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Application of mixed partition–adsorption systems in high-performance liquid chromatography of purines and pyrimidines

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Abstract

Separation of the test mixtures of some purine and pyrimidine derivatives on silicas (types A and B) in adsorption normal-phase (A-NP) and mixed partition–adsorption normal-phase (MPA-NP) mode has been studied. When the A-NP mode is used the peak shapes are unsatisfactory (especially on type A silica). At the same time MPA-NP systems show a good peak symmetry on all silica types. The findings have demonstrated that the MPA-NP mode offers a specific selectivity. This allows MPA-NP systems to supersede reversed-phase systems in some application areas.

Keywords: Partition–adsorption systems; Mobile phase composition; Adsorption; Stationary phases, LC; Purines; Pyrimidines

1. Introduction

Reversed-phase (RP) chromatography has become a widely used method for the separation of purine and pyrimidine derivatives [1–5]. But it is not always the best or only choice for the given analytical task. Normal-phase (NP) chromatography can be a powerful complement to the RP mode [6].

Polar organic substances in NP chromatography conditions require eluents of a high polarity in order to be eluted from unmodified silica. Under these conditions, adsorption is usually the dominating retention mechanism and in such an adsorption normal-phase (A-NP) mode peak shapes of polar solutes are usually unsatisfactory. A peak asymmetry causes a decrease in column efficiency as well as a resolution and detection limits. In order to improve a peak shape on silica one can apply less adsorptive, type B silica (e.g., Zorbax Rx-SIL which is less acidic and highly purified silica especially recommended for analysis of basic solutes [7]) or polar chemically bonded phases [8]. Another approach is based on using mobile phases containing different additives (e.g., a small amount of water). An efficient separation method of some purines on unmodified silica with dichloromethane–methanol–aqueous mixtures as a mobile phase is known [9–11]. The retention process cannot be considered as simple adsorption but rather as a very complex mixed process of adsorption and liquid–liquid partition into a more polar, water-rich liquid stationary phase, developed from the eluent in situ in the column [9]. But it is not a good decision if analytes have a limited solubility in water–organic mixtures or may decompose in aqueous solutions.

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Previous findings [12–16] have demonstrated that the use of nonaqueous mixed partition–adsorption normal-phase (MPA-NP) systems leads to the improvement of a peak shape of some purine and pyrimidine derivatives. According to this method a mixture of two or three solvents with a limited mutual solubility is used as a mobile phase and unmodified silica as a stationary phase. A liquid stationary phase (LSP) consisting mainly of a polar solvent (e.g., ethylene glycol, dimethyl sulfoxide, formamide) is generated dynamically in the silica pores, even in the mobile phase not saturated with the polar component, and this results in a mixed retention mechanism where partition dominates over adsorption. Conditionally, such a system was called MPA [14].

The goal of this work was to compare peak symmetry and system selectivity in A-NP, MPA-NP and RP systems for the same test mixtures (solutes 1–11 in Fig. 1) and to show the applicability of the MPA-NP mode in high-performance liquid chromatography (HPLC) for the separation of purines and pyrimidines.

2. Experimental

2.1. Analysis of the test solutes 1–11 (see Fig. 1)

NP mode: The chromatographic measurements were performed on a Gilson Model 302 HPLC system, equipped with a spectrophotometer (wavelength 254 nm). Zorbax-SIL (a model for type A silica) and Zorbax Rx-SIL (a model for type B silica [7]) columns were used as stationary phases at ambient temperature. Mixtures of ethylene glycol (EG), formamide (FA) and/or methanol (MeOH), isopropanol (IPA) with ethyl acetate (EA) or hexane (HEX) were studied as mobile phases at a flow-rate of 1.5 ml/min.

RP mode: The chromatographic measurements were performed on a Varian ProStar HPLC system, equipped with a spectrophotometer (wavelength 254 nm). A Zorbax SB-C_{18} column was used as a stationary phase at a temperature of 40°C. Mixtures of acetonitrile with 0.1% aqueous phosphoric acid were studied as mobile phases at a flow-rate of 1.5 ml/min.

The samples (injection volume 10–50 μl, sample concentration in mobile phase 0.1 mg/ml) were injected via a Rheodyne 7125 sampling valve (NP mode) or autosampler ProStar Model 410 (RP mode). All Zorbax columns were from Hewlett-Packard and were 150×4.6 mm.

2.2. Analysis of ftorafur (FT) substance

The chromatographic measurements were performed on a Gilson Model 302 HPLC system, equipped with a spectrophotometer (wavelength 270 nm). OmniSpher 5 C_{18} (Varian) 150×4.6 mm (RP mode) and Zorbax-SIL or Zorbax Rx-SIL (MPA mode) columns were used as a stationary phase at ambient temperature. A mixture of acetonitrile–0.1% aqueous phosphoric acid (20:80) at a flow-rate of 1.0 ml/min (RP mode) and a mixture of EG–IPA–HEX (3:18:79) at a flow-rate of 1.5 ml/min (MPA mode) were studied as mobile phases. The samples (injection volume 25–50 μl, sample concentration in mobile phase about 0.5 mg/ml) were injected via a Rheodyne 7125 sampling valve.

In the MPA mode the column was conditioned before each series of retention measurements. Conditioning included flushing with 50 ml of IPA–HEX (50:50) followed by the mobile phase under study. Usually 50 ml of the latter eluent [14–16] was sufficient to obtain constant retention values in the MPA-NP mode except when the mobile phases contained formamide (in this case 150 ml was necessary [13]). The eluents for MPA systems were prepared by slow addition of the polar component (EG, FA) to the vigorously mixed binary solvent alcohol–HEX or ethyl acetate (EA) chloroform (CHL) and dioxane to ensure complete homogenization. All the solvents were purchased from commercial sources and were of analytical grade and used without any pretreatment.

The capacity factors (k') of the solutes under study were calculated according to the usual expression [17]. The system mobile phase volume was regarded to be equal to benzene retention volume. The peak asymmetry (A_s) was calculated by determination of the A/B ratio at 10% of peak height [18].

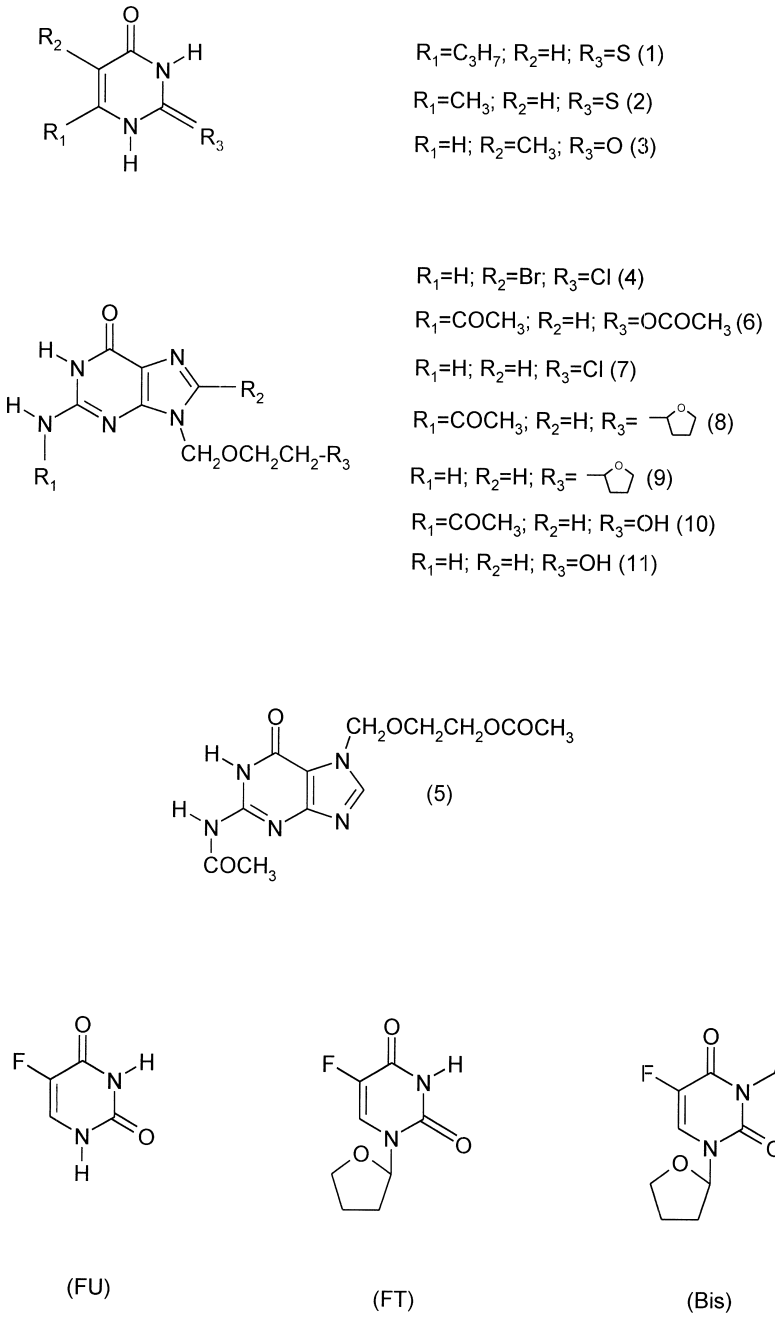

Fig. 1. Molecular structures of the solutes under study.

3. Results and discussion

According to Table 1, the mobile phases studied have a sufficient elution strength for elution polar analytes (solutes 1–11 in Fig. 1) from unmodified silica. However in the A-NP mode on type A silica (Zorbax-SIL column) poor peak shapes are observed (asymmetry factor from 2.0 to 4.5). At the same time

Table 1
Retention and peak symmetry of the solutes studied in the A-NP mode on type A and type B silicas (columns Zorbax-SIL and Zorbax Rx-SIL), in the MPA-NP mode on type A silica (column Zorbax-SIL) and in the RP mode (column Zorbax SB-C_{18})

Column	Mobile phase composition (v/v)	Solutes under study[a] (No.)	Retention (k')	Asymmetry factor (A_s)
Zorbax SIL	EA–HEX	1	1.0	2.0
	(60:40)	2	2.3	2.5
		3	8.1	3.0
	MeOH–EA	4	1.2	2.0
	(10:90)	5	1.8	2.0
		6	4.9	3.5
		7	5.9	4.0
	MeOH–EA	8	1.8	3.5
	(20:80)	9	3.0	4.0
		10	3.2	4.0
		11	5.0	4.5
	FA–IPA–HEX	1	4.2	1.05
	(3:29:68)	2	7.9	1.10
		3	6.7	1.15
	EG–EA	4	4.0	1.05
	(4:96)	5	2.3	1.05
		6	4.6	1.05
		7	10.5	1.15
	EG–MeOH–EA	8	1.6	1.05
	(12:4:84)	9	2.8	1.05
		10	4.4	1.10
		11	7.5	1.15
Zorbax Rx-SIL	EA–HEX	1	0.6	1.3
	(60:40)	2	1.5	1.4
		3	5.4	1.8
	MeOH–EA	4	0.9	1.3
	(5:95)	5	1.5	1.5
		6	2.0	2.0
		7	4.4	2.5
	MeOH–EA	8	1.5	1.5
	(10:90)	9	3.1	2.0
		10	3.2	2.0
		11	6.5	3.0
Zorbax SB-C_{18}	Acetonitrile–0.1% aqueous	1	5.5	1.10
	phosphoric acid (10:90)	2	0.7	1.05
		3	0.4	1.05
	Acetonitrile–0.1% aqueous	4	11.3	1.10
	phosphoric acid (12:88)	5	5.3	1.05
		6	4.4	1.05
		7	1.7	1.05
	Acetonitrile–0.1% aqueous	8	7.0	1.10
	phosphoric acid (5:95)	9	0.8	1.05
		10	7.0	1.10
		11	0.4	1.10

[a] See Fig. 1.

the peak symmetry for the test solutes is much better (asymmetry factor from 1.3 to 3.0) in the A-NP mode on type B silica (Zorbax Rx-SIL column).

Nevertheless peak shape of polar solutes on silica can be improved significantly only if the MPA-NP mode is applied (asymmetry factor<1.15). According to Table 1, the MPA-NP mode is not only useful to improve the peak shapes but also offers a specific selectivity for the pairs 2/3 and 4/5.

The data in Table 1 also show the behavior of the same test mixtures in the RP mode. Besides that the MPA system is the best choice for organic-soluble samples or samples which may decompose in aqueous solutions, it can be useful for the samples which are unretained or too strongly retained by the RP mode. It can be seen that the RP mode is unable to achieve a good separation for the pairs 5/6, 8/10 (separation factor too small) and 1/3, 4/7, 10/11 (too large) and the MPA-NP mode is a good alternative for RP.

The use of MPA-NP separation in a combination with RP can offer additional possibilities for purity control. The impurities 5-fluorouracil (FU) and 1,3-bis(2-tetrahydrofuryl)uracil (Bis) were determined in

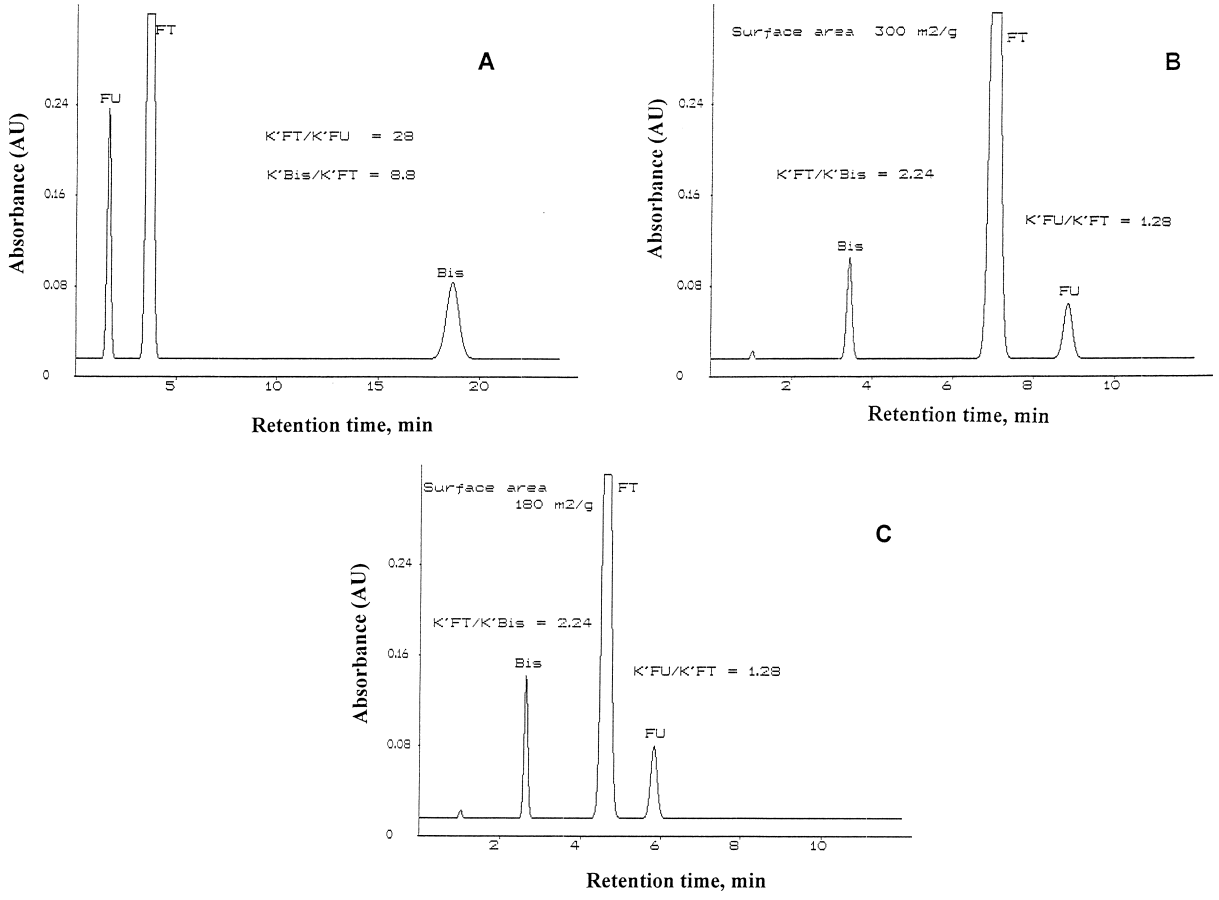

Fig. 2. Determination of impurities in technical-grade ftorafur (FT) substance. (A) Column, OmniSpher 5 C_{18}; 150×4.6 mm, ambient temperature. Mobile phase: acetonitrile–0.1% aqueous phosphoric acid (20:80); flow-rate, 1.0 ml/min. Detection, UV, 270 nm; 0.35 AUFS. Injection: 50 μl of 0.56 mg per ml in mobile phase. (B) Column, Zorbax-SIL; 150×4.6 mm; ambient temperature. Mobile phase EG–IPA–HEX (3:18:79); flow-rate, 1.5 ml/min. Detection, UV, 270 nm; 0.32 AUFS. Injection: 25 μl of 0.55 mg per ml in mobile phase. (C) Column, Zorbax Rx-SIL; 150×4.6 mm; ambient temperature. Mobile phase EG–IPA–HEX (3:18:79); flow-rate, 1.5 ml/min. Detection, UV, 270 nm; 0.32 AUFS. Injection: 25 μl of 0.56 mg per ml in mobile phase. 5-Fluorouracil (FU) and 1,3-bis(2-tetrahydrofuryl)uracil (Bis) were identified by co-elution with standards.

Table 2
Selectivity of MPA-NP systems (70% mobile phase saturation)

N	MPA-NP mobile phase	Mobile phase composition (v/v)	Separation coefficient, k'_{FU}/k'_{FT} [a]
1	EG–IPA–HEX	3:18:79	1.3
2	EG–IPA–HEX	5:24:71	1.3
3	EG–IPA–HEX	7:27:66	1.2
4	EG–IPA–EA	3:1:96	2.2
5	EG–IPA–CHL	8:2:90	8.7
6	EG–dioxane–CHL	3:12:85	13.1

[a] FU=5-Fluorouracil; FT=ftorafur.

technical-grade ftorafur substance in the RP mode (Fig. 2A) and in the MPA-NP mode (Fig. 2B and C). It can be seen that both methods are applicable. But in the RP mode (isocratic elution) a separation factor for pairs FT/FU and Bis/FT is too large, and a gradient elution is preferable in this case. At the same time the MPA-NP system under isocratic conditions has a good selectivity. Moreover, the MPA-NP mode demonstrates good peak shapes of solutes under study on all silica types (Fig. 2B and C). It can be observed that the retention time in MPA systems depends on the packing material (retention is stronger on the silica with larger surface area), but the selectivity does not differ much on different silica columns. According to Table 2, if considerable selectivity alterations are necessary the best approach is to use another MPA system.

4. Conclusions

In A-NP systems the peak shapes of the solutes under study are unsatisfactory (especially on type A silica). The substitution of the A-NP mode for the MPA-NP mode results in a good peak symmetry of polar solutes. Besides, the MPA-NP mode shows a specific selectivity.

Although the RP mode has superseded the NP mode in many application areas, the MPA-NP mode can play a promising role in the separation of purines and pyrimidines.

Definitely, the MPA-NP mode is not always the best or the only way to solve a problem. But the MPA-NP mode is a good choice to enlarge the number of chromatographic systems in HPLC, especially if a system with specific selectivity is needed.

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