

ACD/ChromManager & LC Simulator

Version 6.0 for Windows

Technical Note

Determining LogD Values From Chromatographic Experiments

Advanced Chemistry Development Inc.

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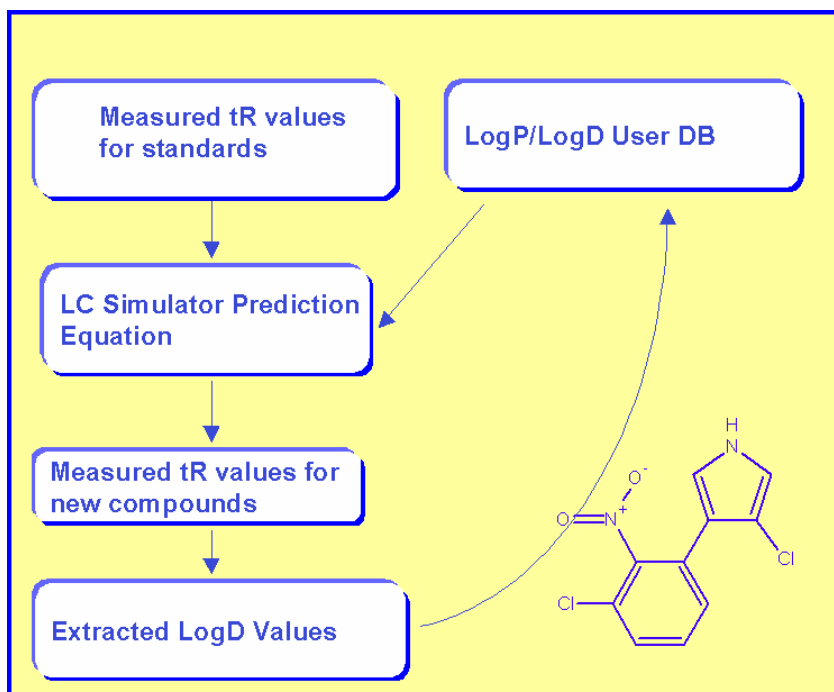
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1. Introduction

With careful design of chromatographic experiments, it is possible to experimentally determine $\log D$ and $\log P$ values by HPLC. ChromManager uses output from LC Simulator, combined with experimental $\log D$ values for some standard compounds, to extract $\log D$ values for new compounds. The technique uses coordination of three different ACD/Labs modules: ChromManager, LC Simulator, and LogP DB. Please note that the user should have the ACD/LogD Suite to be able to put both $\log D$ and $\log P$ values into a User Database. With ACD/LogP DB, the user will only have $\log P$ values in their User Database. The values that ChromManager measures can be written directly to LogP DB user databases to increase the accuracy of predicted values.



In order to use this feature, you need experimental $\log D$ values for a set of standard compounds. If your HPLC method is carefully designed for a primarily hydrophobic retention mechanism, you can use your standards for a wide range of test compounds. For more general HPLC methods, it is better to use standards that have similar functionality to your test compounds. In order to develop a good equation, it is best to have at least five compounds.

The user should also avoid using steep and/or multi-step gradient methods for this technique; if at all possible it is better to use isocratic conditions. A typical approach is to design a number of methods that will apply to a wide variety of compounds.


2. Part 1: Extracting LogD Values from Chromatographic Data—Internal Standard

The most effective way to compensate for small changes in conditions is to use internal standards. Addition of a small amount of each component to the mixture will allow you to generate prediction equations directly in the experiment in question.

A. Attach Structures to Peaks on the Chromatogram in ChromManager, Processor window


Import the chromatogram and peak-pick each component. Switch to ChemSketch and draw or import the structures. Attach the structures to relevant peaks. If you will be using these standards repeatedly with this method, it will be best to create a macro to attach them automatically to the correct peaks. If the chromatographic data format that you use does not contain the chromatographic parameters, you can also create a group macro to attach these. See the online help for ChromManager to get detailed instructions on how to do this.

B. Generate a Prediction Equation for the Chromatogram

In order to generate a prediction equation for the chromatogram, you will first need to transfer the chromatogram to LC Simulator. Click **Transfer to LC Simulator**  and choose Prediction Mode. In order to properly use your internal standards, you need to specify your user database in LC Simulator so that it will use the experimental logD values to calculate the equation. In the input window that opens, unselect the test compound, but leave all of the standard compounds in place.

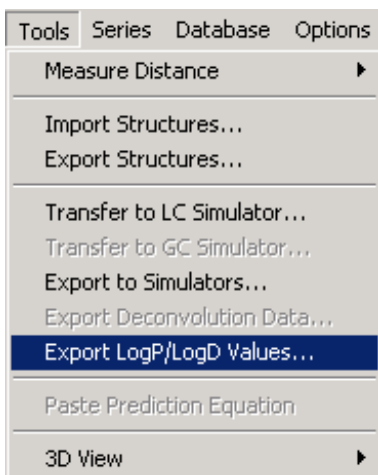
| No | Struc | Use | View | Name | tR_exp |
|----|-------|-----|------|-----------------------|--------|
| 1 | NO | N/A | ✓ | | 0.42 |
| 2 | YES | ✓ | ✓ | amoxicilline | 1.09 |
| 3 | YES | ✓ | ✓ | benzylpenicilline Na | 1.78 |
| 4 | YES | ✗ | ✓ | New structure | 2.25 |
| 5 | YES | ✓ | ✓ | phenethicilline K- | 2.78 |
| 6 | YES | ✓ | ✓ | sodium flucloxacillir | 4.28 |

Now click **OK** to generate the prediction equation. The final step inside LC Simulator is to copy the prediction equation to the Chromatogram. Click **Copy the Prediction Equation to**

SpecManager . ChromManager will retain the prediction equation with the saved or databased chromatogram.

C. Write the Extracted Log D Value to the User DB

From the **ACD/Labs** menu, choose **Log P DB** and open the user database. ChromManager will now access this database. Switch back to SpecManager (using the **ACD/Labs** menu). From the **Tools** menu, choose **Export Log D /Log P Values**.



Log P DB will prompt you to overwrite the values for each of the structures. You should accept only the new structures.

You now have extracted experimental values for one or more new structures. These will be used to increase the accuracy of calculations of log P , log D , and solubility values for new compounds.

It is also useful for LC/GC Simulator.

3. Part 2: Extracting LogD Values from Chromatographic Data—External Standard

As long as your chromatographic method is very stable, it is possible to use external standards in order to determine prediction equations for your chromatographic system. For example, you can inject your standards every 10 or twenty samples to verify that the results are not changing, and then use the same prediction equation for each of the test injections in between.

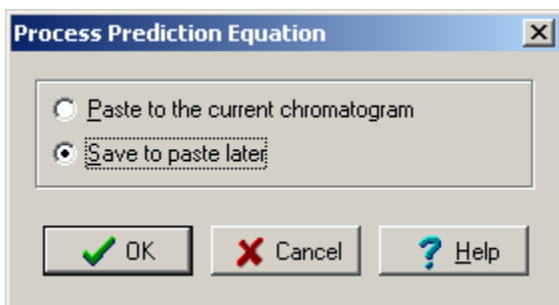
This procedure is exactly like the one that was done in part 1, but in this case, the prediction equation attached to the test chromatogram will come from a different run.

A. Attach Structures to Peaks on the Chromatogram in ChromManager, Processor window

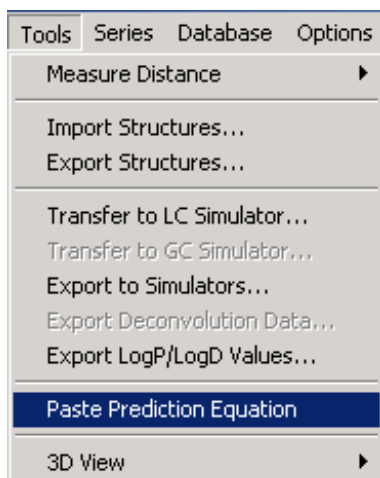
Import the chromatogram and peak-pick each component. For most systems, you will have only one component. Switch to ChemSketch and draw or import the structures. Attach the structures to relevant peaks. Since you will be taking the prediction equation from another chromatogram, it is not necessary to attach chromatogram parameters unless this chromatogram will be saved for later review.

B. Attach a Prediction Equation for the Chromatogram

In this case, you will not be carrying the chromatographic retention times to LC Simulator. Rather, you will be taking the results from the standard run. If you have not already done so, open the most recently collected standard chromatogram and generate a prediction equation as in part 1. Click **Copy Equation to SpecManager** as in Part 1, but this time, save to the clipboard to paste later.



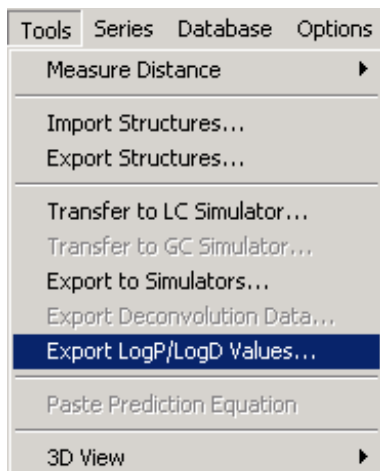
Now you can browse to all of the chromatograms from this session, and attach the same prediction equation to each one; from the **Tools** menu, choose **Paste Prediction Equation**.



The prediction equation for the external standard is now attached to the test chromatogram.

C. Write the Extracted Log D Value to the User DB

From the **ACD/Labs** menu, choose **Log P DB** and open the user database. ChromManager will now access this database. Switch back to SpecManager (using the **ACD/Labs** menu). From the **Tools** menu, choose **Export Log D /Log P Values**.



Log P DB will prompt you to overwrite the values for each of the structures. You should accept only the new structures.

You now have extracted experimental values for one or more new structures. These will be used to increase the accuracy of calculations of log P , log D , and solubility values for new compounds.

4. Conclusion

HPLC is a fast, effective way to measure $\log P$ and $\log D$ values. Using ChromManager and LC Simulator, you can extract values very effectively and automatically write these values to a user database. The user database is then ready to use for new compounds in order to increase accuracy of the predicted values that you get from ACD/LogD and ACD/LogP DB.

For more information please refer to the following references:

1. Franco Lombardo, Marina Y. Shalaeva, Karl A. Tupper, Feng Gao, and Michael H. Abraham, "ELogPoct: A Tool for Lipophilicity Determination in Drug Discovery", in *J. Med. Chem.* 2000, **43**, 2922-2928.
2. K. Valko, P. Slegel, "New Chromatographic Hydrophobicity Index (ϕ_o) Based on the Slope and the Intercept of the LogK' Versus Organic Phase Concentration Plot", in *Journal of Chromatography*, **631**, (1993) pp. 49-61.
3. K. Valko, C. Bevan, D. Reynolds, "Chromatographic Hydrophobicity Index by Fast-Gradient RP-HPLC: A High-Throughput Alternative to LogP/LogD," in *Analytical Chemistry*, **69**, (1997) pp. 2557-2581.