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Introduction

Traditionally in drug discovery, the emphasis has focused primarily on the synthesis of large numbers of compounds and their interaction with a specific target. The other physiochemical properties are typically relegated to a later time in the discovery process. Recent developments in the use of liquid chromatography to correlate specific physiochemical properties with retention time has enabled researchers to increase the success and efficiency of the drug discovery process. The ability to combine several different separation techniques on a single system and to correlate the data into a single report will greatly aid the profiling of new drug candidates in the discovery process.

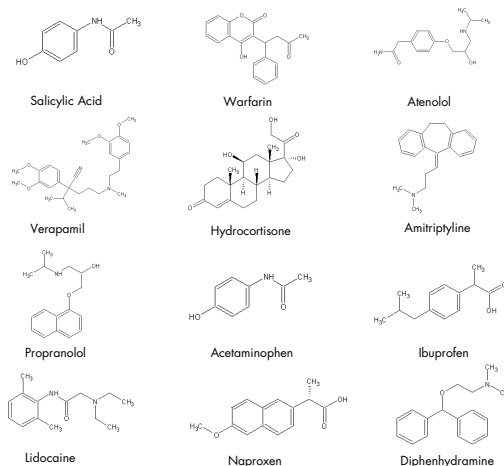
In this specific example two types of properties were calculated using the chromatographic retention characteristic. The first is the K_{IAM} which is the retention factor for Immobilized Artificial Membrane (IAM) columns. IAM columns are silica-based columns with a bonded phospholipid coating. In this case the coating is phosphatidylcholine. The second is the Chromatographic Hydrophobicity Index which is based on the retention on C_{18} columns at a specific pH. In this case the CHI was determined at three different pH values.

This type of data has been shown to be related to adsorption, solubility, ligand affinity, and metabolism of drug candidates.

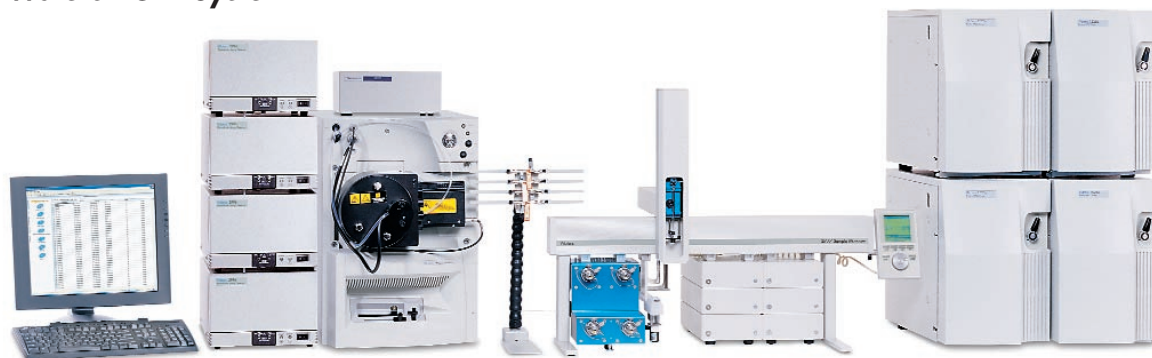
Methods

Pharmaceutical compounds with varying degrees of hydrophobicity and acidity were analyzed using different chromatographic conditions with MS and diode array UV detection. The chromatographic system utilized four independently controlled binary gradient pumps to allow different gradients to be run simultaneously. This is one of the Waters ECP™ Systems for early candidate profiling. Chromatographic Hydrophobicity Index (CHI) was determined at three different pH values and the partition coefficient for an Immobilized Artificial Membrane (K_{IAM}) column was determined at physiological conditions. The values were calculated from the retention time from the chromatographic runs.

Model Analytes



Waters ECP™ System



*1525µ Binary HPLC Pumps shown here

CHI Chromatographic Method

- HPLC System
 - 4 x Waters 1525 Binary HPLC Pumps
 - Waters 2777 Sample Manager with 4 injector valves
 - 4 x Waters 2996 Photodiode Array
- Detectors
- Column
 - Waters XTerra® MS C₁₈ 3.5µm 3.9x20mm
- Mobile Phase
 - A: pH 2.0
 - 0.01% v/v phosphoric acid
 - A: pH 7.4
 - 10 mM ammonium acetate
 - A: pH 10.5
 - 10 mM ammonium acetate
 - B: 100% ACN
- Injection Volume
 - 2 µL
- Gradient

Time	Flow	%A	%B	Curve
0	0.5 mL/min	95%	5%	-
8	0.5 mL/min	0%	100%	6
10	0.5 mL/min	95%	5%	1

MS Method

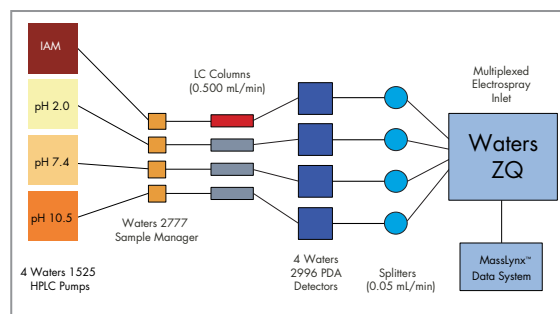
- MS System
 - Waters Micromass® ZQ™ Mass Detector with MUX-technology™
- MS Method
 - ESI+ and ESI-
 - Scan Range: 100 to 600 amu
 - Scan Time: 0.3 s
 - Interscan Delay: 0.1 s
 - Cone Voltage: 30V
 - Capillary Voltage: 3.0 kV
 - Source Temp: 120° C
 - Desolvation Temp: 300° C

IAM Chromatographic Method

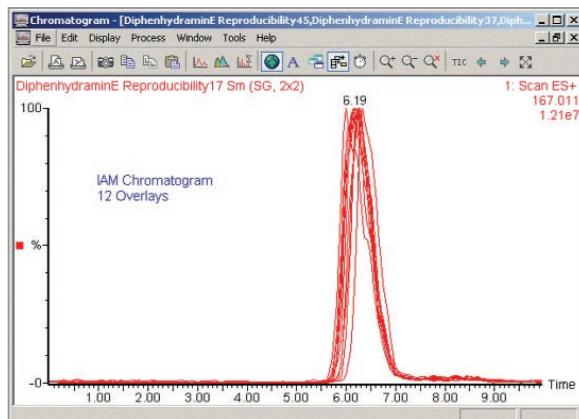
- HPLC System
 - 4 x Waters 1525 Binary HPLC Pumps
 - Waters 2777 Sample Manager with 4 injector valves
 - 4 x Waters 2996 Photodiode Array
- Detectors
- Column
 - Regis Technologies IAM Fast-Screen Mini Column 3x10mm with Ester IAM.PC.C10/C3 packing
- Mobile Phase
 - A: pH 7.4
 - 10 mM phosphate
 - 0.2 g/L potassium chloride
 - 8 g/L sodium chloride
 - B: 100% ACN
- Injection Volume
 - 2 µL
- Gradient

Time	Flow	%A	%B	Curve
0	0.5 mL/min	100%	0%	-
8	0.5 mL/min	60%	40%	6
10	0.5 mL/min	100%	0%	1

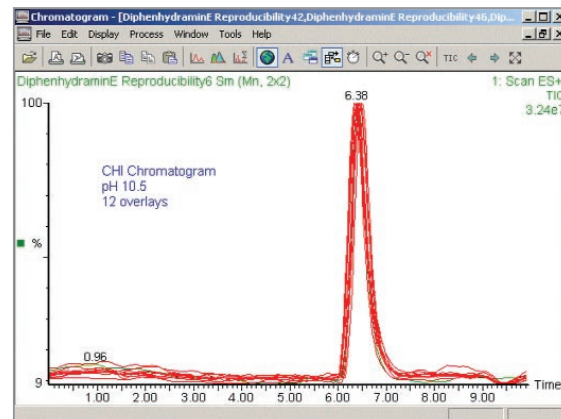
Waters ECP Systems Diagram



Chromatographic Reproducibility

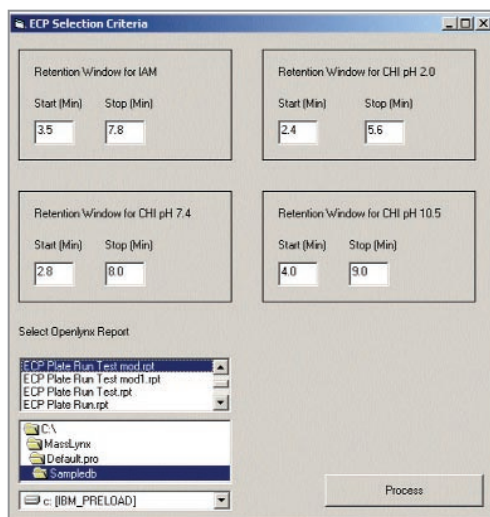


Extracted ion chromatogram of 12 injections of 10 ng of diphenhydramine taken over two days using IAM conditions. Note the chromatographic conditions for the IAM column use extremely high concentrations of non-volatile salts.

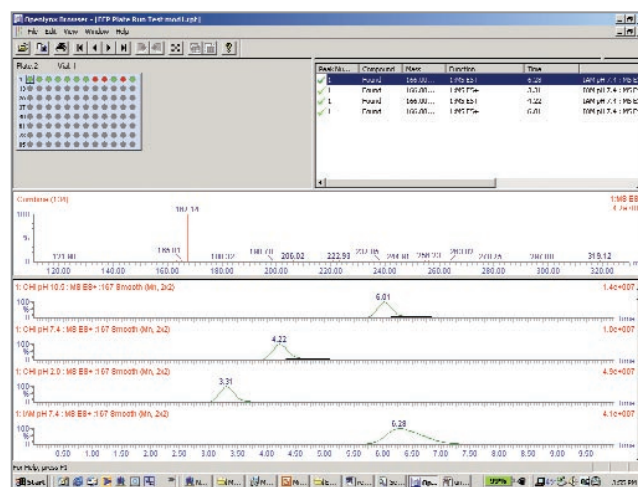


TIC chromatogram of 12 injections of 10 ng of diphenhydramine over two days using CHI conditions at pH 10.5. Note that this separation takes place at high pH. The XTerra MS C₁₈ column is a hybrid column that allows long-term stability at high pH.

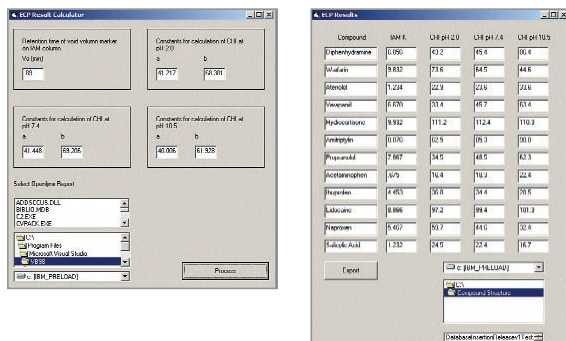
Retention Selection Software



ECP Report



K_{IAM} and CHI Calculation Program



Conclusions

- The Waters ECP Systems are well suited to do multiple physiochemical measurements simultaneously.
- The ECP systems are easy to automate and interface with existing data systems and can report the physiochemical data using a graphical interface.
- The system is robust and reproducible even in the presence of high salt concentration and high pH conditions. The use of a hybrid silica packing material gives the chromatographic stability required at pH above 8.0.

Future Work

- Research into other types of physiochemical measurements that can be done chromatographically such as ligand affinity and other types of membrane permeability.
- Analysis criteria based on quantification as well as the retention-based characteristics. This should allow the inclusion of other properties such as solubility, rates of metabolism and stability studies.
- Development of a more generic software package that will be suitable for a wide range of early candidate profiling analyses.
- Development of database interactivity tools to allow warehousing and retrieval of the physiochemical data.

References

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- Kangas, H.; Kotiaho, T.; Salminen, T.; Kostianinen, R. Rapid Communications in Mass Spectrometry. 2001, 15: 1501-1505.
- Liu, H.; Carter, G. T.; Tischler, M. Rapid Communications in Mass Spectrometry. 2001, 15: 1533-1538.
- Camurri, Giulio; Zaramella, Alessio. Anal. Chem. 2001, 73 (15), 3716-3722.

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