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Review

Analysis of purines and pyrimidines by mixed partition–adsorption normal-phase high-performance liquid chromatography

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Abstract

This paper summarizes the results in the development of mixed partition–adsorption (MPA) normal-phase high-performance liquid chromatography published in the last 10 years. The MPA normal-phase systems are an alternative approach not only to the adsorption normal-phase mode but also to the most widely used reversed-phase mode in the separation area of purine and pyrimidine derivatives. It is shown that the MPA systems are applicable in analytical practice.

Keywords: Mixed-mode chromatography; Partition–adsorption systems; Reviews; Liquid chromatography; Purines; Pyrimidines; Nucleobases

Contents

1. Introduction

High-performance liquid chromatography (HPLC) remains a very popular analytical technique to separate closely related analytes with excellent selectivity and sensitivity. Although reversed-phase liquid chromatography (RPLC) has become a widely used method for the separation of purine and pyrimidine derivatives [1–11], it is not always the best or only choice for the given analytical task. Moreover, for some samples, separation by the reversed-phase method is ineffective (inadequate retention or poor

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selectivity) and such samples must be handled in a different manner.

Normal-phase liquid chromatography (NPLC) has been used to separate both neutral and ionic compounds, but neutral samples predominantly. In general, NPLC is well suited for samples that do not dissolve well in water–organic mixtures and for preparative HPLC. Sample retention in NPLC decreases as the polarity of the mobile phase increases and less polar compounds elute first, while more polar compounds leave the column last (opposite to RPLC). While retention in RPLC is based on a partition process, retention in NPLC is based on an adsorption process [12]. That is why NPLC, being based on a different retention process than RPLC, often provides a greater resolution for positional or stereoisomers [12–14].

NPLC can be a powerful complement to RPLC. Medium-polarity samples are often separated equally well by either RPLC or NPLC, and their combination can offer additional possibilities in the separation process. For instance, a combination of RPLC and NPLC provides efficient separation of the ring-oxidized derivatives of nitro-polycyclic aromatic hydrocarbons. NPLC gives the opposite retention order with different separability among some of the nitro-polycyclic aromatic hydrocarbons as compared to RPLC [15].

Silica is traditionally applied in NPLC with mobile phases of a low or medium polarity [16,17]. Polar samples interacted strongly with the stationary phase and required eluents of a high polarity in order to be eluted from silica. The strength of the mobile phase increases with increasing solvent polarity. Thus, in NPLC, aliphatic alcohols [18,19] or aprotic dipolar solvents with large dipole moments (e.g., dimethyl sulfoxide [20,21]) are used.

A common problem for separations by NPLC is that polar compounds often show broad tailing peaks, especially for basic analytes. Peak asymmetry causes a reduction in column efficiency as well as a decrease in resolution and detection limits. When ionic samples are separated by NPLC, it is usually advisable to add acids (e.g., 0.2% aqueous phosphoric acid [22], acetic acid [23]) to the mobile phase for acidic compounds and amines (diethylamine [24], triethylamine [25]) for basic compounds. Separation of basic analytes (e.g., emepromium, a quaternary ammonium compound) can be obtained using silica columns together with non-aqueous, primarily methanolic solutions of perchloric acid or ammonium perchlorate of an appropriate pH and ionic strength [26].

Another approach is based on alteration of the stationary phase surface. ‘‘Buffered silica gel’’ is used to reduce the tailing of polar compounds in adsorption chromatography with neutral organic mobile phases. The silica has been coated with a crystalline salt. The water used to bring this buffer salt into the pores and to distribute it evenly over the whole surface is evaporated and therefore has no influence on the chromatographic behaviour. The buffer layer affects only the peak symmetry of polar compounds and makes their elution on the adsorption chromatographic system possible [27]. Another way to reduce the peak tailing of polar compounds is to use less adsorptive and better purified (so-called type B) silica. With a type A silica column, benzanilide eluted after phenol as a strongly tailing peak. However, a type B silica column reveals an excellent peak form for benzanilide and on this column benzanilide is eluted prior to phenol [19]. Employment of polar chemically bonded diol, nitrile and amino phases as column packings for NPLC separation allows the elution of polar compounds (e.g., acetylcholine and choline [28]) that are eluted with difficulty from the more retentive unmodified silica.

Over the last years an increasing number of publications demonstrated the utility of using aqueous eluents with nonbonded silica for the analysis of not only basic but also neutral and acidic analytes [29]. An aqueous buffer solution was applied to impregnate the silica surface. This aqueous layer formed a stationary phase and the retention mechanism is partition like under RPLC conditions. An efficient separation method of purines and pyrimidines with such a ‘‘pseudo-reversed-phase’’ mode is known. The chromatographic system consists of a silica column and a mixture of acetonitrile–ammonium acetate buffer (pH 6) with hexadecyltrimethylammonium ion [30].

Water is the most polar common solvent, however, the use of water as a mobile phase component under NPLC conditions is complicated. Hydrocarbons, dichloromethane and chloroform that are generally used in NPLC as less polar components are immisc-

ible with water. It was shown that a mixture of chloroform–methanol 100% saturated with 0.05 *M* ammonium formate is applicable to the analysis of some purines and pyrimidines [31]. In such an in-situ-generated liquid–liquid system an aqueous stationary liquid phase is generated spontaneously on the surface of a stationary phase if one (less polar) of the two coexisting phases of a liquid–liquid system is employed as the mobile phase. Adsorption of mobile phase components on the solid support surface leads to the formation of the liquid phase having the same overall composition as a liquid bulk phase in equilibrium with the mobile phase and this approach allows to create a partition retention mechanism on silica [32]. However, in-situ-generated liquid–liquid systems need more care than other common liquid chromatographic phase systems, such as extensive presaturation of the mobile phase and precise thermostatting of the mobile phase reservoir and column [31].

Good and stable separation conditions for a test mixture of steroids could be obtained by using an eluent (dichloromethane–ethanol–water [33]) that was only partially saturated with water. The use of organic eluents partially saturated with water is a good choice for reducing peak tailing [34]. Water preferentially adsorbed onto strong adsorption sites, leaving a more uniform population of weaker sites which then serve to retain the sample. This ''deactivation'' of the adsorbent leads to a number of improvements in the subsequent separation. The retention process cannot be considered as a simple adsorption but rather as a very complex mixed process of adsorption and liquid–liquid partition into more polar, water-rich liquid stationary phase developed from the eluent in-situ in the column [35]. Such a mixed-mode separation takes advantage of more than one retention process. An efficient separation of some purines and pyrimidines on unmodified silica with a partially water-saturated mobile phase is known [35,36]. Mixtures of dichloromethane, methanol and aqueous ammonium formate–formic acid [35] or potassium butanesulfonate [36] solution were used as a mobile phase. Anhydrous partially acetonitrile-saturated 2,2,4-trimethylpentane–ethanol–acetonitrile mixtures were tested before [37,38]. However, it was observed that the separation selectivity for aromatic hydrocarbons [37] and steroids [38] on silica is better in the chromatographic system with 100% acetonitrile saturation than in the partially saturated mode.

In the last 10 years mobile phases partially saturated with ethylene glycol, formamide or dimethyl sulfoxide were tested to analyze purine and pyrimidine derivatives [39–47]. Conditionally, such a chromatographic system was called the mixed partition–adsorption (MPA) normal-phase mode [42]. MPA systems allow one to separate purine and pyrimidine derivatives on silica with a good selectivity, high column efficiency and peak symmetry.

2. What is the mixed partition–adsorption system?

According to this method unmodified silica is used as a stationary phase and a mixture of two or three solvents with a limited mutual solubility is used as a mobile phase. Contrary to the normal-phase in-situ-generated liquid–liquid chromatography (LLC) where the mobile phase is 100% saturated with a polar component {e.g., ethylene glycol (EG) [48,49], formamide (FA) [31,48] or dimethyl sulfoxide (DMSO) [48,49]} in the MPA system a mobile phase is saturated only partially [39–47]. Such a partially saturated mobile phase has some obvious advantages over a saturated one. The eluent for the LLC mode was mixed vigorously during 12 h at a fixed temperature to ensure a complete demixing, followed by phase separation for 2 h [48,49], whereas the eluent for the MPA mode was prepared during approximately 10 min at ambient temperature by slowly adding the polar component (EG, FA, DMSO) to the vigorously mixed solution in order to ensure a complete homogenization [44,45]. Besides, a precise thermostatting of the mobile phase reservoir and column is necessary in LLC [48,49]. At the same time, the sensitivity to temperature changes in the MPA system is not much higher than in usual adsorption NPLC [41].

Fig. 1 represents the triangular phase diagram for ethylene glycol–isopropanol–hexane (EG–IPA–HEX) ternary system. Point 1 in Fig. 1 corresponds to the binary mobile phase IPA–HEX with adsorption mechanism of retention where separation is based on differences in adsorption and desorption

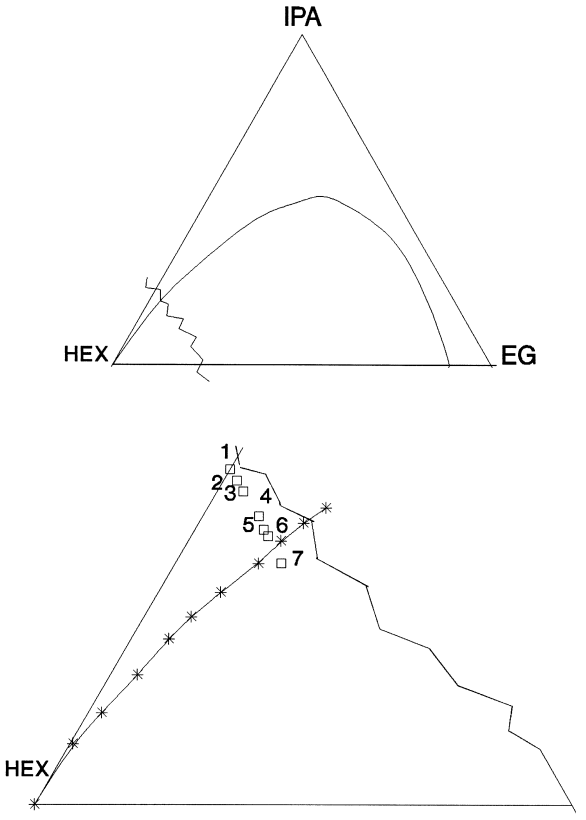

Fig. 1. Triangular phase diagram of ternary system EG–IPA–HEX at ambient temperature (reprinted from Ref. [44]).

rates of analytes on the silica surface. Point 7 below the equilibrium curve corresponds to the composition of ternary systems EG–IPA–HEX which split into two co-existing liquid phases, one of which is presaturated with EG, but the other with HEX. If the first of the coexisting phases is applied as a mobile phase, an in-situ-generated liquid–liquid system is formed. The liquid stationary phase is mechanically held as a bulk film onto a solid support, and the retention mechanism in such a system is partition. Points 2–6 above the equilibrium curve correspond to the compositions which are homogeneous and differ from others by the degree of saturation with EG. It was observed that the bulk liquid stationary phase can be formed in the silica pores not only by the saturated solution but also with partially saturated solution [39–47]. The mechanism of sorption in partially saturated systems is mixed, involving adsorption on the silica surface and partition. The contribution of each process depends on the volume of the deposited liquid phase.

It can be assumed that under MPA system conditions the total volume within the column (apart from the silica) consists of the mobile phase volume and the stationary liquid phase volume:

$$V_{mo} = V_m + V_s \quad (1)$$

where V_{mo} is the total volume, V_m is the mobile phase volume and V_s is the stationary liquid phase volume. Thus accordingly:

$$V_s = V_{mo} - V_m \quad (2)$$

Then it can be assumed that under adsorption NPLC conditions mobile phase did not generate stationary liquid phase on the silica packing ($V_s = 0$), so V_m equals V_{mo}. If under conditions of adsorption NPLC with a strong enough eluent, such as ethyl acetate (EA), benzene is not adsorbed and its retention volume corresponds to V_{mo}. The formation of the dynamically generated liquid stationary phase under MPA NPLC conditions leads to a decrease in the benzene retention volume ($V_s > 0$). This allows the calculation of V_s according to Eq. (2), where V_{mo} is the benzene retention volume under adsorption NPLC conditions and V_m is the benzene retention volume under MPA NPLC conditions. The phase ratio (ϕ) of the column in Table 1 was calculated according to the formula:

Table 1
Column[a] phase ratio and corresponding saturation of the EG–IPA–HEX mobile phase with polar components

No.[b]	Mobile phase EG–IPA–HEX composition (%, v/v)	Column phase ratio	Mobile phase saturation (%)
1	0:34:66	0	0
2	1:33:66	0.01	3
3	2:32:66	0.03	9
4	5:29:66	0.09	25
5	6:28:66	0.17	50
6	7:29:66	0.28	80
7[c]	10:24:66	0.34	100

Source: taken in part from Ref. [44].
[a] Zorbax SIL (150×4.6 mm).
[b] Points 1–7 in Fig. 1.
[c] One of the coexisting phases (saturated with polar components).

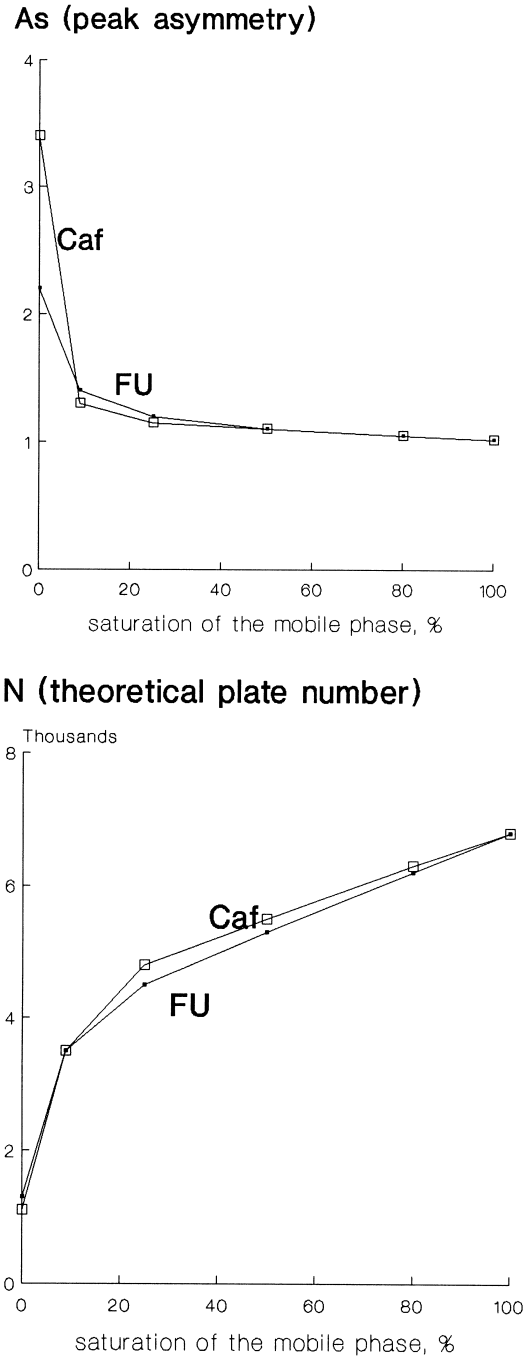

Fig. 2. Relationship between saturation of the mobile phase EG–IPA–HEX with polar component (Table 1) and peak asymmetry (A_s) and theoretical plate number (N). Solutes: caffeine (Caf), 5-fluorouracil (FU). Column: Zorbax SIL, 5 μm, 150×4.5 mm. Data from Ref. [44].

$$\phi = V_s/V_m \quad (3)$$

The effect of the mobile phase saturation on theoretical plate number N and peak asymmetry A_s is shown in Fig. 2. It can be seen that the generation of a liquid stationary phase is accompanied by an improvement of the column efficiency and peak symmetry. If the saturation of the mobile phase is less than 40%, the peak symmetry and the column efficiency for such systems are better than in the adsorption mode, but worse than in the partition mode. However, if the saturation is more than 40%, the systems under study demonstrate a good peak symmetry and column efficiency. Such mobile phases (with column phase ratio >0.1, Table 1) are applicable to create the MPA mode where the influence of the silica matrix is low and the partition retention mechanism prevails over adsorption. The advantages and disadvantages of the MPA mode are summarized in Table 2.

3. Mobile phase in the mixed partition–adsorption system

Mobile phases in the MPA system were binary (EG–EA [39,40,46]; FA–EA [41]) or ternary {EG or FA–methanol (MeOH) or IPA–EA [42,43,46,47]; EG, FA or DMSO–IPA or dioxane–HEX or chloroform [43–47]} solution partially (40–80%) saturated with polar component. Analyte retention in the MPA mode increases as the polarity of the mobile phase decreases. Less polar compounds eluted first, while more polar compounds leave the column last as in traditional NPLC. At the same time, MPA systems show a specific selectivity compared to adsorption NPLC (inversion of the elution order for some solutes studied, e.g., for the adenine/inosine pair [42]).

A binary MPA mobile phase has a restriction in the elution strength (up to 6.5% of EG or up to 3.5% of FA can be dissolved in EA at room temperature). It can be seen [39,40] that the elution strength of binary MPA mobile phase 4% EG in EA (60% saturation of EA with EG) was not sufficient for some of the most polar analytes under study (e.g., inosine, acyclovir, cytarabine). Moreover, the alteration in the concentration of the polar component

Table 2
Characteristics of the MPA mode

Advantages
(1) Extensive presaturation of the mobile phase with the stationary phase is not necessary as in LLC
(2) Temperature sensitivity in the MPA mode is the same as in adsorption NPLC
(3) Liquid stationary phase can be easily stripped from silica surface and recoated in-situ many times
(4) Column is quite stable and the reproducibility of retention time on one type column is satisfactory
(5) Separation selectivity on different silica columns does not differ much
(6) Versatility (very large changes in separation selectivity by changing the mobile phase composition)
Disadvantages
(1) Relatively slow column equilibration (more than 30 column volumes)
(2) After preparative separation the sample is always contaminated by the mobile phase polar component (e.g., EG or DMSO)

(EG) does not affect retention very markedly (e.g., k'_{uracil} is 2.0 in the MPA system with mobile phase 4% EG in EA and 2.6 in the MPA system with mobile phase 2% EG in EA [41]). For this reason ternary mobile phases were tested [42–47]. The MPA system with mobile phase EG–MeOH–EA (10:6:84) has an elution strength sufficient to analyse such solutes as inosine, acyclovir and cytarabine [40]. But for decreasing the elution strength or if detection at wavelengths <240 nm is required the best choice is mobile phases based on hexane (e.g., k'_{uracil} is 6.4 in the MPA system with mobile phase EG–IPA–HEX, 5:24:71 [43]).

In order to estimate the flexibility of the MPA mode the concentration of polar and nonpolar components in MPA mobile phases was varied [42–46]. It can be seen that the selectivity does not change very markedly. So, the selectivity factor α ($k'_{cytarabin}/k'_{acyclovir}$) is 1.07 in the MPA system with the mobile phase EG–MeOH–EA (13:6:81) and 1.18 in the MPA system with the mobile phase EG–MeOH–EA (10:3:87) [42]. If considerable selectivity alterations are necessary the best approach is to use another MPA system by replacing both polar and/or nonpolar components. For example, the selectivity factor α ($k'_{cytarabin}/k'_{acyclovir}$) is 1.34 in the MPA system with the mobile phase FA–MeOH–EA (15:1.5:83.5); 1.62 in the MPA system with the mobile phase EG–MeOH–chloroform (10:2:88) and 2.12 in the MPA system with the mobile phase FA–MeOH–chloroform (10:2:88) [42].

4. Stationary phase in the mixed partition–adsorption system

Various silica columns were tested [42,44,45] as

Table 3
Reproducibility of selectivity coefficient (α) in the MPA system with the DMSO–IPA–HEX (10:18:72) mobile phase[a]

No.	Column, trade name (dimensions, mm)	Packing material		Phase ratio	Retention (k')		Selectivity coefficient (α) (TM/TP)
		Specific surface area (m^2/g)	Specific pore volume (ml/g)		Thymine (TM)	Theophylline (TP)	
1	LiChrospher Si-60 (125×4.0)	700	0.85	0.21	3.80	2.50	1.52
2	Silasorb 600 (250×4.0)	600	1.00	0.21	3.11	2.10	1.48
3	LiChrosorb Si-60 (250×4.0)	500	0.70	0.19	2.89	1.90	1.52
4	Zorbax SIL (150×4.6)	350	0.80	0.20	3.00	2.00	1.50
5	Supelcosil LC-SI (250×4.6)	170	0.60	0.18	2.56	1.69	1.51
6	Nova Pak Silica (150×3.9)	120	0.30	0.13	1.66	1.09	1.52

Source: Ref. [45].
[a] 85% mobile phase saturation.

Table 4
5-Fluorouracil column efficiency, peak asymmetry and selectivity in adsorption[a] (A) and MPA[b] modes

No.	Column		Efficiency (N)		Peak asymmetry (A_s)		Selectivity coefficient (α)[d]	
	No.[c]	Phase ratio (V_s/V_m)	A	MPA	A	MPA	A	MPA
1	2	0.25	1300	6300	2.2	1.05	4.39	0.71
2	4	0.22	1400	6200	2.1	1.05	3.45	0.68
3	4	0.22	1300	6200	2.2	1.05	3.33	0.68
4	5	0.19	1400	4800	2.0	1.2	3.07	0.72
5	6	0.13	1100	2000	2.7	1.5	2.91	0.71

Source: data from Ref. [44].
[a] Mobile phase IPA–HEX (34:66).
[b] Mobile phase EG–IPA–HEX (5:24:71); 65% mobile phase saturation.
[c] See Table 3.
[d] k' (theophylline)/k' (5-fluorouracil).

stationary phases to compare retention and selectivity in MPA systems. The retention value differs from silica to silica because of the difference in the stationary phase loading, but the variation in selectivity is surprisingly small (Table 3).

The reproducibility of the selectivity independent of the silica type is an important peculiarity of the MPA mode as compared to with the adsorption systems where the selectivity differs from column to column. The MPA mode advantage versus adsorption (A) mode in column efficiency, peak symmetry and reproducibility of selectivity on various silica columns is presented in Table 4.

The column phase ratio (V_s/V_m) depends not only on the mobile phase (Table 1, Nos. 4–6) but also on the silica type (e.g., surface area, pore volume). Table 3 shows that on the various silica gels investigated in this study, phase ratios between 0.13 and 0.21 were obtained. In most cases (except column No. 4) the volume of the dynamically generated stationary phase is larger and retention is stronger on silicas with a larger surface area and pore volume (Table 3).

The liquid stationary phase can be easily stripped from the silica surface by flushing with 50 ml of IPA–HEX (40:60). Now the silica column can be used in the adsorption mode (the stationary phase is the same as before MPA mode usage) or with another MPA mobile phase (the column should be flushed with MPA mobile phase, usually 30–40 column volumes, after cleaning to equilibrate the column). It was shown that the retention time reproducibility on one type column under MPA mode is satisfactory [45].

5. Application of the mixed partition–adsorption system

Separation of the test mixtures of some purine and pyrimidine derivatives on type A silica in the NPLC mode has been studied [40–47]. When adsorption systems are used, peak tailing is typical.

In order to improve the peak shape, better-purified type B silica was used [46]. Fig. 3 shows that the peak symmetry of the solutes studied under adsorption normal-phase conditions is better on type B than on type A silica, but significant improvement was achieved only if the MPA mode is applied. Moreover, the selectivity is much better in the MPA mode (Fig. 3C) than in the adsorption mode (Fig. 3A and B).

The chromatogram in Fig. 3D demonstrates the behaviour of the same test mixture under RPLC conditions. It can be seen that the RPLC mode is unable to achieve a good separation for the pairs 1/3 (separation factor too small) and 3/4 (too large), and in this case the MPA mode (Fig. 3C) is a good alternative to RPLC. Other examples of the separation of some purines and pyrimidines under MPA NPLC and RPLC conditions are given in Ref. [46].

It is known that the separation of 5-fluorouracil (FU) and uracil, a probable FU substance impurity, is difficult with C_{18} columns commonly used in

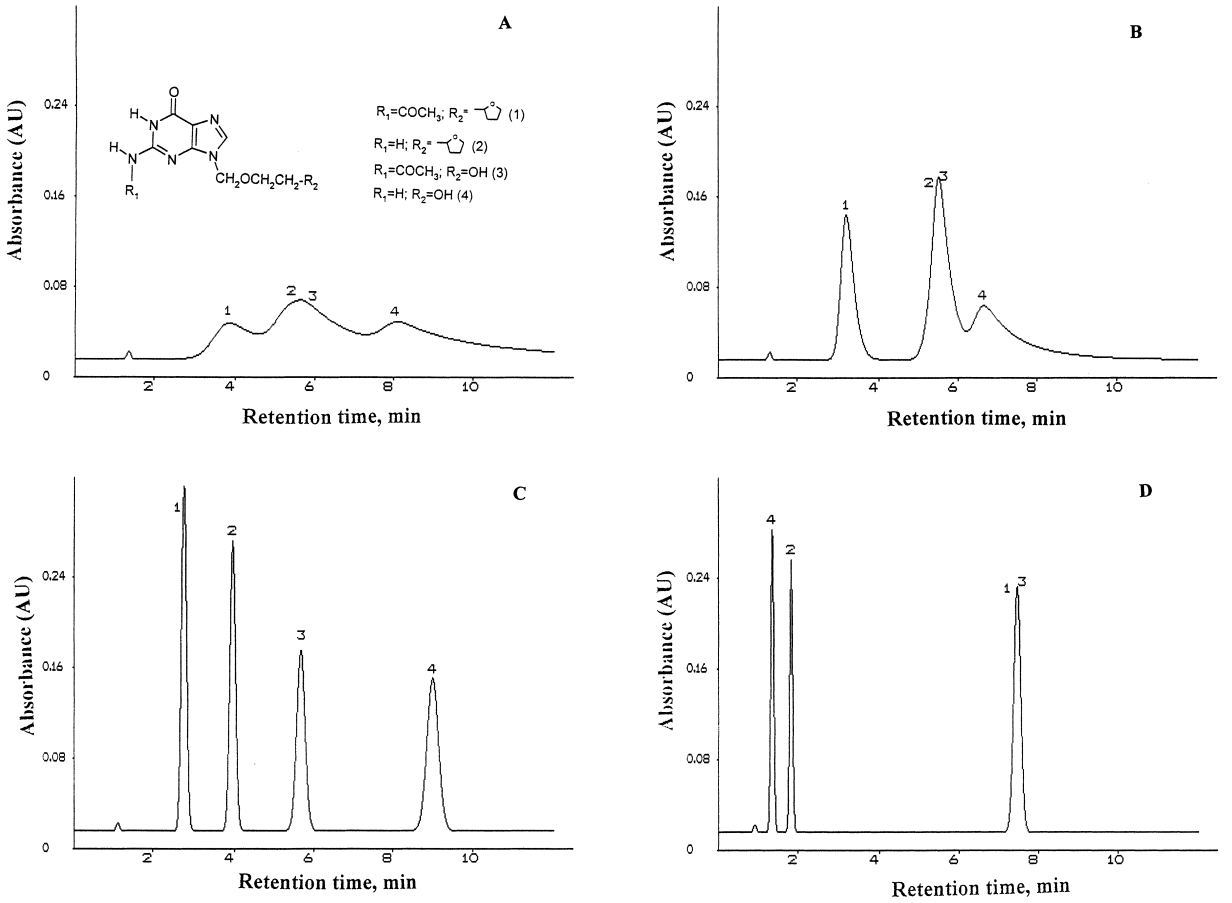

Fig. 3. Chromatograms of a test mixture. (A) Mobile phase MeOH–EA (20:80); column, Zorbax Rx-SIL. (B) Mobile phase MeOH–EA (10:90); column, Zorbax SIL. (C) Mobile phase EG–MeOH–EA (12:4:84); column, Zorbax SIL. (D) Mobile phase 5% acetonitrile in 0.1% solution of phosphoric acid; column, Zorbax SB-C_{18}. All columns are 150×4.6 mm; flow-rate, 1.5 ml/min; detection, UV at 254 nm; ambient temperature; 0.32 AUFS. Injection volume, 10 μl (A–C) and 5 μl (D); sample concentration in mobile phase, 0.1 mg/ml. Data from Ref. [46].

RPLC. A low selectivity (separation factor, $\alpha < 1.2$) for the uracil/FU pair was observed [2]. Special columns are necessary for a good separation of FU and uracil under RPLC conditions {e.g., a PRP-1 column packed with poly(styrene–divinylbenzene) at pH 8.0; separation factor, $\alpha = 3.9$ [50]}. The MPA system (conventional silica column and mobile phase EG–IPA–EA, 3:1:96), being based on a mixed partition–adsorption retention process, provides a good resolution for this pair (separation factor, $\alpha = 2.7$) [43].

Another example where separation is not easily obtained with traditional RPLC is the separation of the guanosine/inosine pair (separation factor, $\alpha < 1.2$ [3,4]). In an adsorption normal-phase system on a silica column for the separation factor, α, for this pair ranged in 12 mobile phases under study from 1.1 to 9.4, but the peak shapes were unsatisfactory ($A_s > 2.5$) in all cases [47]. Chromatograms can be significantly improved on the same silica column if MPA mobile phases are applied ($A_s < 1.3$). It can be seen [47] that in the MPA mode for the guanosine/inosine pair the α value is less than 1.4, but also among 11 MPA systems only two systems possess an α value of less than 1.2.

The impurities FU and 1,3-bis(2-tetrahydrofuryl)uracil (Bis) were determined in ftorafur (FT) under reversed-phase and MPA normal-phase con-

Table 5
Reasons to use the MPA mode

Instead of adsorption normal-phase mode
(1) MPA mode is useful to improve the column efficiency and the peak shapes for polar solutes such as purine and pyrimidine derivatives on unmodified silica
(2) The selectivity obtained in the MPA mode does not differ much on different silica columns
Instead of reversed-phase (RP) mode
(1) MPA mode is useful if RP mode is unable to achieve a good separation ($\alpha < 1.1$)
(2) MPA mode is useful for unretained or too strongly retained RP mode analytes
(3) MPA mode is the best choice for organic soluble samples
(4) MPA mode is the best choice for samples that can decompose in aqueous solutions

ditions [46]. The separation factors for pairs FT/FU and Bis/FT were too large in isocratic elution in the RPLC conditions, and gradient elution was preferable in this case. At the same time the MPA system under isocratic conditions possesses a good selectivity and demonstrates good peak shapes of the solutes under study on all silicas (type A and type B). The use of the MPA normal-phase separation in combination with RPLC can offer additional possibilities to control purity [46].

Table 5 presents the advantages of the MPA mode versus adsorption normal-phase and reversed-phase systems.

6. Conclusions

MPA systems are an alternative approach for the separation of polar solutes such as purines and pyrimidines on silica. Although RPLC supersedes NPLC in many application areas, the MPA normal-phase mode can play a useful role in HPLC analysis. Definitely, the MPA systems are not always the best or only way to solve a chromatographic problem. However, it can be concluded that the MPA normal-phase chromatography method is an applicable method among other HPLC methods and is a good choice to enlarge the number of chromatographic systems in HPLC, especially if a system with specific selectivity is required.

7. Nomenclature

Bis	1,3-Bis(2-tetrahydrofuryl)uracil
Caf	Caffeine
DMSO	Dimethyl sulfoxide
EA	Ethyl acetate
EG	Ethylene glycol
FA	Formamide
FT	Ftorafur
FU	5-Fluorouracil
HEX	Hexane
HPLC	High-performance liquid chromatography
IPA	Isopropanol
LLC	Liquid–liquid chromatography
MeOH	Methanol
MPA	Mixed partition–adsorption
NPLC	Normal-phase liquid chromatography
RPLC	Reversed-phase liquid chromatography
TM	Thymine
TP	Theophylline

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References

[1] M. Ryba, J. Chromatogr. 219 (1981) 245.
[2] A.A. Miller, J.A. Benvenuto, T.L. Loo, J. Chromatogr. 228 (1982) 165.
[3] M. Zakaria, P.R. Brown, J. Chromatogr. 255 (1983) 151.
[4] C. Yi, J.L. Fasching, P.R. Brown, J. Chromatogr. 339 (1985) 75.
[5] T.E.B. Leakey, J. Chromatogr. 507 (1990) 199.
[6] J. Zhao, B. Todd, G.H. Fleet, J. Chromatogr. A 673 (1994) 167.

[7] Z. Liu, T. Li, E. Wang, Analyst 120 (1995) 2181.
[8] W. Hu, K. Hasebe, D.M. Reynolds, H. Haraguchi, Anal. Chim. Acta 353 (1997) 143.
[9] V.Yu. Toshchakov, L.V. Bashkina, N.A. Onishchenko, V.I. Shumakov, Biokhimiya (Moscow) 63 (1998) 259.
[10] M.H. Maguire, I. Szabo, I.E. Valko, B.E. Finley, T.L. Bennett, J. Chromatogr. B 707 (1998) 33.
[11] G. Xu, H. Enderle, H. Liebich, P. Lu, Chromatographia 52 (2000) 152.
[12] L.R. Snyder, J.J. Kirkland, J.L. Glajcht, in: Practical HPLC Method Development, Wiley–Interscience, New York, 1997, p. 268;
L.R. Snyder, J.J. Kirkland, J.L. Glajcht, in: Practical HPLC Method Development, Wiley–Interscience, New York, 1997, p. 731.
[13] H.-L. Jin, Y.-L. Zhang, Y. Guo, S. Jin, Chromatographia 22 (1986) 153.
[14] S.-H. Chen, S.-M. Wu, H.-L. Wu, J. Chromatogr. 595 (1992) 203.
[15] L.S. Von Tungeln, P.P. Fu, J. Chromatogr. 461 (1989) 315.
[16] L. Almela, J.-M. Lopez-Roca, M.E. Candela, M.D. Alcazar, J. Chromatogr. 502 (1990) 95.
[17] S.N. Lanin, Yu. Nikitin, J. Chromatogr. 520 (1990) 315.
[18] P. Jandera, J. Urbanek, B. Prokes, J. Churacek, J. Chromatogr. 504 (1990) 297.
[19] J.J. Kirkland, C.H. Dilks Jr., J.J. DeStefano, J. Chromatogr. 635 (1993) 19.
[20] J. Chmielowiec, Anal. Chem. 55 (1983) 2367.
[21] S.C. Ruckmick, R.J. Hurtubise, J. Chromatogr. 360 (1986) 343.
[22] J. Kirschbaum, J. Noroski, A. Cosey, D. Mayo, J. Adamovics, J. Chromatogr. 507 (1990) 165.
[23] S. Husain, P.N. Sarma, S.M. Sajjad, R. Narsimha, M. Subrahmanyam, J. Chromatogr. 513 (1990) 83.
[24] M. Johansson, S. Eksborg, A. Arbin, J. Chromatogr. 275 (1983) 355.
[25] C.J. Woollam, Anal. Proc. 24 (1987) 187.
[26] R.J. Flanagan, I. Jane, J. Chromatogr. 323 (1985) 173.
[27] R. Schwarzenbach, J. Chromatogr. 334 (1985) 35.
[28] L.R. Treiber, J. Chromatogr. A 696 (1995) 193.
[29] J.A. Adamovics, D.L. Farb, in: Chromatographic Analysis of Pharmaceuticals, 2nd ed., Chromatographic Science Series, Vol. 74, Marcel Dekker, New York, Basel, Hong Kong, 1997, p. 174.
[30] V.D. Shatc, L.A. Brivkalne, Zh. Fiz. Khim. 64 (1990) 2460.
[31] J.P. Crombeen, S. Heemstra, J.C. Kraak, J. Chromatogr. 282 (1983) 95.
[32] J.F.K. Huber, M. Pawlowska, P. Markl, J. Chromatogr. 500 (1990) 257.
[33] J.H.M. Van Den Berg, J. Milley, N. Vonk, R.S. Deelder, J. Chromatogr. 132 (1977) 421.
[34] L.R. Snyder, J.J. Kirkland, in: Introduction to Modern Liquid Chromatography, Wiley–Interscience, New York, 1979, p. 374.
[35] M. Ryba, J. Beranek, J. Chromatogr. 211 (1981) 337.
[36] W.J.Th. Brugman, S. Heemstra, J.C. Kraak, Chromatographia 15 (1982) 282.
[37] J.F. Huber, M. Pawlowska, P. Markl, Chromatographia 17 (1983) 653.
[38] M. Pawlowska, Chem. Anal. 33 (1988) 211.
[39] H.A. Kažoka, V.D. Shatz, Khim.–Farm. Zh. 12 (1989) 1516.
[40] V.D. Shatz, H.A. Kažoka, J. Chromatogr. 552 (1991) 23.
[41] H.A. Kažoka, V.D. Shatz, J. Chromatogr. 626 (1992) 181.
[42] H. Kažoka, V. Shatz, J. Chromatogr. A 732 (1996) 231.
[43] H. Kažoka, Khim.–Farm. Zh. 11 (1995) 49.
[44] H. Kažoka, J. Chromatogr. A 836 (1999) 235.
[45] H. Kažoka, J. Chromatogr. A 874 (2000) 45.
[46] H. Kažoka, J. Chromatogr. A 922 (2001) 187.
[47] H. Kažoka, J. Chromatogr. Sci. 39 (2001) 265.
[48] J.P. Crombeen, S. Heemstra, J.C. Kraak, J. Chromatogr. 286 (1984) 119.
[49] J.P. Crombeen, S. Heemstra, J.C. Kraak, Chromatographia 19 (1984) 219.
[50] G.J. Peters, I. Kraal, E. Laurensse, A. Leyva, H.M. Pinedo, J. Chromatogr. 307 (1984) 464.