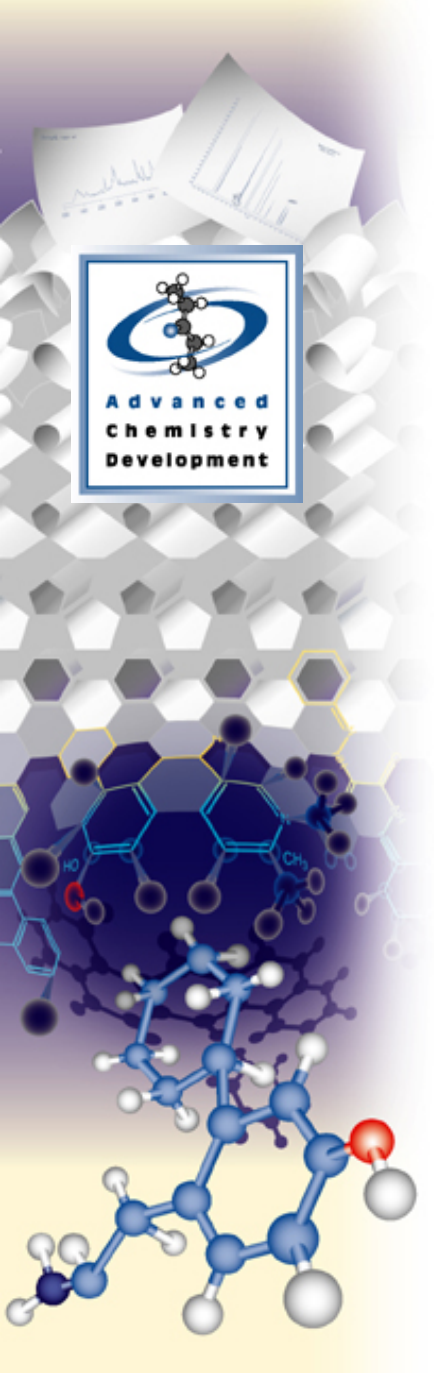


The Impact of pKa prediction and building application databases on Method Development

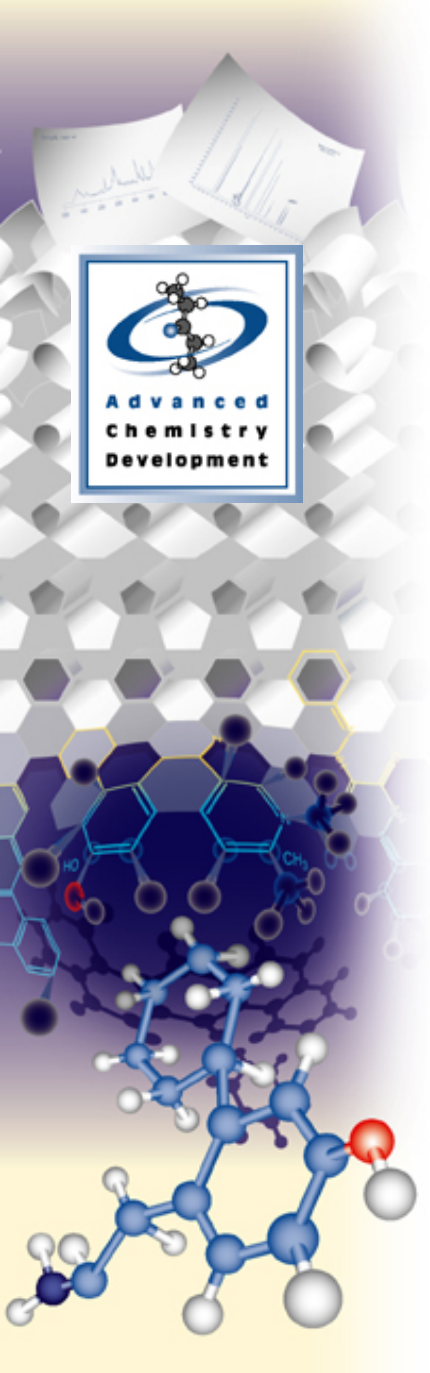
Sherry Gregory
ACD/Labs Account Manager

June 3, 2004

Visionary Software for Scientists

Introduction

- Why is pKa important?
- What is the typical approach to LC Method Development?
- What are some of the obstacles that chromatographers face?
- What solution does ACD/Labs offer?



pKa Prediction Why is it important to chromatographers?

Sherry Gregory

What exactly *is* pKa?

- Acid-base ionization constants.
- pKa values used for ACD/LC Simulator are apparent pKa values

What happens during ionization?

- Henderson – Hasselbach

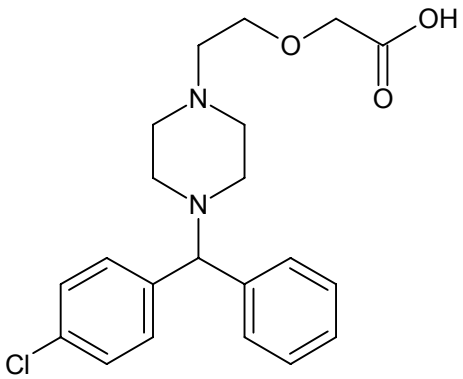
$$pH = pKa - \log([HA]/[A^-])$$

Where the ratio of acid to ionized species is known

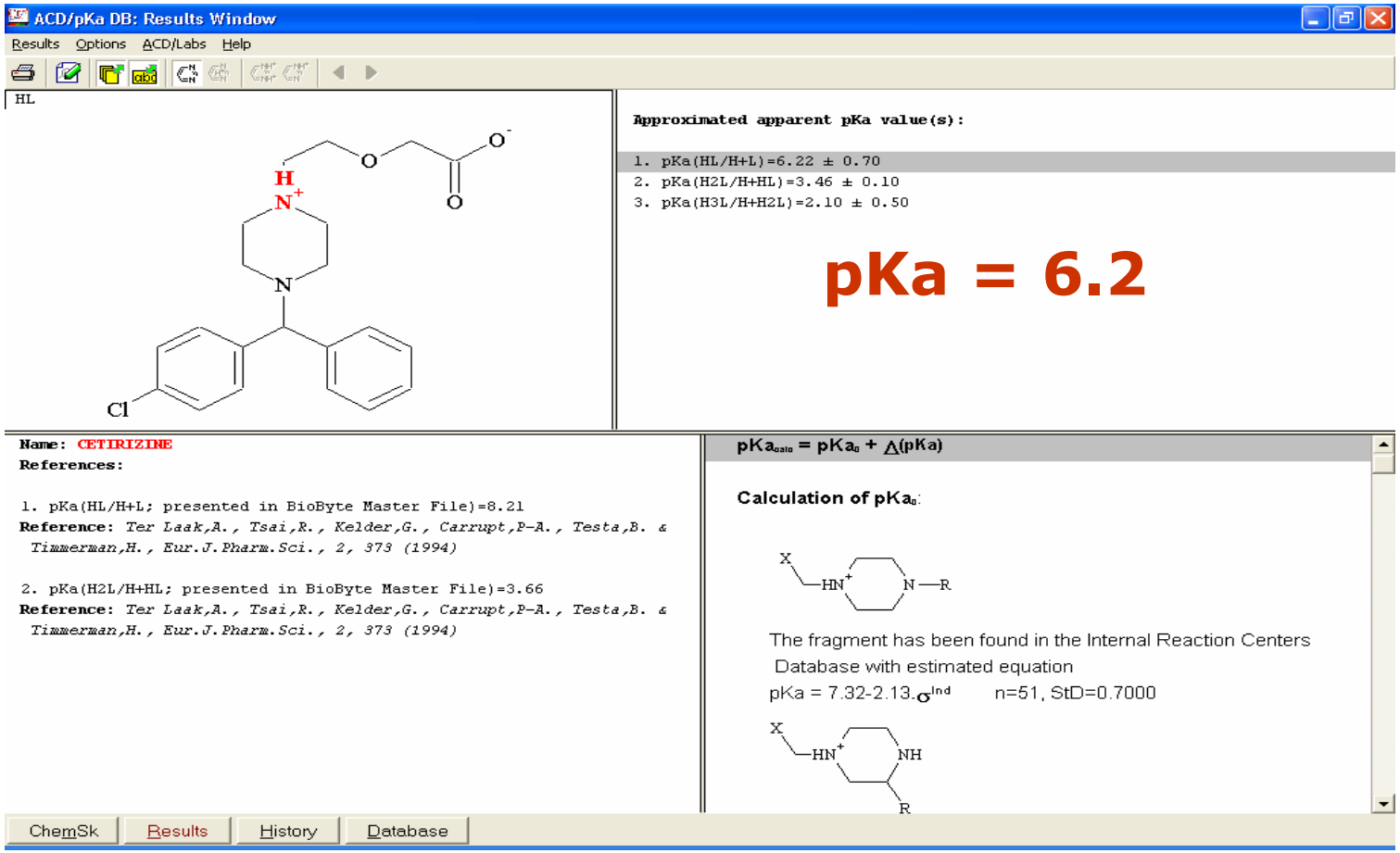
- For example:

Zyrtec

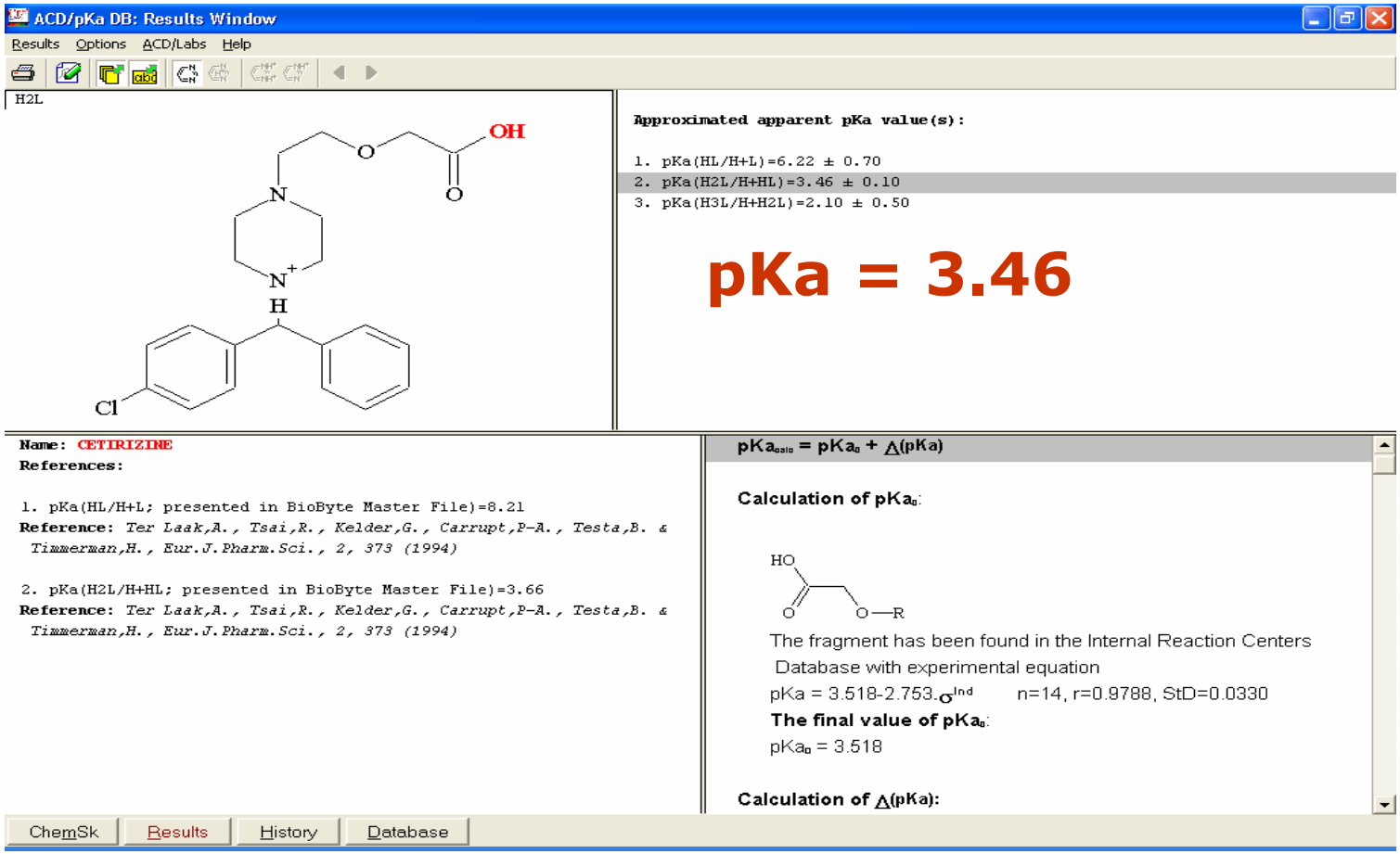
- What's the pka?



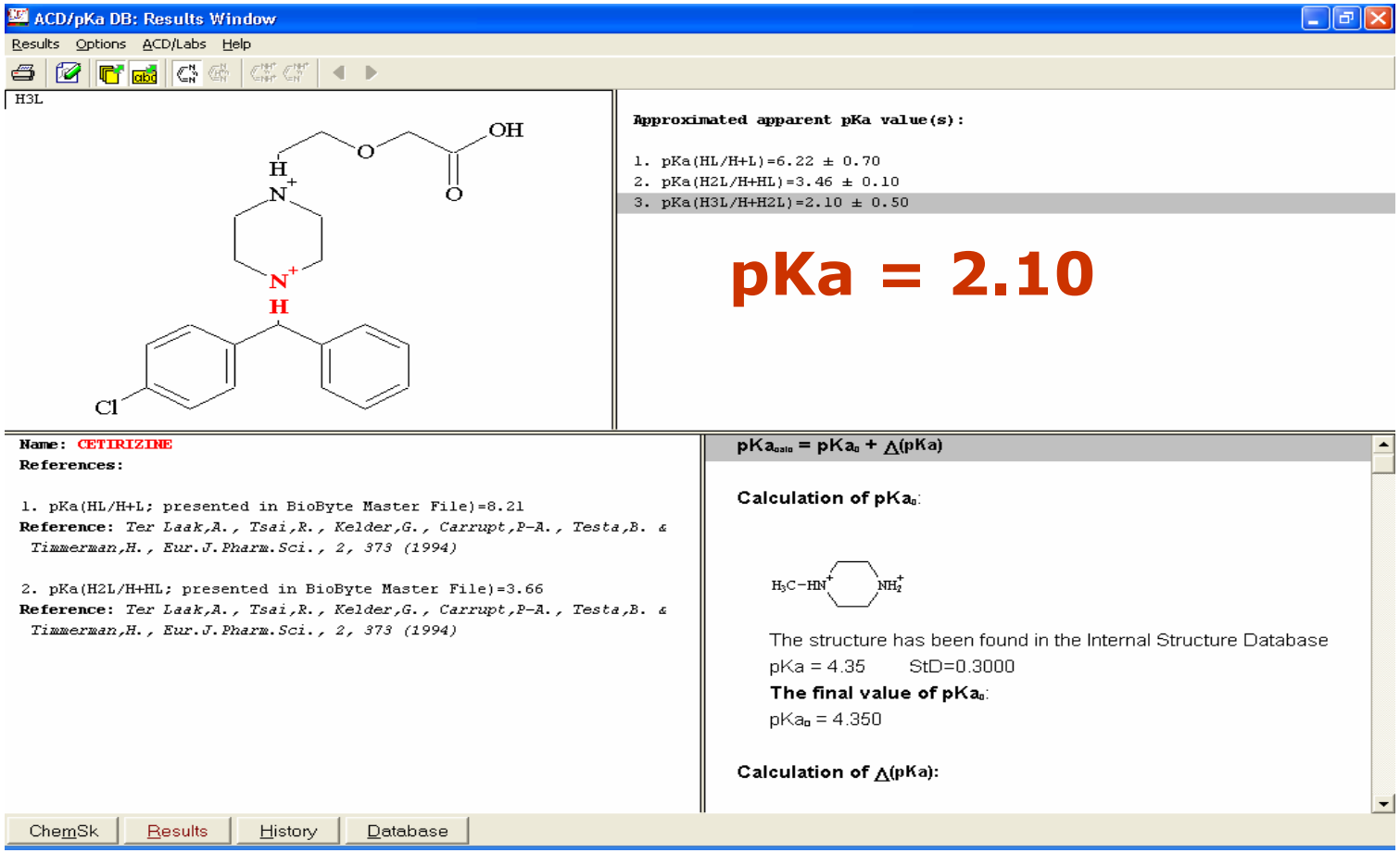
One possible ionic form...



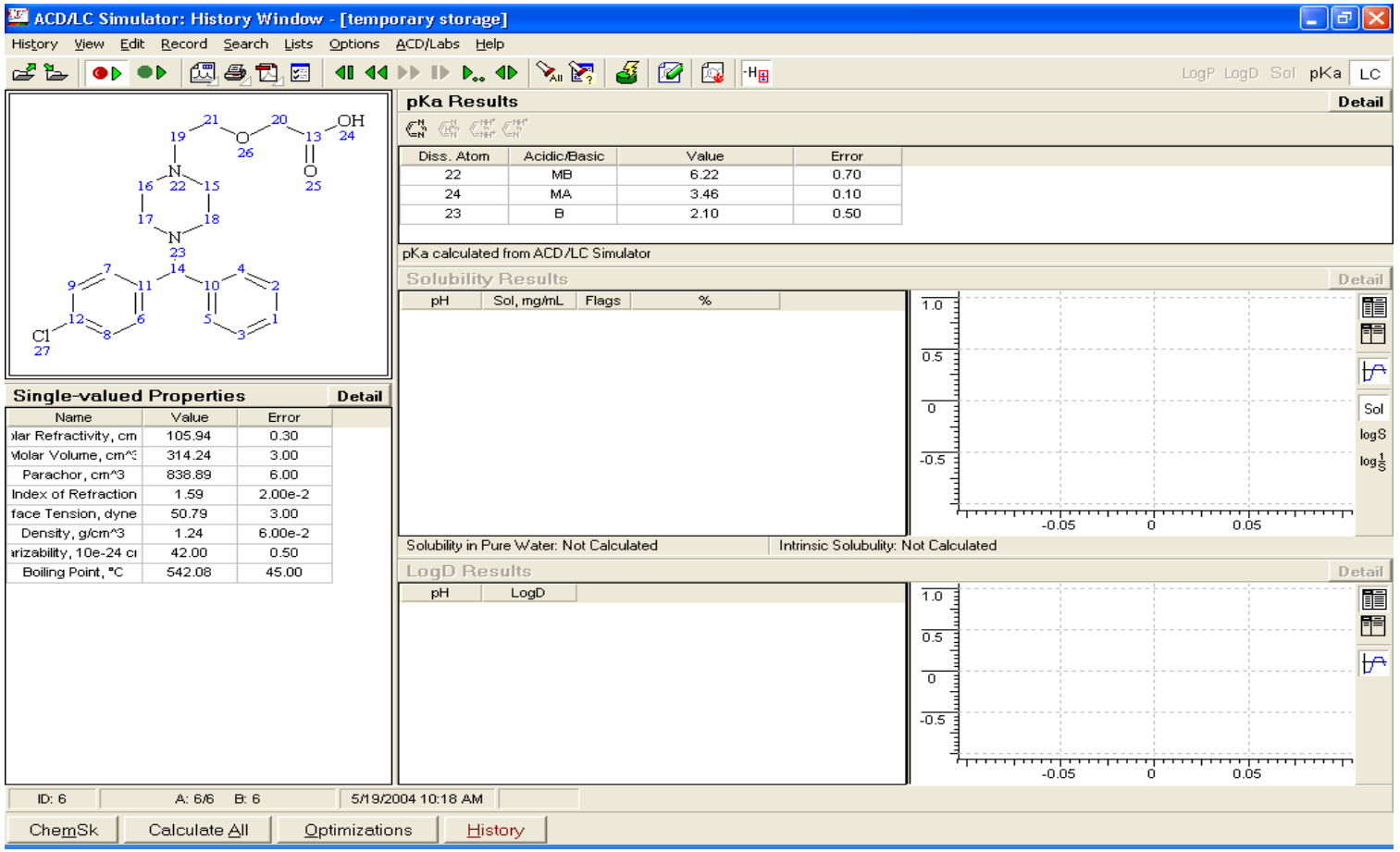
And another ...



…and another.


ACD/pKa DB: Results Window
Results Options ACD/Labs Help
H3L
OH
O
O
H
N+
N+
H
Cl
Approximated apparent pKa value(s):
1. pKa(HL/H+L)=6.22 ± 0.70
2. pKa(H2L/H+HL)=3.46 ± 0.10
3. pKa(H3L/H+H2L)=2.10 ± 0.50
pKa = 2.10
Name: CETIRIZINE
References:
1. pKa(HL/H+L; presented in BioByte Master File)=8.21
Reference: Ter Laak,A., Tsai,R., Kelder,G., Carrupt,P-A., Testa,B. & Timmerman,H., Eur.J.Pharm.Sci., 2, 373 (1994)
2. pKa(H2L/H+HL; presented in BioByte Master File)=3.66
Reference: Ter Laak,A., Tsai,R., Kelder,G., Carrupt,P-A., Testa,B. & Timmerman,H., Eur.J.Pharm.Sci., 2, 373 (1994)
pKacalc = pKa0 + Δ(pKa)
Calculation of pKa0:
H3C–HN+
NH2+
The structure has been found in the Internal Structure Database
pKa = 4.35 StD=0.3000
The final value of pKa0:
pKa0 = 4.350
Calculation of Δ(pKa):
ChemSk
Results
History
Database

As expressed in ACD/LC Simulator



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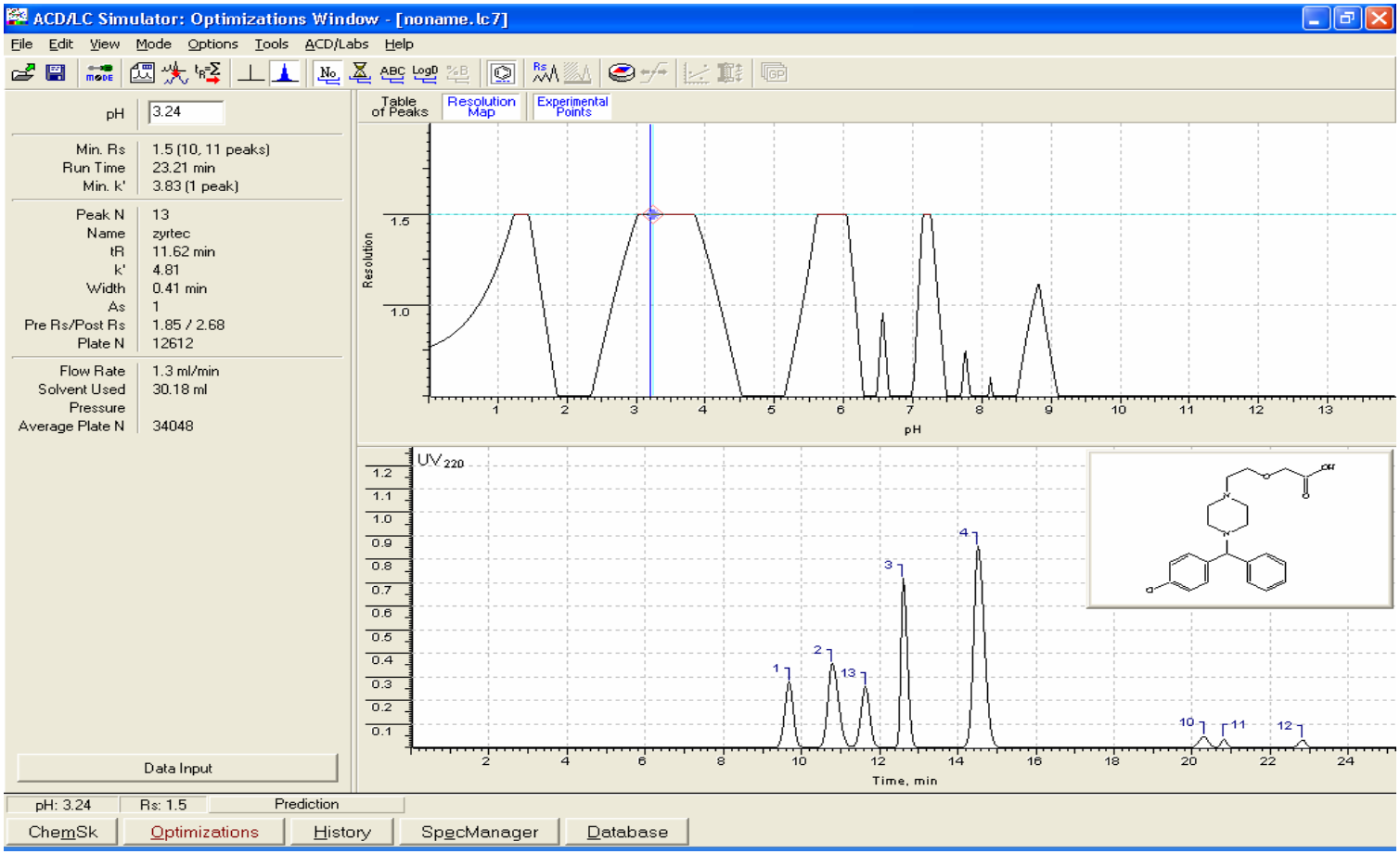
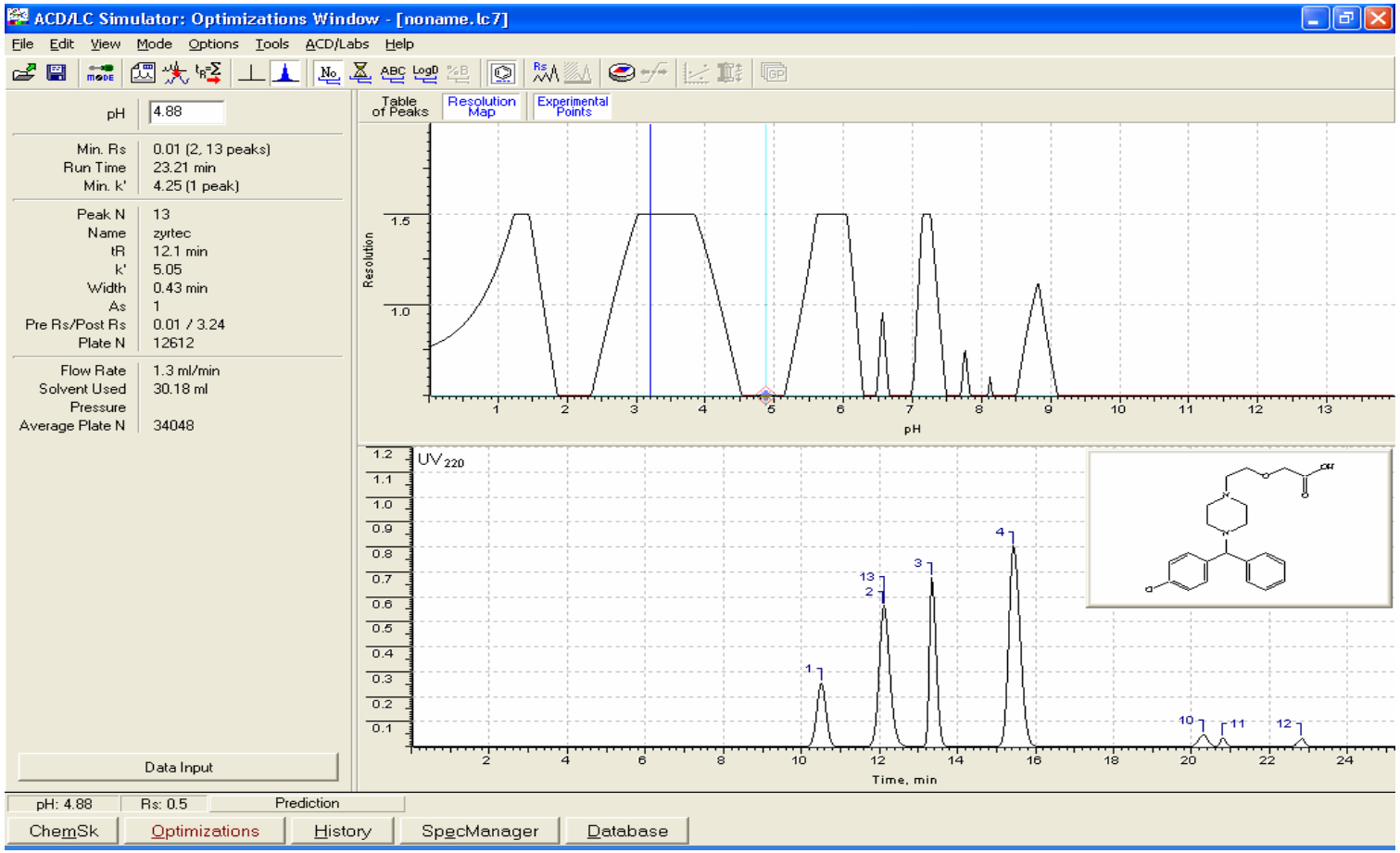
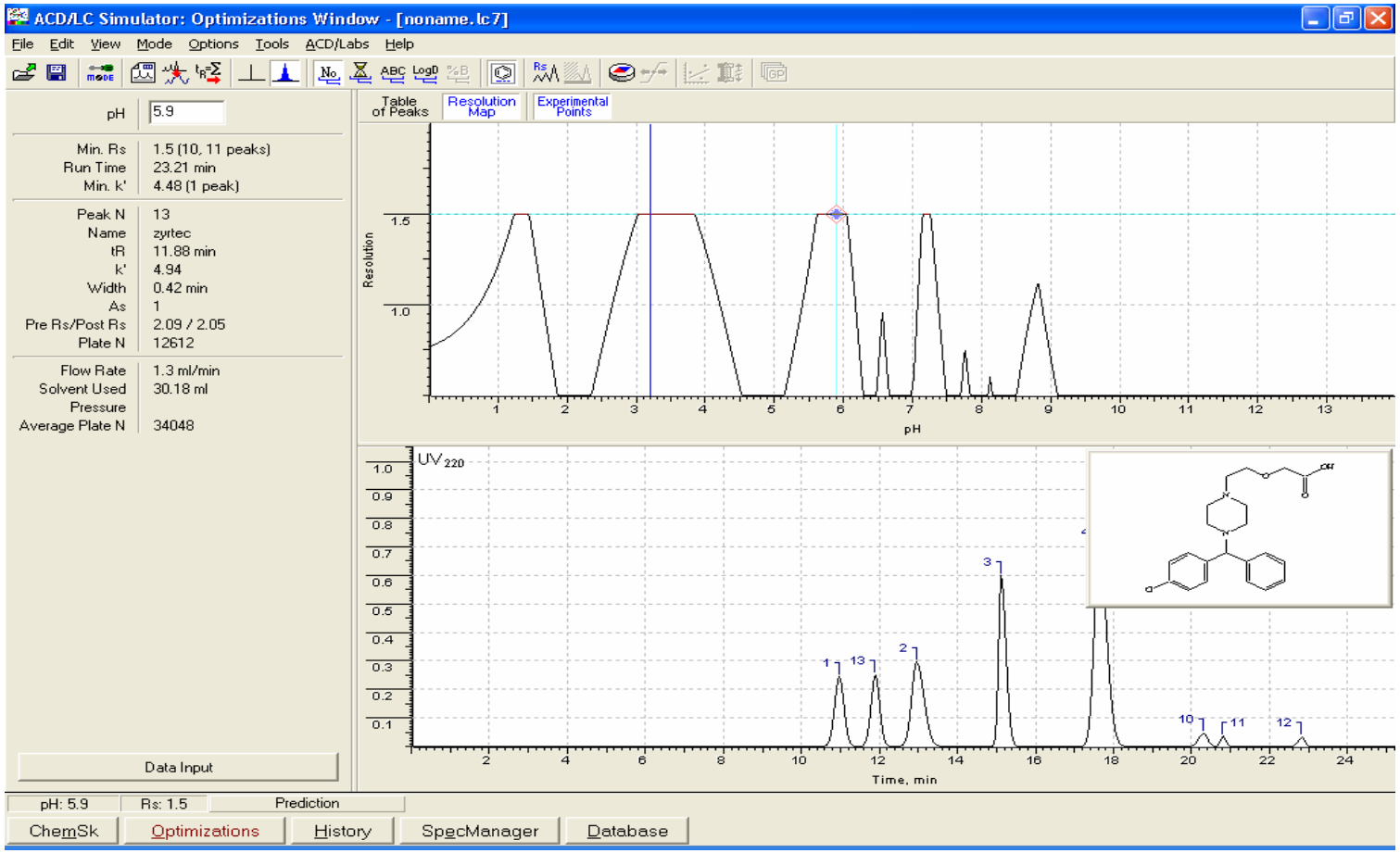
An example of the impact of pKa on retention time. (pH = 3.2)



At pH 5.0…



And now at pH 6.0…



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What's the message?

- Knowing the pKa can save you time and headaches
- All the pKas of compounds in your sample are important, but your target compound is the most important.
- Know where the trouble spots are *before* you inject a sample!

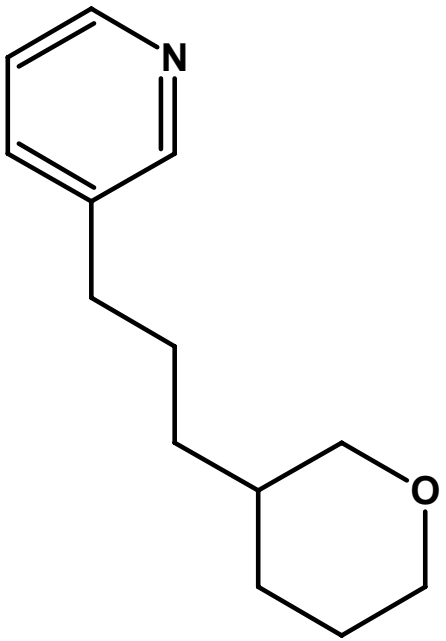
Starting Points
pKa Prediction

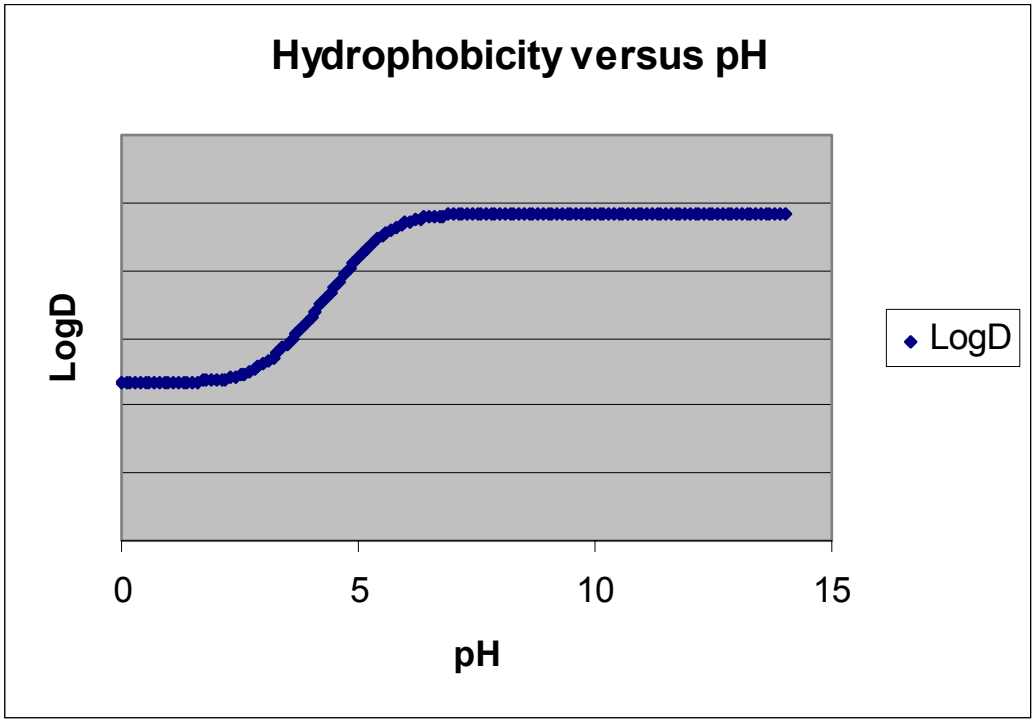
- Known or predicted pKas can aid in the selection of starting pH:
  - The results of pH changes can be hard to interpret
  - Optimization of pH can only be done over small ranges and can still be difficult.

Helpful Hint: When selecting a pH for your solvent system, try to select one that is not near the pKas of your targets.

pKa, LogD and t_R

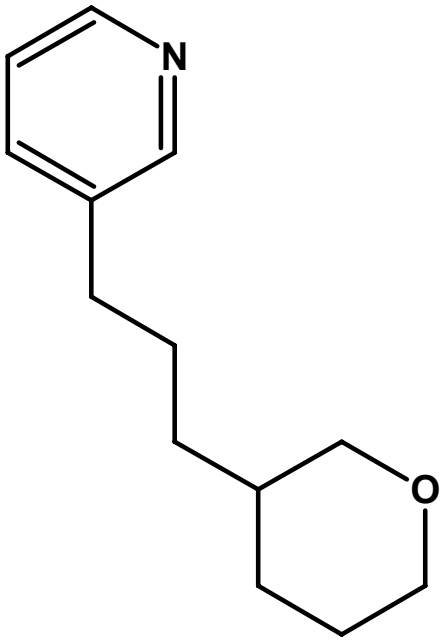
- Hydrophobicity of a Base (pKa = 5.54):

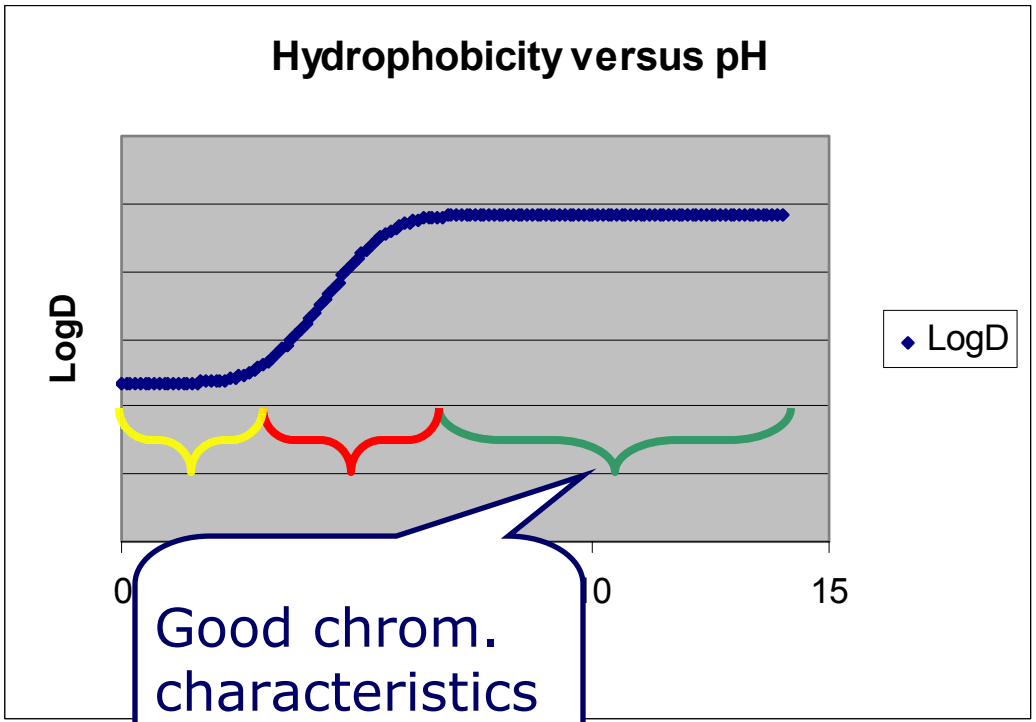




pKa, LogD and tR

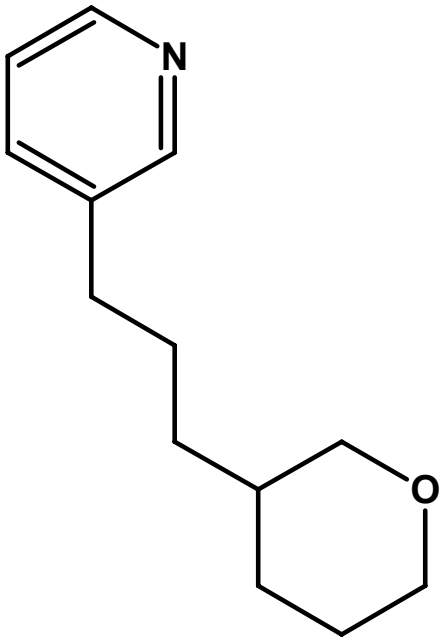
- Reversed phase HPLC:

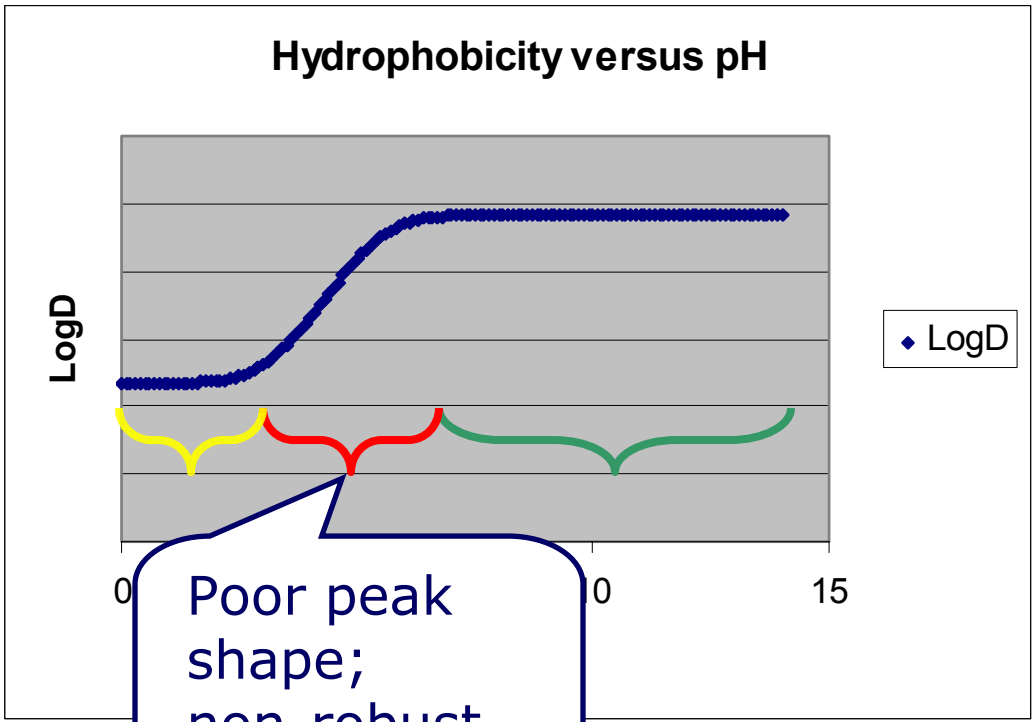




pKa, LogD and tR

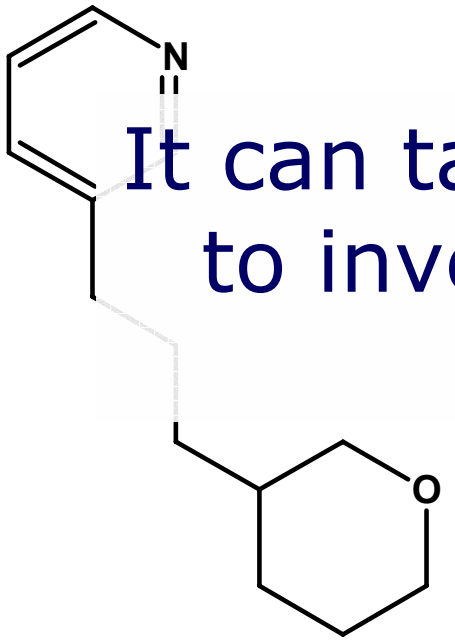
- Reversed phase HPLC:

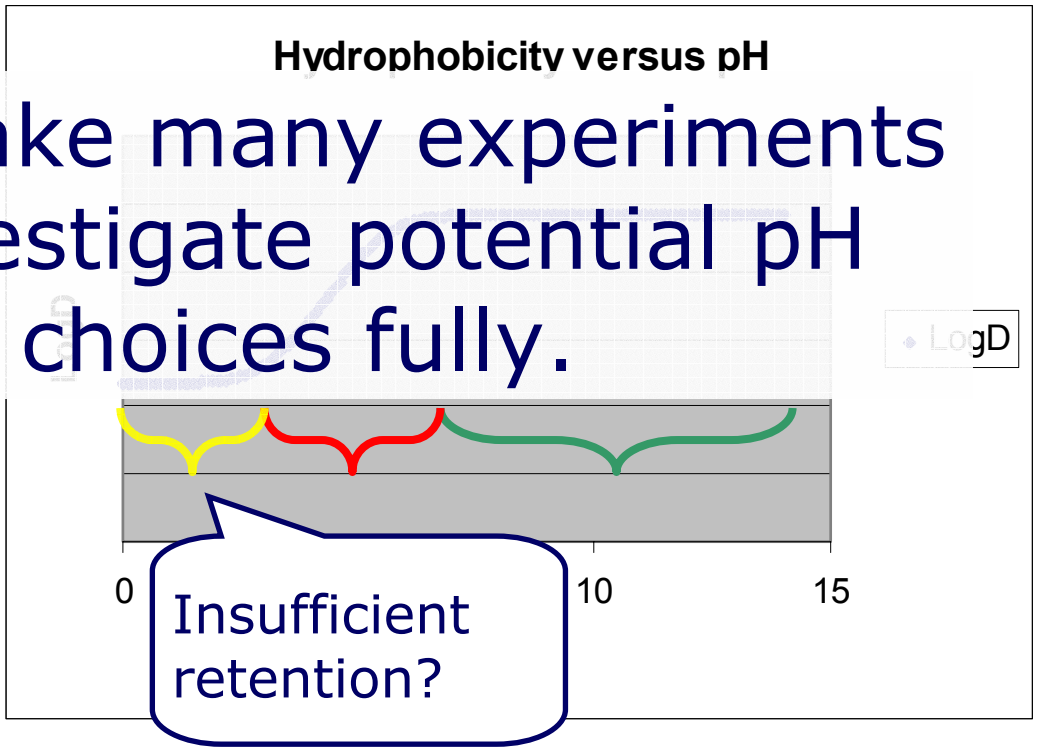




pKa, LogD and tR

- Reversed phase HPLC:





It can take many experiments to investigate potential pH choices fully.

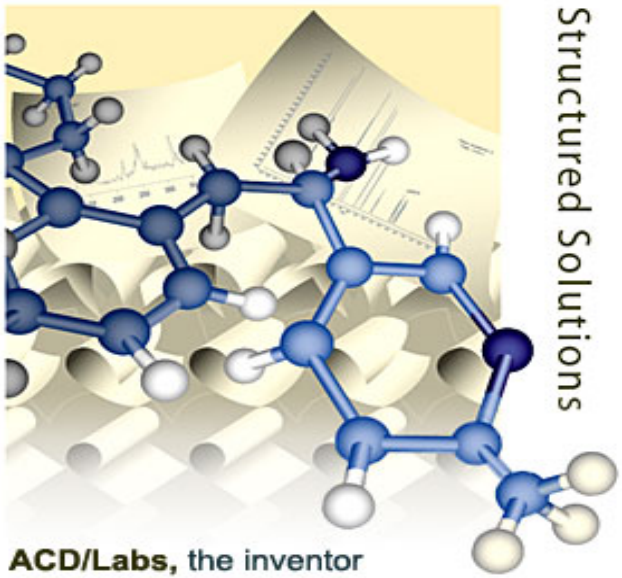
In summary, pKa Prediction

- pKa prediction is important to all small molecule liquid chromatography
- Only Advanced Chemistry Development's method development software contains this capability

pKa Prediction

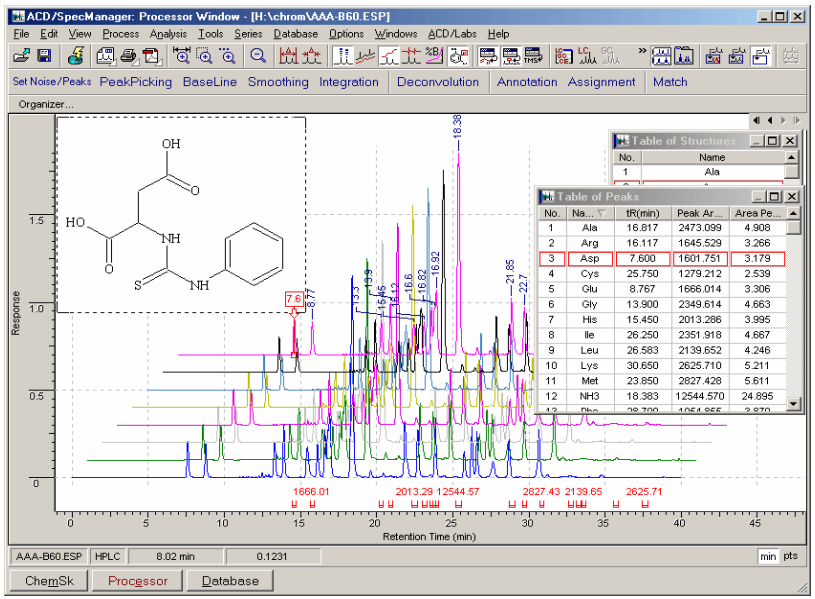
- The value of pKa prediction is underestimated
- A great deal of time is spent optimizing pH with little regard for the pKas of interest.

Switching gears...



Structured Solutions

ACD/Labs, the inventor of ChemAnalytics, develops software that enables the process of discovery for chemists



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Method Development – The typical approach

What is the typical approach to method development?

- Use the same method as last time
- Perform a text based literature search for a method ☹
- Use the hottest new method that you read about in a trade journal!
- Ask the local expert ☺

What's wrong with that approach?

- It might work…but it's not likely
- Text searches based on compound names can be problematic
- You might not have the hottest new column or instrumentation
- The local expert's not talking

What are other obstacles that chromatographers face?

- Limited resources – columns, solvents, detectors, time
- Problems retrieving and sifting through historical data
- Compounds behave differently depending on pH.

What do we suggest?

- Build your own corporate database.
- Have access to a database of commercially available methods that is structure searchable.
- Be able to search those methods intelligently and simultaneously

Once you have that database...

- Search it for a suitable starting point based on *your* requirements –
- Predict retention times based on *your* compounds of interest.
- Select the optimal pH based on the pkas of those compounds

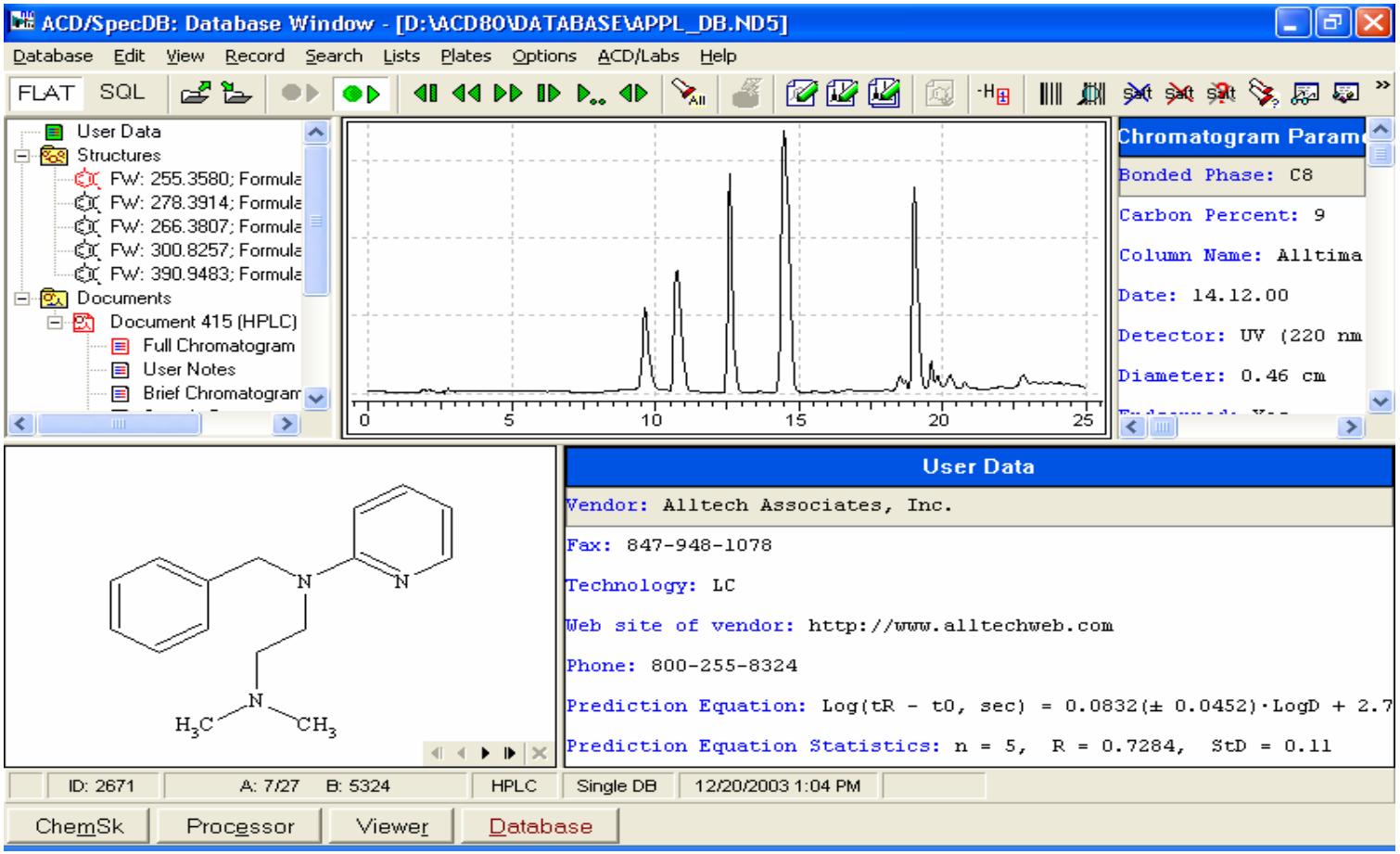
Now we're onto something!

- Predict retention times based on chemical structures.
- Optimize and refine your methods "in silico" as much as possible.
- Select a reasonable pH for the solvent system
- Run your method.
- Update your database with your new method.

The Value of Application Databasing

- Storage of organizational chromatographic information
  - Find developed methods:
    - Avoid redoing the same work
    - Basis for new compound development
  - Structure similarity search is a primary tool.
- Access to commercially available applications

Search the database



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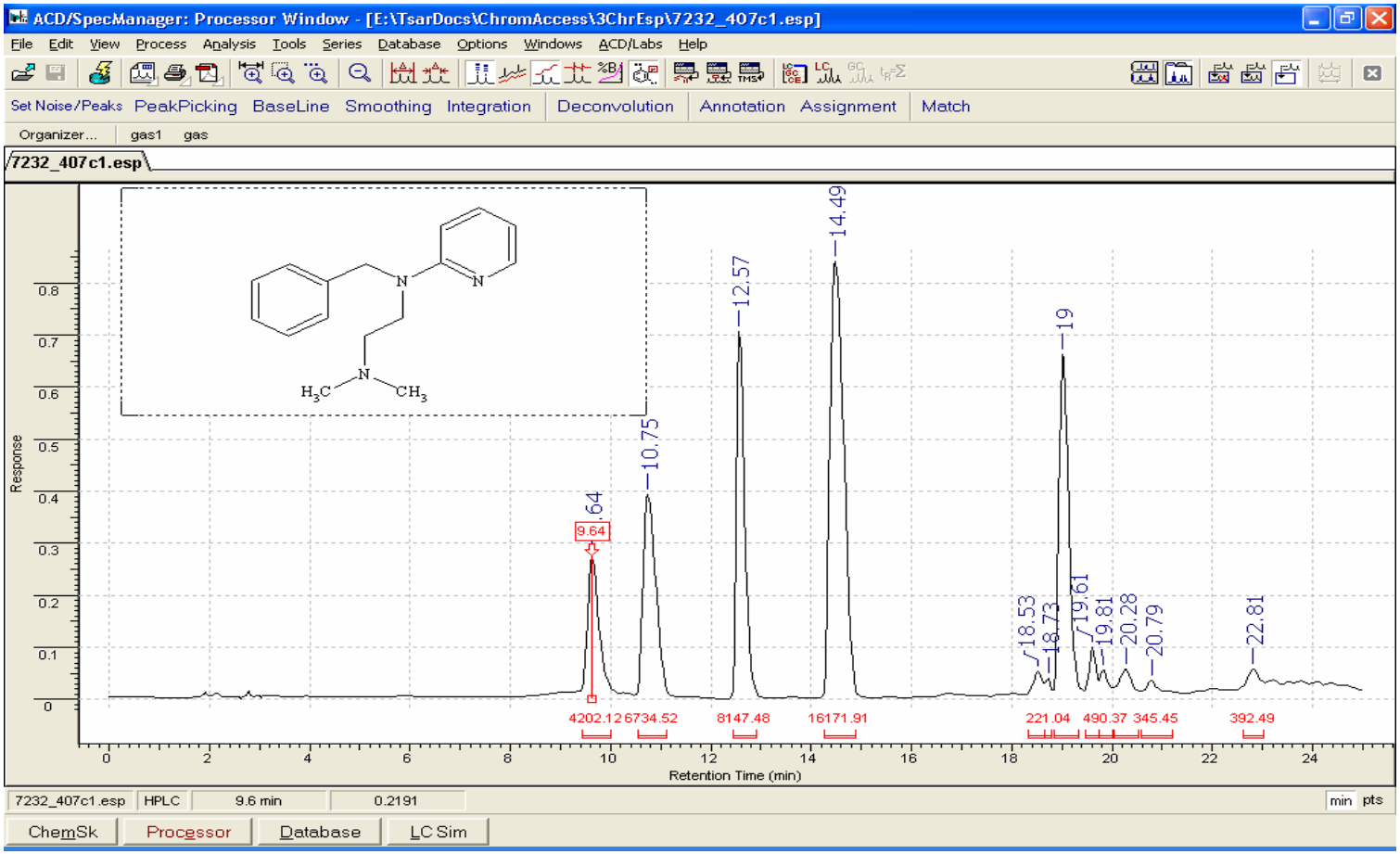
Find a suitable starting point



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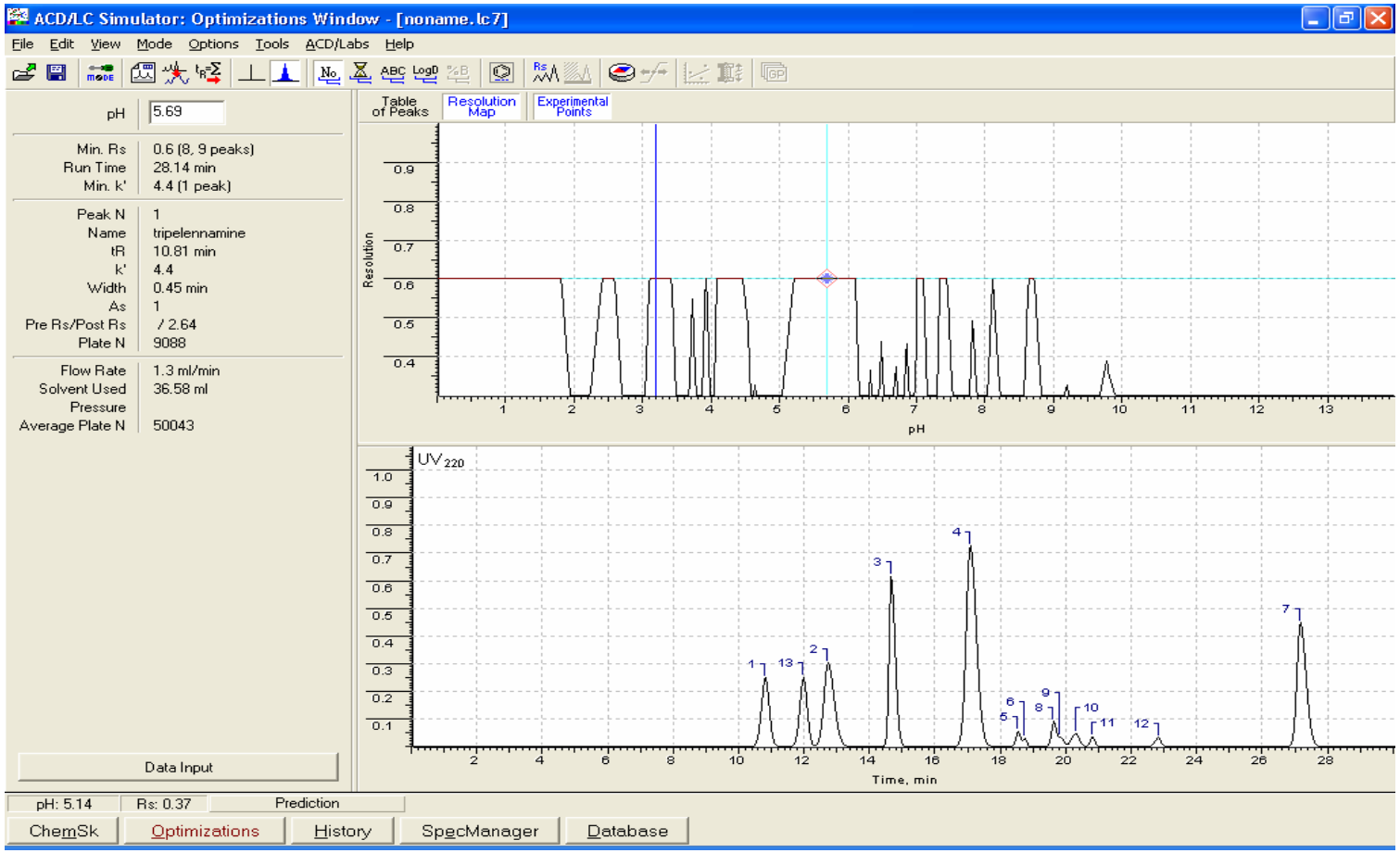
Predict retention times



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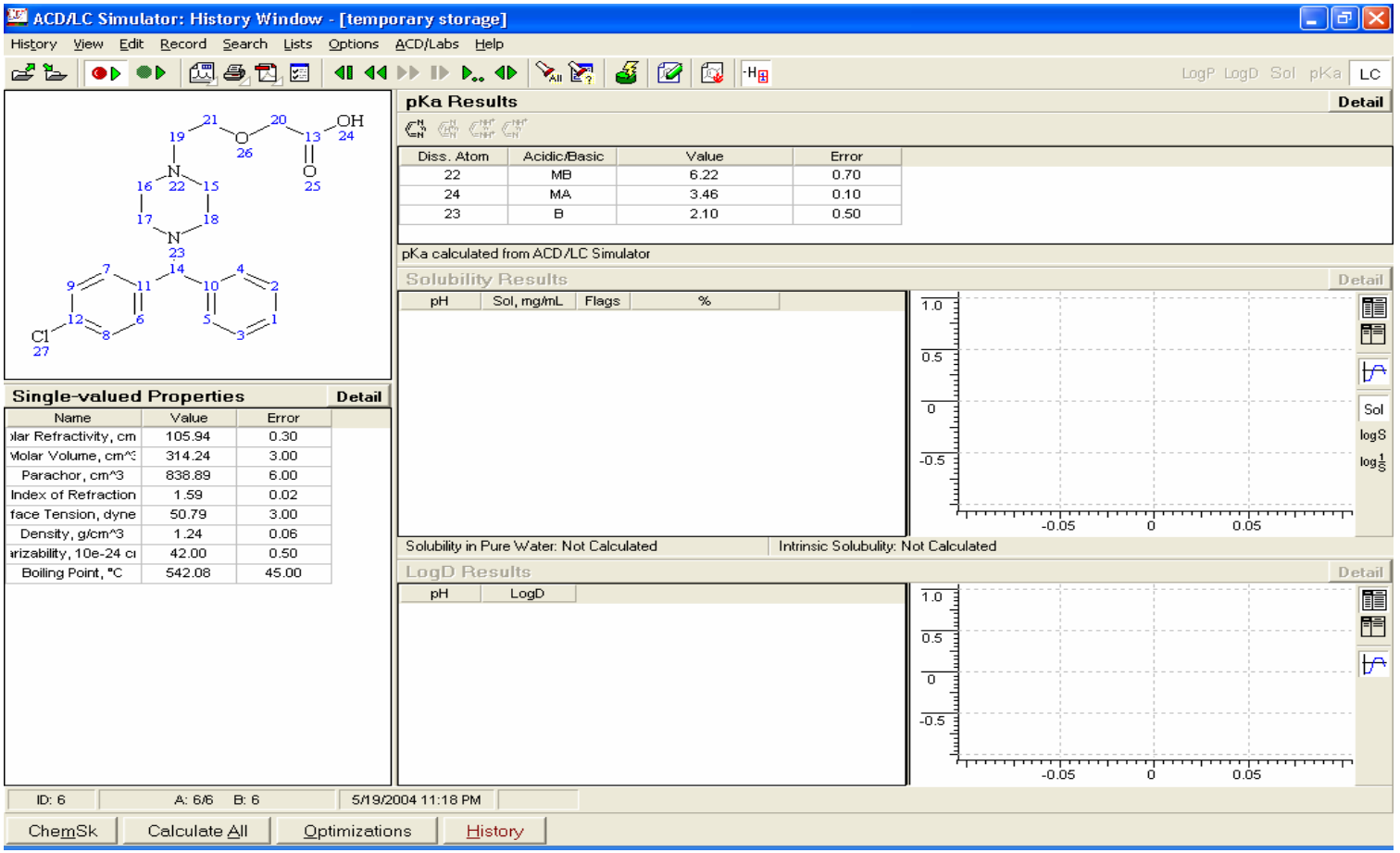
Predict pKas



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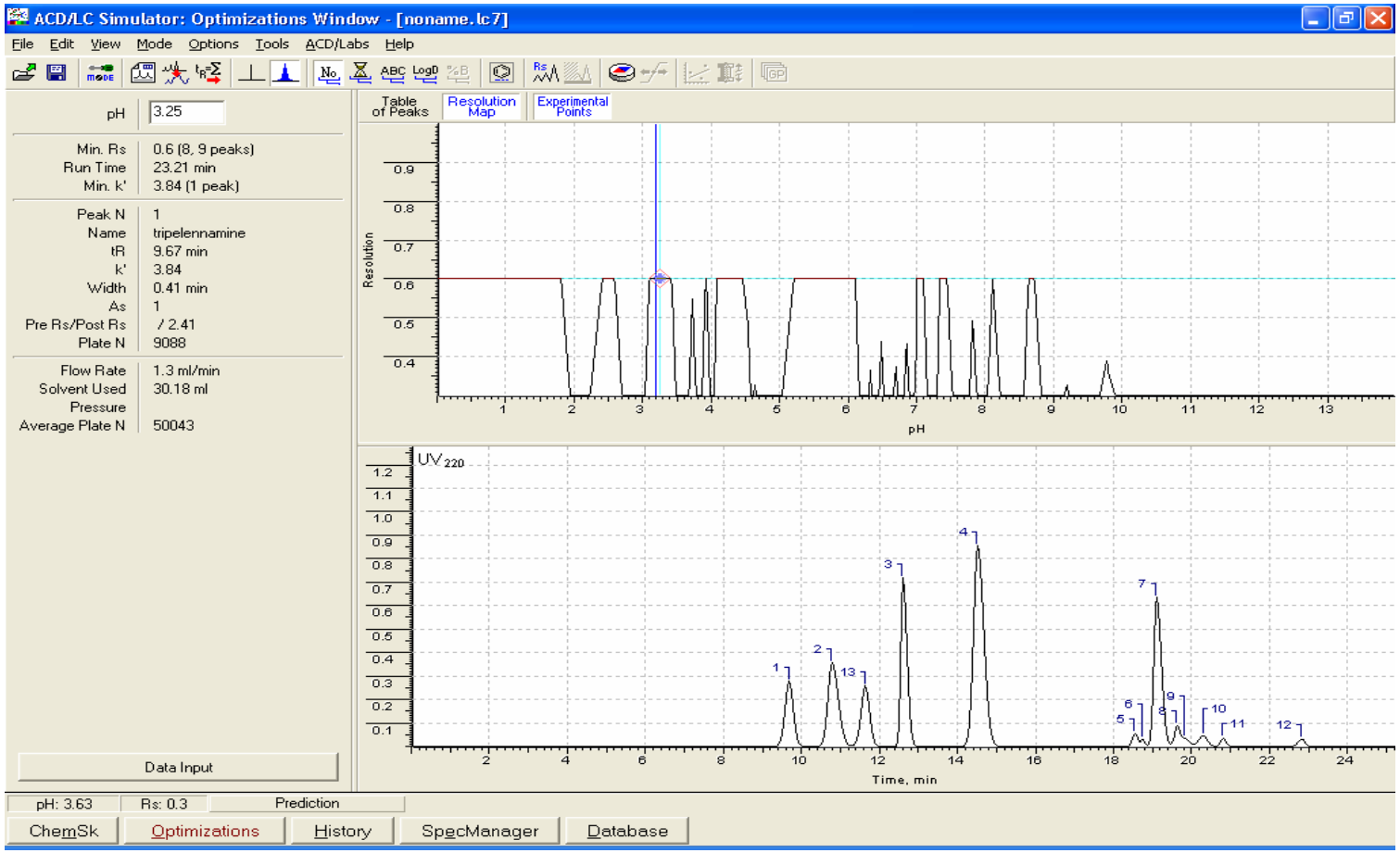
To determine optimal pH



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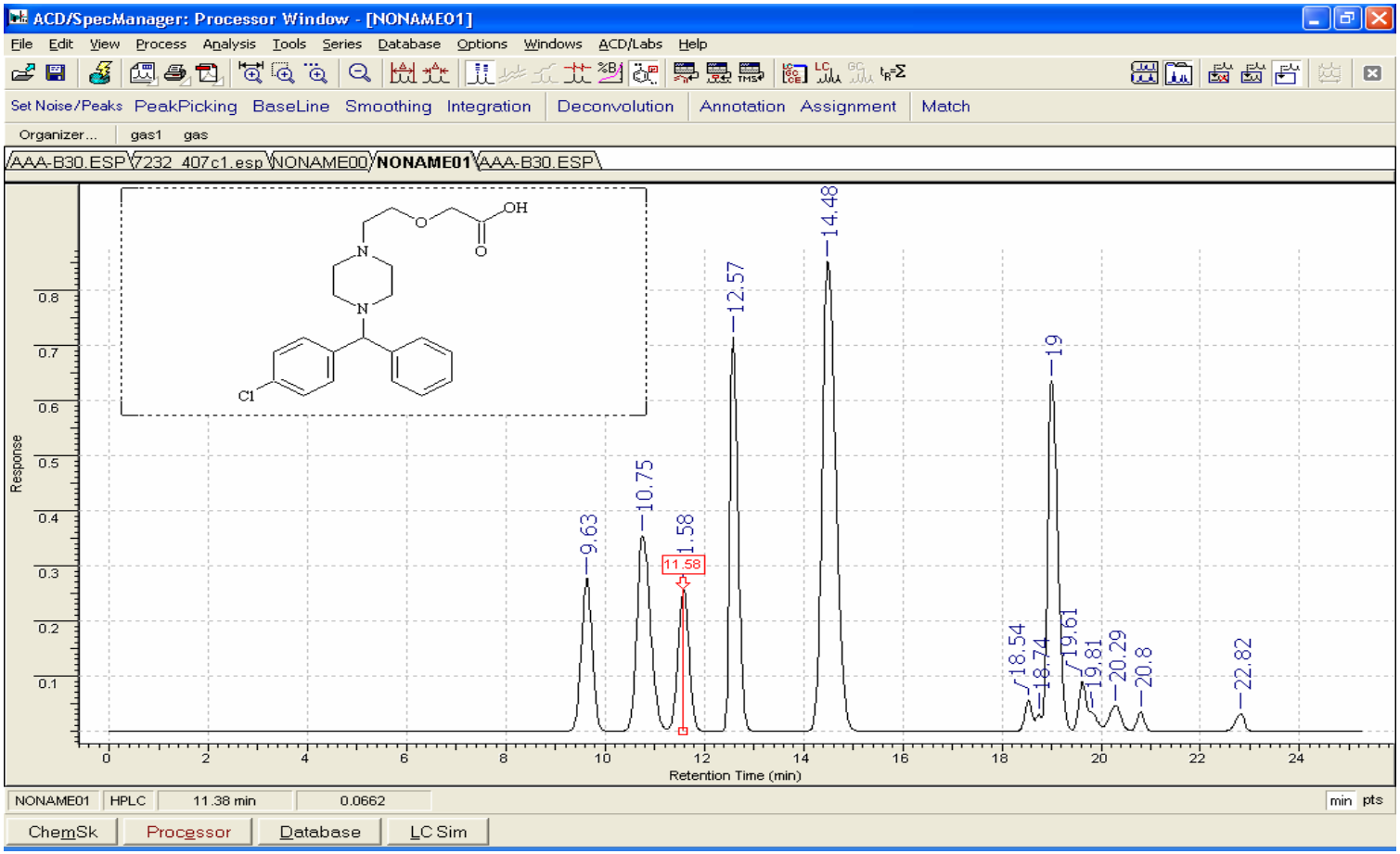
Database your new method!



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Why ACD/ChromManager?

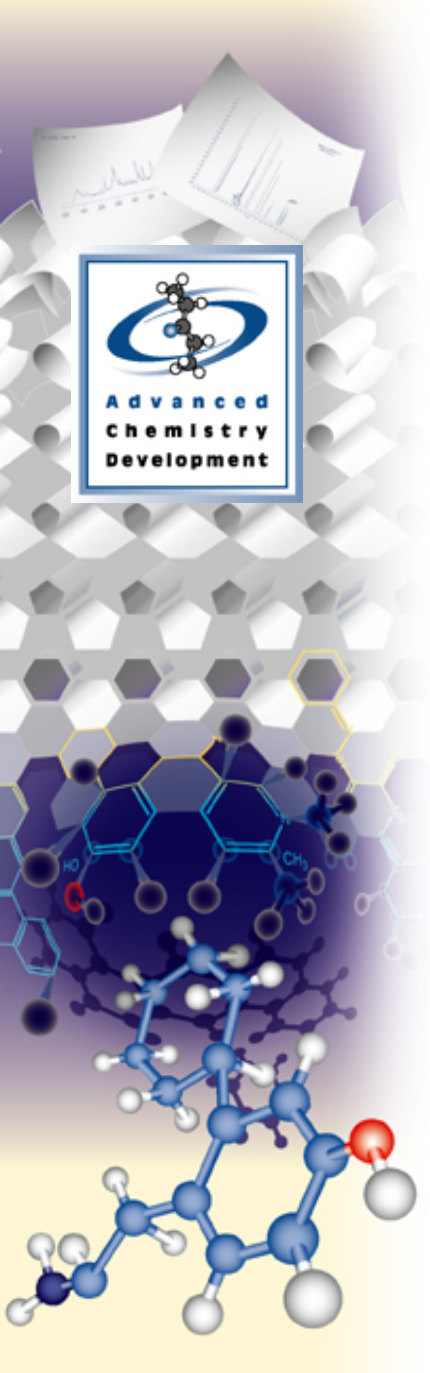
- Advanced Search algorithms
  - Structure Similarity
  - Chromatographic Smart Search
- ACD/Chrom Applications DB
  - Create user DBs as well
  - 18 column vendors
- ACD/ChromProcessor
- ACD/Column Selector
- Web Librarian for “casual users”

Why ACD/LC Simulator?

- Refine methods *before* you inject
- System suitability – resolution maps
- Method Optimization based on experimental runs

Wrapping up

- Don’t overlook the importance of pKas of your compounds.
- Harness the chromatographic knowledge that you have
- Find a suitable starting point
- Optimize and refine
- Update your database



Thanks for your attention!

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