Estimating Bioconcentration Factors for Organic Chemicals Using High Performance Liquid Chromatography (HPLC)

Sean Currens
University of Redlands

Follow this and additional works at: https://inspire.redlands.edu/cas_honors
Part of the Organic Chemistry Commons

Recommended Citation

Creative Commons Attribution-Noncommercial 4.0 License
This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 License
This material may be protected by copyright law (Title 17 U.S. Code).
This Open Access is brought to you for free and open access by the Theses, Dissertations, and Honors Projects at InSPIRe @ Redlands. It has been accepted for inclusion in Undergraduate Honors Theses by an authorized administrator of InSPIRe @ Redlands. For more information, please contact inspire@redlands.edu.
Estimating Bioconcentration Factors for Organic Chemicals Using High Performance Liquid Chromatography (HPLC)

Sean Currens
Research Advisor: Dr. Debra Van Engelen

Submitted to the faculty of the University of Redlands, in partial fulfillment of the requirements for the degree Bachelor of Science of Chemistry
University of Redlands
May 2011
Introduction

Many organic chemicals have the ability to reach high concentrations in organisms relative to their surrounding environment. This tendency becomes an important environmental concern when chemicals found at trace levels in the environment aggregate in an organism and reach toxic levels. The physiological effects of accumulating these compounds often have dire consequences for a species long after the chemicals have been introduced into an ecosystem. As a result of these effects, scientists have studied the degree to which a chemical bioaccumulates, or is taken up by an organism either directly from exposure to a contaminated medium or by eating food containing the chemical. In addition to directly measuring the concentrations of chemicals in organisms above the level in the environment, research has focused on developing models and methods to predict the extent to which new or preexisting chemicals bioaccumulate.

Many organic compounds' hydrophobic properties, or their higher solubility in organic matter than water, contribute to their tendency to concentrate in the fatty material of some species, a process known as bioaccumulation. A common example is the accumulation of nonpolar water contaminates in fish. As water passes through the gills of a fish, some organic molecules will selectively diffuse into the fatty, hydrophobic environment of the gills. For aquatic animals there is a competing process between uptake through respiration and diet and elimination from the organism via respiratory exchange, fecal egestion, metabolic breakdown of the chemical and growth dilution, as shown in figure 1. If these molecules are taken in at a greater rate than they are eliminated, over time the concentration of the chemical in the organism will be higher than the concentration in surrounding environment. Another source of bioaccumulation is through the food chain. If an organism that bioaccumulates a chemical is an
(and their removal from the endangered species act in 2007) has been attributed to the use and ensuing banning of DDT.\footnote{7}

The extent to which a compound accumulates in an animal is given by the bioconcentration factor, or BCF. For fish BCF is the ratio between the concentration of a compound in the fish and its concentration in the surrounding water.

\[
BCF = \frac{[\text{solute}]_{\text{fish}}}{[\text{solute}]_{\text{water}}} \tag{1}
\]

Fish have been the focus of BCF measurements in the past due to their important role as a human food source and the relative ease of measuring the concentrations of chemicals in their tissues.\footnote{8} Unfortunately, there are no universally accepted methods or criteria to measure or assess the quality of bioconcentration data. The reality is many variables exist in BCF measurements which make comparisons of BCF values between different studies unreliable.

One issue in determining BCF is the variability in measuring the concentration of the chemical in the fish. It is widely practiced that the weight of the whole body of the organism is measured, although some studies determine the concentration only for the weight of the lipid portion of the organism. Furthermore, inconsistencies exist between studies which measure the organism’s wet weight or dry weight. The concentration of the chemical throughout the sample is not likely to be uniform among different tissues and hydrophobic compounds accumulate primarily in lipids.\footnote{4} Taking this into account, the lipid content of the organs measured must be considered and the amount of lipid in the tissue determined. Errors may also arise from quantifying compounds using radiolabelled chemicals, where it’s possible to overestimate BCF
Table 1: Ordering organic compounds by increasing log Kow values shows a general trend for higher log BCF values as log Kow increases. These 15 compounds were used in the research presented here.

<table>
<thead>
<tr>
<th>Compound</th>
<th>log Kow</th>
<th>log BCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>0.90</td>
<td>0.41</td>
</tr>
<tr>
<td>2 - Nitroaniline</td>
<td>1.78</td>
<td>0.91</td>
</tr>
<tr>
<td>3 - Chloroaniline</td>
<td>1.88</td>
<td>1.06</td>
</tr>
<tr>
<td>2 - Chloroaniline</td>
<td>1.90</td>
<td>1.18</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.13</td>
<td>1.46</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.79</td>
<td>0.92</td>
</tr>
<tr>
<td>2,4 - Dichloroaniline</td>
<td>2.79</td>
<td>1.98</td>
</tr>
<tr>
<td>3,4 - Dichloroaniline</td>
<td>2.79</td>
<td>1.48</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>3.36</td>
<td>2.20</td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>3.90</td>
<td>2.76</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.45</td>
<td>2.78</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.46</td>
<td>3.16</td>
</tr>
<tr>
<td>2,4,5 - Trichlorobiphenyl</td>
<td>5.51</td>
<td>4.26</td>
</tr>
<tr>
<td>3,3,4,4 - Tetrachlorobiphenyl</td>
<td>5.82</td>
<td>4.59</td>
</tr>
<tr>
<td>Octachloronaphthalene</td>
<td>8.40</td>
<td>2.52</td>
</tr>
</tbody>
</table>

A simple and common model is linear regression of log BCF and logKow shown in equation 3.

\[ \text{log BCF} = a \text{ log Kow} + b \]  

As the value of logKow increases, logBCF increases as well. Qualitatively this suggests that as compounds become increasingly more soluble in octanol than in water, they have a tendency to bioaccumulate. The coefficient \( a \) for this equation is usually positive and less than 1 and the coefficient \( b \) is negative. The disadvantage to this model is that for extremely hydrophobic
negative correlation. For a perfect positive fit, \( r \) has a value of exactly 1 and for two variables with a random, non-linear relationship, \( r \) has a value of 0. The correlation coefficient of the parabolic model (4) \( \log BCF = -0.164 (\log K_{ow})^2 + 2.059 \log K_{ow} - 2.592 \) was \( r = 0.914 \) (\( R^2 = 0.835 \)) and the correlation coefficient for the bilinear model (5) \( \log BCF = 0.910 \log K_{ow} - 1.975 \log(6.8 \times 10^7 K_{ow} + 1) - 0.786 \) was \( r = 0.950 \) (\( R^2 = 0.903 \)). Because the \( r \) value for the bilinear model is higher than the \( r \) values for the linear and parabolic model, this equation is the most suitable model for this application.

\[
\begin{align*}
\text{Figure 1: The relationship between log } K_{ow} \text{ and log BCF for 154 chemicals is modeled using} \\
\text{linear (3), parabolic (4), and bilinear equations (5).}^8
\end{align*}
\]

In order to test the models, BCF and \( K_{ow} \) data were collected from the literature for 29 new chemicals and their \( K_{ow} \)'s were used to generate expected BCF values. Again, the data
dissolution in one of the solvent phases, the formation of colloidal dispersions, volatilization out of the liquid phases, and adsorption of the compounds onto the surface of the flask.\textsuperscript{15}

An older, less-used method for directly measuring $K_{ow}$ is a generator column. In this method a column is packed with a solid support that has been saturated with a fixed concentration of n-octanol. Water is pumped through the column and exits with the concentration of the target compound that is equal to the equilibrium concentration between water and n-octanol.\textsuperscript{13} Slow pumping of water through the stationary phase eliminates the formation of colloidal dispersions yet creates enough interfacial area between the n-octanol and water for an equilibrium to be reached. This method also has the advantage of being more accurate and reproducible for hydrophobic compounds with log$K_{ow}$ values greater than 4, however it is very laborious.\textsuperscript{15,16}

The previously reported procedures are direct methods of measuring $K_{ow}$, that is, they directly measure the concentration of the target compound in one or both liquid phases after a true solvent-solvent partition process. More recently, high performance liquid chromatography (HPLC) has been used to estimate $K_{ow}$.\textsuperscript{17} Figure 3 shows a simple diagram of an HPLC instrument. With HPLC, a small amount of the target compound chemical is injected onto a column around 5mm wide or less. The column is packed with a stationary phase that is usually coated onto a solid support such as silica and can have a variety of chemical functional groups covalently bonded to its surface. The solvent, or mobile phase, is forced through the column at a set speed using a pump exerting pressures as high as 1000 atmospheres.\textsuperscript{18} The compound of interest is constantly partitioning between the mobile and stationary phases based on its attractions to the two phases. If a compound is more soluble in the mobile phase than the
The chromatographic retention time is the elapsed time between when a sample is injected and when the peak maximum of a solute is detected. The void time \((t_v)\) is the time it takes an unretained solute to travel from the injection point to the detector. A more useful term than the retention time for describing the rate a solute migrates through a column is the capacity factor, \(k'\). The capacity factor is easily obtained by subtracting the void time from the retention time and dividing by the void time. The capacity factor is also directly proportional to the concentration of solute in the stationary phase over the concentration of solute in the mobile phase or the partition coefficient between the two phases.

\[
k' = \frac{t_R - t_M}{t_M}
\]  

(6)

Figure ?: The equation for a capacity factor \((k')\) defined by the terms retention time \((t_R)\) and void time \((t_M)\). Note the capacity factor of an unretained peak is 0.

Reversed phase is the most common type of HPLC with a less polar stationary phase and more polar mobile phase. The solid support of the column usually has nonpolar C18 or C8 chains covalently bonded to it and the mobile phase is made up of one or more polar solvents, such as water. Polar molecules will be more attracted to the polar mobile phase and elute from the column quickly, while nonpolar molecules will be less soluble in the mobile phase and spend more time in the stationary phase, causing them to elute from the column later. Normal phase HPLC is similar to reversed phase however the relative polarities of the mobile and stationary phases are reversed. A common normal phase column has cyano or amino functional groups.
higher reproducibility. These features have made HPLC a common means for determining chemical hydrophobicity by correlating retention time data with log $K_{ow}$. The desired result of using HPLC is to optimize the HPLC conditions so that the data collected, which are related to $K_{ow}$, can be used to predict biological processes, such as BCF.

While there are hundreds of studies investigating the relationship between data obtained by reversed phase HPLC and partition coefficients such as $K_{ow}$, with correlation coefficients ranging from 0.5 to 0.999, several papers have tried to estimate BCF directly from retention parameters. Hui et al. investigated the effects of four different reversed phase columns on the correlation between log $k'$ and log BCF. The basis of the investigation is the assumption that the partitioning of a chemical between the polar aqueous-based mobile phase and nonpolar stationary phase in reversed phase LC can model the partitioning of a chemical between water and the nonpolar lipid parts of a fish.

To test the effectiveness of C2, C8, C18 or C-phenyl stationary phases for predicting BCF, the authors obtained from the literature capacity factors for 12 organic compounds using the four columns and 2 mobile phases, acetonitrile/water and methanol/water. For all twelve compounds a simple linear regression of log $k'$ against log BCF having the form log BCF = $a \log k' + b$ was developed for the 8 possible combinations of mobile/stationary phases, as well as a linear regression of log $K_{ow}$ against log BCF. Based on the statistical parameters of the linear regressions, the stationary/mobile phase combination capable of making the best prediction of BCF was a C-phenyl column with methanol/water eluent. The linear correlation coefficient ($r$) and standard error of estimation ($s$), which is used to determine how well a least squares line equation fits a data set, was $r = 0.932\ (R^2 = .869)$ and $s = 0.351$ for the C-phenyl column with
Due to the wide variety and selection of HPLC columns, the ability of many stationary and mobile phases to estimate BCF are untested. To further study the predictive capabilities of HPLC, similar experiments must be done with new columns and instrument conditions. To this end, we selected a small (14), diverse group of organic compounds having a wide range of log $K_{ow}$ and log BCF values and collected $k'$ data on a reverse phased C18 column as well as a normal phase substituted amylose column. Once data has been collected results from both setups will be compared.

We hypothesize that the chiral column will prove to be a good predictor of BCF. Not only does the chiral column have a binary lipophilic/hydrophilic composition similar to the cyano column used by Hui et. al., it may also be more able to model the driving thermodynamic forces of biological partitioning. Passive transport across membranes is mainly entropy driven, unlike partitioning in reversed phase HPLC which is enthalpy driven. The 3D composition of the amylase-based chiral column makes the partitioning of solute molecules between mobile phases and stationary phases more entropy driven like biological partitioning. We hypothesize that this thermodynamic difference could improve the reversed phase HPLC model and make a better correlation between $k'$ and BCF.
5\,\mu m). The mobile phase for the new column was 95% hexane and 5% isopropanol with a flow rate of 1.0 ml/min. Measurements on this column were not done in triplicate for three of fifteen compounds and solutions were not run in a specific order. The data for both reversed and normal phase columns were collected over the course of 5 months.

Once all retention data had been collected from the HPLC, they were analyzed in Excel 2007 along with $K_{ow}$ and BCF data that was compiled from three literature sources.$^{8,12,14}$
log\textsubscript{K\textsc{ow}} = 8.40 from the regression yields the equation log\textsubscript{BCF} = 0.834 log\textsubscript{K\textsc{ow}} - 0.5691 with R\textsuperscript{2} = 0.9458, a substantial improvement from the previous R\textsuperscript{2} and the R\textsuperscript{2} generated by Bintein. This agrees with the conclusion proposed by Bintein that a linear model is best for describing bioconcentration for a model comprising fewer data points with low log\textsubscript{K\textsc{ow}} values.

Figure 5: A graph of log\textsubscript{K\textsc{ow}} vs log\textsubscript{BCF} for 14 organic chemicals included in this study. A linear regression is shown for all data (log\textsubscript{BCF} = 0.4831 log\textsubscript{K\textsc{ow}} - 0.591, r = 0.769) and for all data excluding octachloronaphthalene and the point 8.40, 2.52 (log\textsubscript{BCF} = 0.839 log\textsubscript{K\textsc{ow}} - 0.517, r = 0.967). Table 3 contains all graphed data points.
more than one significant peak. 3,4 - dichlormethane was an exception due to the impurity peak at 4.7 minutes.

**Figure 6**: Chromatogram of absorbance of 3,4 - dichloroaniline (152 ppm in methanol) at 254 nm on a reversed phase C18 column with a mobile phase of 85:15 methanol and water at a flow rate of 1.0 ml/min.

Pure methanol was used to determine the retention time of an unretained peak, $t_m$, used in determining the capacity factor. Although the absorbance of methanol at 254 nm is approximately 60 times less than 3,4 - dichloroaniline, the UV-Vis detector was sensitive enough to obtain both peak maximums. The retention time of the solute and $t_m$ varied greatly between runs due to an increasingly leaky frit delivering eluent to the column. The increase in leaking (over the course of many runs) caused the actual flow rate of eluent through the column to be less than 1.00 ml/min for later runs. The results of benzene in Table 2 illustrate the shift in $t_m$ and
### Table 3: the average and standard deviation of $k'$ for 13 chemicals using a C18 column.

Columns 5, 6 and 7 show the log transformation of $BCF$, average $k'$ and $K_{ow}$ for those chemicals. 

The ability of $k'$ to predict bioconcentration was examined by graphing average log$k'$ vs log$BCF$, shown in Figure 7. A linear regression of the data produced the equation $\log BCF = 3.22 \log k' - 1.11$ with $R^2 = 0.882$ and $r = 0.939$.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k'$ avg</th>
<th>$k'$ stdev</th>
<th>relative stdev</th>
<th>$\log k'$</th>
<th>$\log BCF$</th>
<th>$\log K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>0.764</td>
<td>0.070</td>
<td>9.21</td>
<td>-0.117</td>
<td>0.41</td>
<td>0.90</td>
</tr>
<tr>
<td>2 - Nitro aniline</td>
<td>1.25</td>
<td>0.720</td>
<td>57.3</td>
<td>0.099</td>
<td>0.91</td>
<td>1.78</td>
</tr>
<tr>
<td>3 - chloroaniline</td>
<td>0.874</td>
<td>0.235</td>
<td>26.8</td>
<td>-0.059</td>
<td>1.06</td>
<td>1.88</td>
</tr>
<tr>
<td>2 - chloroaniline</td>
<td>0.957</td>
<td>0.028</td>
<td>2.90</td>
<td>-0.019</td>
<td>1.18</td>
<td>1.90</td>
</tr>
<tr>
<td>3,4 - dichloro aniline</td>
<td>1.00</td>
<td>0.040</td>
<td>3.99</td>
<td>0.004</td>
<td>1.48</td>
<td>2.79</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.24</td>
<td>0.104</td>
<td>8.37</td>
<td>0.096</td>
<td>1.46</td>
<td>2.11</td>
</tr>
<tr>
<td>2,4 - dichloroaniline</td>
<td>1.32</td>
<td>0.068</td>
<td>5.17</td>
<td>0.121</td>
<td>1.98</td>
<td>2.79</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.62</td>
<td>0.164</td>
<td>10.0</td>
<td>0.210</td>
<td>0.92</td>
<td>2.65</td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>1.84</td>
<td>0.057</td>
<td>3.08</td>
<td>0.266</td>
<td>2.76</td>
<td>3.90</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2.17</td>
<td>0.120</td>
<td>5.50</td>
<td>0.337</td>
<td>2.20</td>
<td>3.36</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.40</td>
<td>0.030</td>
<td>0.690</td>
<td>0.644</td>
<td>3.16</td>
<td>4.57</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.91</td>
<td>0.163</td>
<td>3.31</td>
<td>0.692</td>
<td>2.78</td>
<td>4.54</td>
</tr>
<tr>
<td>2,4,5 Trichlorobiphenyl</td>
<td>8.63</td>
<td>0.351</td>
<td>4.06</td>
<td>0.936</td>
<td>4.26</td>
<td>5.51</td>
</tr>
<tr>
<td>3,3,4,4 Tetrachlorobiphenyl</td>
<td>10.5</td>
<td>0.467</td>
<td>4.43</td>
<td>1.02</td>
<td>4.59</td>
<td>5.82</td>
</tr>
</tbody>
</table>
Table 4: a comparison of $r$, the linear correlation coefficient, and $R^2$ for log$k'$ and logBCF collected by Hong and data from this study.\textsuperscript{12} Data from this study is marked with an asterisk.

<table>
<thead>
<tr>
<th>HPLC Column</th>
<th>Mobile Phase Composition</th>
<th>$r$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>acetonitrile - water</td>
<td>0.906</td>
<td>0.821</td>
</tr>
<tr>
<td>C8</td>
<td>acetonitrile - water</td>
<td>0.825</td>
<td>0.681</td>
</tr>
<tr>
<td>C-phenyl</td>
<td>acetonitrile - water</td>
<td>0.916</td>
<td>0.839</td>
</tr>
<tr>
<td>C2</td>
<td>acetonitrile - water</td>
<td>0.857</td>
<td>0.734</td>
</tr>
<tr>
<td>C18</td>
<td>methanol - water</td>
<td>0.927</td>
<td>0.857</td>
</tr>
<tr>
<td>C8</td>
<td>methanol - water</td>
<td>0.821</td>
<td>0.674</td>
</tr>
<tr>
<td>C-phenyl</td>
<td>methanol - water</td>
<td>0.932</td>
<td>0.869</td>
</tr>
<tr>
<td>C2</td>
<td>methanol - water</td>
<td>0.777</td>
<td>0.604</td>
</tr>
<tr>
<td>*C18</td>
<td>*methanol - water</td>
<td>*0.939</td>
<td>*0.882</td>
</tr>
</tbody>
</table>

No capacity factors were included in Table 3 for the reversed phase HPLC study for octachloronaphthalene, the compound which disrupted the linear trend of log$K_{ow}$ vs logBCF. This is due to the compounds very low solubility in the 85% methanol 15% water mobile phase. No analyte peak could be detected for sample runs of less than 100 ppm of the compound in isopropanol, possibly due to the solute molecules precipitating out of solution in the injector port before reaching the column or the detector. It is unknown whether the compound, which did not follow a linear trend when graphed in log$K_{ow}$ vs logBCF, would have also disrupted the linear trend in log$k'$ vs logBCF. It is clear, however, that the mobile phase used was not a practical choice for measuring such strongly hydrophobic compounds. It is possible increasing the percentage of methanol or using a more nonpolar organic solvent could solve this problem.

Without octachloronaphthalene, the linear correlation coefficient of log$k'$ and logBCF is comparable to $r$ from log$K_{ow}$ and logBCF, 0.939 and 0.967, respectively. The $R^2$ from the HPLC
Figure 8: Chromatogram of 3,4-dichloroaniline (152 ppm in methanol) on a normal phase amylose column.

Compounds in Table 5 are listed in the same order they are listed for Table 2, by increasing k' on the C18 normal phase column. Table 5 shows a trend of decreasing k', indicating that compounds that eluted quickly on the reversed phase column tended to elute more slowly on the normal phase column. This is because in reversed phase chromatography hydrophobic molecules will spend more time in the nonpolar stationary phase (C18) than the polar mobile phase (methanol/water), and the opposite is true for more hydrophilic molecules. In normal phase chromatography the polarity of the mobile and stationary phases is reversed, causing hydrophobic molecules to equilibrate more in the mobile phase (hexane/isopropanol) than the stationary phase (amylose/carbamate) and more hydrophilic molecules to do the opposite. Table 6 presents the average k', the standard deviation and relative standard deviation in k' measurements, log k' and log BCF for the amylase based column.
Graphing log$k'$ vs. logBCF for the normal phase produced the linear regression

$logBCF = -1.91 \log K_{ow} + 1.88$ with $R^2 = 0.550$ and $r = -0.742$ shown in Figure 9. The negative r value indicates an inverse correlation with $k'$ and the low $R^2$ indicates a poor correlation between log$k'$ and logBCF for the normal phase configuration and that the reversed phase column is more capable of predicting bioconcentration.

Figure 9: log $k'$ from an amylose column versus log BCF with the equation of a linear regression.
bulk phases that each have uniform physical properties and uniform solute concentration. This is in contrast to a biological membrane made up of interfacial phases where the physical properties of the phases vary with distance from the interface. Interfacial phases can lead to an gradient of solute concentration at equilibrium. For example, a membrane that is more dense towards the exterior head groups can have a gradient of solute concentration at equilibrium where there is a higher concentration of solute molecules at the interface of the bilayer.\textsuperscript{17} We hypothesized that the chiral properties of the amylose-based normal phase column might result in a gradient of disorder more like an interfacial phase, causing the stationary phase to be more similar to the interior of the lipid bilayer.

Another key difference between biological partitioning and reversed phase HPLC is the driving thermodynamic forces behind the two processes. In reversed phase HPLC large nonpolar molecules undergo induced dipole interactions with the nonpolar stationary phase. These are energetically favorable and enthalpy driven. In a biological system the partitioning of a large nonpolar molecules from the aqueous phase to the lipid bilayer results in an increase in disorder of the water molecules which were previously ordered around the nonpolar molecule. This increase in entropy is the driving thermodynamic force in biological partitioning.\textsuperscript{17} Partitioning in the amylose based normal phase HPLC is partly due to the enthalpy driven induced dipole effects found in the reversed phase HPLC system. However, the chiral column also separates solute molecules using 3D inclusion pockets. These pockets make the partitioning entropy driven like biological partitioning.

We hypothesized the thermodynamic similarities between the reversed phase HPLC and biological partitioning would make the chiral reversed phase HPLC a better predictor of
References


Organized by increasing $k'$ on C18 column

Aniline
150 ppm in methanol
logKow = 0.90
log BCF = 0.41
Avg tr C18 = 3.39 min

2 - Nitroaniline
150 ppm in methanol
logKow = 1.78
log BCF = 0.91
Avg tr C18 = 3.60 min

3 - Chloroaniline
150 ppm in methanol
logKow = 1.88
log BCF = 1.06
Avg tr C18 = 3.68 min

2 - Chloroaniline
166 ppm in methanol
logKow = 1.90
log BCF = 1.18
Avg tr C18 = 3.88 min

3,4 - Dichloroaniline
152 ppm in methanol
logKow = 2.79
log BCF = 1.48
Avg tr C18 = 4.06 min

Benzene
150 ppm in methanol
logKow = 2.13
log BCF = 1.46
Avg tr C18 = 4.41 min

2,4 - Dichloroaniline
150 ppm in methanol
logKow = 2.79
log BCF = 1.98
Avg tr C18 = 4.57 min

Toluene
75 ppm in methanol
logKow = 2.73
log BCF = 0.92
Avg tr C18 = 5.09 min

Triphenyl phosphate
159 ppm in methanol
logKow = 3.90
log BCF = 2.76
Avg tr C18 = 5.44 min