary phase, with the positive charge oriented towards the mobile phase. The access to the silanol groups is thus blocked, but in turn, the stationary phase is positively charged and will repel the protonated basic compounds, which unless their polarity is sufficiently low, will elute at very short times. In this work, a comparative study of the performance of a group of amines (butylamine, pentylamine, hexylamine, cyclopentylamine, cycloheptylamine, *N,N*-dimethyloctylamine and tributylmethylammonium chloride), as modifiers of the chromatographic behaviour of basic compounds, is carried out. The behaviour is compared with that obtained with the ionic liquids 1-butyl-3-methylimidazolium chloride and 1-hexyl-3-methylimidazolium chloride, used as additives. The study revealed that the performance of the cationic additives to block the silanol activity is mainly explained by the additive size and its ability to be adsorbed onto the stationary phase.

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## 1. Introduction

A vast majority of alkyl-bonded stationary phases in reversed-phase liquid chromatography (RPLC), using silica as support, corresponds to the group known with the general term octadecylsilane or C18. These columns are popular because they are able to separate a wide variety of compounds through a simple retention mechanism based on solute adsorption on the alkyl-bonded stationary phase [1,2]. Consequently, the more hydrophobic the compound, the longer is its retention. Unfortunately, owing to steric problems in the derivatisation process of silica supports, which varies with the brand and manufacturer, free silanol groups remain on the stationary phase in a non-negligible amount. When these silanols are negatively charged, they give rise to additional ion-exchange interactions with cationic compounds, which increases their retention. Sorption-desorption kinetics of this process is slow, which yields tailed and broad peaks and

affects the peak resolution [3–5]. Most compounds used in the pharmaceutical industry, or compounds with biomedical and biological importance, are bases positively charged at the usual working pH range of typical RPLC columns ( $2 < \text{pH} < 8$ ), which are exposed to silanol interaction. In consequence, the minimisation and/or suppression of the “silanol effect” has been of special consideration throughout the development of RPLC [6,7].

Several strategies have been proposed to reduce or suppress the undesirable effects of residual silanols on the chromatographic performance of basic compounds. One of the most outstanding solutions is the development of a new generation of stationary phases based on “ultrapure” silica, where the influence of surface silanols on the retention of basic compounds is less pronounced [8,9]. However, some tailing is still observed with these compounds, even for the most modern columns. Therefore, alternative solutions are being developed to avoid the silanol effect. Two of the most extended practices are: (i) buffering the pH between 2.5 and 3.5 to protonate the residual silanols, and (ii) masking the silanol interaction through the addition to the mobile phase of

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ionic (cationic or anionic), or even non-ionic reagents, able to be adsorbed on the stationary phase.

Silanol suppression with the assistance of additives is experimentally rather simple, since it does not require the use of additional or alternative hardware (pumps, injectors, etc.), or columns with special characteristics. However, the selection of the most convenient suppressor is not straightforward, due to the wide variety of potential additives and the complexity of the interactions that can take place simultaneously inside the column. These processes may involve direct neutralisation of the anionic silanol sites (only for cationic additives), which blocks ion-exchange processes with analytes, and hydrophobic interaction of the cation or anion in the additive with the alkyl-bonded stationary phase, which creates a charged bilayer that prevents the penetration of the basic compounds to reach the silanol sites. Finally, ion pair formation in the mobile phase can also affect the retention process.

Amines, especially bulky tertiary ones, are among the first assayed mobile phase additives used to mask residual silanols [10–12]. At acidic pH, amines are positively charged and can interact electrostatically with the anionic silanols on the stationary phase. The hydrophobic part of the molecule can also associate with the alkyl-bonded chains bonded to the silica stationary phase, with the ammonium groups oriented away from the surface (Fig. 1a). The access to the silanols is therefore blocked, but the stationary phase adopts a positive charge that repels protonated basic compounds, which elute at short retention times, or even with the void volume. According to Cole and Dorsey [13], “while the choice of stationary phase, organic modifier, and gradient strength can have significant effects on the separations, mobile phase additives can also have a significant effect on the chromatographic selectivity, recovery, efficiency and resolution”. In a pioneer study, these authors demonstrated the beneficial silanol-masking properties of cyclohexylamine compounds (i.e., chloride salts of cyclohexylamine, dicyclohexylamine and dicyclomine) as mobile phase additives, and the cleaner chromatograms in comparison to the most usual triethylamine. These additives were chosen “for their bulky, structurally-rigid cyclohexyl rings, in hopes that better silanol coating could be facilitated by covering the silanol with a bulky substituent, and providing greater stationary phase ordering and coverage”.

More recently, room temperature ionic liquids (RTILs) have been proposed as effective silanol suppressors as an alternative to amines [14–16]. Most RTILs used as additives in RPLC are made of a large imidazolium cation associated with a relatively large anion. Both cation and anion may be able to interact with the stationary phase and participate in the separation process (Fig. 1b). This confers RTILs a dual nature, making the retention mechanism more complex with several equilibria in both stationary and mobile phases [17]. In the literature, RTILs have been considered so satisfactory as silanol suppressors, that the authors frequently comment they can substitute classical amines not only owing to their smaller toxicological or environmental impact, but also because of their superior silanol masking capability [18–22]. However, in a recent study where the silanol suppressing potency of a group of imidazolium-based RTILs was compared with the effect produced by a bulky amine (*N,N*-dimethyloctylamine), highly symmetrical peaks were obtained with the amine, similar to those yielded with some RTILs [23]. Therefore, the performance of basic compounds in the presence of amines deserves a more extensive study.

In this work, a group of amines of different nature are evaluated as mobile phase additives to enhance the chromatographic behaviour of basic compounds. The performance of amines is compared with that obtained with two imidazolium RTILs associated to chloride (an anion without affinity for the stationary phase). The

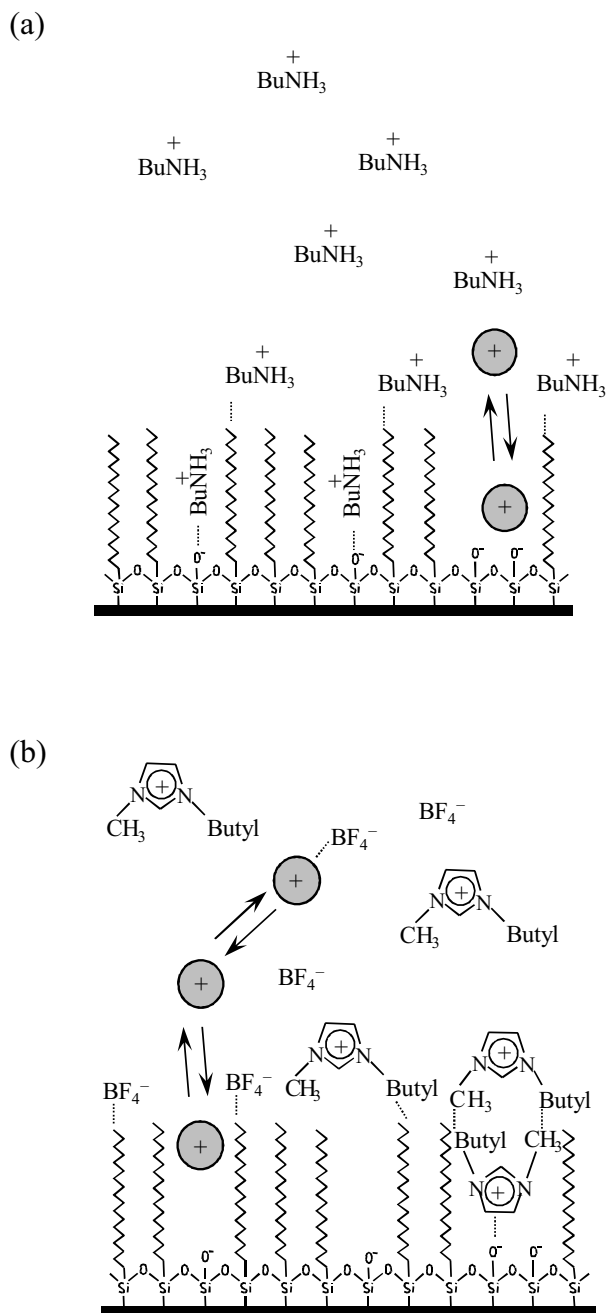


Fig. 1. Solute environment in a C18 stationary phase in the presence of (a) butylamine, and (b) 1-butyl-3-methylimidazolium tetrafluoroborate.

study tries to offer some light on the most suitable additives to analyse basic compounds.

## 2. Experimental

### 2.1. Reagents

Nine  $\beta$ -adrenoceptor antagonists were used as probe compounds: acebutolol, atenolol, carteolol, esmolol, metoprolol, nadolol, oxprenolol, pindolol and timolol (all from Sigma, St. Louis, MO, USA). All these compounds were basic ( $pK_a = 9\text{--}10$ ) [24]. Therefore, at the working pH of the mobile phase ( $\sim 3$ ) they are positively charged. Stock solutions of the drugs were prepared in a small amount of acetonitrile with the aid of an Elmas 15h ultrasonic bath from Elmasonic (Singen, Germany), and diluted with water. The

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