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International collaborative study of the retention reproducibility of basic drugs in high-performance liquid chromatography on a silica column with a methanol–ammonium nitrate eluent*

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Abstract: An international collaborative study between 10 laboratories has been carried out to study the reproducibility of the separation of basic drugs on silica columns. The laboratories used common solutions of drugs on both a common batch of packing material and different batches of the same brand of packing material. These were also compared with separations on other brands of packing material. Variations within-batch, within-brand and between brands have been compared.

The retentions of the drugs were compared using retention times, capacity factors and relative capacity factors compared with an internal standard. The last method was found to give the most reproducibile results. Considerable variations were found between the different brands of silica with a smaller variation between the batches of a single silica brand. However, unlike earlier studies, significant variations were found for separations on a single batch of silica which were partly attributed to differences in eluent preparation and column temperature.

Keywords: *High-performance liquid chromatography; basic drugs; silica columns; collaborative study.*

Introduction

Despite the widespread use of high-performance liquid chromatography (HPLC) as a method for quantitative analysis, its application to the identification of unknown compounds has been very limited. Partly, this arises from differences in the methods used to report retentions, but mainly from the poor reproducibility of retention properties between different laboratories even when using the same type of column packing material and following the same eluent recipe. Small but significant differences

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occur when different equipment is used causing small differences in flow-rates and dead volumes. Slight differences in the operating temperature and/or eluent composition can also have an effect.

These problems with both reversed-phase and normal phase HPLC have meant that it has not been possible to collect sets of retention data, such as those available for thin-layer chromatography (TLC) [1, 2] and gas–liquid chromatography (GLC) [2, 3], which can be transferred between laboratories and used for the tentative identification of an unknown sample. This has led us to undertake a series of studies to investigate the significant factors which influence the reproducibility of retention in HPLC and to develop robust methods to record retention values. Work has concentrated on drug separations of forensic interest, including barbiturates [4–6], local anaesthetics [7] and thiazide diuretics [8] using reversed-phase chromatography on octadecyl-bonded silica (ODS silica). The studies on the barbiturate HPLC system concluded with an interlaboratory collaborative study, which confirmed that, with care, highly reproducible results could be obtained [6]. One important variable was the method used to determine the column void volume. If a relative method of recording retention was used (e.g. relative capacity factors or retention indices) rather than absolute capacity factors, the results were sufficiently robust for reliable interlaboratory comparisons.

These drug analysis studies have recently been extended to the separation of basic drugs on silica columns using a methanol–ammonium nitrate eluent to identify those factors which may limit reproducibility. The effect of changing the eluent composition, operating temperature and the stationary phase have been studied [9] (and unpublished studies by R. M. Smith, T. G. Hurdley, R. Gill and M. D. Osselton). In this case, the proportion of methanol in the eluent and the temperature were important but the largest effects were caused by different silica columns. Major changes occurred if different commercial brands of silica were used but significant differences were also found with different batches of the same brand.

These conclusions were tested in a limited collaborative study between nine UK forensic science laboratories to investigate the reproducibility of retention values measured on a single batch of packing material [10]. The study confirmed that the method could give acceptable results as long as a relative method was used to record retentions, such as relative capacity factors or corrected retention times derived by interpolation between standards. Although this latter method was the most accurate, the additional calculations and standards required meant that it would probably be impractical for routine application. So far, very few other interlaboratory studies of this type have been carried out for HPLC analyses. Apart from our earlier work with the barbiturates [6], the only other published detailed study was designed to investigate the robustness of retention indices as a method of recording the retentions of various drugs on ODS silicas [11].

The present study extends the earlier work on the basic drugs to an international collaborative study between 10 laboratories which examined the separation on columns prepared from a single batch of silica, different batches of the same brand of silica, and separations carried out on a range of different silica brands.

Experimental

Collaborating laboratories

The analyses were carried out in 10 toxicology and forensic science laboratories

located in Australia, Austria, Canada, Netherlands (2), Norway, South Africa and the UK (3).

Test solutions

The drugs came from the reference collection of the Central Research Establishment, Home Office Forensic Science Service. Nine test solutions (**A–I**) were prepared, each containing a drug mixture (including protriptyline as an internal standard), dissolved in ethanol/water 90:10 (v/v). A tenth solution (**J**) consisted of sodium nitrate in methanol/water 90:10 (v/v) for void volume determination. The concentrations of the drugs were chosen so that all the compounds gave similar peak heights at a given detector sensitivity (0.08 AUFS). The components in solutions **A** and **J** were declared to the collaborating laboratories, whilst they were only told the total number of drugs in each of the other solutions (**B–H**) and that each contained protriptyline.

The detailed compositions are given below (concentrations mg ml^{-1} in ethanol/water 90:10 v/v).

- **(A)** Caffeine, 0.05; imipramine hydrochloride, 0.08; morphine hydrochloride, 1.04; methylamphetamine hydrochloride, 3.44; protriptyline hydrochloride, 0.26.
- **(B)** Cocaine hydrochloride, 0.82; phentermine, 2.36; ephedrine, 3.38; protriptyline hydrochloride, 0.19.
- **(C)** Diazepam, 0.04; propranolol, 0.37; nortriptyline hydrochloride, 0.15; protriptyline hydrochloride, 0.24.
- **(D)** Amitriptyline hydrochloride, 0.08; prolintane hydrochloride, 2.44; protriptyline hydrochloride, 0.24.
- **(E)** Nitrazepam, 0.04; chlorpromazine hydrochloride, 0.03; pipazethate hydrochloride, 0.22; protriptyline hydrochloride, 0.28.
- **(F)** Dextropropoxyphene hydrochloride, 1.51; amphetamine sulphate, 2.28; pholcodine, 1.61; protriptyline hydrochloride, 0.2.
- **(G)** Papaverine, 0.04; dipipanone hydrochloride, 0.81; codeine phosphate, 0.80; methdilazine hydrochloride, 0.06; protriptyline hydrochloride, 0.22.
- **(H)** Procaine hydrochloride, 0.12; promazine, 0.03; ethoheptazine citrate, 3.60; protriptyline hydrochloride, 0.28; strychnine, 0.14.
- **(I)** Phenylephrine bitartrate, 1.04; protriptyline hydrochloride, 0.2.
- **(J)** Sodium nitrate, 30 mg ml^{-1} in methanol/water 90:10 (v/v).

The stability of the test solutions on storage was tested to ensure that no changes would occur on distribution to the collaborating laboratories. Only chlorpromazine showed any decomposition when it was exposed to light. The laboratories were therefore asked to store all solutions in the dark before analysis.

Procedure

Each laboratory was asked to use routine HPLC equipment and to work at ambient temperature or under thermostat control, in line with their normal practice. Samples were injected using a valve injector with a 5-μl loop and peaks were detected at 254 nm.

Each laboratory was asked to carry out the study on three columns (25 cm × 4–5 mm,

i.d.). All of the columns were packed specially for the study using the slurry packing methods normally used by the laboratories.

(a) *Spherisorb S5W (batch No.* 5116/1, *Phase Separations, Queensferry, UK).* Studied by all the collaborating laboratories.

(b) *Spherisorb S5W (either batch No.* 5026, 5106, *or* 5123, *Phase Separations, Queensferry, UK).* The Central Research Establishment, Home Office Forensic Science Service examined all three batches while the other laboratories examined only one of these batches.

(c) *A silica packing material usually used in each laboratory.* These were Spherisorb S5W [batches 2752 (two laboratories), 5115 and F5492/1, Phase Separations, Queensferry, UK], Lichrosorb 5 μm (Merck, Darmstadt, FRG), Nucleosil 50–5 5 μm (batch 3061, Machery Nagel Düran, FRG), Waters Radial Pak 10 μm and Waters μ Porasil 10 μm (Millipore Corp, Milford, USA), Syloid 74 4–8 μm (source unknown), Partisil-5 5 μm (Whatman, Maidstone, UK), Perkin–Elmer 10 μm (batch 3201, Perkin–Elmer, Beaconsfield, UK) and Hypersil 5 μm (batch GA 245, Shandon Southern, Runcorn, UK).

The laboratories were instructed to prepare the eluent by mixing HPLC grade methanol (2700 ml) with an aqueous ammonium nitrate buffer (300 ml). The aqueous buffer was prepared by mixing analytical grade ammonium nitrate (27 g), 0.880 concentrated ammonia (90 ml) and distilled water (900 ml). The mobile phase was pumped at 2 ml min^{-1}.

Once the HPLC system had equilibrated, 5-μl samples of the test solutions **A–J** were injected in turn into the column. The sequence was completed by a second injection of solution **A**. The retention times for all of the peaks were recorded in seconds, either from a chart recorder (at a chart speed of at least 40 mm min^{-1}) or electronically using an integrator. The raw data were then collected for analysis.

Calculation of retention parameters

The retention time results from each laboratory were used to calculate a series of parameters to describe the retention properties of the basic drugs on the HPLC system.

Capacity factors (k') were determined as $k' = (t_R - t_o)/t_o$,

where t_R is the retention time of the analyte and t_o is the retention time of sodium nitrate (test solution **J**).

The relative capacity factors were calculated for each drug as k'/k'_P, where k'_P is the capacity factor for the protriptyline internal standard in the same test solution. Relative capacity factors can also be called relative adjusted retention times.

Principal components analysis were carried out using GENSTAT IV on a Multics computer at Loughborough University.

Results and Discussion

In order to determine the reproducibility of the retentions of the basic drugs, each laboratory was asked to carry out the study on three columns. In addition, the Central Research Establishment of the Home Office Forensic Science Service conducted the separations on a wider range of packing materials. A single batch of Spherisorb was used

by all laboratories to compare the variation between laboratories under operational conditions. Groups of laboratories also examined different batches to determine if the between-batch variation was significant, and in addition, each laboratory was asked to carry out the separation on another silica commonly used in their laboratory to investigate the between-brand variation. Unlike the UK forensic science laboratories involved in the previous collaborative study [10], the laboratories in the present study had no standardization agreement to use a specific brand or batch of silica.

The test solutions used in the study were very similar to those employed for the previous collaborative study [10] except that phenylephrine, which had previously been in solution **D** was examined separately (solution **I**), as in trial separations it has been unresolved from prolintane on some column materials. All collaborating laboratories were able to complete the study as requested and could resolve and detect all the components of the test samples.

Each laboratory measured the pH of the eluent before use. The values showed a wide variation from pH 8.5 to 10.18 with a mean pH of 9.36, whereas in the earlier study, a much more restricted range of pH (pH 9.3 to 9.5) had been obtained [10]. This variation was surprising as in the preliminary evaluation study, changing the concentrations of the buffer components had shown little effect on the pH of the eluent [9]. However, there has been concern that because of evaporation on standing, the concentration of ammonia solutions may differ markedly from the nominal value and this could alter the ionic strength of the mobile phase. This aspect is being investigated in further studies.

Temperature is often not controlled in HPLC laboratories, however, it has been found to have a significant effect on the absolute retention and selectivities of the present separation [9]. In the collaborative study, none of the laboratories used a thermostated column and the room temperatures varied from 19 to 28°C and changed over the period of the study.

Retention times

Each laboratory reported retention times on each column and subsequent calculations were carried out centrally. The retention times measured on a single batch of silica (Spherisorb S5W, 5116/1) showed considerable variation with relative standard deviation (RSD) of 6–12% (Table 1). These differences can largely be attributed to differences in the internal diameter of the column between laboratories (reported 4.0–5.0 mm). Because they should be independent of column dimensions, the corresponding capacity factors and relative capacity factors were then calculated based on the retention of sodium nitrate as the column void volume marker. Although there has been considerable debate on the accuracy of different void volume test samples, this method has been found previously to give reproducible results, which was the main criterion in the present study.

The variations in the capacity factors on the common batch of silica (Batch 5116/1, Table 1) ranged from 8 to 50% and were generally greater than those found in the earlier collaborative study [10]. However, it was known from the previous work that the discrimination ability of both retention times and capacity factors were very poor and that relative measurements were required for satisfactory discrimination [11].

Relative capacity factors on batches of Spherisorb S5W silica

The relative capacity factors were calculated by comparison with the protriptyline peak in each sample chromatogram. These results showed considerably less variation

Table 1
Comparison of retention times, capacity factors and relative capacity factors of basic drugs measured in 10 laboratories using a single batch of silica gel in a collaborative study. Results on S5W Spherisorb batch 5116/1 from all 10 laboratories

Test compound solution (Listed in order of injection)		Retention times			Capacity factors			Relative capacity factors		
		Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)
A	Caffeine	106	6	5.7	0.11	0.01	9.1	5.2	0.5	9.6
	Imipramine	158	15	9.5	0.67	0.10	14.9	30.6	2.5	8.2
	Morphine	199	17	8.5	1.09	0.11	10.1	50.4	2.7	5.4
	Methylamphetamine	265	26	9.8	1.78	0.20	11.2	82.2	3.4	4.1
	Protriptyline	301	25	8.3	2.17	0.20	9.2	100.0	0.0	0.0
B	Cocaine	109	7	6.4	0.14	0.03	21.4	6.5	1.0	15.4
	Phentermine	162	13	8.0	0.71	0.08	11.3	32.6	1.2	3.7
	Ephedrine	241	21	8.7	1.54	0.16	10.4	70.9	1.8	2.5
	Protriptyline	301	26	8.6	2.17	0.21	9.7	100.0	0.0	0.0
C	Diazepam	98	6	6.1	0.03	0.01	33.3	1.4	0.5	35.7
	Propranolol	144	11	7.6	0.51	0.07	13.7	23.4	1.4	6.0
	Nortriptyline	219	20	9.1	1.30	0.14	10.8	60.0	1.9	3.2
	Protriptyline	301	26	8.6	2.17	0.20	9.2	100.0	0.0	0.0
D	Amitriptyline	134	11	8.2	0.41	0.06	14.6	18.8	2.0	10.6
	Prolintane	212	25	11.8	1.22	0.21	17.2	56.2	5.6	10.0
	Protriptyline	301	26	8.6	2.17	0.21	9.8	100.0	0.0	0.0
E	Nitrazepam	97	6	6.2	0.02	0.01	50.0	1.1	0.6	54.5
	Chlorpromazine	139	11	7.9	0.47	0.06	12.8	21.4	1.6	7.5
	Pipazethate	223	29	13.0	1.35	0.23	17.0	61.9	6.1	9.9
	Protriptyline	301	26	8.6	2.17	0.21	9.7	100.0	0.0	0.0

F	Dextropropoxyphene	106	7	6.6	0.12	0.03	25.0	5.3	0.8	15.1
	Amphetamine	171	14	8.2	0.80	0.08	10.0	36.5	1.9	5.2
	Pholcodine	225	24	10.1	1.47	0.17	11.6	66.9	3.8	5.7
	Protriptyline	303	25	8.3	2.19	0.18	8.2	100.0	0.0	0.0
G	Papaverine	101	5	5.0	0.06	0.02	33.3	2.8	0.6	21.4
	Dipipanone	153	16	10.5	0.61	0.12	19.7	28.1	3.6	12.8
	Codeine	192	18	9.4	1.01	0.12	11.9	46.7	2.5	5.4
	Methdilazine	234	26	11.1	1.46	0.19	13.0	67.1	4.3	6.4
	Protriptyline	301	27	9.0	2.17	0.22	10.1	100.0	0.0	0.0
H	Procaine	113	8	7.1	0.20	0.04	20.0	9.1	1.0	11.0
	Promazine	171	16	9.4	0.80	0.11	13.8	36.9	2.6	7.0
	Ethoheptazine	223	25	11.2	1.34	0.19	14.2	61.9	4.1	6.6
	Protriptyline	301	27	9.0	2.16	0.21	9.7	100.0	0.0	0.0
	Strychnine	405	52	12.8	3.25	0.42	12.9	150.1	9.1	6.1
I	Phenylephrine	228	19	8.4	1.40	0.14	10.0	64.5	1.8	2.8
	Protriptyline	301	27	9.0	2.16	0.21	9.7	100.0	0.0	0.0
J	Sodium nitrate	95	5	5.3	—	—	—	—	—	—
A	Caffeine	105	6	5.7	0.10	0.02	16.4	4.7	0.3	7.4
	Imipramine	156	15	9.5	0.65	0.10	15.9	30.6	2.3	7.4
	Morphine	198	19	9.4	1.07	0.13	12.1	50.5	2.6	5.2
	Methylamphetamine	263	29	10.9	1.74	0.22	12.4	82.2	3.5	4.3
	Protriptyline	299	29	9.7	2.12	0.22	10.2	100.0	0.0	0.0

Separation conditions	Reported range	
Temperature	19–28°C	
Eluent pH	8.5–10.18	Mean 9.36
Column i.d. (mm)	4.0–5.0	

Table 2
Comparison of relative capacity factors (× 100) of basic drugs measured on different batches of Spherisorb S5W in a collaborative study (protriptyline = 100.0 in each injection)

Test compound (Listed in order of injection)	Batch of Spherisorb (number of laboratories)														
	5116/1 (10)			5026 (4)			5106 (4)			5123 (4)			Others (4)*		
	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)
A Caffeine	5.2	0.5	9.6	4.7	1.0	21.3	4.9	0.4	7.5	4.4	0.4	9.2	5.2	0.5	9.7
Imipramine	30.6	2.5	8.2	31.1	2.8	9.0	29.9	2.2	7.4	31.6	2.1	6.6	32.9	2.1	6.3
Morphine	50.4	2.7	5.4	50.2	1.8	3.6	50.5	1.3	2.6	51.5	2.0	3.9	53.0	2.0	3.8
Methylamphetamine	82.2	3.4	4.1	83.3	3.5	4.2	81.9	1.5	1.8	84.8	2.7	3.2	83.7	2.0	2.3
B Cocaine	6.5	1.0	15.4	6.9	1.3	18.8	8.2	2.3	28.3	6.9	0.6	8.8	7.0	0.8	12.1
Phentermine	32.6	1.2	3.7	33.3	1.5	4.5	32.7	1.3	3.9	33.5	1.1	3.2	32.7	0.9	2.6
Ephedrine	70.9	1.8	2.5	70.8	1.7	2.4	71.3	1.5	2.2	71.5	1.2	1.7	71.7	0.7	1.0
C Diazepam	1.4	0.5	35.7	1.3	0.7	53.8	1.8	1.2	67.7	1.5	0.3	21.6	1.9	0.9	48.7
Propranolol	23.4	1.4	6.0	24.5	1.9	7.8	23.4	1.3	5.4	24.6	1.0	4.1	24.3	1.9	7.9
Nortriptyline	60.0	1.9	3.2	60.1	2.2	3.7	59.4	1.3	2.1	60.0	1.0	1.7	61.7	1.2	1.9
D Amitriptyline	18.8	2.0	10.6	19.6	1.7	8.7	19.2	1.3	6.9	20.6	1.5	7.4	20.9	1.3	6.1
Prolintane	56.2	5.6	10.0	63.9	7.4	11.6	58.6	5.5	9.4	65.1	6.5	9.9	56.8	9.8	17.2
E Nitrazepam	1.1	0.6	54.6	1.2	0.8	66.7	1.4	0.5	33.9	1.3	0.3	24.1	1.4	1.0	73.2
Chlorpromazine	21.4	1.6	7.5	21.5	2.0	9.3	20.9	1.3	6.4	22.0	1.4	6.2	23.3	1.0	4.4
Pipazethate	61.9	6.1	9.9	68.8	8.4	12.2	67.4	5.6	8.3	69.8	5.4	7.8	64.6	9.4	14.6
F Dextropropoxyphene	5.3	0.8	15.1	5.8	1.3	22.4	5.3	0.9	16.4	5.4	0.7	12.6	5.7	0.8	14.1
Amphetamine	36.5	1.9	5.2	35.9	1.7	4.7	35.9	0.7	2.1	36.5	1.0	2.9	36.1	0.5	1.4
Pholcodine	66.9	3.8	5.7	69.7	4.5	6.5	68.5	1.6	2.3	70.3	3.0	4.3	70.1	4.9	7.0
G Papaverine	2.8	0.6	21.4	3.3	0.9	27.3	2.9	0.8	26.4	2.5	0.9	35.3	2.8	0.1	3.8
Dipipanone	28.1	3.6	12.8	33.8	6.1	18.0	29.6	4.3	14.6	32.3	3.9	12.0	27.8	6.6	23.7
Codeine	46.7	2.5	5.4	46.3	2.8	6.0	47.0	1.6	3.3	48.3	1.7	3.5	49.3	1.9	3.8
Methdilazine	67.1	4.3	6.4	68.1	4.3	6.3	66.6	3.4	5.0	70.2	3.0	4.2	72.4	3.9	5.4
H Procaine	9.1	1.0	11.0	9.8	1.2	12.2	9.5	0.8	8.1	9.9	0.6	5.9	9.1	1.3	14.3
Promazine	36.9	2.6	7.0	37.0	2.9	7.8	36.8	2.3	6.1	38.1	1.9	5.1	39.9	1.9	4.8
Ethoheptazine	61.9	4.1	6.6	64.0	5.0	7.8	61.8	3.2	5.2	65.2	2.9	4.5	66.5	3.8	5.7
Strychnine	150.1	9.1	6.1	161.9	12.9	8.0	156.0	7.7	4.9	163.1	8.6	5.3	158.8	17.0	10.7
I Phenylephrine	64.5	1.8	2.8	63.3	1.6	2.5	66.8	6.2	9.2	62.8	1.1	1.8	65.2	3.6	5.6

*Batch 2752 (2), 5115, F5492/1.

(Tables 1 and 2) and were more comparable to the results obtained previously [10]. The mean values for a number of the drugs measured on the common 5116/1 batch differed markedly from those obtained in the earlier collaborative study on the older 2752 batch. For example, prolintane changed from 46.3 (on Batch 2752) to 56.2 (on Batch 5116/1), pipazethate from 53.1 to 61.9, dipipanone from 21.4 to 28.1, and strychnine from 137.1 to 150.1. These compounds also all showed particularly high RSDs. This was not unexpected as these four drugs had also been shown to be particularly sensitive to changes in the separation conditions in the highly controlled initial studies [9]. Clearly, there was a significant difference between these two batches of the same packing material brand, which had been manufactured some time apart.

Three further batches of Spherisorb S5W were also each examined by groups of three collaborating laboratories and by the Central Research Establishment. In addition, four other Spherisorb columns were examined in the collaborative study, two of which were, by chance, Batch 2752, which had been used previously [9]. The relative capacity factors were calculated in each case (Table 2). The laboratories reported similar variations in eluent pH and column temperature with these materials as in their work with a single batch. Again for most of the drugs the results were comparable but prolintane, pipazethate, dipipanone and strychnine showed considerable variation both within each batch or group and between groups. Dipipanone seemed to be particularly sensitive to column variations.

Relative capacity factors on different brands of silica

The collaborators also carried out the separation of the basic drugs on columns packed with the silica materials normally used in their laboratories. These comprised two batches of Spherisorb, which have already been discussed, and eight other brands. The relative capacity factors of the drugs on these latter columns showed a very wide variation (Table 3). These were particularly large for the "sensitive" compounds, for example pipazethate (37.6–64.3) but were also significant for other normally more reproducible compounds. These results clearly demonstrate the need to specify the brand of packing material to be used for a particular separation.

In order to be able to visualize these differences in brands and batches of packing material, the relative capacity factors of all the drugs on all the columns examined in this study were examined using a multivariate principal components analysis [12]. The plot of the weighting of the first two principal component scores (Fig. 1) shows the relationships between the different column materials. Two factors are noticable, firstly, all the results for Spherisorb batches form a single group distinct from all the other brands. This emphasizes the differences between brands. However, the variation within the Spherisorb region shows that the results for the different batches overlap considerably. Thus the between-batch differences are apparently less significant than the interlaboratory variations on a single batch (i.e. 5116/1).

These conclusions contrast with our unpublished observations made under more controlled conditions of temperature and eluent composition within one laboratory when batch-to-batch variations appeared to be significant. The spread of the within-batch differences is also much larger than in the previous collaborative study [10]. It may, therefore, be that the different batches cannot be discriminated in the present study because the underlying between laboratory variation is too large, masking such differences.

Table 3
Relative capacity factor of basic drugs on eight other silica gel column packing materials studied in a collaborative study. Relative capacity factors (×100) compared with protriptyline

		Column material							
		a	b	c	d	e	f	g	h
A	Caffeine	6.0	5.4	4.5	4.3	5.2	7.1	7.4	4.6
	Imipramine	28.9	31.9	26.5	27.6	34.4	31.5	30.7	28.1
	Morphine	50.1	61.4	47.7	48.3	55.2	54.8	53.4	46.2
	Methylamphetamine	77.7	91.0	77.3	75.0	89.6	82.1	80.1	76.2
B	Cocaine	3.1	3.5	4.5	3.4	3.9	4.2	5.1	3.5
	Phentermine	29.4	31.8	29.3	29.1	29.9	31.0	31.2	29.3
	Ephedrine	60.6	79.4	68.4	73.5	74.7	73.8	71.6	69.1
C	Diazepam	0.3	1.2	0.8	0.0	0.0	1.2	1.7	0.0
	Propranolol	19.1	21.8	20.5	18.8	27.9	20.7	21.5	18.1
	Nortriptyline	59.4	63.5	58.3	63.2	61.7	63.9	62.1	60.2
D	Amitriptyline	18.6	20.6	16.7	18.8	20.3	21.3	20.2	17.4
	Prolintane	35.1	37.1	39.4	29.1	57.5	33.1	36.0	32.8
E	Nitrazepam	0.3	1.2	0.8	0.0	0.0	1.2	1.1	0.0
	Chlorpromazine	20.2	32.8	18.8	21.4	23.4	23.1	21.5	19.8
	Pipazethate	37.6	42.9	44.4	38.5	64.3	41.4	40.7	39.1
F	Dextropropoxyphene	1.4	1.7	6.8	0.9	0.0	3.0	2.9	1.6
	Amphetamine	34.2	39.0	34.1	35.9	35.7	37.9	37.1	34.1
	Pholcodine	59.0	67.8	59.8	56.4	65.4	66.9	64.0	55.0
G	Papaverine	1.4	2.8	1.5	0.0	0.0	1.8	3.4	0.8
	Dipipanone	9.9	11.9	16.9	7.8	14.9	10.6	11.4	10.1
	Codeine	45.6	54.5	43.9	44.0	50.6	51.6	49.4	42.6
	Methdilazine	63.7	71.6	61.4	67.2	85.1	70.6	65.9	64.0
H	Procaine	8.2	8.8	7.5	6.8	12.3	8.8	9.2	6.2
	Promazine	35.9	38.6	33.1	36.8	42.9	39.8	37.9	34.7
	Ethoheptazine	58.5	66.7	54.7	61.5	74.0	64.9	60.9	57.1
	Strychnine	107.6	120.5	122.6	107.7	124.0	121.6	114.9	113.1
I	Phenylephrine	67.3	83.1	63.6	74.6	73.2	74.3	71.3	65.3

Columns: a, Lichrosorb 5 μm; b, Nucleosil 50–5 5 μm; c, Waters Radial Pak 10 μm; d, Syloid 74; e, μ Porasil; f, Partisil-5; g, Perkin–Elmer; h, Hypersil 5 μm.

Conclusions

The results demonstrate the importance of standardizing on specific brands of HPLC packing materials when trying to achieve reproducible HPLC separations in different laboratories. Nominally equivalent brands of silica gave large differences in retention and selectivity, while particular compounds (e.g. dipipanone, prolintane) suffered particularly large variations. Clear differences between batches of a given brand were not observed in the present collaborative study, however, this probably arises from the relatively large interlaboratory variations. It appears that differences in ambient temperature and in eluent pH and composition between the collaborating laboratories were sufficient to cause significant differences in the results. Arising from this, the results emphasize the requirement of any specified HPLC method to involve a thermostated column and an eluent recipe which can be followed with high reproducibility.

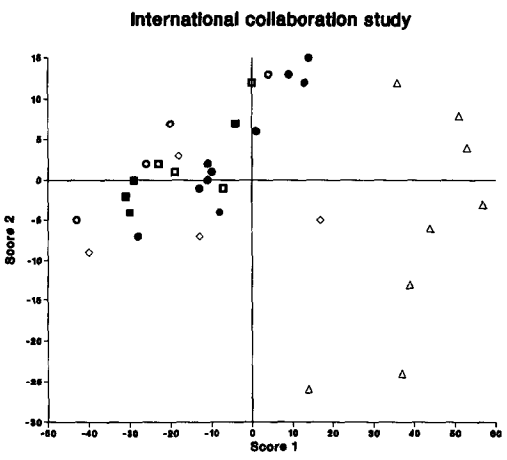

Figure 1
First and second principal components scores from the principal components analysis of the relative capacity factors of 27 basic drugs on different batches and brands of silica. ●, Spherisorb batch 5116/1; ○, Spherisorb batch 5026; □, Spherisorb batch 5106; ■, Spherisorb batch 5123; ◇, other Spherisorb batches; △, other brands of silica (see Table 3).

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