



# Determination of true octanol-water partition coefficients by means of solvent generated liquid-liquid chromatography\*

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**Abstract:** Compared to other methods for the determination of octanol-water partition coefficients chromatography offers a number of advantages: sample purification is unnecessary, the partition coefficients of the components of a mixture can be measured simultaneously and a minimum amount of sample is needed. In the past these determinations were almost exclusively carried out by liquid-solid chromatography (LSC) on alkyl bonded silica as stationary phase (conventionally described as 'reversed-phase liquid chromatography; RPLC). Such systems based on liquid-solid distribution are, however, a poor simulation of liquid-liquid partition. On the other hand liquid-liquid chromatographic columns loaded with high amounts of water-saturated octanol are unstable since they suffer from "column bleeding" — a loss of the stationary liquid octanol phase caused by erosion. It is shown that the technique of solvent generated liquid-liquid chromatography (SGLLC) leads to stable columns with liquid-liquid partition as the predominant retention mechanism, if systematic errors due to specific adsorption effects are avoided by the selection of an "inert" solid support. This is demonstrated by the comparison of LSC and SGLLC data. SGLLC significantly reduces the scattering of the retention data of calibration standards around the calibration line. The use of SGLLC thus significantly improves the accuracy and precision of the resulting octanol-water partition coefficients.

**Keywords:** Octanol-water partition coefficient; solvent generated liquid-liquid chromatography; alkyl-bonded silica; reversed-phase liquid chromatography; liquid-solid chromatography.

## Introduction

The octanol-water partition coefficient,  $K_{ow}$ , is defined as the equilibrium concentration of a dissolved substance in octan-1-ol divided by its concentration in water. The  $K_{ow}$  value of a compound is a measure of its lipophilic character. It is widely used to predict its distribution between polar and non-polar compartments in an organism or in the environment.

The measurement of octanol-water partition coefficients is traditionally carried out by a batch method equilibrating the liquid-liquid system, separating the two phases and measuring the concentrations of the solute [1]. This method is a direct method, no reference system is necessary but it has several disadvantages: it is time-consuming, only applicable to pure substances and problems with the phase separation lead to systematic errors particularly for lipophilic substances with very high  $K_{ow}$

values. To avoid these problems alternative experimental methods such as so-called counter current chromatography [2-4] or generator columns [5] have been applied. The accuracy of computer assisted methods which allow the estimation of  $\log K_{ow}$  values from the chemical structure of a substance [6, 7] critically depends on the accuracy and precision of the partition coefficients of the calibration compounds needed.

Numerous studies have been published [8-13] dealing with the correlation between chromatographic retention data and  $\log K_{ow}$  values. Until now the most convenient technique has been adsorption chromatography on alkyl silica (commonly referred to as reversed phase liquid chromatography (RPLC)) in spite of the fact that liquid-solid distribution (i.e. RPLC) is a poor simulation of liquid-liquid partition. In addition, chemically bonded stationary phases of the same type vary from one manufacturer to another and sometimes

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even from batch to batch, leading to differences in the retention of the calibration standards. These adsorption characteristics are specific for different classes of compounds and affect the goodness of the linear fit between the capacity factors and the true  $K_{ow}$  values of the calibration standards, thereby introducing systematic errors in the  $K_{ow}$  values of the test substances.

With retention data measured by liquid-liquid chromatography close correlations with corresponding  $K_{ow}$  values have been obtained [14–16], but liquid-liquid columns produced by the conventional methods suffer from the loss of stationary phase during operation. Such columns are inherently unstable because they are not loaded with octanol to a steady state.

These disadvantages can be overcome by the technique of solvent generated liquid-liquid chromatography [17–19]. In SGLLC a mobile phase in equilibrium with the intended stationary liquid phase (octanol in the present work) is pumped through a column packed with a suitable solid support material. The loading of the column packing is mediated by the mobile phase and starts with the adsorption of octanol, finally leading to a stationary phase which has the same distribution properties as the bulk liquid phase in equilibrium with the mobile phase. Thus the solid support is loaded with the maximum amount of stationary liquid phase which can be immobilized on the support material without erosion of the stationary liquid.

## Experimental

### Apparatus

A modular liquid chromatograph was used consisting of a high pressure pump (Model L-6200, E. Merck, Darmstadt, FRG), a column thermostat (Model bfo-04 dt, W.O. Electronics, Langenzersdorf, Austria), a sampling valve equipped with a 20- $\mu$ l injection loop (Model 7161, Rheodyne, Cotati, USA), a UV detector (Model L-4000, E. Merck) and an integrator (Model HP 3396 A, Hewlett Packard, Little Falls, DE, USA). For saturating the mobile phase with octan-1-ol a water bath (Model S, Haake, Berlin, FRG) controlled by a thermostat (Model F3, Haake) was used.

### Chemicals and materials

*Chemicals.* All chemicals were of analytical grade and used without any further treatment

(cf Tables 1 and 2). The following chemicals were used:  $H_3PO_4$ ,  $NaH_2PO_4 \cdot H_2O$ , NaOH, octan-1-ol (p.a., E. Merck) (referred to as octanol in this work), methanol (LiChrosolv, E. Merck). Aqueous mobile phases were prepared with doubly distilled water.

*Columns.* The columns used in this study are listed in Table 3. The packing materials were alkyl bonded silica, namely LiChrospher Si 100 RP-8 (5  $\mu$ m), Si 100 RP-18 (5  $\mu$ m), Si 2000 RP-8 (7  $\mu$ m), Si 4000 RP-8 (10  $\mu$ m) (E. Merck) and Nucleosil Si 100 C 8 (5  $\mu$ m) (Machery, Nagel & Co., Düren, FRG).

### Procedures

*Solvent generated liquid-liquid chromatography.* The following aqueous mobile phases [8], and their corresponding pH values, were used for acidic and basic compounds. To suppress dissociation dilute phosphoric acid (pH 2) was used for acidic compounds. For neutral and basic compounds the mobile phase consisted of a 0.01 molar phosphate buffer (pH 7.5) prepared by dissolving  $NaH_2PO_4 \cdot H_2O$  in doubly distilled water and adjusting the pH with aqueous sodium hydroxide solution.

Both mobile phases were saturated with octanol. Aliquots of 1 l were added to a few millilitres of octanol in glass flasks equipped with glass stoppers. To start the equilibration and to avoid the formation of emulsions the glass flasks were inverted manually approximately 100 times in about 5 min, as proposed by Leo *et al.* [1]. The temperature of the flasks was then maintained in a water bath at  $25.0 \pm 0.1^\circ\text{C}$  for at least 48 h. Most of the excess octanol (upper layer) was then removed with a Pasteur pipette. Residual octanol droplets on the surface served to maintain the saturation. The filled flasks were thermostatted in a water bath and used as the mobile phase reservoir. The tube from the mobile phase to the pump was inserted close to the bottom of the flask, thereby preventing octanol droplets from reaching the column, which was also thermostatted at  $25.0 \pm 0.1^\circ\text{C}$ .

*Liquid-solid chromatography.* Two alkyl-bonded silica materials, LiChrospher Si 100 RP-8 and Nucleosil Si 100 C 8, were used as adsorbents. Methanol was used as organic modifier since the best linear regressions of  $\log \kappa$  values (measured by reversed-phase adsorption chromatography) on  $\log K_{ow}$  values have

**Table 1**  
Set of calibration compounds and best estimate of their log  $K_{ow}$  values selected from the literature

Calibration compounds	log $K_{ow\text{cal}}^*$	Range of data†	Number of data	References
<b>Neutral compounds</b>				
Benzamide	0.64	0.64–0.65	3	[1, 6, 20]
Cyclohexanone	0.81	0.81	2	[1, 20]
Benzyl alcohol	1.05	0.93–1.16	6	[1, 6, 8, 20, 21]
Acetanilide	1.16	1.00–1.21	6	[1, 6, 8, 20]
2-Phenylethanol	1.36	1.36	2	[1, 20]
Benzaldehyde	1.48	1.45–1.48	3	[1, 6, 20]
Benzonitrile	1.56	1.50–1.56	5	[1, 6, 8, 20]
Benzylcyanide	1.56	1.56	1	[6]
Acetophenone	1.63	1.58–1.75	6	[1, 6, 8, 20, 21]
Nitrobenzene	1.85	1.79–1.90	9	[1, 6, 8, 20, 21]
Anisole	2.11	2.08–2.11	5	[1, 6, 8, 20]
Benzene	2.13	2.01–2.16	13	[1, 6, 8, 20, 21]
Methyl benzoate	2.20	2.10–2.20	6	[1, 6, 8, 20, 21]
Toluene	2.73	2.59–2.74	11	[1, 6, 8, 20, 21]
Chlorobenzene	2.80	2.80–2.84	5	[1, 6, 8, 20, 21]
Bromobenzene	3.00	2.99–3.02	5	[1, 6, 8, 20, 21]
Benzophenone	3.20	3.18–3.20	4	[1, 6, 8, 20]
Biphenyl	4.00	3.78–4.09	10	[1, 6, 8, 20, 21]
<b>Basic compounds</b>				
Aniline	0.90	0.90–1.03	8	[1, 6, 8, 20, 21]
<i>o</i> -Toluidine	1.32	1.29–1.32	3	[1, 20]
<i>p</i> -Toluidine	1.39	1.39–1.49	5	[1, 6, 20, 21]
<i>m</i> -Toluidine	1.40	1.40–1.43	4	[1, 6, 20]
4-Chloroaniline	1.80	1.73–1.83	5	[1, 6, 8, 21]
Indole	2.14	2.00–2.25	6	[1, 6, 20, 21]
<i>N,N</i> -Dimethylaniline	2.31	2.30–2.62	4	[1, 6, 20]
Diphenylamine	3.40	3.22–3.50	6	[1, 6, 8, 20]
<b>Acidic compounds</b>				
Hydroquinone	0.55	0.55–0.97	5	[1, 6, 21]
Catechol	0.95	0.95	1	[1]
Phenobarbital	1.42	1.42	1	[1]
Phenol	1.50	1.46–1.54	9	[1, 6, 8, 20, 21]
2,4-Dinitrophenol	1.50	1.50–1.54	4	[1, 6, 8]
2-Nitrophenol	1.80	1.73–1.80	3	[1, 8]
Benzoic acid	1.87	1.79–1.95	7	[1, 6, 8, 20, 21]
<i>m</i> -Cresol	1.97	1.96–2.01	3	[1, 20]
<i>p</i> -Cresol	1.97	1.90–2.03	7	[1, 6, 8, 20, 21]
3-Nitrophenol	2.00	2.00	1	[1]
2,4-Dimethylphenol	2.35	2.30–2.35	2	[6, 20]
3,5-Dimethylphenol	2.35	2.35	2	[1, 20]
2,6-Dimethylphenol	2.36	2.36	3	[1, 6, 20]
2-Naphthol	2.70	2.70–2.84	2	[1, 20]
Thymol	3.30	3.30	3	[1, 6, 8]

\* log  $K_{ow\text{cal}}$ ; best estimate of log  $K_{ow}$  used for calibration in this study.

† Range: range of log  $K_{ow}$  values found in the cited references.

been obtained with mobile phases consisting of methanol and aqueous buffers [13]. A water content of at least 25% v/v has been recommended [8]. Eluents were, therefore, prepared by adding 400 ml methanol to 600 ml of each of the mobile phases used in SGLLC, resulting in a nominal 40% v/v methanol content in each eluent. All were degassed before use.

*Determination of elution volumes.* In both the SGLLC and LSC mode the solutes were dissolved in the mobile phase in concentrations which permitted their UV detection after

injection of a 20- $\mu$ l sample. All elution volumes could be measured with a precision  $\leq 0.1\%$ , and are reported as the arithmetic mean of three measurements. The mobile phase volume  $V_m$ , was determined for each column by means of formamide, an unretarded substance.

In order to check the stability of the phase system a mixture of formamide and a retarded compound (acetophenone for neutral and basic compounds, 2-nitrophenol for acidic compounds) was injected periodically throughout these experiments.

**Table 2**  
Logarithms of the capacity factors of the selected calibration compounds determined by solvent generated liquid-liquid chromatography on different solid supports

Calibration compounds	Log $\kappa^*$ Solid support†				
	(1)	(2)	(3)	(4)	(5)
Benzamide	0.39	0.33	—	—	0.35
Cyclohexanone	0.59	0.59	—	—	0.55
Benzyl alcohol	0.78	0.70	—	—	0.72
Acetanilide	0.87	0.80	—	-0.85	0.88
2-Phenylethanol	1.04	0.96	—	-0.72	1.00
Benzaldehyde	1.21	1.23	—	-0.65	1.17
Benzonitrile	1.27	1.26	—	-0.60	1.22
Benzylcyanide	1.25	1.20	—	-0.58	1.21
Acetophenone	1.36	1.37	—	-0.47	1.34
Nitrobenzene	1.55	1.55	-0.21	-0.34	1.50
Anisole	1.84	1.81	0.05	-0.05	1.78
Benzene	1.84	1.81	0.03	-0.04	1.78
Methyl benzoate	1.86	1.84	0.09	0.01	1.82
Toluene	2.37	2.35	0.52	—	—
Chlorobenzene	—	—	0.66	—	—
Bromobenzene	—	—	0.80	—	—
Benzophenone	—	—	0.89	—	—
Biphenyl	—	—	1.75	—	—
Aniline	0.66	0.61	—	—	0.64
<i>o</i> -Toluidine	1.06	1.03	—	—	1.05
<i>p</i> -Toluidine	1.15	1.10	—	-0.46	1.15
<i>m</i> -Toluidine	1.12	1.07	—	-0.58	1.11
4-Chloroaniline	1.53	1.46	0.00	-0.17	1.50
Indole	1.88	1.79	0.08	-0.08	1.80
N,N-Dimethylaniline	1.93	2.00	0.14	0.13	1.98
Diphenylamine	—	—	1.15	—	—
Hydroquinone	0.09	0.18	—	—	-0.08
Catechol	0.48	0.49	—	—	0.35
Phenobarbital	1.02	1.01	—	-0.73	0.93
Phenol	1.10	1.10	—	-0.85	1.01
2,4-Dinitrophenol	1.24	1.27	—	-0.71	1.17
2-Nitrophenol	1.45	1.52	-0.18	-0.44	1.39
Benzoic acid	1.50	1.50	-0.16	-0.40	1.41
<i>m</i> -Cresol	1.57	1.56	-0.16	-0.39	1.47
<i>p</i> -Cresol	1.58	1.57	-0.11	-0.33	1.48
3-Nitrophenol	1.61	1.59	-0.18	-0.47	1.49
2,4-Dimethylphenol	2.07	2.07	0.27	0.11	—
3,5-Dimethylphenol	2.03	2.02	0.19	0.04	—
2,6-Dimethylphenol	1.92	1.92	0.10	-0.05	—
2-Naphthol	2.46	2.45	0.69	0.48	—
Thymol	—	—	0.87	—	—

\*log  $\kappa$ : logarithm of the capacity factor. Mobile Phase: Phosphate buffer saturated with octanol.

†Support material: (1) LiChrospher Si 100 RP-8; (2) LiChrospher Si 100 RP-18; (3) LiChrospher Si 2000 RP-8; (4) LiChrospher Si 4000 RP-8; (5) Nucleosil Si 100 C 8.

## Results and Discussion

### Selection of calibration compounds

Since the accuracy of any indirect method depends primarily on the accuracy of the calibration procedure it is necessary to select calibration compounds with accurately known  $K_{ow}$  values. The following criteria were used for the selection of the calibration set:

- The set of calibration compounds must consist of chemically different classes of

substances: neutral, basic and acidic compounds.

- The calibration compounds must cover a wide range of  $K_{ow}$  values.
- More than one  $K_{ow}$  value should be available for each calibration compound selected. These values should have been determined by different methods yielding results within a narrow range.

The calibration compounds selected were taken from the list of recommended calibration

**Table 3**  
Estimation of the contribution of residual adsorption to retention in solvent generated liquid-liquid chromatography by linear regression of retention volumes on octanol-water partition coefficients of calibration substances (see equation 2)

Support materials/column dimensions	$n$	$r$	$SD_{y,x}$ ( $cm^3$ )	$V_{m,REG}$ ( $cm^3$ )	$c_L(V_{m,REG})$ ( $cm^3$ )	$V_{m,EXP}$ ( $cm^3$ )	$V_{s,REG}$ ( $cm^3$ )	$c_L(V_{s,REG})$ ( $cm^3$ )	$q$
LiChrospher Si 2000 RP-8 25 mm × 4 mm i.d.	23	0.99638	0.26297	0.32	0.12	0.26	0.001	0.00006	0.004
LiChrospher Si 100 RP-8 25 mm × 4 mm i.d.	19	0.99189	0.52857	0.30	0.39	0.20	0.09	0.006	0.45
LiChrospher Si 100 RP-18 25 mm × 4 mm i.d.	19	0.98726	0.61243	0.37	0.45	0.19	0.09	0.01	0.47
Nucleosil Si 100 C 8 25 mm × 4 mm i.d.	30	0.98724	0.82453	0.24	0.46	0.23	0.10	0.006	0.43
LiChrospher Si 4000 RP-8 250 mm × 4 mm i.d.	27	0.97085	0.38986	2.75	0.22	2.64	0.01	0.002	0.004

$n$ : number of compounds;  $r$ : correlation coefficient;  $SD_{y,x}$ : standard error of estimate;  $V_{m,REG}$ : volume of the mobile phase calculated from the regression line;  $c_L(V_{m,REG})$ : confidence limit of  $V_{m,REG}$ ;  $V_{m,EXP}$ : volume of the mobile phase determined with a non-retarded compound;  $V_{s,REG}$ : volume of the stationary phase calculated from the regression line;  $c_L(V_{s,REG})$ : confidence limit of  $V_{s,REG}$ ;  $q$ : phase ratio  $V_s/V_m$ .

compounds elaborated in an OECD Laboratory Intercomparison Test [8]. To extend the number of calibration compounds  $K_{ow}$  values of substances given in other papers [1, 6, 20, 21] have been included.

The set of calibration compounds selected, the best estimate of their log  $K_{ow}$  values (log  $K_{ow,cal}$ ) used for calibration in this study, and the range of the data found in the literature cited are given in Table 1.

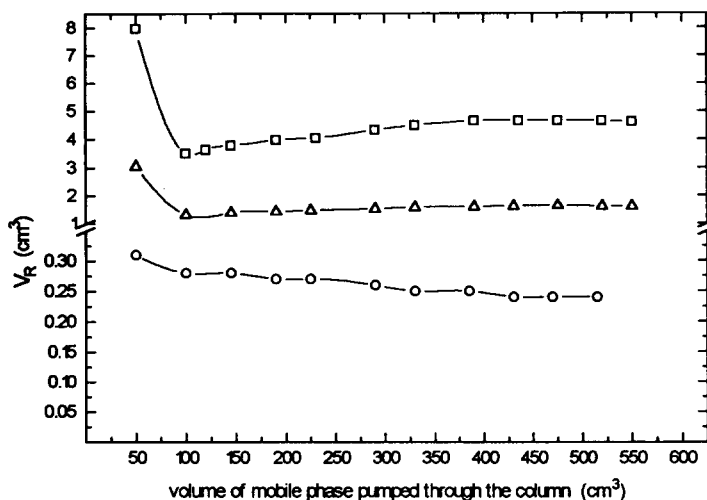
#### Solvent generated liquid-liquid chromatography

*Generation of the stationary liquid phase.* Phosphate buffer presaturated with octanol was pumped through the columns packed with alkyl silica as support material. The formation of the stationary liquid phase was followed and the stability of the columns checked during the measuring period by measuring the retention volumes of two retarded compounds (acetanilide and acetophenone) and of formamide as a non-retarded compound. Figure 1 shows the changes in retention during the generation of the stationary liquid phase. Initially substances are retarded more strongly because they are adsorbed onto the solid support. The saturation of the adsorption sites by octanol leads to a decrease in retention volume until a minimum is achieved. Beyond this minimum liquid-liquid partitioning increasingly dominates solute retention. The hold-up volume of formamide is reduced due to increased filling of the pores of the particles of the packing by stationary liquid. Owing to the low solubility of octanol in the phosphate buffer, the generation of the stationary phase is slow. After pumping through approximately 1500 interparticle volumes of the column constant retention was achieved. All liquid-liquid phase systems formed in this way were stable during the measurement of the data sets; deviations in retention volume due to column instability (column bleeding) were not observed.

The capacity factor,  $\kappa_i$ , was calculated from the retention volume,  $V_{Ri}$ , and the mobile phase volume,  $V_m$ , according to the conventional equation

$$\kappa_i = \frac{V_{Ri} - V_m}{V_m} \quad (1)$$

The logarithms of the capacity factors, log  $\kappa_i$ , determined for the calibration compounds on



**Figure 1**

Formation of the stationary phase by solvent generated liquid-liquid chromatography. Support material: LiChrospher Si 100 RP-8; column: 2.5 cm × 4 mm i.d.; mobile phase: phosphate buffer presaturated with octanol. Key: ○ formamide (unretarded compound); △ acetanilide; □ acetophenone.

various support materials by SGLLC are summarized in Table 2.

*Estimation of adsorption contributions to retention.* Substances which are retarded in a chromatographic system by liquid-liquid partition show a linear relationship between the retention volume,  $V_{Ri}$ , and the liquid-liquid partition coefficient,  $K_i^{(L/L)}$ , according to the equation

$$V_{Ri} = V_m + V_s K_i^{(L/L)}, \quad (2)$$

where  $V_m$  is the mobile phase volume and  $V_s$  is the stationary phase volume. In order to verify the liquid-liquid partition mechanism the correlation between the retention volumes,  $V_{Ri}$ , and the corresponding octanol-water partition coefficients,  $K_{ow\text{cal}}$ , was investigated. Differences between the mobile phase volume,  $V_{m\text{REG}}$ , as calculated from the regression line, and the experimental mobile phase volume,  $V_{m\text{EXP}}$ , as determined by injection of formamide, indicate the magnitude of residual adsorption contributions to retention. In Table 3 the calculated and the experimental values of  $V_m$ , and the calculated value of  $V_s$ , are listed together with the confidence limits and the phase ratio,  $q = V_s/V_m$ .

The precision of  $V_{m\text{REG}}$  is determined by the scattering of retention values around the calibration function and/or the number of calibration compounds used. In all cases the  $V_{m\text{EXP}}$  values measured are within the con-

fidence limits for  $V_{m\text{REG}}$ . Since the regression line has been calculated from a large number of calibration compounds residual adsorption contributions to the overall retention are negligible.

The phase ratio determines the range of the log  $K_{ow}$  values accessible by the SGLLC method. The amount of stationary liquid phase and thus the phase ratio,  $q$ , depends on the type of solid support (surface area, pore volume, pore size). The phase ratio was calculated from  $V_{m\text{EXP}}$  rather than  $V_{m\text{REG}}$  because of the latter's relatively wide confidence limits. With the support materials investigated in this study phase ratios from 0.004 to 0.53 were obtained thus making a range from  $0.5 \leq \log K_{ow} \leq 4$  accessible.

*Reproducibility of the phase system.* Repeated loading of the same column packing resulted in retention volumes which were reproducible within  $\pm 0.02 \text{ cm}^3$ . The regression data for the calibration line obtained by linear regression of  $\log \kappa$  on  $\log K_{ow\text{cal}}$  agreed within experimental limits.

#### *Liquid-solid chromatography*

The retention volumes of the calibration compounds were also determined in LSC systems in order to allow a comparison of log  $K_{ow}$  values determined by LSC and SGLLC. Columns 1 and 2 of Table 4 present the log  $\kappa$  values determined in the LSC mode in this study using reversed-phase materials.

**Table 4**  
Logarithms of the capacity factors of calibration compounds determined by liquid–solid chromatography

Calibration compounds	log $\kappa$ for adsorbents								
	Experimental data			Literature data					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Benzamide	—	0.12	−0.26	—	—	0.98	1.04	0.81	1.24
Cyclohexanone	0.24	0.25	—	—	—	—	—	—	—
Benzyl alcohol	0.21	0.20	0.15	—	1.47	1.39	1.32	1.05	1.33
Acetanilide	0.18	0.21	0.10	—	—	1.38	1.45	1.17	1.52
2-Phenylethanol	0.48	0.44	—	—	1.80	—	1.73	1.42	1.93
Benzaldehyde	0.51	0.48	—	—	1.80	1.74	1.67	1.54	1.74
Benzonitrile	0.55	0.53	0.36	—	1.77	1.83	1.85	1.51	2.35
Benzylcyanide	0.53	0.51	—	—	—	—	—	—	—
Acetophenone	0.64	0.62	0.45	—	1.92	2.02	—	—	—
Nitrobenzene	0.78	0.74	—	1.91	2.03	1.91	2.00	1.70	2.62
Anisole	0.97	0.89	0.80	—	2.15	2.23	2.20	2.01	2.46
Benzene	0.97	0.87	0.83	2.05	2.16	2.11	2.08	1.91	2.40
Methyl benzoate	1.02	0.97	0.79	—	2.28	2.44	2.26	2.15	2.48
Toluene	1.35	1.25	1.16	2.59	2.72	2.74	2.62	2.62	3.25
Chlorobenzene	1.40	1.30	1.14	2.71	2.80	2.80	2.75	2.72	3.25
Bromobenzene	1.51	1.40	1.22	—	—	—	—	—	—
Benzophenone	1.57	1.54	1.22	—	3.15	—	3.11	3.45	3.71
Biphenyl	—	—	1.77	—	3.89	4.17	3.88	3.92	4.63
Aniline	—	0.06	0.02	0.86	1.21	1.05	1.13	0.95	1.46
<i>o</i> -Toluidine	0.41	0.38	—	—	—	—	—	—	—
<i>p</i> -Toluidine	0.45	0.43	0.31	1.05	—	—	—	—	—
<i>m</i> -Toluidine	0.44	0.40	—	—	—	—	—	—	—
4-Chloroaniline	0.62	0.59	—	1.80	—	1.92	—	—	—
Indole	0.74	0.67	0.55	—	—	—	—	—	—
N,N-Dimethylaniline	1.18	1.10	—	—	—	2.57	2.36	2.28	2.87
Diphenylamine	1.70	—	—	—	—	—	—	—	—
Hydroquinone	—	—	—	0.29	—	—	—	—	—
Catechol	—	−0.24	−0.22	—	—	—	—	—	—
Phenobarbital	0.31	0.34	—	—	—	—	—	—	—
Phenol	0.11	0.12	—	1.35	1.34	1.27	1.30	1.28	1.81
2,4-Dinitrophenol	0.50	0.52	—	—	—	—	—	—	—
2-Nitrophenol	0.66	0.63	—	—	1.90	—	—	—	—
Benzoic acid	0.46	0.44	—	1.92	—	—	—	—	—
<i>m</i> -Cresol	0.49	0.46	—	—	—	—	—	—	—
<i>p</i> -Cresol	0.47	0.46	0.43	1.91	—	—	—	—	—
3-Nitrophenol	0.44	0.43	—	—	1.80	—	—	—	—
2,4-Dimethylphenol	0.85	0.80	0.74	—	2.31	—	—	—	—
3,5-Dimethylphenol	0.81	0.77	—	—	—	—	—	—	—
2,6-Dimethylphenol	0.80	0.76	0.70	—	—	—	—	—	—
2-Naphthol	0.97	0.90	—	—	—	—	—	—	—
Thymol	1.47	—	1.28	—	—	—	—	—	—

log  $\kappa$ : logarithm of the capacity factor. Adsorbents: (1) LiChrospher Si 100 RP-8. (2) Nucleosil Si 100 C 8. (3)–(9) literature data: (3) Alltech RP-18 [24]; (4) Hypersil RP-18 [12]; (5) LiChrosorb RP-18 [25]; (6) LiChrosorb RP-18 [26]; (7) LiChrosorb RP-18 [27]; (8) LiChrosorb RP-18 [27]; (9) octadecyl-polyvinyl copolymer gel [27].

#### Correlation of SGLLC and LSC retention data with log $K_{ow,cal}$ values

The log  $\kappa$  values of the calibration compounds were measured in both the SGLLC and LSC mode and correlated with the corresponding logarithms of the octanol–water partition coefficients.

Linear regression yields a number of parameters which characterize the regression: the correlation coefficient,  $r$ , the intercept,  $a$ , and slope,  $b$ , of the regression line and their standard deviations; the standard error of the estimate,  $SD_{y,x}$ , which describes the spread of

the calibration points around the regression line; and the standard deviation of the method,  $SD_{x_0} = SD_{y,x}/b$ , which reflects the influence of uncertainties of the calibration line on the standard deviation of log  $K_{ow}$  values determined by the method [22]. Both the correlation coefficient and the standard error of the estimate can be used to quantify the goodness of the linear fit. These parameters depend on the number of calibration compounds used and the proportion of calibration substances taken from different classes of compounds, such as neutral, basic or acidic substances. Table 5 is a

**Table 5**Linear regression parameters according to  $y = a + b x$  ( $y = \log \kappa$ ,  $x = \log K_{ow\text{cal}}$ ) for SGLLC and LSC

Support/adsorbent	<i>n</i>	<i>a</i>	<i>b</i>	<i>SD<sub>a</sub></i>	<i>SD<sub>b</sub></i>	<i>r</i>	<i>SD<sub>y,x</sub></i>	<i>SD<sub>xo</sub></i>
<b>SGLLC</b>								
LiChrospher Si 100 RP-18	35	-0.325	0.994	0.033	0.019	0.99415	0.06138	0.06175
LiChrospher Si 100 RP-8	35	-0.300	0.989	0.037	0.021	0.99255	0.06894	0.06971
LiChrospher Si 2000 RP-8	23	-1.761	0.848	0.084	0.033	0.98418	0.09294	0.10960
Nucleosil Si 100 C 8	30	-0.366	0.996	0.066	0.041	0.97741	0.10457	0.10499
LiChrospher Si 4000 RP-8	27	-1.844	0.814	0.112	0.059	0.93964	0.11881	0.14596
<b>LSC</b>								
LiChrospher Si 100 RP-8	40	-0.532	0.648	0.073	0.032	0.95553	0.15497	0.23915
Nucleosil Si 100 C 8	38	-0.496	0.603	0.076	0.038	0.93401	0.15310	0.25390

*n*: number of calibration compounds; *SD<sub>a</sub>*: standard deviation of intercept; *SD<sub>b</sub>*: standard deviation of slope; *r*: correlation coefficient; *SD<sub>y,x</sub>*: standard error of estimate; *SD<sub>xo</sub>*: standard deviation of the method; (for  $\log \kappa$  and  $\log K_{ow\text{cal}}$  values used for linear regression see Tables 1, 2 and 4).

**Table 6**Linear regression parameters according to  $y = a + b x$  ( $y = \log \kappa$ ,  $x = \log K_{ow\text{cal}}$ ) for SGLLC and LSC on each of two materials

Support/adsorbent	Class of compounds	<i>n</i>	<i>a</i>	<i>b</i>	<i>SD<sub>a</sub></i>	<i>SD<sub>b</sub></i>	<i>r</i>	<i>SD<sub>y,x</sub></i>	<i>SD<sub>xo</sub></i>
<i>LiChrospher</i>									
<i>Si 100 RP-8</i>									
SGLLC	ne	13	-0.214	0.953	0.025	0.014	0.99875	0.02646	0.02776
LSC			-0.442	0.656	0.064	0.037	0.98331	0.06728	0.10256
SGLLC	ba	6	-0.146	0.921	0.077	0.044	0.99552	0.04155	0.04511
LSC			-0.466	0.641	0.230	0.130	0.92657	0.12347	0.19262
SGLLC	ac	12	-0.459	1.053	0.097	0.048	0.98986	0.06334	0.06015
LSC			-0.515	0.548	0.192	0.095	0.87697	0.12590	0.22974
SGLLC	ne, ba, ac	31	-0.240	0.959	0.040	0.022	0.99260	0.05644	0.05885
LSC			-0.346	0.536	0.114	0.061	0.85058	0.15930	0.29720
<i>Nucleosil</i>									
<i>Si 100 C 8</i>									
SGLLC	ne	13	-0.250	0.950	0.025	0.016	0.99851	0.02695	0.02837
LSC			-0.433	0.625	0.062	0.040	0.07866	0.06807	0.10891
SGLLC	ba	7	-0.188	0.936	0.027	0.016	0.99924	0.01999	0.02136
LSC			-0.466	0.612	0.150	0.090	0.95048	0.10922	0.17846
SGLLC	ac	9	-0.576	1.060	0.129	0.076	0.98240	0.07561	0.07133
LSC			-0.637	0.594	0.289	0.170	0.79635	0.16909	0.28466
SGLLC	ne, ba, ac	29	-0.281	0.948	0.063	0.038	0.97854	0.09071	0.09569
LSC			-0.452	0.579	0.109	0.066	0.85889	0.15681	0.27083

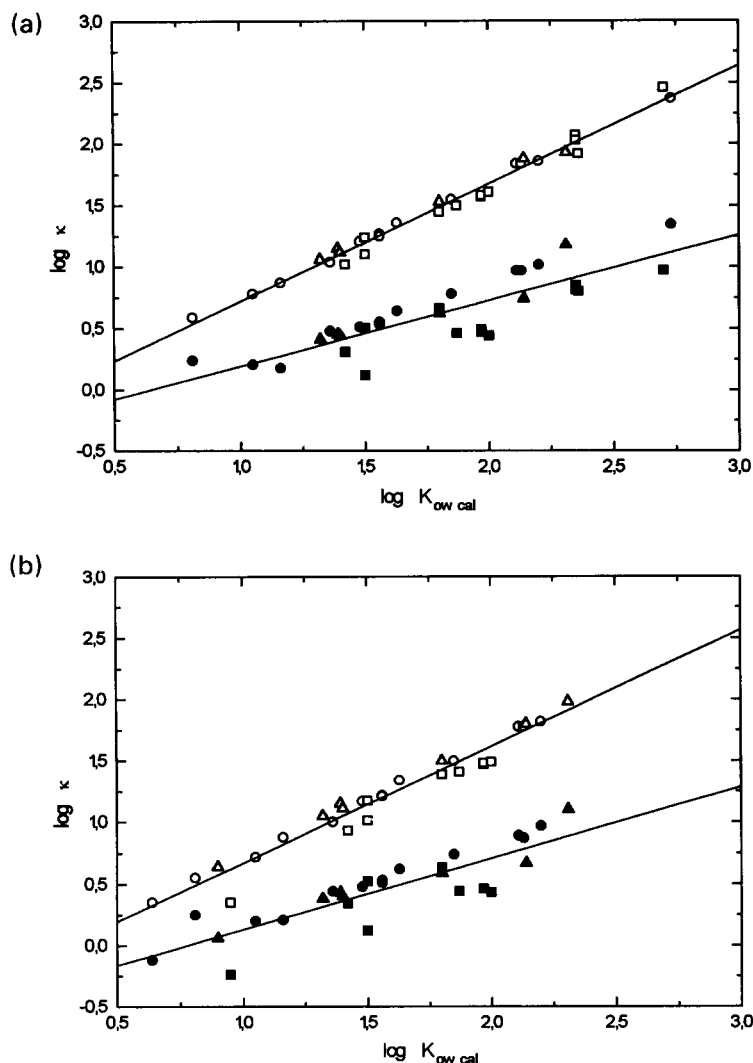
*n*: number of calibration compounds of the consistent data set used for linear regression; class of compounds: ne: neutral, ba: basic, ac: acidic; *SD<sub>a</sub>*: standard deviation of intercept; *SD<sub>b</sub>*: standard deviation of slope; *r*: correlation coefficient; *SD<sub>y,x</sub>*: standard error of estimate; *SD<sub>xo</sub>*: standard deviation of the method; (for  $\log \kappa$  and  $\log K_{ow\text{cal}}$  values used for linear regression see Tables 1, 2 and 4).

compilation of the regression data obtained in SGLLC and LSC by linear regression of the  $\log \kappa$  values on the  $\log K_{ow\text{cal}}$  values. Linear regression was carried out for the full set of neutral, acidic and basic calibration compounds. The highest correlation coefficients ( $r \geq 0.993$ ) were observed for the correlation of  $\log K_{ow\text{cal}}$  values and  $\log \kappa$  values measured on LiChrospher Si 100 RP-8 and RP-18 in the SGLLC mode. With the exception of LiChrospher Si 4000 RP-8, which is a bimodal porous material, SGLLC shows better correlations than does LSC. For all the phase systems

investigated the standard error of the estimate and the standard error of the method were smaller in SGLLC than in LSC.

In order to compare the results of different operating modes on the same column, or to assess the suitability of different packing materials for SGLLC, it is necessary to evaluate consistent data sets obtained with the same calibration compounds. In addition, class-specific effects can be shown by calculating regression data separately for each class of compounds. Table 6 and Fig. 2 (a and b) show the results of the linear regression for con-





**Figure 2**

Plot of  $\log \kappa$  vs  $\log K_{ow,cal}$  determined in the SGLLC and in the LSC mode (for  $\log \kappa$  and  $\log K_{ow,cal}$  values used for linear regression see Tables 1, 2 and 4). Key: open symbols: SGLLC; solid symbols: LSC;  $\circ$  neutral;  $\triangle$  basic;  $\square$  acidic compounds. (a) Support material/adsorbent: LiChrospher Si 100 RP-8; (b) Support material/adsorbent: Nucleosil Si 100 C 8.

sistent sets of calibration compounds measured in both the SGLLC and LSC mode on LiChrospher Si 100 RP-8 and on Nucleosil Si 100 C 8. Table 6 summarizes the correlation data for each individual class of compounds separately, as well as for the full set of calibration compounds. Figure 2 (a and b) shows plots of  $\log \kappa$  vs  $\log K_{ow,cal}$  in both modes for the full consistent set of neutral, basic and acidic calibration substances used. For both support materials retention data measured in the SGLLC mode were more closely correlated to  $\log K_{ow,cal}$  values than were the corresponding LSC data. This holds for the full set of calibration compounds as well as for corre-

lations calculated for each individual class of compounds separately.

In SGLLC the differences between the three classes of compounds were small: very high correlation coefficients were obtained with SGLLC data for neutral and basic compounds ( $r \geq 0.995$ ) and slightly lower ones for acidic compounds. By contrast the correlation coefficients obtained with LSC show a strong dependence on the class of compound. The correlation coefficients found were higher for neutral compounds than for basic compounds. Very low correlation coefficients were obtained for the regression data on acidic compounds. This can be attributed to class-

specific interactions with the adsorbent surface of the reversed-phase materials. All correlations based on SGLLC retention data yielded much lower standard errors of estimate and lower standard deviations of the method, than did the correlations based on LSC data. SGLLC largely eliminates systematic errors and reduces the spread of the calibration points about the regression line.

*Pairwise comparison of SGLLC and LSC data and test of significance of the differences observed*

Correlation coefficients and standard errors of estimate were calculated for each of the pairs of SGLLC/LSC data sets by correlating the log  $\kappa$  values, obtained respectively by SGLLC and LSC, of the consistent data set with the corresponding log  $K_{ow,cal}$  values. Statistical tests were applied to show the significance of differences in the correlation

coefficients and the standard errors of estimate.

In order to enlarge the data base for comparison of the SGLLC and the LSC methods, additional LSC data from the literature were included [24–27]. In LSC the best published correlations were obtained using methanol as modifier, so the comparison was restricted to adsorption data measured with mobile phases containing methanol. Since statistical significance tests need a rather large data base, the consistent data sets used for pairwise comparison comprised log  $\kappa$  values for neutral, basic and acidic compounds. Combining each of the five SGLLC data sets (see Table 2) with each of the nine LSC data sets (see Table 4) results in 45 pairs of data sets. Table 7 presents the correlation coefficients and standard errors of estimate calculated by linear regression for these 45 pairs of SGLLC/LSC systems.

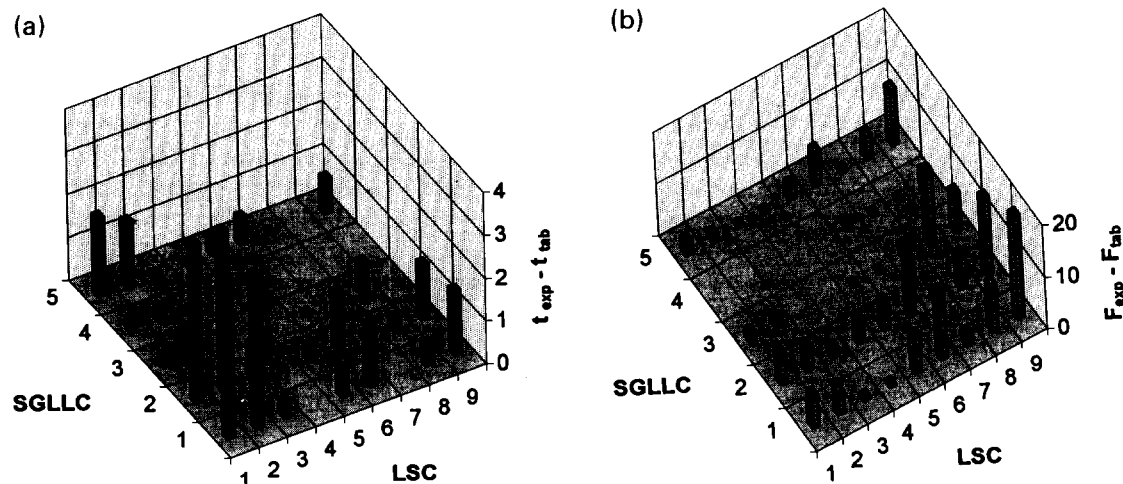
Figure 3(a) shows the result of the *t*-test [28]

**Table 7**

Correlation coefficients and standard errors of estimate for the pairwise comparison of the consistent SGLLC/LSC data sets

SGLLC mode		LSC mode			
		LiChrospher Si 100 RP-8	Nucleosil Si 100 C 8	Alltech RP-18 [24]	Hypersil RP-18 [12]
LiChrospher Si 100 RP-8	ne+ba+ac	13+6+12	14+7+13	9+3+4	3+3+4
	$r_{LLC}$	0.99260	0.99274	0.99381	0.99305
	$r_{LSC}$	0.85058	0.88531	0.96022	0.98399
	$SD_{y,x(LLC)}$	0.05644	0.06347	0.07080	0.07887
	$SD_{y,x(LSC)}$	0.15930	0.15391	0.11601	0.12819
LiChrospher Si 100 RP-18	ne+ba+ac	13+6+12	14+7+13	9+3+4	3+3+4
	$r_{LLC}$	0.99218	0.99338	0.99578	0.99759
	$r_{LSC}$	0.85058	0.88531	0.96022	0.98399
	$SD_{y,x(LLC)}$	0.05924	0.06181	0.05939	0.04506
	$SD_{y,x(LSC)}$	0.15930	0.15391	0.11601	0.12819
LiChrospher Si 2000 RP-8	ne+ba+ac	8+4+10	8+3+9	8+1+4	4+1+2
	$r_{LLC}$	0.97777	0.97008	0.98942	0.96518
	$r_{LSC}$	0.90826	0.88680	0.95475	0.99267
	$SD_{y,x(LLC)}$	0.08935	0.08729	0.07947	0.09875
	$SD_{y,x(LSC)}$	0.16756	0.15591	0.11345	0.04848
Nucleosil Si 100 C 8	ne+ba+ac	12+6+8	13+7+9	8+3+2	2+3+4
	$r_{LLC}$	0.97689	0.97783	0.98395	0.97573
	$r_{LSC}$	0.80089	0.85889	0.95132	0.98228
	$SD_{y,x(LLC)}$	0.07755	0.09155	0.10213	0.13374
	$SD_{y,x(LSC)}$	0.16242	0.15681	0.11712	0.12249
LiChrospher Si 4000 RP-8	ne+ba+ac	10+5+12	10+5+12	6+2+3	2+2+3
	$r_{LLC}$	0.93964	0.93964	0.95544	0.74666
	$r_{LSC}$	0.79615	0.79333	0.90088	0.96150
	$SD_{y,x(LLC)}$	0.11881	0.11881	0.09495	0.18597
	$SD_{y,x(LSC)}$	0.16104	0.14539	0.10997	0.11080

ne+ba+ac: number of neutral, basic and acidic calibration compounds of the consistent data set;  $r_{LLC}$ : correlation coefficient obtained in the SGLLC mode;  $r_{LSC}$ : correlation coefficient obtained in the LSC mode;  $SD_{y,x(LLC)}$ : standard error of estimate obtained in the SGLLC mode;  $SD_{y,x(LSC)}$ : standard error of estimate obtained in the LSC mode.



**Figure 3**  
 Statistical significance of the differences in the regression parameters for SGLLC and LSC. SGLLC: Support materials: (1) LiChrospher Si 100 RP-8; (2) LiChrospher Si 100 RP-18; (3) LiChrospher Si 2000 RP-8; (4) LiChrospher Si 4000 RP-8; (5) Nucleosil Si 100 C 8. LSC: Adsorbents: (1) LiChrospher Si 100 RP-8; (2) Nucleosil Si 100 C 8; (3)–(9) taken from literature: (3) Alltech RP-18 [24]; (4) Hypersil RP-18 [12]; (5) Lichrosorb RP-18 [25]; (6) LiChrosorb RP-18 [26]; (7) LiChrosorb RP-18 [27]; (8) LiChrosorb RP-18 [27]; (9) Octadecyl-polyvinyl copolymer gel [27]. (a) Significance of the difference in the correlation coefficients determined by the  $t$ -test; (b) significance of the difference in the standard errors of estimate determined by the  $F$ -test.

**Table 7**  
 Continued

LSC mode				
Lichrosorb RP-18 [25]	Lichrosorb RP-18 [26]	Lichrosorb RP-18 [27]	Lichrosorb RP-18 [27]	Octadecyl-polyvinyl copolymer [27]
7+0+4	11+3+1	11+2+1	11+2+1	11+2+1
0.98806	0.99746	0.99767	0.99767	0.99767
0.83748	0.95857	0.98713	0.98033	0.95404
0.05083	0.04060	0.04081	0.04081	0.04081
0.16107	0.15966	0.08952	0.13867	0.19214
7+0+4	11+3+1	11+2+1	11+2+1	11+2+1
0.98447	0.99732	0.99723	0.99723	0.99723
0.83748	0.95857	0.98713	0.98033	0.95404
0.05590	0.04320	0.04630	0.04630	0.04630
0.16107	0.15966	0.08952	0.13867	0.19214
7+0+4	7+2+0	8+1+0	8+1+0	8+1+0
0.99517	0.98803	0.99686	0.99686	0.99686
0.98930	0.98896	0.98718	0.91772	0.97622
0.06137	0.09798	0.05124	0.05124	0.05124
0.09575	0.10973	0.12616	0.21616	0.17028
9+1+3	10+3+1	10+2+1	10+2+1	10+2+1
0.98510	0.99443	0.99486	0.99486	0.99486
0.87404	0.94767	0.98264	0.97434	0.93649
0.06932	0.05453	0.05460	0.05460	0.05460
0.16215	0.16522	0.09087	0.14390	0.19836
7+0+4	8+2+1	8+1+1	8+1+1	8+1+1
0.91963	0.96153	0.97703	0.97616	0.97616
0.83748	0.90294	0.96783	0.94982	0.88428
0.12031	0.10219	0.08639	0.08639	0.08639
0.16107	0.18016	0.10050	0.16437	0.21712

applied to the paired correlation coefficients. One axis represents the 5 SGLLC data sets and the other one the nine LSC data sets. The appearance of a column in the SGLLC/LSC field indicates that the  $\log \kappa$  values measured by SGLLC are significantly better correlated with the  $\log K_{ow,cal}$  values than are the  $\log \kappa$  values measured by LSC. The significance of these differences is represented by the height of the column, which corresponds to the difference between  $t_{exp}$ , calculated from the data, and the significance limit,  $t_{tab}$ , tabulated for 95% probability. It was found that in the SGLLC mode the correlation coefficients were significantly higher in 29 of the 45 paired cases investigated. For the other pairs the difference between SGLLC and LSC data was not significant. In no case was a significantly higher correlation coefficient observed in the LSC mode.

Figure 3(b) shows the significance of the differences in the standard errors of estimate by the *F*-test [28]. The difference between the

experimental value,  $F_{exp}$ , and the significance limit tabulated for 95% probability,  $F_{tab}$ , is plotted on the *z*-axis. In 37 of the 45 paired cases investigated the standard error of estimate was significantly lower in SGLLC than in LSC. In the other cases no significant differences were found. The same general picture emerges if LSC data determined with other mobile phases are included in the comparison [23].

#### Estimation of $\log K_{ow}$ values of test compounds

The spread of calibration points about the regression line (see Fig. 2) reflects the limits of reproducibility of the retention data of calibration compounds and also systematic errors due to specific adsorption effects. As the present results show in SGLLC specific adsorption contributions to solute retention are substantially reduced. This results in a reduction of the standard deviation of the method and a corresponding reduction in the standard error of the estimate.

**Table 8**

Set of test compounds with their retention data ( $\log \kappa$  values) measured on LiChrospher Si 100 RP-8, the estimated  $\log K_{ow}$  values and  $\log K_{ow}$  values found in literature [1, 20]

Test compounds	$\log \kappa$		$\log K_{ow,est}$		$\log K_{ow}$	
	SGLLC	LSC	SGLLC	LSC	[20]	[1]
Chloramphenicol	0.77	0.30	1.08 ± 0.15	0.99 ± 0.48	—	1.14
Cortisol	1.16	—	1.48 ± 0.14	—	—	1.49
1,3-Dinitrobenzene	1.21	0.66	1.53 ± 0.14	1.55 ± 0.51	—	1.49
4-Nitrotoluene	2.04	—	2.37 ± 0.15	—	2.42 ± 0.10	2.37
						2.42
2-Nitrotoluene	2.07	1.09	2.40 ± 0.15	2.21 ± 0.53	2.30 ± 0.25	2.30
Ethyl benzoate	2.37	1.36	2.70 ± 0.15	2.63 ± 0.57	2.64 ± 0.20	—
3-Nitroaniline	1.06	0.28	1.38 ± 0.14	0.96 ± 0.48	—	1.37
4-Nitroaniline	1.07	0.14	1.39 ± 0.14	0.75 ± 0.48	—	1.39
2-Nitroaniline	1.48	0.53	1.80 ± 0.14	1.35 ± 0.51	—	1.44
						1.79
						1.83
3-Chloroaniline	1.59	0.64	1.91 ± 0.14	1.52 ± 0.51	—	1.83
						1.90
2-Chloroaniline	1.61	0.66	1.93 ± 0.14	1.55 ± 0.51	—	1.90
						1.92
Isoquinoline	1.97	1.06	2.30 ± 0.15	2.17 ± 0.53	2.08 ± 0.20	2.08
Barbital	0.28	—	0.59 ± 0.15	—	—	0.65
Resorcinol	0.41	—	0.72 ± 0.15	—	—	0.80
						0.77
2-Nitrobenzoic acid	0.75	—	1.06 ± 0.15	—	—	—
Allobarbitol	0.75	—	1.06 ± 0.15	—	—	1.19
2,6-Dihydroxybenzoic acid	0.92	—	1.23 ± 0.15	—	—	2.20
Phenylacetic acid	1.03	0.35	1.34 ± 0.14	1.07 ± 0.48	1.41 ± 0.15	1.41
4-Hydroxybenzoic acid	1.11	—	1.43 ± 0.14	—	—	1.58
Hexobarbital	1.13	—	1.45 ± 0.14	—	—	1.49
3-Nitrobenzoic acid	1.47	0.49	1.79 ± 0.14	1.29 ± 0.48	—	1.83
4-Nitrobenzoic acid	1.50	0.54	1.82 ± 0.14	1.37 ± 0.51	—	1.89
4-Nitrophenol	1.58	0.36	1.90 ± 0.14	1.09 ± 0.48	—	1.91

$\log \kappa$ : logarithm of the capacity factor;  $\log K_{ow,est}$ :  $\log K_{ow}$  value ± the confidence limit at 95% probability level calculated according to [22, p. 113] (for regression parameters see Table 5).

Table 8 illustrates these advantages offered by SGLLC using a number of test compounds as examples. Log  $K_{ow}$  values for these compounds are to be found in the literature but their quality is not such as to fulfill the criteria given above for the selection of calibration compounds. Table 8 presents the capacity factors measured on LiChrospher Si 100 RP-8 by both SGLLC and LSC, the log  $K_{ow}$  values calculated from the regression line obtained with the calibration compounds (for regression parameters see Table 5) and the corresponding 95% confidence limits calculated from the standard deviation of the method. These data have been compared with literature data measured by the batch method [1] and with data contained in a critical compilation of data obtained by several different methods [20]. This comparison shows that the use of SGLLC significantly reduces the uncertainty in determining  $K_{ow}$  values.

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