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The characterization of silica-based sorbents in reversed phase liquid chromatography with application to method development in solid-phase extraction

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THE CHARACTERIZATION OF SILICA-BASED SORBENTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH APPLICATION TO METHOD DEVELOPMENT IN SOLID-PHASE EXTRACTION

by

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DISSERTATION

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Colin F Poole 12/9/97

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DEDICATION

To my mother, Mary Ann, whose patience and love
have never failed, though I have tested them many times;
to the memory of my father, Andrew, who would have said
I stayed in school too long--every day I find I'm more like him;
and to Keith Agdanowski, on his thirtieth birthday,
although I'm sure he was hoping for a slightly more exciting gift.

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1. Introduction

1.1. Sample preparation

Rarely can real-world samples be analyzed by chromatographic means without first undergoing some sample preparation steps. The goal of sample preparation is to remove matrix components and/or particulate matter that may interfere with analysis or cause damage or excessive wear to the analytical instrumentation. Sample preparation techniques are also used to concentrate analytes present in trace amounts in large samples, and can be made highly selective for specific analytes, thus improving detectibility and the quality of the analytical results. Advances in sample preparation have been relatively slow compared to the rapid development of sophisticated separation and detection techniques, and sample preparation remains the most laborious part of typical analyses [1-5]. Most chromatographic techniques are relatively mature. A high degree of automation is available, and great emphasis has been placed in recent years on increasing laboratory productivity and sample throughput. Together, these factors underscore the need for a sound fundamental understanding of sample preparation techniques. Such an understanding will inevitably lead to the streamlining of procedures and a reduction in the time required for sample preparation, method development, and method optimization.

1.2. Liquid-liquid extraction

Traditional liquid-liquid extraction (LLE) using separatory funnels has been considered the simplest and most reliable method of isolating and concentrating analytes from a variety of matrices. LLE functions through the partitioning of analytes and interferences between two immiscible phases. Relatively hydrophilic compounds will partition into the polar aqueous phase while less hydrophilic compounds favor partition in an organic solvent [6]. In practice LLE requires virtually no expensive equipment and only minimal technician training. LLE suffers, however, from being labor intensive, time consuming and difficult to automate. It also requires large volumes of high-purity, high-

cost solvents. The selectivity of LLE is limited by the narrow range of immiscible solvent combinations and the formation of emulsions which prevent the necessary separation into two distinct solvent layers [1,4,7,8]. Exhaustive extractions are often only attainable through multiple extractions; the number of extractions necessary for quantitative transfer of a solute from one solvent to another is dependent on the value of the solute's distribution constant between the two phases [6,7]. Some of these problems can be resolved by moving to solid-phase extraction (SPE) techniques.

1.3. Solid-phase extraction

The business-driven interest in increasing sample throughput while reducing analysis costs has sparked a vigorous effort toward automation. Because of its relatively low cost and its ease of automation, SPE has gained in popularity in recent years, and has been widely accepted as an alternative to LLE [1,4,7]. The number of journal references for SPE has drastically increased since the mid 1980s [1,4], two books have been published as part of the trade literature [9,10], and meetings dedicated solely to SPE have been held in recent years. SPE methods have been developed for numerous environmental and clinical applications and have become the basis for several official or regulatory methods. **Table 1.1** gives some examples of EPA-approved methods involving SPE and gas chromatographic (GC) analysis. Sections of the German DIN 38407 methods for organophosphorus and nitrogen pesticides and phenoxyalkyl carbonic acids also involve SPE sample preparation [2].

In an era where new legislation bans the use of some chlorinated solvents, and solvent purchase, transport, and disposal costs are high, SPE also has the advantage of minimizing solvent consumption. Lower solvent consumption translates into less exposure to toxic solvents for the analyst as well. Field sampling can also be facilitated through the use of SPE. Samples collected in the field can be extracted on site with the aid of a simple vacuum system. Only the extraction device containing the analytes adsorbed

Table 1.1. Examples of EPA-approved methods involving SPE.

EPA method No.	*Analytes*
506	phthalates, adipates
508.1	organochlorine/nitrogen pesticides
515.2	chlorinated herbicides
525.2	semivolatile organics
548.1	endothal
552.1	haloacetic acids, dalapon
1631B	TCDD

on the sorbent bed needs to be physically transported back to the laboratory for analysis, eliminating the need to transport bulk samples [7].

SPE is a means of sample preparation for chromatographic analysis that can function in terms of matrix simplification and/or trace enrichment. It is used to isolate and concentrate selected analytes from a gas, fluid or liquid by transfer to and interaction with an immobilized liquid or solid phase. Compounds retained on the solid phase can be removed in subsequent steps by elution with an appropriate solvent. While the majority of samples are eluted into one final volume, fractionation of samples can be accomplished through stepwise elution with solvents of increasing strength to obtain aliquots of sample containing specific compounds or groups of compounds [11,12]. Derivatization of analytes retained on the solid phase can also be carried out [13,14].

Compared to LLE, SPE methods offer more complete isolation of the analytes without the need for successive extractions to achieve exhaustive extraction. SPE methods also provide more efficient separation of interferences and analytes through the possibility of selectively removing interferences in a wash step [1,6]. Despite its advantages over other extraction methods, SPE does present its own set of attendant problems. Surprisingly little, however, has been done to characterize these systems; SPE

cartridge design has evolved little since its inception and method development for SPE remains largely a labor-intensive, time-consuming trial and error process [4,5,8,10]. The purpose of this research project is to identify some of the performance shortcomings of cartridge SPE devices and to investigate the kinetic and retention properties of some common SPE sorbents with the intention of generating a rational means of method development.

1.4. Summary of background information

Background information will be provided to give a comprehensive view of solid-phase extraction sorbents, equipment, methods, and approaches for method development. Alternatives to experimental approaches in the form of systems that allow the prediction of extraction conditions will be discussed, along with mathematical and theoretical descriptions of the models used to build a new predictive method. Elements of this new method provide insight into liquid chromatographic retention mechanisms, a standardized means of characterizing sorbent selectivity, as well as ways in which sorbent materials and extraction devices could be improved or optimized. Sufficient background for the understanding of these assertions will be provided, along with background in the field of steroid analysis. Discussion of this latter field, specifically the analysis of estrogens in complex matrices, is included as these types of samples are of considerable clinical interest and will be used to evaluate the accuracy of the predictive method developed here.

1.5. Solid-phase extraction formats

A variety of formats for SPE are commercially available and each comes with its own set of advantages and disadvantages. Relatively recently, traditional cartridge devices have been augmented by the introduction of other SPE formats including disk technology and solid-phase microextraction.

1.5.1. Cartridge devices

Figure 1.1 depicts a cross section of a typical SPE cartridge device. The basic design consists of an open syringe barrel containing a sorbent with an average particle size of 40-60 µm packed between porous plastic or metal frits. Common barrel volumes are between 1 and 6 ml containing from 250 mg to 1 g of packing, although both larger and smaller cartridge sizes are available. The dimensions of cartridge devices are intentionally miniaturized to allow sample processing by gravity or mild suction and to reduce the volumes of solvents consumed in conditioning the sorbent and in recovering the analyte from the sorbent.

The cartridge format has several disadvantages. The small cross-sectional area of the sorbent bed results in slow sample-processing rates and a propensity toward blockage by particles and adsorbed matrix components. Channelling reduces the capacity of the column to retain analytes and is the dominant cause of poor reproducibility of sampling with cartridges [15]. The reproducibility of sorbent properties from batch to batch and

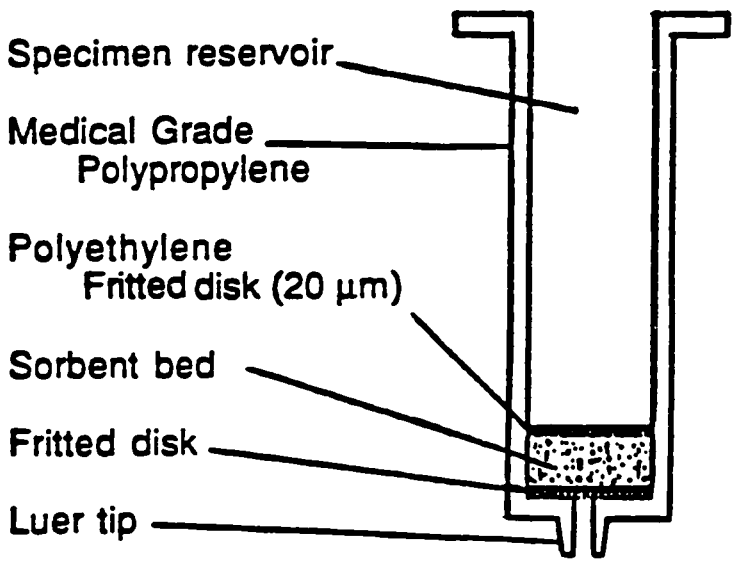


Figure 1.1. Cross section of a typical cartridge device

between manufacturers is poor and packing materials are subject to many of the same production problems associated with HPLC packings [7]. Furthermore, the recovery of analytes is reduced by incomplete reversibility of sorption to active sites and the presence of impurities originating from manufacturing and packaging processes may contaminate samples and interfere with analysis [15]. Despite these problems, cartridges remain one of the most popular formats for SPE devices.

1.5.2. Disk technology

Particle-loaded membranes (PLMs) [16-18] and particle-embedded glass fiber disks (PEGFDs) [19,20] are alternative formats to cartridge devices in SPE. PLMs come in a range of diameters from 4 to 96 mm, are 0.5 mm thick, and consist of a web of PTFE microfibrils in which sorbent particles of about 8 μm in diameter are suspended [7]. The membranes have a homogeneous structure and are flexible, requiring the use of sintered-glass or other supports. PEGFDs contain sorbent particles embedded in a glass fiber supporting matrix. Small diameter disks can be used without additional support, but the larger diameter disks are normally used with a supporting structure.

Disk technology claims several advantages over SPE using traditional cartridge devices [21]. The larger cross-sectional area of the disk allows higher flow rates, lower pressure drops and faster sample processing times. The larger cross-sectional area also makes disks less susceptible to plugging and therefore good choices for processing large samples containing suspended particulate matter (such as environmental water samples). The tendency toward channeling is reduced through the use of smaller particle sizes and the greater stability of the sorbent bed. Optimization of the bed mass leads to the reduction of non-specific matrix adsorption resulting in cleaner extracts with lower levels of interferences.

1.5.3. Solid-phase microextraction

Solid-phase microextraction (SPME) is a relatively new technique that utilizes a polymer-coated fused silica fiber to sample aqueous solutions either directly by insertion of the fiber into the solution or indirectly by suspending the fiber in the headspace above the sample solution in a closed vessel [22]. Reusable fibers are commercially available coated with polydimethylsiloxane, polyacrylate, and carbowax/divinylbenzene layers. **Figure 1.2** shows a typical fiber/housing assembly. The active sampling area is a cylindrical fiber typically 1 cm long with a radius of about 0.015 cm coated with a 10-100 μm thick film of immobilized polymer. The coated fiber is encased in a syringe needle that can be retracted to expose the fiber. Analytes diffuse to and partition into the fiber coating and are subsequently eluted in the injection port of a GC by thermal desorption or an HPLC by displacement with a strong solvent.

The major advantage of SPME is the (usually) solventless extraction of analytes from solution, while the major disadvantage is that the extent of extraction depends on the analyte's distribution constant between the two phases. Since sampling by SPME is an equilibrium process, exhaustive extraction is usually not achieved. For the direct sampling of liquids, assuming that the sample volume is much greater than the volume of the fiber coating, the amount of material extracted by the fiber is simply expressed in equation 1 where C_0 is the initial concentration of the analyte in the sample solution,

$$w_1 = C_0 V_1 K_c \qquad (1)$$

V_1 is the volume of the fiber coating and K_c is the distribution constant for the analyte between the fiber coating and the sample solution [23,24] which equals the ratio of the analyte concentration in the sample solution to its concentration in the fiber coating. This linear relationship is valid only at equilibrium and does not take into account the time required to reach equilibrium, which can be long for compounds with large distribution constants. Extraction times can be reduced by agitating the sample solution by stirring or

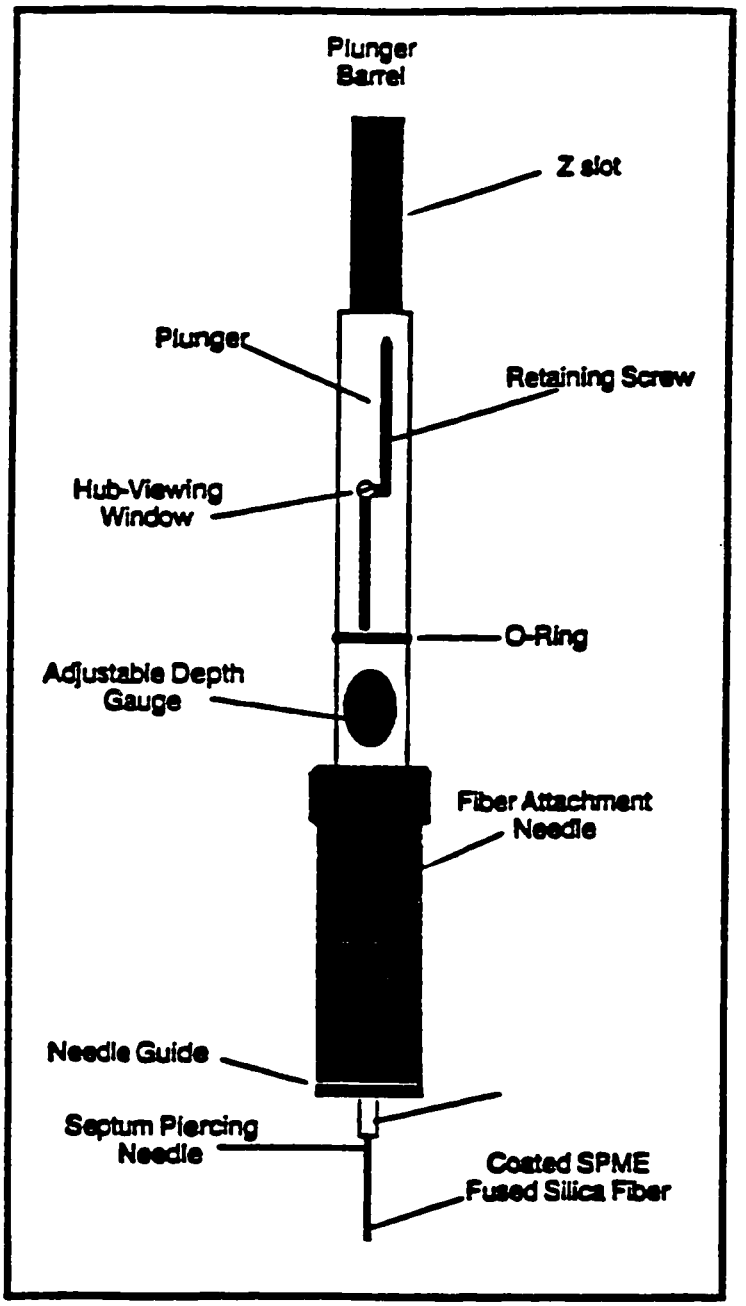


Figure 1.2. Solid phase microextraction device

sonication. Even with adequate agitation of the sample solution, the analytes must still diffuse through a small static layer of solution at the fiber's surface, thus limiting the application of SPME for analytes with large distribution constants.

Sampling the headspace above a solid sample or sample solution can greatly reduce extraction time since diffusion coefficients in the vapor phase are greater than in solution [25] and rapid equilibrium between the solvent and vapor phase can be achieved. Headspace extraction works well for volatile compounds, but compounds with low volatility and high solvent solubility transfer only slowly from the sample matrix to the headspace. Since distribution constants are temperature dependent, an optimum temperature should be sought where release of analytes from the sample matrix is favorable but absorption of the analytes by the coated fiber from the vapor phase still occurs. The distribution constants involved in SPME could be modeled by the solvation parameter model in a similar fashion to the work presented here for cartridge extraction devices, but no publications have appeared on this topic to date.

1.6. Sample processing using cartridge devices

Sample processing by SPE using traditional cartridge devices can be performed in one of two different modes, as illustrated in **Figure 1.3**. Either the impurities are retained on the column with compounds of interest passing through unretained, or the compounds of interest are retained and the impurities are allowed to pass through or are washed through the column. This latter mode is the most frequently employed approach, generally involving four steps. The conditioning step consists of washing the packing material with an appropriate solvent (*e.g.*, methanol) to solvate and activate the functional groups, to increase the surface area of the bonded phase, and to remove any impurities present in the sorbent bed [4]. Further conditioning with a solvent similar to the sample matrix is required before the sample is applied to the cartridge. Without letting the sorbent bed dry out, the sample is then introduced into the barrel of the cartridge and

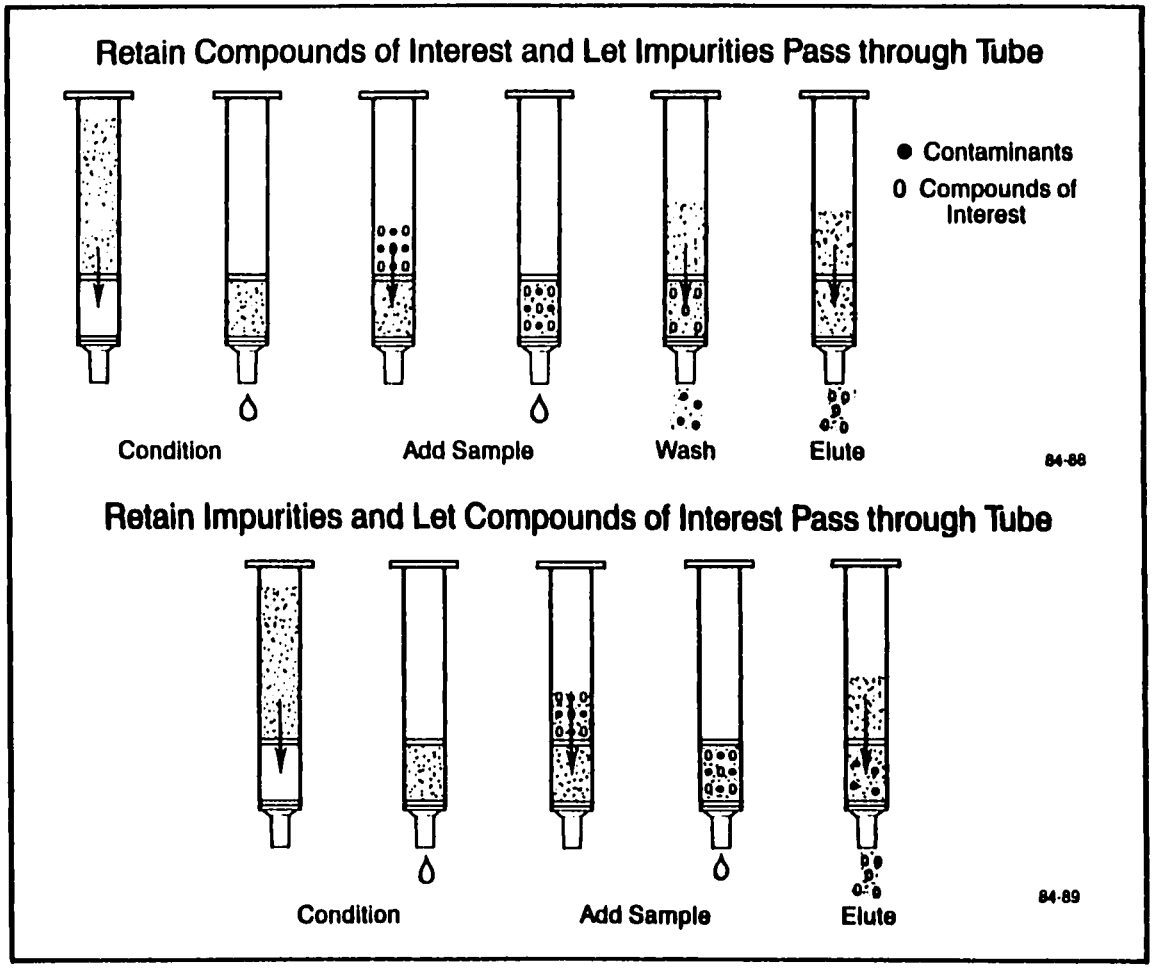


Figure 1.3. General procedures for solid-phase extraction.

forced through the packed bed by suction from an aspirator, by positive pressure from a syringe, or by centrifugation. After the sample has passed through the bed, a wash solvent is used to remove matrix interferences and the sorbent bed is sucked dry by passing air through the cartridge for a short time (usually 1-2 minutes). Finally, the analytes are recovered from the cartridge with a small volume of elution solvent. Minimizing the volume of the elution solvent serves to maximize the concentration effect in relation to the original sample volume.

1.6.1. Automation of SPE procedures

Sample processing in SPE has been greatly simplified through the introduction of various levels of automation. Vacuum manifolds allow the manual processing of samples through up to 24 cartridges simultaneously, but manipulating solvents and controlling flow for a large number of cartridges with variable flow rates present many difficulties for the analyst [2,3]. Off-line automated systems alleviate these difficulties by performing programmed extraction steps in sequence and can be classified as semi-automated or fully automated. The degree of automation is distinguished by the method of transfer of the extracted sample to the analytical chromatographic system. In a fully automated system, cartridge eluates are transferred to the chromatographic system without the need for human intervention, allowing unattended sample preparation and analysis [1,2]. On-line SPE systems are often referred to as "column switching" devices in which a portion of the effluent from a low-efficiency extraction column is directed to an analytical column for separation and subsequent quantitation. Column switching techniques have been extensively reviewed (*e.g.*, [26-28]). One of the greatest advantages of on-line techniques is that they make use of the entire eluent volume. In off-line methods only an aliquot of the cartridge eluent is transferred to the analytical system, thus negating to some extent the concentration effect gained during extraction [8].

1.6.2. Method development using cartridge devices

Method development in solid-phase extraction requires the selection of extraction conditions in terms of the type and amount of sorbent used, safe sampling conditions and the composition and volume of wash and elution solvents. Most analysts using SPE cartridges follow instructions provided by the manufacturer and do not engage in method development or optimization for the specific sample at hand. Those who do develop their own methods rely largely on prior experience, previously published methods, or other empirical means to determine extraction conditions [2].

A typical procedure for sorbent and solvent selection involves many steps, beginning with defining the analytical problem. The analyte type, sample matrix type and likely interferences and/or impurities must be delineated along with their relative polarities, pK_a values, solubilities and molecular weights. The extent of sample concentration needed and any solvent constraints should be considered in terms of the capabilities of the intended analysis and detection systems [2,10]. Manufacturers usually supply long lists of compound types and suggested sorbents for initially narrowing down the choice of sorbents, but for a given compound type more than one retention mechanism and several sorbents within a type can be listed as choices. Solvent strength and polarity indices are generally used for estimating solvent compositions that are likely to work as wash and elution solvents, but the general rule of "like dissolves like" tends to be the best piece of advice given to customers.

Once a preliminary set of extraction conditions has been chosen, a systematic process of method optimization shown in **Figure 1.4** (adapted from [10]) should be undertaken. The first step should be optimization of the elution solvent, accomplished by loading small, known amounts of analytes onto cartridges and testing recovery using various solvent compositions and volumes. To ensure complete elution, at least 2-3 times the dead volume of the cartridge should be used. The second step is verification that the analytes are retained by the selected sorbent from a standard solution with the same pH,

DEFINE EXTRACTION PROBLEM
characterize sample
evaluate analytical requirements

PROPOSE PRELIMINARY METHOD
select extraction mode
select extraction sorbent
select elution solvent
select sample volume
select matrix modification method

OPTIMIZE METHOD

1. Verify elution of analytes

   possible problems:
   improper column polarity
   elution solvent not strong enough
   elution volume too small
   irreversible adsorbtion

2. Extract analytes from standard solution

   possible problems:
   column not properly conditioned
   sample solution needs adjustment of polarity, pH or ionic strength
   sorbent deactivated by large aqueous samples

3. Extract analytes from spiked matrix

   possible problems:
   strong analyte-matrix interactions
   matrix components interfere with solute-sorbent interactions
   matrix components diminish capacity available to analytes

4. Check for co-elution of interferences

   possible problems:
   wash step not effective

5. Validate method

Figure 1.4. Method development scheme for SPE

ionic strength and solvent strength as the intended sample solution. At this point, manufacturers recommend evaluating several sorbent types within a given separation mechanism (*e.g.*, for a reversed-phase system, try octadecyl, octyl, phenyl and cyano phases). Adjustment of sample solvent strength, pH or ionic strength may help to optimize retention. The next step is to attempt the extraction of analytes from the spiked sample matrix to assess possible matrix interferences, strong matrix interactions with the analytes, or strong matrix interactions with the sorbent that reduce the capacity of the sorbent to interact with the analytes. If co-elution of interferences with the analytes occurs, a series of wash solvents with a range of solvent strengths should be evaluated for the removal of the interferences with minimal loss of analytes. Overall, the selection of SPE conditions is far from straightforward, and even with prior experience and common sense, the development of a reliable method for SPE remains an empirical, trial and error process.

1.7. SPE sorbents

Packing materials for SPE function under a variety of retention mechanisms. The most popular adsorbants are the reversed-phase silica-based bonded materials, but porous polymer, normal phase, adsorption, ion exchange, and size exclusion packing materials are also commercially available in cartridge devices. More recently, mixed modal packings that function simultaneously in more than one retention mechanism (*e.g.*, combining ion exchange and reversed phase mechanisms in one packing material) as well as packings designated for specific applications (*e.g.*, pesticide and drugs of abuse extraction) have also appeared on the market [2,10]. **Table 1.2** summarizes the general retention mechanisms and analyte and sorbent types associated with each. Within the group of silica-based bonded reversed-phased packings (applications listed in **Table 1.3**), sorbents differ mainly by the length of the hydrocarbon chain (*e.g.*, C_2, C_4, C_8, C_{18}) and any functional group attached (*e.g.*, cyclohexyl, phenyl, cyanopropyl, diol). Some of the

Table 1.2. Retention mechanisms in SPE

Separation mechanism	*Analyte type*	*Sorbents*
Normal phase	Slightly to moderately polar	kieselguhr, silica gel, florisil, alumina
Normal phase (polar bonded phase)	Moderately to strongly polar	cyano, amino, diol
Reversed phase (nonpolar bonded phase)	Nonpolar to moderately polar	octadecyl, octyl, butyl, ethyl, cyclohexyl, phenyl, cyano, diol
Reversed phase	Nonpolar	porous polymers
Anion Exchange (SAX, WAX)	Ionic acid	amino, quaternary amine
Cation Exchange (SCX, WCX)	Ionic base	carboxylic acid, propyl or aromatic sulfonic acid
Size Exclusion	Proteins	sephadex
Specified application	pesticides narcotics from urine	$-(CH_2)_nCH_3$ methylester, cation/HIC

Table 1.3. Reversed-phase silica-based bonded phase sorbents.

Sorbent	*Analyte type*	*Typical applications*
Octadecyl (C_{18})	nonpolar to moderately nonpolar, hydrophobic	most applications...some examples: abused drugs, antibiotics, essential oils, fatty acids, phenols, priority pollutants, steroids, tricyclic antidepressants, etc.
Octyl (C_8)	non polar to moderately nonpolar, hydrophobic	priority pollutants, pesticides, and other compounds retained too much by C_{18}
Phenyl (C_6H_5), Cyclohexyl (C_6H_{11})	moderately nonpolar, intermediate hydrophobicity	offer less retention of hydrophobic compounds
Butyl (C_4), Cyano (CN), Diol (COHCOH)	slightly polar to moderately nonpolar	work well for compounds retained too much by C_{18}

bonded phases, such as the cyano, amino, and diol phases, can be used in both normal and reversed-phase modes [1]. With the abundance of sorbents from which to choose, the question arises as to whether sorbents that differ only slightly in their bonded-phase chemistry really offer significant differences in selectivity. Likewise, with the proliferation of new sorbent materials, the need arises for a standardized method of classifying sorbents and describing retention mechanisms that allows significant differences in selectivity to be easily recognized.

1.7.1. Characterization of sorbents

Manufacturers generally provide values for some characteristic properties of the sorbent contained in cartridge extraction devices. Typical properties include particle size distribution determined by laser granulometry, specific surface area by the BET method, the surface coverage of bonded phases determined by combustion, an apparent sorbent pH determined from an aqueous suspension of the sorbent in water, UV absorbance of a solvent extract, and a nominal mean pore diameter by calculation or size exclusion chromatography. These values can be useful for comparing sorbents from different sources or lot numbers, but give little information about how the sorbent will perform in SPE. For maximum performance of extraction devices, a homogeneously packed bed and a reasonable quality sorbent are recommended. In practice, the quality of SPE sorbents is not maximized in order to keep the price of extraction devices low. Certain tests, developed for evaluating chromatographic columns, can be used to assess whether the low quality of the packing material can be expected to unduly affect extraction results.

1.7.2. Physical/functional measurements

Porosity measurements are an indication of the mobile phase volume accessible to the solute as it travels through the sorbent. High values of interparticle porosity can indicate voids or regions of low packing density in the sorbent bed. Low proportions of

intraparticle porosity in relation to interparticle porosity indicate the choking of pores by the bonded ligand or by fines (particles with diameters less than the average particle diamater), conditions that result in unfavorable mass transport characteristics of the sorbent that are not necessarily deleterious to the use of the sorbent in SPE. The total porosity of a column, ε_t, is defined as the sum of the interparticle porosity, ε_u, and the intraparticle porosity, ε_i. In the absence of significant extracolumn volumes, the total porosity of a column can be determined by the elution volume of a totally permeating and unretained substance as indicated by equation 2 where F_V is the volumetric flow rate

$$\varepsilon_t = F_V t_m / \pi r^2 L \qquad (2)$$

through the column, t_m is the retention time of an unretained substance, r is the internal column radius and L is the column length. Problems with stationary phase sorption and various normal and ionic exclusion mechanisms make the choice of the unretained solute difficult in reversed-phase liquid chromatography and many methods for the determination of column holdup (or dead) volume have been developed [29-32]. Injection of sodium nitrate at a high concentration (*e.g.*, 25 mg/ml) can be used to measure dead volume for the determination of total porosity [15,33]. A lower concentration (*e.g.*, 1 mg/ml) of the same unretained compound can be used to estimate the interparticle volume, with the assumption that at low concentrations sodium nitrate is excluded from the intraparticle volume. The intraparticle porosity is the difference between the total and interparticle porosity values.

From Darcy's law (equation 3), the specific permeability of the column, B, is

$$u = B\Delta P / \varepsilon_t L \eta \qquad (3)$$

obtained from the slope of a plot of u against $\Delta P/\varepsilon_t L\eta$ where ΔP is the pressure drop across the column, u is the mobile phase velocity, and η is the viscosity of the mobile phase (evaluated from tables [34,35]). The apparent chromatographic average particle

diameter, d_p, can be obtained from the specific permeability using the semi-empirical Carman-Kozeny equation (equation 4) [33,36] where ψ^2 is a shape factor assumed to

$$B = (d_p^2/180\psi^2)[\varepsilon_u^3/(1-\varepsilon_u)^2] \quad (4)$$

have a value of 1.7 for irregular porous particles. In order to reduce channeling and achieve homogeneous flow through the sorbent bed, particle diameters should be tightly clustered around the average value.

The pressure drop across a column is an important factor in determining the column flow resistance parameter, ϕ, a sensitive test for the presence of particles smaller

$$\phi = \Delta P d_p^2 t_m/\eta L^2 \quad (5)$$

than the average particle diameter in the sorbent material [7]. As calculated by equation 5, acceptible values of the unitless ϕ range from 500 to 1000.

The hydrophobicity index is a measure of the volume of accessible stationary phase. Hydrophobicity increases with carbon loading, *i.e.*, with increasing the chain length of the bonded ligand, increasing the bonding density, endcapping the phase, and increasing the surface area of the silica substrate [7]. Hydrophobicity indices have been developed based on the measurement of retention factors for various compounds [37-40]. Based on one measure of hydrophobicity [41], fairly large differences were observed among C_{18} bonded phases reflecting the differences in their preparation and also indicating the large difference in retention that can be anticipated when C_{18} sorbents from different manufacturers are selected for the same application. Scant data for the hydrophobicity index for bonded phases other than C_{18} are available.

Chemically bonded phases are generally prepared by reacting the silanol groups of a silica substrate with a reactive organosilane reagent. Residual, or unreacted, silanol groups in bonded phases have been associated with undesirable interactions with polar solutes such as excessive peak tailing, irreproducible retention times, and excessively long

retention times [7]. The silanophilic index indicates the extent of the presence of unreacted silanol groups and the propensity of the sorbent toward non-specific adsorption interactions. Many of the same groups generating hydrophobicity indices also develop silanophilicity measures [37-39], and literature values for the silanophilic index of several C_{18} sorbents are available.

1.8. Breakthrough volume

Under typical experimental conditions, a sample of fixed concentration enters the sorbent bed with a constant velocity and is quantitatively retained by the sorbent up to the point where the sample volume exceeds the retention capacity of the sorbent. Further sample entering the sorbent bed will not be quantitatively retained and eventually the sample concentration entering and exiting the sorbent bed will be identical. **Figure 1.5** illustrates this process, and is an example of both a frontal chromatogram and a breakthrough curve. The breakthrough volume, V_b, can be defined at different levels, but is generally the point on the curve at which a defined amount (*e.g.*, 0.1%, 1%, etc.) of sample is detected at the outlet of the sorbent bed. A second point, V_M, corresponds to

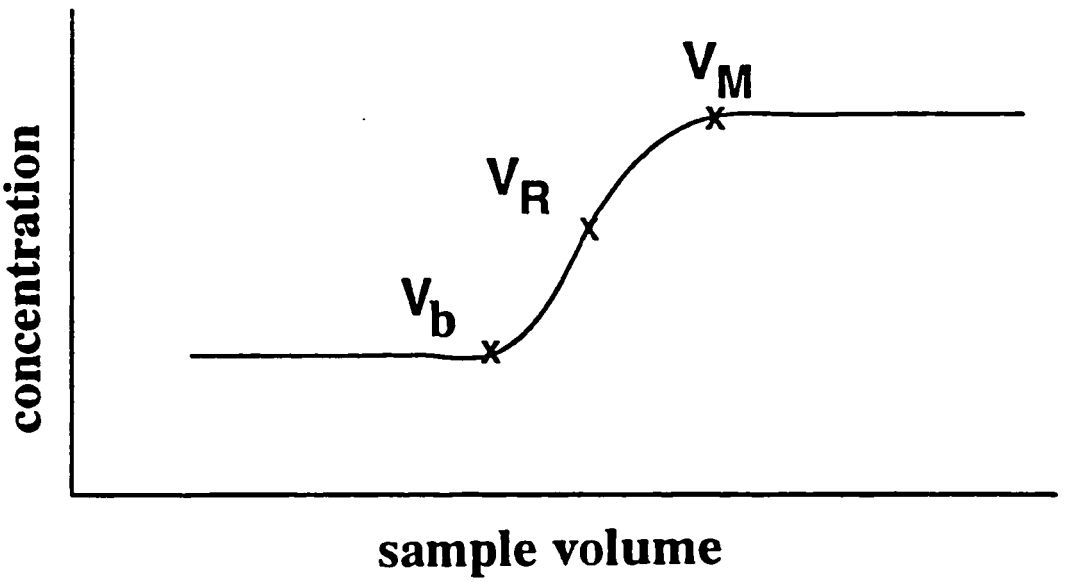


Figure 1.5. Breakthrough curve

the volume at which the sampling capacity of the sorbent is exceeded and the flux of analyte entering and leaving the sorbent bed are nearly identical. The inflection point in the curve is defined as the retention volume, V_R, of the analyte [4,8]. The retention volume is analogous to the maximum in a Gaussian peak in elution chromatography.

Breakthrough volume can be used as an indicator of the ability of an SPE device to effectively isolate an analyte from a sample solution. It can be defined as the volume of sample of a constant concentration that can be passed through an extraction device before the concentration at the outlet reaches a certain fraction of the concentration at the inlet. In an SPE scenario, the breakthrough volume then corresponds to the largest sample volume that can be processed without significant loss of analyte and for which, in the absence of irreversible sorbent interactions, recovery after elution for all sample volumes less than the breakthrough volume will be 100%.

1.8.1. Measurement of breakthrough volume

Breakthrough volumes can be determined directly with on-line detection by recording a frontal chromatogram produced by passing a dilute solution of an analyte through the sampling device at a constant flow rate and monitoring the appearance of the analyte at the outlet of the sorbent bed or by collecting and analyzing fractions of eluent to determine where breakthrough occurs [8,41,42]. Breakthrough volumes can also be determined off-line by measuring recovery of analytes extracted from dilute solutions of varying volumes after elution from individual extraction devices [8,21]. Recoveries are plotted against the volume of solution processed and the initial high recovery drops off once the breakthrough volume has been exceeded. These approaches for measuring breakthrough volume are tedious and time consuming, especially when the breakthrough volumes of multiple analytes must be determined. Furthermore, since all of these measurements include random error, these methods also depend on subjective, and sometimes difficult, decisions regarding the point at which breakthrough occurs [8].

Gelencser *et al.* [5] proposed an alternative method for determining breakthrough volumes in SPE that involves equilibrating an analyte solution of known volume and concentration with an SPE cartridge by recycling the solution through the cartridge in a closed loop system. Retention factors were calculated from the adsorbed amount determined by other chromatographic methods after elution and then related to breakthrough volume. Equilibration time between the cartridge and analyte solution is long (up to 16 hours) but can be accomplished unattended.

1.8.2. Prediction of breakthrough volume

As an alternative to the measurement of breakthrough volumes, several predictive models have been developed with varying degrees of success. Breakthrough volume can be defined as in equation 6 [43] where σ_V corresponds to the degree of band broadening

$$V_b = V_R - 2\sigma_V \tag{6}$$

represented as variance in a Gaussian peak shape model. The variance can also be determined from equation 7 where k_W is the retention factor value in pure water and V_O is

$$\sigma_V = V_O(1 + k_W)/N^{1/2} \tag{7}$$

the interparticle volume of the sorbent bed. The number of theoretical plates, N, can be determined by frontal analysis using equation 8 [44]. Frontal analysis can also be used to

$$N = V_R (V_R - \sigma_V)/ \sigma_V^2 \tag{8}$$

determine k_W, but many k_W values are difficult to determine, as retention is high and front shapes can be distorted when pure water is used as a mobile phase. Various relationships have been explored for predicting k_W [4].

Early work toward predicting k_W consisted of studying the relationship between

aqueous molar solubility and log k_W on porous polymer sorbents [45,46]. These methods generally produce good correlations which successfully explain experimental findings, but they ignore stationary phase contributions to retention and are limited by the availability of quality solubility data. Log k_W can also be estimated by extrapolation of reversed-phase retention measurements over a range of solvent compositions. Data generated by extrapolation must be treated carefully; this caution is further discussed in section 1.9.5. The relationship between log k_W and the octanol-water partition coefficient, log P_{Oct}, is generally linear and large collections of log P_{Oct} values are available. When log k_W values are unavailable, log P_{Oct} values can be used as rough substitutes in determining breakthrough volumes [7,47]. Literature values for log P_{Oct}, however, contain uncertainty stemming from the variety of methods used for their measurement or calculation [8].

The Lovkist-Jonsson model [48] is a general retention model based on frontal analysis which has proven to be useful in predicting breakthrough volumes. Expressed formally, equation 9 describes sampling in systems with low numbers of theoretical

$$V_b = V_m(1+k_s)\left(a_0 + \frac{a_1}{N} + \frac{a_2}{N^2}\right)^{-1/2} \qquad (9)$$

plates. This model can be used to relate the breakthrough volume, V_b, to the properties of extraction cartridges. Breakthrough volume is dependent upon the cartridge holdup volume (V_m), a retention term where k_S is the capacity factor (or retention factor) value of a solute, and a kinetic term. The constants a_0, a_1 and a_2 depend on the selected breakthrough level (b) and are tabulated in the literature for various values of b [48]. N is the number of theoretical plates provided by the cartridge.

Mol *et al.* [49] demonstrated for open-tubular traps that for $N < 1.5$ the breakthrough volume cannot be increased by increasing retention, indicating that a threshold value for minimum efficiency is required for quantitative trapping. Pankow *et*

al. [50] have suggested that premature breakthrough for sorbent traps with low plate numbers occurs because of poor transport, that is, a lack of sufficient time for all of the analyte molecules to reach the surface of a particle before exiting the sorbent bed, and is independent of the retention capacity of the sorbent. These studies suggest that there may be a lower limit on the number of theoretical plates required for acceptable performance of an extraction device, but this number is not well defined. Fernando *et al.* [51] have shown that equation 9 provides a reasonable interpretation of sampling properties for sorbent traps with more than 4 theoretical plates.

The kinetic and retention information required to calculate breakthrough volumes using equation 9 is easily obtained under the highly controlled and easily varied conditions of high performance liquid chromatography (HPLC) using columns packed with sorbent excised from SPE cartridges. Weidolf and Henion [52] used HPLC to select the optimum solvents and solvent volumes for recovery and matrix simplification of sulfaconjugated steroids and their hydrolysis products. These compounds were isolated from equine urine with a column prepared from the same sorbent material as that used for SPE cleanup. The volume and composition of the wash solvent was determined from the retention factor of the peak front of the analyte and the composition for elution from the retention factor of the peak tail in appropriate mobile phases. Casas *et al.* [53] optimized the solvent composition for the recovery of 1,4-benzodiazepines from urine by determining their elution curves (plots of recovery against solvent composition) for a sorbent cartridge. The relationship between the retention factor and the inflection points in the curve can be used to predict suitable compositions of wash and elution solvents for compounds similar to the standards used to generate the elution curves.

1.8.2.1. Kinetic contribution to breakthrough volume

The influence of kinetic properties on the breakthrough volume is contained in the last term on the right-hand side of equation 9. The homogeneity of the sorbent bed, the

quality of the sorbent and the sample flow rate will influence the breakthrough volume through the dependence of N on these parameters. The kinetic contribution can be evaluated numerically for different breakthrough levels as illustrated in **Figure 1.6.** At about N = 100 the kinetic term reaches about 95% of its asymptotic value for a 1% breakthrough level, N = 25 for a 5% breakthrough level, and N = 10 for a 10% breakthrough level [54]. For typical cartridges and PLMs, extractions are performed with such low values of N that the breakthrough volume is less than the maximum possible. In fact, experimental conditions fall in the region where the breakthrough volume is most strongly influenced by kinetic properties. These undesirable features are tolerated in practice to preserve the simplicity of sample processing by gravity or suction, and to minimize the cost of the sampling device. A cartridge packed with a 60 μm-diameter

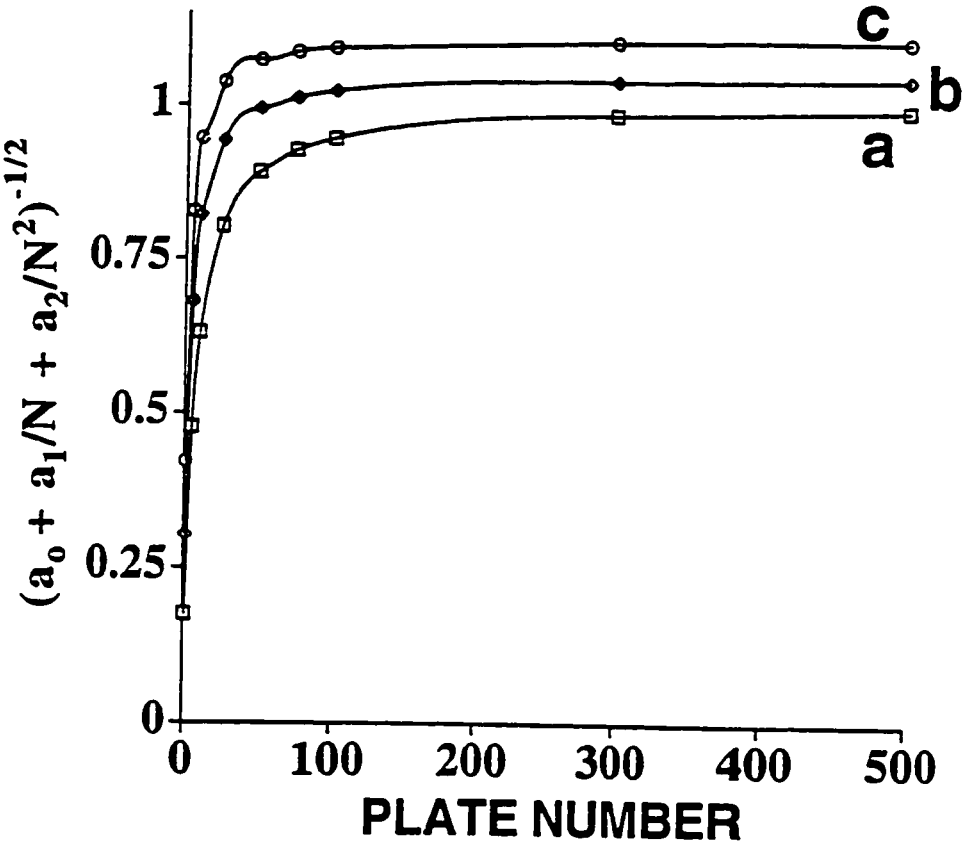


Figure 1.6. Contributions of kinetic term to V_b for various breakthrough levels, a = 1%, b = 5%, c = 10% breakthrough level.

sorbent, such that it provided a plate height of 2-3 particle diameters, need only have a bed height of 1.2 - 1.8 cm to provide 100 theoretical plates [54], illustrating the importance of maximizing the packing density of sorbent cartridges as a simple way to improve their sampling performance.

Miller and Poole showed that the flow rate dependence on breakthrough volume for an octadecylsiloxane bonded cartridge sorbent is more significant than for PLMs [15]. The nature of the flow rate dependence for the two sorbents is illustrated in **Figure 1.7**. While the cartridge shows a continuous decrease in breakthrough volume with an increase in flow rate, the breakthrough voume for the PLM is not strongly affected by flow rate in this range. These results underscore the need to control the flow rate within reasonable limits in order to obtain reproducible results with cartridge SPE devices.

The efficiency of a chromatographic column can be expressed as either the number of theoretical plates (N) or as the height equivalent of a theoretical plate (HETP). Values

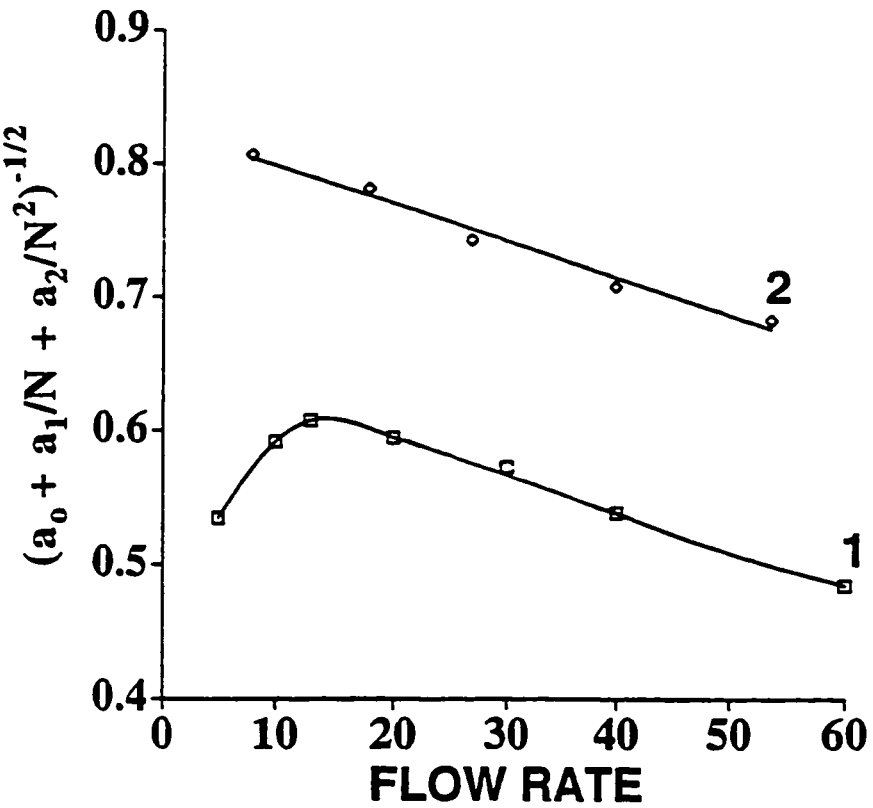


Figure 1.7. Flow rate dependence for ODS PLM and cartridge sorbents.

for N can be obtained from the peak retention time and the variance in a Gaussian peak shape model and are affected by band broadening processes through the effect that these processes have on variance. A plot of HETP against mobile phase velocity (**Figure 1.8,** known as a van Deemter plot) is useful for identifying the contributions to band broadening and the optimum flow rate needed to achieve the highest number of theoretical plates (*i.e.*, the point at which plate height reaches a minimum).

$$HETP = A + B/u + (C_S + C_m)u \qquad (10)$$

Equation 10 is the van Deemter equation where u is the mobile phase velocity, A represents the contribution to band broadening from flow anisotropy, B is the contribution from longitudinal diffusion and the C terms are the contributions from mass transfer in the stationary and mobile phases [7].

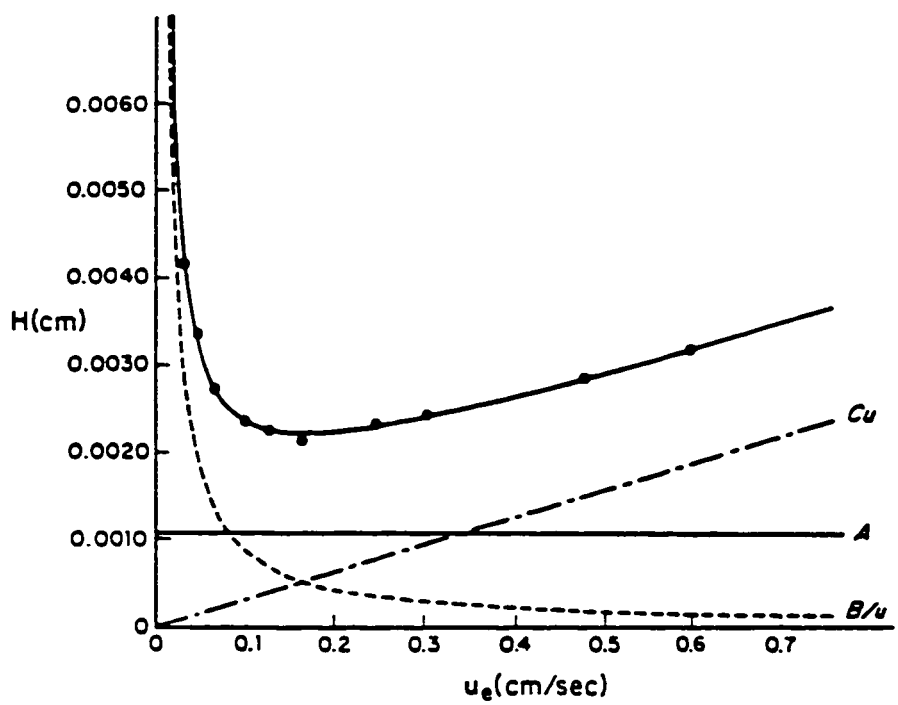


Figure 1.8. Van Deemter plot

1.8.2.2. Retention contribution to breakthrough volume

The influence of retention on breakthrough volume is by far the largest of the three contributions and takes the form of dependence upon the retention factor (k_S) value. Because the measurement of retention in SPE is time consuming, tedious and requires complex experimental setups, methods that allow the prediction of retention with a minimal number of experiments are particularly attractive. Predictive methods require retention models that adequately describe the partition and adsorption processes occuring between solutes and the solvated stationary phase in chromatographic systems.

Retention in liquid chromatography, *i.e.*, the transfer of a solute between a liquid mobile phase and a stationary phase, is not easily defined because of the difficulty in defining the boundary between these regions. In a conditioned column, the stationary phase boundary is better described as an interphase region where solvent is selectively absorbed from the mobile phase. This selective uptake of solvent leads to uncertainty in the active volume and composition of the stationary phase, both of which also vary in an unknown way with changes in mobile phase composition. Selective sorption of organic components of the mobile phase leads to uncertainty in the mobile phase composition itself, and even unsolvated stationary phases are unlikely to be homogeneous, leading to sites with different sorption characteristics. Retention models based on solvophobic theory and lattice statistical thermodynamic theory have been successful at explaining a number of experimental findings, but are generally mathematically complex and limited by a lack of knowledge of all the variables required in the equations [7, 55-58].

Retention in liquid chromatography occurs according to the combination of several contributions from intermolecular interactions between the solvated stationary phase and the solute. Rarely (if ever) can a solute be said to participate in only one type of interaction within a chromatographic system, thus requiring a retention model that has the capability of deconvoluting the sum total of retention into separate contributions from each type of interaction. Abraham's solvation parameter model [59] has the advantage of

providing fundamental information about clearly defined and quantifiable intermolecular interactions. This model has been used in characterizing the retention properties of gas chromatographic stationary phases [60-66], predicting octanol-water partition coefficients [67,68], determining sorption properties of sorbents [69-71], including graphitic carbon adsorbants [72], and determining breakthrough volumes in solid-phase extraction [22,73-75]. Abraham *et al.* [68] and Miller and Poole [15] applied the model to retention on octadecylsiloxane-bonded silica-based sorbents under typical reversed-phase liquid chromatographic conditions. Further application of the model has included the elucidation of factors governing water/micelle partition in MEKC [76-79], the ability of drugs to diffuse through the blood/brain barrier [80], the skin permeation of drugs [81], the adsorption properties of acoustic wave sensors [82-84], and the partitioning of organic compounds between water and soil [85].

1.9. Solvation parameter model

The solvation parameter model grew out of LSER theory "solvatochromic parameters" [86] but differs from the previous model in some fundamental ways. In the solvatochromic equations, several of the solute descriptors are derived from solvent properties whereas all of the terms in the solvation parameter model are exclusively solute properties [87]. Care should be taken to distinguish between descriptors and system constants used and/or generated with the two models; for most systems the two models will generate different results.

The solvation parameter model is based on a cavity model of solution that represents the transfer of a solute from one phase to another as occurring by three independent operations: a) the creation of a cavity in the solvent of a suitable size to accommodate the solute; b) reorganization of the solvent molecules around the cavity; and c) interaction of the solvent molecules with the surrounding solvent molecules. The transfer from one phase to another occurs with the formation of a cavity in the acceptor

phase and the collapse of a cavity of the same size in the donor phase with the breaking of the interactions between the solute and the donor solvent. For transfer to occur, the difference in the free energy of solution (or sorption) between the two phases must be favorable. Under typical extraction conditions the sample solutions can be reasonably assumed to be infinitely dilute such that all interactions that are formed in solution are of the solute-solvent type. These interactions can be characterized as dispersion, orientation, induction and complexation. Dispersion interactions are non-selective and solute-solvent dispersion interactions will be approximately equal in the sample solvent and solvated sorbent and are not a significant contributor to the change in free energy accompanying the transfer process. The capacity of a solvent or solvated sorbent for selective polar interactions is more important in solute transfer. Molecules with a permanent dipole moment can interact with each other by the cooperative alignment of their dipoles (orientation interactions) and by their capacity to induce a temporary complementary dipole in a polarizable molecule (induction interactions). Other selective polar interactions include complexation interactions involving the sharing of electron density or a hydrogen atom between molecules (*e.g.*, charge transfer and hydrogen bonding). Those properties which affect the transfer of a solute between phases are the availability of complementary intermolecular forces to those of the solute in each phase as well as differences in the cohesion of the phases (*e.g.*, solvent-solvent interactions) which determine the contribution of cavity formation to the transfer process.

Set forth in a form suitable for use with liquid chromatographic data, the solvation parameter model is formally expressed by equation 11 where SP is some free energy-

$$SP = mV_X + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^0 + c \qquad (11)$$

related property of the system, such as the retention factor (log k), distribution constant, or the breakthrough volume (log V_b). V_X is the solute characteristic molar volume, R_2 is the solute's excess molar refraction, π_2^H is the ability of the solute to stabilize a

neighboring dipole by virtue of its capacity for orientation and induction interactions, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^0$ are parameters characteristic of the solute's effective hydrogen-bond acidity and basicity, respectively. For the solute descriptors, the subscript 2 indicates that the values are applicable to solute-solvent interactions where the solute and solvent are not identical. Solute-solute interactions, where the solutes are identical, are given subscripts of 1. The coefficients m, r, s, a, b, and c are referred to as system constants.

1.9.1. Determination of solute descriptors

A large collection of solute descriptors is available in the literature and, more recently, estimation schemes for determining the descriptors for novel compounds have been developed [80,81]. Literature values for solute descriptors were originally measured by liquid-liquid distribution, or chromatographic or spectroscopic methods until a consistent set of system constants were determined. Further descriptors were then back calculated using retention data generated on well-characterized chromatographic systems.

The V_X descriptor is a measure of a solute's characteristic molar volume. McGowan and Abraham [88] outlined a simple arithmetic means of calculating V_X (in units of (ml/mol)/100) based on summing contributions for each atom in the molecular structure and subtracting a fixed contribution for each bond. Dividing by 100 is necessary to bring V_X into a numerical range similar to the other descriptors. V_X represents the exoergic process of disrupting solvent-solvent interactions to create a cavity of suitable size to accommodate the solute and the endoergic processes of solvent reorganization around the solute.

The R_2 descriptor is a solute's excess molar refraction which represents polarizability contributions to retention from n- and π-electrons. Molar refraction (MR) can be calculated from refractive index measurements according to equation 12 where η

$$MR = (\eta^2 - 1)(\eta^2 + 2) V_X \qquad (12)$$

is the refractive index for the sodium D-line at 298K and V_X is McGowan's characteristic volume described above. R_2 then is the molar refraction of the solute less the molar refraction of an alkane with the same characteristic volume [89]. Lists of fragment values of R_2 (in units of $cm^3/10$) are available, enabling the simple estimation of this descriptor through summation.

The π_2^H , $\Sigma\alpha_2^H$ and $\Sigma\beta_2^0$ descriptors are the solute's dipolarity/polarizability, hydrogen bond acidity and hydrogen bond basicity, respectively, which must be determined by experiment. All three descriptors can be determined by gas-liquid chromatography (GLC; π_2^H on polar, nonacidic stationary phases, $\Sigma\alpha_2^H$ on highly basic stationary phases and $\Sigma\beta_2^0$ on highly acidic stationary phases) or by the use of partition coefficients for various water/solvent systems. The latter has become the most common method and involves measuring partition coefficients for solutes in many different solvent systems and calculating the set of descriptors that best describes the partition coefficients (as judged by low standard deviations) [87].

Both the hydrogen-bond acidity, α_2^H, and hydrogen-bond basicity, β_2^H, scales are generated based on 1:1 complexation in tetrachloromethane. Solutes that undergo multiple hydrogen bond interactions are not adequately characterized by the scales based on monofunctional solutes, however, and summation values, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$, must be used [59]. One further alteration in the basicity scale (replacing $\Sigma\beta_2^H$ with $\Sigma\beta_2^0$) is required when dealing with predominantly aqueous systems because some solutes exhibit variable basicity in the presence of appreciable amounts of water. The two descriptors are identical for the majority of solutes, but differ for solutes such as pyridines, anilines, and sulphoxides [90].

1.9.2. System constants

The system constants m, r, s, a, b, and c are generated by multiple linear regression using retention measurements for a set of varied compounds with known solute

descriptors. This set of solutes is referred to as the "training set" and is chosen to contain solutes with sufficient variation of properties to define all interactions in equation 11. The system constants are:

a) unambiguously defined;

b) ideally independent of the solute identity;

c) characteristic of the stationary/mobile phase composition for which they are generated; and,

d) allow the prediction of retention for compounds outside the training set for which descriptors are known.

Evaluation of system constants allows expression of retention in liquid chromatography in terms of individual intermolecular interactions and can be invaluable in sorbent and solvent composition selection in SPE. System constants indicate the relative tendencies of the stationary and mobile phases in a chromatographic system to enter into specific interactions with a solute. The sign of a given system constant indicate whether the contribution favors transfer of the solute to the stationary phase (positive constant) or favors solubility in the mobile phase (negative constant).

Previous application of the solvation parameter model to a high-loaded octadecylsiloxane-bonded phase [15] shows that the driving force for retention in the stationary phase is the ease of cavity formation (large, positive m-constant) with a smaller contribution to retention from n- and π-electron interactions (small, positive r-constant). All other contributions are negative and favor solubility in the mobile phase. The most significant of these interactions is the b-constant, with a large, negative value indicating that solutes that are hydrogen-bond bases will be less retained than their non-basic counterparts. The general picture of retention that emerges from these results confirms the findings of Roses and Bosch [91] for the reversed-phase liquid chromatography of a series of phenols in methanol-water mixtures, although it should be noted that these latter workers used the solvatochromic model which differs from the

model used here and in the published work done on the HL C_{18} sorbent.

The c-constant is a fitting constant that encompasses any interactions or effects not accounted for in the other terms in the model. The phase ratio is contained in this term as well as any contributions from electrostatic interactions. The latter interactions arise from ionized silanol groups that may retain bases by an ion-exchange mechanism and cause increased retention that would not be predicted by the solvation parameter model. Electrostatic interactions are a cause of poor analyte recovery that has been noted for pharmaceutical compounds necessitating the addition of a competing base to the elution solvent [92,93].

1.9.3. Retention mapping

Mapping retention over a range of mobile phase compositions provides a simple graphical representation of the change in system constants with a change in the amount of organic solvent in the mobile phase (*e.g.*, see Appendix B). These plots allow comparison between solvent compositions and, if generated for more than one solvent, aid in identifying changes in selectivity that can be effected by changing solvent identity. The plots can serve to provide an educated first guess for the selection of solvents and solvent compositions used in sampling, washing and elution steps in SPE. The left-hand side of the plots provides the system constants for the chosen sampling conditions and allows the breakthrough volumes to be estimated for the analytes. The right-hand side of the plots indicates the range of solvent compositions at which the same analytes can be eluted with low retention factors from the sorbent bed ($k_S < 1$ preferred). Solvent compositions in the central regions of the plots can be useful for identifying wash solvents to achieve matrix simplification. Retention maps can be constructed for different sorbents and/or co-solvents; and the breakthrough volume, wash solvent volume, and elution volume can be predicted in each case for all analytes to establish the working range of a method and to compare findings with extraction requirements, thus avoiding experiments that cannot be

successful.

1.9.4. Solvent effects on selectivity

Mobile phase components are absorbed into the interphase region during column equilibration in liquid chromatography or cartridge conditioning in SPE. The interphase region defies definition, having neither the characteristics of the unsolvated stationary phase nor those of the bulk mobile phase, but indeed, some unknown combination of the two. Because of their unique solvating abilities, different organic solvents are unlikely to be absorbed into the interphase region in like manner to each other, and changes in selectivity are expected with changes in the identity of the organic solvent in the mobile phase. Large solvent effects have been observed for a porous polymer PLM that showed considerable changes in breakthrough volume that depended on the identity of the co-solvent (at 1% v/v organic solvent in water) [73]. Solvent effects for macroreticular porous polymer sorbents (PLRP-S) have been shown to exist, but to a smaller extent than with the PLM [94]. The PLM contains a less rigid poly(styrene-divinylbenzene) sorbent than the PLRP-S sorbent which is more highly cross-linked. No prior work using the solvation parameter model to assess solvent effects for silica-based bonded phases has appeared in the literature.

1.9.5. Extrapolation methods

Sampling of large aqueous solutions in SPE is often accomplished through the addition of small amounts of organic solvent. This processing solvent, typically 1-5% v/v organic solvent in an aqueous sample, ensures that the stationary phase remains wetted throughout sample application, improves extraction efficiency and decreases sample processing times significantly [17]. In addition, small amounts of residual conditioning solvent can be expected to remain in the interphase region during sample application even when the sample is entirely aqueous. Retention factors at 1% v/v organic solvent have

been shown to be numerically quite similar to retention factors with pure water (k_W) for porous polymer sorbents [94]. Retention measurements at low organic compositions are, therefore, of particular interest in determining the sampling characteristics of a sorbent and as estimates for k_W.

In previous work, excessive retention prevented measurements of retention factors on a C_{18} bonded phase below about 15% v/v organic solvent, requiring that extrapolation methods be used to estimate retention at lower organic compositions. Plots of log k against % v/v methanol for the system show both linear and curved relationships, with no apparent means of knowing which type of relationship to expect for a given solute. When plotted against solvent compostion, system constants at low organic compositions calculated from extrapolated log k_W values show marked discontinues from the smooth functions found at higher organic solvent compositions [15]. The relationship between log k and % v/v organic composition for porous polymer phases shows considerable curvature [95], leading to different results depending on which part of the curve is used for extrapolation. Rolliet and Poole [94] found similar curvature in the plots from 50-1% v/v organic solvent for macroreticular porous polymer sorbents. Values for k_W obtained through extrapolation from specified higher ranges differed considerably from the measured values at 1% v/v organic solvent. These studies show that the quality of data estimated for low organic compositions through extrapolation methods from data gathered at higher organic compositions cannot be guaranteed. Furthermore, at low organic solvent compositions, the selective uptake of organic solvent components may result in variations in the interphase region not accounted for by extrapolation methods, underscoring again that a cautious approach should be taken when using data generated by extrapolation.

1.9.6. Multiple linear regression analysis

Multiple linear regression is a means of data analysis employed when the dependent variable is affected by more than one independent variable. In mathematical

terms, this dependency can be expressed as equation 13. To determine the unknown

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots b_m x_m \quad (13)$$

parameters ($\beta_0,\ldots,\beta_m$), a series of observations are made of the dependent variable y ($y_1,\ldots,y_n$) with the corresponding values for the independent variables x ($x_1,\ldots,x_m$) where n is the number of observations made and m is the number of terms in the equation (excluding the β_0 term) [96]. The similarity of this equation to the solvation parameter model (equation 11) is not difficult to see. The dependent variable, y, corresponds to SP. The independent variables, x, correspond to the solute descriptors and the β values correspond to the system constants. When two of the three parameters (or sets of parameters) are known, the third can be determined using an appropriate statistical software package.

1.10. Other methods for selecting extraction conditions in SPE

Although the vast majority of SPE methods are developed without any systematic attempt to preselect conditions, several other methods for pinpointing appropriate conditions have been developed. Early work by Thurman *et al.* [45] entailed the prediction of retention factors on porous polymer sorbents through correlations with aqueous solubility data. These correlations allowed for matching the cartridge and sample sizes for preconcentration techniques. Solubility data can be used to estimate the extent to which a solute is expelled from the aqueous mobile phase through the hydrophobic effect, but these methods ignore stationary phase contributions to retention.

Other groups have made use of octanol-water partition (log P_{oct}) coefficients to estimate k_w and breakthrough volume. This information can be used to assess the suitability of sorbents based on estimates of V_R [8]. Nakamara *et al.* [97] made use of log P_{oct} as a measure of hydrophobicity and developed a complex flow chart to aid in choosing appropriate extraction conditions in terms of sorbent selection, mobile phase

(sample) additive selection, and elution solvent selection. Methods based on correlations with log P_{oct} depend on the quality of literature values for this parameter.

Gelencser *et al.* [98] calculated expected recoveries over a range of sample volumes, thus approximating breakthrough curves through the prediction of retention factors. This method allows for the estimation of enrichment factors as well as the minimum concentration of an analyte that can be determined by SPE. This method is based upon the equilibration of sample and sorbent and is unsuitable for use with solutes that have large retention factors because of the excessively long time needed to reach equilibrium.

Hughes and Gunton [99] proposed a graphical method where the slope, shape and intercept of plots of $-\ln(1-R_t)$, where R_t is retention time, versus mobile phase volume can provide information regarding selectivity, extent of irreversible binding of analyte, loading and type of elution profile in SPE. Comparison of the slopes of the plots for analytes and impurities allows favorable wash and elution solvent compositions and volumes to be recognized. Favorable conditions for matrix simplification are those in which the slope (*i.e.*, extraction efficiency) is low for the analytes and high for the matrix in the wash step. The slope should be high for the analytes in the elution step. In many cases the plots prove to be non-linear, but this behavior does not prevent a qualitative assessment of the recovery and matrix simplification procedure from being evaluated graphically from the experimentally derived curves.

1.11. Objectives

The previously discussed solvation parameter model (equation 11) and the Lovkist-Jonsson retention model (equation 9) can be combined to formulate a new approach for the prediction of extraction conditions, thus furthering the goal of generating a rational means of method development in SPE. A stepwise approach can be used to realize this goal:

1) study the physical/functional, kinetic, and retention properties of SPE cartridges and sorbent materials using HPLC;

2) apply the solvation parameter model to generate system constants for popular SPE sorbents over wide ranges of solvent compositions and for various solvent identities;

3) use data from steps 1 and 2, to predict breakthrough volumes of solutes in SPE for compounds outside (and significantly different from) the training set;

4) use breakthrough volumes produced in step 3, to predict extraction conditions likely to succeed;

5) apply predictions to the extraction of analytes from complex matrices to check the feasibility of predicted extraction conditions with real-world samples; and

6) test predictions for accuracy and efficacy.

1.12. Estrogen analysis

For evaluation of the proposed predictive method for the selection of extraction conditions, a sample system was sought where the analytes show significant differences from the set of solutes used to generate the system constants. Because of widespread clinical interest, prior experience in estrogen analysis in this research group, the availability of estrogen solute descriptors, and the availability of a well-characterized sample in the form of a pharmaceutical product, the analysis of estrogens in urinary matrices was chosen as an application.

Table 1.4 lists the steroids of significance in human beings [100]. Steroid profiling allows the determination of levels of many of these compounds concurrently and a complete profile can be helpful in the recognition and diagnosis of certain disease states and disorders through the study of the overall picture of possibly interrelated fluctuations in steroid levels. Profiling usually involves fractionation of the steroids into convenient groupings for analysis in multiple chromatographic runs [100]. The approach taken for analyzing a specific class or group of steroids varies significantly from profiling, although many of the experimental steps are similar.

Table 1.4. Steroids of interest in human beings

Primary hormonal steroids:	*Typical metabolites:*
Cortisol	*Mainly glucuronides:*
Aldosterone	Tetrahydrocortisone
Progesterone	Tetrahydrocortisol
Testosterone	5α-tetrahydrocortisol
Dihydrotestosterone	Cortoic acids
Estradiol	Tetrahydroaldosterone
	Pregnanediol
	Androsterone, etiocholanolone
	Estrone, estriol
	Unconjugated:
	Cortisol
	20-Dihydrocortisol
	6β-Hydroxycortisol
Secondary hormonal steroids:	*Typical metabolites:*
17α-Hydroxyprogesterone	*Mainly glucuronides:*
21-Deoxycortisol (S)	17α-Hydroxypregnanolone
Corticosterone (B)	Pregnanetriol
Deoxycorticosterone (DOC)	Pregnanetriolone
18-Hydroxy-DOC	Tetrahydro-substance S
18-Hydroxy-B	Tetrahydro-A
18-Hydroxycortisol	Tetrahydro-B
Androstenedione	5α-Tetrahydro-B
Dehydroepiandrosterone (DHA)	Tetrahydro-DOC
	18-Hydroxytetrahydro-DOC
	18-Hydroxytetrahydro-A
	Androsterone
	Etiocholanolone
	16α-Hydroxy-DHA
	5-Androstene-3β,16α,17β-triol
Hormonal precursors:	*Typical metabolites:*
DHA sulfate (DHAS)	16α-Hydroxy-DHAS
Pregnenolone sulfate	Androstenetriol sulfate
17α-Hydroxyprenenenolone sulfate	Pregnenediol sulfate
	16α-Hydroxypregnenolone sulfate
	Pregnenetriol sulfate
	Androstenediol disulfate
	21-Hydroxypregnenolone disulfate

The measurement of estrogen concentrations in body tissues or fluids has many clinically important applications including the diagnosis and management of certain diseases, and the investigation and treatment of post-menopausal medical problems. Estrogen levels change dramatically during the course of pregnancy and are routinely monitored to detect possible complications or fetal abnormalities. For example, patients with gestosis show lower overall estrogen levels [101] and placental sulfatase deficiency can be diagnosed by monitoring estrogen levels [102]. Characteristic changes in estrogen levels in both plasma and urine can be seen during the course of a normal menstrual cycle. These defined changes can be used to locate the start and end of the probable fertile period, leading to obvious applications to the treatment of human infertility.

1.12.1. Estrogen structures and associated conditions

Estrogens are a class of steroid hormones that exhibit estrogenic activity, *i.e.*, the ability to induce uterine growth or vaginal cornification in an immature or ovariectomized rodent. Not all compounds that exhibit such activity possess the characteristic C18 steriod nucleus, but all of the compounds discussed here are derivatives of that structure. The C18, cyclopentanoperhydrophenanthrene, skeleton shown in **Figure 1.9** is modified by the addition of hydrophilic groups (hydroxy and oxo groups) at the C3, C16 and C17 positions. The presence of a phenolic A ring is characteristic of estrogens and renders them acidic.

Figure 1.10 shows the structures of the three most common estrogens in humans: estrone (E1); 17β-estradiol (E2); and estriol (E3). E2 is an essential hormone for maintenance of the female reproductive system and bone density. E2 is metabolized to both E1 and E3. Levels of these three estrogens in various body tissues and fluids serve as markers for septic shock, myocardial infarction in women, fetal Down's Syndrome, premature delivery, alcoholism in pregnant women, hypertension in pregnant women, breast cancer, and other conditions and diseases [102].

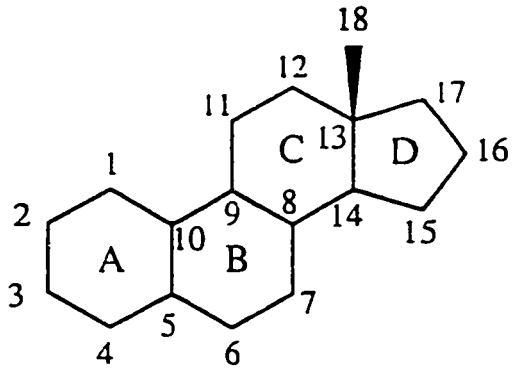
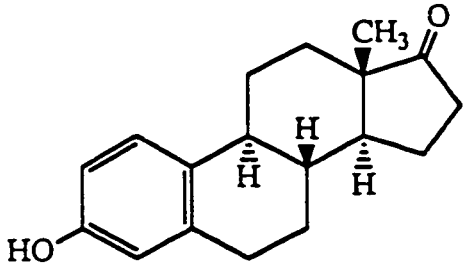
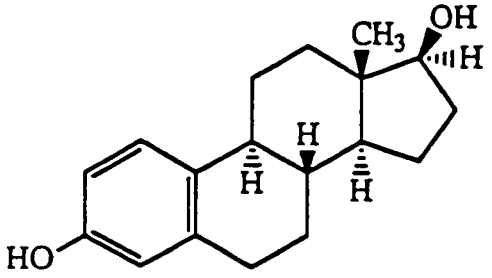


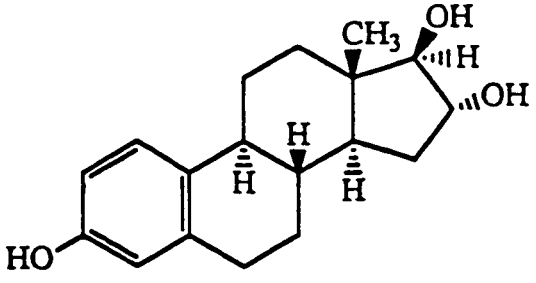
Figure 1.9. Skeleton steroid structure.



Estrone
1,3,5-Estratriene-3-ol-17 one
$C_{18}H_{22}O_2$ MW 270.36



β-Estradiol
Estra-1,3,5(10)-triene-3,17β-diol
$C_{18}H_{24}O_2$ MW 272.37



Estriol
Estra-1,3,5(10)-triene-3,16,17-triol
$C_{18}H_{24}O_3$ MW 288.39

Figure 1.10. Structures of three important human estrogens

1.12.2. Estrogen synthesis, excretion and metabolism

Steroid hormones secreted *in vivo* are transported in the bloodstream bound to plasma proteins; estrogens are commonly bound by sex hormone-binding globulin (SHBG). The principal estrogen in human females, 17β-estradiol, is produced in and secreted by the ovaries under the control of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Large amounts of estrogens are produced by the placenta during pregnancy. Estrogen metabolism (outlined in **Figure 1.11** [103]) takes place primarily in the liver where 17β-estradiol readily interconverts with estrone which is eventually metabolized to estriol. Further metabolism includes conjugation with sulfuric acid and derivatives of monosaccharides (mainly glucose) to form sulfates and glucuronides as shown in **Figure 1.12**. Most steroids form monosulfates, monoglucuronides, or disulfates, with mixed conjugates being rare. Indeed, estrogens are the only steroids for which mixed conjugation of sulfates and glucuronides has been reported [100]. Conjugation renders steroids much more hydrophilic and allows them to be eliminated through excretion into urine or bile, along with other related compounds.

Concentrations of the three principal estrogens in late pregnancy urine can vary significantly between individuals, but some example cases are documented in the literature. For three individuals between the 18th and 38th week of pregnancy, urinary E1 and E2 levels remain relatively constant and range from 0.2 to 3.8 mg/day for E1 and from 0.1 to 1.1 mg/day for E2. E3 levels showed a dramatic increase in the last several weeks of pregnancy and ranged from 0.9 to 24.2 mg/day [104]. Serum estrogen levels fluctuate rapidly while urinary levels indicate an average excreted value over a period of time.

1.12.3. Premarin

Premarin is a pharmaceutical preparation used in estrogen replacement therapy to alleviate the symptoms of menopause, in suppressive therapy to counter the effects of

other hormones, to minimize bone loss due to osteoporosis, and for treatment of the advanced stages of breast and prostate cancer [105]. The preparation contains conjugated and esterified estrogens derived from pregnant mares' urine.

Since biologically-derived substances tend to vary in composition, absolute

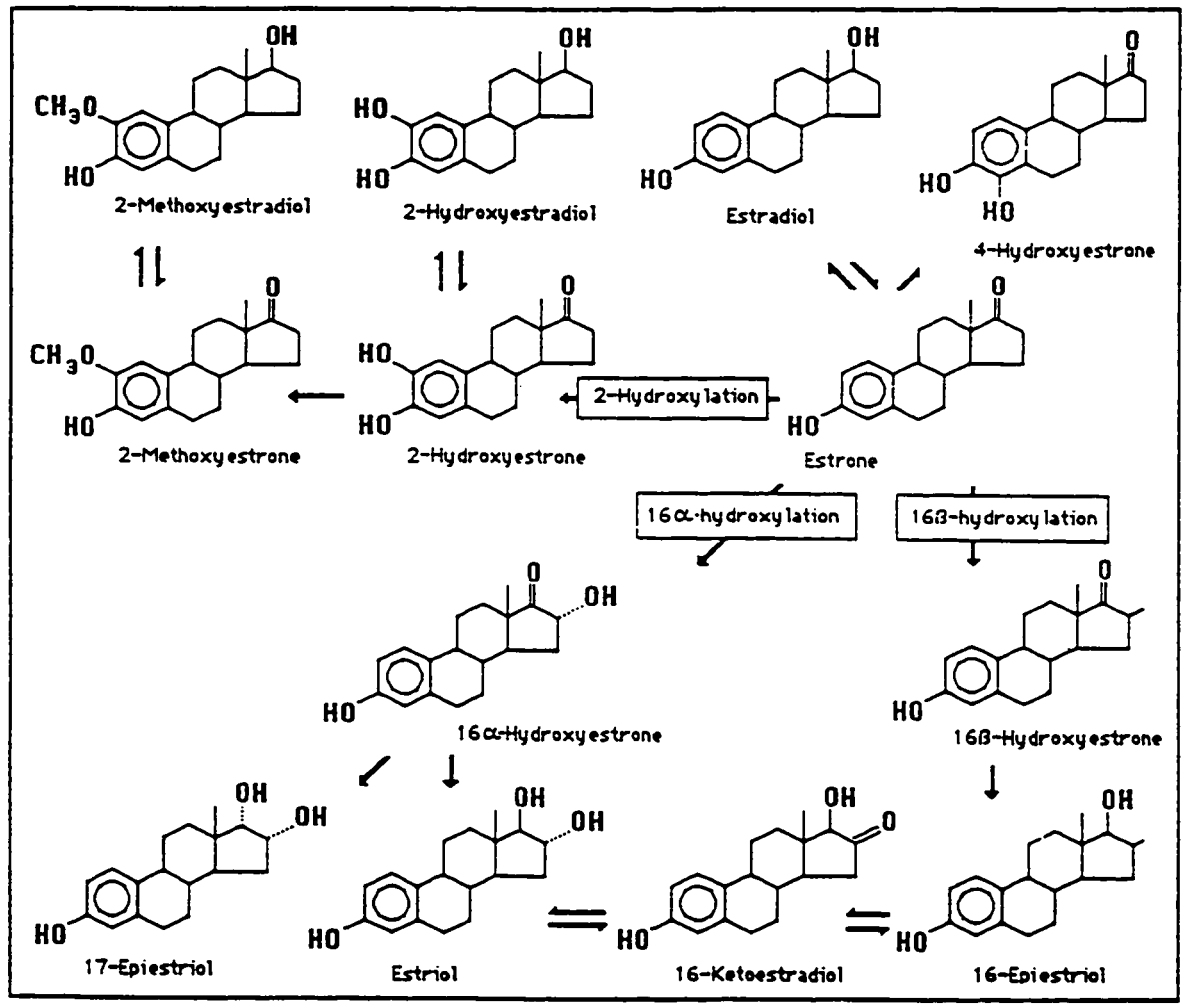
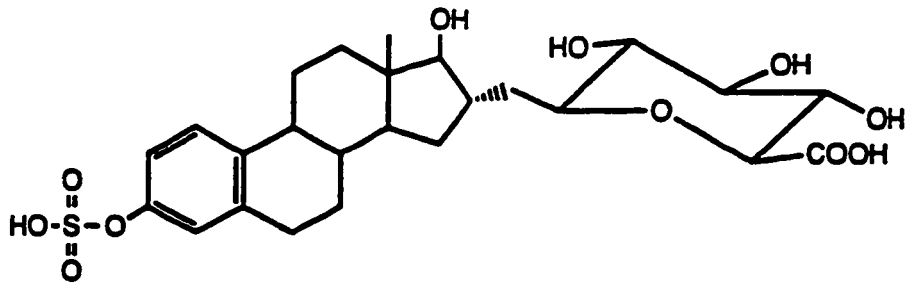


Figure 1.11. Metabolism of estrogens



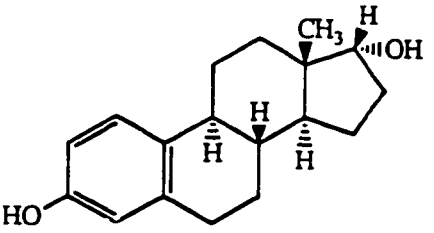
estriol 3-sulfate 16-glucuronide

Figure 1.12. Conjugation of estrogens

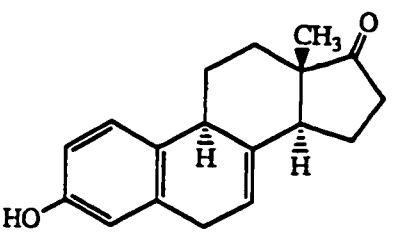
amounts of conjugated estrogens in the tablets cannot be claimed. The U.S. Pharmacopeia stipulates that conjugated estrogen tablets contain not less than 73% and not more than 95% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate in the tablets should be not less than 0.35 and not more than 0.65 [106]. In addition to the sulfate conjugates of estrone and equilin, other components, including the conjugates of equilenin, 17α- and 17β-estradiol, 17α- and 17β-dihydroequilin, 17α- and 17β-dihydroequilenin, and estriol, are present in lesser amounts. The structures of these compounds are shown in **Figure 1.13**.

1.12.4. Methods of estrogen analysis

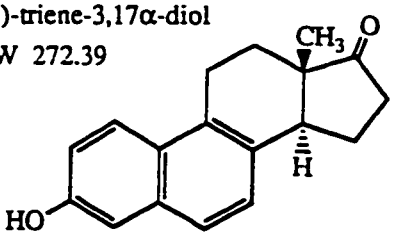
Techniques used for estrogen analysis appear in **Table 1.5**. The early techniques of colorimetry and fluorimetry [107,108] have seen limited usage because they lack specificity. Protein binding methods [109] can be useful as a screening tool that gives a



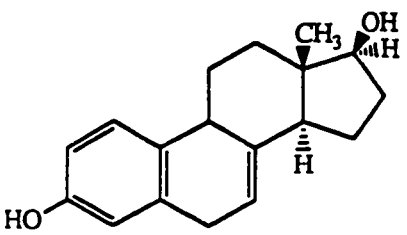
α-Estradiol
Estra-1,3,5(10)-triene-3,17α-diol
$C_{18}H_{24}O_2$ MW 272.39



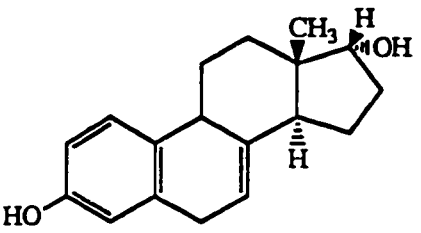
Equilin
3-Hydroxyestra-1,3,5(10),7-tetraen-17-one
$C_{18}H_{20}O_2$ MW 268.34



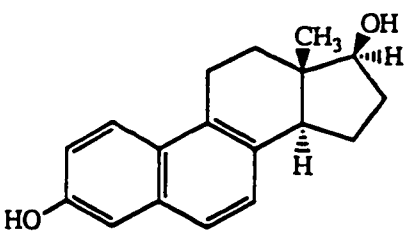
Equilenin
3-Hydroxyestra-1,3,5(10),7,9-pentaen-17-one
$C_{18}H_{18}O_2$ MW 266.32



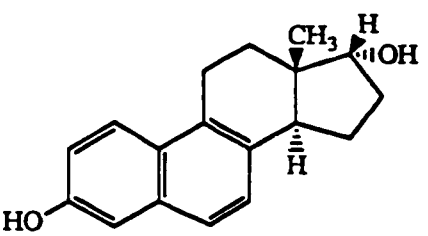
17β–Dihydroequilin
Estra-1,3,5(10),7-tetraene-3,17β-diol
$C_{18}H_{22}O_2$ MW 270.36



17α–Dihydroequilin
Estra-1,3,5(10),7-tetraene-3,17α-diol
$C_{18}H_{22}O_2$ MW 270.36



17β–Dihydroequilenin
Estra-1,3,5(10),6,8(9)-pentaene-3,17β-diol
$C_{18}H_{20}O_2$ MW 268.36



17α–Dihydroequilenin
Estra-1,3,5(10),6,8(9)-pentaene-3,17α-diol
$C_{18}H_{20}O_2$ MW 268.36

Figure 1.13. Structures of equine estrogens in addition to estrone, β-estradiol, and estriol.

simple positive/negative result for estrogens at a defined concentration. With the proliferation of radioimmunoassay (RIA) techniques [110,111], the development of other methods proceeded only slowly. RIA, however, also suffers from a lack of specificity as well as the requirement of handling and disposing of radiolabeled substances. Because of these problems, analysts have sought alternatives to RIA and have generally turned to chromatographic techniques [112].

1.12.4.1. Chromatographic techniques

For routine screening, thin-layer chromatography (TLC) is the simplest and most economical means of estrogen analysis [113,114]. Reversed-phase liquid chromatography (RPLC) methods for separating intact conjugated estrogens have been developed [115-117] as well as methods for complete separation of all ten of the equine estrogens [118-121]. Mobile phases for these latter separations are complex, however, leading to the limited use of these methods. Lamparczyk and Zarzicki and Spencer and Purdy have investigated β-cyclodextrans as mobile phase additives to improve RPLC separations [122,123]. A capillary zone electrophoresis (CZE) method to separate the major three human estrogens has been developed [124] and micellar electrokinetic chromatography (MEKC) has been used to separate complex mixtures of up to ten estrogens [100,125]. Poole and Poole resolved ten estrogens from Premarin extracts by MEKC [126]. Gas chromatography coupled with a mass selective detector (GC-MS) is considered the definitive technique in estrogen analysis [127,128], allowing positive identification of structure as well as quantitation, but GC-MS methods remain too technically challenging for use in routine analysis.

1.12.4.2. GC analysis and derivatization

Underivatized estrogens can be separated by capillary gas chromatography (GC) [129,130] but more complete separations can be achieved through the formation of oxime-

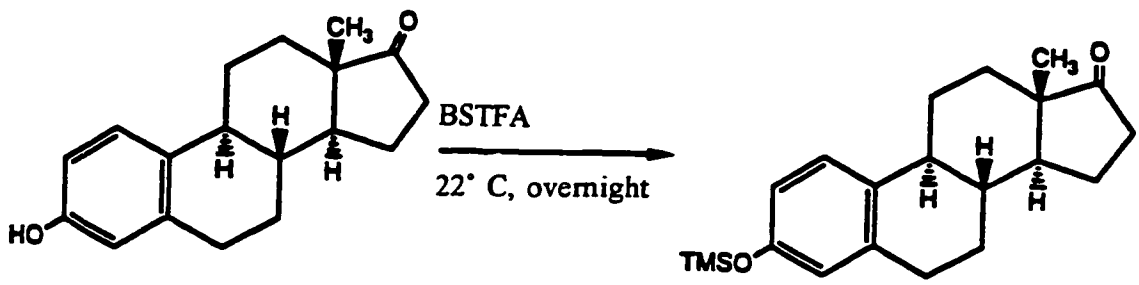
Table 1.5. Methods of estrogen analysis

Method	*Comments*	*ref.*
colorimetry	nonspecific	107
fluorimetry	nonspecific	108
protein binding methods (competitive or noncompetitive)	low-tech, can be adjusted to give simple positive/negative for presence of estrogens	109
RIA	crossreactivity and radioactivity	110,111
TLC	simple screening method	113,114
HPLC	complex mobile phases	115-123
CZE, MEKC	rapid, good resolution	124-126
GLC	requires derivatization for complete resolution	129-133
GC-MS	benchmark technique, but challenging	127,128

trimethysilyl, trimethylsilyl, or *tert*-butyldimethylsilyl derivatives prior to GC analysis [131-133]. For the separations of estrogens in biological fluids and Premarin extracts presented here, this latter approach is taken, making use of derivatization to trimethylsilyl (TMS) ethers as shown in **Figure 1.14**. Quantitation is achieved through the use of internal standards which serves to minimize problems associated with variation in the injection volume and to improve precision when using split injection.

1.12.5. Sample preparation

Chromatographic methods generally require sample preparation steps prior to analysis. Estrogens can be extracted from biological matrices both as free estrogens and in their conjugated forms.



TMS = trimethylsilane
BSTFA = bis(trimethylsilyl)trifluoroacetamide

Figure 1.14. Derivatization of estrogens to TMS ethers

1.12.5.1. Hydrolysis of conjugates

Disruption of conjugation can be achieved through enzymatic digestion [134-137], acid digestion [132,138,139], or through solvolysis, in which steroid conjugates are hydrolyzed by a solvent in the presence of H^+ [140]. Solvolysis is particularly effective for cleaving estrogen sulfates. For enzymatic hydrolysis, four major β-glucuronides are commercially available: a) bacterial (*E. coli*) enzymes; b) Roman snail (*Helix pomatia*) digestive juices; c) limpet (*Patella vulgata*) enzymes; and d) beef liver enzymes. Of the four enzymes, beef liver β-glucuronidases are the most commonly used [100]. Hydrolysis methods can generally be accelerated through digestion at elevated temperatures. SPE or LLE of intact conjugates before enzymatic hydrolysis has been shown to improve recoveries of estrogens [136]. This preliminary extraction step apparently removes substances that would otherwise inhibit the action of the hydrolysis enzyme [100].

1.12.5.2. Extraction

Solvent extractions typically employ dichloromethane or diethyl ether and suffer mainly from emulsion formation and low recoveries of polar estrogen conjugates [134,141]. Because of this latter problem, LLE is mainly used for extraction of the free estrogens and, even then, the more polar estriol requires different extraction conditions than estrone and β-estradiol [112]. SPE methods using various retention mechanisms for extracting estrogens from complex matrices have been developed. Ager and Oliver reviewed size exclusion methods for extracting conjugated estrogens using Sephadex [142]. Ion exchange SPE using Amberlite XAD-2, a strong anion exchanger, proved to be a useful technique although changes in the sorbent over time required gradual modifications in extraction procedures. The sorbent also suffers from low capacity and allows only low flow rates to be used [141, 143].

The use of silica-based bonded phases eliminated the capacity and flow rate problems associated with ion exchange media. C_{18} has been shown to retain steroids and their conjugates effectively and the majority of SPE methods for steroid extraction now rely on C_{18} bonded phases. Heikkinnen *et al.* [141] extracted radiolabelled estrogens and conjugates from spiked late pregnancy and male urine samples on C_{18} SPE cartridges according to the procedure in **Figure 1.15**. Recoveries were high except for the most polar of the estrogen conjugates. Suppressing ionization by buffering the sample solution and wash solution to pH 3 gave recoveries between 85.8 and 99.1% for all estrogens and conjugates. Shackleton [100] used a dual extraction technique prior to steroid profiling in which a C_{18} SPE step was incorporated both before and after enzymatic hydrolysis. This procedure is outlined in **Figure 1.16**. The preliminary extraction of conjugated estrogens serves to remove enzyme inhibitors. No recovery data were available for this method. Ji *et al.* [102] used a combination post-hydrolysis LLE and C_{18} SPE procedure (**Figure 1.17**) in a preliminary method for MEKC analysis of urinary estrogens. LLE was accomplished at low pH with dichoromethane and was followed by several wash

Cartridges: Waters Sep-Pak C_{18}, 360 mg of packing		
	Not buffered	Buffered
Conditioning:	10 ml water 5 ml methanol 10 ml water	10 ml water 5 ml methanol 10 ml water
Sampling:	10 ml spiked urine	9 ml spiked urine, 1 ml pH 3 buffer
Washing:	10 ml water	10 ml pH 3 buffer
Elution:	3 ml methanol	3 ml methanol

Figure 1.15. Extraction procedures used by Heikkinnen *et al.* [141] for buffered and non-buffered spiked urine samples

Cartridges: brand name and size not available	
Conditioning:	5 ml methanol 5 ml water
Sampling:	30 ml centrifuged urine
Washing:	5 ml water
Elution:	4 ml methanol
Hydrolysis:	Enzymatic digestion
Re-extraction:	Same procedure as above

Figure 1.16. Sample preparation scheme employing dual SPE used by Shackleton [100].

Cartridges: Varian BondElut C_{18}, 200 mg of packing	
Hydrolysis:	Acid digestion
LLE:	10 ml dichloromethane
Washing:	alkaline solutions
Solvent switch:	organic phase evaporated under N_2 reconstituted in methanol
Conditioning:	3 ml water 3 ml acetonitrile 3 ml water
Sampling:	reconstituted extract from above
Washing:	2 ml water 2 ml 22% v/v acetonitrile/water (eluent dicarded)
Elution:	1 ml 30% v/v acetonitrile/water (eluent collected, contains E3)
Washing:	1 ml 40% v/v acetonitrile/water (eluent discarded)
Elution:	1 ml 55% v/v acetonitrile/water (eluent collected, contains E1, E2, and the internal standard, d-equilenin)
Analysis:	Eluents combined and analyzed by MEKC

Figure 1.17. Sample preparation scheme employing both LLE and SPE steps used by Ji *et al* [102].

steps with basic solutions intended to remove acidic components. The wash and elution steps in the SPE procedure constitute an attempt at fractionating the analytes using successively stronger solvents to selectively remove sample components. No quantitation or recovery information for this preliminary method was provided, nor was

the method of arriving at the wash and elution conditions explained. Schmidt *et al.* [144] extracted urinary steroids using C_{18} SPE both before and after enzymatic hydrolysis and found that adding an amino bonded phase SPE cartridge in series with the final extraction cartridge is an effective replacement for alkaline wash steps used by others. The recovery of free steroids added to urine ranged from 71 to 103%. Their procedure is summarized in **Figure 1.18**.

These four procedures are examples of extraction methods that vary in their degree of sophistication. Certain steps in each of these methods would benefit from further optimization. For example, it is doubtful that the full sampling capacity of the cartridges has been exploited in any of the methods and loading a larger sample in combination with

Cartridges:	Bakerbond C18, 500 mg of packing Bakerbond amino, 500 mg of packing
Conditioning:	2 x 5 ml methanol 2 x 5 ml water
Sampling:	10 ml centrifuged urine
Washing:	2 x 5 ml water
Elution:	2 x 1 ml methanol
Hydrolysis:	Enzymatic digestion
Re-extraction:	conditioned as above amino column inserted before C_{18} cartridge
Elution:	2 x 1.5 ml ethyl acetate

Figure 1.18. Sample preparation scheme employing dual SPE steps and an amino SPE cartridge used by Schmidt *et al* [144].

a reduction in elution volume would enhance the concentration effect between the original sample and the cartridge eluent. The wash steps, with the exception of those used in the method by Ji *et al.*, have not been selected to remove the maximum amount of interferences. In fact, washing with pure water does little more than flush the sorbent bed to remove any non-retained or weakly adsorbed substances. In itself, flushing these substances out is helpful, as inorganic acids will harm GC columns, but the potential for cleaning up the sample is not fully taken advantage of when water by itself is used as a wash solvent.

2. Experimental

2.1. Materials

Solid-phase extraction cartridges were obtained from J.T. Baker (Philipsburg, NJ, USA). Extraction cartridges were packed with 500 or 1000 mg of one of four silica-based sorbents: a cyanopropylsiloxane-bonded phase (product number 7021-07, lot number H02571); a spacer-bonded propanediol phase (product number 7094-03, lot number H41551); a butylsiloxane-bonded phase (product number 7216-06, lot number H14574); or a light-loaded octadecylsiloxane-bonded phase (product number 7189-03, lot numbers H03558 and L20553). The sorbent material was excised from several cartridges by cutting the barrel with a scalpel slighty above the top of the sorbent bed and removing the upper frit with forceps.

Methanol, acetonitrile, tetrahydrofuran, 2-propanol and water were Omnisolv grade from EM Science (Gibbstown, NJ, USA). Test solutes were reagent grade or better and were obtained from various sources. Dichloromethane and N,N-dimethylformamide were obtained from Fisher (Fair Lawn, NJ, USA). Bis(trimethylsilyl)trifluoracetamide and estriol were purchased from Aldrich (Milwaukee, WI, USA). Estrone, equilin, 17α-estradiol and 17β-estradiol were obtained from Sigma (St. Louis, MO, USA). Equilenin was obtained from Steraloids, Inc. (Wilton, NH, USA). 17α-Dihydroequilin, 17β-dihydroequilin, 17α-dihydroequilenin and 17β-dihydroequilenin were gifts from Wyeth-Ayerst Research (Princeton, NJ, USA). Premarin tablets, containing 2.5 mg of conjugated estrogens per tablet, were obtained at a local pharmacy. Late pregnancy urine samples and non-pregnant urine samples were donated by willing individuals.

2.2. Instrumentation, apparatus and methods

2.2.1. HPLC

The HPLC system consisted of a Varian 5000 pump module (Walnut Creek, CA, USA), Valco 10 µl rotary injection valve (Houston, TX, USA), and Kratos Spectroflow

773 UV-visible absorption detector (Westwood, NJ, USA). A Nelson Analytical 9000 series interface (PE Nelson, Cupertino, CA, USA) and an Equity II+ computer running under PE Nelson 2100 PC integrator software (revision 5.1) were used for data acquisition, along with a Waters 730 data module and a Hewlett Packard 3392 integrator.

Test solutes were dissolved in a small amount of methanol and the retention time was recorded for each solute with each stationary phase/mobile phase system. Sodium nitrate (26 mg/ml) was used as a dead time marker with a lower concentration (1 mg/ml) being used for the determination of interparticle porosity. Columns were prepared by packing 25 x 4.6 i.d. column blanks with sorbent excised from SPE cartridges using the tap and fill (dry packing) procedure [7,145]. Before packing, fines were removed from the sorbent material by suspending it in methanol and siphoning off the top portion of the solvent. After filling, the columns were consolidated and freed from air by pumping methanol through the column until the pressure stabilized and no further gas bubbles were observed at the detector outlet. Prior to data collection, the columns were equilibrated with the appropriate mobile phase until retention times for consecutive injections of the same solute remained stable.

The hydrophobicity index was determined as the ratio of retention factors for n-pentylbenzene and n-butylbenzene in methanol-water (4:1 v/v). The silanophilic index was determined as the ratio of the retention factors for caffeine and phenol in methanol-water (3:7 v/v). Both the hydrophobicity and silanophilic indices are described by Kimata *et al.* [37].

2.2.2. Data processing

The Dorsey-Foley model was selected for the variance measurements [146] used to generate plate height values. Revision 5.0 of SPSS/PC+ (SPSS, Inc., Chicago, IL, USA) was used for multiple linear regression analysis. Descriptors for the 27 test solutes in the training set were obtained by calculation (V_X) or from the literature (all others) and are

listed in **Table 2.1**. Solute descriptors for the three estrogens are literature values provided in **Table 2.2** [81]. Excel (version 4.0) and CricketGraph (version 1.5) were used for performing calculations and plotting results, respectively.

2.2.3. Solid-phase extraction

Solid-phase extraction was performed using a Baker spe-12G column processor

Table 2.1. Solute descriptors for the training set

Compound	V_x	R_2	π_2^H	α_2^H	β_2^0
Naphthalene	1.085	1.340	0.92	0.00	0.20
n-Propylbenzene	1.139	0.604	0.50	0.00	0.15
Chlorobenzene	0.839	0.718	0.65	0.00	0.07
1,2-Dichlorobenzene	0.961	0.872	0.78	0.00	0.04
Bromobenzene	0.891	0.882	0.73	0.00	0.09
Benzaldehyde	0.873	0.820	1.00	0.00	0.39
Benzonitrile	0.871	0.742	1.11	0.00	0.33
Anisole	0.916	0.708	0.75	0.00	0.29
Acetophenone	1.014	0.818	1.01	0.00	0.49
Phenol	0.775	0.805	0.89	0.60	0.31
Nitrobenzene	0.891	0.871	1.11	0.00	0.28
Benzyl alcohol	0.916	0.803	0.87	0.33	0.56
2-Phenylethanol	1.057	0.811	0.91	0.30	0.65
2-Chlorophenol	0.897	0.853	0.88	0.32	0.31
2-Hexanone	0.968	0.136	0.68	0.00	0.51
Benzamide	0.973	0.990	1.50	0.49	0.67
Acetanilide	1.114	0.870	1.40	0.50	0.67
4-Cresol	0.916	0.820	0.87	0.57	0.32
1-Naphthol	1.144	1.520	1.05	0.61	0.37
4-Phenylphenol	1.383	1.560	1.41	0.59	0.45
1-Nitroaniline	0.990	1.180	1.37	0.30	0.36
Ethylphenylketone	1.155	0.804	0.95	0.00	0.51
2-Octanone	1.252	0.108	0.68	0.00	0.51
Benzene	0.716	0.610	0.52	0.00	0.14
Methylbenzoate	1.073	0.733	0.85	0.00	0.48
2-Cresol	0.916	0.822	0.88	0.57	0.34

Table 2.2. Estrogen descriptors

compound	V_x	R_2	π_2^H	α_2^H	β_2^0
estrone	2.156	1.73	3.1	0.56	0.91
estradiol	2.199	1.8	3.3	0.88	0.95
estriol	2.258	2	3.36	1.4	1.22

(J.T. Baker, Philipsburg, NJ, USA) capable of supplying vacuum from a water aspirator to 12 cartridges simultaneously. Cartridges were conditioned with 2-3 barrel volumes of methanol. For breakthrough volume measurements, cartridges were conditioned further with 2-3 barrel volumes of solvent matching the solvent composition of the sample being processed. Without letting the cartridge run dry, the sample was applied with a pipette to measure exact volumes. The cartridge was washed with 2 barrel volumes of water, sucked dry for 2 min., and eluted with 3 x 0.50 ml of methanol. The internal standard, equilenin, was added to the eluted samples and the methanol was evaporated off under nitrogen. For extraction of estrogens from urinary matrices, the cartridges were further conditioned with 1% v/v methanol after the initial methanol wash. Without letting the sorbent bed run dry, the sample was poured into the cartridge barrel from a small conical flask and the flask was rinsed three times with small volumes of 1% v/v methanol to ensure quantitative transfer. The rinse solutions were poured into the cartridge barrel after the majority of the sample had been processed. The cartridge was washed with 6.0 ml of 40% v/v methanol, sucked dry for 2 min. and eluted with 3 x 0.5 ml methanol. The eluted samples were evaporated to a residue under nitrogen.

2.2.4. Liquid-liquid extraction

Extractions by LLE were performed in separatory funnels by adding 10.0 ml of

chloroform, shaking gently and collecting the bottom organic layer. The organic layer was passed through a short column of sodium sulfate (prepared in a Pasteur pipette) to remove residual moisture and then evaporated down to a small volume under nitrogen. The extraction was repeated twice more to ensure complete extraction, the dried organic layers were added to the same vial as the first extract, and the combined extracts were evaporated to a residue under nitrogen.

2.2.5. Derivatization

The residue resulting from SPE or LLE was dissolved in 0.300 ml DMF and 0.050 ml BSTFA was added to derivatize the estrogens to their TMS ethers. The solutions were allowed to stand at room temperature overnight and GC analysis was performed the next day.

2.2.6. Gas chromatography

Separations of derivatized estrogen mixtures were performed on a Hewlett Packard 6890 gas chromatograph running under Chemstation software (revision A.04.02) on a Hewlett Packard Vectra XM Series 4 computer. The GC was equipped with a split-splitless injector held at 250 °C; run in the split mode (split ratio 10:1), and a flame ionization detector, which was held at 300 °C. A fused silica open tubular column, HP-5 (crosslinked 5% phenyl methylsiloxane), 30 m x 0.32 mm i.d., 0.25 μm film thickness, was used. The temperature program consisted of an initial isothermal period of 18 minutes at 200 °C, followed by a temperature ramp of 5 °C/min. to 280°C. The final temperature was held until the end of the separation. Nitrogen carrier gas flow was maintained at 1.0 ml/min. The sample volume injected was 1 μl.

2.3. Sample preparation

2.3.1. Preparation of solutions for breakthrough volume measurements

A stock solution containing known concentrations (approximately 1 mg/ml) of estriol, 17β-estradiol and estriol was prepared in 80% v/v aqueous methanol. Appropriate dilutions were made with 80% v/v methanol to produce solutions of varying volumes with known and approximately equal amounts of the three estrogens. All sample volumes were processed in triplicate. After processing by SPE, the internal standard, equilenin, was added to all samples in equal amounts. The same procedure was followed for solutions at 70%, 60% and 50% v/v methanol.

2.3.2. Premarin

2.3.2.1. Preparation of Premarin stock solution

The outer coating of twelve Premarin tablets was carefully removed with a moist paper towel down to the shellac layer and the tablets were weighed. The tablets were then crushed and ground to a powder in a mortar and pestle and the equivalent mass of ten tablets was weighed into a test tube. Conjugated estrogens were extracted from the powder by sonication with methanol. The test tube was centrifuged and the supernatant was collected and concentrated on a rotary evaporator. The extraction was repeated twice more to ensure complete recovery of the conjugated estrogens from the tablet matrix. The resulting methanol solution was diluted with an equal volume of water and 4 ml of concentrated HCl were added. Hydrolysis of the conjugated estrogens to form the free estrogens was accomplished by heating the mixture to reflux for 25 minutes. The resulting solution was cooled to room temperature, neutralized with 10 M NaOH to pH 7, and diluted up to 250.0 ml with methanol in a volumetric flask. The solution was stored at 4 °C until needed and allowed to warm to room temperature before use. From this solution, 20 ml aliquots were mixed with 80 ml of water, the internal standard was added and the samples were processed by either SPE or LLE.

2.3.2.2. Preparation of Premarin standards

A standard solution containing 0.5 mg/ml of the 9 components analyzed in Premarin tablets (estrone, equilin, equilenin, 17α-estradiol, 17β-estradiol, 17α-dihydroequilin, 17β-dihydroequilin, 17β-dihydroequilenin, and estriol) was prepared by dissolving 12.5 mg of each of the pure compounds in a small amount of methanol and diluting up to 25.0 ml with methanol in a volumetric flask. The solution was stored at 4 °C until needed and allowed to warm to room temperature before use. Appropriate volumes of this solution were evaporated, derivatized and analyzed by GC.

2.3.3. Urine samples

2.3.3.1 Preparation of non-pregnant urine samples

Urine samples (45 ml) were mixed with 10 ml of methanol and 4 ml concentrated HCl and refluxed for 25 min. After hydrolysis, the samples were cooled to room temperature, neutralized with 10 M NaOH, filtered through 0.20 μm filters (Gelman Science, Ann Arbor, MI, USA), and pooled. The pooled solution was separated into individual 65 ml samples, spiked with known amounts (approximately 150 μg each) of estriol, 17β-estradiol, estriol, and the internal standard, equilenin, and extracted by SPE.

2.3.3.2. Preparation of late pregnancy urine samples

To 60 ml of late pregnancy urine, 10 ml of methanol and 4 ml concentrated HCl were added. The mixture was refluxed for 25 min., cooled, neutralized with 10 M NaOH, and filtered through 0.20 μm filters. The internal standard, equilenin, was added and the samples were processed by SPE.

2.3.3.3. Preparation of urinary estrogen standards

A standard solution containing 0.5 mg/ml of the urinary estrogens (estrone, 17β-estradiol and estriol) was prepared by dissolving 12.5 mg of each compound in a small

amount of methanol and diluting up to 25.0 ml with methanol in a volumetric flask. The solution was stored at 4 °C until needed and allowed to warm to room temperature before use. Appropriate volumes of this solution were used to spike non-pregnant urine samples or evaporated, derivatized and analyzed by GC as standards.

2.3.3.4. Preparation of internal standard solution

The internal standard solution containing 0.5 mg/ml of equilenin was prepared by dissolving 12.5 mg of the pure compound in a small amount of methanol and diluting up to 25.0 ml with methanol in a volumetric flask. The solution was stored at 4 °C until needed and allowed to warm to room temperature before use. Identical volumes of this solution were pipetted into all sample and standard solutions prior to extraction by LLE or SPE.

3. Results and discussion

3.1. Sorbent properties

The characteristic properties of the bonded phase sorbents investigated, as disclosed by the manufacturer, are summarized in **Table 3.1**. Three of the four sorbent types are small-pore, high surface area materials. The exception is the butylsiloxane bonded phase (C_4) which has a wide-pore substrate designed for the extraction of higher molecular weight compounds. By its nature as a wide-pore material, this substrate has a lower surface area than the small-pore materials, and can be expected to provide less retention than a small-pore sorbent with the same bonded ligand [7]. The cyanopropylsiloxane (CN), propanediol (DIOL), and butylsiloxane bonded phases all have high loadings of bonded ligands while the octadecylsiloxane (LL C_{18}) bonded phase was manufactured to have a low loading of bonded ligand of approximately 12% carbon. High-loaded C_{18} bonded phases from the same manufacturer have a typical loading of 17.2% carbon. The apparent particle diameter is marketed in the trade literature as 40 μm but consistently registers at 50 μm for individual lots of sorbents. Inspection of the specifications for the two lots of LL C_{18} sorbent reveals slight differences in the sorbent from lot to lot, indicating variations in the manufacturing process that may have small effects on the performance of cartridge extraction devices. The structures of the bonded ligands are shown in **Figure 3.1**.

Table 3.2 summarizes the physical dimensions and packing densities of the cartridge sorbent beds and packed HPLC columns. The packing density of the cartridges varies between individual cartridges in the same lot and is consistently smaller than the packing density of the corresponding HPLC column. The columns were packed by the tap-and-fill dry packing procedure which requires no special high-pressure equipment, nor are high pressures used in their evaluation. Therefore, the column packing densities can be considered an easily obtainable upper limit for cartridge packing density. A rough calculation shows that the sorbent cartridges contain about 15-25% additional empty

Table 3.1. Manufacturer's specifications for cartridge devices. All sorbents were obtained from J.T. Baker.

Sorbent	*CN*	*DIOL*	*C_4*	*LL C_{18}*	
Product number	7021-07	7094-03	7216-06	7189-03	
Lot number	H02571	H41551	H14574	H03558	L20553
Particle size distribution					
APD, µm	50	50	53	50	51
<31 µm, wt %	16	16		16	14
Mesh					
On U.S. No. 200 sieve, %	0.1	0.5		0.1	<0.2
Specific surface area, m^2/g	519	516	278	519	515
Carbon (C), %	9.8	8.4	5.9	12.3	12.5
Hydrogen (H), %	1.9	2.1	1.4	2.9	2.6
Nitrogen (N), %	2.5				
Surface coverage, $\mu g/m^2$					
Based on % C	188	158	213	237	228
Based on % N	49				
Mean pore diameter, µm	0.6	0.6	2.5	0.6	0.6
pH	6.9	6.3	6.3	3.9	4.1
Extraction residue, %	0.1	0.02	0.01	0.1	0.04

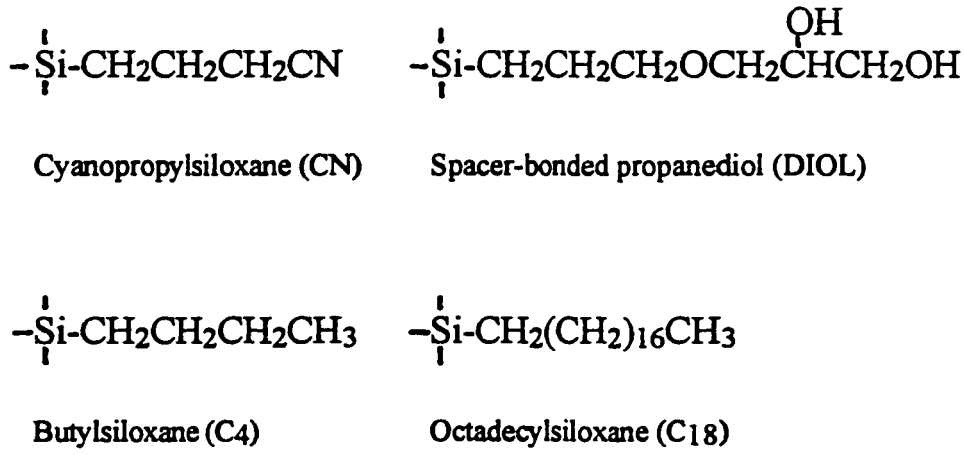


Figure 3.1. Structures of the bonded ligands.

Table 3.2. Cartridges and column characteristics.

	CN	*DIOL*	*C_4*	*LL C_{18}*
Cartridge				
diameter, cm	1.25	0.90	1.30	0.95
bed height, cm	1.40	1.40	1.20	1.30
packing density, g/ml				
mean	0.599	0.557	0.322	0.532
range	0.584-0.619	0.546-0.571	0.316-0.328	0.527-0.537
HPLC column (long)				
diameter, cm	0.46	0.46	0.46	0.46
length, cm	25.0	25.0	25.0	25.0
packing density, g/ml	0.634	0.577	0.453	0.639
HPLC column (short)				
diameter, cm			0.46	0.46
length, cm			4.0	4.0
packing density, g/ml			0.343	0.620

space on average compared to the stable column beds prepared by the tap-and-fill method. The effects of inadequate packing density on SPE are: a) variations in flow rates between cartridges because of differences in permeability; b) a dependence of recovery on sample flow rate; and c) typical bed heights used in cartridge devices being greater than required to compensate for the heterogeneous packing structure, thereby increasing contamination of the sample extracts due to increased matrix adsorption.

Sorbent properties determined by chromatographic means are summarized in **Table 3.3.** The intraparticle porosity values indicate high loadings of the bonded phases. A previously obtained value for intraparticle porosity (0.06) for a high-loaded C_{18} [15], indicates a virtually non-porous packing with pore openings largely choked by the high loading of bonded ligands. The bonded phases summarized below show slightly more accessible pore volume, indicating more favorable mass transfer characteristics over those

Table 3.3. Chromatographically measured properties of sorbents.

	CN	*DIOL*	*C_4*	*LL C_{18}*
porosity				
total	0.52	0.58	0.74	0.48
interparticle	0.41	0.41	0.60	0.36
intraparticle	0.11	0.17	0.14	0.12
specific permeability, 10^{13} m^2	2.84	2.47	3.73	2.54
flow resistance parameter	1402	1122	281	2192
hydrophobicity index	1.04	1.14	1.27	1.76
silanophilic index	0.64	0.61	0.74	1.90
apparent particle diameter, μm	27.6	21.8	12.0	34.1

for the high-loaded C_{18}. The intraparticle porosity for the wide-pore C_4 bonded phase is unexpectedly similar to the values for the small-pore materials. This discrepancy may be caused by an assumption in the measurement process that dilute $NaNO_3$ is excluded from the pore volume. With a wide-pore substrate, the unretained, and supposedly excluded, compound may indeed explore the pore volume and part of the intraparticle porosity is actually contained in the value for the interparticle porosity. Since the apparent particle diameter, the flow resistance parameter, and the specific permeability are all dependent upon porosity measurements, the values for those parameters are also anomalous for the C_4 sorbent.

The flow resistance parameter is a sensitive indicator for the presence of fines in the sorbent bed. A high value indicates a significant portion of particles with diameters less than the average size. Expected values of this unitless property range from 500 to 1000. The CN and LL C_{18} bonded phases exhibit unacceptable proportions of fine particles, resulting in poor cartridge permeability. The chromatographically determined mean particle diameters deviate significantly from the manufacturer's claims. These

measurements already exclude a portion of the fines, as the sorbent material was washed with methanol and fines removed by sedimentation prior to packing the column. The low values for particle size then suggest a skewed particle size distribution with an appreciable fraction of the sorbent particles significantly smaller than the specified particle size diameter. The achievement of adequate flow during sample processing depends on having a particle size distribution that is tightly clustered around a mean particle diameter.

The hydrophobicity index is a measure of the accessible volume of stationary phase inferred to be proportional to the concentration of organic ligands bonded to the silica substrate. As measured by Kimata *et al.* [37], the typical range for HPLC packings is 1.3-1.52. The three shorter alkyl chain sorbents have relatively low hydrophobicity indices as expected, but the LL C_{18} sorbent has an unusually high hydrophobicity index indicating a higher phase ratio for this sorbent. The normal range for the silanophilic index for HPLC packings is 0.4-1.29. The silanophilic index indicates, at least qualitatively, that the sorbents have low non-specific sorption characteristics with a capacity for silanophilic interactions in the low range based on the properties of C_{18} silica-based sorbents [15,20,37,75]. The one exception is the LL C_{18} sorbent which registers a significantly higher presence of unreacted silanol groups and, thus, a propensity toward non-specific adsorption interactions.

Overall, these studies suggest that typical cartridges show variations in sampling performance that result from an inadequate packing density and an heterogeneous particle size distribution. A simple remedy to reduce the variation in cartridge performance is to consolidate each cartridge bed prior to sampling by vibration and repositioning of the upper frit if possible. From the manufacturing perspective, the end user has the right to expect tighter control of the particle size range, a higher packing density, and a tighter range of packing densities. There is no evidence that a smaller average particle diameter is required for cartridges of a conventional bed height to provide acceptable sampling

performance; indeed, as long as suction remains the primary means of sample processing, decreasing the particle size would be counterproductive. On the other hand, the typical bed heights used in cartridge devices are probably greater than required to compensate for the heterogeneous packing structure of the bed, in turn contributing to increased contamination of sample extracts by elevating the concentration of nonspecifically adsorbed sample matrix components.

3.2. Kinetic properties of sorbents

A Van Deemter plot of the variation of plate height with mobile phase velocity for the four sorbents is given in **Figure 3.2** (data tabulated in **Table 3.4**). None of the trends show minima indicating that the optimum flow rate for operation at the highest possible efficiency was not reached and that, for the flow rates measured (and those commonly used in practice), the efficiency is controlled by resistance to mass transfer and flow anisotropy. The following linear relationships were obtained for three of the sorbents shown in the Van Deemter plot:

CN	$H = 0.844 + 0.202\ u_e$	$r^2 = 0.990$, n = 7, u_e range = 1.3-3.5
DIOL	$H = 0.471 + 0.172\ u_e$	$r^2 = 0.992$, n = 9, u_e range = 1.0-5.0
LL C_{18}	$H = 0.287 + 0.082\ u_e$	$r^2 = 0.964$, n = 7, u_e range = 1.6-5.0

The relationship for the C_4 sorbent is best described with the second order fit:

C_4 $\quad H = 0.724 + 5.46 \times 10^{-2} u_e + 4.32 \times 10^{-2} u_e^2$

$r^2 = 0.999$, n = 7, u_e range = 0.5-3.6

Of the four sorbents, the C_{18} sorbent offers the highest efficiency in terms of the greatest number of theoretical plates per unit of bed height. For most applications sorbent cartridges are used above the optimum velocity in a flow-rate range where the plate height is variable.

The kinetic data were generated using HPLC columns at higher packing densities than the extraction cartridges. The plate heights obtained are, therefore, elevated with

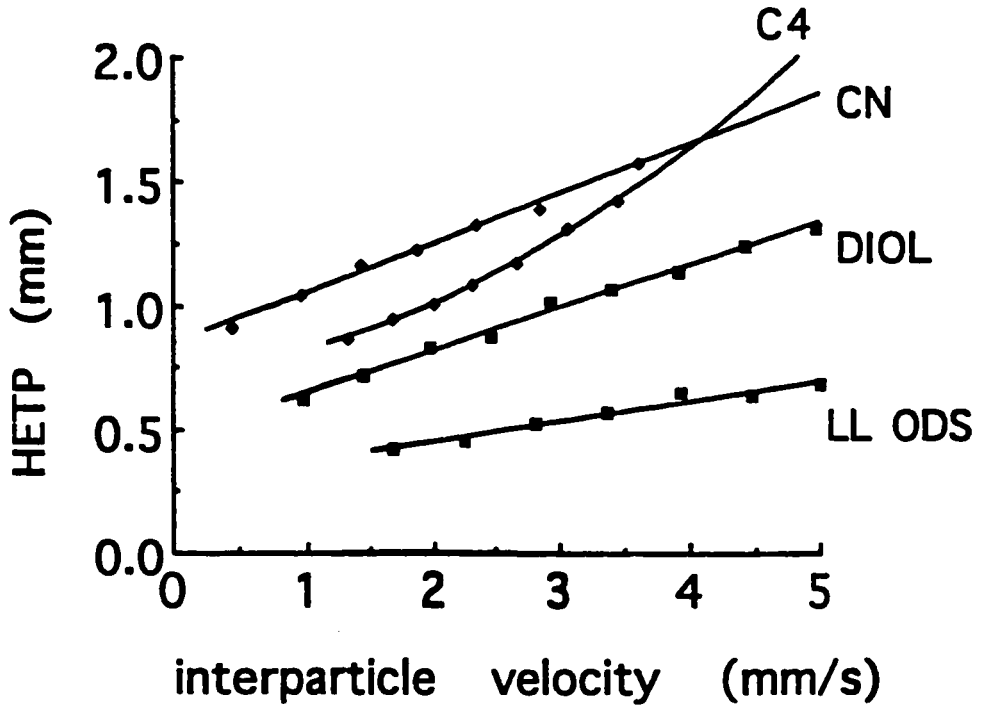


Figure 3.2. Van Deemter plots for the four sorbents investigated.

Table 3.4. Measured plate height, H (μm), for HPLC columns.

Flow rate (ml/min)	*CN*	*DIOL*	*C_4*	*LL C_{18}*
0.4	1043			
0.6	1166			420
0.8	1222	2083	869	454
1.0	1332	2180	945	526
1.2	1391	2404	1004	566
1.4	1574	2729	1080	644
1.6		2836	1171	637
1.8		3000	1307	680
2.0		3273	1425	740
2.2				729
2.4				776

Table 3.5. Number of theoretical plates provided by a LL C_{18} cartridge device (adjusted for bed dimensions and packing density).

Flow rate (ml/min)	*N*
0.6	25.8
0.8	23.8
1.0	20.6
1.2	19.1
1.4	16.8
1.6	17.0
1.8	15.9
2.0	14.6
2.2	14.9
2.4	14.0

respect to the plate heights expected with cartridge devices and should be considered as upper boundaries for the numbers of theoretical plates obtainable with cartridge devices. In the flow rate region likely to be used during sample processing, cartridges are probably only able to provide 5-10 theoretical plates per centimeter of bed height. **Table 3.5** provides an estimate of the number of plates provided by a C_{18} cartridge device adjusted from column measurements for the cartridge bed dimensions and the differences in packing density between the cartridges and columns.

3.3. Retention measurements

Retention factor values for the four sorbents over wide composition ranges with aqueous solutions of four different organic solvents are given in Appendix A. Generally, retention factor values increase with a decrease in the organic solvent content of the mobile phase and, for simple hydrocarbons with no other functional groups, increase with the length of the alkyl chain in the bonded ligand. A plot of log k vs. % composition for

some representative test solutes is shown in **Figure 3.3**. Since no uniform or easily discernable relationship exists between log k and % composition (some solutes show linearity, others must be fitted with polynomial functions), data estimated by extrapolation from higher organic compositions to lower organic compositions must be treated carefully. Previous work was done on a high loaded C_{18} sorbent [15] and, since measurements below about 15% v/v methanol were not feasible, obtaining estimates for log k necessitated extrapolation from data obtained at higher methanolic compositions. Through the use of bonded phases with shorter alkyl chains, measurements of retention factors were attainable over the entire composition range all the way down to 1% v/v organic solvent. By using shorter column lengths, measurements could also be made at all compositions for a light-loaded C_{18} phase, thus eliminating the need for error-prone extrapolation methods.

Although error from extrapolation can be eliminated from these studies, the

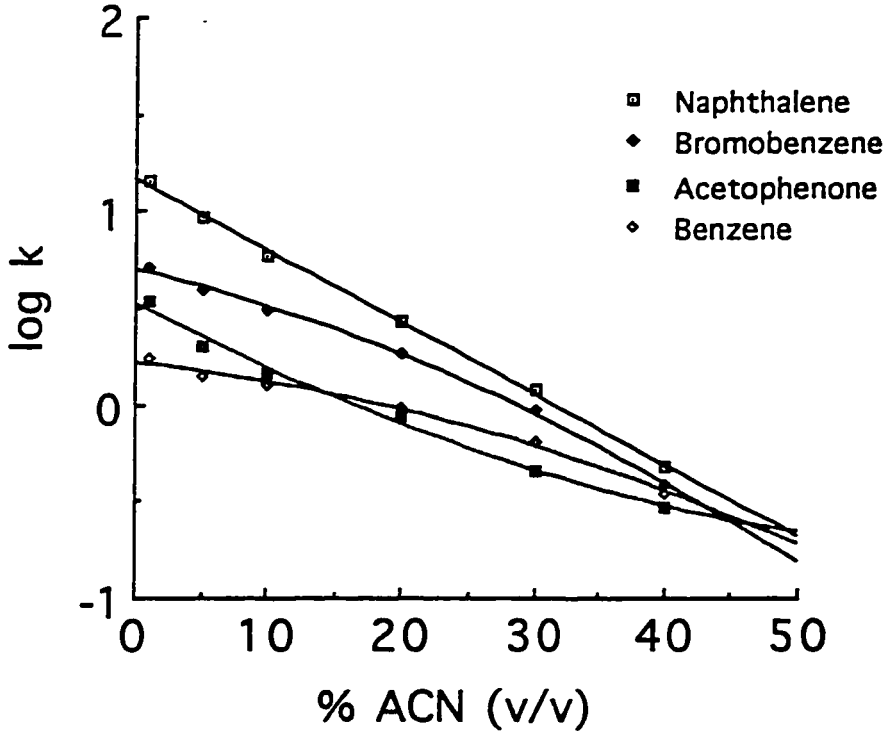


Figure 3.3. Change in retention with mobile phase composition change.

Table 3.6. Influence of mobile phase composition (% v/v organic solvent) on the determination of column holdup time for the CN phase.

solvent	*50 %*	*40 %*	*30 %*	*20 %*	*10 %*	*5 %*	*1 %*
methanol	1.46	1.48	1.55	1.51	1.58	1.64	1.83
acetonitrile	0.90	0.93	0.97	1.03	1.08	1.10	1.12
isopropanol	1.05	1.02	1.05	1.08	1.09	1.12	1.12
tetrahydrofuran	0.96	0.98	1.04	1.10	1.10	1.12	1.15

measurement of retention factor cannot be divorced from the accuracy with which the column holdup time can be determined. **Table 3.6** shows the influence of mobile phase composition on the determination of holdup time for a column packed with CN sorbent. The elution time of sodium nitrate slightly declines with increasing organic solvent in the mobile phase and is significantly greater for methanol-water as mobile phase compared to the other three solvents. These changes could be artifacts of the measurement process or actual changes in the stationary phase volume. When calculating retention factors, replacing the holdup time for methanol with average values obtained for other solvents results in significant changes in the system constants generated, particularly in the m- and b- constants. These observations confirm the importance of an exact knowledge of the column holdup time if accurate system constants are to be obtained.

3.4. Generation of system constants

Capacity factor values (Appendix A) were substituted into the solvation parameter model (equation 11) along with solute descriptors for the training set (**Table 2.1**). Multiple linear regression analysis was performed using retention data for the set of test solutes at each mobile phase composition. The resulting system constants are tabulated together with the statistics for the fit in **Tables 3.7-3.21**. Generally, the fit to

the model is good (multiple r > 0.990, low standard error, high F statistic), with the best fits to the equation occurring in medium to low organic solvent compositions. The quality of the fit deteriorates to some extent at high and extremely low organic solvent compositions. At high organic compositions, retention times are small, increasing the effect of error in their measurement, while at low organic compositions, peak shapes tend to be distorted making retention times more difficult to measure at these compositions. The loss in the quality of the fit in these regions is not egregious, however, and the data are still sufficiently well-determined for use in further calculations.

The change in system constants over ranges of mobile phase composition are represented graphically in Appendix B. Data at some high organic compositions have been omitted from these plots because capacity factor values at those compositions are small and difficult to measure with certainty. The plots consist of smooth functions over the ranges studied with no unexpected discontinuities. In addition to allowing the calculation of retention factors for any compound outside the training set for which solute descriptors are available, the sign and magnitude of the system constants yield valuable information about the difference in solvating behavior of the mobile and stationary phases. System constants for each sorbent will be discussed separately.

3.4.1. Cyanopropylsiloxane bonded phase

For the CN bonded phase with methanol as the organic component, only the cavity term (m-constant) and interactions with lone pair electrons (r-constant) are positive and favor retention in the stationary phase. The cavity term is the dominant term favoring retention, particularly at high water compositions, indicating that forming a cavity in the solvated stationary phase is not as difficult as forming a cavity of the same size in the mobile phase. This difference in the ease of cavity formation increases as the water composition increases reaching a near plateau value as the concentration of methanol reaches a minor fraction of the mobile phase composition.

Table 3.7. System constants, cyanopropylsiloxane bonded phase, methanol.

MeOH % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
80	0	0.09	0	-0.23	-0.13	-0.29	0.971	0.02	77.2
	0	(0.02)	0	(0.02)	(0.03)	(0.02)			
70	0.30	0.12	0	-0.21	-0.35	-0.45	0.974	0.03	55.7
	(0.08)	(0.04)	0	(0.04)	(0.05)	(0.08)			
60	0.58	0.13	0	-0.18	-0.63	-0.48	0.972	0.03	103.0
	(0.09)	(0.04)	0	(0.04)	(0.05)	(0.08)			
50	0.84	0.21	0	-0.20	-0.88	-0.48	0.991	0.03	184.7
	(0.09)	(0.04)	0	(0.04)	(0.05)	(0.08)			
40	1.09	0.24	0	-0.22	-1.13	-0.47	0.997	0.02	623.2
	(0.06)	(0.03)	0	(0.03)	(0.03)	(0.05)			
30	1.45	0.32	0	-0.24	-1.36	-0.62	0.998	0.02	846.9
	(0.06)	(0.03)	0	(0.03)	(0.04)	(0.06)			
20	1.65	0.35	0	-0.25	-1.54	-0.60	0.999	0.02	1088.1
	(0.06)	(0.03)	0	(0.03)	(0.04)	(0.06)			
10	1.78	0.41	0	-0.29	-1.60	-0.64	0.997	0.04	508.7
	(0.10)	(0.04)	0	(0.05)	(0.06)	(0.09)			
5	1.87	0.46	0	-0.32	-1.63	-0.69	0.996	0.04	431.9
	(0.11)	(0.05)	0	(0.05)	(0.06)	(0.10)			
1	2.06	0.53	0	-0.51	-1.45	-0.88	0.991	0.06	175.4
	(0.18)	(0.07)	0	(0.08)	(0.10)	(0.16)			

The s-constant for the CN bonded phase is not statistically significant at any methanol composition. Under similar conditions, C_{18} sorbents have negative s-constants [15,17,73]. In this case a value of zero for the s-constant does not indicate that the cyanopropyl group has no influence on retention, but rather that it effectively competes with methanol-water mixtures in dipole-type interactions while the C_{18} sorbents do not.

Table 3.8. System constants, cyanopropyl bonded phase, acetonitrile

ACN % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
50	0.40	0.05	0	-0.18	-0.54	-0.11	0.995	0.02	320.2
	(0.05)	(0.02)	0	(0.02)	(0.03)	(0.04)			
40	0.64	0.09	0	-0.21	-0.80	-0.11	0.996	0.02	448.0
	(0.05)	(0.02)	0	(0.03)	(0.03)	(0.05)			
30	0.98	0.15	0	-0.24	-1.06	-0.23	0.995	0.03	368.1
	(0.08)	(0.03)	0	(0.04)	(0.04)	(0.07)			
20	1.27	0.24	0	-0.25	-1.27	-0.42	0.996	0.03	420.7
	(0.09)	(0.04)	0	(0.04)	(0.05)	(0.08)			
10	1.62	0.33	0	-0.26	-1.43	-0.65	0.998	0.03	784.6
	(0.07)	(0.03)	0	(0.03)	(0.04)	(0.06)			
5	1.78	0.36	0	-0.28	-1.50	-0.72	0.997	0.03	608.5
	(0.08)	(0.04)	0	(0.04)	(0.05)	(0.08)			
1	1.95	0.40	0	-0.34	-1.53	-0.77	0.997	0.04	477.8
	(0.10)	(0.04)	0	(0.05)	(0.06)	(0.09)			

The fact that a balance in dipole-type interactions between the two phases exists is surprising considering that retention in the CN phase is generally regarded as occurring as a result of dipole-type interactions.

The capacity of a solute to act as a hydrogen-bond acid or base favors retention in the mobile phase at all solvent compositions. The hydrogen-bond basicity of the system (a-constant) is virtually constant until low concentrations of methanol are reached while the hydrogen-bond acidity (b-constant) increases with increasing concentration of water until the region of low methanol concentration is reached. These results are similar to those observed in reversed-phase liquid chromatography with C_{18} packings [15,68,147]. Water is a significantly stronger hydrogen-bond acid than the components of the solvated

Table 3.9. System constants, cyanopropyl bonded phase, isopropanol

IPA % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
50	0	0.15	0	-0.27	-0.10	-0.21	0.964	0.02	91.7
	0	(0.02)	0	(0.02)	(0.03)	(0.02)			
40	0.29	0.16	0	-0.27	-0.41	0.12	0.975	0.03	107.1
	(0.05)	(0.02)	0	(0.03)	(0.04)	(0.04)			
30	084	0.20	0	-0.29	-1.05	-0.17	0.992	0.03	333.7
	(0.05)	(0.03)	0	(0.03)	(0.05)	(0.04)			
20	1.54	0.29	0	-0.26	--1.55	-0.50	0.994	0.04	411.4
	(0.07)	(0.03)	0	(0.04)	(0.06)	(0.06)			
10	1.79	0.39	0	-0.28	-1.59	-0.75	0.993	0.05	376.4
	(0.08)	(0.04)	0	(0.05)	(0.07)	(0.06)			
5	1.87	0.44	0	-0.29	-1.59	-0.81	0.991	0.06	287.0
	(0.09)	(0.05)	0	(0.06)	(0.08)	(0.08)			
1	1.86	0.43	0	-0.38	-1.54	-0.68	0.991	0.06	251.2
	(0.06)	(0.05)	0	(0.06)	(0.08)	(0.08)			

stationary phase and this property is more significant than the observed polar interactions.

Comparison of the system constants among the four organic solvents shows that the general trends hold true from solvent to solvent, but some differences appear in the plots, reflecting the differences in characteristic properties of the organic solvents. For the cavity term, the hydrogen-bond forming organic solvents show different behavior than acetonitrile. The cavity terms with the former solvents show steep curves flattening out in the range 1-10% v/v organic solvent, while the m-constant for acetonitrile-water mixtures increases approximately linearly with increasing water content. For the b-constant, the behavior with acetonitrile once again differs from that of the other solvents;

Table 3.10. System constants, cyanopropyl bonded phase, tetrahydrofuran

THF % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
50	0.47	0	0	-0.11	-0.67	-0.08	0.987	0.02	258.0
	(0.03)	0	0	(0.02)	(0.03)	(0.03)			
40	0.70	0	0	-0.06	-0.93	0.03	0.991	0.02	330.8
	(0.03)	0	0	(0.02)	(0.03)	(0.03)			
30	1.18	0	0	0	-1.45	-0.02	0.986	0.04	370.4
	(0.07)	0	0	0	(0.06)	(0.06)			
20	1.64	0.20	0	0	-1.91	-0.31	0.992	0.05	339.7
	(0.08)	(0.04)	0	0	(0.07)	(0.08)			
10	1.89	0.33	0	-0.16	-1.81	-0.71	0.998	0.03	1179.0
	(0.04)	(0.02)	0	(0.03)	(0.04)	(0.04)			
5	1.93	0.40	0	-0.20	-1.73	-0.83	0.997	0.04	749.6
	(0.06)	(0.03)	0	(0.04)	(0.05)	(0.05)			
1	1.82	0.41	0	-0.30	-1.60	-0.68	0.989	0.06	214.6
	(0.10)	(0.05)	0	(0.07)	(0.09)	(0.09)			

the change in the b-constant as a function of organic solvent composition is essentially linear. With the other solvents, a minimum occurs around 10-20% v/v organic solvent followed by a shallow increase in the b-constant at lower amounts of organic solvent. These small changes at low organic solvent composition are probably occur as a result of intermolecular hydrogen bond formation as the water structure builds up in competition with solute-water interactions.

The s-constant is effectively zero for the four solvents at all solvent compositions. Since water is by far the most polar of the solvents, it is probable that water molecules are localized around the cyano groups of the stationary phase, effectively neutralizing their influence on the retention of dipolar and polarizable solutes in the

Table 3.11. System constants, spacer-bonded diol phase, methanol

MeOH % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
60	0.46	0.21	0	-0.19	-0.68	-0.67	0.969	0.05	86.1
	(0.07)	(0.03)	0	(0.05)	(0.06)	(0.06)			
50	0.80	0.21	0	-0.16	-0.94	-0.70	0.993	0.03	353.3
	(0.05)	(0.02)	0	(0.03)	(0.04)	(0.04)			
40	1.02	0.30	0	-0.17	-1.11	-0.74	0.992	0.04	325.2
	(0.06)	(0.03)	0	(0.04)	(0.05)	(0.05)			
30	1.25	0.31	0	-0.15	-1.19	-0.78	0.990	0.04	263.5
	(0.07)	(0.03)	0	(0.04)	(0.06)	(0.06)			
20	1.54	0.37	0	-0.20	-1.24	-0.95	0.989	0.05	217.3
	(0.08)	(0.04)	0	(0.05)	(0.07)	(0.07)			
10	1.53	0.48	0	-0.29	-1.07	-0.98	0.981	0.07	118.8
	(0.11)	(0.06)	0	(0.07)	(0.11)	(0.09)			
5	1.65	0.50	0	-0.31	-1.04	-1.05	0.980	0.07	121.5
	(0.12)	(0.06)	0	(0.08)	(0.11)	(0.10)			
1	1.57	0.61	0	-0.45	-0.80	-1.05	0.982	0.07	123.8
	(0.11)	(0.06)	0	(0.07)	(0.11)	(0.09)			

interphase region. For any other reason, one would anticipate changes in the numerical value of the s-constant with changes in the solvent type and composition.

The a-constant shows only a small variation with solvent composition from 50-10% v/v acetonitrile, methanol, and isopropanol, and from 50-20% v/v tetrahydrofuran. For less than 10% v/v organic solvent there is a small decrease in the a-constant (larger negative value). These changes probably result from alteration in the solute-solvent environment related to structural changes in the water hydrogen-bonding network and are not easily interpreted as gross solvent effects. In general, the numerical range of a-

Table 3.12. System constants, spacer-bonded diol phase, acetonitrile.

ACN % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
40	0.15	0.12	0	-0.09	-0.29	-0.60	0.921	0.03	30.6
	(0.05)	(0.03)	0	(0.03)	(0.04)	(0.05)			
30	0.56	0.13	0	-0.09	-0.70	-0.58	0.971	0.04	85.8
	(0.06)	(0.03)	0	(0.04)	(0.06)	(0.06)			
20	0.95	0.19	0	-0.11	-0.97	-0.67	0.988	0.04	199.9
	(0.06)	(0.03)	0	(0.04)	(0.05)	(0.05)			
10	1.37	0.30	0	-0.14	-1.19	-0.90	0.990	0.04	250.2
	(0.07)	(0.04)	0	(0.05)	(0.06)	(0.06)			
5	1.5	0.44	0	-0.17	-1.23	-1.01	0.995	0.06	166.6
	(0.09)	(0.05)	0	(0.06)	(0.08)	(0.08)			
1	1.62	0.45	0	-0.30	-1.02	-1.02	0.981	0.07	115.6
	(0.11)	(0.06)	0	(0.08)	(0.11)	(0.10)			

constant values is small when impressed on the the b-constant scale, and, although by no means unimportant, the hydrogen-bond basicity of the phase is far less influential in effecting selectivity changes to retention than the hydrogen-bond acidity.

3.4.2. Spacer-bonded propanediol phase

The retention properties of the DIOL sorbent were determined over the composition range 1-50% v/v organic solvent for tetrahydrofuran and isopropanol, 1-60% v/v for methanol and 1-40% for acetonitrile. Inadequate solute retention prevents the exploration of retention with mobile phases containing more organic solvent than the largest amount indicated for each solvent. The system constants for the DIOL phase show similar trends to those of the CN phase and, likewise, show some solvent-specific

Table 3.13. System constants, spacer-bonded diol phase, isopropanol.

IPA % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
50	0 0	0.22 (0.02)	-0.07 (0.03)	-0.18 (0.02)	-0.29 (0.03)	-0.33 (0.02)	0.987	0.02	196.4
40	0.39 (0.04)	0.19 (0.03)	-0.14 (0.03)	-0.14 (0.02)	-0.61 (0.04)	-0.25 (0.03)	0.992	0.02	234.9
30	1.01 (0.08)	0.21 (0.06)	-0.17 (0.07)	-0.18 (0.05)	-1.12 (0.10)	-0.30 (0.07)	0.985	0.05	130.8
20	1.56 (0.08)	0.31 (0.06)	-0.13 (0.07)	-0.17 (0.05)	-1.55 (0.10)	-0.54 (0.07)	0.991	0.05	218.2
10	1.89 (0.10)	0.30 (0.05)	0 0	-0.18 (0.06)	-1.52 (0.09)	-0.94 (0.08)	0.989	0.06	213.6
5	1.83 (0.12)	0.39 (0.06)	0 0	-0.18 (0.08)	-1.34 (0.10)	-1.04 (0.10)	0.983	0.07	136.6
1	1.80 (0.13)	0.44 (0.07)	0 0	-0.26 (0.09)	-1.20 (0.12)	-1.03 (0.12)	0.976	0.08	101.5

behavior. The general picture of retention remains the same as for the CN phase; the m-constant dominates in favoring retention with a small contribution from the r-constant. The s-constant is zero or small and negative for all mobile phase compositions, and the hydrogen-bonding interactions favor retention in the mobile phase with the b-constant dominating those effects.

The dominant term in reducing retention is the b-constant, representing the relative capacity of the solvated sorbent and mobile phase to participate in hydrogen-bonding interactions as a hydrogen-bond acid. As seen with the CN phase, water's strong hydrogen bond acidity ensures that the DIOL phase does not compete with it as a hydrogen bond acid; but the shape of the b-constant trend is characteristic across the four

Table 3.14. System constants, spacer-bonded diol phase, tetrahydrofuran.

THF % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
50	-0.04	0.04	0	-0.16	-0.14	-0.52	0.971	0.02	86.0
	(0.02)	(0.01)	0	(0.02)	(0.02)	(0.02)			
40	0.28	0.03	-0.06	-0.04	-0.45	-0.36	0.994	0.01	314.8
	(0.02)	(0.01)	(0.02)	(0.01)	(0.02)	(0.02)			
30	0.57	0.14	-0.10	-0.03	-0.84	-0.30	0.992	0.03	228.4
	(0.04)	(0.03)	(0.07)	(0.03)	(0.05)	(0.04)			
20	1.19	0.24	-0.10	0	-1.41	-0.53	0.992	0.04	297.5
	(0.07)	(0.04)	(0.06)	0	(0.07)	(0.06)			
10	1.58	0.35	-0.10	0	-1.49	-0.85	0.993	0.05	314.0
	(0.08)	(0.05)	(0.07)	0	(0.08)	(0.07)			
5	1.71	0.35	0	-0.07	-1.42	-1.05	0.990	0.05	232.1
	(0.09)	(0.04)	0	(0.06)	(0.08)	(0.08)			
1	1.54	0.58	-0.25	-0.20	-0.82	-0.95	0.985	0.06	114.5
	(0.11)	(0.08)	(0.11)	(0.07)	(0.17)	(0.10)			

solvents. In the region representing less than 10% v/v organic solvent, the b-constant becomes more positive, indicating that the solvated sorbent is increasingly more competitive with the mobile phase as a hydrogen-bond acid. Presumably, the interphase region associated with the sorbent may become richer in water as the composition of the mobile phase becomes increasingly aqueous at low concentrations of organic solvent in a manner that is different from that observed in the composition range above about 10% v/v organic solvent.

The s-constant, describing the capacity of the system for interactions of a dipole type with dipolar or polarizable solutes, is either zero or small and negative. For the CN phase, the s-constant was zero for the same four organic solvents over all compositions,

whereas for C_{18} sorbents negative values have been found [15,68,90,148]. Water is the most dipolar and polarizable of the solvents used in these studies and the results for the C_{18} phases are as expected, given the nonpolar and nonpolarizable nature of the phase. The DIOL and CN phases should be more competitive than the solvated C_{18} phases for dipole-type interactions, but a characteristic dependence on the type and composition of the mobile phase is expected. That this is not generally the case suggests that short-range dipole forces of the polar functional groups of the bonded sorbent are effectively quenched by preferential association with solvent, presumably water. The association of water around the polar functional groups limits their participation in solute-sorbent interactions and maintains a near equality of dipole-type interactions for the solute in the mobile phase and sorbent interphase regions.

3.4.3. Butylsiloxane bonded phase

The overall trends in the system constants described for the CN and DIOL phases hold true also for the C4 phase; retention is driven by a large m-constant, supported in some cases by a small r-constant and counteracted by the s-constant and hydrogen-bond interactions. The most notable difference between the polar phases and the C4 phase is the wider range of m-constant values, indicating the increased facility of cavity formation in the solvated C4 phase over the polar phases. The b-constant also demonstrates the characteristic upward curvature (smaller negative numbers) with decreasing proportions of organic solvent, once again reflecting the increasingly organized water structure at low organic compositions. The s-constant is small and negative at most compositions in contrast with the balance between these interactions in the mobile and solvated polar stationary phase, indicating that dipole-type interactions favor the mobile phase over the solvated C4 phase. With the absence of polar groups in the interphase region of the C4 phase, the negative s-constant indicates that solvent is taken up to a lesser extent in the interphase region resulting in less cohesion and a more favorable transfer of solutes to the

Table 3.15. System constants, butylsiloxane bonded phase, methanol.

MeOH % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
60	1.36	0	-0.26	-0.27	-1.13	-0.87	0.995	0.03	527.2
	(0.05)	0	(0.03)	(0.03)	(0.04)	(0.04)			
50	1.65	0	-0.24	-0.26	-1.33	-0.78	0.995	0.04	464.4
	(0.05)	0	(0.04)	(0.03)	(0.05)	(0.05)			
40	2.04	0	-0.25	-0.30	-1.51	-0.94	0.995	0.04	460.6
	(0.06)	0	(0.04)	(0.04)	(0.06)	(0.06)			
30	2.45	0	-0.23	-0.32	-1.69	-0.98	0.994	0.05	383.0
	(0.08)	0	(0.05)	(0.05)	(0.07)	(0.08)			
20	2.81	0	-0.16	-0.36	-1.76	-1.02	0.992	0.06	276.4
	(0.11)	0	(0.07)	(0.06)	(0.09)	(0.10)			
10	3.08	0	-0.12	-0.39	-1.65	-1.23	0.985	0.08	150.2
	(0.15)	0	(0.09)	(0.08)	(0.11)	(0.12)			
5	3.17	0	-0.12	-0.43	-1.57	-1.25	0.985	0.08	143.3
	(0.15)	0	(0.09)	(0.08)	(0.12)	(0.13)			
1	3.32	0	0	-0.48	-1.56	-1.39	0.980	0.09	152.0
	(0.16)	0	0	(0.09)	(0.13)	(0.15)			

stationary phase.

System constant values at low organic compositions approach similar values among the four solvents indicating that the properties of water dominate retention at these compositions; however, as organic solvent is added to the aqueous phase, differences in the shapes of the curves indicate changes in system retention behavior affected through differences in solvent properties. The most notable changes in this case are seen in the curvature of the b-constant. For methanol the curvature is slight but becomes increasingly more significant with acetonitrile, isopropanol and tetrahydrofuran,

Table 3.16. System constants, butylsiloxane bonded phase, acetonitrile.

ACN % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
40	1.35	0	-0.10	-0.29	-1.43	-0.43	0.993	0.04	389.7
	(0.06)	0	(0.04)	(0.04)	(0.05)	(0.06)			
30	1.99	0	-0.19	-0.31	-1.82	-0.60	0.993	0.05	375.0
	(0.07)	0	(0.05)	(0.05)	(0.07)	(0.07)			
20	2.53	0.21	-0.35	-0.30	-2.04	-0.71	0.996	0.05	481.6
	(0.10)	(0.06)	(0.07)	(0.05)	(0.10)	(0.08)			
10	2.92	0.23	-0.33	-0.28	-2.01	-0.91	0.996	0.05	457.8
	(0.10)	(0.07)	(0.07)	(0.06)	(0.10)	(0.08)			
5	3.15	0.15	-0.25	-0.29	-1.89	-1.05	0.994	0.06	297.0
	(0.13)	(0.08)	(0.09)	(0.07)	(0.12)	(0.10)			
1	3.24	0.12	-0.23	-0.42	-1.48	-1.15	0.992	0.07	214.0
	(0.14)	(0.09)	(0.10)	(0.08)	(0.14)	(0.11)			

Table 3.17. System constants, butylsiloxane bonded phase, isopropanol.

IPA % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*r*	*stand'd error*	*F statistic*
40	1.00	0.14	-0.40	-0.27	-1.06	-0.40	0.988	0.05	152.5
	(0.08)	(0.05)	(0.07)	(0.05)	(0.09)	(0.07)			
30	1.93	0	-0.42	-0.27	-1.72	-0.51	0.994	0.05	392.9
	(0.08)	0	(0.05)	(0.05)	(0.08)	(0.07)			
20	2.71	0.19	-0.45	-0.28	-2.30	-0.78	0.994	0.07	295.8
	(0.14)	(0.09)	(0.10)	(0.07)	(0.13)	(0.11)			
10	3.03	0.21	-0.31	-0.33	-2.27	-0.96	0.992	0.08	230.3
	(0.16)	(0.10)	(0.11)	(0.08)	(0.10)	(0.12)			
5	3.15	0.23	-0.37	-0.30	-1.87	-1.04	0.991	0.08	187.8
	(0.16)	(0.11)	(0.11)	(0.08)	(0.17)	(0.12)			
1	3.27	0	0	-0.40	-1.60	-1.27	0.989	0.08	138.2
	(0.12)	0	0	(0.07)	(0.11)	(0.11)			

Table 3.18. System constants, butylsiloxane bonded phase, tetrahydrofuran.

THF % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*r*	*stand'd error*	*F statistic*
40	0.99	0.10	-0.24	0	-1.25	-0.18	0.983	0.05	146.0
	(0.08)	(0.05)	(0.07)	0	(0.08)	(0.07)			
30	1.82	0	-0.23	-0.16	-2.09	-0.32	0.990	0.06	238.6
	(0.09)	0	(0.06)	(0.06)	(0.09)	(0.09)			
20	2.56	0.20	-0.31	-0.13	-2.72	-0.59	0.993	0.07	252.3
	(0.12)	(0.09)	(0.10)	(0.08)	(0.14)	(0.11)			
10	2.75	0.45	-0.46	-0.16	-2.72	-0.61	0.995	0.07	326.1
	(0.15)	(0.10)	(0.10)	(0.08)	(0.14)	(0.11)			
5	2.90	0.45	-0.48	0	-2.38	-0.72	0.993	0.08	310.0
	(0.16)	(0.09)	(0.11)	0	(0.14)	(0.13)			
1	3.25	0.25	-0.33	-0.13	-1.93	-1.12	0.991	0.08	198.8
	(0.16)	(0.11)	(0.11)	(0.09)	(0.16)	(0.13)			

indicating the relative abilities of the organic solvent/water combinations to compete with the stationary phase for hydrogen-bond acidic interactions.

3.4.4. Light-loaded octadecylsiloxane bonded phase.

The LL C_{18} phase shows characteristics very similar to the C_4 phase, with the most notable difference being the larger m-constant values reflecting the increased retention with increasing ligand alkyl chain length. The increase in retention was sufficient to allow measurement of retention data for higher organic proportions (up to 80% v/v organic solvent) than was possible with the shorter-chain phases. The LL C_{18} phase reflects the same trends as seen with the C_4 phase in the b-constant; the curvature is more pronounced for acetonitrile than with methanol. Numerically, the b-constants for the C_4 and C_{18} phases are very similar--a finding that makes sense because a simple

Table 3.19. System constants, light loaded octadecylsiloxane bonded phase, methanol.

MeOH % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
80	1.10	0	-0.16	-0.30	-0.80	-0.58	0.979	0.05	91.2
	(0.09)	0	(0.06)	(0.06)	(0.08)	(0.08)			
70	1.40	0.07	-0.24	-0.33	-1.11	-0.52	0.991	0.04	230.2
	(0.07)	(0.05)	(0.06)	(0.05)	(0.08)	(0.06)			
60	1.71	0.12	-0.33	-0.34	-1.32	-0.42	0.993	0.05	268.7
	(0.10)	(0.07)	(0.07)	(0.05)	(0.09)	(0.07)			
50	2.03	0.16	-0.40	-0.34	-1.51	-0.35	0.992	0.06	232.6
	(0.12)	(0.08)	(0.08)	(0.06)	(0.11)	(0.09)			
40	2.46	0.21	-0.42	-0.37	-1.77	-0.40	0.994	0.06	330.8
	(0.12)	(0.08)	(0.08)	(0.06)	(0.11)	(0.09)			
30	2.95	0.18	-0.42	-0.38	-1.92	-0.48	0.995	0.06	407.7
	(0.12)	(0.08)	(0.08)	(0.06)	(0.11)	(0.09)			
20	3.21	0.18	-0.36	-0.42	-1.90	-0.52	0.996	0.06	451.9
	(0.11)	(0.08)	(0.08)	(0.06)	(0.11)	(0.09)			
10	3.47	0.17	-0.32	-0.47	-1.71	-0.61	0.996	0.06	454.9
	(0.11)	(0.08)	(0.08)	(0.06)	(0.11)	(0.09)			
5	3.82	0	-0.18	-0.48	-1.68	-0.80	0.991	0.07	242.8
	(0.16)	0	(0.09)	(0.07)	(0.11)	(0.14)			
1	3.92	0	-0.11	-0.54	-1.53	-0.90	0.991	0.07	250.3
	(0.15)	0	(0.08)	(0.07)	(0.10)	(0.13)			

change in the alkyl chain length of the bonded ligand should not affect hydrogen bonding interactions. At higher organic solvent compositions, the ranges of system constants with methanol are wider than with acetonitrile, indicating that acetonitrile offers more potential for changing selectivity by changing the mobile phase composition than is attainable by making the same change in composition with methanol.

Table 3.20. System constants, light loaded octadecylsiloxane bonded phase, acetonitrile.

ACN % comp (v/v)	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*
80	0.58 (0.16)	0.15 (0.11)	-0.14 (0.11)	-0.39 (0.08)	-0.45 (0.15)	-0.44 (0.12)	0.925	0.08	22.5
70	0.82 (0.07)	0 0	-0.10 (0.04)	-0.35 (0.04)	-0.71 (0.05)	-0.27 (0.06)	0.983	0.04	147.4
60	0.96 (0.06)	0.08 (0.04)	-0.23 (0.04)	-0.39 (0.03)	-0.94 (0.05)	-0.09 (0.04)	0.995	0.03	336.2
50	1.20 (0.72)	0.08 (0.05)	-0.16 (0.05)	-0.43 (0.04)	-1.14 (0.07)	-0.06 (0.06)	0.994	0.04	334.5
40	1.56 (0.09)	0.12 (0.06)	-0.22 (0.06)	-0.46 (0.05)	-1.48 (0.08)	-0.02 (0.07)	0.994	0.04	355.6
30	2.11 (0.10)	0.16 (0.07)	-0.28 (0.07)	-0.49 (0.05)	-1.89 (0.09)	-0.07 (0.07)	0.996	0.05	470.5
20	2.74 (0.10)	0.15 (0.07)	-0.34 (0.07)	-0.46 (0.05)	-2.18 (0.10)	-0.23 (0.08)	0.997	0.05	581.3
10	3.35 (0.09)	0.22 (0.06)	-0.41 (0.06)	-0.42 (0.04)	-2.22 (0.08)	-0.49 (0.06)	0.998	0.04	934.0
5	3.51 (0.13)	0.22 (0.09)	-0.30 (0.08)	-0.43 (0.06)	-1.96 (0.11)	-0.67 (0.11)	0.996	0.06	445.6
1	3.92 (0.15)	0 0	-0.16 (0.08)	-0.48 (0.07)	-1.68 (0.10)	-0.91 (0.13)	0.992	0.07	271.2

3.5. Comparison of system constants at 1% v/v methanol

The system constants at 1% v/v methanol for the four silica-based bonded phases are collected in **Table 3.21**. This mobile phase composition has been chosen as a reference solvent since the addition of a small amount of an organic solvent considerably improves the sample processing speed when large sample volumes are extracted. Exact

Table 3.21. System constants at 1% methanol for four sorbents.

sorbent	*m*	*r*	*s*	*a*	*b*	*c*
LL C_{18}	3.92	0	-0.11	-0.54	-1.53	-0.90
C_4	3.36	0	0	-0.46	-1.53	-1.38
CN	2.06	0.53	0	-0.51	-1.45	-0.88
DIOL	1.57	0.61	0	-0.45	-0.80	-1.05

measurements of the retention factor with 1% v/v organic solvent can be made by HPLC, while, when pure water is used, peak profiles are often highly distorted. The presence of a small amount of organic solvent is probably also closer to true experimental conditions since the interphase region will generally contain some residual organic solvent from the conditioning step, even when pure water is used as the sample solvent. With a small amount of organic solvent in the sample solvent, the sampling conditions remain stable while the breakthrough volume is not significantly affected [17]. Measurements of retention at 1% v/v organic solvent can, therefore, serve as rough estimates of k_W.

3.5.1. Retention in reversed-phase systems

The general picture that emerges from **Table 3.21** for sorbent extraction from predominantly aqueous solution is that the dominant contribution to retention is the ease of cavity formation in the solvated sorbent with a smaller contribution from lone-pair electron interactions (positive m-constants supported by positive r-constants in some cases). The driving force for retention is expulsion of the analyte from the hydrogen-bonded water network; and the characteristic property that distinguishes individual solvated sorbents is their cohesion. The CN and DIOL groups are more cohesive than those with simple bonded alkyl chains resulting in less favorable conditions for solute transfer from aqueous solution (smaller m-constant). The CN and DIOL phases are also

more able to participate in n- and π-electron interactions (positive r-constant) than the hydrocarbon phases.

Both hydrogen-bond bases and hydrogen-bond acids are less well retained than neutral molecules of a similar size because none of the solvated sorbents are competitive with water for these interactions (a- and b-constants are always negative). In particular, water is such a strong hydrogen-bond acid that the conditions are always less favorable for the retention of hydrogen-bond bases than for nonpolar analytes. Of all the sorbents, the DIOL phase competes most effectively with water for these interactions, but even the presence of two hydroxyl groups cannot match the hydrogen-bonding capabilities of water. The increased hydrogen-bond acidity of the CN phase over the C_4 and C_{18} phases is possibly a result of selective sorption of methanol or could reflect the presence of acidic amide and carboxylic acid groups introduced into the bonded phase during synthesis [149].

The most surprising result is the influence of dipole-type interactions on retention. For three sorbents (CN, DIOL, and C_4) the s-constant is zero while for the LL C_{18} it is negative. For the two sorbents generally regarded as polar phases, there is no preference for dipole-type interactions in the solvated sorbent or sample solution, a balance of properties that is difficult to explain, but suggests that the polar functional groups of the solvated sorbent are selectively solvated by water in the interphase region, negating their influence on sorbent selectivity. In the case of the LL C_{18} sorbent, the negative s-constant suggests that there is less uptake of water in the interphase region in this case and dipole-type interactions favor the most polar entity in the system, water, resulting in reduced retention in the sorbent.

3.5.2. Sorbent selection

The solvation parameter model allows the influence of individual intermolecular interactions on retention to be identified and quantified. Representative data for the

individual interaction contributions to retention for some varied solutes on the four cartridge sorbents are summarized in **Table 3.22**. Data for a high-loaded C_{18} sorbent [15], a macroreticular porous polymer sorbent [94], and a porous graphitic carbon sorbent [150] have been included for comparison. The results for the HL C_{18} and porous graphitic carbon sorbents were obtained via extrapolation methods and are not likely to be as well defined as the constants for the other sorbents.

Knowledge of the system constants provides knowledge of how any given compound with known descriptors will behave with a given solvated stationary phase. Inspection of the tabulated data leads to the conclusion that there are no general circumstances for which selection of one of the polar bonded sorbents would provide increased retention compared to one of the C_{18} sorbents with water as the sample solvent. The higher cohesion of the polar bonded sorbents is never adequately compensated by the capacity of the sorbent to compete with water for polar interactions. Therefore, when the desire is to increase retention (obtain a larger breakthrough volume) for extraction from water using bonded phases, a C_{18} sorbent is the best choice. On the other hand, for larger molecules which have a breakthrough volume well in excess of the sample volume, a polar bonded sorbent might be a better choice to enhance recovery in a small volume of solvent. The porous graphitic carbon sorbent provides more retention than any of the bonded phases and the macroreticular porous polymer sorbent generally gives the greatest retention of all. These latter sorbents would be useful for extracting small, relatively polar molecules for which retention on bonded phases is insufficient.

3.5.3. Solvent effects

Under reversed-phase conditions, porous polymer sorbents have been shown to undergo significant changes in selectivity with different processing solvents due to their propensity to selectively adsorb the organic solvent from water [73, 94]. The system constants for the CN phase for 1% v/v organic solvent in **Table 3.23** indicate that these

Table 3.22. Contribution of intermolecular interactions to retention of some varied solutes on various sorbents at 1% v/v methanol. HL C_{18} = high loaded octadecylsiloxane bonded phase [15]; PLRP-S = poly(styrene-divinylbenzene) macroporous polymer sorbent [94]; PGC = porous graphitic carbon calculated from data in [150].

compound	*sorbent*	*contribution to log k*						*predicted*
		mV_x	rR_2	$s\pi_2^H$	$a\alpha_2^H$	$b\beta_2^H$	c	*log k*
n-propylbenzene	HL C_{18}	6.44	0.42	-0.38		-0.49	-1.18	4.81
	LL C_{18}	4.46		-0.06		-0.23	-0.90	3.27
	C4	3.82				-0.23	-1.38	2.22
	CN	2.20	0.33			-0.22	-0.88	1.44
	DIOL	1.79	0.37			-0.12	-1.05	0.99
	PLRP-S	5.95	0.51	-0.25		-0.60	-0.18	5.43
	PGC	5.29				-0.20	-1.60	3.49
benzonitrile	HL C_{18}	4.92	0.52	-0.84		-1.08	-1.18	2.34
	LL C_{18}	3.41		-0.12		-0.50	-0.90	1.89
	C4	2.93				-0.51	-1.38	1.04
	CN	1.68	0.40			-0.49	-0.88	0.71
	DIOL	1.37	0.45			-0.26	-1.05	0.51
	PLRP-S	4.55	0.62	-0.54		-1.32	-0.18	3.13
	PGC	4.04				-0.44	-1.60	2.00
acetanilide	HL C_{18}	6.29	0.61	-1.06	-0.20	-2.18	-1.18	2.28
	LL C_{18}	4.37		-0.15	-0.27	-1.03	-0.90	2.02
	C4	3.74			-0.23	-1.03	-1.38	1.11
	CN	2.15	0.47		-0.26	-0.98	-0.88	0.50
	DIOL	1.75	0.53		-0.23	-0.54	-1.05	0.47
	PLRP-S	5.81	0.73	-0.69	-0.70	-2.69	-0.18	2.28
	PGC	5.16			0.28	-0.89	-1.60	2.95

Table 3.21 continued on next page

Table 3.22. (continued) Contribution of intermolecular interactions to retention of some varied solutes on various sorbents at 1% v/v methanol. HL C_{18} = high loaded octadecylsiloxane bonded phase [15]; PLRP-S = poly(styrene-divinylbenzene) macroporous polymer sorbent [94]; PGC = porous graphitic carbon calculated from data in [150].

compound	*sorbent*	*contribution to log k*						*predicted*
		mV_x	rR_2	$s\pi_2^H$	$a\alpha_2^H$	$b\beta_2^H$	c	*log k*
phenol	HL C_{18}	4.38	0.56	-0.78	-0.24	-0.98	-1.18	1.76
	LL C_{18}	3.04		-0.10	-0.32	-0.47	-0.90	1.25
	C4	2.60			-0.28	-0.47	-1.38	0.47
	CN	1.49	0.43		-0.31	-0.44	-0.88	0.29
	DIOL	1.22	0.49		-0.27	-0.24	-1.05	0.15
	PLRP-S	4.05	0.68	-0.44	-0.83	-1.24	-0.18	2.04
	PGC	3.60			0.34	-0.41	-1.60	1.93
2-hexanone	HL C_{18}	5.47	0.09	-0.52		-1.66	-1.18	2.20
	LL C_{18}	3.79		-0.07		-0.78	-0.90	2.04
	C4	3.25				-0.78	-1.38	1.09
	CN	1.87	0.07			-0.75	-0.88	0.31
	DIOL	1.52	0.08			-0.41	-1.05	0.15
	PLRP-S	5.05	0.11	-0.33		-2.05	-0.18	2.60
	PGC	4.49				-0.68	-1.60	2.21

Table 3.23. System constants at 1% v/v organic solvent for four bonded phases and porous polymer sorbents [73,94].

a) CN

solvent	*m*	*r*	*s*	*a*	*b*	*c*
methanol	2.06	0.53	0	-0.51	-1.45	-0.88
acetonitrile	1.95	0.40	0	-0.34	-1.53	-0.77
isopropanol	1.86	0.43	0	-0.38	-1.54	-0.68
tetrahydrofuran	1.83	0.41	0	-0.30	-1.60	-0.68

b) DIOL

solvent	*m*	*r*	*s*	*a*	*b*	*c*
methanol	1.57	0.61	0	-0.45	-0.80	-1.05
acetonitrile	1.62	0.45	0	-0.30	-1.02	-1.02
isopropanol	1.80	0.44	0	-0.26	-1.20	-1.03
tetrahydrofuran	1.54	0.58	-0.25	-0.20	-0.82	-0.95

c) C_4

solvent	*m*	*r*	*s*	*a*	*b*	*c*
methanol	3.32	0	0	-0.48	-1.56	-1.39
acetonitrile	3.24	0.12	-0.23	-0.42	-1.48	-1.15
isopropanol	3.27	0	0	-0.40	-1.60	-1.27
tetrahydrofuran	3.25	0.25	-0.33	-0.13	-1.93	-1.12

d) LL C_{18}

solvent	*m*	*r*	*s*	*a*	*b*	*c*
methanol	3.92	0	-0.11	-0.54	-1.53	-0.90
acetonitrile	3.92	0	-0.16	-0.48	-1.68	-0.91

e) PLRP-S porous polymer sorbent

solvent	*m*	*r*	*s*	*a*	*b*	*c*
methanol	5.15	1.04	-0.38	-1.52	-3.84	-0.21
acetonitrile	5.11	0.68	-0.50	-1.22	-3.85	-0.03
isopropanol	5.31	0.88	-0.57	-1.40	-4.17	-0.15

f) porous polymer membrane disk

solvent	*m*	*r*	*s*	*a*	*b*	*c*
methanol	5.16	0.81	-0.65	-1.85	-2.93	-0.77
acetonitrile	5.72	-0.26	-0.35	-1.17	-2.81	-1.19
isopropanol	7.20	0.41	-0.34	-1.50	-4.53	-2.10
tetrahydrofuran	5.09	0	0	-0.92	-4.19	-1.00

effects are much smaller for silica-based sorbents. Application of the Student t-test to the system constants for the four organic solvents at a composition of 1% v/v in water indicates that the numerical differences between constants, although small, are significant at the 95% confidence level except for the b-constant (isopropanol and acetonitrile) and the m- and r-constants (isopropanol and tetrahydrofuran). In general, these differences seem to be too small to state confidently whether they represent differences in the selective solvation of the stationary phase by the organic solvent or simply modulation of the mobile phase selectivity due to characteristic differences in the solvent properties of the organic solvent; they may in fact, represent some combination of the two. For the DIOL phase, the small differences in the m- and c-constants between the four solvents are statistically significant at the 95% confidence level. Selectivity differences are more apparent, however, in the values of the b- and a-constants. These differences are only roughly in agreement with the capacity of the organic solvent for the indicated interactions. This is not surprising, since the composition of the interphase region cannot be defined and is unlikely to be identical for each co-solvent. The data for 1% v/v organic solvent in water for all the bonded phases stand in stark contrast to the results obtained with porous polymer sorbents and are a clear indication of a fundamental difference in behavior between sorbents of the porous polymer type and those with similar properties to the silica-based sorbents.

3.6. Normal phase systems

In a few instances retention data have been reported with a sufficient number of varied solutes with a single mobile phase composition [151-153] that a comparison to the reversed-phase data reported here for the polar bonded phases can be made. In the cases where the solvation parameter model can be applied, the system constants based on the solutes for which descriptors were available are summarized in **Table 3.24**. The driving force for retention under normal-phase conditions is the capacity of the solvated sorbent

Table 3.24. System constants for normal-phase separations on polar bonded stationary phases with 20% v/v diethyl ether-pentane as the mobile phase.

sorbent	*m*	*r*	*s*	*a*	*b*	*c*	*mult. r*	*stand'd error*	*F stat.*	*n*
amino	0	0.35 (0.08)	0	1.79 (0.17)	0.45 (0.21)	-0.65 (0.16)	0.941	0.096	43	21
CN	0	0	0.63 (0.06)	0	0.28 (0.14)	-0.91 (0.10)	0.916	0.083	57	25
CN	0	0	0.59 (0.06)	0	0	-0.74 (0.04)	0.900	0.088	98	25
DIOL	0	0	0.33 (0.08)	0.47 (0.14)	0.74 (0.18)	-0.29 (0.12)	0.831	0.107	16	26

for polar interactions. In contrast to reversed-phase systems, there is a levelling of the ease of cavity formation in the solvated stationary phase and the mobile phase such that the m-constant is generally insignificant. As would be expected, the driving force for retention on the aminopropylsiloxane-bonded phase is the high basicity of the solvated sorbent (a-constant). For the CN phase, the driving force for retention is the capacity of the solvated stationary phase for dipole-type interactions (s-constant). For the DIOL phase, the capacity of the sorbent to participate in hydrogen-bond interactions, particularly as a hydrogen-bond acid (b-constant) drives retention combined with a significant capacity for dipole-type interactions (s-constant). In effect, the reasons for selecting a particular sorbent for separation or extraction in reversed- and normal-phase systems can be seen to be completely different, with most of this difference attributable to the unique properties of water. There remains, however, a critical need for further studies in normal-phase chromatography with a wider range of mobile phase compositions to adequately characterize the influence of solvent type on selectivity.

3.7. Prediction of breakthrough volumes

Of the four sorbents investigated, the LL C_{18} provides the most retention and will, therefore, provide the largest breakthrough volumes in an SPE application. For this reason, it was chosen as the test sorbent for evaluating predictions of breakthrough volume and extraction conditions. The extraction of urinary estrogens was chosen as an application because of the complex matrix involved, the clinical interest in estrogen analysis, and because the estrogen structures and descriptors differ significantly from those of the compounds in the training set.

System constants for the LL C_{18} phase with methanol (**Table 3.19**) and descriptors for three estrogenic compounds (**Table 2.2**) were substituted into the solvation parameter model (equation 11) to give estimates of retention factor values for each compound at each mobile phase composition measured from 80% to 1% v/v methanol. The results are listed in logarithmic form in **Table 3.25**. Retention is greatest for estrone and decreases for β-estradiol and estriol, corresponding most apparently to

Table 3.25. Predicted retention for estrogens

MeOH % (v/v)	*log k_S estrone*	*log k_S β-estradiol*	*log k_S estriol*
80	0.400	0.287	-0.032
70	0.571	0.441	0.035
60	1.054	0.909	0.481
50	1.496	1.345	0.889
40	2.228	2.077	1.571
30	2.916	2.771	2.237
20	3.630	3.498	2.969
10	4.344	4.221	3.731
5	5.077	4.985	4.498
1	5.538	5.452	4.984

the increase in the number of hydroxyl groups in the estrogen structures.

Retention factor values were used to estimate breakthrough volumes for the three estrogens using the model set forth by Lovkist and Jonsson (equation 9) for sampling at low theoretical plate numbers. The other two measured parameters required by the model, the cartridge dead volume (V_m) and the number of theoretical plates in the system (N), were calculated from HPLC data by adjusting for differences in packing density and total volume between the column and cartridge. The adjusted dead volume for the cartridge is 0.548 ml and the number of theoretical plates over a range of flow rates is given in **Table 3.5**. The resulting estimates of breakthrough volumes at three different levels (0.1%, 1% and 10% breakthrough) are given in **Table 3.26**.

3.8. Measurement of breakthrough volume

To assess the accuracy of the predicted values, breakthrough volumes were determined experimentally for four methanol/water mobile phase compositions (80%, 70%, 60% and 50% v/v MeOH). Breakthrough volumes at mobile phase compositions below 50% v/v MeOH become very large (as expected from **Table 3.26**) and difficult to measure with the relatively small SPE cartridge devices. Measurement of breakthrough volumes was accomplished by passing varying amounts of solutions containing known amounts of the three estrogens and measuring percent recovery upon elution by internal standard quantitation.

3.8.1. Verification of elution volume

Since breakthrough depends on the amount of analytes recovered, the elution step in sample processing must be evaluated and optimized for complete recovery within a small volume of the chosen solvent. Samples with volumes well below the predicted breakthrough volumes and with known estrogen content were loaded onto SPE cartridges and eluted with varying volumes of methanol in 0.50 ml increments. **Table 3.27** shows

Table 3.26. Predicted breakthrough volumes, V_b (ml)

a) 0.1% breakthrough at 2.4 ml/min

% MeOH	*estrone*	*β-estradiol*	*estriol*
80	1.05	0.88	0.58
70	1.41	1.12	0.62
60	3.67	2.71	1.20
50	9.64	6.90	2.60
40	50.6	35.9	11.4
30	261	187	54.9
20	1350	995	295
10	7360	5540	1790
5	39800	32200	10500
1	115000	94300	32100

b) 1% breakthrough at 2.4 ml/min

% MeOH	*estrone*	*β-estradiol*	*estriol*
80	1.35	1.13	0.74
70	1.81	1.44	0.80
60	4.72	3.49	1.54
50	12.4	8.87	3.35
40	65.1	46.2	14.7
30	335	240	70.6
20	1740	1280	379
10	9470	7130	2310
5	51200	41400	13500
1	148000	121000	41300

c) 10% breakthrough at 2.4 ml/min

% MeOH	*estrone*	*β-estradiol*	*estriol*
80	1.90	1.59	1.04
70	2.56	2.04	1.13
60	6.67	4.92	2.18
50	17.5	12.5	4.73
40	91.9	65.1	20.7
30	473	339	99.6
20	2450	1800	535
10	13400	10100	3250
5	72200	58400	19000
1	209000	171000	58300

Table 3.27. Recovery of estrogens with varying volumes of elution solvent.

compound	*elution volume*	*expected recovery (μg)*	*average recovery (μg)*	*average percent recovery*
estrone	2 x 0.50 ml	60.0	54.0	89.9
	3 x 0.50 ml	60.0	61.5	102.5
	4 x 0.50 ml	60.0	60.0	100.0
	5 x 0.50 ml	60.0	62.6	104.3
β-estradiol	2 x 0.50 ml	61.2	51.9	84.8
	3 x 0.50 ml	61.2	62.5	102.1
	4 x 0.50 ml	61.2	60.8	99.3
	5 x 0.50 ml	61.2	63.7	104.1
estriol	2 x 0.50 ml	60.7	58.1	95.7
	3 x 0.50 ml	60.7	63.2	104.1
	4 x 0.50 ml	60.7	59.9	98.7
	5 x 0.50 ml	60.7	62.4	102.8

that the percent recovery is low when two 0.50 ml portions of methanol are used but reaches a plateau when three or more 0.50 ml portions are used. For subsequent extractions, the elution volume was held at 3 x 0.50 ml of methanol.

3.8.2. Comparison of measured and predicted breakthrough volumes

The breakthrough curves in Appendix C were established by plotting percent recovery against volume of solution passed through the cartridge. At low sample volumes, the recovery of estrogens is high, but as the breakthrough volume for each compound is exceeded, recovery falls of rapidly. Since pinpointing an exact volume where breakthrough occurs is difficult, **Figures 3.4-3.6** compare measured breakthrough volumes to predicted breakthrough volumes (**Table 3.26.c**) at the 10% level. Agreement between measured and estimated values is quite good for estrone and estriol except at the higher methanol percent mobile phases where the breakthrough volume is presumably too

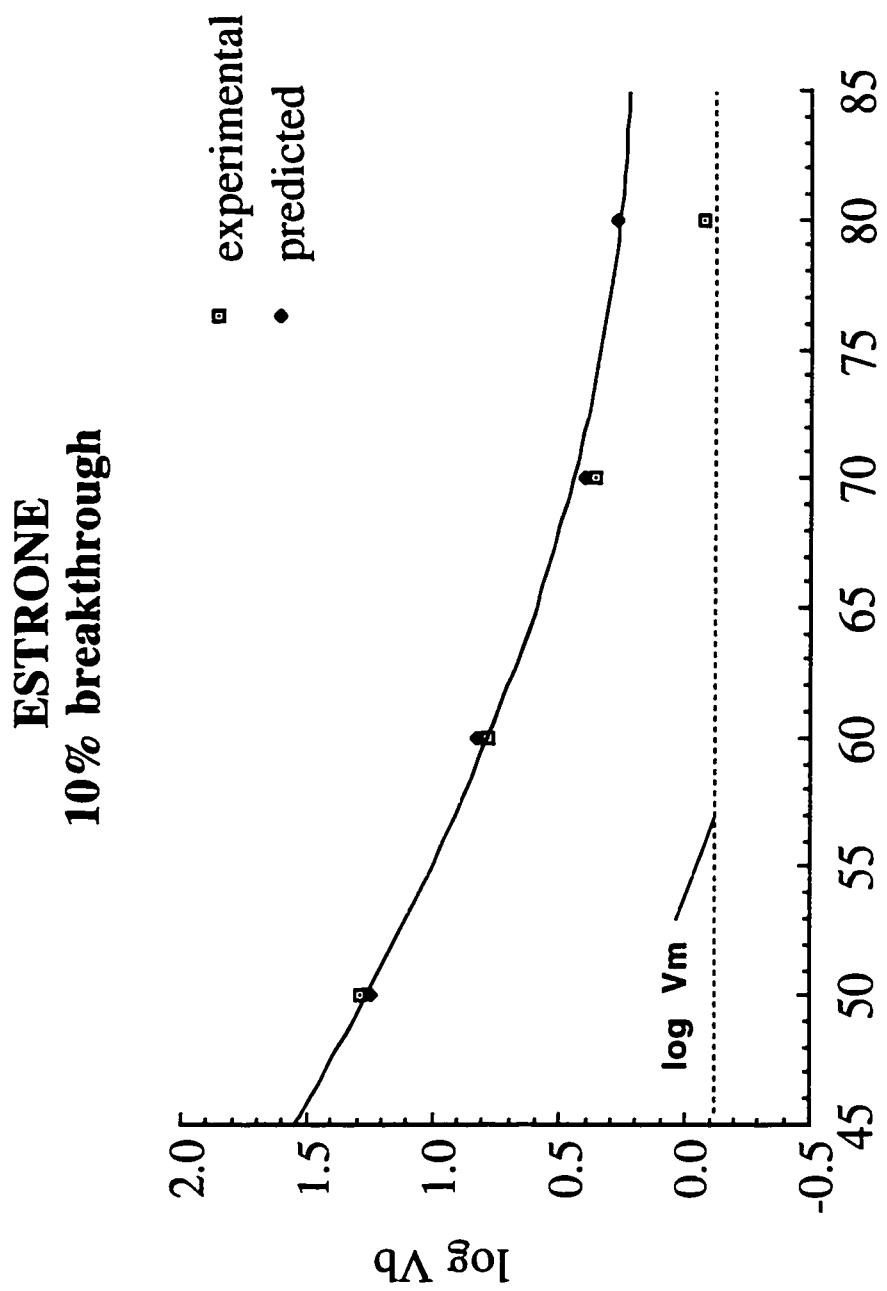


Figure 3.4. Comparison of estimated and predicted breakthrough volumes for estrone.

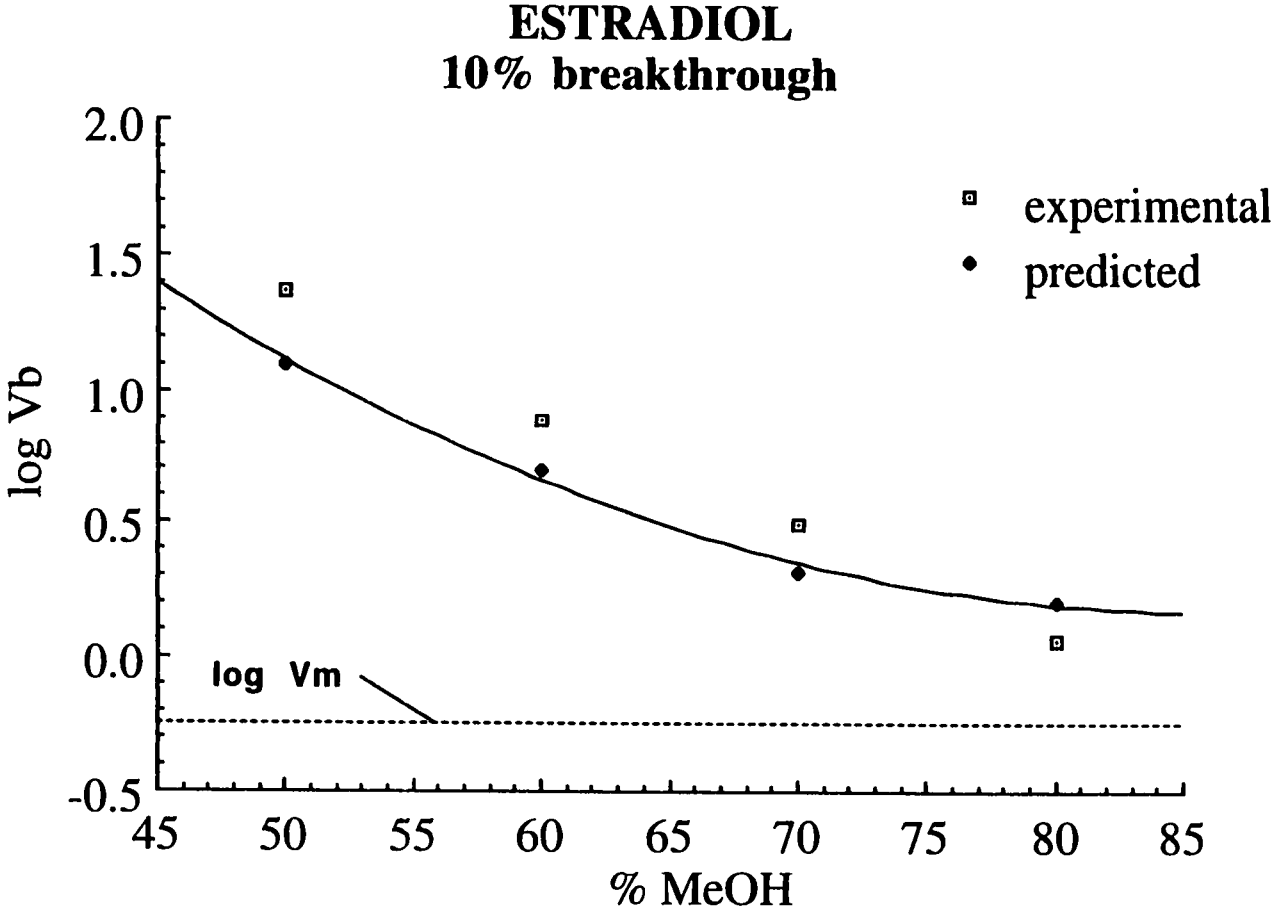


Figure 3.5. Comparison of estimated and predicted breakthrough volumes for β-estradiol.

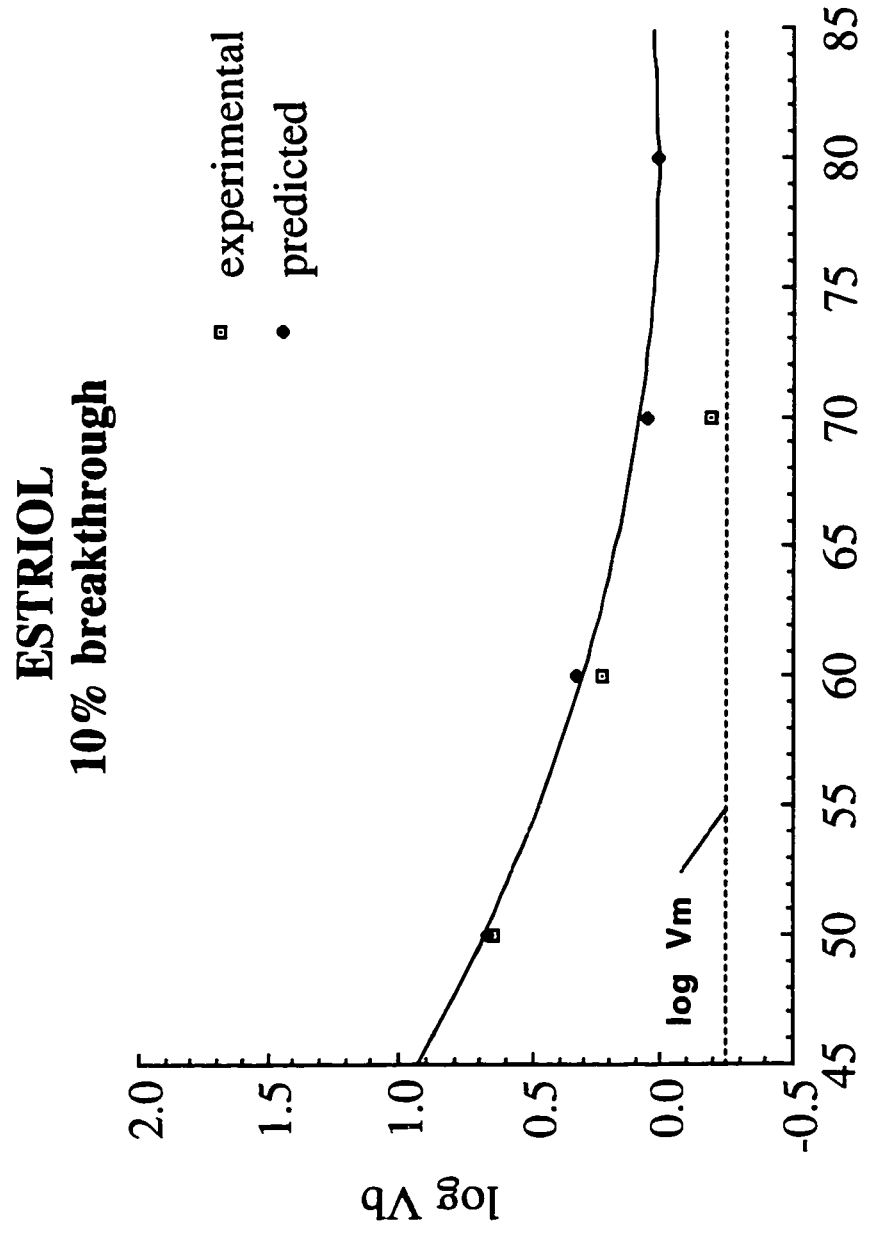


Figure 3.6. Comparison of estimated and predicted breakthrough volumes for estriol.

close to the dead volume of the cartridge to measure with certainty. Measured breakthrough volumes for estradiol are consistently higher than predicted values. The deviation of measured values from the predicted values for only one of the three compounds suggests that an error in the solute descriptors exists for that compound. The differences in the solute descriptors between the three compounds, however, appear to be logical based on structural differences.

3.9. Prediction of extraction conditions

The breakthrough volumes in **Table 3.26** can be used to predict conditions that are likely to yield a successful extraction of the estrogens from mainly aqueous solutions. The set of conditions chosen for the extraction of urinary estrogens are summarized in **Figure 3.7**. Any mobile phase where the breakthrough volume is significantly larger than the sample volume can be used for sample application to the cartridge (*e.g.*, referring to the table, for an intended sample volume of 50 ml, any mobile phase between 20% and 1% v/v methanol is suitable).

For wash solutions, a mobile phase with breakthrough volumes large enough to

Cartridges: BakerBond LL C18, 500 mg packing weight	
Conditioning:	6 ml methanol 6 ml 1% v/v methanol
Sampling:	45 ml hydrolyzed urine
Wash:	6 ml 40% v/v methanol
Elution:	3 x 0.5 ml methanol

Figure 3.7. Predicted conditions for extracting urinary estrogens.

ensure that a small volume of the mobile phase will not elute the components of interest should be chosen. Under this constraint, the mobile phase with the highest methanol content should be chosen to remove the maximum amount of matrix components, thus achieving the highest degree of matrix simplification possible. Using these criteria, 40% v/v MeOH was chosen for a wash solvent for the extraction of estrogens from mainly aqueous media. The wash solvent volume was chosen to be within a safe margin of the lowest predicted breakthrough volume at the 0.1% breakthrough level. For estriol, the least retained of the three estrogens, the predicted breakthrough volume is 11.4 ml, and 6.0 ml was chosen as the wash solvent volume.

Elution solvents should be mobile phases where the breakthrough volume is as small as possible to achieve the highest sample concentration effect. Although retention factors were not measured above 80% v/v methanol on the LL C_{18} sorbent, mobile phases in this region can be expected to have very small breakthrough volumes for the analytes and pure methanol was chosen for elution in this case. A rule of thumb for elution solvents is that the volume used should be 3-5 times the dead volume of the extraction device. Verification of the efficacy of 3 x 0.50 ml methanol as the elution solvent has been previously discussed.

3.10. Extraction of urinary estrogens

Although the above measurements of breakthrough volume agree well with the estimated values, a test of whether the same is true for real-world samples is necessary. In order to see if a complex matrix (here, urine) has any effect on the prediction of breakthrough volumes, estrogens were extracted from late pregnancy urine samples, spiked urine samples and Premarin tablets (processed pregnant mares' urine packaged for use in estrogen replacement therapy).

Table 3.28. Recovery of estrogens from spiked urine samples.

	estrone recovered (mg)	*estrone % rec*	*estradiol recovered (mg)*	*estradiol % rec*	*estriol recovered (mg)*	*estriol % rec*
spike level	0.150		0.156		0.150	
ml wash						
6.0 (ave, n=9)	0.143	95.6	0.153	97.9	0.152	101.2
6.0 (s.d.)	4.9 e-3	3.27	5.4 e-3	3.5	5.0 e-3	3.3
6.0 (r.s.d.)	3.4	3.4	3.5	3.5	3.3	3.3
8.0	0.147	98.0	0.147	94.2	0.143	95.3
10.0	1.445	96.3	0.157	100.5	0.151	100.7
12.0	0.140	93.3	0.135	86.5	0.085	56.7

3.10.1. Spiked urine samples

Recoveries of estrogens in spiked samples with varying volumes of wash solvent are shown in **Table 3.28**. Chromatograms for a spiked sample and the corresponding standards used for quantitation are shown in **Figures 3.8** and **3.9**, respectively. **Figure 3.10** is a chromatogram of a non-pregnant urine sample taken from the same pool as the spiked samples, in which a small amount of estradiol was detected. The estradiol was quantified and the amount found in non-spiked samples was subtracted from the amount found in the spiked samples to give the amount listed in **Table 3.28**. With 6.0 to 10.0 ml of wash solvent, acceptable recoveries were obtained indicating that appreciable analyte loss during sample application does not occur and that the analytes are fully eluted from the cartridge. These findings confirm that the predicted conditions are indeed appropriate for the sampling and elution processes. **Figures 3.11** and **3.12** are chromatograms of the original sample solution and the wash solution collected from the SPE process. These solutions were re-extracted by LLE and no estrogens were detected in either solution, underscoring the efficacy of the extraction process and confirming the hypothesis that the

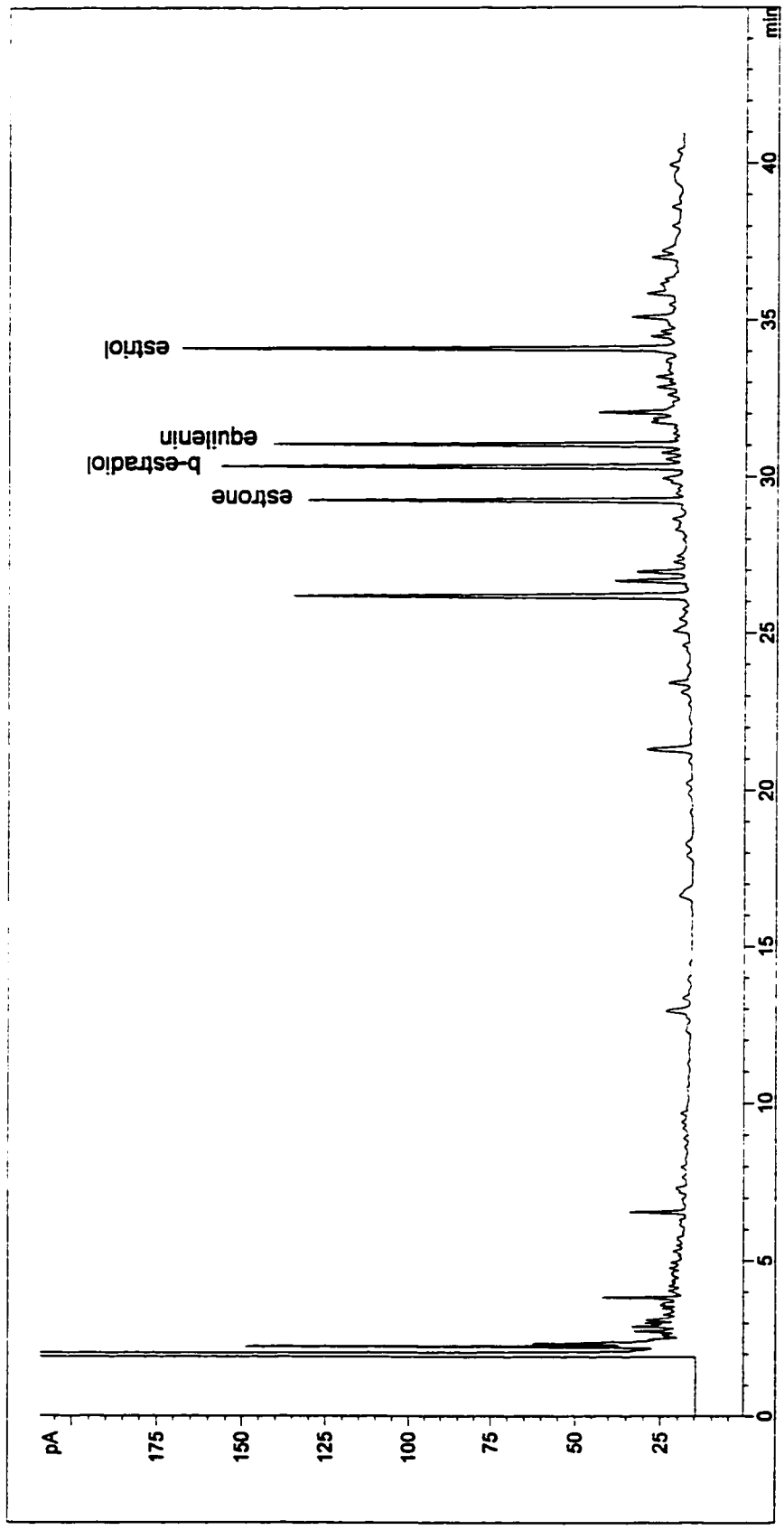


Figure 3.8. Chromatogram of a spiked non-pregnant urine sample.

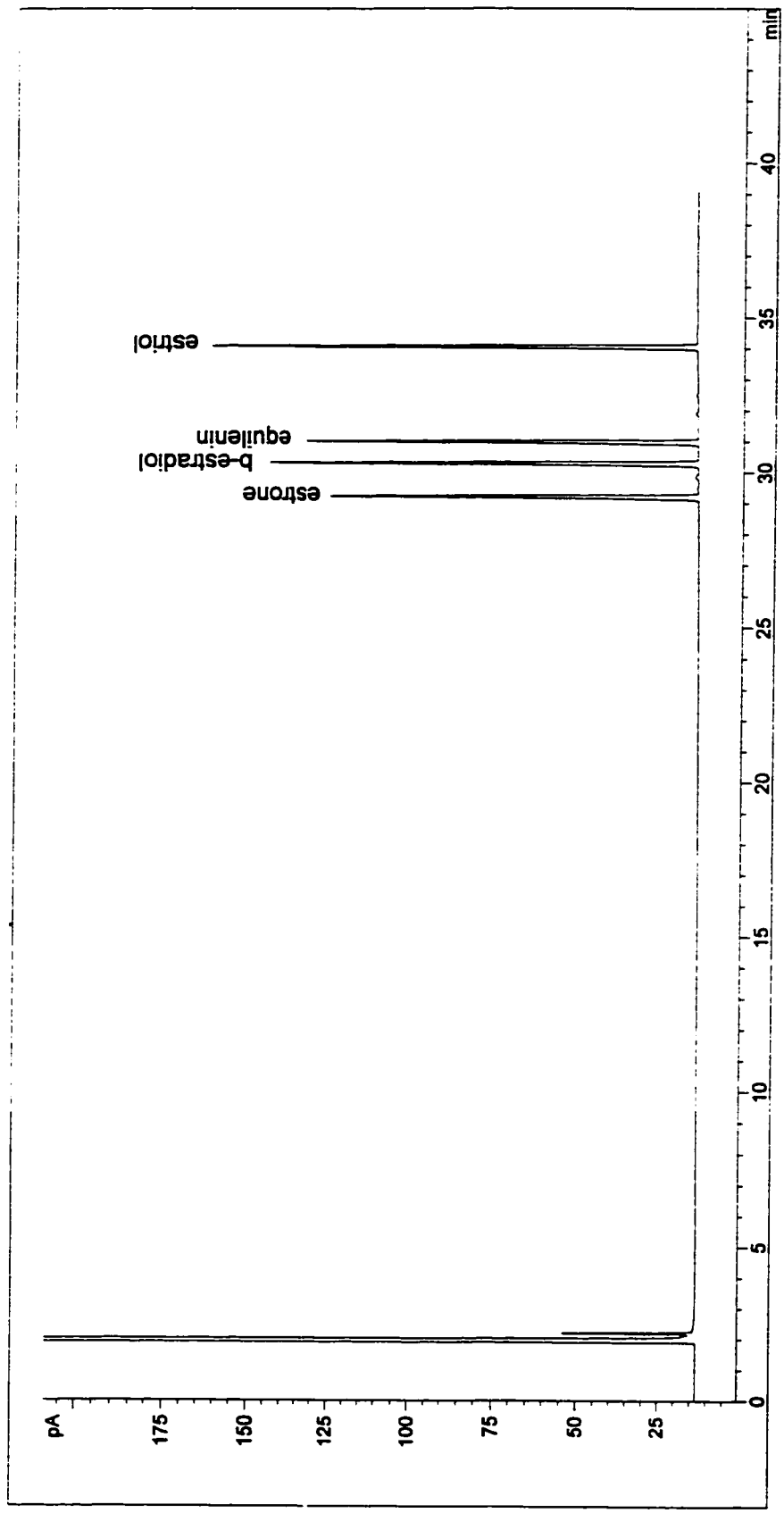


Figure 3.9. Chromatogram of standards used for quantitation for spiked urine samples.

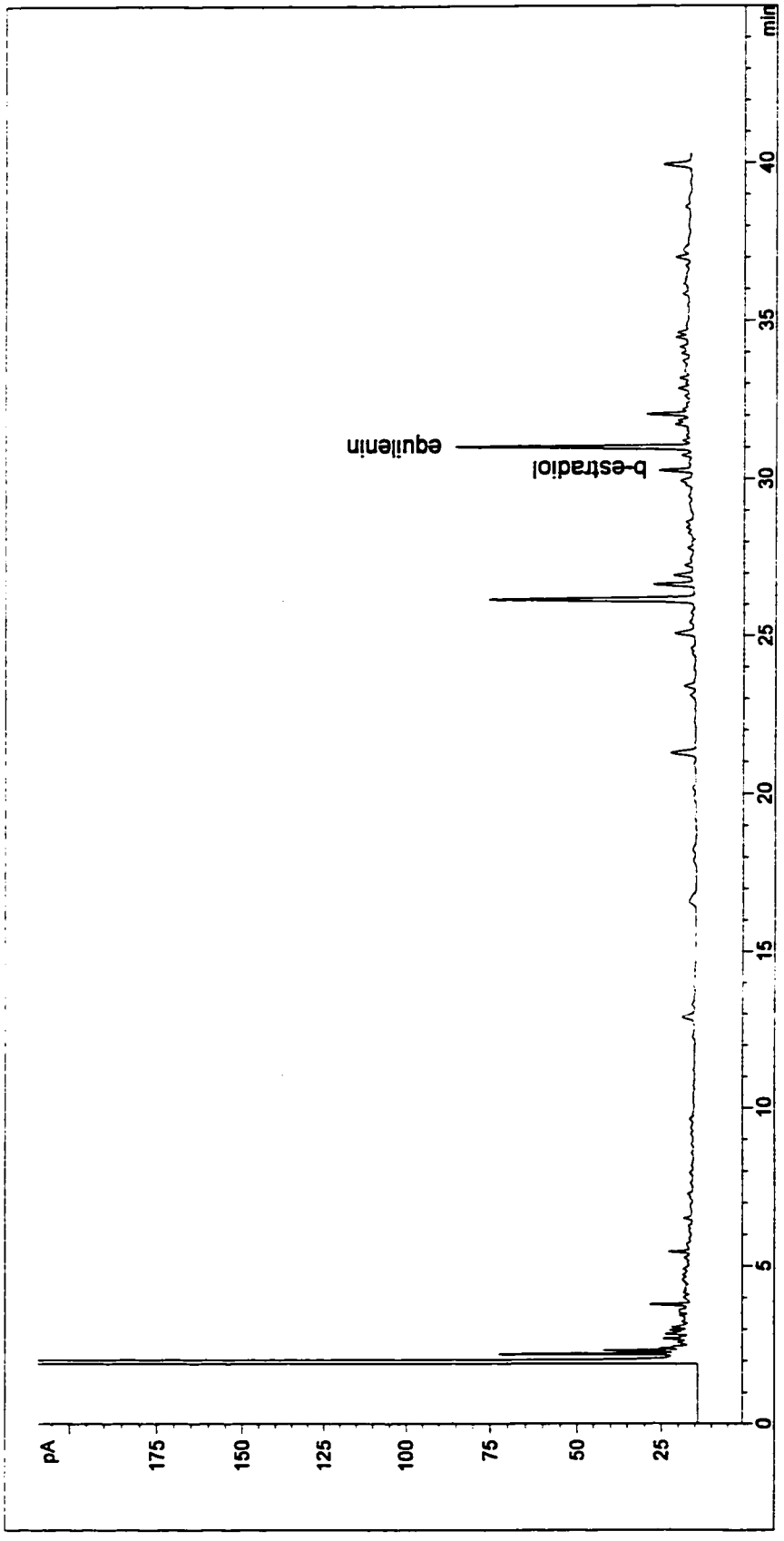


Figure 3.10. Chromatogram of urine sample prior to spiking. β-estradiol was detected in small amounts.

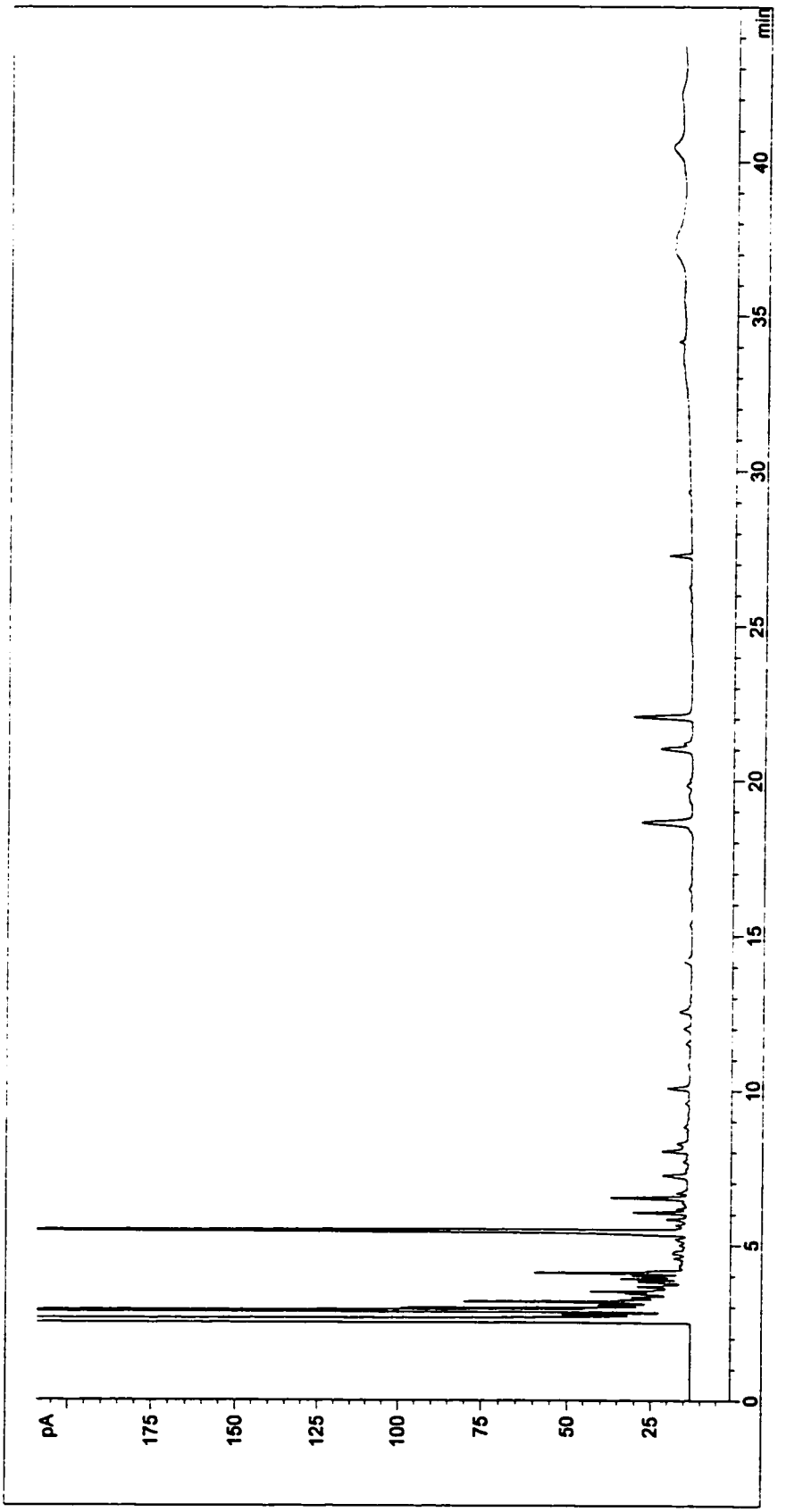


Figure 3.11. Sample solution collected from SPE re-extracted by LLE. No estrogens were detected.

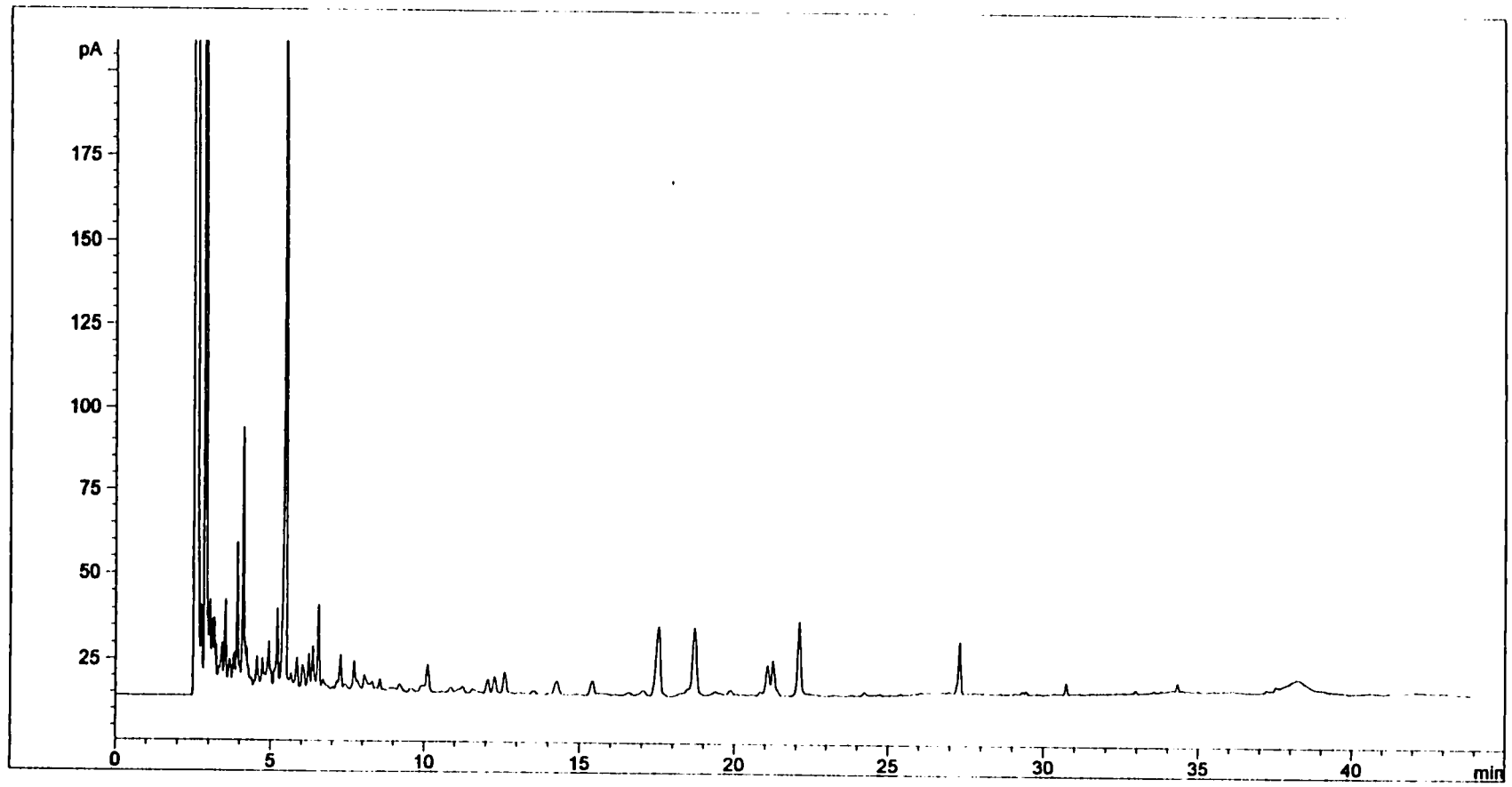


Figure 3.12. Wash solution collected from SPE re-extracted by LLE. No estrogens were detected.

wash solvent (volume = 6.0 ml) does not, in fact, elute the analytes to any significant extent.

The prediction of the composition and volume of suitable wash solvents is not as straightforward as the prediction of sampling and elution conditions. With 12.0 ml of wash solvent, recoveries for estriol and estradiol decreased by about 43% and 13%, respectively. Referring to **Table 3.26**, for the case of estriol, 12.0 ml falls between the 0.1% and 1% breakthrough levels and significant breakthrough is not expected. With estradiol, the volume of wash solvent is well within the predicted breakthrough volume at all levels, but significant breakthrough is still observed. From these observations, the conclusion can be drawn that, while the selection of an appropriate mobile phase composition through the calculation of breakthrough volumes is easily accomplished, prediction of an exact volume of the chosen composition for the wash step is not a perfect process. While some fine tuning of this particular parameter is still necessary to achieve an optimized SPE method, the overall estimation of extraction conditions does provide valuable input on a starting point that is likely to succeed. Moreover, such predictions identify for the analyst conditions that most definitely will not work, eliminating the majority of the trial and error associated with method development.

3.10.2. Late pregnancy urine samples

Chromatograms for a late pregnancy urine sample and the standards used for its quantitation are shown in **Figures 3.13** and **3.14**, respectively. Results from the quantitation show that the sample contained 1.73 μg/ml estriol and 0.54 μg/ml estradiol. Estrone was not detected. For three cases reported in literature [104], amounts found are slightly higher for estriol but about the same for estradiol. Small amounts of estrone were detected in the cases reported in the literature. The chromatograms show many unidentified peaks that have similar properties to the estrogens (they respond similarly to extraction conditions and elute in the same region of the chromatogram). These

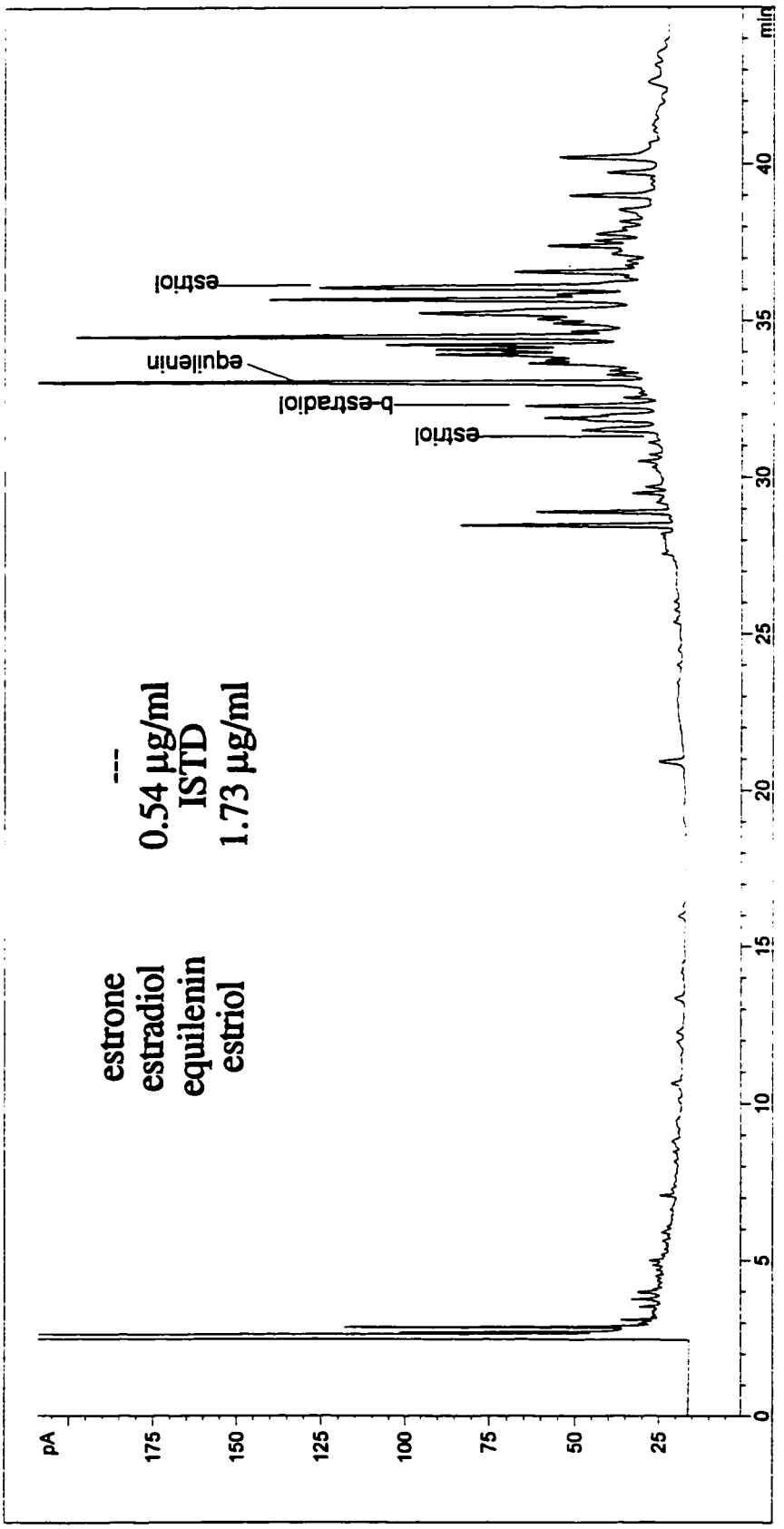


Figure 3.13. Chromatogram of late pregnancy urine. β-estradiol and estriol were detected at concentrations shown.

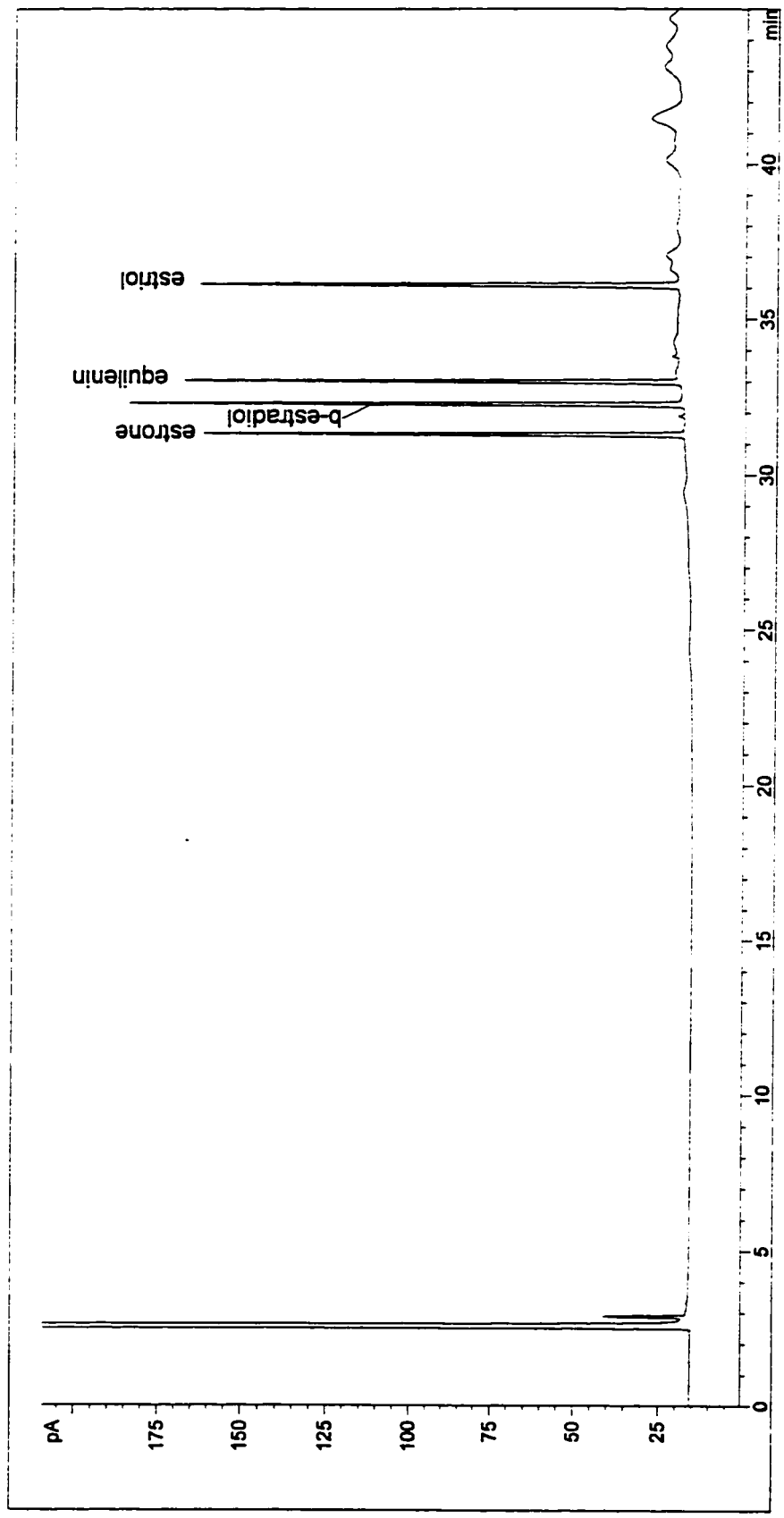


Figure 3.14. Chromatogram of standards used for quantitation of estrogens in late pregnancy urine samples.

compounds are presumably other steroid hormones and related compounds produced by the body during pregnancy. With positive identification of these substances comes the possibility of removing them during sample preparation through the same procedure of predicting breakthrough volumes used to characterize the behavior of the analytes.

3.10.3. Premarin tablets

Conjugated estrogens in Premarin tablets were hydrolyzed and extracted by SPE as well as by LLE. Chromatograms for a sample extracted by SPE and the corresponding standards are shown in **Figures 3.15** and **3.16**. Those for a sample extracted by LLE and the corresponding standards are shown in **Figures 3.17** and **3.18**. Baseline resolution for some critical pairs of compounds was not achieved. Despite the lack of optimization in the separation of all of the components, the chromatograms can still be useful; the response factors for the estrogens are all similar and the sample is well-characterized through prior investigation [111,117]. Quantitation results making use of known ratios of estrone and equilin are given in **Table 3.29**. The results for both methods fall within the USP guidelines for the pharmaceutical preparation and are similar to each other, confiming that the hydrolysis step, in particular (since estrogens are present as conjugates in the preparation), and the overall experimental procedure, in general, are suitable for analyzing these samples. The original sample solution and wash solution from the SPE procedure were collected for the Premarin samples and, as with the late pregnancy urine samples, no estrogens were detected in either solution after they were re-extracted by LLE.

3.11. Assessment of extraction conditions

Overall, the use of the predicted extraction conditions has lead to full recovery of spiked estrogens in a complex matrix, the successful extraction of analytes from real samples, and the successful extraction of analytes present in a well-characterized pharmaceutical product. Solvent consumption in this method has been reduced in

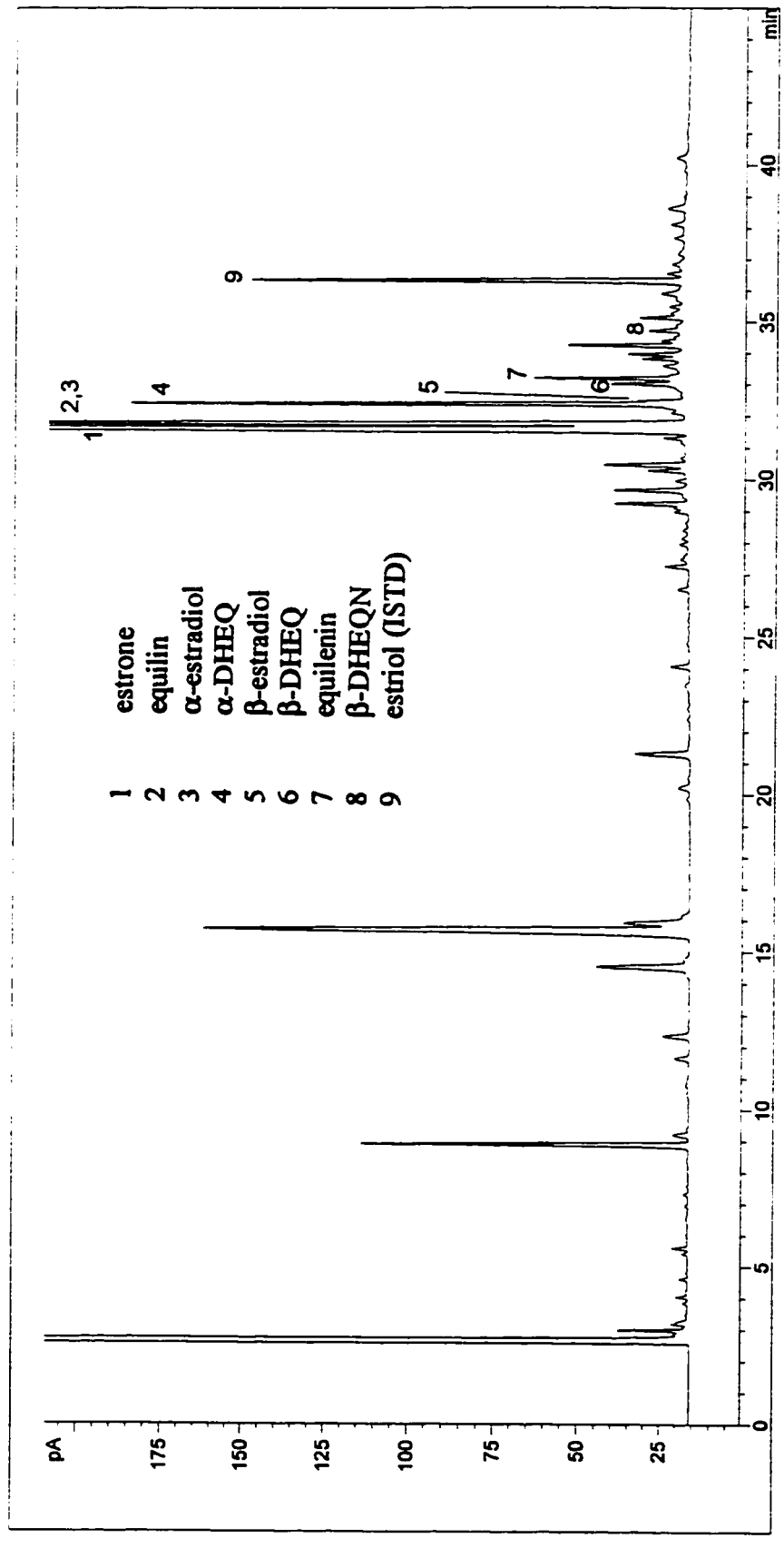


Figure 3.15. Chromatogram of estrogens extracted from Premarin tablets by SPE.

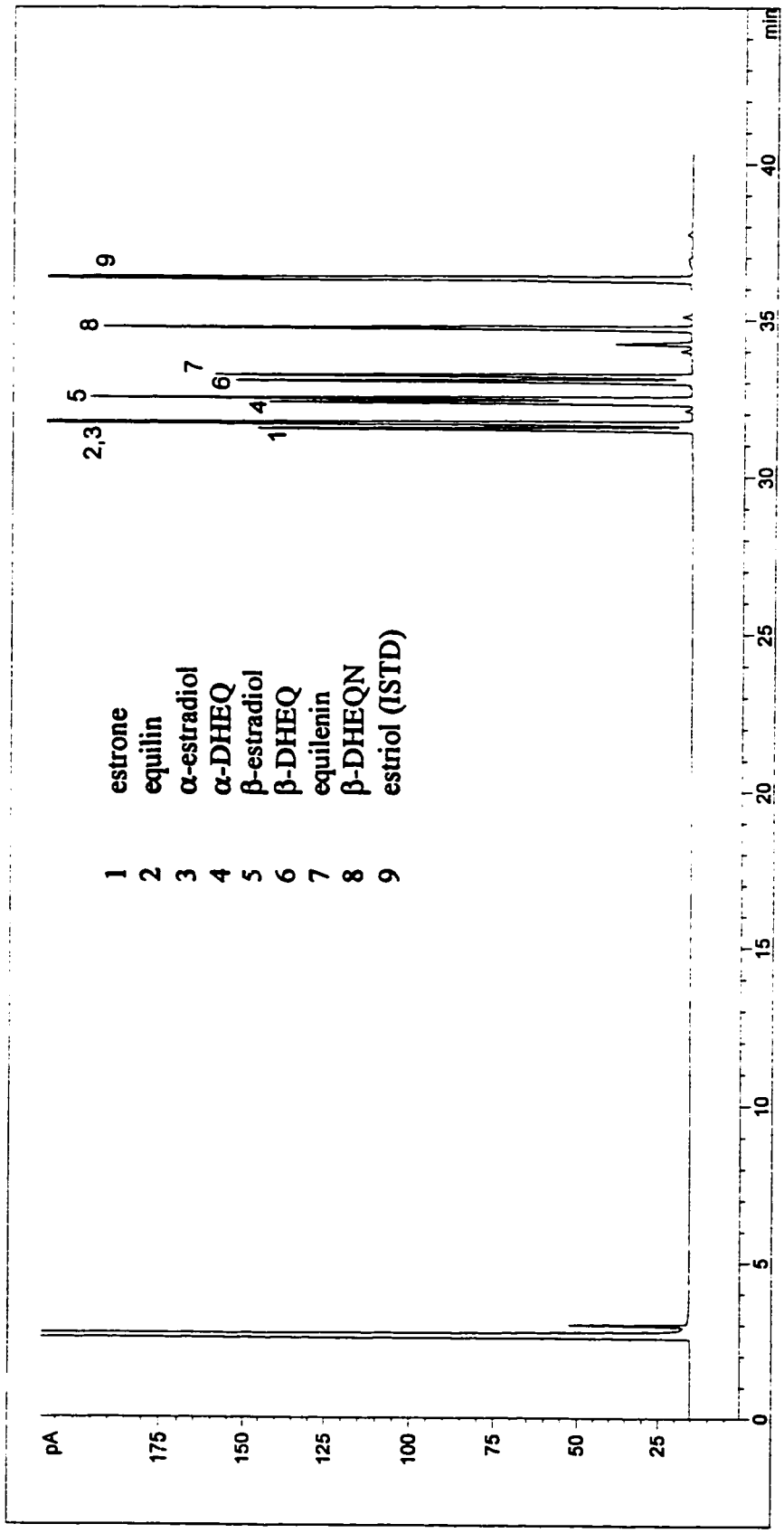


Figure 3.16. Standards used for quantitation of estrogens extracted from Premarin tablets by SPE.

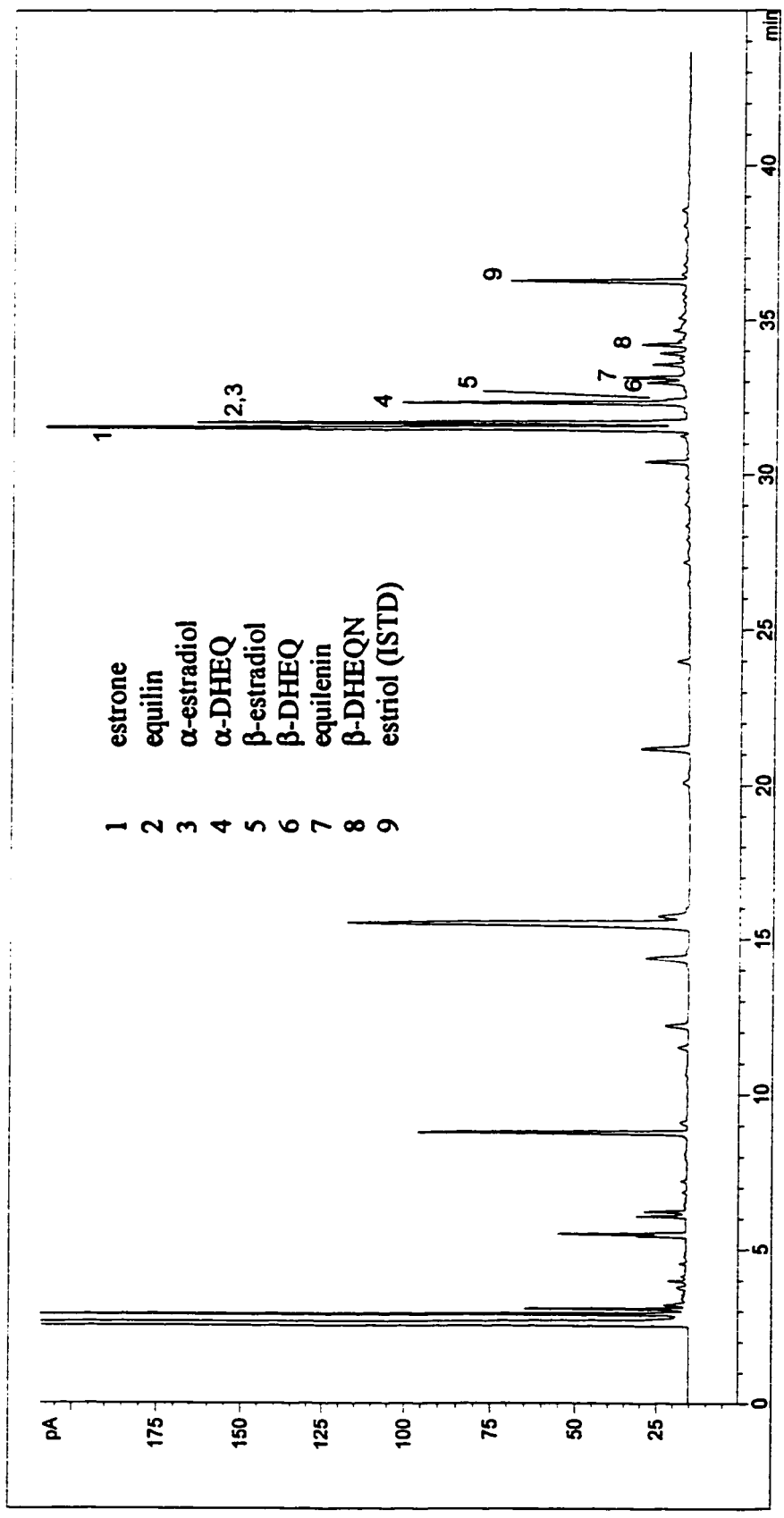


Figure 3.17. Chromatogram of estrogens extracted from Premarin tablets by LLE.

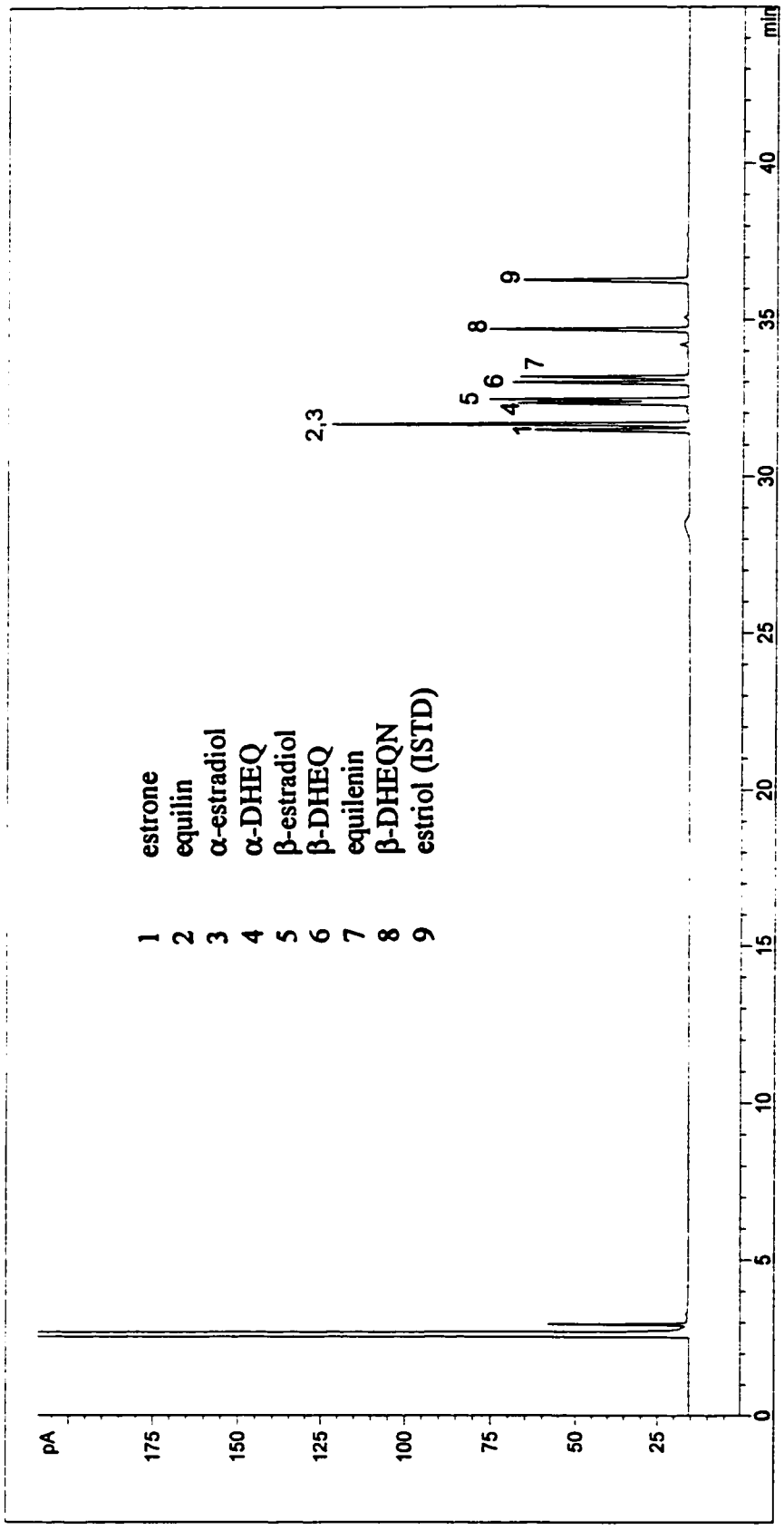


Figure 3.18. Standards used for quantitation of estrogens extracted from Premarin tablets by L.L.E.

Table 3.29. Estrogen quantitation in Premarin tablets (mg per tablet of conjugated estrogens).

compound	*LLE/GC*	*SPE/GC*
estrone	1.250	1.328
equilin	0.809	0.703
α-estradiol	0.014	0.012
α-dihydroequilin	0.441	0.401
β-estradiol	0.022	0.018
equilenin	0.098	0.077
β-dihydroequilin	0.077	0.067
β-dihydroequilenin	0.057	0.023
estriol	---	---
α-dihydroequilenin	---	---
total:	2.767	2.623 s.d. = 0.058 r.s.d. = 2.2% n = 4

comparison to previously published methods in that conditioning solvent volumes are not excessive and wash and elution solvents have been optimized. No chlorinated solvents are used. A glance at the chromatograms for the late-pregnancy urine samples, however, demonstrates that the method could stand to be optimized further to provide cleaner extracts. One approach to removing interferences is through the fractionation of sample components.

3.12. Fractionation of analytes

The significantly lower breakthrough volume predicted for estriol (see **Table 3.26**) suggests the possibility of adopting an extraction procedure based on fractionating the estrogens by stepwise elution with successively stronger solvents. For such a procedure

to be effective in practice, however, the difference between the breakthrough volumes of the components being separated should be so large that the entire amount of one analyte can be eluted while other analytes remain in the sorbent bed. For instance, inspection of the breakthrough volumes at 50% methanol for estriol at the 0.1 and 10% levels and for estradiol at the 0.1% level reveals that not much more than 10% of the first compound would be eluted before seeing breakthrough of the second. These circumstances would not lead to clean fractionation of the two compounds. Indeed, according to the predicted breakthrough volumes with methanol as a solvent, no set of conditions exists where a successful separation of estriol from the other sample components could be achieved.

In a sample preparation method proposed by Ji *et al.* [102], the preliminary extraction scheme calls for fractionation of the analytes (**Figure 1.17**), but no description of the method used to arrive at the experimental conditions was provided. System constants for the LL C_{18} sorbent with acetonitrile were used to make rough estimates of breakthrough volumes (**Table 3.30**) to assess the feasibility of the proposed method. The breakthrough volumes shown here should be treated carefully, as the amount and type of sorbent used to make the estimates differs from those used in the experiment. The dead volume and number of theoretical plates were estimated by adjustment from measured values for the LL C_{18} phase. For a truly reliable assessment, however, these measurements (along with retention factors and system constants) should be made for the particular sorbent used (which probably has a higher loading of C_{18} bonded ligand). Despite these differences, the tabulated estimates can still be useful, for if the breakthrough volumes are too large to achieve the extraction on the LL C_{18} cartridge, then using the same conditions with a packing with a higher carbon loading (which inherently offers more retention) will not work either.

At first glance, fractionation may seem possible in this case because the breakthrough volumes are significantly different for the three sample components. The proposed initial wash step involving 2 ml of 22% v/v acetonitrile is acceptable. The

Table 3.30. Estimated breakthrough volumes for estrogens on the LL C18 sorbent.

a) 0.1% breakthrough at 2.4 ml/min

% ACN (v/v)	*Vb estrone*	*Vb β-estradiol*	*Vb estriol*
80	0.15	0.13	0.11
70	0.24	0.20	0.13
60	0.24	0.19	0.12
50	0.67	0.49	0.22
40	1.47	1.01	0.35
30	6.03	3.95	0.99
20	33.4	22.4	4.99
10	294	210	52.7
5	1600	1240	376
1	13400	11100	3660

b) 1% breakthrough at 2.4 ml/min

% ACN (v/v)	*Vb estrone*	*Vb β-estradiol*	*Vb estriol*
80	0.22	0.19	0.15
70	0.35	0.29	0.19
60	0.34	0.27	0.17
50	0.95	0.70	0.31
40	2.10	1.44	0.50
30	8.60	5.64	1.42
20	47.6	31.9	7.13
10	419	300	75.2
5	2290	1780	537
1	19200	15800	5220

c) 10% breakthrough at 2.4 ml/min

% ACN (v/v)	*Vb estrone*	*Vb β-estradiol*	*Vb estriol*
80	0.37	0.32	0.26
70	0.59	0.49	0.33
60	0.58	0.46	0.29
50	1.62	1.18	0.54
40	3.58	2.46	0.85
30	14.7	9.60	2.42
20	81.1	54.4	12.1
10	714	510	128
5	3890	3020	914
1	32700	26900	8890

breakthrough volumes are high enough at this solvent composition that the small volume of wash solvent is not likely to elute the analytes. The first elution step (1 ml 30% v/v acetonitrile), however, is not likely to elute all of the first component, estriol. Indeed, estriol is more likely to be lost in the next step, a wash step using 40% v/v acetonitrile from which the eluent is discarded. Even this wash step is not likely to remove all of the estriol, and some of this component will likely be eluted along with the other sample components in the final fraction. Elution with 55% v/v acetonitrile does seem possible, at least for the LL C_{18} sorbent, because the breakthrough volume is very small at this solvent composition. It is likely that complete elution would take more than the 1 ml prescribed, however, and the final eluate would be more concentrated if a smaller volume of a stronger solvent were used.

While stepwise elution potentially offers more extensive removal of unwanted sample components, development of a set of conditions that will give complete elution of one or more components while leaving others securely retained is a challenging task. SPE, with its low numbers of theoretical plates, does not generally function in a separation mode as is required for fractionation of analytes. It is more effective when used in an on/off retention/elution mode, as is seen in the vast majority of its applications. Still, with careful optimization in cases where large differences in breakthrough volume for sample components exist, appropriate schemes for fractionation can be devised. The prediction of breakthrough volumes can save substantial amounts of time by steering analysts away from wash and elution conditions that will not work.

3.13. Conclusions

Solid-phase extraction has developed as a largely empirical method of sample preparation viewed in contemporary terms as an economical replacement for liquid-liquid extraction with favorable characteristics for automation. The theoretical models presented in this dissertation provide a more purposeful approach to understanding the fundamental

aspects of the technique. They provide a rational basis for the design of sampling devices, a simple means to standardize sorbents based on their retention characteristics, the basis on which to build models to predict retention for different sorbents, a means of identifying new sorbents with sampling properties that set them apart from materials already available, and an approach to developing expert systems to predict the optimum sampling conditions for any analyte. Although automation of SPE methods has seen significant progress in recent years, no attempt to use computer-aided software for the selection of extraction conditions or to automate the method development process itself has appeared. This research could serve as a model upon which to build a hardware/software system that is capable of independent sorbent evaluation, method development, and method optimization.

APPENDIX A: Retention factor values

Retention factor values for the four silica-based bonded phases with methanol, acetonitrile, isopropanol, and tetrahydrofuran as mobile phase organic solvents in water.

cyanopropylsiloxane	Table A.1.1 to A.1.4
propanediol	Table A.2.1 to A.2.2
butylsiloxane	Table A.3.1 to A.3.4
LL octadecylsiloxane	Table A.4.1 to A.4.2

Table A.1.1. Capacity factor values, cyanopropylsiloxane bonded phase, methanol

Solute	*100% MeOH*	*90% MeOH*	*80% MeOH*	*70% MeOH*	*60% MeOH*	*50% MeOH*	*40% MeOH*
naphthalene	0.23	0.34	0.66	0.96	1.64	3.35	6.35
2-hexanone	0.20	0.26	0.45	0.47	0.59	0.80	1.06
benzaldehyde	0.21	0.32	0.54	0.60	0.76	1.11	1.61
anisole	0.21	0.32	0.56	0.70	1.00	1.59	2.35
benzonitrile	0.21	0.32	0.54	0.62	0.83	1.29	1.90
bromobenzene	0.17	0.30	0.59	0.81	1.29	2.43	4.11
chlorobenzene	0.15	0.29	0.57	0.75	1.21	2.13	3.57
benzyl alcohol	0.14	0.23	0.44	0.45	0.56	0.79	1.02
chlorophenol	0.12	0.24	0.42	0.47	0.72	1.30	2.05
propylbenzene	0.15	0.25	0.56	0.82	1.42	2.93	5.46
acetophenone	0.20	0.31	0.52	0.58	0.78	1.21	1.78
1,2-dichlorobenzene	0.16	0.31	0.59	0.83	1.44	2.91	5.34
1-phenylethanol	0.13	0.25	0.44	0.49	0.71	0.98	1.29
4-cresol	0.11	0.23	0.43	0.50	0.72	1.07	1.61
phenol	0.13	0.24	0.40	0.47	0.63	0.92	1.23
benzamide	0.16	0.24	0.36	0.41	0.47	0.67	0.86
acetanilide	0.17	0.26	0.40	0.48	0.62	0.96	1.27
nitrobenzene	0.22	0.37	0.58	0.80	1.22	1.86	2.68

Solute	*30% MeOH*	*20% MeOH*	*10% MeOH*	*5% MeOH*	*1% MeOH*
naphthalene	13.03	22.50	34.38	45.06	58.54
2-hexanone	1.34	1.87	2.22	2.54	3.12
benzaldehyde	2.22	3.27	4.21	4.99	6.53
anisole	3.43	5.05	6.83	7.06	8.43
benzonitrile	2.60	3.85	4.67	5.07	6.44
bromobenzene	6.68	10.68	13.91	17.28	17.81
chlorobenzene	5.40	8.56	9.99	12.01	12.85
benzyl alcohol	1.32	1.62	1.87	2.05	2.44
chlorophenol	2.98	4.84	5.95	7.03	8.34
propylbenzene	10.33	17.32	22.50	27.83	32.34
acetophenone	2.66	4.18	5.61	6.96	9.28
1,2-dichlorobenzene	10.33	17.32	22.75	28.56	35.76
1-phenylethanol	1.77	2.30	2.65	2.88	3.39
4-cresol	2.26	3.51	4.43	4.69	5.48
phenol	1.65	2.13	2.44	2.64	2.98
benzamide	1.14	1.59	1.92	2.30	2.93
acetanilide	1.75	2.38	3.30	3.46	4.50
nitrobenzene	3.86	5.39	6.73	8.15	9.47

Table A.1.2. Capacity factor values, cyanopropylsiloxane bonded phase, acetonitrile

Solute	*50% ACN*	*40% ACN*	*30% ACN*	*20% ACN*	*10% ACN*	*5% ACN*	*1% ACN*
naphthalene	1.92	3.38	6.30	9.84	18.17	25.58	38.71
2-hexanone	0.99	1.26	1.53	1.59	1.78	2.08	2.63
benzaldehyde	1.17	1.67	2.09	2.37	2.89	3.46	4.58
anisole	1.40	2.08	2.97	3.48	4.32	5.02	7.25
benzonitrile	1.26	1.80	2.45	2.83	3.33	3.77	5.64
bromobenzene	1.76	2.88	4.62	6.37	9.52	11.18	14.50
chlorobenzene	1.69	2.75	4.39	5.68	7.32	8.75	11.21
benzyl alcohol	0.83	1.08	1.30	1.43	1.59	1.76	2.03
chlorophenol	1.12	1.66	2.37	2.94	3.96	4.69	6.08
n-propylbenzene	1.98	3.70	6.99	10.10	14.58	18.75	27.25
acetophenone	1.16	1.59	2.16	2.61	3.57	4.36	6.28
2-phenylethanol	0.93	1.22	1.58	1.83	2.15	2.45	2.89
4-cresol	1.04	1.46	1.94	2.21	2.91	3.31	4.54
phenol	0.94	1.25	1.59	1.71	1.93	2.16	2.47
benzamide	0.71	0.85	1.04	1.19	1.58	1.81	2.40
acetanilide	0.88	1.15	1.47	1.75	2.28	2.72	3.70
nitrobenzene	1.44	2.18	3.13	3.72	4.60	5.24	6.86
1,2-dichlorobenzene	1.86	3.31	5.49	8.42	14.67	18.89	25.71
1,2-dibromobenzene	2.02	3.75	7.10	11.58	21.87	32.24	49.00

Table A.1.3. Capacity factor values, cyanopropylsiloxane bonded phase, isopropanol

Solute	*50% IPA*	*40% IPA*	*30% IPA*	*20% IPA*	*10% IPA*	*5% IPA*	*1% IPA*
naphthalene	1.03	2.22	6.70	18.63	26.45	33.11	44.68
2-hexanone	0.59	0.91	1.30	1.63	1.72	1.95	2.96
benzaldehyde	0.74	1.20	1.85	2.85	3.17	3.63	5.14
anisole	0.80	1.45	2.69	4.28	4.88	5.52	7.29
benzonitrile	0.78	1.30	2.25	3.46	3.75	4.11	4.88
bromobenzene	0.85	1.76	4.75	8.41	8.98	9.39	11.29
chlorobenzene	0.81	1.66	4.52	9.81	11.17	12.68	15.29
benzyl alcohol	0.56	0.87	1.22	1.52	1.61	1.68	2.00
chlorophenol	0.61	1.10	2.13	3.86	4.50	5.24	6.71
n-propylbenzene	0.75	1.75	5.73	15.33	18.19	21.11	27.57
acetophenone	0.73	1.21	2.01	3.32	4.00	4.87	6.71
2-phenylethanol	0.57	0.95	2.38	2.07	2.15	2.33	2.74
4-cresol	0.54	0.95	1.70	2.74	3.11	3.55	4.61
phenol	0.53	0.90	1.40	1.99	2.11	2.25	2.54
benzamide	0.51	0.74	1.02	1.34	1.69	1.98	2.47
acetanilide	0.58	0.92	1.41	2.07	2.50	2.83	3.76
nitrobenzene	0.92	1.70	3.13	4.93	5.28	5.71	7.26
1,2-dichlorobenzene	0.77	1.70	5.29	15.63	19.29	22.07	26.57
1,2-dibromobenzene	0.88	1.96	6.24	23.00	33.39	40.29	59.18
1-naphthol	0.72	1.44	3.70	9.96	15.15	19.36	25.18
4-phenylphenol	0.68	1.46	4.24	15.48	27.66	37.25	53.79
2-nitroaniline	0.81	1.53	2.70	5.15	6.16	7.10	8.89
ethylphenylketone	0.78	1.40	2.57	4.67	6.49	7.57	11.79
2-octanone	0.59	1.17	2.47	5.04	5.57	6.07	7.96
benzene	0.78	1.35	2.67	3.62	3.53	3.89	4.61
methyl benzoate	0.69	1.25	2.45	4.69	5.79	6.65	8.87
3-cresol	0.55	1.00	1.73	3.04	3.20	3.55	4.57

Table A.1.4. Capacity factor values, cyanopropylsiloxane bonded phase, tetrahydrofuran

Solute	*50% THF*	*40% THF*	*30% THF*	*20% THF*	*10% THF*	*5% THF*	*1% THF*
naphthalene	1.99	4.20	9.81	20.93	26.31	29.18	38.30
2-hexanone	0.98	1.32	1.43	1.72	1.65	1.68	2.10
benzaldehyde	1.20	1.85	2.79	3.27	3.12	3.11	4.02
anisole	1.50	2.59	4.43	5.60	5.09	4.94	6.11
benzonitrile	1.31	2.15	3.46	4.30	3.83	3.60	4.31
bromobenzene	1.86	3.65	8.11	13.98	12.89	12.29	14.30
chlorobenzene	1.81	3.43	7.11	11.68	10.27	9.11	10.58
benzyl alcohol	0.91	1.31	1.74	1.86	1.68	1.63	1.86
chlorophenol	1.29	2.37	4.63	6.59	5.98	5.43	6.37
n-propylbenzene	2.33	5.00	13.23	24.85	23.04	21.36	24.96
acetophenone	1.17	1.79	2.72	3.46	3.65	3.80	5.46
2-phenylethanol	0.94	1.44	2.14	2.55	2.30	2.21	2.60
4-cresol	1.23	2.09	3.42	4.45	3.75	3.31	4.30
phenol	1.08	1.81	2.80	3.13	2.47	2.24	2.46
benzamide	0.69	0.85	1.13	1.38	1.58	1.66	2.11
acetanilide	0.81	1.21	1.84	2.34	2.45	2.53	3.26
nitrobenzene	1.52	2.73	5.01	6.59	5.55	5.39	6.47
1,2-dichlorobenzene	1.81	3.76	9.27	20.27	22.67	21.86	24.89
1,2-dibromobenzene	1.96	4.16	10.97	26.85	36.89	37.43	47.46
1-naphthol	1.50	3.05	6.87	13.95	15.87	17.93	24.16
4-phenylphenol	1.58	3.39	9.23	21.62	31.25	34.43	25.53
2-nitroaniline	1.34	2.42	4.81	6.90	6.72	6.33	8.23
ethylphenylketone	1.46	2.45	4.10	5.55	5.71	6.02	9.42
2-octanone	1.50	2.49	5.19	7.07	6.20	5.68	7.04
benzene	1.75	3.90	9.88	17.63	3.81	3.46	3.87
methyl benzoate	1.33	2.22	3.82	5.41	5.41	5.48	7.59
3-cresol	1.22	2.11	3.88	4.86	3.78	3.93	4.14

Figure A.2.1. Capacity factor values, spacer-bonded propanediol phase, methanol.

Solute	*60% MeOH*	*50% MeOH*	*40% MeOH*	*30% MeOH*	*20% MeOH*
naphthalene	0.88	1.75	3.39	5.13	9.48
2-hexanone	0.26	0.41	0.54	0.75	1.02
benzaldehyde	0.39	0.59	0.88	1.29	1.72
anisole	0.47	0.80	1.14	1.64	2.14
benzonitrile	0.37	0.94	0.98	1.44	1.94
bromobenzene	0.70	1.19	1.84	2.67	3.42
chlorobenzene	0.73	1.28	2.11	3.21	4.22
benzyl alcohol	0.27	0.44	0.58	0.76	0.88
chlorophenol	0.43	0.71	1.20	1.77	2.64
n-propylbenzene	0.78	1.50	2.52	4.10	5.53
acetophenone	0.39	0.64	0.95	1.46	2.10
2-phenylethanol	0.35	0.51	0.70	0.96	1.17
4-cresol	0.39	0.64	0.94	1.38	1.78
phenol	0.35	0.52	0.70	0.96	1.19
benzamide	0.26	0.37	0.54	0.80	1.10
acetanilide	0.30	0.47	0.65	0.93	1.24
nitrobenzene	0.52	0.85	1.25	1.79	2.28
1,2-dichlorobenzene	0.85	1.66	2.85	4.97	7.30
1,2-dibromobenzene	1.03	2.06	3.74	7.11	11.69
1-naphthol	0.70	1.31	2.70	4.73	7.33
4-phenylphenol	0.74	1.53	3.23	6.33	12.02
2-nitroaniline	0.54	0.89	1.33	1.93	2.80
ethylphenylketone	0.44	0.77	1.21	1.97	3.01
2-octanone	0.41	0.72	1.09	1.68	2.67
benzene	0.52	0.72	1.07	1.37	1.51
methyl benzoate	0.48	0.81	1.31	2.08	2.97
3-cresol	0.39	0.64	0.93	1.34	1.79

Table A.2.1 continued on next page

Figure A.2.1. (continued) Capacity factor values, spacer-bonded propanediol phase, methanol.

Solute	*10% MeOH*	*5% MeOH*	*1% MeOH*
naphthalene	13.10	16.19	18.31
2-hexanone	1.31	1.60	2.87
benzaldehyde	2.03	2.50	3.21
anisole	2.31	2.74	3.18
benzonitrile	2.21	2.65	3.02
bromobenzene	4.07	4.17	4.69
chlorobenzene	5.00	5.28	5.60
benzyl alcohol	0.93	1.12	1.19
chlorophenol	3.02	3.50	3.86
n-propylbenzene	6.70	7.56	8.19
acetophenone	2.89	3.69	5.08
2-phenylethanol	1.24	1.43	1.49
4-cresol	1.89	2.29	2.41
phenol	1.25	1.35	1.42
benzamide	1.48	1.72	1.96
acetanilide	1.58	1.89	2.31
nitrobenzene	2.76	3.83	5.08
1,2-dichlorobenzene	9.36	9.94	10.83
1,2-dibromobenzene	16.41	19.36	22.17
1-naphthol	9.49	13.48	14.97
4-phenylphenol	16.95	22.21	26.64
2-nitroaniline	3.17	3.82	4.98
ethylphenylketone	4.35	5.52	8.16
2-octanone	2.48	3.33	3.93
benzene	1.60	1.83	1.87
methyl benzoate	3.80	4.27	5.14
3-cresol	1.97	2.29	2.40

Table A.2.2. Capacity factor values, spacer-bonded propanediol phase, acetonitrile.

Solute	*50% ACN*	*40% ACN*	*30% ACN*	*20% ACN*	*10% ACN*	*5% ACN*	*1% ACN*
naphthalene	0.139	0.473	1.182	2.700	5.893	9.265	14.228
2-hexanone	0.104	0.250	0.418	0.591	0.795	1.044	1.616
benzaldehyde	0.104	0.295	0.518	0.818	1.232	1.575	2.446
anisole	0.104	0.321	0.609	1.018	1.545	1.903	2.589
benzonitrile	0.104	0.295	0.545	0.882	1.321	1.655	2.420
bromobenzene	0.130	0.402	0.873	1.609	2.571	3.106	4.384
chlorobenzene	0.130	0.384	0.936	1.827	3.054	3.920	5.116
benzyl alcohol	0.113	0.286	0.436	0.600	0.741	0.867	1.089
chlorophenol	0.096	0.321	0.618	1.082	1.750	2.310	3.089
n-propylbenzene	0.113	0.411	1.091	2.455	4.571	5.584	7.893
acetophenone	0.096	0.295	0.455	0.873	1.438	2.018	3.420
2-phenylethanol	0.113	0.277	0.464	0.682	0.920	1.062	1.321
4-cresol	0.096	0.295	0.536	0.864	1.304	1.619	2.071
phenol	0.113	0.277	0.464	0.682	0.920	1.071	1.295
benzamide	0.165	0.295	0.436	0.609	0.893	1.168	1.696
acetanilide	0.130	0.286	0.455	0.682	1.000	1.283	1.768
nitrobenzene	0.104	0.312	0.609	1.000	1.536	1.973	3.054
1,2-dichlorobenzene	0.148	0.464	1.136	2.500	4.705	7.000	9.464
1,2-dibromobenzene	0.165	0.527	1.364	3.327	7.964	11.885	19.643
1-naphthol	0.113	0.393	0.918	1.991	4.625	7.885	12.250
4-phenylphenol	0.087	0.357	0.936	2.373	6.821	11.257	19.607
2-nitroaniline	0.122	0.330	0.618	1.064	1.786	2.451	3.536
ethylphenylketone	0.104	0.312	0.618	1.091	1.973	2.894	5.143
2-octanone	0.087	0.286	0.600	1.055	1.696	2.018	3.321
benzene	0.130	0.348	0.636	0.964	1.250	1.389	1.732
methyl benzoate	0.104	0.312	0.609	1.073	1.830	2.504	3.902
3-cresol	0.087	0.304	0.536	0.864	1.304	1.611	2.098

Table A.2.3. Capacity factor values, spacer-bonded propanediol phase, isopropanol.

Solute	*50% IPA*	*40% IPA*	*30% IPA*	*20% IPA*	*10% IPA*	*5% IPA*	*1% IPA*
naphthalene	0.667	1.455	4.661	12.770	15.786	16.094	18.261
2-hexanone	0.306	0.545	0.911	1.372	1.521	15.47	1.950
benzaldehyde	0.252	0.664	1.500	2.088	2.470	2.444	3.034
anisole	0.495	0.909	1.759	3.027	3.188	3.026	3.412
benzonitrile	0.441	0.727	1.375	2.460	2.735	2.675	3.059
bromobenzene	0.586	1.245	3.536	6.823	6.214	5.120	5.227
chlorobenzene	0.613	1.318	3.857	8.451	7.615	6.291	6.378
benzyl alcohol	0.360	0.573	0.884	1.221	1.231	1.162	1.269
chlorophenol	0.441	0.818	1.929	3.566	4.162	4.231	4.378
n-propylbenzene	0.577	1.382	5.179	13.265	12.094	9.803	9.857
acetophenone	0.396	0.691	1.286	2.363	3.103	3.325	4.303
2-phenylethanol	0.333	0.591	1.179	1.611	1.658	1.530	1.655
4-cresol	0.396	0.709	1.420	2.469	2.726	2.590	2.790
phenol	0.360	0.627	1.063	1.619	1.675	1.564	1.571
benzamide	0.324	0.482	0.723	1.124	1.444	1.547	1.966
acetanilide	0.324	0.455	0.866	1.416	1.769	1.821	2.193
nitrobenzene	0.514	0.909	1.812	30.97	3.162	3.085	3.815
1,2-dichlorobenzene	0.622	1.409	4.786	13.973	14.487	10.248	12.412
1,2-dibromobenzene	0.676	1.582	5.786	18.823	23.171	20.915	23.874
1-naphthol	0.532	1.100	3.000	8.522	12.675	13.735	15.445
4-phenylphenol	0.477	1.045	3.313	11.876	21.188	24.709	28.076
2-nitroaniline	0.477	0.836	1.696	3.602	4.368	3.957	5.084
ethylphenylketone	0.441	0.800	1.679	3.425	4.521	5.094	6.798
2-octanone	0.306	0.764	2.286	3.496	5.222	4.436	4.714
benzene	0.532	0.991	1.938	2.673	2.214	1.932	2.025
methyl benzoate	0.441	0.818	1.804	3.761	4.880	4.650	5.454
3-cresol	0.378	0.709	1.420	2.522	2.718	2.556	2.697

Table A.2.4. Capacity factor values, spacer-bonded propanediol phase, tetrahydrofuran.

Solute	*50% THF*	*40% THF*	*30% THF*	*20% THF*	*10% THF*	*5% THF*	*1% THF*
naphthalene	0.304	0.667	1.637	4.897	8.932	9.625	13.182
2-hexanone	0.232	0.333	0.513	0.664	0.805	0.917	1.314
benzaldehyde	0.232	0.468	0.726	1.112	1.525	1.542	1.950
anisole	0.277	0.541	0.991	1.672	2.059	2.125	2.479
benzonitrile	0.250	0.486	0.779	1.241	1.551	1.650	2.025
bromobenzene	0.304	0.667	1.416	3.121	3.881	3.550	3.818
chlorobenzene	0.304	0.676	1.522	3.621	4.915	4.592	4.934
benzyl alcohol	0.232	0.396	0.593	0.793	0.873	0.900	0.992
chlorophenol	0.232	0.514	1.035	2.043	2.907	2.958	3.091
n-propylbenzene	0.277	0.757	1.876	5.276	6.661	6.350	6.380
acetophenone	0.250	0.441	0.690	1.112	1.593	1.900	2.661
2-phenylethanol	0.214	0.396	0.637	0.948	1.102	1.133	1.256
4-cresol	0.232	0.486	0.867	1.500	1.915	1.942	2.107
phenol	0.214	0.450	0.752	1.164	1.314	1.250	1.281
benzamide	0.214	0.333	0.469	0.664	0.932	1.083	1.421
acetanilide	0.205	0.360	0.558	0.862	1.144	1.292	1.603
nitrobenzene	0.277	0.541	1.018	1.716	1.949	1.958	2.372
1,2-dichlorobenzene	0.295	0.694	1.743	5.224	8.534	8.292	8.405
1,2-dibromobenzene	0.304	0.721	1.867	6.284	12.780	13.550	15.388
1-naphthol	0.214	0.577	1.310	3.862	7.551	8.933	11.174
4-phenylphenol	0.214	0.559	1.389	4.466	11.424	14.400	18.223
2-nitroaniline	0.250	0.514	0.991	1.966	2.602	2.792	3.215
ethylphenylketone	0.250	0.486	0.858	1.500	2.280	2.800	4.066
2-octanone	0.232	0.532	0.885	1.750	2.364	2.633	2.868
benzene	0.295	0.586	1.106	1.586	1.669	1.483	1.545
methyl benzoate	0.250	0.486	0.867	1.664	2.449	2.758	3.471
3-cresol	0.214	0.486	0.867	1.526	1.932	1.925	2.107

Table A.3.1. Capacity factor values, butylsiloxane bonded phase, methanol.

Solute	*60% MeOH*	*50% MeOH*	*40% MeOH*	*30% MeOH*	*20% MeOH*
naphthalene	1.365	3.247	5.28	12.84	32.60
2-hexanone	0.426	0.801	1.04	1.96	4.32
benzaldehyde	0.405	0.781	1.00	1.96	4.40
anisole	0.655	1.301	1.68	3.20	6.60
benzonitrile	0.453	0.925	1.24	2.48	5.84
bromobenzene	1.068	2.329	3.20	6.00	12.20
chlorobenzene	1.203	2.664	4.28	9.56	16.24
benzyl alcohol	0.284	0.527	0.60	1.04	2.04
chlorophenol	0.453	0.952	1.28	2.52	5.32
n-propylbenzene	2.297	6.315	11.32	27.32	66.04
acetophenone	0.459	0.952	1.32	2.72	7.20
2-phenylethanol	0.385	0.753	0.96	1.76	3.76
4-cresol	0.419	0.822	1.08	2.08	4.44
phenol	0.284	0.541	0.60	1.08	2.04
benzamide	0.149	0.288	0.36	0.68	1.44
acetanilide	0.250	0.507	0.64	1.20	2.60
nitrobenzene	0.588	1.178	1.48	2.76	6.20
1,2-dichlorobenzene	1.622	3.932	6.00	14.68	34.04
1,2-dibromobenzene	1.892	4.685	7.88	19.56	53.24
1-naphthol	0.676	1.603	2.56	5.96	16.32
4-phenylphenol	0.959	2.562	4.52	13.24	45.40
2-nitroaniline	0.426	0.870	1.20	2.32	5.40
ethylphenylketone	0.696	1.534	2.32	4.84	14.80
2-octanone	1.250	2.637	4.60	12.12	---
benzene	0.676	1.267	1.52	2.52	4.40
methyl benzoate	0.730	1.637	2.48	5.32	13.92
3-cresol	0.419	0.849	1.08	2.00	4.44

Table A.3.1 continued on next page

Table A.3.1. (continued) Capacity factor values, butylsiloxane bonded phase, methanol.

Solute	*10% MeOH*	*5% MeOH*	*1% MeOH*
naphthalene	47.32	54.84	74.20
2-hexanone	5.88	7.72	10.08
benzaldehyde	6.20	7.64	10.56
anisole	8.56	9.88	12.04
benzonitrile	7.40	8.80	11.40
bromobenzene	12.32	15.64	17.72
chlorobenzene	17.68	21.40	23.80
benzyl alcohol	2.64	3.16	3.80
chlorophenol	6.60	7.72	9.20
n-propylbenzene	---	---	---
acetophenone	10.56	13.52	19.40
2-phenylethanol	4.80	5.88	7.88
4-cresol	5.76	6.36	8.16
phenol	2.40	2.48	3.16
benzamide	2.20	2.84	3.76
acetanilide	3.92	5.00	6.80
nitrobenzene	7.52	8.56	12.64
1,2-dichlorobenzene	41.24	44.92	51.96
1,2-dibromobenzene	68.04	77.72	104.28
1-naphthol	21.92	29.40	41.88
4-phenylphenol	82.68	98.68	173.56
2-nitroaniline	7.36	8.84	11.28
ethylphenylketone	23.84	34.20	52.60
2-octanone	---	---	---
benzene	4.44	4.76	5.28
methyl benzoate	21.96	28.44	42.04
3-cresol	5.72	6.60	7.96

Table A.3.2. Capacity factor values, butylsiloxane bonded phase, acetonitrile

Solute	*40% ACN*	*30% ACN*	*20% ACN*	*10% ACN*	*5% ACN*	*1% ACN*
naphthalene	4.609	10.833	36.333	67.96	84.60	100.28
hexanone	1.000	1.417	2.625	4.44	7.64	12.76
benzaldehyde	1.174	1.750	3.458	5.64	7.36	11.32
anisole	2.043	3.292	7.542	11.48	13.24	16.76
benzonitrile	1.478	2.333	4.750	7.24	1028	14.36
chlorobenzene	3.304	5.708	16.000	23.32	24.28	23.48
bromobenzene	3.478	7.000	20.333	31.00	33.56	33.24
benzyl alcohol	0.696	0.833	1.625	2.64	3.36	5.24
chlorophenol	1.348	2.333	5.958	9.32	11.96	11.80
n-propylbenzene	7.174	20.833	72.167	130.36	161.24	184.92
acetophenone	1.217	1.917	4.500	8.00	12.48	22.36
phenylethanol	0.783	1.208	2.833	5.00	7.28	10.68
4-cresol	1.174	1.875	4.042	6.96	9.08	11.32
phenol	0.826	1.125	2.208	3.08	3.76	3.88
benzamide	0.478	0.542	0.917	1.760	2.60	3.92
acetanilide	0.652	0.875	1.917	3.60	5.32	8.76
nitrobenzene	1.870	3.167	6.083	8.64	11.32	13.56
1,2-dichlorobenzene	4.652	12.167	35.833	61.560	72.60	74.68
1,2-dibromobenzene	5.304	13.833	50.667	95.16	123.60	---
1-naphthol	2.087	4.542	15.542	34.04	46.84	57.08
4-phenylphenol	2.826	6.833	36.167	93.72	149.40	202.04
2-nitroaniline	1.348	2.125	4.667	8.920	11.64	15.16
octanone	3.217	8.167	---	---	---	---
benzene	2.130	3.375	5.792	7.04	6.96	7.48
methyl benzoate	1.870	3.333	9.083	18.68	27.16	43.32
3-cresol	1.174	1.875	4.375	7.40	9.40	11.16

Table A.3.3. Capacity factor values, butylsiloxane bonded phase, isopropanol

Solute	*40% IPA*	*30% IPA*	*20% IPA*	*10% IPA*	*5% IPA*	*1% IPA*
naphthalene	1.826	8.217	32.833	63.96	81.08	83.32
hexanone	0.522	1.000	2.000	3.84	5.96	11.48
benzaldehyde	0.522	1.087	2.375	4.64	6.36	11.16
anisole	0.913	2.261	5.833	9.56	12.60	15.96
benzonitrile	0.565	1.348	3.125	6.60	8.56	13.40
chlorobenzene	1.609	5.174	17.500	24.76	26.36	21.88
bromobenzene	1.783	6.391	21.500	32.44	35.80	30.68
benzyl alcohol	0.391	0.739	1.208	2.040	2.96	3.96
chlorophenol	0.739	1.478	4.250	9.040	11.48	11.96
n-propylbenzene	2.957	16.565	74.667	125.88	161.40	156.76
acetophenone	0.565	1.261	3.083	6.96	12.12	21.24
phenylethanol	0.522	0.957	2.125	3.48	6.84	9.40
4-cresol	0.565	1.348	3.208	5.56	8.60	10.52
phenol	0.435	0.870	1.625	2.60	3.24	3.72
benzamide	0.087	0.348	0.667	1.40	2.28	3.80
acetanilide	0.304	0.609	1.292	2.64	4.20	6.64
nitrobenzene	0.739	1.826	4.083	7.08	9.08	11.96
1,2-dichlorobenzene	1.913	9.957	44.167	79.96	130.68	133.88
1,2-dibromobenzene	2.217	11.522	61.000	---	---	---
naphthol	0.826	2.826	12.500	30.52	43.16	51.00
4-phenylphenol	0.957	4.000	25.833	77.88	130.20	174.52
2-nitroaniline	0.522	1.174	3.083	7.16	10.04	13.88
octanone	1.174	5.609	7.167	16.60	---	---
benzene	1.174	2.739	5.042	6.32	6.56	6.28
methyl benzoate	0.826	2.348	6.708	17.56	27.64	41.72
3-cresol	0.565	1.391	3.292	5.88	8.92	10.20

Table A.3.4. Capacity factor values, butylsiloxane bonded phase, tetrahydrofuran

Solute	*40% THF*	*30% THF*	*20% THF*	*10% THF*	*5% THF*	*1% THF*
naphthalene	3.227	10.304	35.500	90.84	119.16	100.28
hexanone	0.818	0.957	1.250	2.08	3.24	7.00
benzaldehyde	1.045	1.565	2.417	4.08	5.08	7.32
anisole	1.773	3.609	6.667	11.64	14.04	14.36
benzonitrile	1.182	2.043	3.417	5.36	7.00	9.88
chlorobenzene	2.864	7.043	20.333	37.24	38.04	26.20
bromobenzene	3.045	7.696	24.333	47.48	51.16	39.16
benzyl alcohol	0.818	1.087	1.542	2.40	3.16	3.96
chlorophenol	1.909	4.565	11.667	21.40	24.12	16.44
n-propylbenzene	5.455	20.739	67.333	154.52	197.56	168.44
acetophenone	1.000	1.609	2.583	5.000	7.44	13.40
phenylethanol	0.864	1.435	2.458	4.12	5.88	8.28
4-cresol	1.500	3.217	6.167	11.64	13.24	11.32
phenol	1.273	2.174	3.625	5.56	5.72	1.52
benzamide	0.136	0.522	0.667	1.32	2.00	3.00
acetanilide	0.591	0.957	1.667	3.08	4.40	5.88
nitrobenzene	1.727	3.565	6.583	9.36	10.20	10.16
1,2-dichlorobenzene	3.500	12.565	44.500	112.76	115.32	93.56
1,2-dibromobenzene	3.773	12.565	57.500	187.16	---	---
1-naphthol	2.455	7.522	26.500	77.72	86.68	69.40
4-phenylphenol	2.864	10.826	44.500	99.32	121.08	231.88
2-nitroaniline	1.545	3.391	7.667	12.76	14.36	12.92
octanone	2.182	5.609	12.833	---	---	---
benzene	2.045	4.000	6.458	8.28	8.40	6.56
methyl benzoate	1.409	2.826	5.833	12.48	20.44	28.92
3-cresol	1.591	3.348	6.375	11.96	13.88	12.28

Table A.4.1. Capacity factor values, light-loaded octadecylsiloxane bonded phase, methanol

Solute	*80% MeOH*	*70% MeOH*	*60% MeOH*	*50% MeOH*	*40% MeOH*	*30% MeOH*	*20% MeOH*
naphthalene	2.235	4.824	11.059	27.706	72.647	159.67	319.44
2-hexanone	0.824	1.235	2.118	3.412	6.353	12.333	23.667
benzaldehyde	1.059	1.118	1.941	3.412	6.471	11.611	21.556
anisole	1.176	2.000	3.706	6.882	13.765	24.111	42.556
benzonitrile	0.706	1.118	1.941	3.588	7.176	13.167	25.889
chlorobenzene	1.647	3.118	6.647	13.765	32.176	57.667	96.333
bromobenzene	1.059	3.647	7.824	17.824	41.118	77.000	130.78
benzyl alcohol	0.588	0.824	1.353	2.235	4.000	6.389	11.222
chlorophenol	0.706	1.176	2.235	4.294	8.647	15.667	29.000
n-propylbenzene	3.059	7.235	18.471	47.941	137.82	337.44	590.78
acetophenone	0.824	1.353	2.412	4.353	9.059	18.778	38.778
2-phenylethanol	0.706	1.059	1.941	3.471	6.941	12.111	21.889
4-cresol	---	1.059	1.941	3.824	7.000	13.444	24.556
phenol	0.471	0.765	1.235	2.059	3.647	5.667	8.722
benzamide	0.412	0.529	0.765	1.235	2.235	3.833	7.333
acetanilide	0.647	0.824	1.412	2.412	4.588	8.167	17.000
nitrobenzene	1.059	1.706	3.059	5.529	11.118	18.33	30.944
1,2-dichlorobenzene	2.294	4.941	11.647	29.353	72.412	152.56	279.22
1,2-dibromobenzene	---	6.118	14.706	38.294	104.41	234.33	512.56
1-naphthol	1.059	2.059	4.412	9.706	27.000	56.778	125.89
4-phenylphenol	1.353	2.941	7.118	18.118	57.353	154.78	348.33
2-nitroaniline	0.765	1.353	2.353	4.235	8.647	14.889	30.111
ethylphenylketone	---	2.000	3.941	2.176	18.882	39.778	94.556
octanone	---	3.412	---	---	---	---	---
benzene	1.118	1.941	3.647	6.471	11.588	17.778	23.889
methyl benzoate	1.118	2.000	3.941	8.235	18.706	38.611	84.778
3-cresol	0.647	1.059	1.941	3.706	7.529	12.722	25.222

Table A.4.1 continued on next page

Table A.4.1. (continued) Capacity factor values, light-loaded octadecylsiloxane bonded phase, methanol.

Solute	*10% MeOH*	*5% MeOH*	*1% MeOH*
naphthalene	561.95	748.47	902.58
2-hexanone	45.947	---	---
benzaldehyde	43.211	64.895	88.053
anisole	73.526	101.95	126.58
benzonitrile	46.579	67.421	83.421
chlorobenzene	121.74	138.79	167.84
bromobenzene	172.26	211.42	232.05
benzyl alcohol	18.789	26.368	32.895
chlorophenol	47.421	56.474	73.316
n-propylbenzene	1004.3	---	---
acetophenone	83.842	132.68	180.05
2-phenylethanol	39.000	54.789	79.842
4-cresol	43.211	54.158	65.316
phenol	13.947	17.737	18.579
benzamide	13.684	21.947	36.053
acetanilide	32.895	53.947	87.211
nitrobenzene	51.895	71.421	101.21
1,2-dichlorobenzene	429.32	477.32	548.05
1,2-dibromobenzene	791.42	976.47	1251.6
1-naphthol	228.47	353.32	450.37
4-phenylphenol	885.53	---	---
2-nitroaniline	51.158	84.263	110.58
ethylphenylketone	226.58	---	---
octanone	---	---	---
benzene	34.368	37.316	41.526
methyl benzoate	178.16	267.84	394.79
3-cresol	41.737	54.158	67.000

Table A.4.2. Capacity factor values, light-loaded octadecylsiloxane bonded phase, acetonitrile

Solute	*80% ACN*	*70% ACN*	*60% ACN*	*50% ACN*	*40% ACN*	*30% ACN*	*20% ACN*
naphthalene	1.462	2.593	4.856	9.836	22.841	65.769	174.80
2-hexanone	0.436	1.120	1.664	2.322	3.331	5.692	13.659
benzaldehyde	0.679	1.100	1.692	2.603	4.205	7.455	11.898
anisole	0.885	1.433	2.466	4.233	7.695	15.545	27.972
benzonitrile	0.641	1.133	1.815	2.932	5.066	9.288	15.688
chlorobenzene	1.173	2.060	3.630	6.582	13.907	34.064	66.955
bromobenzene	0.699	2.260	4.034	7.267	15.623	39.321	89.875
benzyl alcohol	0.538	0.800	1.082	1.473	2.046	3.314	5.068
chlorophenol	0.551	0.947	1.452	2.315	4.238	7.712	15.830
n-propylbenzene	1.846	3.527	6.945	14.110	38.603	123.62	345.36
acetophenone	0.846	1.287	1.801	2.808	4.669	8.647	15.710
2-phenylethanol	0.699	0.907	1.260	1.822	2.881	4.923	9.011
4-cresol	0.506	0.907	1.356	2.103	3.510	6.897	11.608
phenol	0.449	0.707	1.068	1.568	2.377	3.917	6.034
benzamide	0.506	0.640	0.842	1.021	1.311	1.897	3.040
acetanilide	0.558	0.807	1.116	1.548	2.285	3.673	6.483
nitrobenzene	0.795	1.307	2.219	3.836	7.086	14.109	21.875
1,2-dichlorobenzene	1.583	2.800	5.021	9.945	22.709	64.769	152.66
1,2-dibromobenzene	1.795	3.233	5.952	11.932	28.483	88.673	256.73
1-naphthol	0.679	1.167	2.027	3.740	7.801	19.372	53.403
4-phenylphenol	0.756	1.347	2.466	4.842	11.318	36.038	112.75
2-nitroaniline	0.603	1.040	1.671	2.781	4.841	9.321	18.02
ethylphenylketone	0.955	1.567	2.568	4.390	7.940	17.590	33.886
benzene	0.923	1.513	2.582	4.404	7.974	14.750	23.188
methyl benzoate	1.000	1.493	2.068	4.055	7.391	15.731	32.835
3-cresol	0.532	0.833	1.322	2.089	3.530	6.705	12.324

Table A.4.2 continued on next page

Table A.4.2. (continued) Capacity factor values, light-loaded octadecylsiloxane bonded phase, acetonitrile

Solute	*10% ACN*	*5% ACN*	*1% ACN*
naphthalene	434.03	609.70	771.51
2-hexanone	---	---	---
benzaldehyde	22.185	35.733	64.308
anisole	50.937	66.673	102.41
benzonitrile	27.677	39.653	65.445
chlorobenzene	117.85	130.53	140.99
bromobenzene	164.82	189.00	207.06
benzyl alcohol	9.952	14.842	25.204
chlorophenol	33.942	46.327	60.517
n-propylbenzene	804.82	1010.3	---
acetophenone	36.751	65.188	140.04
2-phenylethanol	21.540	34.342	58.654
4-cresol	25.772	38.208	53.692
phenol	10.090	13.084	16.777
benzamide	9.767	11.970	24.308
acetanilide	15.296	27.812	59.948
nitrobenzene	40.275	50.718	73.028
1,2-dichlorobenzene	362.36	453.06	489.65
1,2-dibromobenzene	675.19	893.65	1030.4
1-naphthol	146.37	240.68	375.11
4-phenylphenol	477.84	---	---
2-nitroaniline	36.143	52.861	85.919
ethylphenylketone	91.593	166.43	---
benzene	32.228	32.807	35.900
methyl benzoate	82.492	145.63	272.74
3-cresol	26.407	38.361	54.829

APPENDIX B: System constant plots

Plots of system constants against mobile phase composition for the four silica-based bonded phases for methanol, acetonitrile, isopropanol, and tetrahydrofuran as mobile phase organic solvents in water.

cyanopropylsiloxane	Figure B.1.1 to B.1.4
propanediol	Figure B.2.1 to B.2.4
butylsiloxane	Figure B.3.1 to B.3.4
LL octadecylsiloxane	Figure B.4.1 to B.4.2

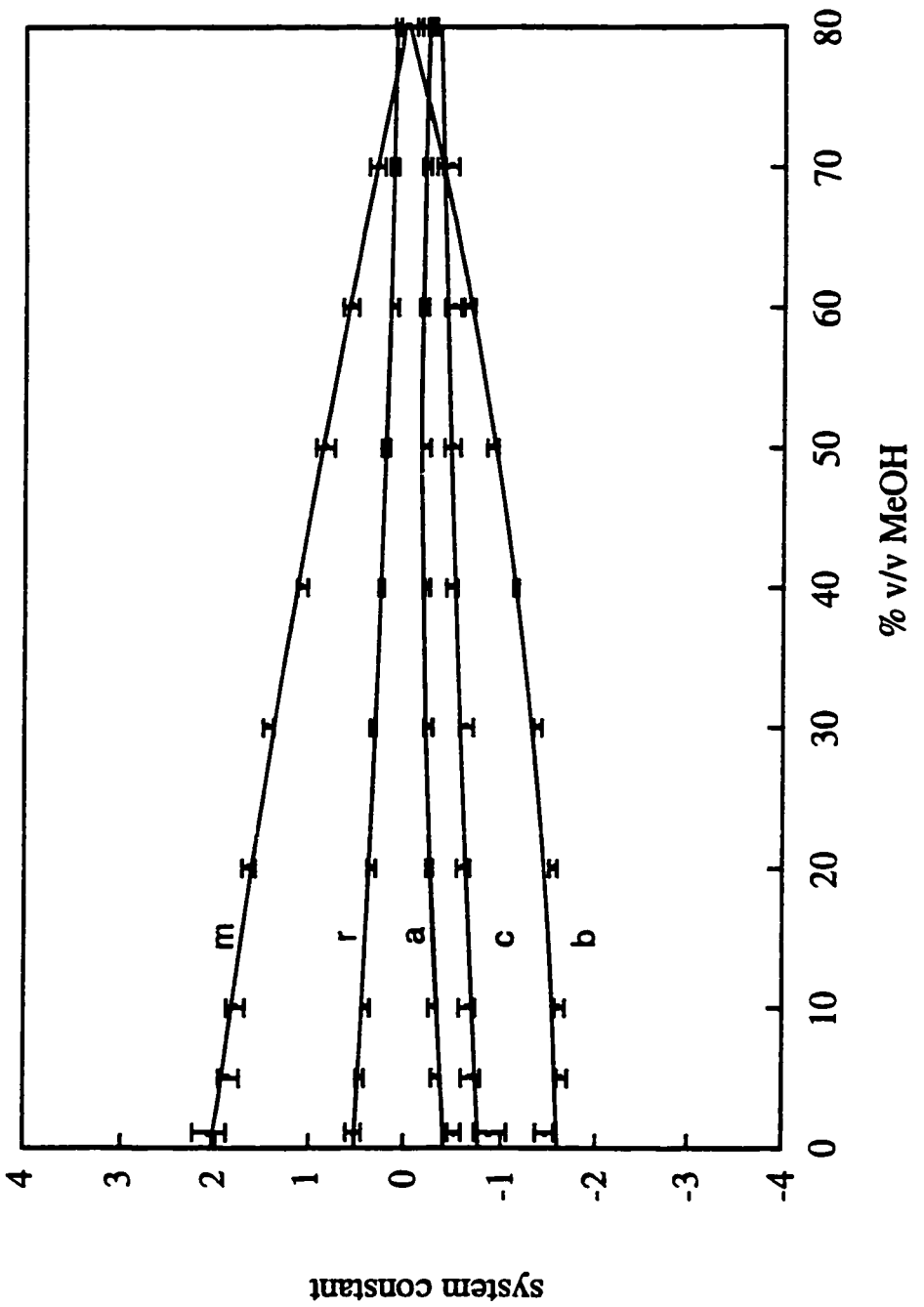


Figure B.1.1. System constants for the cyanopropylsiloxane bonded phase with methanol/water mobile phases. The s-constant is insignificant at all compositions.

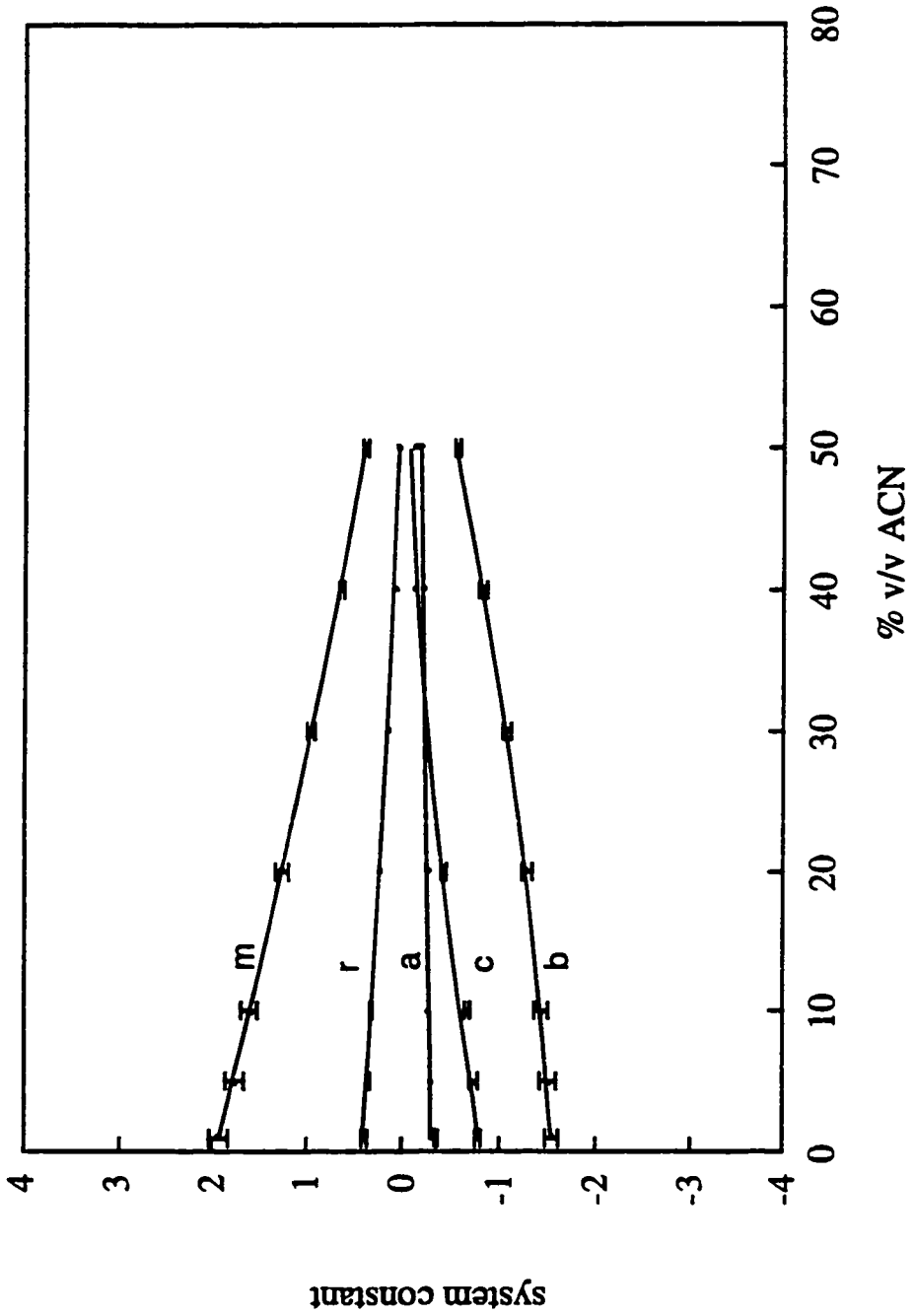


Figure B.1.2. System constants for the cyanopropylsiloxane bonded phase with acetonitrile/water mobile phases. The s-constant is insignificant at all compositions.

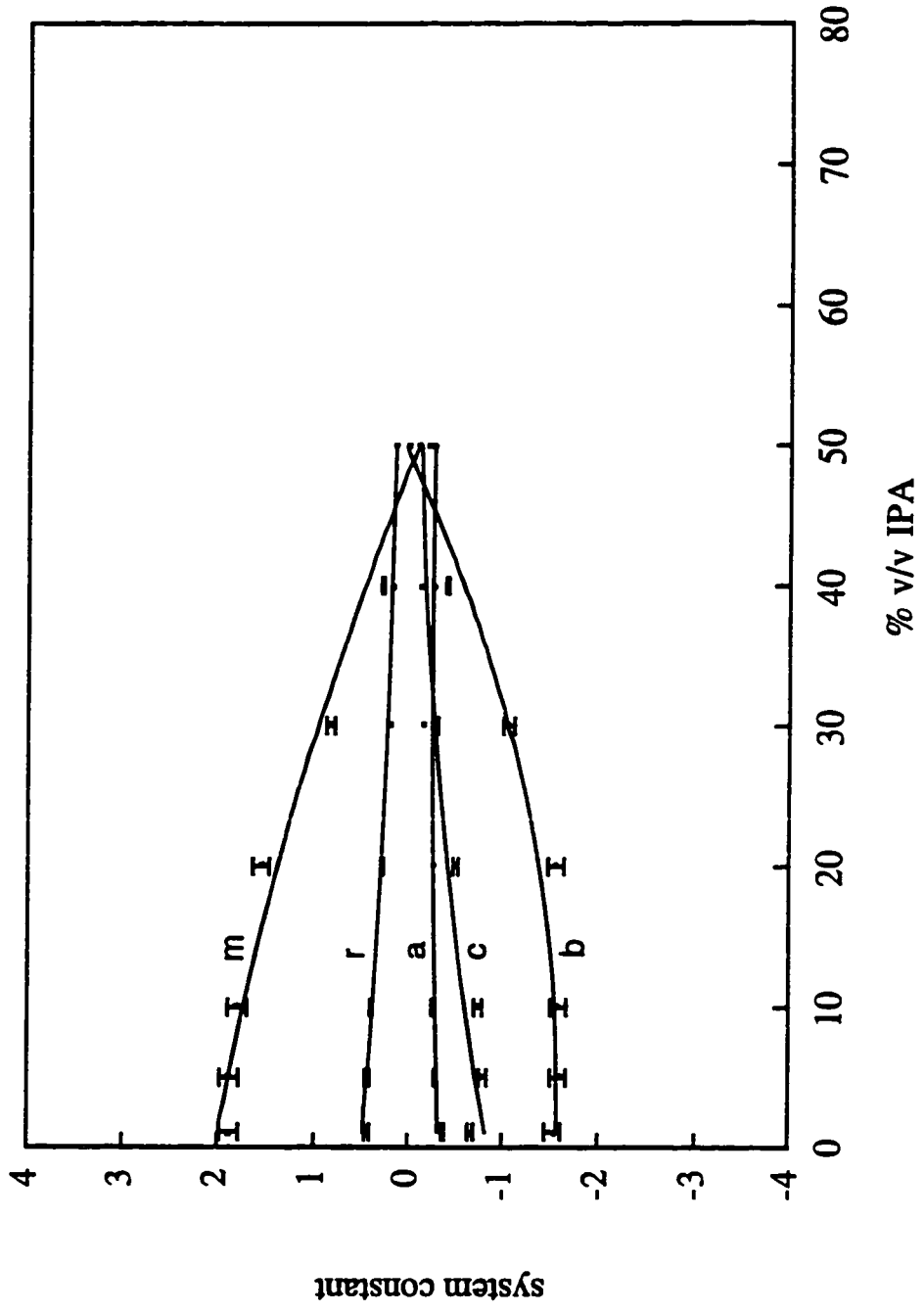


Figure B.1.3. System constants for the cyanopropylsiloxane bonded phase with isopropanol/water mobile phases. The s-constant is insignificant at all compositions.

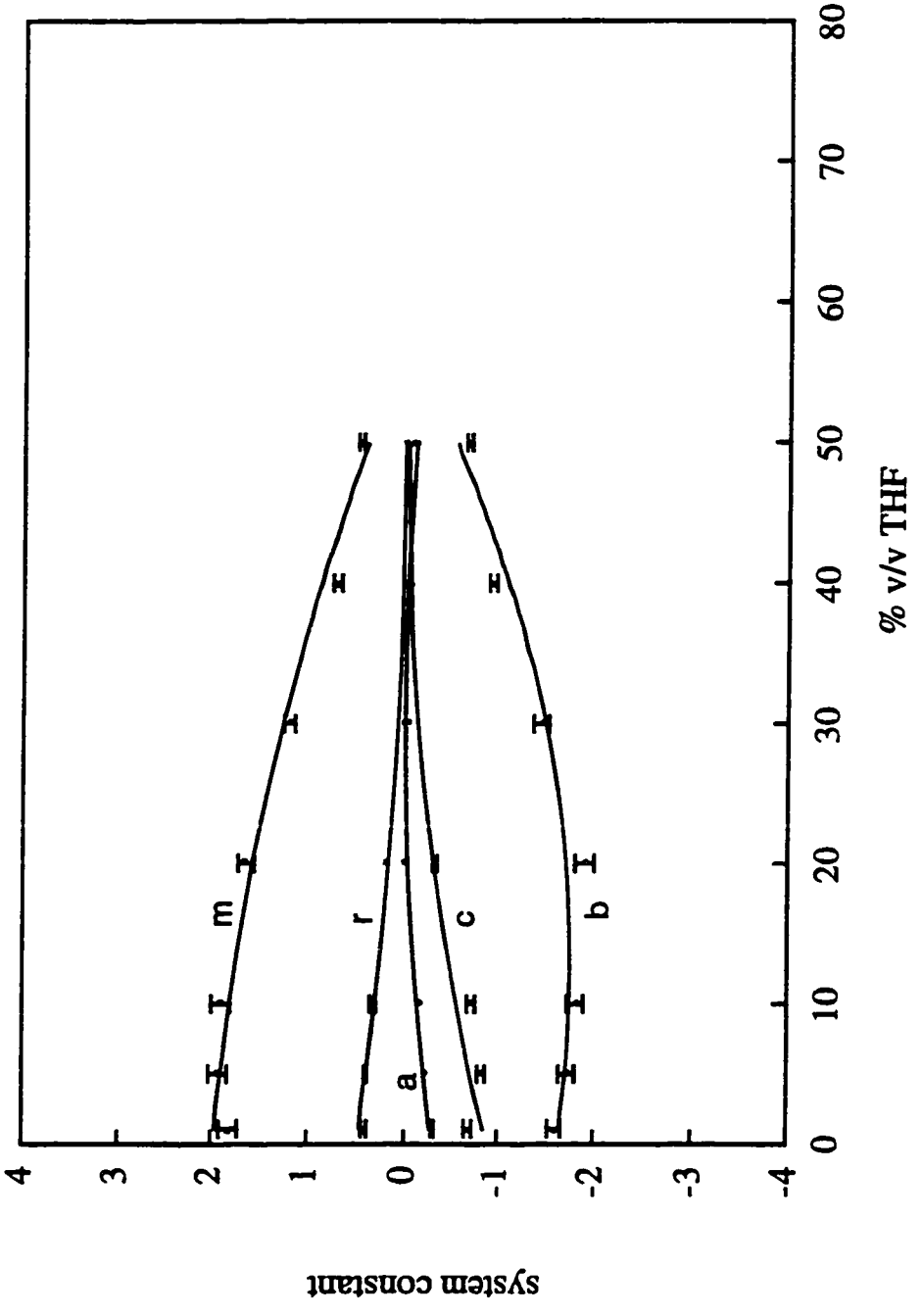


Figure B.1.4. System constants for the cyanopropylsiloxane bonded phase with tetrahydrofuran/water mobile phases. The s-constant is insignificant at all compositions.

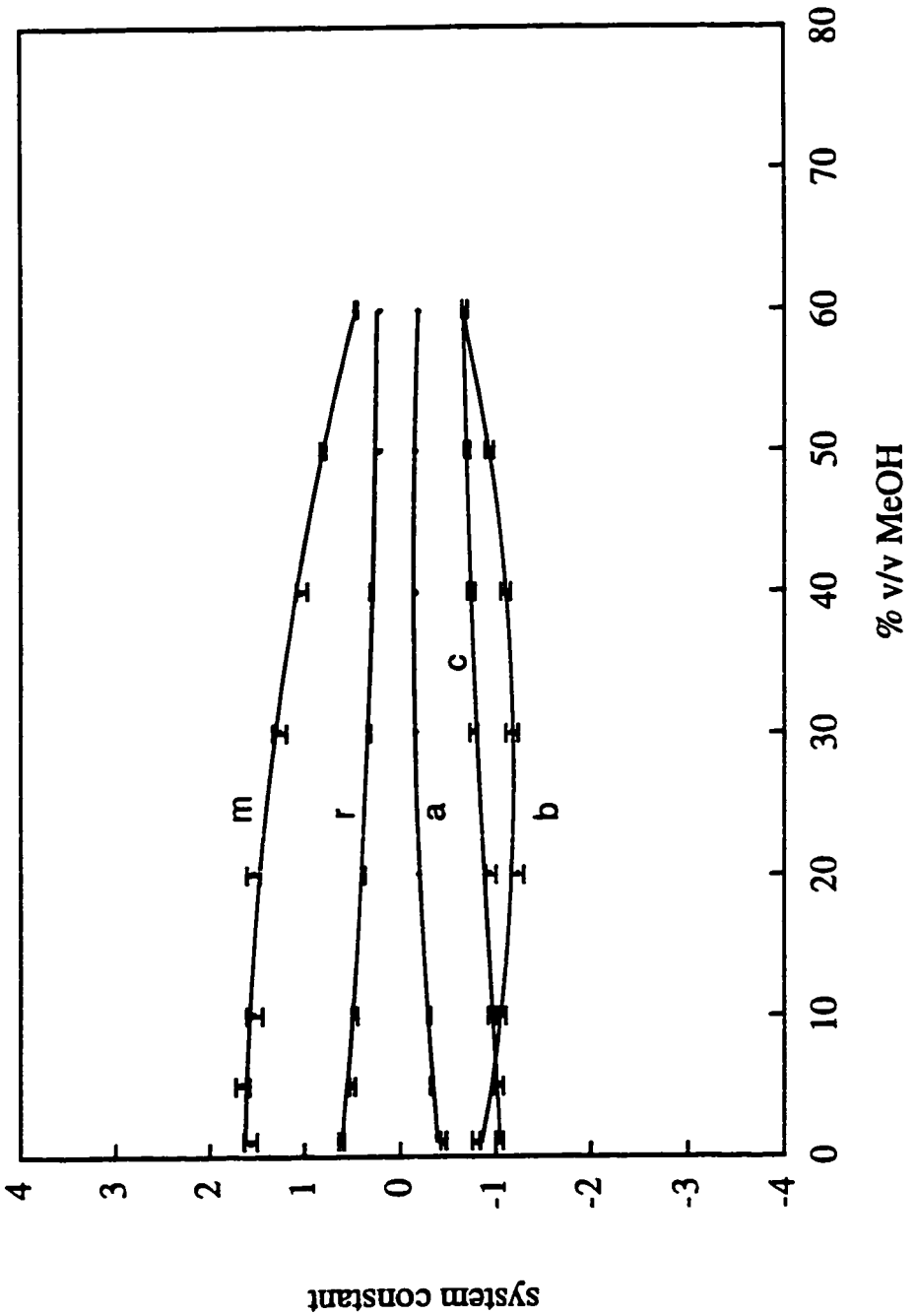


Figure B.2.1. System constants for the propanediol bonded phase with methanol/water mobile phases. The s-constant is insignificant at all compositions.

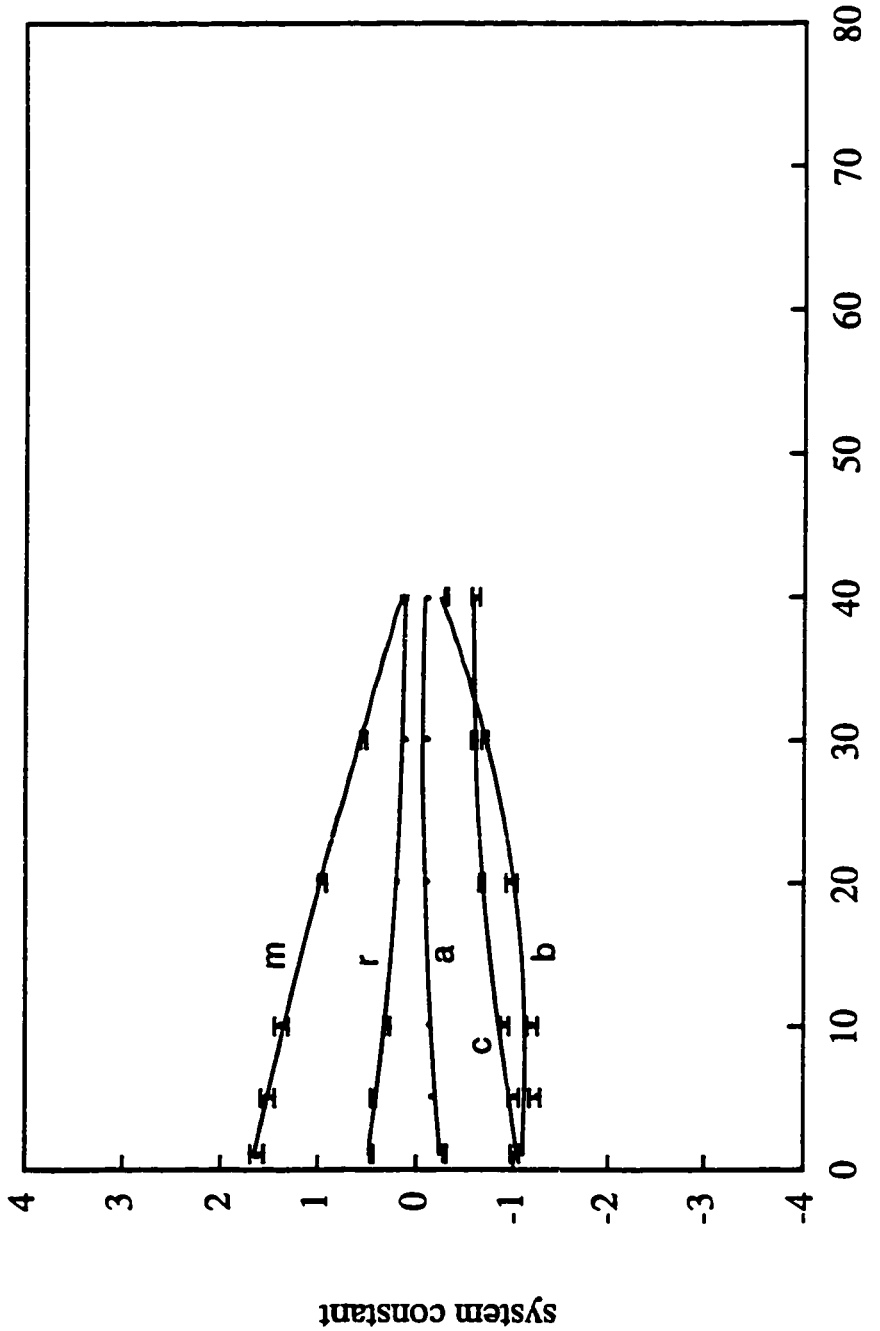


Figure B.2.2. System constants for the propanediol bonded phase with acetonitrile/water mobile phases. The s-constant is insignificant at all compositions.

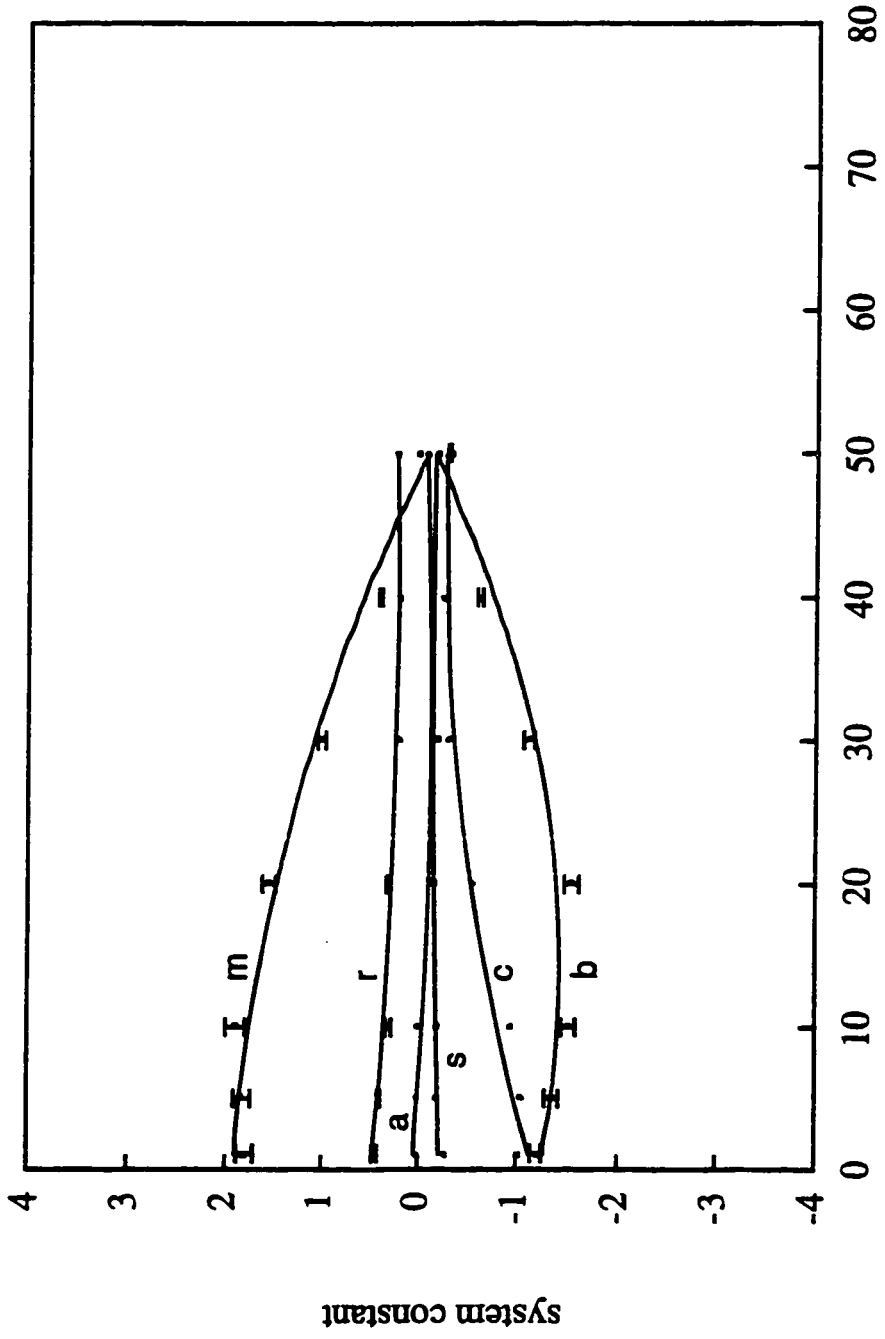


Figure B.2.3. System constants for the propanediol bonded phase with isopropanol/water mobile phases.

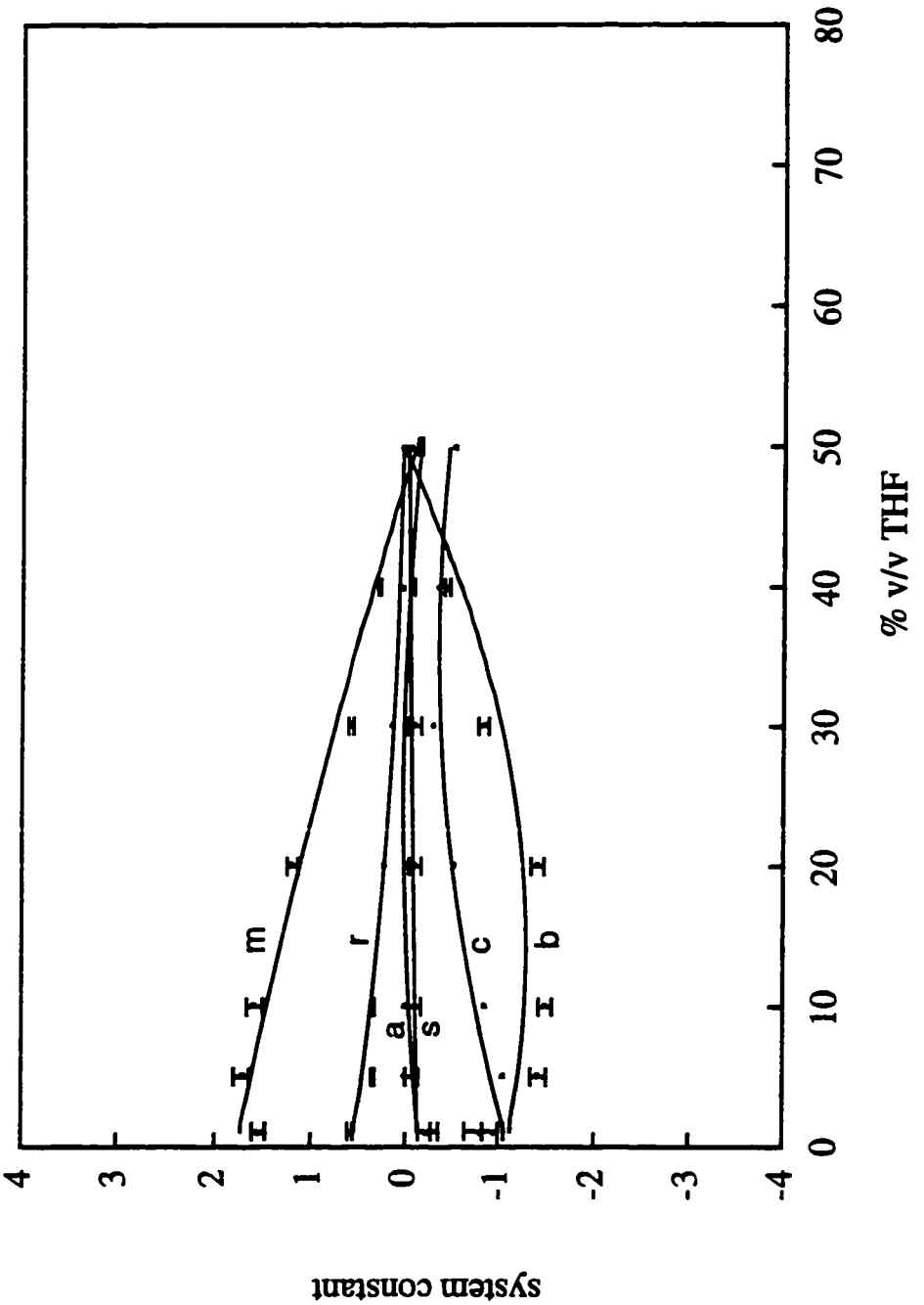


Figure B.2.4. System constants for the propanediol bonded phase with tetrahydrofuran/water mobile phases.

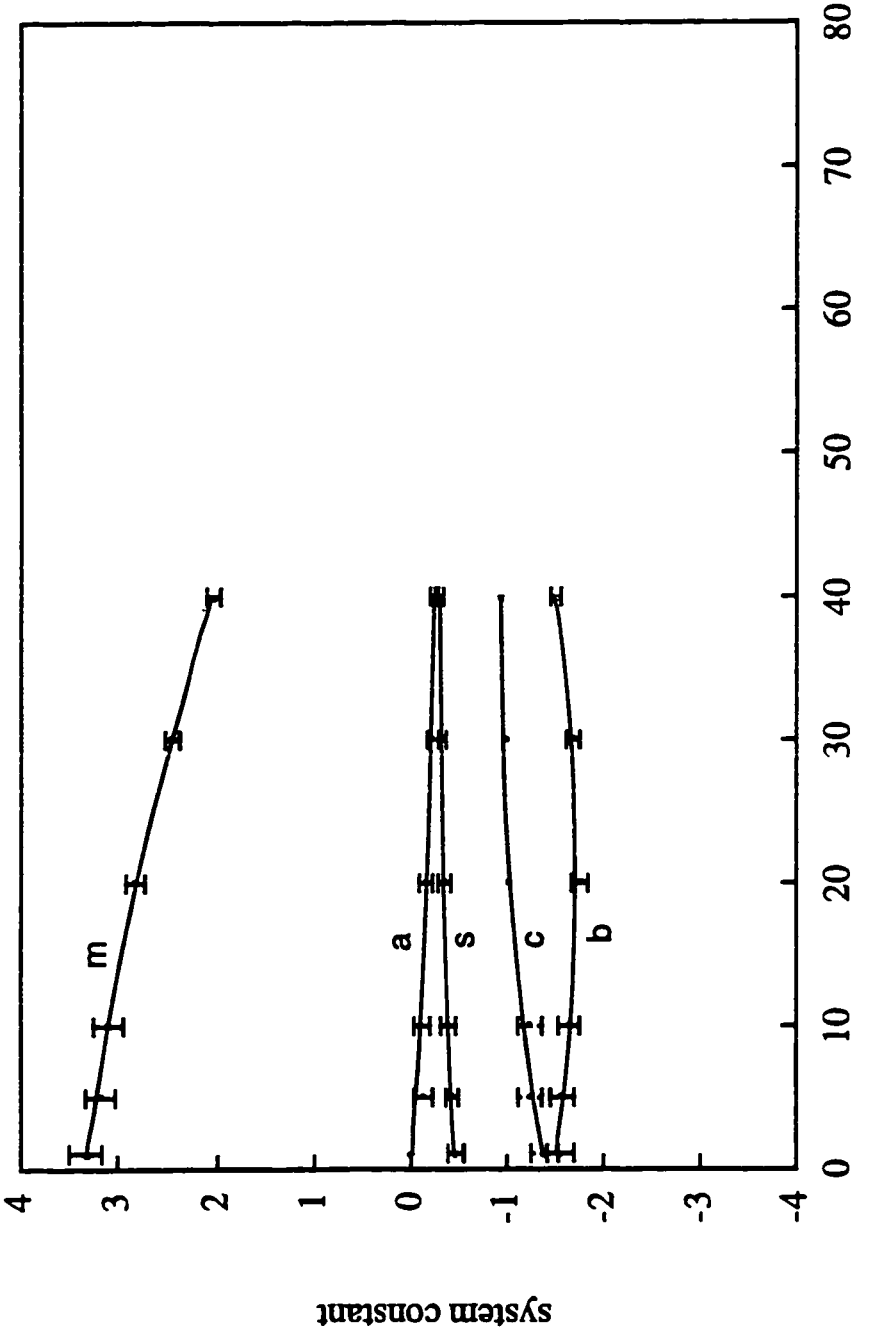


Figure B.3.1. System constants for the butylsiloxane bonded phase with methanol/water mobile phases. The r-constant is insignificant at all compositions.

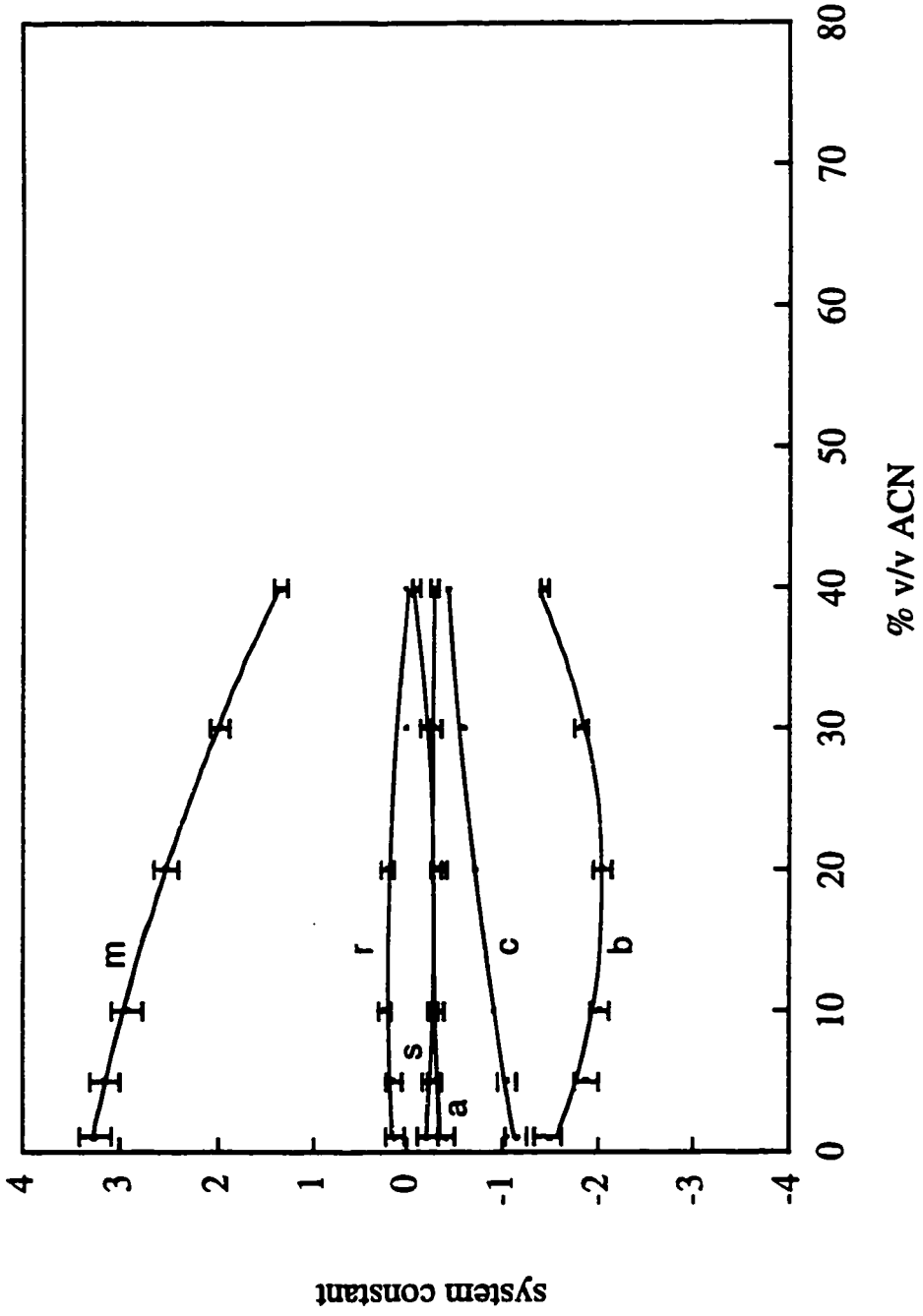


Figure B.3.2. System constants for the butylsiloxane bonded phase with acetonitrile/water mobile phases.

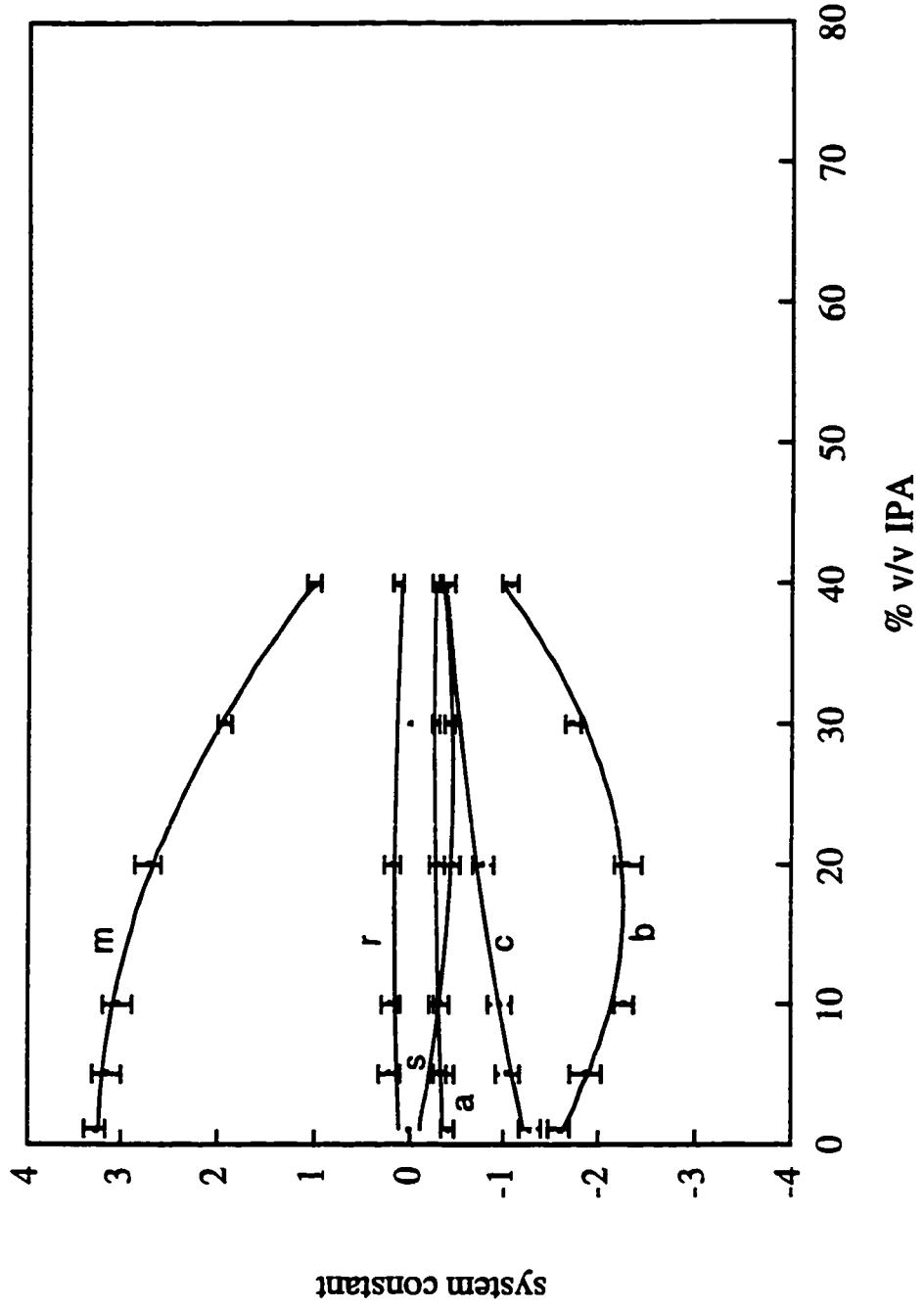


Figure B.3.3. System constants for the butylsiloxane bonded phase with isopropanol/water mobile phases.

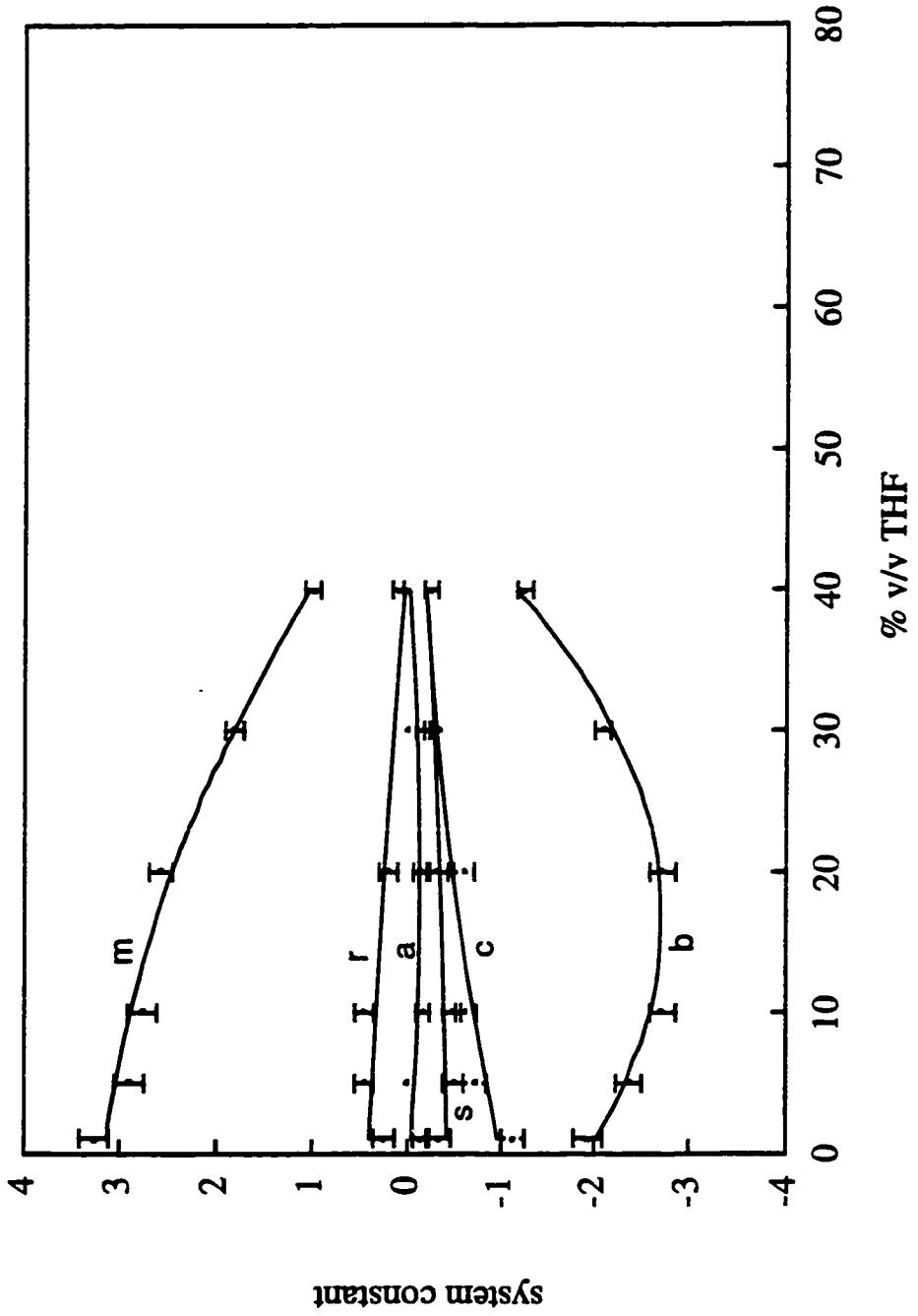


Figure B.3.4. System constants for the butylsiloxane bonded phase with tetrahydrofuran/water mobile phases.

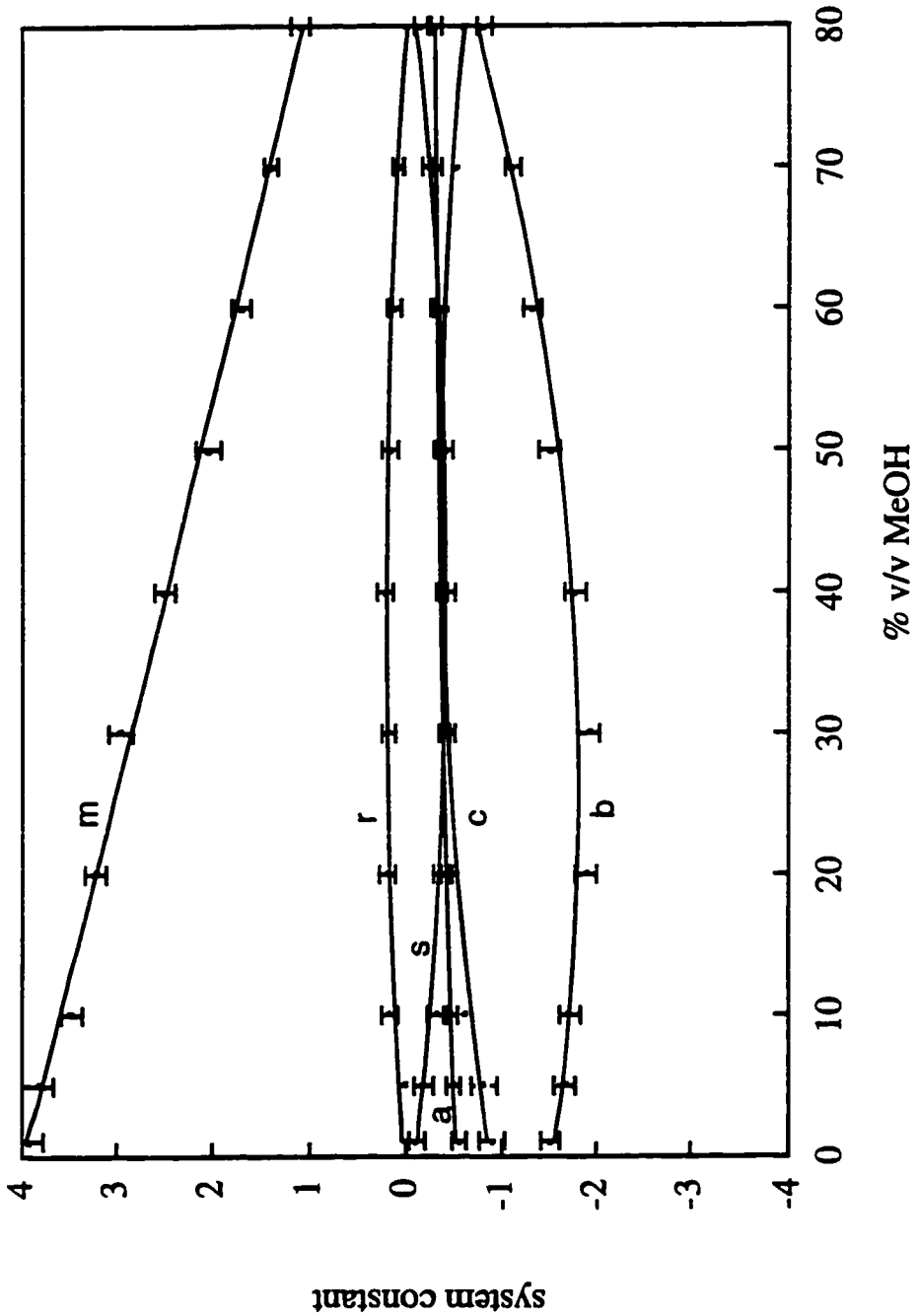


Figure B.4.1. System constants for the light-loaded octadecylsiloxane bonded phase with methanol/water mobile phases.

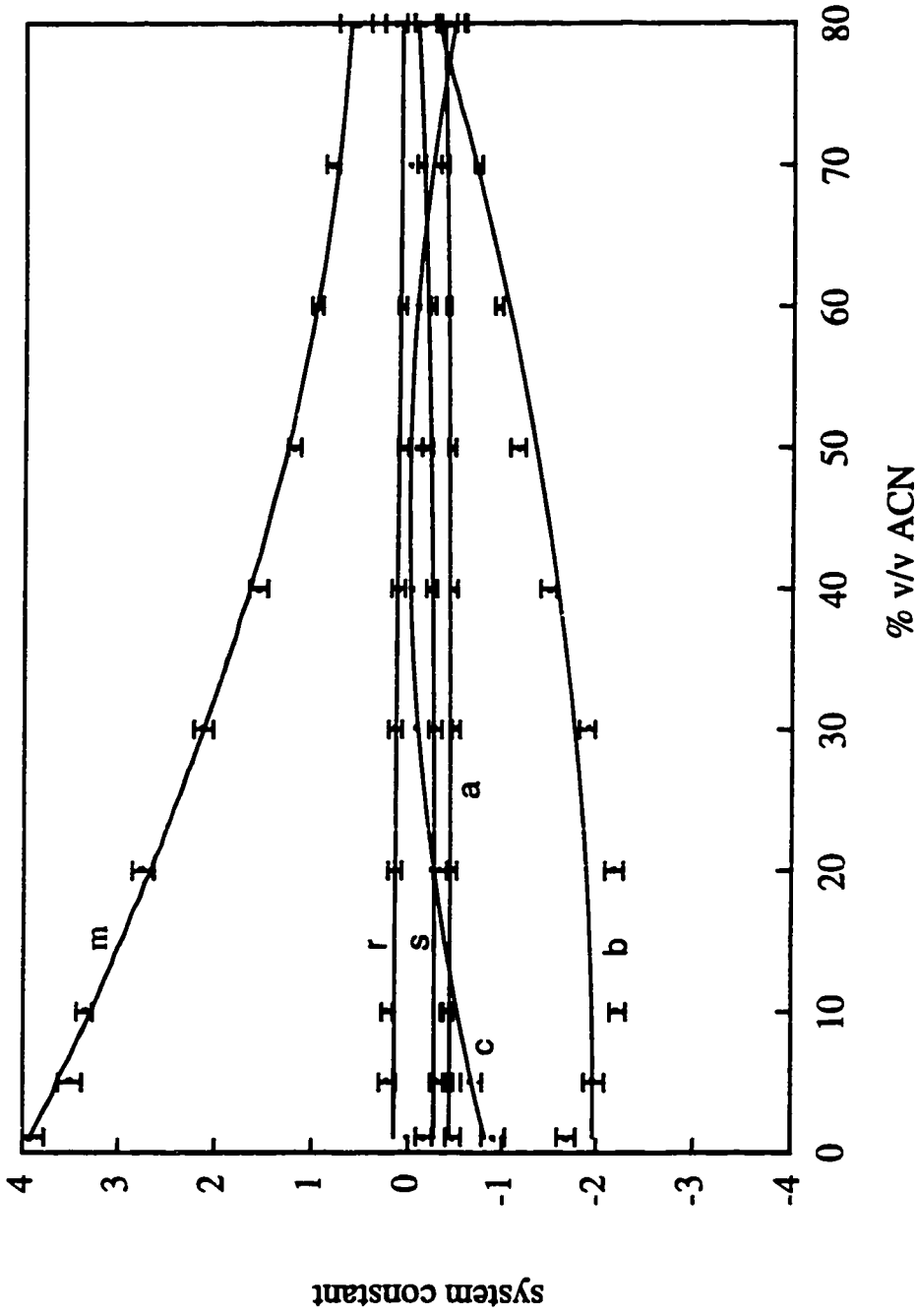


Figure B.4.2. System constants for the light-loaded octadecylsiloxane bonded phase with acetonitrile/water mobile phases.

APPENDIX C: Breakthrough curves

Measured breakthrough curves for estrone, β-estradiol, and estriol at 80%, 70%, 60%, and 50% v/v methanol.

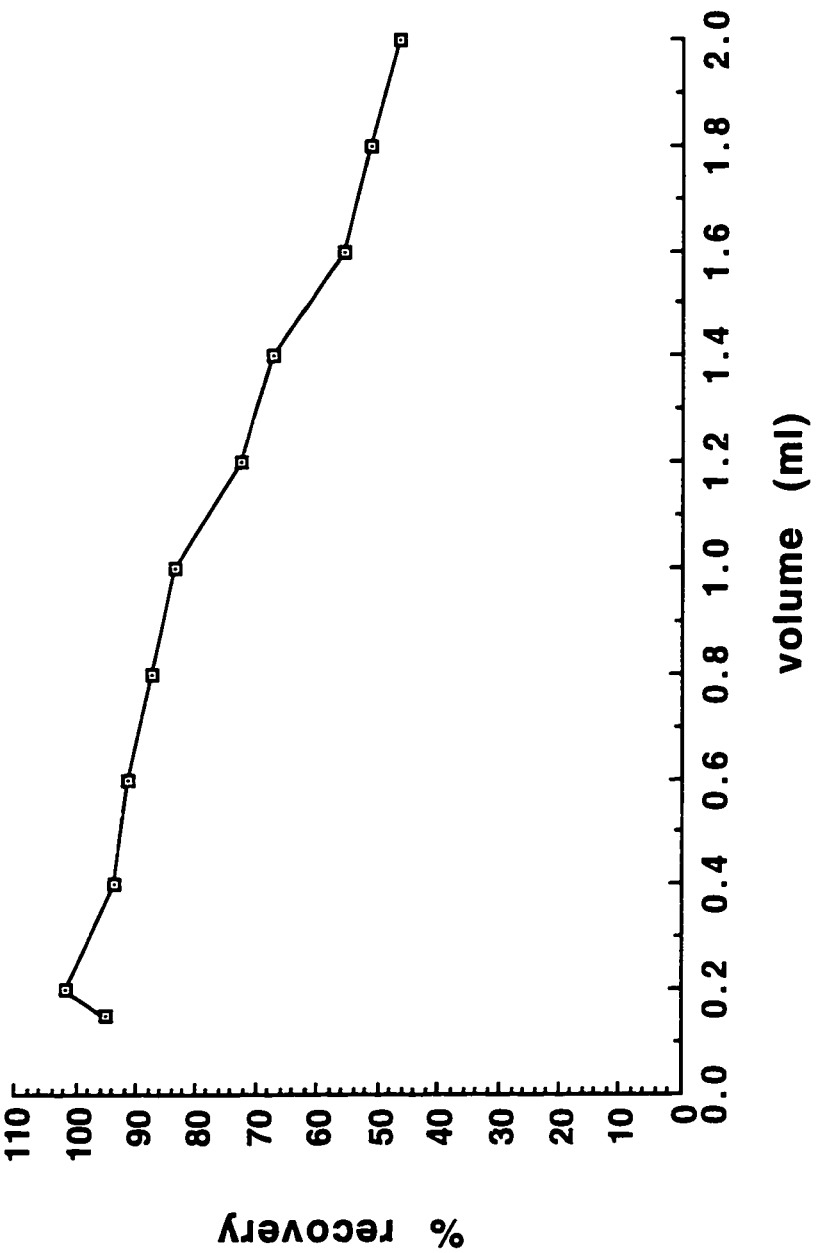


Figure C.1.1. Breakthrough curve for estrone in 80% v/v methanol.

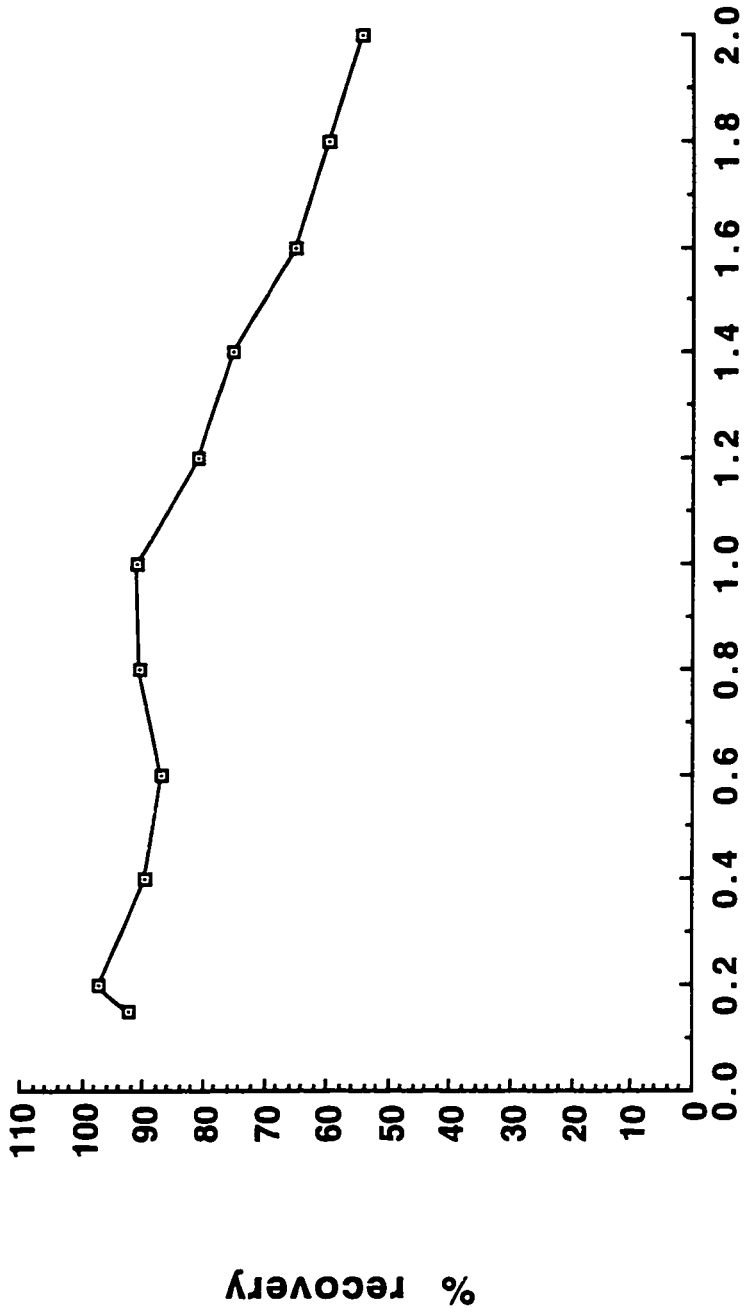


Figure C.1.2. Breakthrough curve for β-estradiol in 80% v/v methanol.

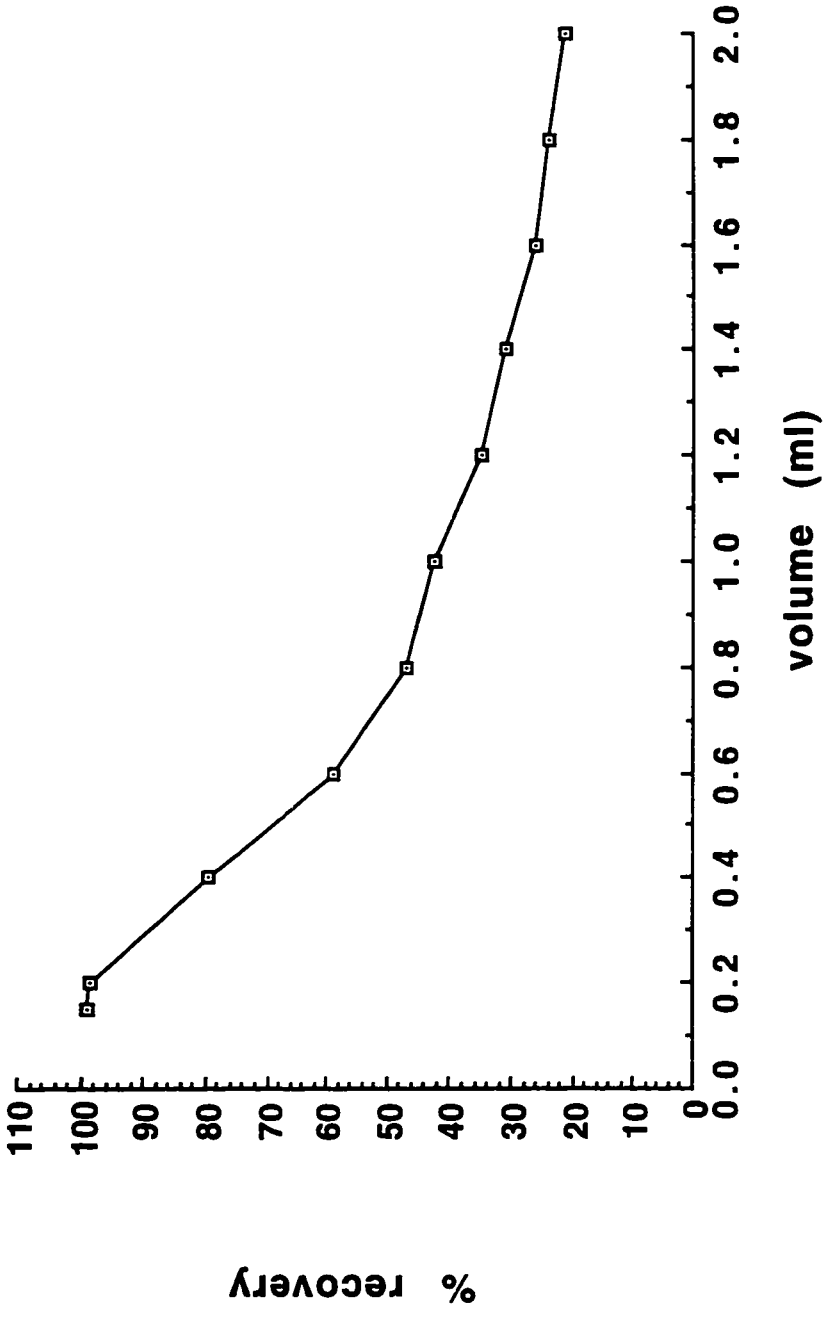


Figure C.1.3. Breakthrough curve for estriol in 80% v/v methanol.

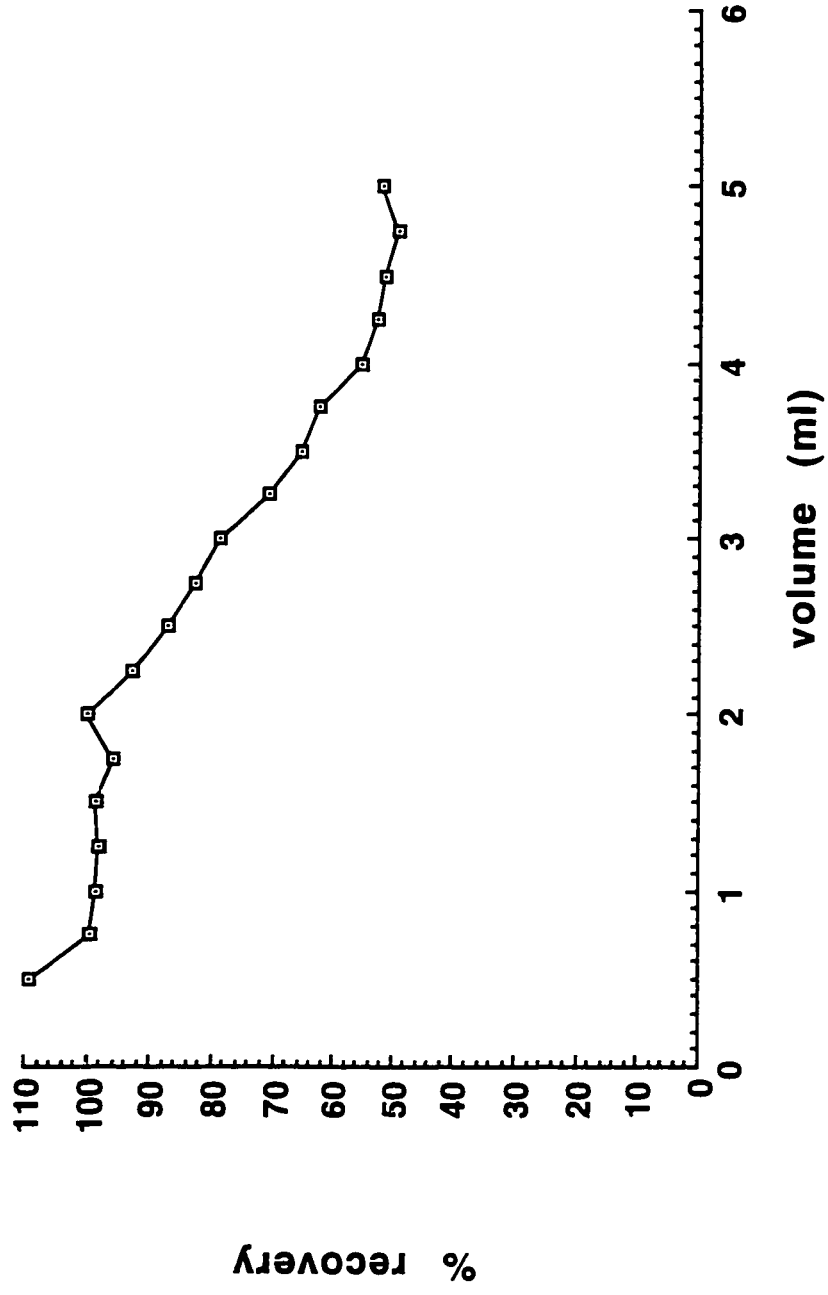


Figure C.2.1. Breakthrough curve for estrone in 70% v/v methanol.

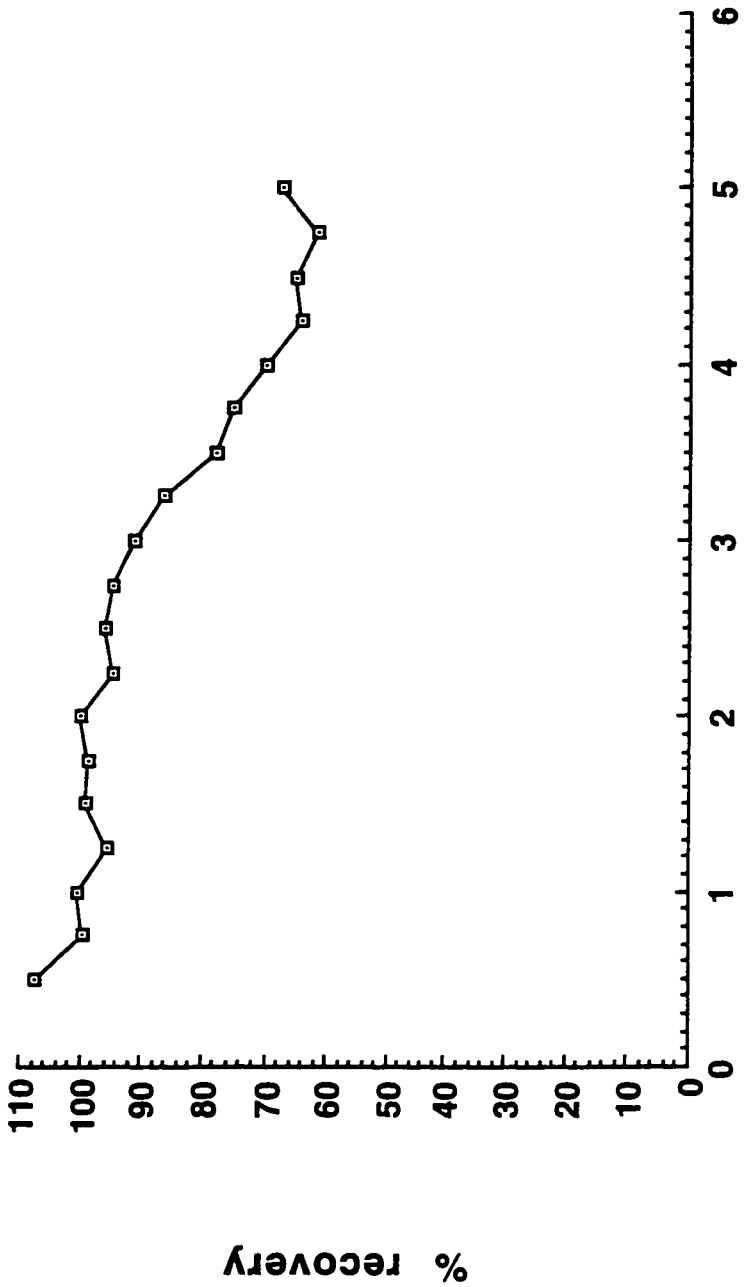


Figure C.2.2. Breakthrough curve for β-estradiol in 70% v/v methanol.

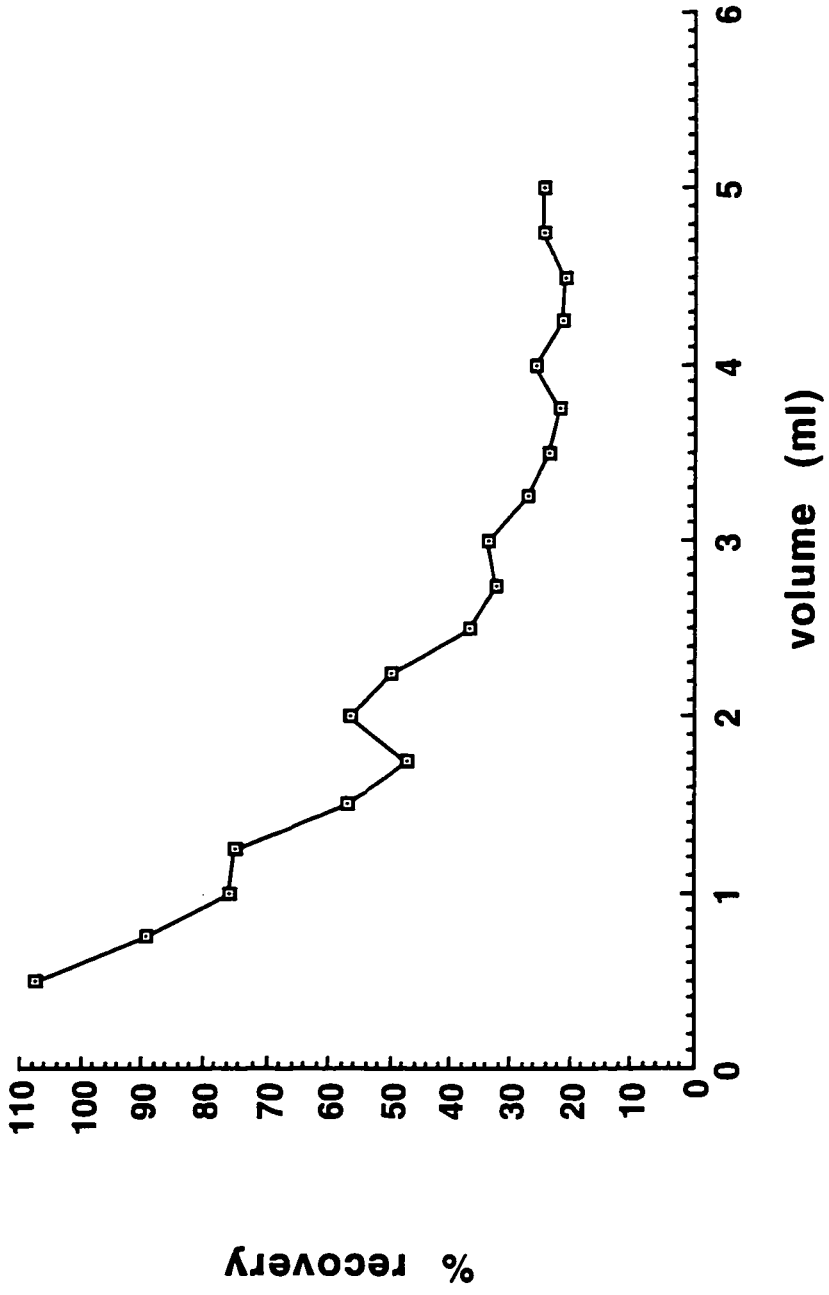


Figure C.2.3. Breakthrough curve for estriol in 70% v/v methanol.

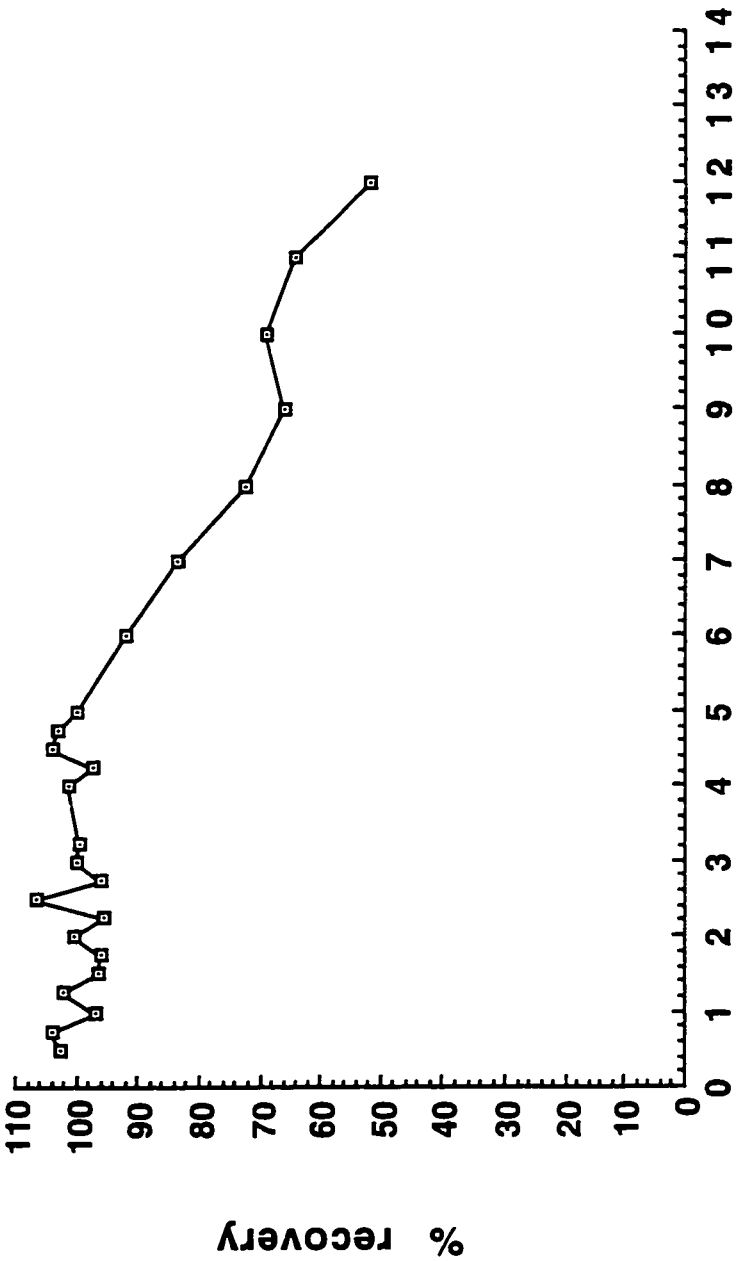


Figure C.3.1. Breakthrough curve for estrone in 60% v/v methanol.

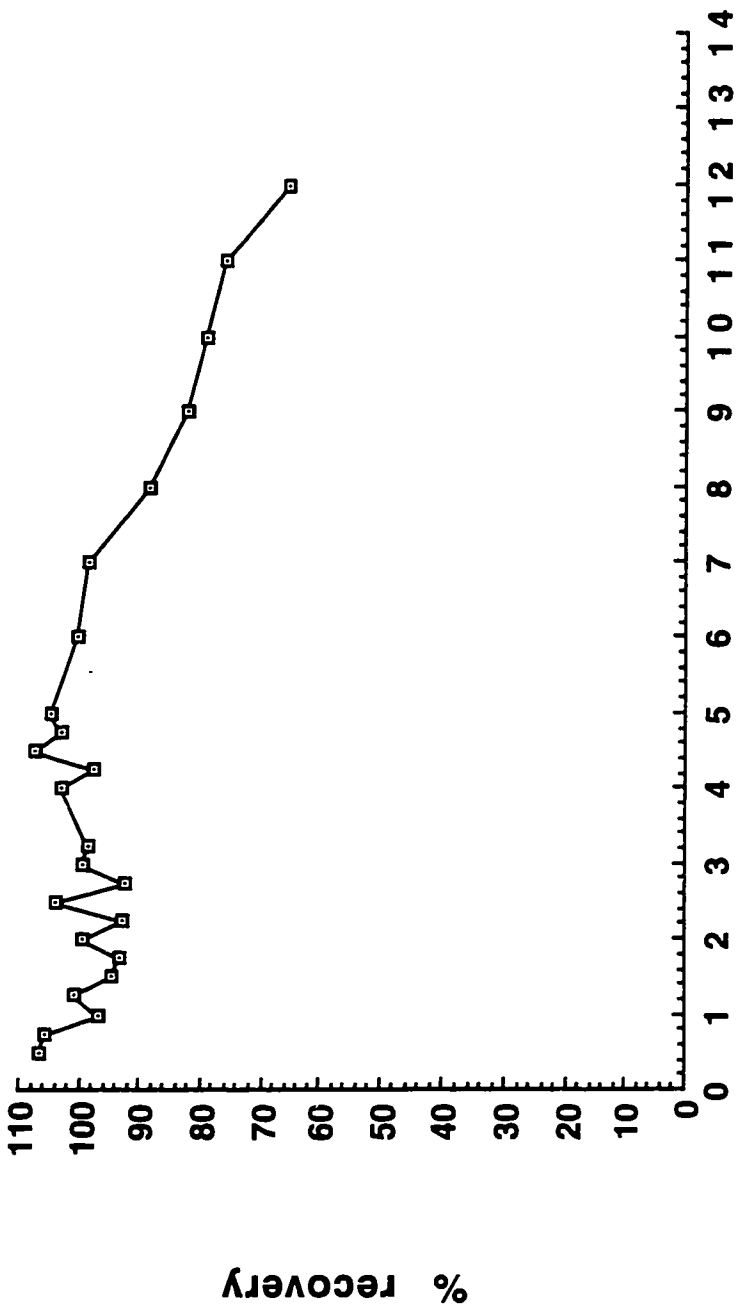


Figure C.3.2. Breakthrough curve for β-estradiol in 60% v/v methanol.

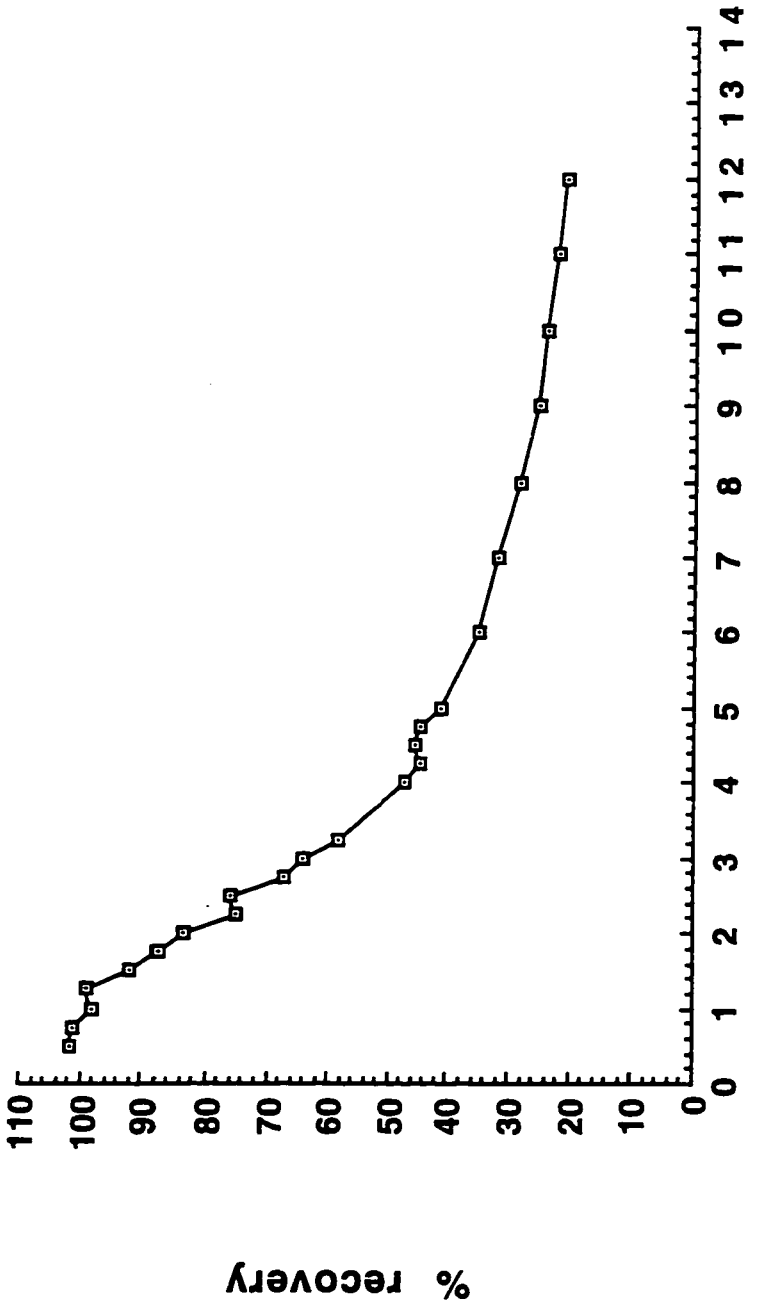


Figure C.3.3. Breakthrough curve for estriol in 60% v/v methanol.

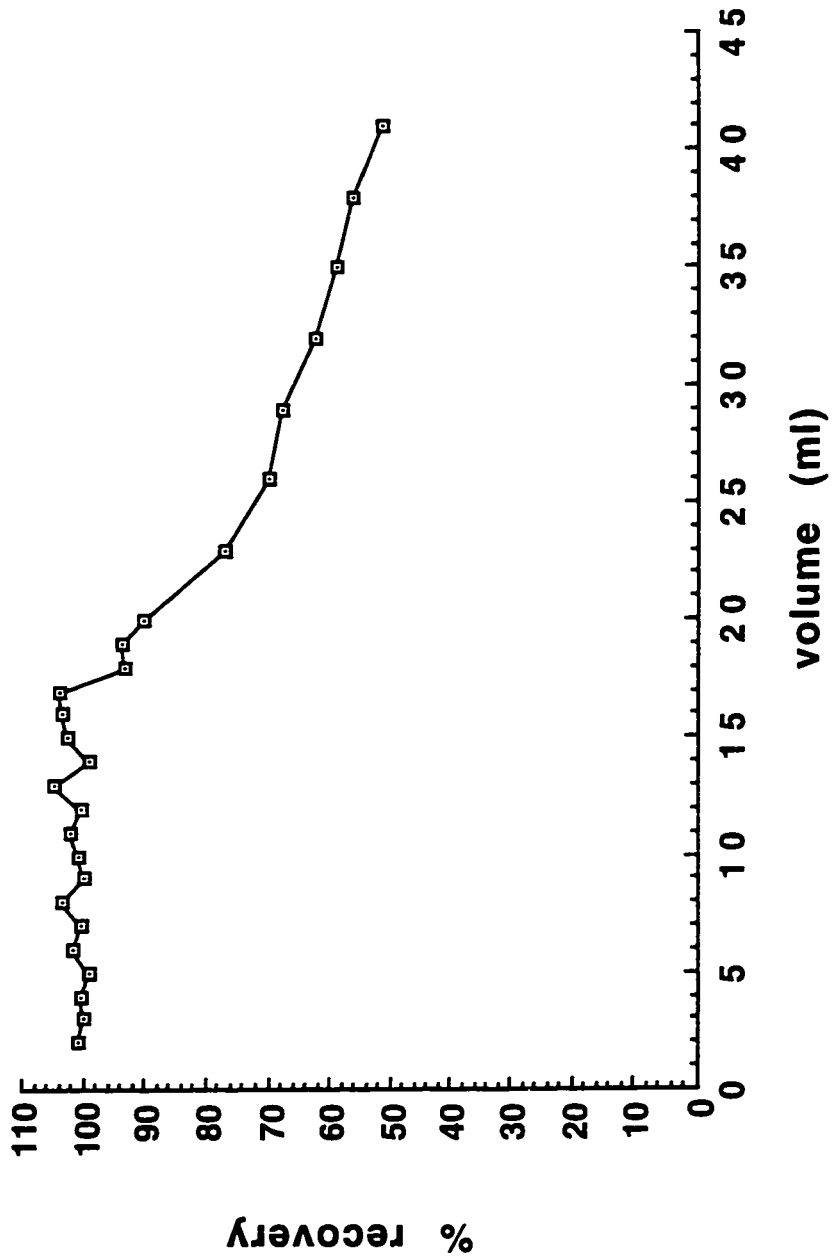


Figure C.4.1. Breakthrough curve for estrone in 50% v/v methanol.

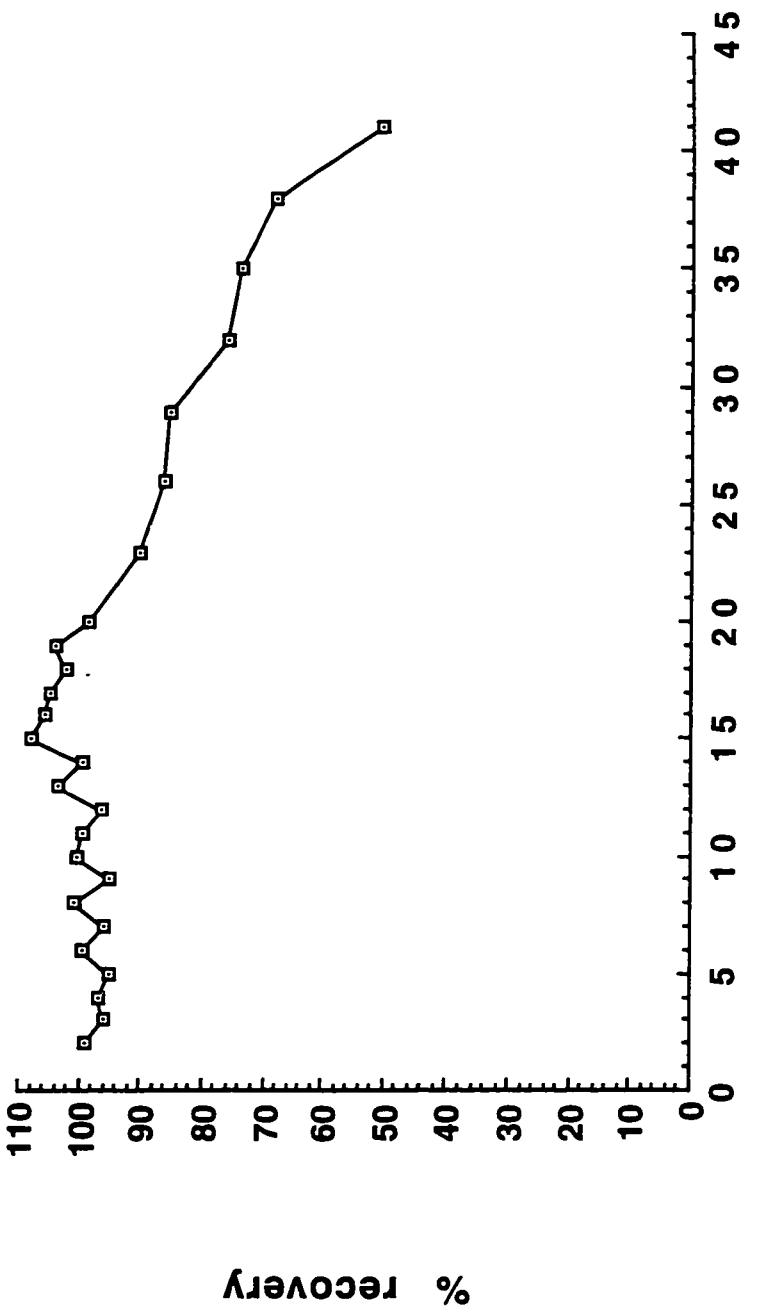


Figure C.4.2. Breakthrough curve for β-estradiol in 50% v/v methanol.

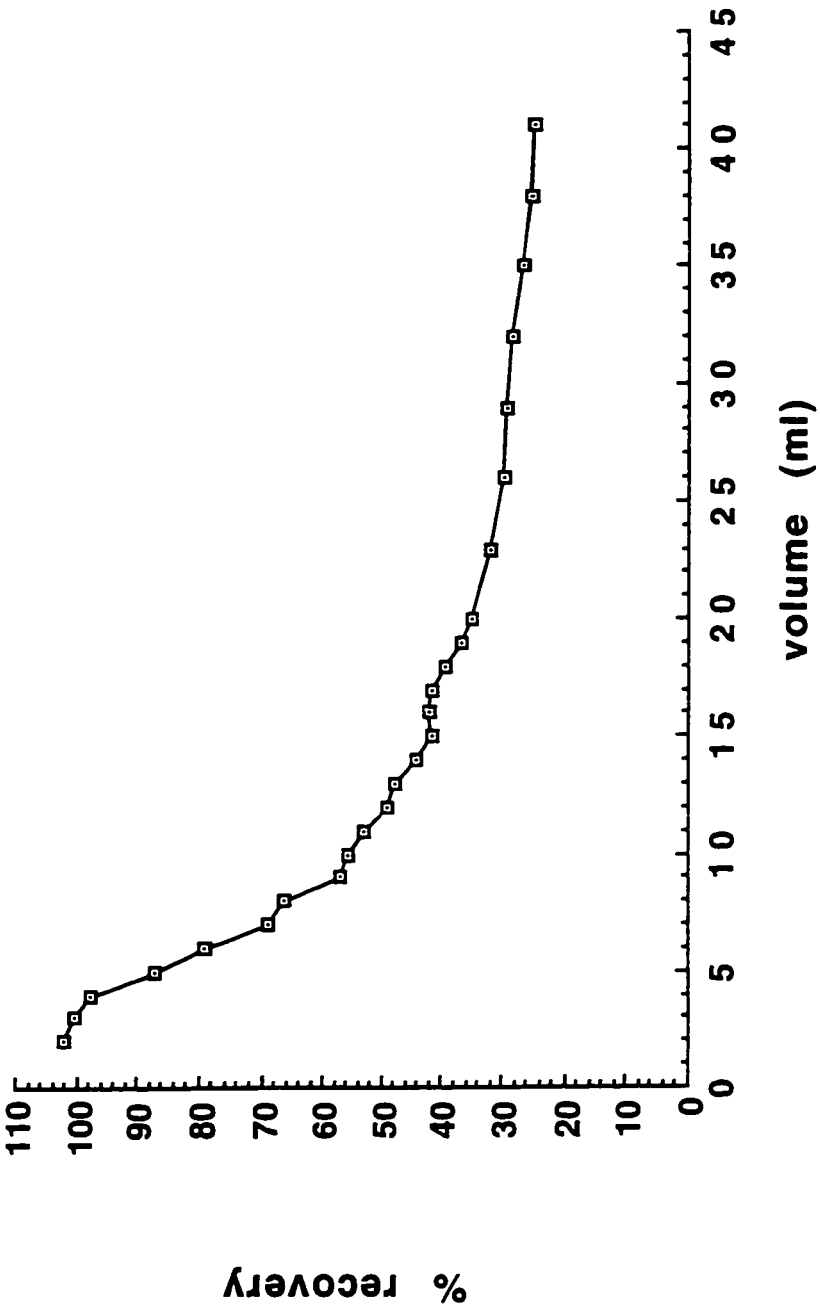


Figure C.4.3. Breakthrough curve for estriol in 50% v/v methanol.

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ABSTRACT

THE CHARACTERIZATION OF SILICA-BASED SORBENTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH APPLICATION TO METHOD DEVELOPMENT IN SOLID-PHASE EXTRACTION

by

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May 1998

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Despite the wide application of solid-phase extraction (SPE) in the environmental, clinical and pharmaceutical fields, method development for this technique has remained largely an empirical process of trial and error. In an effort to provide a systematic approach to method development, the kinetic and retention properties of four popular siloxane-bonded SPE sorbents have been determined. Kinetic measurements allow the evaluation of the performance of extraction devices and reveal areas in which small changes in design or manufacture could improve sampling characteristics.

Application of the solvation parameter model to retention data yields a set of system constants that describe the ability of the solvated stationary phase to compete with the bulk mobile phase for specific, clearly defined, intermolecular interactions with the solute. Once established, system constants can be used for the prediction of retention for any compound for which solute descriptors are available, the identification of new sorbents with sampling properties that set them apart from materials already available, and the prediction of breakthrough volume in SPE. Knowledge of breakthrough volume is a key parameter for predicting extraction conditions which are likely to be successful, thus eliminating a large portion of the guesswork involved in method development.

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2. Influence of Solvent Effects on Retention in Reversed-phase Liquid Chromatography and Solid-phase Extraction using a Cyanopropylsiloxane-bonded Silica-based Sorbent, D.S. Seibert and C.F. Poole, Chromatographia 41 (1995) 51.

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