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# AN INTERACTIVE PHYSICOCHEMICAL PROPERTY PROFILING SOFTWARE FOR EARLY CANDIDATE ANALYSIS IN DRUG DISCOVERY

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## INTRODUCTION

- Full characterization of the physicochemical properties of new chemical entities (NCE's) typically takes place in pharmaceutical development laboratories. However, in an effort to minimize drug development costs, pharmaceutical companies are now pushing physicochemical screening back into the drug discovery laboratories so that unsuitable compounds fail earlier in the process.
- By generating retention time and quantitative data, LC/UV/MS provides a mechanism by which indicators of compounds' physicochemical properties—such as solubility, hydrophobicity, membrane permeability, stability, and protein binding—can be modeled and screened.
- The large number of compounds that require screening can be analyzed by high throughput parallel LC/UV/MS methods. This technology allows the analysis of more samples in the same time, or the analysis of multiple physicochemical properties at the same time. While many compounds can be tested, large volumes of data from these parallel analyses must still be processed and reviewed.
- The new software application presented here, ProfileLynx™ Application Manager, handles sample set management, data processing and results browsing, as well as provides exporting capabilities and interactivity with other software packages to simplify physicochemical property screening.
- The multiple streams of acquisition data from parallel LC/UV/MS systems are integrated into an easily managed and interpreted format by ProfileLynx.

## PHYSICOCHEMICAL PROPERTY PROFILING

Compounds were screened for their physicochemical properties using three different indicators:

- Chromatographic hydrophobicity index using $C_{18}$ column retention (CHI) at various pH's
  - The CHI relates directly to the log D of a compound, which indicates the compound's solubility and permeability
- Chromatographic hydrophobicity index using immobilized artificial membrane (IAM) columns ($CHI_{IAM}$)
  - The $CHI_{IAM}$ is measured on IAM columns under gradient conditions and relates to the membrane permeability of a compound
- Solubility

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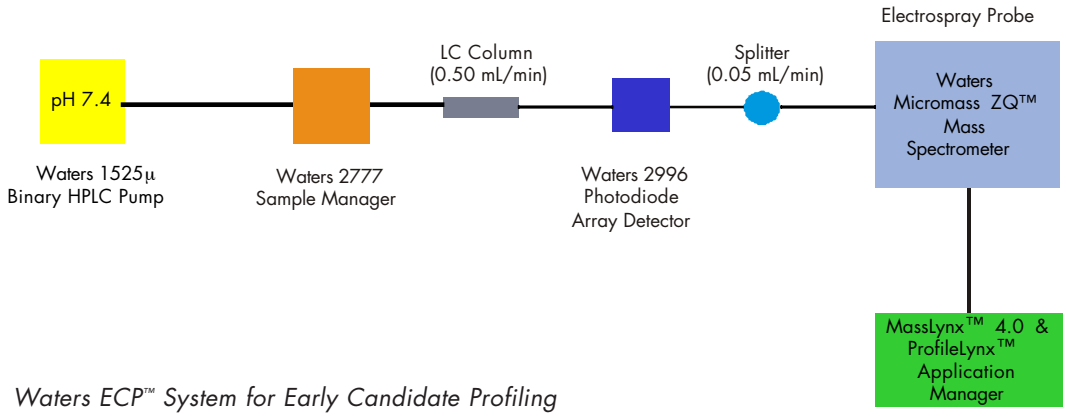
## EXPERIMENTS

### Single Chromatographic System




*Waters ECP™ System for Early Candidate Profiling*

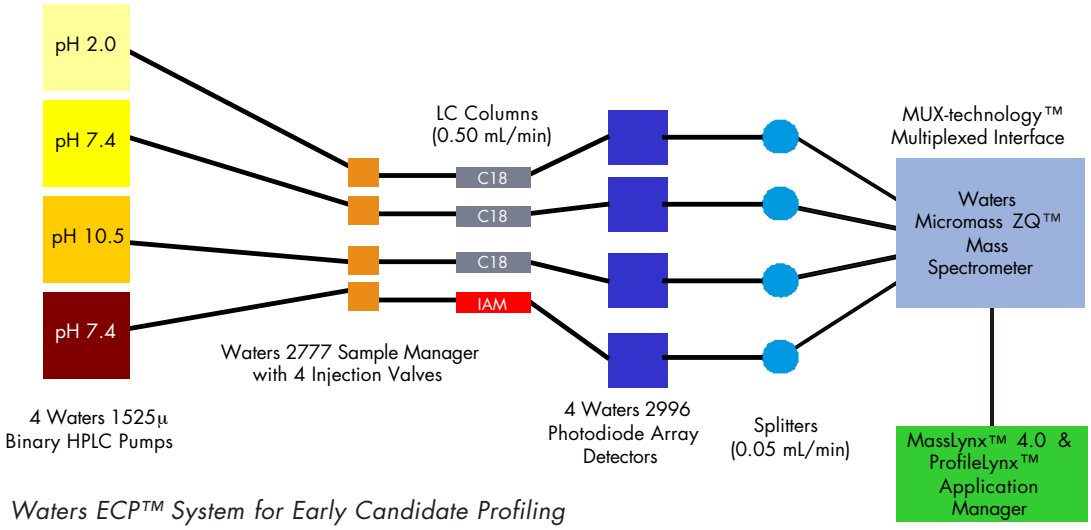
### Experiment 1: CHI Screening

- Standards with known CHI values were run to determine the correlation between retention time and CHI
- Pharmaceutical compounds were then analyzed and their CHI values were calculated from the compounds' chromatographic retention time at pH 7.4

### Experiment 2: Simulated Solubility Screening

- A wide set of pharmaceutical compounds were used to simulate a solubility study
- The analysis used a plate of standards of known concentration and then a plate of samples with varying concentrations
- Calibration curves were developed using the standards, and compounds were quantified to determine their "solubility"

## Parallel Chromatographic System




*Waters ECP™ System for Early Candidate Profiling*

### Experiment 1: CHI and $CHI_{IAM}$ Screening

- The parallel system used four independent binary gradient pumps for simultaneous compound analysis under four different separation conditions
- Standards with known CHI and $CHI_{IAM}$ values were run to determine the correlation between retention time and CHI or $CHI_{IAM}$
- Pharmaceutical compounds were then analyzed and their CHI or $CHI_{IAM}$ values were calculated from the chromatographic retention time
- CHI values were calculated based on compound retention on $C_{18}$ columns at three different pH conditions (pH 2.0, pH 7.4, and pH 10.5)
- $CHI_{IAM}$ values were calculated based on compound retention under simulated physiological conditions (pH 7.4)

### Experiment 2: Simulated Solubility Screening

- The parallel system used four independent binary gradient pumps for the simultaneous analysis of four different compounds under the same conditions, resulting in a four-fold increase in sample throughput over the single chromatographic system.
- Calibration curves were developed using the standards, and compounds were quantified to determine their "solubility".

## METHODS

### Columns

- CHI
  - Waters XTerra® MS $C_{18}$ 3.5mm 3.9x50mm
- $CHI_{IAM}$
  - Regis Technologies IAM Fast-Screen Mini Column 3x10mm with Ester IAM.PC.C10/C3 packing
- Simulated Solubility
  - Waters XTerra® MS $C_{18}$ 3.5mm 3.9x50mm

### Mobile Phases

- CHI & $CHI_{IAM}$
  - A: CHI pH 2.0, 0.01% v/v phosphoric acid
  - A: CHI pH 7.4, 10 mM ammonium acetate
  - A: CHI pH 10.5, 10 mM ammonium acetate
  - A: $CHI_{IAM}$, pH 7.4, 10 mM ammonium acetate
  - B: 100% ACN
- Simulated Solubility
  - A: $H_2O$
  - B: 100% ACN

### HPLC Gradients

CHI and $CHI_{IAM}$

| Time | Flow | %A | %B | Curve |
|---|---|---|---|---|
| 0 | 0.5 mL/min | 100% | 0% | - |
| 10 | 0.5 mL/min | 0% | 100% | 6 |
| 12 | 0.5 mL/min | 0% | 100% | 6 |
| 14 | 0.5 mL/min | 100% | 0% | 1 |

Simulated Stability

| Time | Flow | %A | %B | Curve |
|---|---|---|---|---|
| 0 | 0.5 mL /min | 100% | 0% | - |
| 4 | 0.5 mL /min | 0% | 100% | 6 |
| 6 | 0.5 mL /min | 100% | 0% | 6 |

### Injection Volume

- 2.0 µL

### MS Conditions

- ESI+ and ESI-
- 100 to 600 amu
- Scan time 0.3 sec
- Interscan delay 0.1 sec
- Cone voltage 30V
- Capillary voltage 3.0 kV
- Source temp 120 °C
- Desolvation temp 300 °C

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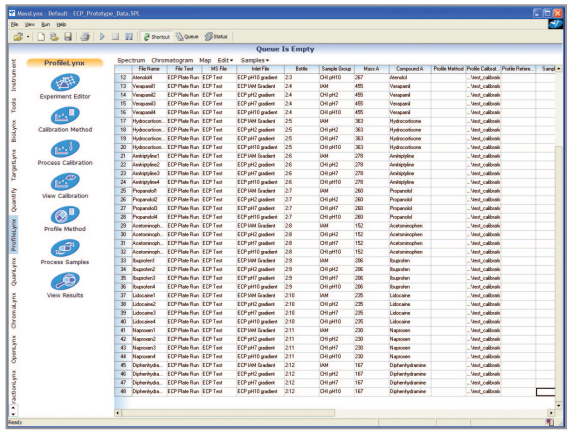
## PHYSICOCHEMICAL PROPERTY PROFILING SOFTWARE

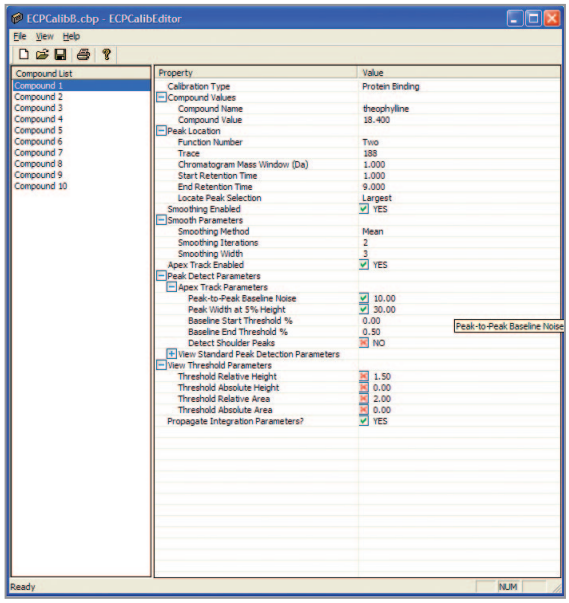
### Step 1: Define the physicochemical property experiment




*Sample List*

Using the MassLynx sample list, the physicochemical experiment parameters can be associated with appropriate samples. From the Sample List, all of the ProfileLynx Application Manager tools can be accessed.

### Step 2: Define and set experimental criteria for physicochemical property calibration




*Calibration Editor*

Developing the relationship between retention times and physicochemical property indicators is easily enabled through the Calibration Editor in ProfileLynx. In the Calibration Editor the user simply selects the type of retention time calibration to be used (CHI, $CHI_{IAM}$, IAM, or Protein Binding) and then provides a customizable name for the specific experiment. The user then sets criteria such as the equation to use for calculating the physicochemical property indicator, integration parameters, and retention time ranges for the retention time calibration.

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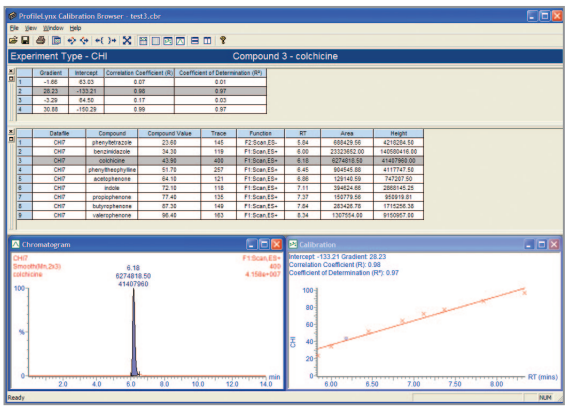
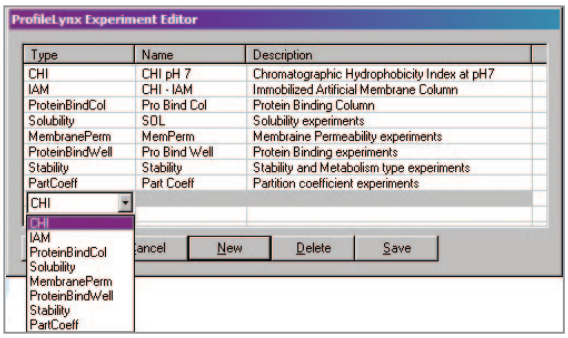
## Step 3: Review physicochemical property calibration



*Data Review Tool*

Retention time calibration review is facilitated through the Calibration Review Tool. Chromatograms can be manually integrated, peaks can be reassigned, and points can be ignored. The calibration is then stored and can be used during compound analysis.

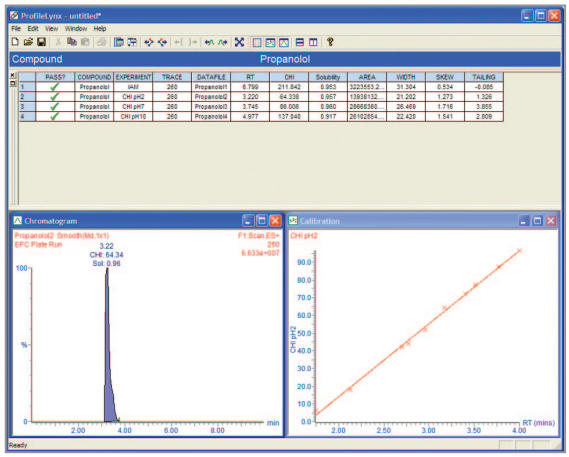
## Step 4: Select the type of experiment to be run and set pass/fail criteria



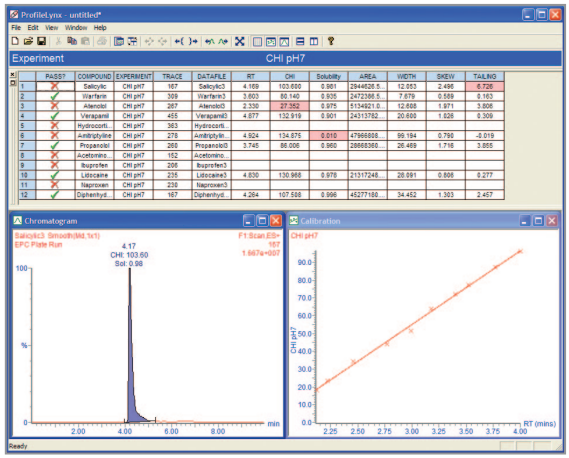

*Experiment Editor*

The type of physicochemical property screening experiment to be run is selected in the Experiment Editor. Options include CHI, IAM, solubility, permeability, stability, protein binding, partitioning, and metabolic inhibition.

## Step 5: Review physicochemical property analysis results by compound or experiment



*Data Review By Compound*



*Data Review By Experiment*

Processed analysis results can be viewed by experiment or by compound using the data review tool. The sample table shows the calculated physicochemical property indicator values for the samples, as well as markings to indicate whether the sample passed or failed the test based on threshold criteria. The first column in the display shows a summary of all the pass/fail criteria. If the mouse indicator is passed over a failed field, a pop-up box indicates the reason for the failure. Manual adjustments to peak integration and peak assignments may also be made in this view. Physicochemical property analysis results may also be sorted and viewed by experiment.

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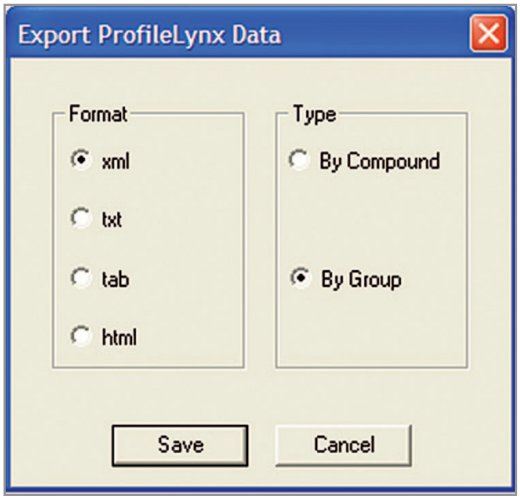
### Step 6: Export data




*Data Export Manager*

Data export is facilitated through the Data Export Manager. The format of the exported data file is defined in the data review window. The export file itself can be either xml, comma delimited, tab delimited or html format.

## REFERENCES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## CONCLUSIONS

- The ProfileLynx Application Manager is well suited to do multiple physicochemical property indicator measurements simultaneously.
- The ProfileLynx Application Manager easily interfaces with existing data systems and can report physicochemical data using a graphical interface.
- The ProfileLynx Application Manager allows easy data management and review and allows full automation of physicochemical indicator measurements with little or no operator intervention.

## FUTURE WORK

- Open Access walk-up type analysis for fully automated analysis with automated reporting and e-mailing of results to chemists.
- Inclusion of time-based metabolic and stability calculations.
- Integration of a fully customizable sample import tool to allow connection with any type of sample list format.
- Development of database interactivity tools to allow warehousing and retrieval of the physicochemical data.

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