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Article

Ionic Liquids as Mobile Phase Additives for Feasible Assay of Naphazoline in Pharmaceutical Formulation by HPTLC–UV–Densitometric Method

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A specific and reliable high-performance thin layer chromatography method with densitometry detection has been developed for the determination of naphazoline nitrate in nasal drops. The best separation of the basic analyte, without spot tailing, was achieved by using a mobile phase composed of acetonitrile–water (60:40, v/v), adding 1.5 % (v/v) imidazolium-class ionic liquid and covering the plates with a stationary phase based on RP-18 with F_{254S} (10 × 20 cm). The presented results confirm that imidazolium tetrafluoroborate ionic liquids are efficient suppressors of free silanols, which are considered to be responsible for troublesome and irreproducible chromatographic determinations of basic compounds. The developed chromatographic system was found to be convenient in use and to provide a repeatable assay of naphazoline nitrate in nasal drops, which could not be obtained with the use of standard silanol suppressing mobile phase additives such as triethylamine or dimethyloctylamine.

Introduction

Thin-layer chromatography (TLC) used to be a leading chromatographic technique that is still very popular in pharmaceutical analysis. Unfortunately, the chromatographic analysis of basic drugs is often complicated because of the effect of free silanols on retention (1, 2). This is probably due to the ionic interactions of the positively charged analytes with the free silanol groups on silica or alkyl-bonded-silica stationary phases. The ion-exchange interaction causes a strong retention of basic analytes, resulting in poor peak or spot shape and tailing. Hence, the addition of different buffer salts and various amines such as triethylamine (TEA) or dimethyloctylamine (DMOA) is often needed to improve chromatographic results (3).

Studies described in the last decade show that ionic liquids (ILs) can be used in a great variety of applications. Because of their unique and flexible physical and chemical properties, they are innovative solvents and are often considered in green chemistry ideas. Numerous reviews have described recent efforts in the application of ILs in almost all areas of analytical chemistry (4–8). ILs are organic salts with low melting points, called neoteric solvents, that are also finding new applications as additives in chromatographic techniques (9–11). The most commonly employed ILs in liquid chromatography are composed of alkylammonium and imidazolium cations, which are soluble in common chromatographic solvents (12–14). Also, the ILs based on the BF_4^-, Cl^- and $MeSO_4^-$ anions are water-stable compounds, which dissolve in typical chromatographic mobile phases.

Recently, ILs have been proposed as silanol suppressing agents (15, 16). The significant effects of imidazolium-based ILs as mobile phase modifiers in thinlayer chromatography (TLC) and high-performance liquid chromatography (HPLC) on the retention of basic compounds have been studied and described elsewhere (13). The addition of 0.5–2.5% (v/v) of some types of ILs composed of 1,3-dimethylimidazolium, 1-ethyl-3-methylimidazolium, 1-propyl-3-methylimidazolium, 1-butyl-3-methylimidazolium, 1-hexyl-3-methylimidazolium, 1-octyl-3-methylimidazolium or 1-butyl-4-methylpyridinium cations and bromide chlorate, hexafluorophosphate, methyl sulphate, tetrafluoroborate or tosylate anions more markedly decreased the retention of basic analytes than other alkylamines (17–21). The experiments have been evaluated by primarily using octadecyl-silica stationary phases with a strong or moderate silanol activity. The retention mechanism of studied compounds is very complex because of the dual nature of ILs. Both parts of the salts, cation and anion, can affect the chromatographic retention. The oppositely charged ions can cause synergistic or antagonistic effects (22). It has been demonstrated that both cations and anions of ILs can be adsorbed on hydrophobic stationary phases. However, the authors' previous studies demonstrated the significant influence of 1-alkyl-3-methylimidazolium cation on the silanol suppressing properties of ILs (14). The 1-alkyl-3-methylimidazolium tetrafluoroborate IL provided better silanol blocking activity on the bare silica or octadecyl silica TLC plates than ammonia and ternary amines (TEA, DMOA). Hence, the silanol suppressing effect was successfully used for the qualification of basic drugs and for the optimization of separation of peptides in TLC plates (14, 23).

Until now, most studies describing the use of ILs as mobile phase modifiers in LC have been related to qualification analysis with regard to the evaluation of their silanol suppressing potency. The primary aim of this study is the application of previously selected ILs in quantification HPTLC analysis of naphazoline nitrate in nasal drops. Naphazoline nitrate is a basic drug that causes analytical problems during the chromatographic process on silica based stationary phasess. Several methods for the determination of naphazoline and other α-adrenergic agents have been validated and described in literature. Naphazoline, phenylephrine, tetrahydrozoline, tramazoline,

tymazoline and xylomethazoline were determined as single active compounds or mixtures in commercial preparations in nasal and ophthalmic solutions using HPLC and capillary electrophoresis (CE) (24). Also, except for the pharmaceutical compound analysis, the determination of the α-adrenergic drugs in the presence of other chemicals as preservatives in pharmaceutical preparations have been described (25, 26). Numerous silica-based stationary normal and reverse phases have been tested to improve the chromatographic separation and qualification of 2-imidazolidine drugs. However, most of the tested chromatographic systems were based on ammonia and adsorbable amino quenchers (e.g., triethylamine or tetramethylammonium bromide) as mobile phase modifiers to suppress free silanol effects. This study describes an application of imidazolium-based ILs to reduce the deleterious effects of free silanols on LC separation of naphazoline nitrate. Replacement of the commonly used alkylamine additives with the readily available ILs may improve chromatographic separations of the problem-causing chemicals. The use of ILs is justified and can be recommended instead of the currently employed environmentally harmful agents. Finally, in the present study, a novel, simple HPLC–ultraviolet (UV)–densitometric method was developed for the determination of naphazoline nitrate in commercially available pharmaceutical preparation.

Experimental

Chemicals

1-Ethyl-3-methylimidazolium tetrafluoroborate ([emim][BF_4]) and 1-hexyl-3-methylimidazolium tetrafluoroborate ([hmim][BF_4]) were purchased from Fluka Chemika (Buchs, Switzerland) (Figure 1). The reference standard of naphazoline nitrate was acquired from POCh (Gliwice, Poland). Rhinazin drops consisting of 1 mg/mL naphazoline nitrate were from Polfa Warszawa (Warsaw, Poland). Acetonitrile, methanol, sodium chloride, tetrahydrofuran and water were purchased from POCh. The water used in the study was prepared using a Milli-Q Water Purification System (Millipore, Bedford, MA).

Methods

HPTLC was performed on glass plates covered with silica gel plates with F_{254S} and RP-18 with F_{254S} (10cm x 20cm) purchased from Merck (Darmstadt, Germany). Spotting of the analytes onto the HPTLC plates was performed by Desaga AS 30 HPTLC applicator (Wiesloch, Germany) with injection volume of 1 μL/spot and application rate of 14 s/μL. The resulting spot size was 4 mm and the distance between center spots was 13 mm. The distance between the start line and the bend line of the plate was 1 cm.

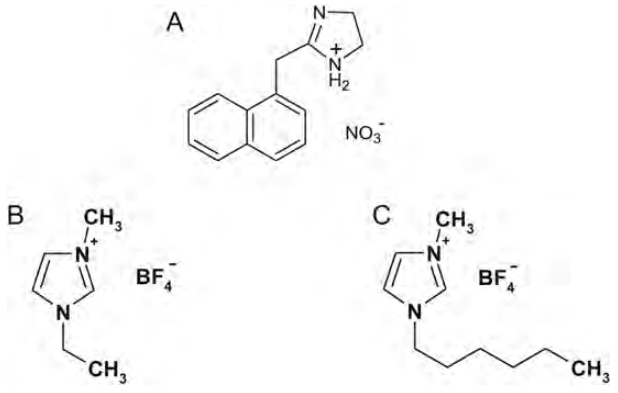

Figure 1. Structural formulas: naphazoline nitrate (A); [emim][BF_4] (B); [hmim][BF_4] (C).

The plates were developed in a horizontal chamber (Modin, Lublin, Poland) that was saturated with the respective mobile phases. The mobile phase consisted of acetonitrile–water (6:4, v/v) with and without the addition of [emim][BF_4] or [hmim][BF_4]. Visualization was performed by a CabUV-Vis (Desaga, Wiesloch, Germany), and documented using Canon Power Shot G5 connected to a PC with ProViDoc 3.0 software (Desaga). The determination of naphazoline nitrate was executed using CD 60 HPTLC densitometer connected to a PC with ProQuant software (Desaga) at a wavelength of 276 nm. The size of the scanning light beam was set at 0.02 mm for the slit height and 0.4 mm for slit width. The 10 mm/s scanning rate was used to obtain resolution of 25 μm.

The wavelength of maximum absorption was determined by a scan of the assayed drug spot (1 μL/spot) on the HPTLC plate (Figure 2).

Measurements of mobile phase UV absorbance were performed using a Shimadzu Prominence system (Kyoto, Japan) composed of a diode array detector (SPD-M20A). The UV spectra of ILs ([emim][BF_4] and [hmim][BF_4]) were investigated by HPLC using acetonitrile–water (60/40 v/v) mobile phase. Studied modifiers showed absorbance maxima at approximately 208 nm, which did not overlap with the maximum absorbance of the naphazoline (276 nm) (Figure 3).

Standard solutions and analytical procedure for the assay of naphazoline nitrate

A stock solution of naphazoline nitrate (1.2 mg/mL) was prepared in methanol. The calibration samples were prepared in methanol in triplicate in the following concentrations: 0.20, 0.30, 0.45, 0.60, 0.75, 0.90 and 1.05 mg/mL. The developed and dried plates were scanned with a densitometer in the absorbance mode at 276 nm. The calibration curves were set by plotting the peak area against the drug quantity per spot. The equations of calibration curves were estimated using linear regression. Naphazoline nitrate nasal drops (750 μL; 1 mg/mL) were diluted with 250 μL of methanol and water, respectively.

Results and Discussion

Studies on the retention behavior of naphazoline nitrate with the use of [emim][BF_4] and [hmim][BF_4] as novel modifiers of the mobile phase were performed in both normal and reversed-HPTLC stationary phase systems. Based on the previously reported results, the optimal composition of the mobile phase for the application of ILs in the chromatography of selected phenothiazine-derived drugs is determined by acetonitrile–water 60/40 (v/v) (14). The concentration of ILs was adjusted by the selection of a retardation coefficient (R_f) value and better shapes of spots. The exemplary HPTLC plates with naphazoline nitrate, developed at different conditions, are

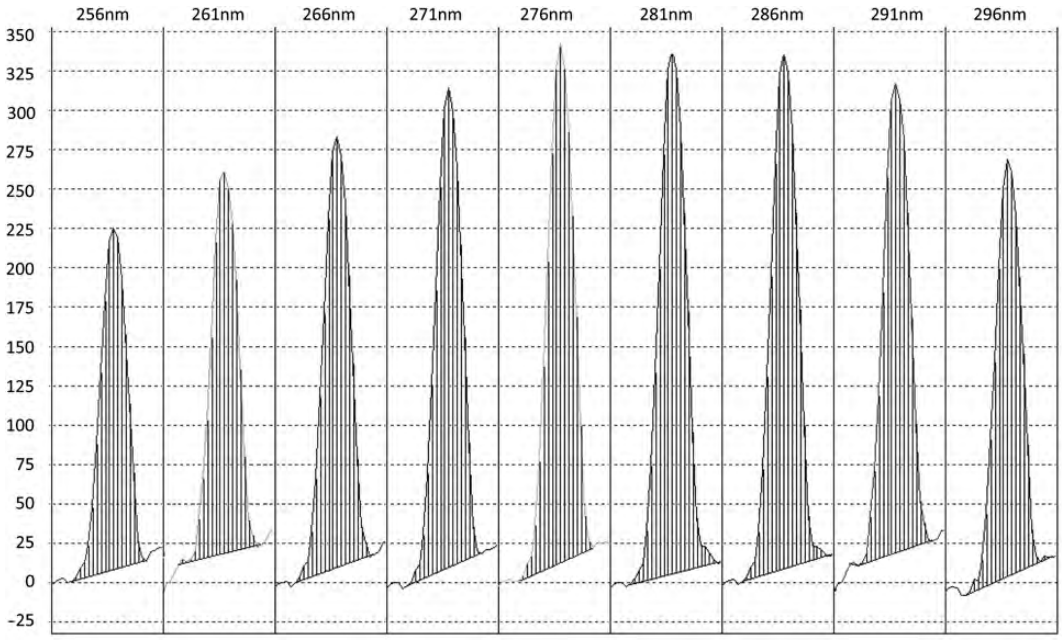

Figure 2. UV–densitometric spectra of naphazoline nitrate determined on an RP-18 HPTLC plate.

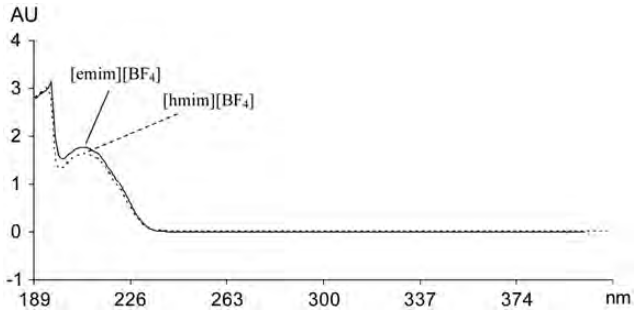

Figure 3. UV spectra of mobile phase modifiers with maximum absorbance at a wavelength of 208 nm.

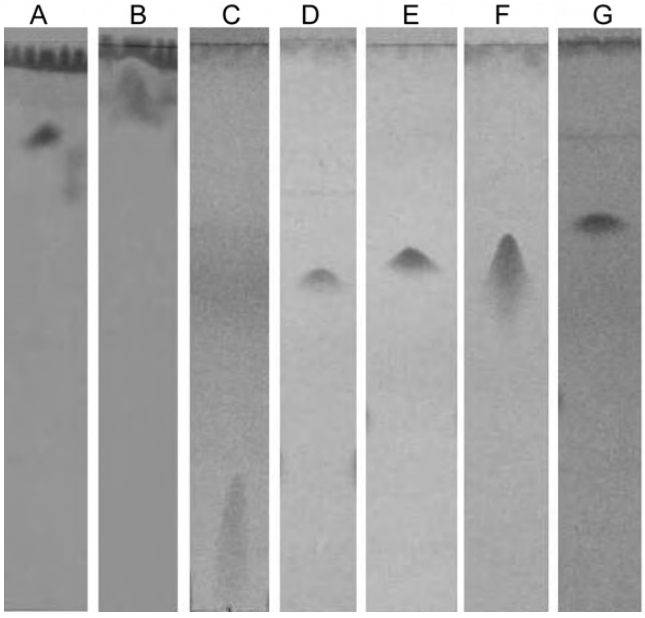

Figure 4. Representative HPTLC chromatograms of naphazoline nitrate from different chromatographic systems: separation on an F_{254} silica plate in acetonitrile–water, 60:40 (v/v) (A); separation on an F_{254} silica plate in acetonitrile–water, 60:40 (v/v) with 1.5% (v/v) of [emim][BF_4] (B); separation on an RP-18 plate with F_{254S} in acetonitrile–water, 60:40 (v/v) (C); the same eluent but with the addition of 0.5% (v/v) [emim][BF_4] (D); the same eluent but with the addition of 1.5% (v/v) [emim][BF_4] (E); the same eluent but with the addition of [emim][BF_4] and 0.5% (v/v) [hmim][BF_4] (F); the same eluent but with the addition of [emim][BF_4] or 1.5% (v/v) (G) of [hmim][BF_4] (G).

presented in Figure 4. The plates were developed with acetonitrile–water eluent, either neat or containing 0.5 and 1.5% (v/v) of [emim][BF_4] and [hmim][BF_4]. The chromatograms in Figures 4A and 4B were obtained using the silica gel and [emim][BF_4]. The addition of IL to the mobile phase significantly distorted the shape of the spots. Therefore, the normal stationary phase was discarded from further study. The next chromatograms show the distinct influence of the proposed modifiers on the retention of the drug. The strong retention of the basic drug with the unmodified mobile phase and spot tailing in the case of an insufficient concentration of IL [0.5% (v/v)] provide evidence for strong silanol interactions with the solute (Figures 4C–G). The shape of the spot is mostly symmetrical, without tailing for an IL concentration of 1.5% (v/v). However, an excess IL concentration over 1.5% (v/v) causes no further improvement in the chromatograms of naphazoline. The similar chromatographic behavior of naphazoline with a regular spot shape was obtained with the use of a mobile phase consisting of tetrahydrofuran–methanol–sodium chloride (58 g/L), 10:45:45 (v/v/v) (chromatogram not given). The comparison of R_f and standard deviations (SD) of

measurements confirmed that the chromatographic systems are suitable for qualification analysis (Table I).

Next, the mobile phases consisted of acetonitrile–water 60:40 (v/v) with 1.5% (v/v) of [emim][BF_4] or [hmim][BF_4] and tetrahydrofuran–methanol–sodium chloride (58 g/L), 10:45:45 (v/v/v) were used to compare applied chromatographic systems for quantitative analysis of naphazoline nitrate. The HPTLC–UV–densitometric method was used at an optimal wavelength of 276 nm to obtain densitograms of naphazoline developed in three different chromatographic systems. The representative chromatogram and densitogram of a developed plate with the use of 1.5% (v/v) of [emim][BF_4] is presented in Figure 5. Parallel with standard solutions, the three spots with both methanolic and aqueous solutions of rhinazin drops were developed on the same plates. The calibration curve equations

Table I
Retardation Coefficients for Naphazoline Nitrate (NT) in Studied HPTLC Systems

Concentration of NT (mg/mL)	Mobile phase A*		Mobile phase B†		Mobile phase C‡	
	R_f mean ($n = 3$)	SD	R_f mean ($n = 3$)	SD	R_f mean ($n = 3$)	SD
0.30	0.597	0.0201	0.700	0.000	0.433	0.022
0.45	0.597	0.0201	0.693	0.015	0.427	0.021
0.60	0.597	0.0201	0.693	0.015	0.427	0.027
0.75	0.597	0.0150	0.690	0.005	0.430	0.023
0.90	0.600	0.0200	0.690	0.015	0.443	0.022
1.05	0.600	0.030	0.687	0.107	0.450	0.017
1.20	0.603	0.0250	0.680	0.011	0.450	0.026

*Acetonitrile–water, 60:40 (v/v) with 1.5% (v/v) of [emim][BF_4].
†Acetonitrile–water, 60:40 (v/v) with 1.5% (v/v) of [hmim][BF_4].
‡Tetrahydrofuran–methanol–sodium chloride (58 g/L), 10:45:45 (v/v/v).

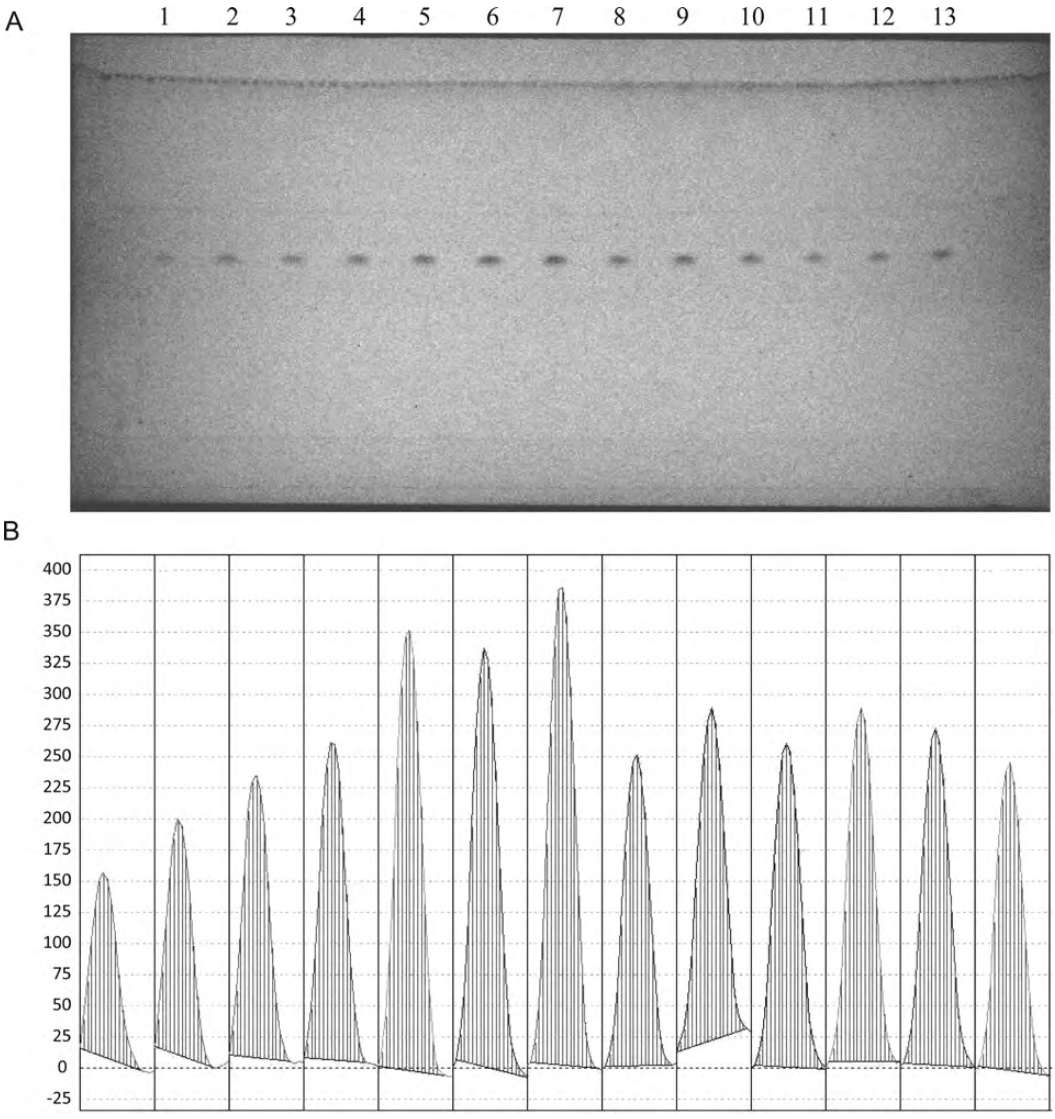

Figure 5. Determination of naphazoline nitrate in nasal drops by the HPTLC–UV–densitometric method using the RP-18 (10 × 20 cm) with F_{254S} fluorescent indicator stationary phase and a mobile phase of acetonitrile–water (6:4, v/v) with the addition of 1.5% (v/v) [hmim][BF_4]: chromatogram in which columns 1–7 correspond to standards (0.20, 0.30, 0.45, 0.60, 0.75, 0.90 and 1.05 mg/mL, respectively), columns 8–10 refer to nasal drops in dissolved with methanol and columns 11–13 are for aqueous solutions (A); respective densitograms (B).

Table II
Calibration Curves and Recovery for the Assay of Naphazoline Nitrate in Nasal Drops

Mobile phase	Calibration line	r	Determination in methanol				Determination in water			
			Declared mean content (mg)	Found mean content (mg)	Recovery (%)	RSD (%)	Declarated mean content (mg)	Found mean content (mg)	Recovery (%)	RSD (%)
Acetonitrile–water (6:4) with [emim][BF_4]	$y = 1212.9x + 42.4$	0.997	0.75	0.812	108.21	6.99	0.75	0.761	101.48	5.42
Acetonitrile–water (6:4) with [hmim][BF_4]	$y = 513.4x + 208.8$	0.968	0.75	0.771	102.74	50.74	0.75	0.682	90.87	40.55
Tetrahydrofuran–methanol–sodium chloride (58 g/L) (10:45:45)	$y = 2273.8x + 779.9$	0.892	0.75	0.168	22.41	5.70	0.75	0.139	18.58	6.02

(peak area versus drug quantity per spot) were estimated using linear regression analysis, with correlation coefficients presented in Table II. When the mobile phase was composed of 1.5% (v/v) of [emim][BF_4], the highest correlation coefficient was determined at $r = 0.997$. An unacceptable value of correlation coefficient ($r = 0.892$) was noted for the tetrahydrofuran–methanol–sodium chloride (58 g/L), 10:45:45 (v/v/v) mobile phase.

The results of the determination of naphazoline nitrate in nasal drops were characterized by acceptable recovery in the range of 90.87 to 108.21%, when [emim][BF_4] or [hmim][BF4] were used as mobile phase modifiers. However, statistically low precision for [hmim][BF_4], with mean relative standard deviations (RSDs) of 50.74 and 40.55%, exclude the use of the IL for accurate analysis. In view of the good specificity of both ILs, the high values of RSD can be attributed to hexyl chains in the imidazolium ring, which probably hinder the densitometric detection. Moreover, the previously evaluated silanol-suppressing potency (K_A) showed the greatest effect of octyl- and hexyl-imidazolium-based ILs on the deleterious effect of free silanols (15). The higher silanol-masking potency of [hmim][BF_4] is likely caused by hydrophobic interaction between the alkyl chain of the IL and the octadecyl-bonded silica phase. Hence, the more stable imidazolium-silanol complex can also distort the densitomertic measurements. A higher viscosity and density of [hmim][BF_4] ($d = 1.14$), in comparison with [emim][BF_4] ($d = 1.28$), can also affect precision. The lowest recovery (22.41 and 18.58) was achieved with the use of a common mobile phase for basic drugs.

Analyzing the data in Table II shows that the dilution of aqueous nasal drops with methanol provides a higher naphazoline recovery due to the contraction effect. Therefore, the aqueous solutions of the studied formulation should be used for accurate determination and quantification of the component drug, naphazoline.

Because the determination of naphazoline nitrate in nasal drops was analyzed, the other components, i.e., preservatives, were expected. What is surprising is that no additional spots were detected during the TLC densitometric method. However, based on the reported studies, preservatives like benzalconium chloride are present in very low concentration, while active ingredients are present in considerably greater concentrations. Additionally, the lack of the detection can also be due to the lower absorptivity of benzalkonium chloride than the active ingredient (naphazoline nitrate) (22, 24).

Conclusion

The presented study demonstrates the application of ILs in TLC–densitometric methods. The use of ILs as modifiers of the mobile phase allowed problems normally encountered in LC of basic analytes to be solved. The results demonstrate that the alkyl-imidazolium class ILs with short alkyl-chain lengths are particularly suitable as modifiers of the mobile phase in the HPTLC determination of naphazoline. The new method can be applied in HPTLC pharmaceutical analysis of basic drugs in their pharmaceutical formulations. The developed approach was demonstrated to provide a reproducible assay of naphazoline nitrate in nasal drops that could not be obtained with the use of standard silanol-suppressing mobile phase additives, like TEA or DMOA. Naturally, the method should be validated for the routine analysis of the known drug analyte in pharmaceutical formulations.

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