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Development of a standardized analysis strategy for basic drugs, using ion-pair extraction and high-performance liquid chromatography — II. Selection of preferred HPLC-systems

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Abstract: A test set of 100 basic drugs has been chromatographed on 16 preselected HPLC-systems using four different types of stationary phase (Si-, NH_2-, CN- and C_{18}-). A numerical treatment of the chromatographic data, based on the discriminating power concept, results in the selection of two preferred HPLC systems for basic drugs, both using the CN-bonded phase. The preferred eluents are *n*-heptane–dichloromethane–acetonitrile–propylamine (25:50:25:0.1) and acetonitrile–water–propylamine (90:10:0.01). The two preferred HPLC systems are adopted in a standardized analysis strategy.

Keywords: *Basic drug analysis; standardized analysis strategy; HPLC of basic drugs; ion pair extraction; discriminating power; information content.*

Introduction

High-performance liquid chromatography (HPLC) is now a well established technique in pharmaceutical and biomedical analysis. Major technological advances have resulted in the availability of a wide spectrum of column packings. Along with the use of ternary or even quaternary mobile phases, this has facilitated efficient separations of complex multi-component mixtures. However, column selection and the choice of mobile phase are often difficult. This problem, and a need to improve the understanding of separation mechanisms, has stimulated the development of systematic optimization strategies in the selection of optimal separation conditions. Pioneering work in this area has been carried out by the Glajch–Kirkland–Snyder group, whose work [1–4] has resulted in an optimization routine yielding a prechosen level of resolution for all pairs of components in a complex mixture of structurally related compounds. Emphasis is laid on the optimization of the 4-component mobile phase, the selection of which is based on the Snyder selectivity classification triangle concept. The ultimate goal is to predict an

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optimum solvent mixture that would achieve complete isocratic separation of all components in only a limited number of experiments. The optimum solvent composition is predicted by applying an overlapping resolution mapping (ORM) technique of data analysis.

The purpose of the present study is to select a limited number of efficient HPLC systems (combinations of a particular stationary phase with a particular mobile phase) which should produce the highest overall discrimination between the members of a large set of basic drugs. The advantages of a limited set of preferred, efficient HPLC systems generally applicable to basic drugs are:

(1) Stationary and mobile phase selection is facilitated,
(2) Separation of both closely related compounds and solutes with very different structures should be possible in one experiment,
(3) The chances that basic compounds not belonging to the test set (e.g. a newly developed drug, unknown basic compounds) can efficiently be chromatographed in one of the preferred HPLC systems are high, and
(4) The time needed for method development for a quantitative analysis is greatly reduced since it involves fine-tuning the mobile phase composition for the particular problem.

The test set used here consists of one hundred basic drugs which are highly representative of the entire population of basic drugs, since it contains compounds belonging to the most frequently encountered chemical and pharmacological classes. This set was originally proposed by Moffat [5, 6], and has been used for the development of an optimal set of qualitative TLC systems [7–9].

The optimization of qualitative systems requires their effectiveness to be adequately assessed, using formal concepts such as information content [10, 11] and discriminating power [12]. These two concepts are compared elsewhere [13]; here only the latter method is applied to the evaluation of HPLC systems. The search for optimized HPLC systems is mainly based on the numerical treatment of chromatographic data, i.e. retention time and bandwidth. The optimization strategy has been performed in two successive steps: (a) a preliminary investigation on a reduced set of 10 drugs and (b) a complete and systematic investigation of the 'candidate' HPLC systems from (a), using the entire set of 100 basic drugs. Two HPLC systems were selected and are proposed for priority use in the HPLC analysis of basic drugs.

Experimental

Apparatus

A Varian 8500 liquid chromatograph equipped with a Varian Stop Flow injector, a Varian Varichrom Variable Wavelength detector and a Varian 9176 recorder were used.

The following columns were used:

MicroPak Si-5 (dp = 5 μm), 2.1 × 250 mm;
MicroPack CN-10 (dp = 10 μm), 2.1 × 250 mm;
MicroPak NH_2-10 (dp = 10 μm), 2.1 × 250 mm;
Partisil-10 ODS (dp = 10 μm), 4.6 × 250 mm.

Reagents

All eluents were mixtures of reagent grade solvents purchased from E. Merck (Darmstadt, FRG). Heptane sulfonate was used as PIC-B7 reagent obtained from Waters Ass.

Procedure

Aliquots (10 μl) of 0.05–0.1% solutions of the drugs dissolved in the bulk solvent of the mobile phase were chromatographed to produce a reasonable detector signal. From the elution chromatogram of three replicate injections of each drug solution, the mean retention time and mean bandwidth at half peak height were recorded.

Table 1
The candidate systems

System	Ratio (v/v)
A. Microsilicacolumn (MicroPak Si-5)	
1. *n*-Heptane/isopropanol/propylamine	100/3/0.5
2. *n*-Heptane/methylenechloride/isopropanol/propylamine	75/25/3/0.3
3. *n*-Heptane/methylenechloride/isopropanol/propylamine	50/50/3/0.3
4. *n*-Heptane/methylenechloride/isopropanol/propylamine	25/75/3/0.3
5. Methylenechloride/isopropanol/propylamine	100/3/0.3
B. Nitrile bonded phase (MicroPak CN-10)	
1. Methylenechloride/isopropanol/propylamine	100/3/0.1
2. *n*-Heptane/methylenechloride/isopropanol/propylamine	50/50/3/0.2
3. *n*-Heptane/methylenechloride/acetonitrile/propylamine	50/50/25/0.1
4. *n*-Heptane/isopropanol/propylamine	100/25/0.5
5. Methanol/water/propylamine	90/10/0.01
6. Acetonitrile/water/propylamine	90/10/0.01
C. Amino bonded phase (MicroPak NH_2-10)	
1. Methylenechloride/isopropanol/propylamine	100/3/0.1
2. *n*-Heptane/methylenechloride/isopropanol/propylamine	50/50/3/0.2
3. *n*-Heptane/methylenechloride/acetonitrile/propylamine	50/50/25/0.1
4. *n*-Heptane/isopropanol/propylamine	100/25/0.5
D. Reversed phase paired ion chromatography (Partisil-10 ODS)	
Methanol/water 60/40 containing 5×10^{-3} M heptane sulphonate.	

Table 2
Discriminating power of the 16 candidate systems

B6	0.912	C2	0.884
B3	0.911	C1	0.880
A1	0.906	A4	0.877
C3	0.905	D	0.872
A2	0.900	B4	0.867
B2	0.893	A5	0.860
B1	0.892	B5	0.851
A3	0.886	C4	0.839

Results and Discussion

The initial step of the optimization procedure was based on a reduction of the set of 100 basic drugs, using a non-hierarchical clustering method called MASLOC. This program selects out of a collection of N objects, each characterized by a (multivariate) data profile, a prechosen number (p) of objects as the 'centrotypes' of p clusters or

Table 3
Chromatography of various basic drugs using the preferred HPLC-systems

Drug	Column	Mobile phase	Flow rate	t_R	Application	Ref.
Papaverine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (50:25:25:0.1)	2	3.4	Determination in blood	19
Aprindine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (0:10:90:0.1)	2	4.1	Determination in plasma	20
Desethylaprindine				9.1		
Amylocaine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (50:75:20:0.1)	1	3.1	Separation of local anesthetics	20
Lidocaine				3.6		
Benzocaine				4.0		
Mepivacaine				5.8		
Piperocaine				9.2		
Procaine				16.5		
Tetracaine				22.2		
Orphenadrine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (25:50:25:0.1)	1	5.3	Separation of orphenadrine from its demethylderivative	20
Diphenhydramine				5.9		
Metoclopramide	Lichrosorb-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (70:15:15:0.1)	2	8.4	Determ. in Primperan® syrup	20
Tiapride (as IS)				6.5		
Metoclopramide	Lichrosorb-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (10:10:80(0.1)	2	4.1	Determination in Primperan® suppository	21
Tiapride (as IS)				2.3		
Lidocaine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (50:75:5:0.1)	1	4.4	Determination in Xylocaine® gel	20
Diphenhydramine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (50:25:25:01)	1.5	2.1	Determination in Caladryl® cream	20
Various basic drugs	Lichrosorb-CN				Determination in pharmaceutical dosage forms	21

Table 3 (continued)

Drug	Column	Mobile phase	Flow rate	t_R	Application	Ref.
2-Nitro-*p*-phenylene diamine	Micropak-CN	C_6H_{14}-CH_2Cl_2-CH_3CN-$C_3H_7NH_2$ (70:50:25:0.1)	1	7.0	Separation and determination of aminophenols and phenylene diamine derivatives in hair dye products	21
4-Nitro-*o*-phenylene diamine				7.8		
o-Aminophenol				11.3		
4-Methoxy-*m*-phenylene diamine				12.8		
m-Aminophenol				13.6		
p-Aminophenol				15.3		
p-Phenylenediamine				17.4		
Veratric acid	Lichrosorb-CN	CH_3CN-H_2O-$C_3H_7NH_2$ (80:20:0.01)	2	1.1	Separation of mebeverine from its hydrolysis products	20
Mebeverine				3.6		
4-Ethyl(*p*-methoxy-α-methylphenethyl) amino-1-butanol				7.8		
Various antihistamines	Micropak-CN				Separation of antihistaminic drugs	22
Tiapride	Lichrosorb-CN	CH_3CN-H_2O-$C_3H_7NH_2$ (80:20:0.01)	2	4.4	Determination in plasma	20
Metoclopramide				5.7		
Amitriptyline	Lichrosorb-CN	CH_3CN-H_2O-$C_3H_7NH_2$ (80:20:0.01)	2	3.5	Determination in plasma	20
Nortriptyline				11.9		
Imipramine				4.8		
Desipramine				23.5		

subsets. This selection of centrotypes is carried out so that the sum of Euclidean distances calculated between each object and the nearest centrotype is minimal. In this way each of the $(N\text{-}p)$ objects is allocated to a centrotype to which its distance is minimal, a procedure leading to the formation of p clusters [14–16]. In this work ten drug substances were selected out of the original collection of 100 drugs. The selection procedure was based on the TLC R_f values of the 100 drugs reported by Moffat [5, 6]. Each of the 10 drugs selected, also called probes, is representative of the other members of the cluster of which it is the centrotype and the subset of the 10 probes is in turn the most representative for the entire set. The following 10 probes were selected: caffeine, carbinoxamine, desipramine, dextropropoxyphene, diazepam, diphenhydramine, levallorphan, naphazoline, phenmetrazine and yohimbine. These are strictly speaking, TLC probes; the relationship between different chromatographic techniques and the lack of adequate HPLC data banks explain their use as HPLC probes. These 10 probes have been chromatographed on three different stationary phases A, B and C which are based on silica microparticulate packings (A, silicagel; B, nitrile-; C, amino bonded phase). A large series of solvent mixtures were applied as mobile phases in order to select those yielding the most favorable retention patterns for the 10 probes. The eluents included a restricted number of solvents: heptane, methylene chloride, acetonitrile, isopropanol, methanol and water. The choice of these solvents was determined by the need to obtain a large diversity of chromatographically exploitable physicochemical properties (polarity, selectivity), taking into account characteristics such as viscosity and UV cut-off wavelength.

To all mobile phases tested a small amount of an alkaline moderator, propylamine, was added to improve the kinetic properties of the systems. Visual inspection of the retention profiles of the 10 probes allowed the preselection of 15 systems in which at least four of the 10 probes were effectively separated. These systems (Table 1) were then used as candidate systems for the complete and systematic investigation of the entire test set. A 16th system (system D) was evaluated to meet the general characteristics of reversed phase paired ion chromatography, a method which has gained much interest in recent years. Heptane sulfonic acid was used as the ion-pairing reagent since it is the most frequently used pairing agent for cationic substances. All 100 drugs were then systematically chromatographed in each of the 16 candidate systems. For each compound chromatographed in each of the candidate systems two characteristic values were measured as the mean of three replicate injections, the retention time and the bandwidth at half peak height. These experimental data were then used to evaluate the systems with respect to their discriminatory effectiveness.

For an objective evaluation of qualitative analytical systems two procedures based on numerical data are available, the information content, I, and discriminating power, DP. The principles and practice of both methods have been described and discussed by Massart [9–11] and Moffat [5, 6, 8, 12]. In a comparison of the application of I and DP [13] it was concluded that the models are equally effective. The present study used only the discriminating power, the probability that two substances, taken at random from a series of N substances, are discriminated. The DP is calculated from the formula

$$\mathrm{DP} = 1 - \frac{2M}{N(N-1)}$$

where M is the total number of different undiscriminated pairs of substances. The discrimination of two substances in chromatography uses a resolution criterion which

defines that two substances i and j are discriminated or resolved if their resolution exceeds unity, i.e. if $|t_i - t_j| > 2\ (\sigma_i + \sigma_j)$, where t_i and t_j are the retention times and σ_i and σ_j the corresponding standard deviations of the elution bands calculated from the measured bandwidth at half peak height. This corresponds to a 4 σ separation of two Gaussian bands.

In Table 2 the systems are classified in order of decreasing discriminating power. It can be seen that systems B6 and B3 have the highest DP scores. Both systems use the nitrile bonded phase, either in the reversed phase (B6) or the normal phase (B3) mode. The efficiency of the nitrile column is confirmed by the appearance of four B-systems in the 'top ten'. The C-systems (NH_2-column) are less effective while A1 and A2 (Si-column) score quite well. Although Hoogewijs *et al.* [17] and Massart and Hoogewijs [18] showed ion-pair chromatography to be less suitable for separating compounds with widely differing structures, it is still surprising that system D exhibits a rather low DP score; however, the C_{18}-column was evaluated only with a single mobile phase.

The most important conclusion is that the two systems with highest DP score (B6 and B3) use the same column which has the further advantage that it can be used in both normal and reversed phase modes. These systems are not necessarily the optimal systems for the separation of any mixture of basic drugs, but they constitute preferred initial investigation conditions when basic drugs have to be chromatographed. Consequently, in the establishment of a standardized analysis strategy both systems (B3 and B6) have been adopted. They use a single column, and a very limited number of solvents (acetonitrile, water, *n*-heptane or *n*-hexane, and dichloromethane). Several years of routine use of these systems (without or in combination with the selected ion-pair extraction techniques) in our laboratory have demonstrated their efficiency in a wide variety of applications (Table 3), some of which will be reported in subsequent papers.

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