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Ionic liquids as mobile phase additives for feasible assay of naphazoline in pharmaceutical formulation by HPTLC UV-densitometric method

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ABSTRACT

A specific and reliable High-Performance Thin Layer Chromatography (HPTLC) with densitometry detection method has been developed for determination of naphazoline nitrate in nasal drops. The best separation of basic analyte, without spot tailing, was achieved using the mobile phase composed of acetonitrile:water (60:40,v/v) with 1.5 % (v/v) imidazolium-class ionic liquid added and the plates covered with stationary phase based on the RP-18 with F_{254S} (10cm x 20cm). 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 at the same time providing a repeatable assay of naphazoline nitrate in nasal drops, which could not be obtained with the use of standard silanol suppressing mobile phase additives, like triethylamine (TEA) or dimethyloctylamine (DMOA).

KEYWORDS: High-performance thin-layer chromatography (HPTLC); Ionic liquids; Mobile phase additives; Silanol deactivation; Naphazoline; Nasal drops

Introduction

Thin-layer chromatography (TLC) used to be a leading chromatographic technique and still is 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 the silica or alkyl-bonded-silica stationary phases. The ion-exchange interaction causes a strong retention of basic analytes resulting in poor peak or spots shape and tailing. Hence, the addition of different buffer salts and various amines such as triethylamine (TEA) or dimethyloctylamine (DMOA) is often needed for improvement of chromatographic results [3].

Studies described in last decade show that ionic liquids (ILs) can be used in a great variety of applications. Due to their unique and "flexible" physical and chemical properties they are innovative solvents and often are considered in "green chemistry" idea. Numerous reviews 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 a new application 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 effect of imidazolium based ILs as mobile phase modifiers in Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) on the retention of basic compounds has been studied and described elsewhere [13]. The addition of 0.5-2.5 % v/v of some types 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 decreased the retention of basic analytes more markedly than other alkylamines [17-21]. The experiments have been evaluated using mainly 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 the synergistic or antagonistic effect [22]. It was demonstrated that both cations and anions of ILs could be adsorbed on hydrophobic stationary phase. However, in our previous studies we demonstrated the significant influence of 1-alkyl-3-methylimidazolium cation on the silanol suppressing properties of ILs [15]. The 1-alkyl-3-methylimidazolium tetrafluoroborate IL had a better silanol blocking activity on the bare silica or on the octadecyl silica TLC plates than ammonia and ternary amines (TEA, DMOA). Hence, the silanol suppressing effect was successfully used for qualification of basic drugs and optimization of separation of peptides in TLC plates [15, 23].

Up to now, most described studies with the use of ILs as a mobile phase modifiers in liquid chromatography have been related to qualification analysis with regard to evaluation of their silanol suppressing potency. The main aim of the study is application of previously selected ILs in quantification HPTLC analysis of naphazoline nitrate in nasal drops. Naphazoline nitrate is a basic drugs that causes analytical problem during the chromatographic process on the silica based stationary phases. Several methods for the determination of naphazoline and other α-adrenergic agents were validated and described in literature. Naphazoline, phenylephrine, tetrahydrozoline, tramazoline, tymazoline and xylomethazoline were determined as a single active compound or mixture in commercial

preparations in nasal and ophthalmic solutions using HPLC and capillary electrophoresis (CE) [24]. Also, except the pharmaceutical compound analysis the determination of the α-adrenergic drugs in the presence of other chemicals as preservatives in pharmaceutical preparations were 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 the ammonia and adsorbable amino quenchers (i.e. triethylamine or tetramethylammonium bromide) as a mobile phase modifiers to suppress free silanol effect. This study describes an application of imidazolium based ionic liquids to reduce deleterious effects of free silanols on liquid chromatographic separation of naphazoline nitrate. We believe that replacement of the commonly used alkylamine additives with the readily available ionic liquids may improve chromatographic separations of the problem-causing chemicals. Use of ionic liquids is justified and can be recommended instead of the conventional environmentally harmful agents currently employed. Finally, in the present study a novel simple high performance thin layer chromatography UV-densitometric method for the determination of naphazoline nitrate in commercially available pharmaceutical preparation was developed.

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). Reference standard of naphazoline nitrate was from POCh (Gliwice, Poland). Rhinazin drops consisting of 1 mg/mL naphazoline nitrate were from Polfa Warszawa S.A.(Warsaw, Poland). Acetonitrile, methanol, sodium chloride, tetrahydrofuran and water were purchased from POCh (Gliwice, Poland). The water used in the study was prepared using a Milli-QWater Purification System (Millipore, Bedford, USA).

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 analytes on to HPTLC plates was performed by Desaga AS 30 HPTLC applicator (Wiesloch, Germany) with injection volume of 1µL/spot and application rate of 14 s/µl. The resulting spot size was 4 mm and the distance between centre spots was 13 mm. The distance between the start line and the bend line of the plate was 1cm.

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

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

Measurements of mobile phase UV absorbance were performed using Shimadzu Prominence system (Kyoto, Japan) composed of diode array detector (SPD-M20A). The UV spectra of used ionic liquids ([emim][BF_4] and [hmim][BF_4]) were investigated by HPLC using the acetonitrile: water (60/40 v/v) mobile phase. Studied modifiers showed absorbance

maxima at about 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

Stock solution of naphazoline nitrate (1.2 mg/ml) was prepared in methanol. The calibration samples were prepared in methanol in triplicates 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 up by plotting peak area against the drug quantity per spot. The equations of calibration curves were estimated using linear regression. 750 µl of naphazoline nitrate nasal drops (1mg/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 a novel modifiers of mobile phases were performed in both normal and reversed HPTLC stationary phase systems. Based on the previously reported results, the optimal composition of mobile phase for ionic liquids application in chromatography of selected phenothiazine-derived drugs is determined by acetonitrile:water 60/40 (v/v) [15]. The concentration of used ionic liquids was adjusted by the selection of retardation coefficient (R_f) value and a better shape of spots. The exemplary HPTLC plates with naphazoline nitrate, developed at different conditions, are 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]. First two chromatograms from the left were obtained using the silica gel and [emim][BF_4] (Figure 4a,b). The addition of IL to mobile phase significantly distorted the shape of spots. Therefore, the normal stationary phase was discarded from further study. Next chromatograms show distinct influence of proposed modifiers on the retention of the studied drug. The strong retention of basic drug for unmodified mobile phase and spot tailing in the case of insufficient concentration of IL (0.5 % (v/v)) is an evidence for strong silanol interactions with solute (Figure 4c-g). The shape of spot is most symmetrical and without tailing for ILs concentration of 1.5 % (v/v). However, the excess ILs concentration over the 1.5 % (v/v) do not cause any further improvement of chromatograms of naphazoline. The similar chromatographic behavior of naphazoline with regular shape of spot was obtained with the use of mobile phase consisted 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 1).

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 optimal wavelength of 276 nm to obtain densitograms of naphazoline developed in three different chromatographic systems. The representative chromatogram and densitogram of 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 solution of Rhinazin drops were developed on the same plates. The equation of calibration curve (peak area *vs*. drug quantity per spot) were estimated using linear regression analysis with correlation coefficients presented in Table 2. When the mobile phase was composed of 1.5% v/v of [emim][BF_4], the highest correlation coefficient was determined (r=0.997). 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 the [emim][BF_4] or [hmim][BF_4] were used as mobile phase modifiers. However, statistically low precision for [hmim][BF_4], with mean RSD of 50.74 and 40.55%, exclude the use of the ionic liquid for accurate analysis. In view of good specificity of both ionic liquids, the high values of RSD can be due to hexyl chains in imidazolium ring which probably provide certain hindrance in 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 ionic liquid 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 2, one can conclude 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 of the determination of naphazoline nitrate in nasal drops were analyzed, the other components i.e. preservatives were expected. What is surprising, that no additional spots during the TLC densitometric method were detected. But based on the reported studies the preservatives like benzalconium chloride are present in very low concentration, while active ingredients are present in considerably greater concentration. Additionally, the lack of the detection can be also due to the lower absorptivities of benzalkonium chloride than active ingredient (naphazoline nitrate) [25].

Conclusion

The presented study demonstrates the ability of application of ionic liquids in TLC-densitometric methods. The use of ionic liquids as modifiers of mobile phase allowed to solve problems normally encountered in liquid chromatography of basic analytes. The results demonstrate that the alkyl-imidazolium class ionic liquids with short alkyl-chain length are particularly suitable as a modifiers of mobile phase in HPTLC determination of naphazoline. The new method can be applied in HPTLC pharmaceutical analysis of basic drugs in their pharmaceutical formulations. The approach here developed 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 triethylamine (TEA) or dimethyloctylamine (DMOA). Naturally, the method should be validated for the routine analysis of the known drug analyte in pharmaceutical formulations.

Figure legends:

Figure 1. Structural formula of (A) naphazoline nitrate, (B) 1-ethyl-3-methylimidazolium tetrafluoroborate ([emim][BF_4]) and (C) 1-hexyl-3-methylimidazolium tetrafluoroborate ([hmim][BF_4]) ionic liquids.

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

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

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

Figure 5. Determination of naphazoline nitrate in nasal drops by HPTLC-UV densitometric method using the RP-18 (10cm x 20cm) with F_{254S} fluorescent indicator stationary phase and a mobile phase acetonitrile:water (6:4,V/V) with addition of 1.5 % v/v [hmim][BF_4]. A – 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; B – respective densitograms.

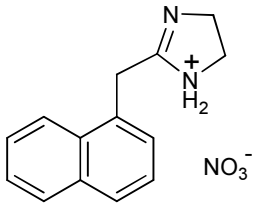
References

1. Nawrocki, J; The silanol groups and its role in liquid chromatography; *Journal of Chromatography A,* (1997); 779: 29–71.
2. Vervoort, R.J.M., Maris F.A., Hindriks, H.; Comparison of high-performance liquid chromatographic methods for the analysis of basic drugs; *Journal of Chromatography A*, (1992); 623: 207-220.
3. Claessens, H.A.; Trends and progress in the characterization of stationary phases for reversed-phase liquid chromatography; *Trends in Analytical Chemistry*, (2001); 20: 563-583.
4. Liu, J.F., Jonsson, J.A., Jiang, G.B; Application of ionic liquids in analytical chemistry; *Trends in Analytical Chemistry,* (2005)**;** 24: 20–27.
5. Koel, M.; Ionic liquids in chemical analysis; *Critical Review in Analytical Chemistry,* (2005); 35: 177–192.
6. Pandey, S; Analytical application of room-temperature ionic liquids:A review of recent efforts; *Analytica Chimica Acta,* (2006); 556: 38–45.
7. Sun, P., Armstrong, D.W.; Ionic Liquids in Analytical Chemistry; *ChemInform*, (2010); 41: 661.
8. Berthod, A., Ruiz-Angel, M.J., Carda-Broch, S.; Ionic Liquids in Separation Techniques; *Journal of Chromatography A,* (2008); 1184: 6-18.
9. Marszałł, M.P., Kaliszan, R.; Application of Ionic Liquids in Liquid Chromatography; *Critical Review in Analytical Chemistry*, (2007); 37: 127–140.
10. Polyakova, Y., Koo, Y.M., Row, K.H.; Application of ionic liquids as mobile phase modifier in HPLC; *Biotechnology and Bioprocess Engineering,* (2006); 11: 1-6.
11. Buszewski, B.; Studzinska, S.; A Review of Ionic Liquids in Chromatographic and Electromigration Techniques; *Chromatographia*, (2008); 68: 1-10.
12. Poole, C.F., Kersten, B.R., Ho, S.S.J., Coddens, M. E., Furton, K.G.; Organic salts, liquid at room temperature, as mobile phases in liquid chromatography; *Journal of Chromatography A*, (1986); 352*:* 407–425.
13. Molíková M., Markuszewski M.J., Kaliszan R., Jandera P.; Chromatographic behaviour of ionic liquid cations in view of quantitative structure-retention relationship; *Journal of Chromatography A*, (2010); 1217: 1305-1312.

14. Petruczynik, A., Waksmudzka-Hajnos, M.; Effect of chromatographic conditions on the separation of selected alkaloids on phenyl stationary phase by an HPLC metod; *Journal of Liquid Chromatography*, (2006); 29: 2807-2822.
15. Kaliszan, R., Marszałł, M.P., Bączek, T., Markuszewski, M.J., Pernak, J.; Suppression of deleterious effects of free silanols in liquid chromatography by imidazolium tetrafluoroborate ionic liquids; *Journal of Chromatography A,* (2004); 1030: 263-271.
16. Marszałł, M.P., Kaliszan, R., Bączek, T.; Reduction of silanophilic interactions in liquid chromatography with the use of ionic liquids; *Analytica. Chimica Acta,* (2005); 542: 172-178.
17. Marszałł, M.P., Kaliszan, R., Bączek T.; Evaluation of the silanol-suppressing potency of ionic liquids; *Journal of Separation Science*, (2006); 29: 1138-1145.
18. Martin-Calero, A**.,** Tejral, G. Ayala, J.H., Gonzalez, V., Afonio, A.M.; Suitability of ionic liquids as mobile-phase additives in HPLC with fluorescence and UV detection for the determination of heterocyclic aromatic amines; *Journal of Separation Science*, (2010); 33: 182-190.
19. Fernadez-Navarro, J.J., Garcia-Alvarez-Coque, M.C., Ruiz-Angel, M.J; The role of the dual nature of ionic liquids in the reversed-phase liquid chromatographic separation of basic drugs; *Journal of Chromatography A,* (2011); 1218: 398-407.
20. Giaginis, C., Tsantili-Kakoulidou, A.; The performance of 1-ethyl-3-methylimidazolium tetrafluoroborate ionic liquid as mobile phase additive in HPLC-based lipophilicity assessment; *Biomedical Chromatography,* (2011); 25: 606-612.
21. Fernández-Navarro, J.J., Torres-Lapasió, J.R., Ruiz-Ángel, M.J., García-Álvarez-Coque, M.C.; Silanol suppressing potency of alkyl-imidazolium ionic liquids on C18 stationary phases; *Journal of Chromatography A*, (2011); 1232: 166-175.
22. Berthod, A., Ruiz-Angel, M.J., Huguet, S.; Nonmolecular Solvents in Separation Methods: Dual Nature of Room Temperature Ionic Liquids; *Analytical Chemistry*, (2005); 77: 4071- 4080.
23. Bączek, T., Marszałł M.P., Kaliszan, R., Walijewski, L., Makowiecka, W., Sparzak, B., Grzonka Z., Wisniewska, K., Juszczyk, P.; Behavior of peptides and computer-assisted optimization of peptides separations in a normal-phase thin-layer chromatography system with and without the addition of ionic liquid in the eluent; *Biomedical Chromatography*, (2005)**;** 19: 1-8.
24. Marchesini, A.F., Williner, M.R., Mantovani, V.E., Robles, J.C., Goicoechea, H.C.; Simultaneous Determination of Naphazoline, Dophenhydramine and Phenylephrine in Nasal Solutions by Capillary Electrophoresis; *Journal of Pharmaceutical and Biomedical Analysis*, (2003); 31: 39-46.
25. Ambrus, G., Takahashi, L.T., Marty, P.A.; Direct Determination of Benzalkonium Chloride in Ophthalmic Systems by Reserved-Phase High-Performance Liquid Chromatography; *Journal of Pharmaceutical Sciences*, (1987); 76: 174-176.
26. Antoniou, C.G., Markopoulou, C.K., Kouskoura, M.G., Koundourellis, J. E.; Study and Development of Determination of 2-Imidazolines in the Presence of Preservatives in Pharmaceutical Preparations. *Journal of AOAC International,* (2011); 94: 703-712.

Figure 1. Structural formula of (**A**) naphazoline nitrate, (**B**) 1-ethyl-3-methylimidazolium tetrafluoroborate ([emim][BF_4]) and (**C**) 1-hexyl-3-methylimidazolium tetrafluoroborate ([hmim][BF_4]) ionic liquids.

A



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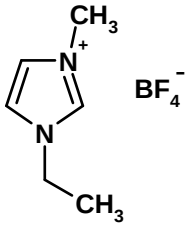

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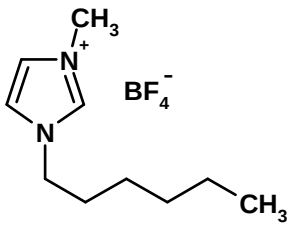

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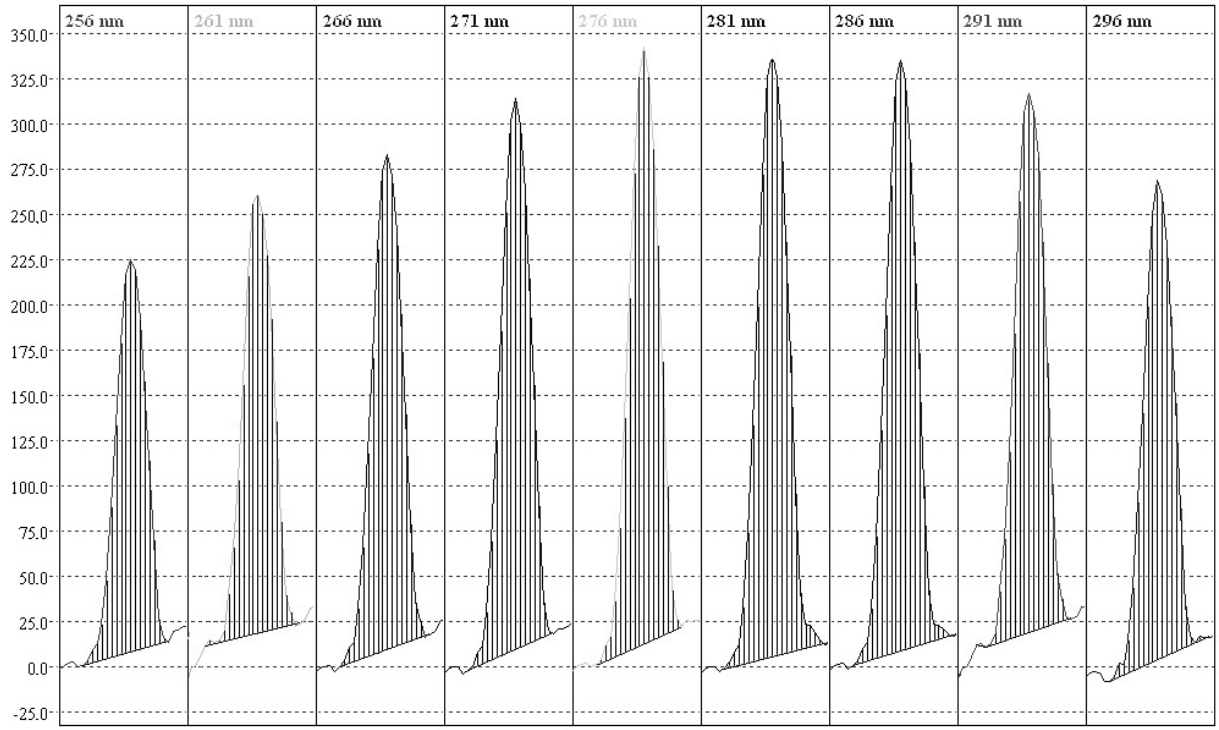

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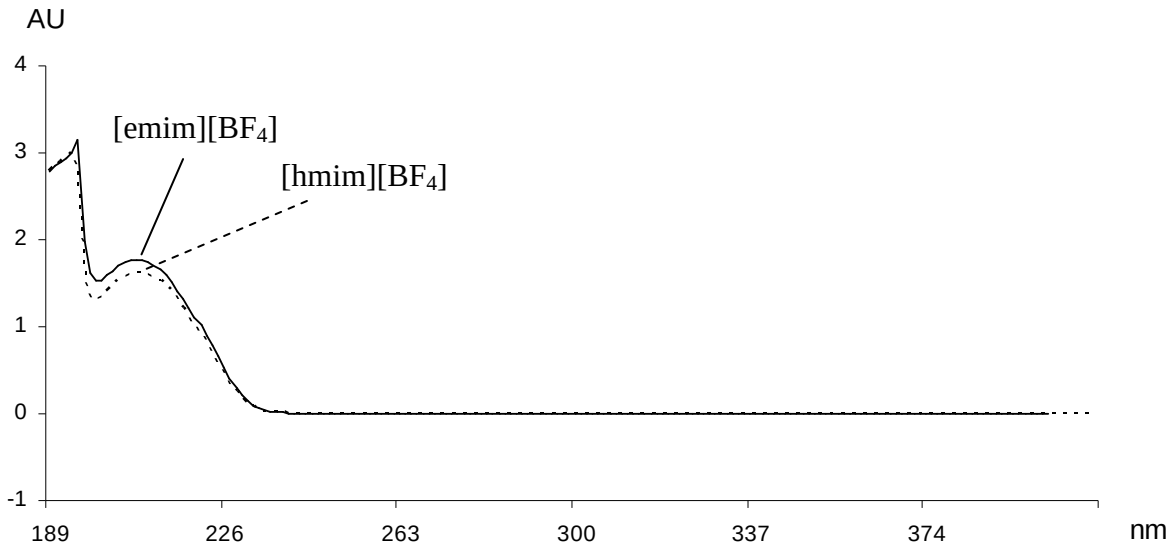

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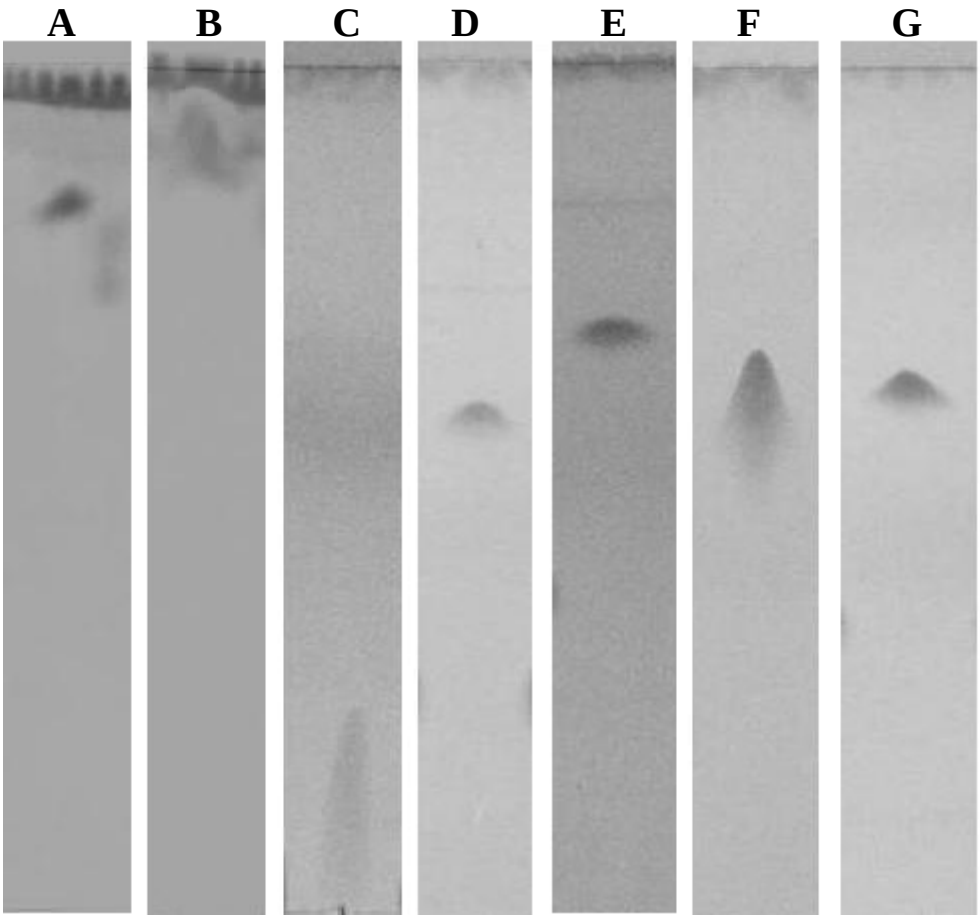

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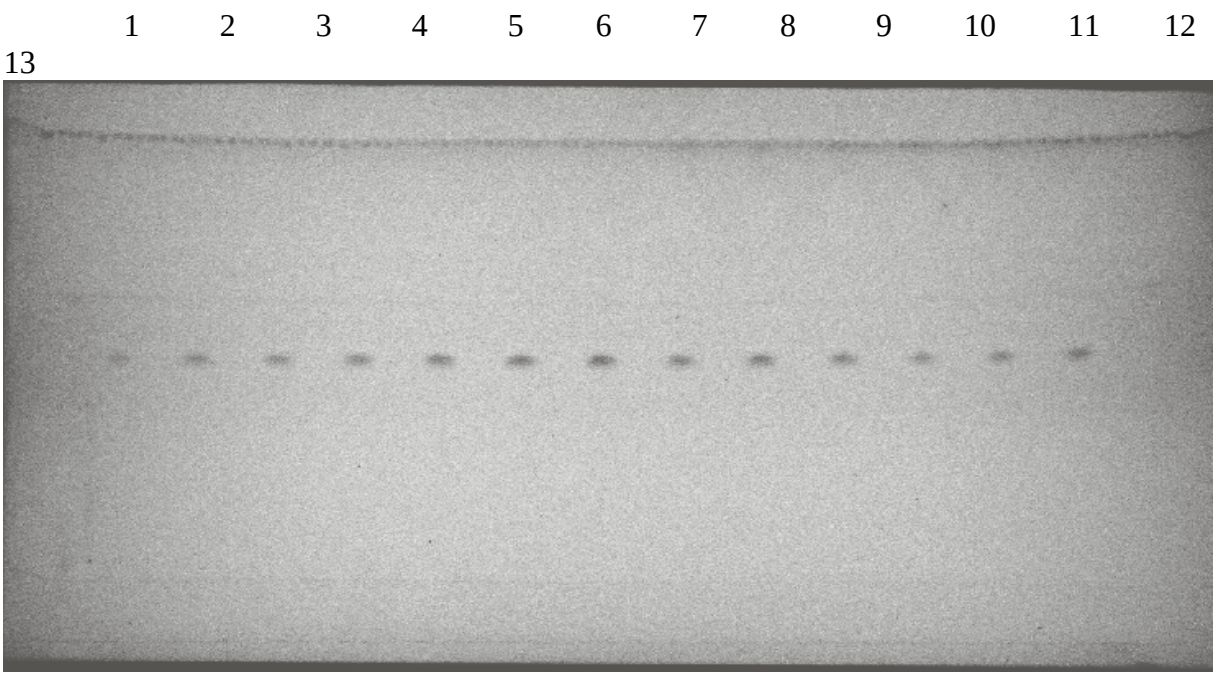
A



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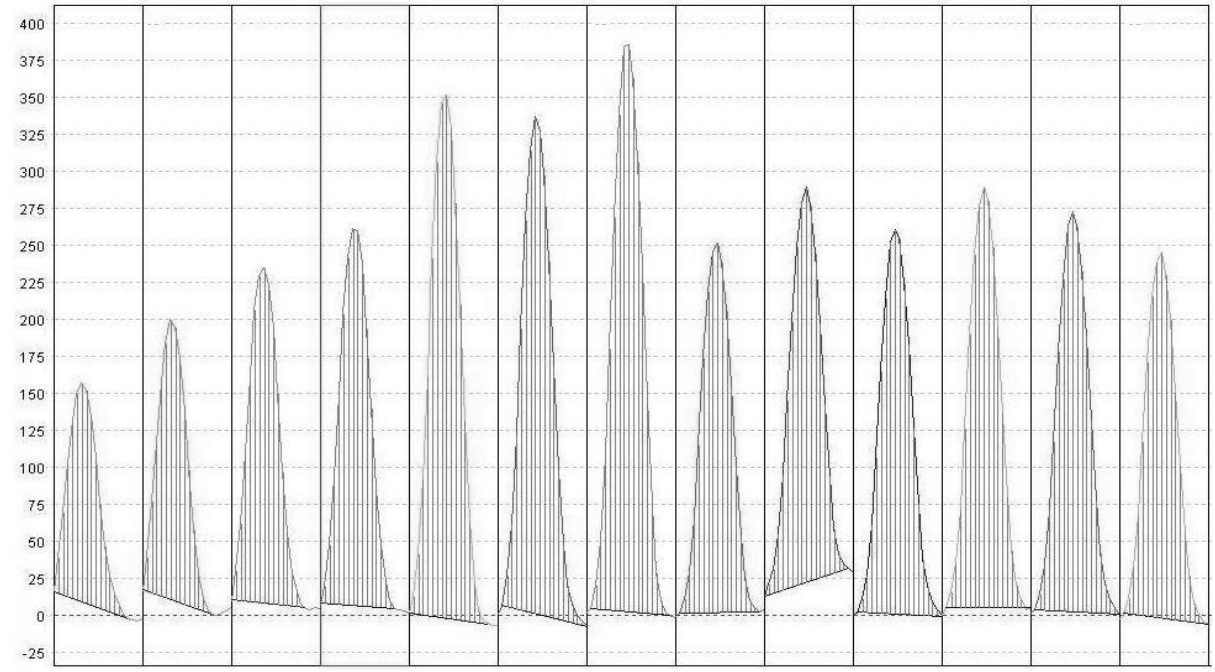

Table 1 . The retardation coefficients (R_f) for naphazoline nitrate (NT) in studied chromatographic HPTLC systems.

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

Mobile phase: A – acetonitrile:water 60:40 (v/v) with 1.5 % (v/v) of [emim][BF_4]
B - acetonitrile:water 60:40 (v/v) with 1.5 % (v/v) of [hmim][BF_4]
C - tetrahydrofuran:methanol:natrium chloride (58 g/L) 10:45:45 (v/v/v)

Table 2. Calibration curve and recovery for the assay of naphazoline nitrate in nasal drops.

			Determination in methanol				Determination in water			
Mobile phase	**Calibration line**	**r**	**Declarated mean content (mg)**	**Found mean content (mg)**	**Recovery (%)**	**RSD (%)**	**Declarated mean content (mg)**	**Found mean content (mg)**	**Recovery (%)**	**RSD (%)**
Acetonitrile:water (6:4) with 1-ethyl-3-methylimidazolium tetrafluoroborate	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 1-hexyl-3-methylimidazolium tetrafluoroborate	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