Behavior of Haloperidol and Various Phenothiazines on Several Alkyl Bonded Phases

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Abstract

Haloperidol and phenothiazines are present in psychiatrical treatments. An analysis in body fluids is tedious because of the presence of demethylated (DM) derivatives of phenothiazines. The behavior of some interfering solutes on alkyl bonded phases has been studied. Phenothiazines and DM derivatives exhibit a very similar behavior with a binary eluent (phosphate buffer–acetonitrile), which precludes an optimization with this system. When a ternary phase is used (phosphate buffer–acetonitrile–methanol), haloperidol and reduced haloperidol behave differently as compared with phenothiazines. In this mode it is possible to unambiguously detect haloperidol that would otherwise interfere. Phenothiazine peaks are characterized by a large tailing. An interesting feature is the comparison between cyclohexyl bonded and octadecyl bonded phases, the former being much more efficient.

Introduction

An accurate and unambiguous determination of drugs in body fluids is essential for therapic drug monitoring. The problem is tedious in psychiatric hospitals because of polytherapy and the wide range of compounds that may be encountered (i.e., benzodiazepines, phenothiazines, and tricyclic antidepressants).

Literature shows a wide variety of employed analytical techniques for the separation of one set of compounds. For example, benzodiazepines can be analyzed by gas chromatography (GC), liquid chromatography (LC), or thin-layer chromatography (1–6). Literature concerning the separation of phenothiazines and their derivatives is less abundant. Separations can be carried out by GC in the polar phase (7), but LC is the preferred method. Thomas et al. (8) have published an optimization procedure for the resolution of the adjacent peaks of several selected phenothiazines on bare silica. However, reversed-phase LC (RPLC) is the method of choice because it involves a water–organic modifier eluent that is compatible with the usual extraction procedure. A computer-assisted high-performance LC (HPLC) method with multiwavelength detection was published in 1990 in which several phenothiazines were considered (9). In an extensive study, Aymard et al. (10) listed the retention factors of 36 solutes, including some phenothiazines. The retention factors were separately determined and the best chromatogram was obtained with solutes of different functionalities. In a recent study on the post-column oxidative derivatization of phenothiazines, Diehl and Karst (11) displayed a separation of seven phenothiazines on a C18 phase. It should be pointed out that in an early study De Smet et al. (12) advocated the use of a cyanopropyl phase. As a result of capillary electrophoresis (CE) gaining importance, Wang et al. (13) separated four phenothiazines by CE, and Muijselaar et al. (14) published the separation of 14 phenothiazines by micellar electrokinetic chromatography.

All of these studies focused on the separation of standards and did not consider metabolites. Phenothiazines can undergo demethylation of the $N(CH_3)_2$ group, and two metabolites are possible according to the substitution of one or two methyl by a hydrogen atom (shown in Figure 1). The monomethyl derivative was only considered as totally demethylated (DM) and is generally not present in plasma. Haloperidol (a substituted fluorobutyrophenone) is a potent antipsychotic drug that is often associated with certain phenothiazines in therapy. In plasma analysis, haloperidol and phenothiazines can be found together with reduced haloperidol and phenothiazine metabolites (i.e., the DM species) (Figure 1).

The purpose of this study was to investigate the behavior of these solutes in RPLC, compare a C18 bonded phase with a cyclohexyl (CH) one, and demonstrate that selectivity is dramatically changed by a ternary mixture. Vervoort et al. (15,16) pointed out that the analysis of compounds with a basic nitrogen atom in the chemical structure is often hampered by poor peak shape or irreproducible retention. A nitrogen atom can be protonated depending on the pK_a of the analyte and the pH of the eluent. An ion-exchange retention mechanism occurs together with the

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classical partitioning. Very large differences in peak shapes were observed by Law (17) because efficiencies span from 173 (!) to 70,000 theoretical plates. In order to improve peak shape the analyst can either select a mobile phase in which the pH is less than the pK_a of the residual silanols, add a silanol blocker such as triethylamine (TEA) in the eluent, or modulate the ionic strength of the mobile phase.

Experimental

Chromatographic measurements were performed on a TSP (Thermo Separation Products, San Jose, CA) equipped with a Rheodyne 7125 injection valve (100-µL sample loop) (Touzart et Matignon, Courtaboeuf, France). This unusual injection volume is mandatory for the determination of very small amounts of solute in plasma samples. Detection was performed with a diode-array (UV–vis) detector (Spectrafocus, Thermo Separation Products). The selected wavelength was 220 nm. A PC 1000 connected to a M86 Bx2 Getek (Thermo Separation Products) was used for data acquisition. Chromatography was performed with a 150- × 4.6-mm column packed with CH bonded silica (Varian Associates, Palo Alto, CA) called a CH column, a 100- × 3.0-mm packed column with a C18 Omnisphere (Varian), and a C18 RP Select B from Merck (Darmstadt Germany).

For all cases the particle diameter was 5 µm. The columns were used as received; no information was available on the number of µmol/m^2 from the manufacturer. The retention time of the unretained solute was measured by the injection of uracil solution. Columns were thermostatted at 30°C in an oven (Cluzeau, Sainte Fay la Grande, France).

Samples were kindly supplied by manufacturers and were as follows: Haloperidol and reduced haloperidol were provided by Janssen-cilag (Issy les Moulineaux, France) and cyamemazine, levomepromazine, chlorpromazine, and the corresponding DM products were provided by Specia RhonePoulenc Rorer (Aventis, Paris, France).

Stock solutions were prepared in order to achieve 20-mg/L concentrations.

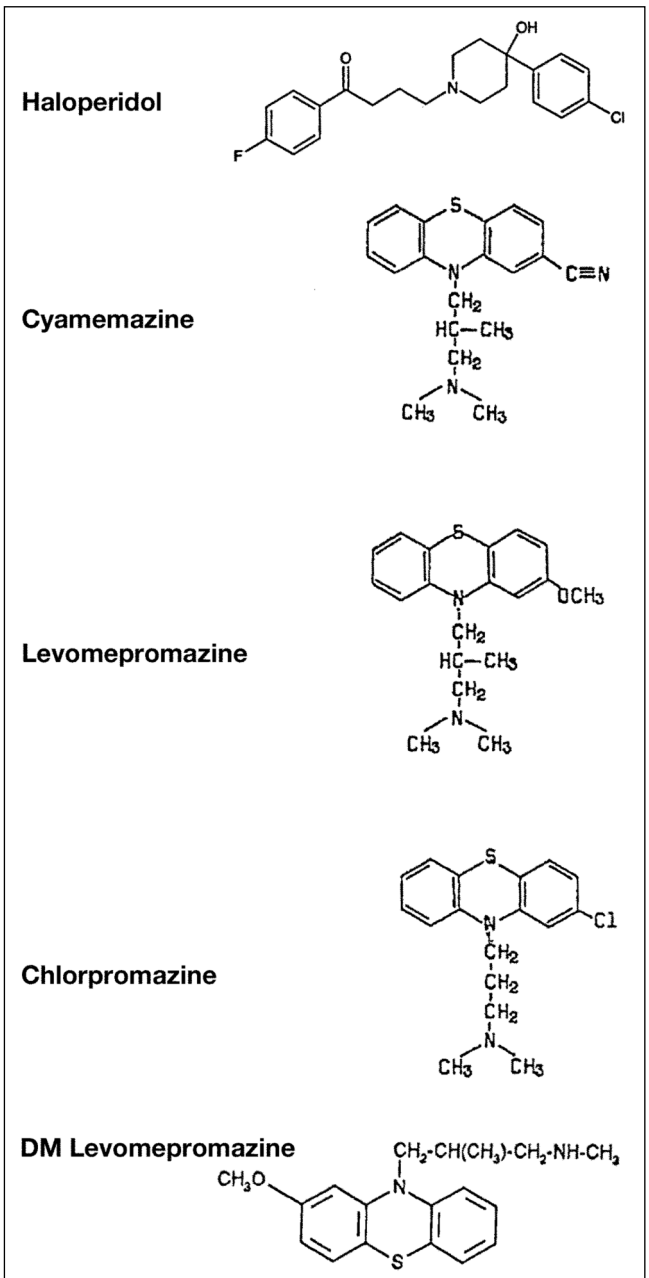

Figure 1. Formulas of the studied solutes.

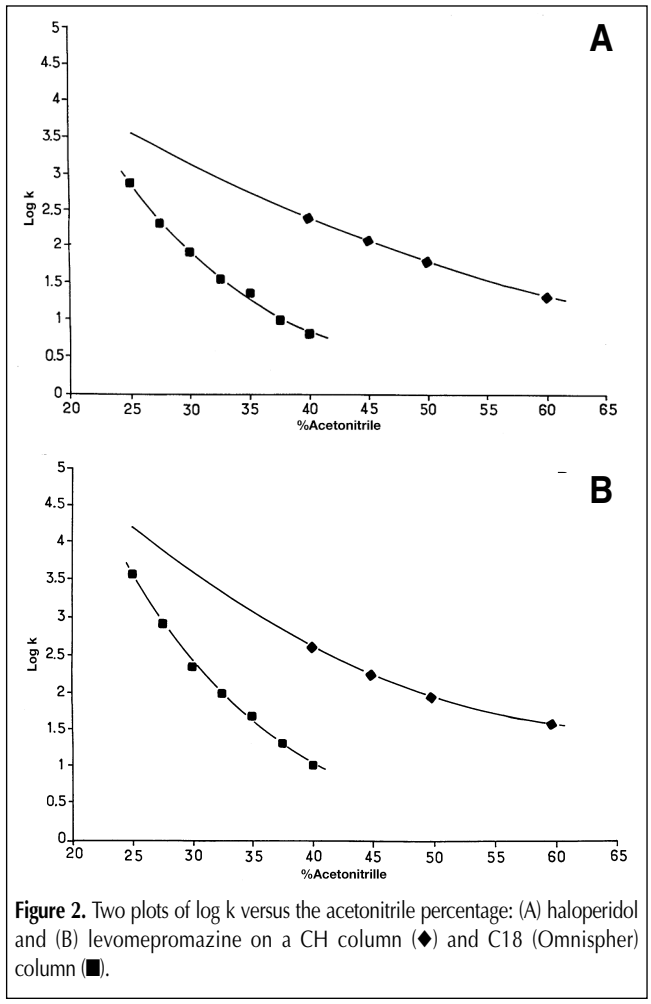

Figure 2. Two plots of log k versus the acetonitrile percentage: (A) haloperidol and (B) levomepromazine on a CH column (◆) and C18 (Omnispher) column (■).

The solvents (HPLC-grade) were acetonitrile and methanol from Merck (Darmstadt, Germany), and water was from Baxter (Versailles, France). Buffers were prepared by first using 6.8 g KH_2PO_4 and 1N HCl to obtain the desired pH by dissolving it in water up to 1000 mL. Buffers were then mixed with organic modifiers. The pH of the mixture was not measured.

Results and Discussion

Behavior in the binary mixture

Potassium phosphate was selected as the aqueous mobile phase because potassium can act as an ion-exchange counterion to reduce silanol activity. Potassium is a stronger counterion than sodium. A preliminary experiment demonstrated the difference between sodium and potassium. With the same salt concentration, the retention factor of haloperidol was divided by two (4.34 to 2.38) and the phenothiazine retention factor experienced a 1/3 decrease (6.47 to 4.09 with levomepromazine and 5.59 to 4.63 with cyamemazine). Acetonitrile was selected as the organic modifier because it is a low viscosity solvent, thus increasing the diffusion coefficients of the analytes in the mobile phase.

Figure 2 displays the plots of the experimental log k versus the acetonitrile volume percentage on both the C18 (Omnispher) and CH bonded phases. For all cases, the variation of the retention factor can be described by a quadratic equation:

$$\log k = a\phi^2 + b\phi + c \qquad \text{Eq. 1}$$

where ϕ is the acetonitrile volume percentage. The percentage was kept in the 30% to 60% range in order to get acceptable retention times. As can be predicted, metabolites were eluted before the corresponding species. The plots were parallel (Figure 3), which meant that selectivity remained constant. As far as phenothiazines are considered, coefficients of the quadratic regression equation did not vary too much (Table I).

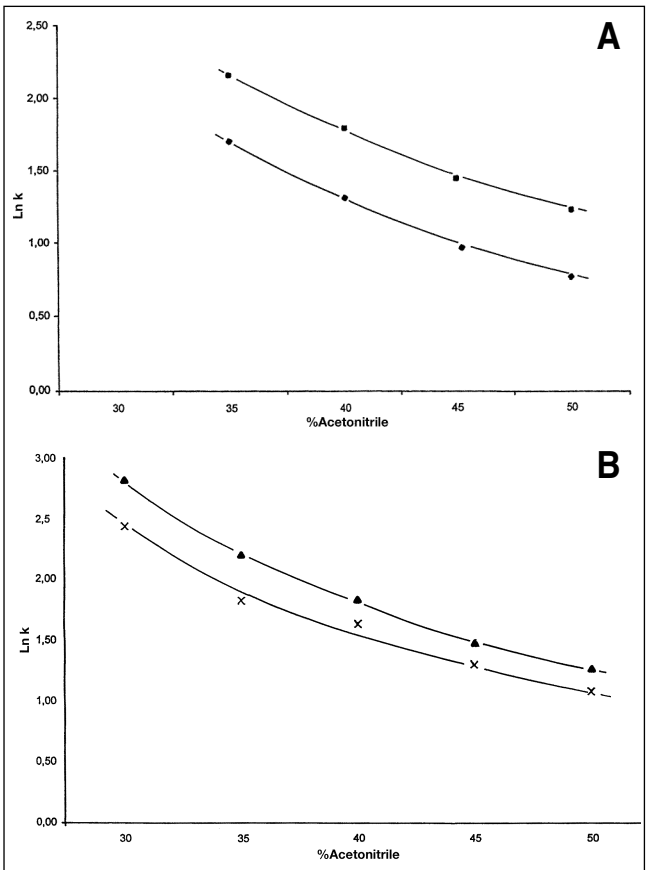

Figure 3. Plot of log k versus the acetonitrile percentage of two phenothiazines and their corresponding DM derivatives: (A) levomepromazine (■) and DM levomepromazine (♦) and (B) chlorpromazine (▲) and DM chlorpromazine (×).

It can be noticed that the gap between levomepromazine and the DM derivative was larger than the gap between chlorpromazine and DM chlorpromazine and the gap between cyamemazine and DM cyamemazine (Figure 3). The average selectivity between levomepromazine and DM levomepromazine was $\alpha = 1.6$, and the average selectivity was $\alpha = 1.20$ between chlorpromazine and DM chlorpromazine and $\alpha = 1.15$ between cyamemazine and DM cyamemazine. Haloperidol (and also reduced haloperidol) exhibited a very similar behavior. The selectivity between haloperidol and phenothiazines slightly increased with a very low acetonitrile content, which lead to a very long analysis time.

The c term in equation 1 corresponds to $\log k_w$, the hypothetical retention in pure water. It is highly correlated to the octanol–water partition coefficient log P. The c term increases in the order of cyamemazine < levomepromazine < chlorpromazine, which is in accordance with the observed retention. An observation of the plots reveals that the CH bonded phase was more retentive than the C18 one. A decrease in retention was steeper with the C18 phase than with the CH one when the percentage of acetonitrile increased.

Table II lists the retention factors in the binary mixture buffer–acetonitrile (60:40, v/v). It can be seen that the retention factors were very close and the resolution of the solutes would need a very efficient column. The retention order was different on both phases and the separation on the C18 phase seemed impossible. The problem was even more difficult to solve because of the peak tailing.

Asymmetry coefficients

Peak asymmetry factors were measured according to the usual procedure (19). All peaks exhibited very high skew—at least two even on base-deactivated silica (shown in Table III). Working with

Table I. Coefficients of the Relationship of Equation 1

	a (× 10^3)		b (× 10^1)		c	
	CH	C18	CH	C18	CH	C18
Haloperidol	0.6	4.7	1.2	4.4	6.1	10.9
Reduced haloperidol	0.4		1.2		5.7	
DM levomepromazine	1.6		2.0		5.7	
Levomepromazine	1.4	5.3	1.9	5.0	8.2	12.9
DM cyamemazine	1.7		1.9		6.1	
Cyamemazine	1.7		1.9		6.6	
DM chlorpromazine	1.9		2.1		6.7	
Chlorpromazine	2.4		2.6		11.9	

the C18 Omnispher, we could decrease the asymmetry by the addition of TEA in the eluent. In this mode the asymmetry factor was reduced to 1.70 with Haloperidol and 2.1 with levomepromazine. However, the addition of TEA does not suppress peak tailing. The drawback of TEA addition is the observed increase in retention and pH monitoring. The most striking feature was the difference observed in the efficiency calculation from the measurement of the peak width at half height. The CH bonded phase was much more efficient. By consequence, resolutions were better. Nevertheless, there were peak overlaps with the binary eluent.

Influence of the anion

We checked the nature and concentration of the salt in the mobile phase. Three anions were selected—chloride, perchlorate, and phosphate. Experiments were performed on the C18 column, and the pH was set at 5.77. This value was not the buffering range of potassium phosphate, but the pH was fixed for the purpose of comparison with chloride. Because the partition coefficient increased with the dielectric constant, we may expect a decrease in retention when increasing the salt concentration, but too high of a salt concentration may preclude the use of a gradient. By keeping constant the acetonitrile volume percentage (40%), we can observe that retention decreases rapidly in a quite linear shape when the chloride or the perchlorate concentration increases. The retention factor (k) was roughly divided by two with a two-fold increase in concentration. When sodium chloride was used, phenothiazines and their metabolites were not separated. A poor separation was noticed with perchlorate.

Table II. Retention Factors in Phosphate Buffer–Acetonitrile (60:40, v/v)

Solute	k
CH column	
Reduced haloperidol	2.93
DM levomepromazine	3.65
DM cyamemazine	4.10
Haloperidol	4.61
Cyamemazine	4.80
DM chlorpromazine	5.12
Levomepromazine	5.92
Chlorpromazine	6.22
C18 column	
Reduced haloperidol	1.47
DM cyamemazine	2.11
Haloperidol	2.21
Cyamemazine	2.30
DM levomepromazine	2.54
Levomepromazine	2.80

Table III. Asymmetry Factors and Peak Width at Half Height on Different Columns

	CH column	C18 column	RP Select B
Asymmetry			
Haloperidol	2.0	2.09	2.0
DM levomepromazine	2.0	2.24	2.35
Levomepromazine	2.0	2.33	2.83
Peak width			
Haloperidol	0.24	0.48	0.64
DM levomepromazine	0.34	0.98	0.90
Levomepromazine	0.42	1.24	1.02

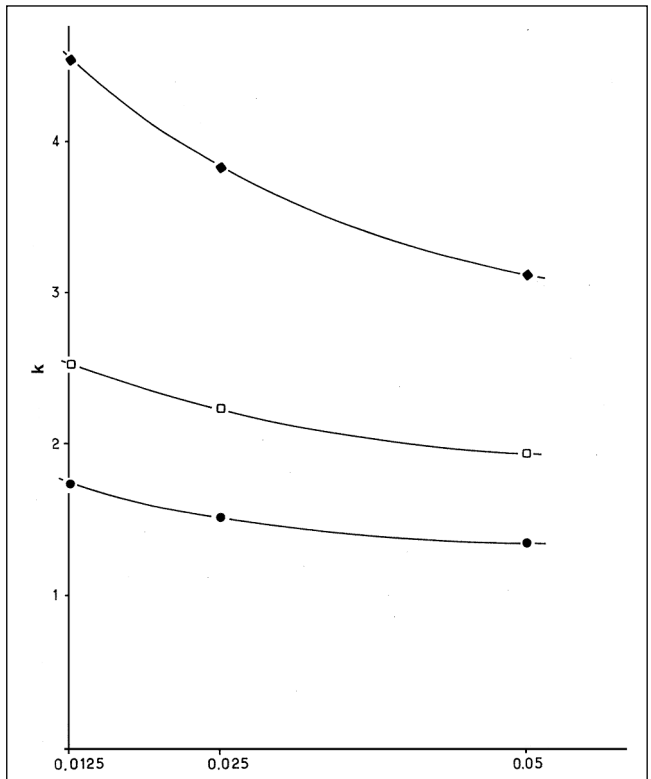

Figure 4. Plot of k versus the molarity of phosphate buffer on a C18 (Omnispher) column: cyamemazine (♦), DM cyamemazine (□), and reduced haloperidol (●).

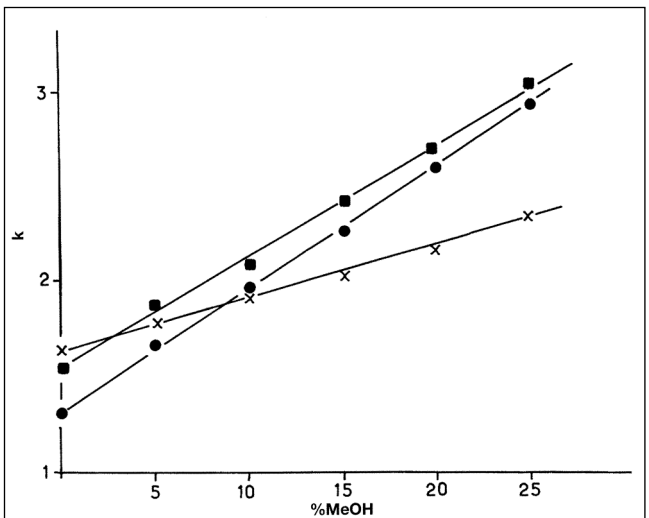

Figure 5. Plot of the retention of haloperidol (■), DM cyamemazine (●), and cyamemazine (×) with an increasing volume of methanol in the eluent. The CH column's starting eluent was phosphate buffer–acetonitrile (55:45, v/v) (0.125M buffer, pH 3.5).

With phosphate anion, solutes were less retained, the plot shape was different, and retention did not vary too much when the phosphate concentration was beyond 0.025 mol/L. Phenothiazines exhibited a slightly different curve, but the situation was roughly identical (Figure 4). It can be noticed that the DM derivative curve was more flattened when compared with the nondemethylated compound. Within the concentration range, selectivity between one compound and its metabolite slightly increases when the phosphate buffer is less concentrated. When considering the selectivity between levomepromazine and DM levomepromazine, selectivity increased from 1.47 to 1.78. In conclusion, there was no need to increase the phosphate concentration too much.

Behavior of the solutes in methanol–acetonitrile–buffer

Because methanol and acetonitrile did not belong to the same group in Snyder's solvent classification (18), it may be supposed that solutes may undergo different interactions when methanol is used. A fine selectivity tuning may be obtained with ternary mixtures. By keeping constant both the buffer concentration and the pH (3.5) and increasing the methanol content, we observed a linear variation in the retention (Figure 5).

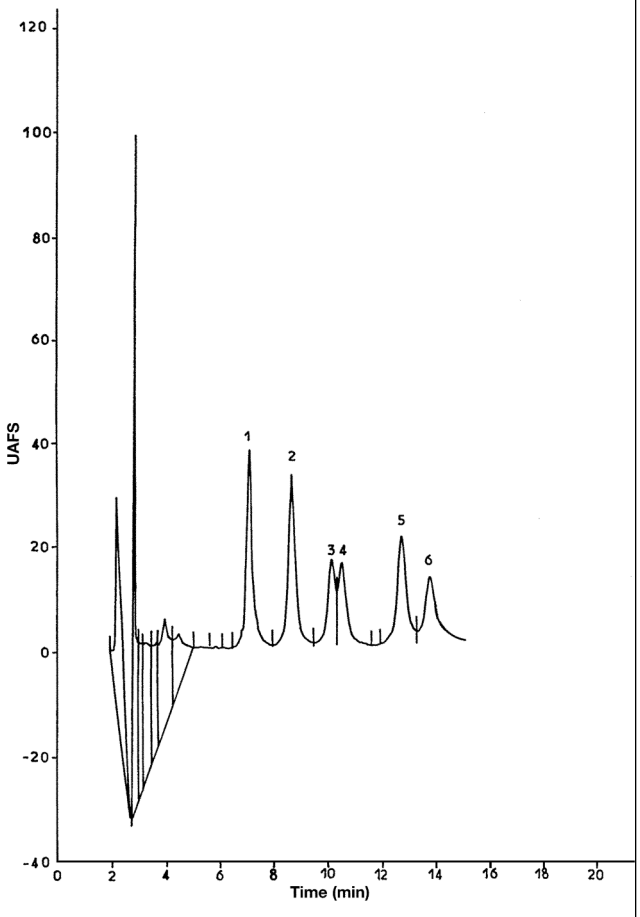

Figure 6. Separation of selected solutes with the ternary mixture phosphate buffer (pH 3.5)–methanol–acetonitrile (50:25:25, v/v) showing the shift of phenothiazine versus haloperidol at 30°C: reduced haloperidol, 1; haloperidol, 2; DM cyamemazine, 3; cyamemazine, 4; DM chlorpromazine, 5; and chlorpromazine, 6.

As could be expected, an increase in the methanol content produced an increase in retention. One striking feature was the difference in the behavior of phenothiazines and their DM derivatives.

If we consider the C18 column, the selectivity between haloperidol and reduced haloperidol did not vary when methanol content increased. Conversely, selectivity between one phenothiazine and its corresponding metabolite decreased with the increase in the methanol percentage, and peaks coalesced when 10% of methanol was added. This phenomenon can be observed with any column. The retention of haloperidol linearly increased at a lesser extent than the retention of one phenothiazine and its metabolite. We can take advantage of this feature. With 10% to 25% methanol volume in the mobile phase (depending on the column being CH or octadecyl), the selectivity between reduced haloperidol and haloperidol remained identical and DM cyamemazine and cyamemazine were coeluted (Figure 5).

Two experiments that were performed without the addition of methanol in the mobile phase and with the addition of 10% (v/v) methanol permitted the drawing of the linear plot and the determination of when haloperidol would be completely separated from phenothiazines and when phenothiazine and its metabolite would be coeluted. In this mode an unambiguous detection was carried out.

Displayed in Figure 6 is a chromatogram of such a separation with a ternary mobile phase. Haloperidol was well ahead of DM cyamemazine and cyamemazine, which would interfere in a binary eluent with the sole acetonitrile as a mobile phase.

It should be pointed out that we performed some experiments with a change in the temperature. Van't Hoff plots were parallel and no selectivity change could be obtained in this way.

Conclusion

Peaks of phenothiazines and their metabolites in RPLC were characterized by an important tailing that was only partially reduced by the addition of TEA in the eluent. Interestingly, the CH bonded phase was much more efficient than a C18 one. In binary mixtures (phosphate buffer–acetonitrile), these solutes behaved similarly, and the log k followed a quadratic relationship with the acetonitrile content. Haloperidol interfered with some of the DM phenothiazines. The convenient way to unambiguously separate and detect haloperidol was to use a ternary mixture with the addition of methanol in the mobile phase. With ternary mixtures, DM phenothiazines did not follow the same trend as their parent compounds.

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