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1-ALKYL-3-METHYLIMIDAZOLIUM TETRAFLUOROBORATE AS AN ALTERNATIVE MOBILE PHASE ADDITIVES FOR DETERMINATION OF HALOPERIDOL IN PHARMACEUTICAL FORMULATION BY HPTLC UV DENSITOMETRIC METHOD

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□ *An alternative thin-layer chromatography (TLC) method for the determination of haloperidol in pharmaceutical formulation against the method proposed by European Pharmacopeia (7.0) has been compared and described. The proposed method uses a mobile phase composed of acetonitrile/water, 60:40, with the addition of 1.5% (v/v) 1-ethyl-3-methylimidazolium tetrafluoroborate ionic liquid. The ionic liquid modifiers of a mobile phase have been shown to be suitable suppressor of silanol ionization in the TLC of basic drugs. Besides the silanol-suppressing potency of the 1-ethyl-3-methylimidazolium tetrafluoroborate, the lack of interaction and interference with UV densitometric detection was observed. These properties open a new possibilities in the application of alky-imidazolium-based ionic liquids in chromatographic techniques.*

Keywords haloperidol, ionic liquids, mobile phase additives, solanol deactivation, thin-layer chromatography

INTRODUCTION

The thin-layer chromatography (TLC) of basic drugs is often unfeasible because of the effect of free silanols on their chromatographic retention.[1–3] The undesirable effect is probably due to the ionic interactions of the positively charged analytes with the free silanol groups on the surface of silica or alkyl-bonded silica stationary phases. The ion-exchange interactions cause a strong retention of basic analytes resulting in poor peak or spots shape and tailing. Hence, the numerous mobile-phase modifiers have been tested to suppress undesirable silanol effect in liquid chromatography, e.g., different buffer salts, ammonia, and various amines such as

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triethylamine (TEA), diethylamine (DEA), dimethyloctylamine (DMOA), or tetrabutylamine (TBA) and other primary, secondary, tertiary, and quaternary amine additives.[4] Nahum and Horvath recommended them as additives to the eluent in liquid chromatography that can be successfully used in reversed phase chromatography to suppress the deleterious effect of free silanolic groups in stationary phase.[5] The modifiers are very popular and are often recommended by pharmacopoeias for qualitative and quantitative analysis of some drugs with the use of TLC method or are used to improve any chromatographic process of basic compounds.[6,7]

In the last decade, ionic liquids have opened a new possibility to improve chromatographic systems, also TLC. The role of the ionic liquids is the interaction with silanol sites to reduce its deleterious effect on basic compounds. Due to their unique and "flexible" physical and chemical properties, ionic liquids are often called as "green chemistry" solvents and presently have potential application in pharmaceutical and chemical industry.[8] A numerous reports described recent efforts in the application of the "green solvents" in almost all areas of analytical chemistry.[9–13] Ionic liquids are organic salts with low melting points that have also a great opportunity for a new application as solvents in chromatographic techniques.[14–16] The most commonly used ionic liquids in liquid chromatography are salts composed of alkylammonium and imidazolium cations which are soluble in common chromatographic solvents.[17,18] Also the ILs based on the BF_4, Cl, and $MeSO_4$ anions are water-stable compounds, which dissolve in commonly used chromatographic mobile phases.

In most performed studies, ionic liquids have been proposed as silanol suppressing additives to mobile phase.[19,20] The advantage of these suppressing modifiers of mobile phase in TLC and high-performance liquid chromatography (HPLC) is that their addition of 0.5–2.5% v/v to mobile phase decreased the retention of basic analytes more markedly than other alkylamines.[21–25] The desirable effect of new additives probably based on the dual nature of ionic liquids. Both parts of the ionic salts, cation and anion, can affect the chromatographic retention.[26] It was demonstrated that both cations and anions of ILs could be adsorbed on hydrophobic stationary phase. Our previous studies showed the significant influence of 1-alkyl-3-methylimidazolium cation on the silanol suppressing properties of ILs [19]. The 1-alkyl-3-methylimidazolium tetrafluoroborate IL had a better silanol blocking activity on different silica-based TLC plates than ammonia and ternary amines (TEA, DMOA) and was successfully used for quantification of basic drugs and optimization of separation of peptides with TLC systems.[19,27]

The main aim of the study is the comparison of a suggested pharmacopeial TLC method (European Pharmacopeia 7.0) for determination of haloperidol with the chromatographic system composed of previously selected ionic liquids [21]. The comparison involves the quantification HPTLC analysis

of haloperidol in oral drops that causes an analytical problem during the chromatographic process on silica-based stationary phases.

EXPERIMENTAL

Chemicals

The Haloperidol Reference Standard Was Obtained from POCh (Gliwice, Poland)

Both ionic liquids used in the study, 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). Acetonitrile, tetrahydrofuran, methanol, water, and sodium chloride with high analytical grade were from POCh (Gliwice, Poland).

The Pharmaceutical Formulation

Haloperidol Unia (oral drops containing 2 mg of haloperidol in 1 mL solution) was from pharmaceutical company, Zakłady Farmaceutyczne UNIA Spółdzielnia Pracy (Warszawa, Poland).

Apparatus

The HPTLC system was comprised of a microsyringe from Innovative Labor Systeme GmBH (Stützerbach, Germany), HPTLC applicator AS 30 made by Desaga (Wiesloch, Germany), and HPTLC CD 60 densitometer (Desaga, Wiesloch, Germany) assisted by a computer with a ProQuant software (Desaga, Wiesloch, Germany). Visualization of the chromatographic

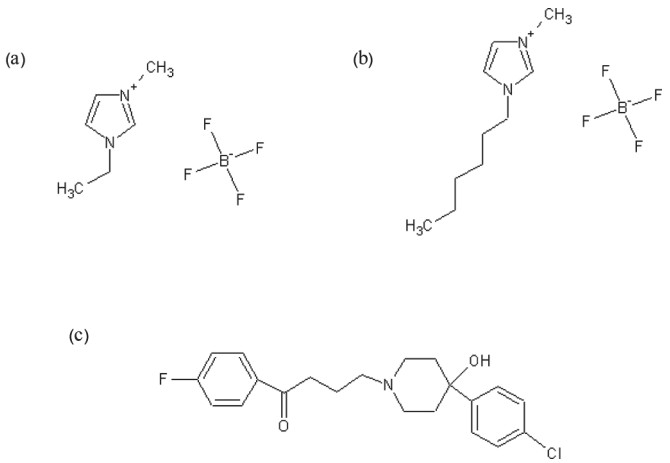

FIGURE 1 Chemical structures of (a) [emim][BF_4], (b) [hmim][BF_4], and (c) haloperidol.

separation was carried out by CabUV-VIS (Desaga, Wiesloch, Germany) and documented using a Canon Power Shot G5 digital camera with ProViDoc 3.0 (Desaga, Wiesloch, Germany). The chromatographic process was performed using horizontal developing chambers from Modin (Lublin, Poland) and HPTLC RP-18 glass plates (10 cm×20 cm) precoated with silica gel 60 F_{254} (Fluka, Germany).

Methods

Quantitative analysis of haloperidol by HPTLC was conducted using densitometer and applicator. A 1 μL of stock solution in appropriate concentration and test solutions of haloperidol were applied on the glass plates RP-18 of 10 cm×20 cm in size, 10 mm from the bottom edge. Further, the HPTLC plates were placed in chromatographic chamber, previously saturated with the mobile phase suggested by European Pharmacopeia (7.0) for TLC haloperidol identity testing (tetrahydrofuran/methanol/sodium chloride solution (58 g/L), 10:45:45 v/v/v). Afterwards, developed and dried at room temperature, plates were subjected to densitometric analysis at a wavelength of 245 nm. The length of chromatogram run was 8 cm.

Identical procedure and analysis were also performed for other plates, placed in separate chambers saturated with vapor of chromatographic mobile phases consisting of acetonitrile/water/[emim][BF_4] (60:40:1.5 v/v/v) and acetonitrile/water/[hmim][BF4] (60:40:1.5 v/v/v).

Canon Power Shot G5 digital system in combination with ProViDoc 3.0 (Desaga, Wiesloch, Germany) was used for visualization, imaging, and archiving developed high-performance thin-layer plates.

Standard Solution and Analytical Procedure for the Assay of Haloperidol

Standard solution was prepared by dissolving 5.0 mg of haloperidol in 5 mL methanol to obtain the concentration of 1 mg/mL. The calibration curves were plotted from seven dilutions (0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mg/mL of haloperidol) of the standard solution of haloperidol. Working solutions of the studied compound (0.5 mg/mL) were prepared by dilution of haloperidol drops with methanol or water with an analytical HPLC grade. For the calibration curves, 1 μL of all standards and samples were applied to the HPTLC RP-18 plates by means of the HPTLC Applicator AS 30 equipped with a 25-μL microsyringe. Thirteen bands per plate were applied 10 mm from the bottom edge, 13 mm apart. The rate of application was constant and was set at 14 s/μL. 250 μL of haloperidol oral drops (2 mg/mL) were diluted with 750 μL of methanol or water to reach the final concentration of 0.50 mg/mL.

RESULTS AND DISCUSSION

Preliminary study with the use of [emim][BF_4] as a TLC mobile phase additive was performed in order to compare the effect of imidazolium-based ionic liquid on the chromatographic retention of haloperidol. The TLC plates were developed with acetonitrile/water (60:40 v/v) eluent, either neat or with 0.5, 1.5, or 2.0% (v/v) of [emim][BF_4]. The strong retention of the tested analyte was observed in the mobile phase without the ionic liquid (Table 1). The "tailing" spot (R_f=0.05) of haloperidol on a octadecyl-silica plate confirms the undesirable interactions of base analyte with free silanols on the surface of stationary phase. However, when the small amount of IL was added (0.50% v/v), the retention of the haloperidol decreased but the shape of the spot was still irregular. The addition of threefold concentration of [emim][BF_4] to the mobile phase caused a further decrease in chromatographic retention of the drug. The excess of studied IL over 1.5% (v/v) did not improve the chromatographic process (Table 1). Next, based on the observation the 1.5% (v/v) of [emim][BF_4] and [hmim][BF_4] as a mobile phase, additives were investigated and compared with chromatographic method recommended by the European Pharmacopoeia (7.0).

The comparative analysis with a mobile phase suggested by European Pharmacopeia (7.0) involves the TLC determination of haloperidol with the use of tetrahydrofuran/methanol/sodium chloride solution (58 g/L) (10:45:45 v/v/v) as an eluent. Seven various concentrations of standard stock solution of haloperidol were spotted on octadecyl-silica plates (0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mg/mL) and developed using three different mobile phase: (1) Pharmacopeial, (2) 1.5% (v/v) of [emim][BF_4], and (3) 1.5% (v/v) of [hmim][BF_4] (Figure 2). Parallel, the three spots with methanol and aqueous solution of haloperidol oral drops were developed on the same TLC plate. Based on the R_f values and shape of spots, one can conclude that all studied eluents were acceptable for TLC separation and determination of haloperidol. Also, the comparison of R_f values with acceptable standard deviation (SD) can be taken as evidence of the useful of all three chromatographic systems for the quantitative analysis of haloperidol (Table 2).

TABLE 1 Rf Values of Haloperidol Using Different Concentrations of [emim][BF4] as Modifier of Mobile Phase (Acetonitrile/Water, 60:40 v/v)

	Haloperidol			
Concentration of [emim][BF_4] in (acetonitrile/water, 60:40 v/v)	0% (v/v)	0.5% (v/v)	1.5% (v/v)	2% (v/v)
R_f value of haloperidol	0.05*	0.55*	0.57	0.57

*Tailing spot.

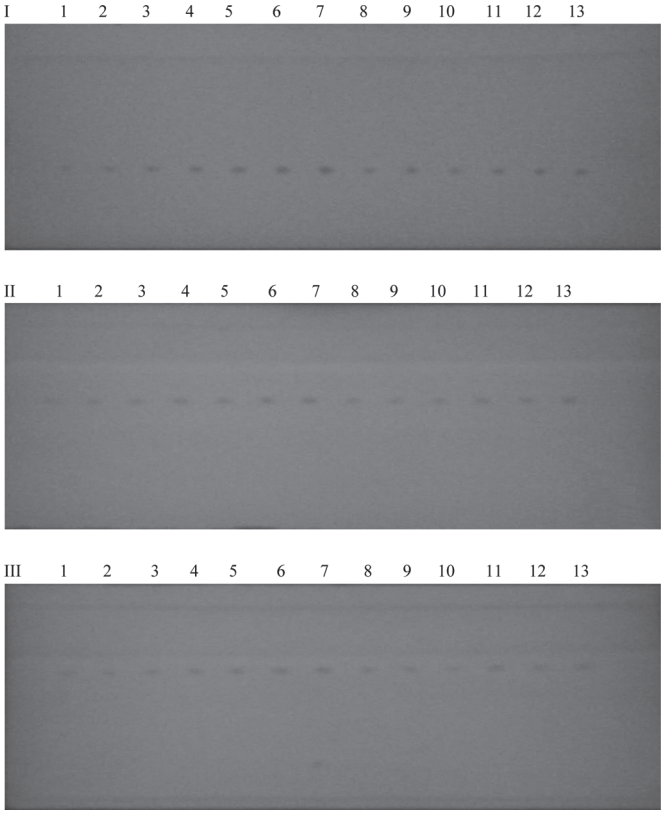

FIGURE 2 Representative HPTLC plates of developed HPTLC plate in different chromatographic system. Tracks 1–7, the increasing concentrations of haloperidol (0.3–0.9 mg/mL); Tracks 8–10, 0.5 mg/mL (aqueous solutions); Tracks 11–13, 0.5 mg/mL (methanol solutions).
Mobile phase: I—tetrahydrofuran/methanol/sodium chloride (58 g/L), 10:45:45 (v/v/v).
II—acetontrile/water, 60:40 (v/v), with 1.5 % (v/v) of [emim][BF_4].
III—acetonitrile/water 60:40 (v/v), with 1.5 % (v/v) of [hmim][BF_4].

Next, the three studied mobile phases were compared as a potential thin-layer chromatographic system for the quantification of haloperidol in pharmaceutical formulations. For the quantitative analysis, the HPTLC-UV densitometric method was used. The optimal conditions of UV densitometric scanning were determined in the range of 190 to 400 nm and the analytical wavelength was adjusted at λ=245 nm. The representative densitograms are presented in Figure 3. The use of tetrahydrofuran/methanol/sodium chloride as a mobile phase and the 1.5% (v/v) of [emim][BF_4] additive to acetonitrile/water did not influence the strength of the signal**.** The significant decrease in signal intensity was observed for densitogram from the chromatographic system with acetonitrile/water, 60:40 (v/v), mobile phase and with 1.5% (v/v) of [hmim][BF_4] as an additive. That can

TABLE 2 Comparison of Studied Chromatographic HPTLC Systems with Regard to the Retardation Coefficient (Rf) and Standard Deviation for Haloperidol ($n=3$)

Concentration of haloperidol (mg/mL)	Mobile phase 1	Mobile phase 2	Mobile phase 3
	R_f	R_f	R_f
0.30	0.177±0.063	0.513±0.035	0.400±0.127
0.40	0.177±0.063	0.503±0.050	0.407±0.119
0.50	0.180±0.061	0.510±0.041	0.470±0.061
0.60	0.180±0.061	0.513±0.040	0.497±0.061
0.70	0.183±0.058	0.520±0.046	0.497±0.055
0.80	0.183±0.058	0.517±0.042	0.463±0.084
0.90	0.183±0.058	0.520±0.046	0.460±0.101

Mobile phase: 1—tetrahydrofuran/methanol/sodium chloride (58 g/L), 10:45:45 (v/v/v).
2—acetonitrile/water, 60:40 (v/v), with 1.5% (v/v) of [emim][BF_4].
3—acetonitrile/water, 60:40 (v/v), with 1.5% (v/v) of [hmim][BF_4].

be due to the hexyl chain in imidazolium ring, which probably hinder the densitometric detection.

The calculated standard curves were evaluated by linear regression ($y=ax+b$) using the least squares approach (Table 3). The calibration curve equations were estimated by linear regression analysis (peak area versus drug quantity per spot). Linear correlation between the peak area and the

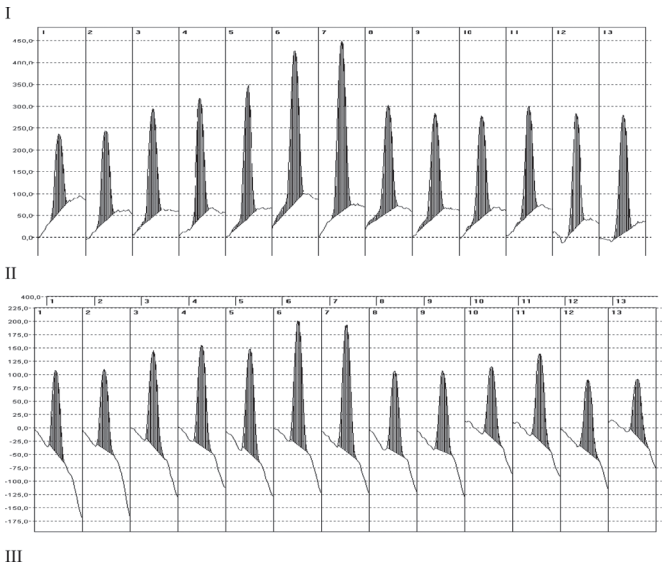

FIGURE 3 Representative densitograms obtained by scanning HPTLC plates with the wavelength at 245 nm in different chromatographic systems. Tracks 1–7, the increasing concentrations of haloperidol (0.3–0.9 mg/mL); Tracks 8–10, 0.5 mg/mL (aqueous solutions); Tracks 11–13, 0.5 mg/mL (methanol solutions).
Mobile phase: I–tetrahydrofuran/methanol/sodium chloride (58 g/L) 10:45:45 (v/v/v).
II–acetonitrile/water 60:40 (v/v) with 1.5 % (v/v) of [emim][BF_4].
III–acetonitrile/water 60:40 (v/v) with 1.5 % (v/v) of [hmim][BF_4].

TABLE 3 Calibration Curve and Comparison of the Recovery for the Assay of Haloperidol in Oral Drops Determined in Methanol and Aqueous Solutions Using Different Mobile Phase Systems

				Methanol solutions			Aqueous solutions		
Mobile phase	Calibration line	r	Declared mean content (mg)	Found mean content (mg)	Recovery (%)	RSD (%)	Found mean content (mg)	Recovery (%)	RSD (%)
Tetrahydrofuran/methanol/sodium chloride (58 g/L) (10:45:45)	$y=1353x+103.0$	0.995	0.50	0.511	102.23	11.96	0.499	99.789	7.86
Acetonitrile/water (60:40) with 1-ethyl-3-methylimidazolium tetrafluoroborate	$y=1071x+158.2$	0.994	0.50	0.532	106.50	13.86	0.497	99.36	10.53
Acetonitrile/water (60:40) with 1-hexyl-3-methylimidazolium tetrafluoroborate	$y=550.4x+417.7$	0.907	0.50	0.428	85.52	73.86	0.415	82.96	67.80

concentration of haloperidol in the range of 0.3 to 0.9 mg/mL was found with a highest correlation coefficient of 0.995 for tetrahydrofuran/methanol/sodium chloride mobile phase and the lowest (0.907) for the mobile phase composed of 1.5% (v/v) of [hmim][BF_4]. The efficiency of the tetrahydrofuran/methanol/sodium chloride and [emim][BF_4] based mobile phase is confirmed by the acceptable recovery in the range of 99.36% to 106.50%. However, the value of recovery for the methanol dilution of haloperidol oral drops is significantly higher than the aqueous dilution. The differences in assays are probably due to the methanol—water contraction phenomena occurring in resulted solutions. As a result of intermolecular interactions between the components of the mixture (methanol as solvent and purified water from the droplets), agglomerate occurs, resulting in a change of volume and thus the concentration of obtained methanol solution is slightly higher than that in the case of water solution.

The unsatisfactory recovery (82.96–85.52) for [hmim][BF_4] used as an mobile phase additive was observed, regardless of chemical solution. The unacceptable value is probably related with low value of correlation coefficient (0.907) and low precision with mean of relative standard deviation. One of the explanation of the phenomena is that the efficient suppression of the free silanols interaction increased with the length of the alkyl chain at position C-1 of the imidazolium ring [21]. Thus the hexyl chain attached to the C1 position can stabilize the silanol–imidazolium complex by hydrophobic interaction between the alkyl chain of the ionic liquid and the octadecyl-bonded silica phase. Such stable imidazolium–silanol complex and long chain can distort the chromatographic process and densitomertic measurements.

CONCLUSION

The addition of 1.5% (v/v) of ionic liquids as mobile phase modifiers appears to give an equivalent separation of haloperidol compared to the mobile phase suggested by European Pharmacopeia 7.0. The results with [emim][BF_4] as the mobile phase additive give similar separation and quantitative results with no peak tailing, confirming the positive effect of ionic liquids on the chromatographic behavior of basic drugs. However, the longer chain length ionic liquid [hmim][BF_4] when added as a 1.5% modifier, gives a satisfactory separation but lacks the precision for it to be recommended as a suitable additive for haloperidol mobile phase.

The application of ionic liquids as additives of mobile phase allows to solve problems of silanol interaction in liquid chromatography of basic compounds. The results demonstrate that the alkyl-imidazolium class ionic liquids with short alkyl-chain length are particularly suitable modifier of mobile phase in HPTLC and the use with acetonitrile/water compostion

can be an alternative mobile phase in determination of haloperidol in comparison with tetrahydrofuran/methanol mixture. Undoubtedly, the individual conditions should be optimized to respective determination of compound.

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