



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

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June 3, 2004





# 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?

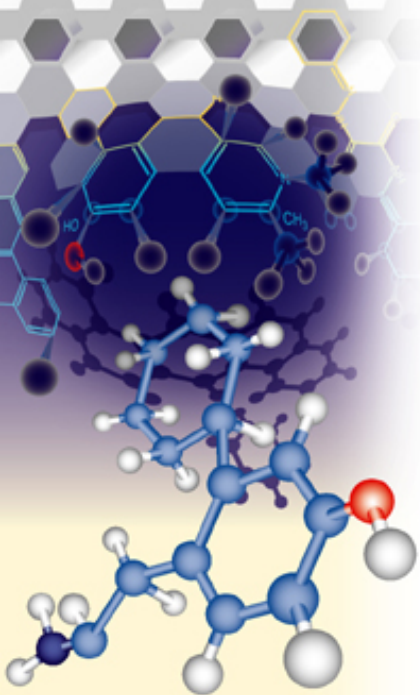
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# pKa Prediction

## 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

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# What happens during ionization?

🌀 Henderson – Hasselbach

$$\text{pH} = \text{pKa} - \log([\text{HA}]/[\text{A-}])$$

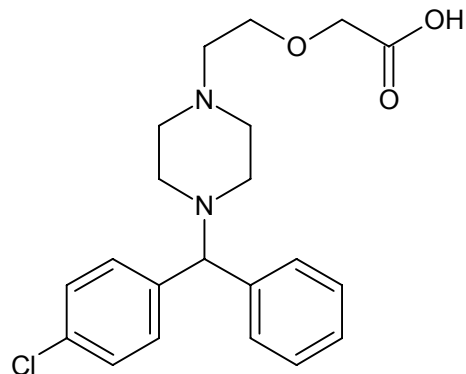
Where the ratio of acid to ionized species is known

🌀 For example:



# Zyrtec

🌀 What's the pka?



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# One possible ionic form...

HL

**Approximated apparent pKa value(s):**

1.  $pK_a(HL/H+L)=6.22 \pm 0.70$
2.  $pK_a(H2L/H+HL)=3.46 \pm 0.10$
3.  $pK_a(H3L/H+H2L)=2.10 \pm 0.50$

**pKa = 6.2**

**Name:** CETIRIZINE

**References:**

1.  $pK_a(HL/H+L)$ ; 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.  $pK_a(H2L/H+HL)$ ; 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)

**$pK_{a_{calc}} = pK_{a_0} + \Delta(pK_a)$**

**Calculation of  $pK_{a_0}$ :**

The fragment has been found in the Internal Reaction Centers Database with estimated equation  
 $pK_a = 7.32 - 2.13 \cdot \sigma^{ind}$      $n=51$ ,  $StD=0.7000$

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# And another ...

ACD/pKa DB: Results Window

Results Options ACD/Labs Help

H2L

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 = 3.46**

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)
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Reference: Ter Laak, A., Tsai, R., Kelder, G., Carrupt, P.-A., Testa, B. & Timmerman, H., Eur. J. Pharm. Sci., 2, 373 (1994)

$pK_{a_{calc}} = pK_{a_0} + \Delta(pKa)$

Calculation of pKa<sub>0</sub>:

The fragment has been found in the Internal Reaction Centers Database with experimental equation

$pKa = 3.518 - 2.753 \cdot \sigma^{Ind}$  n=14, r=0.9788, StD=0.0330

The final value of pKa<sub>0</sub>:

pKa<sub>0</sub> = 3.518

Calculation of  $\Delta(pKa)$ :

ChemSk Results History Database

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# ...and another.

ACD/pKa DB: Results Window

Results Options ACD/Labs Help

H3L

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)

**pKa<sub>calc</sub> = pKa<sub>0</sub> + Δ(pKa)**

**Calculation of pKa<sub>0</sub>:**

The structure has been found in the Internal Structure Database  
pKa = 4.35    StD=0.3000  
**The final value of pKa<sub>0</sub>:**  
pKa<sub>0</sub> = 4.350

**Calculation of Δ(pKa):**

ChemSk

Results

History

Database

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# As expressed in ACD/LC Simulator

ACD/LC Simulator: History Window - [temporary storage]

History View Edit Record Search Lists Options ACD/Labs Help

LogP LogD Sol pKa LC

**Chemical Structure:** A complex molecule with a central nitrogen-containing ring system, a carboxylic acid group, and a chlorine atom. Atoms are numbered 1 through 27.

**Single-valued Properties**

Name	Value	Error
Molar Refractivity, cm <sup>3</sup>	105.94	0.30
Molar Volume, cm <sup>3</sup>	314.24	3.00
Parachor, cm <sup>3</sup>	838.89	6.00
Index of Refraction	1.59	2.00e-2
Surface Tension, dyne	50.79	3.00
Density, g/cm <sup>3</sup>	1.24	6.00e-2
Wizability, 10e-24 c	42.00	0.50
Boiling Point, °C	542.08	45.00

**pKa Results**

Diss. Atom	Acidic/Basic	Value	Error
22	MB	6.22	0.70
24	MA	3.46	0.10
23	B	2.10	0.50

pKa calculated from ACD/LC Simulator

**Solubility Results**

pH	Sol, mg/mL	Flags	%
Solubility in Pure Water: Not Calculated			

**LogD Results**

pH	LogD
Intrinsic Solubility: Not Calculated	

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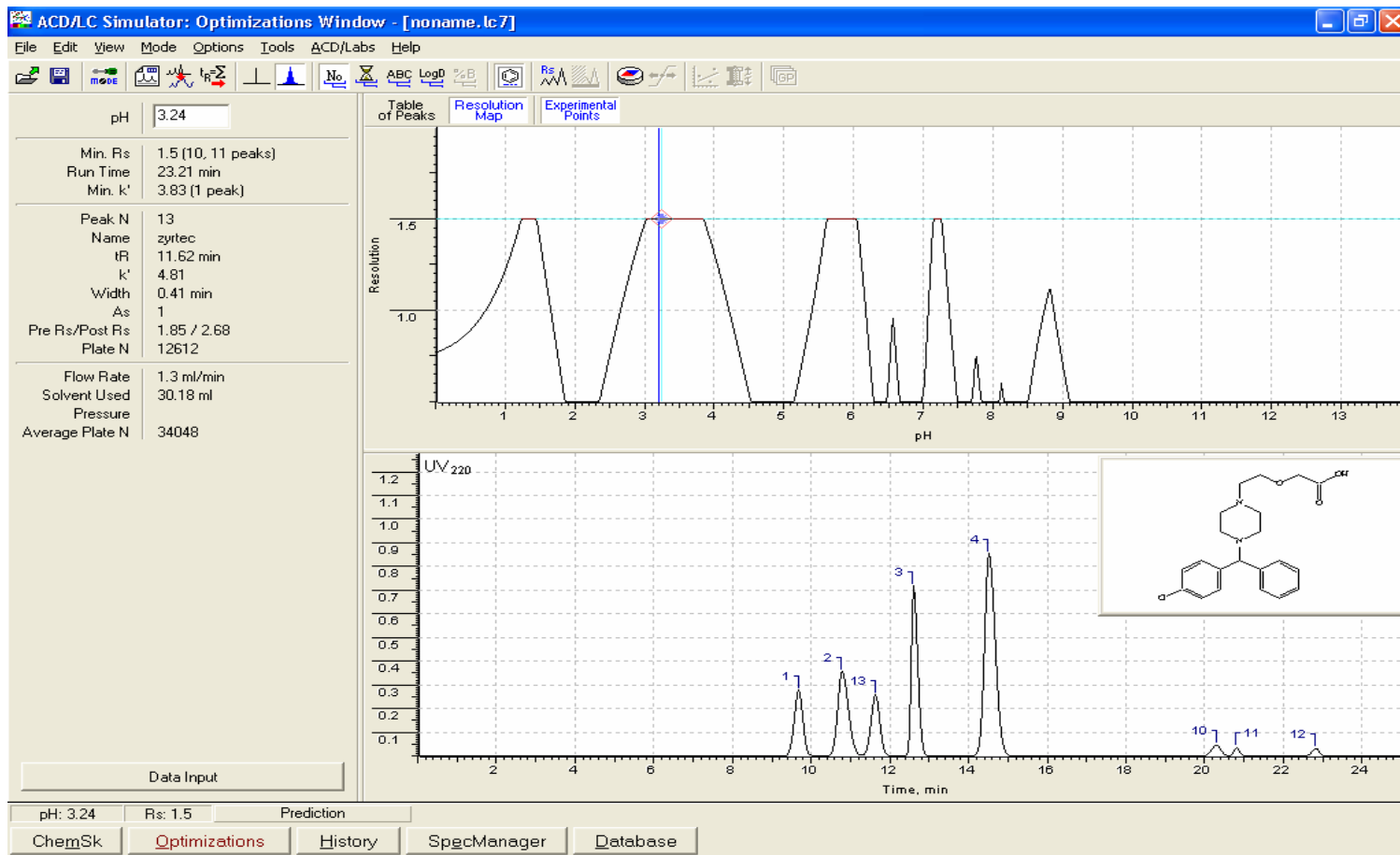
ChemSk Calculate All Optimizations History

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

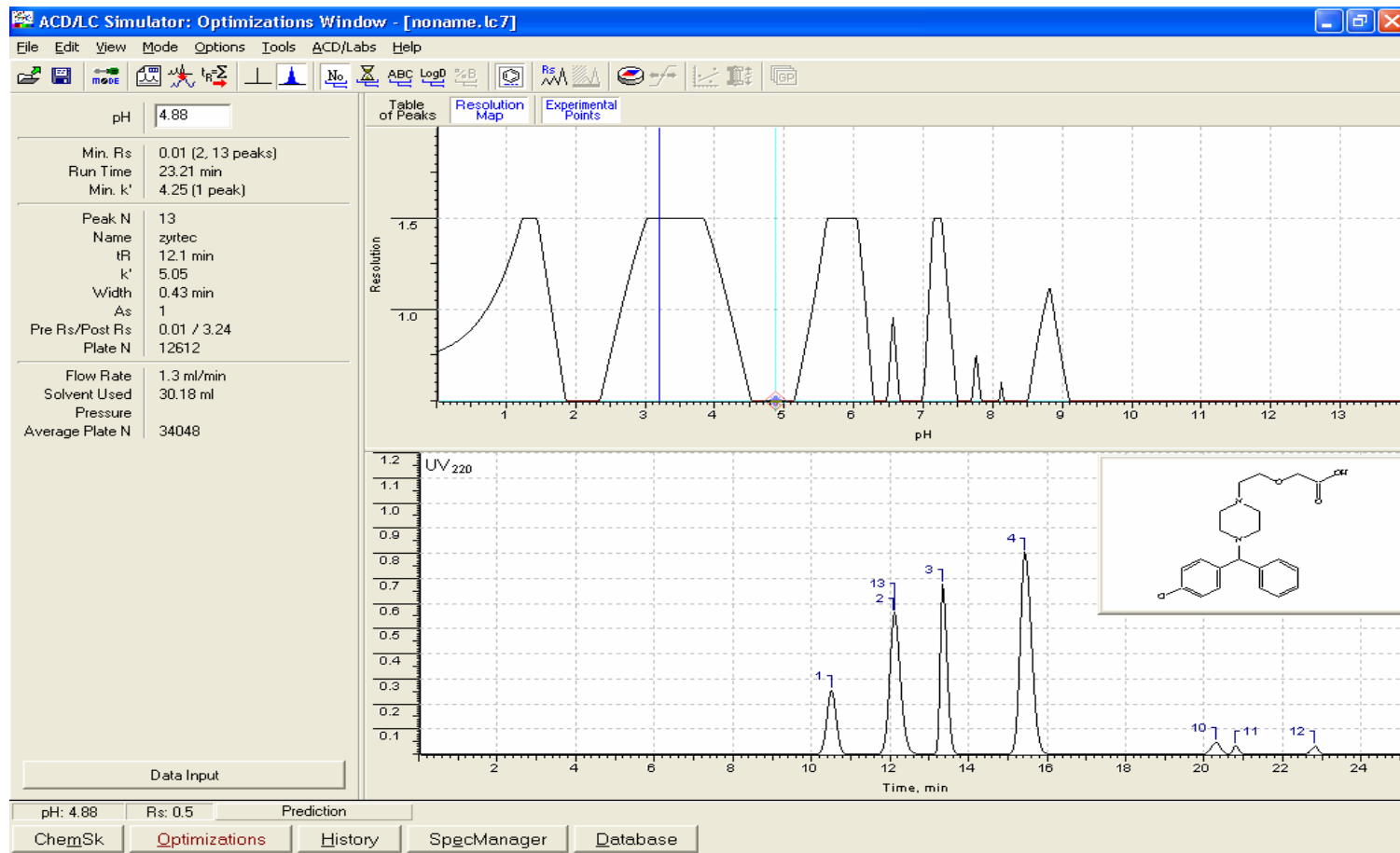


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# At pH 5.0...

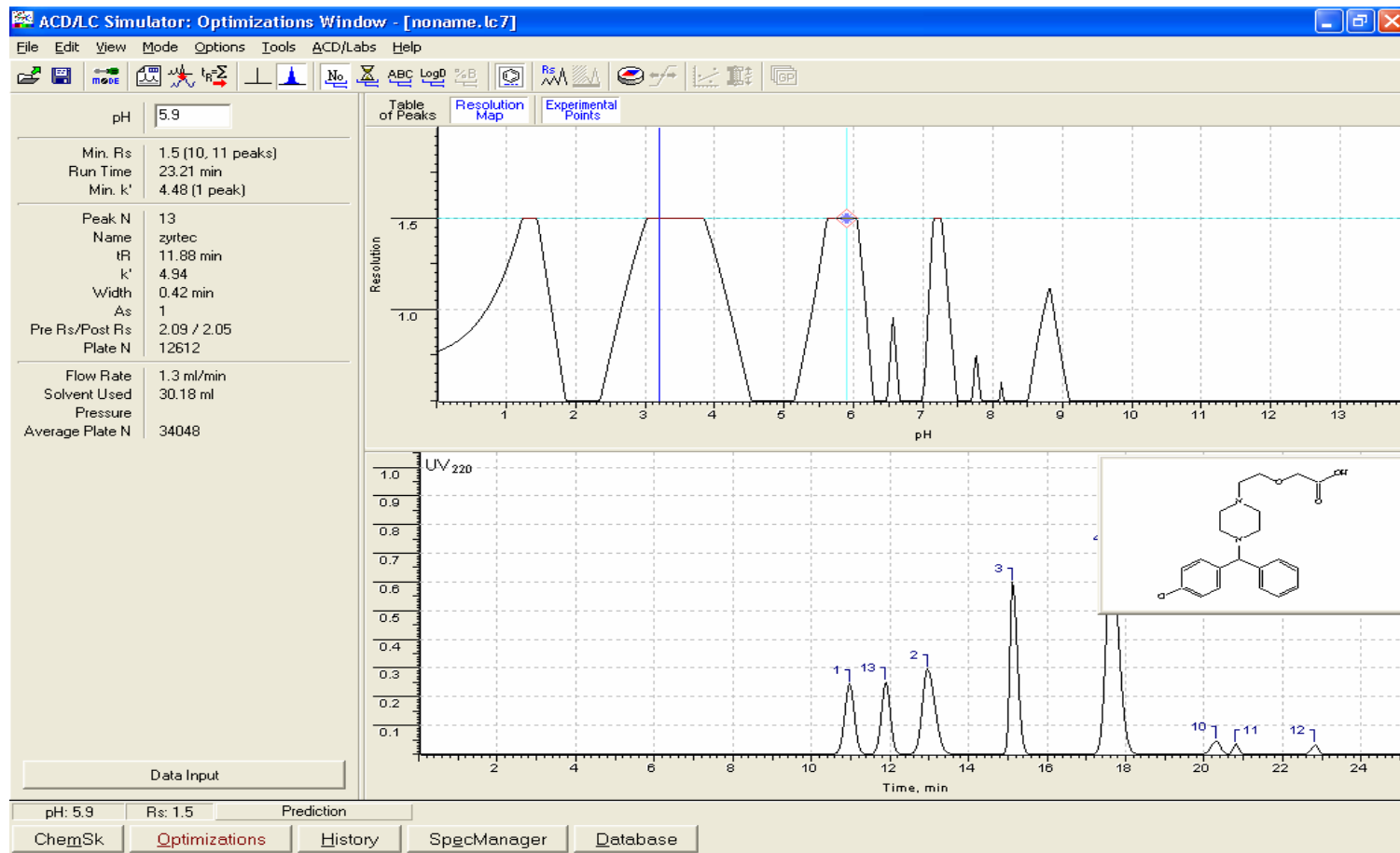


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# And now at pH 6.0...



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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!

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# 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.

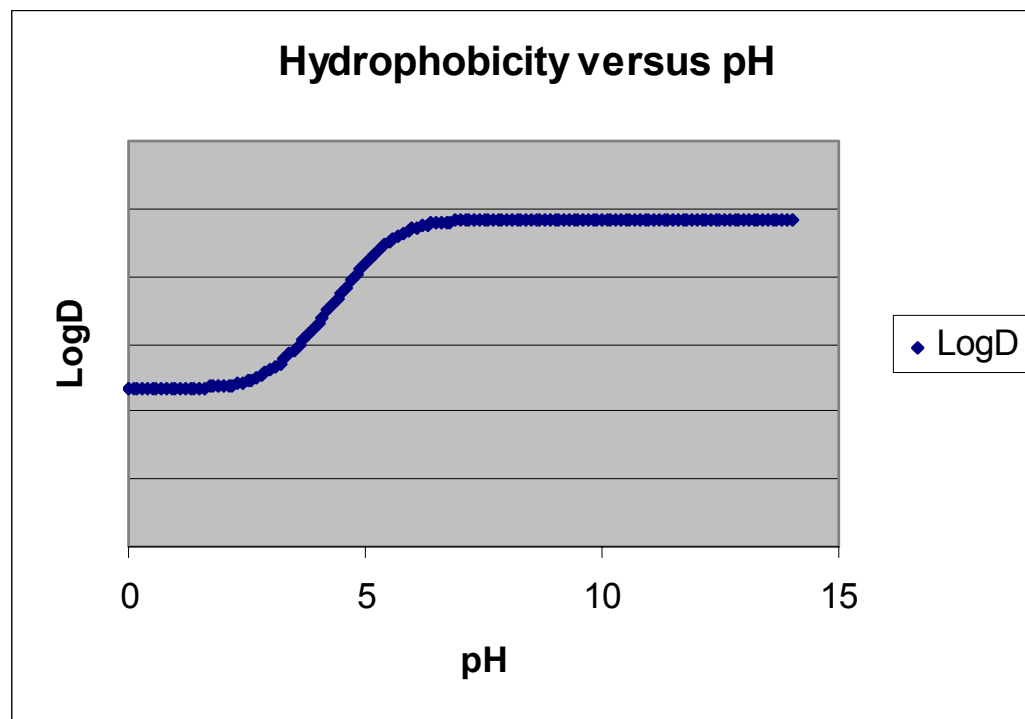
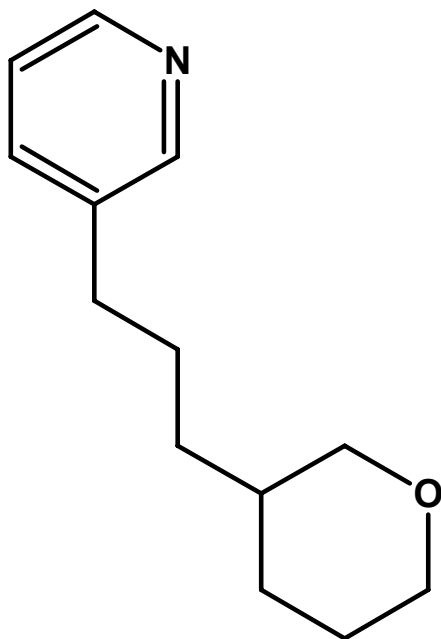
Helpful Hint: When selecting a pH for your solvent system, try to select one that is not near the pKas of your targets.

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# pKa, LogD and $t_R$

Hydrophobicity of a Base  
(pKa = 5.54):



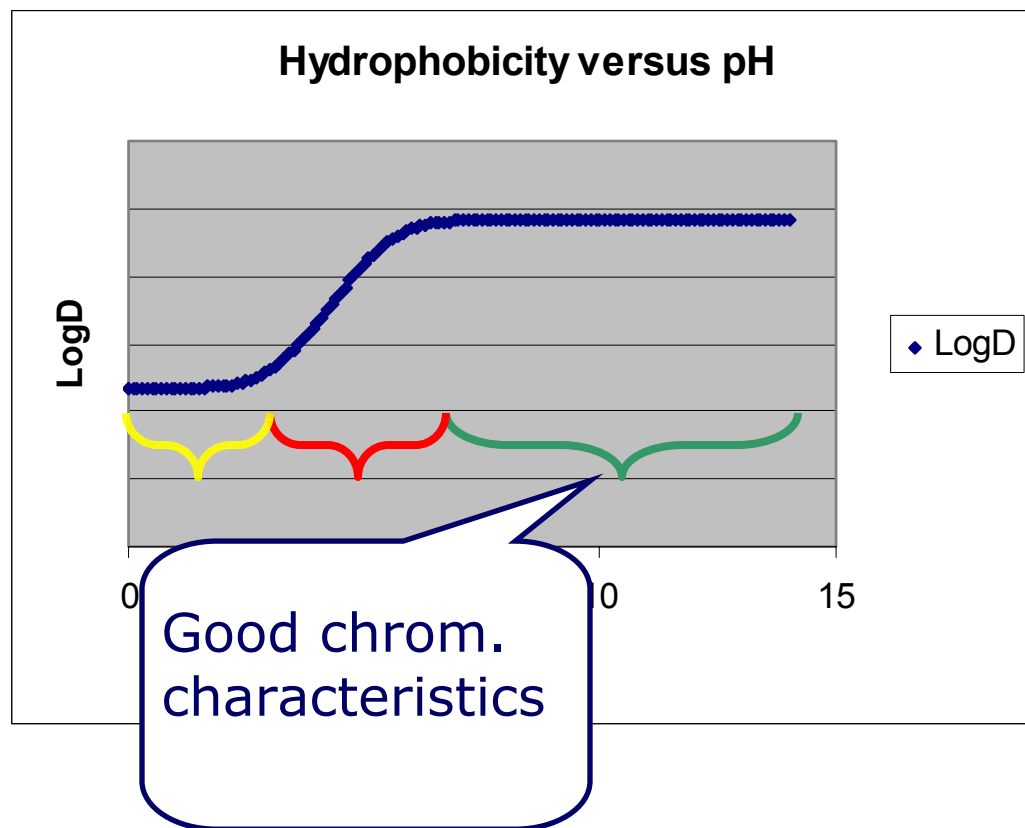
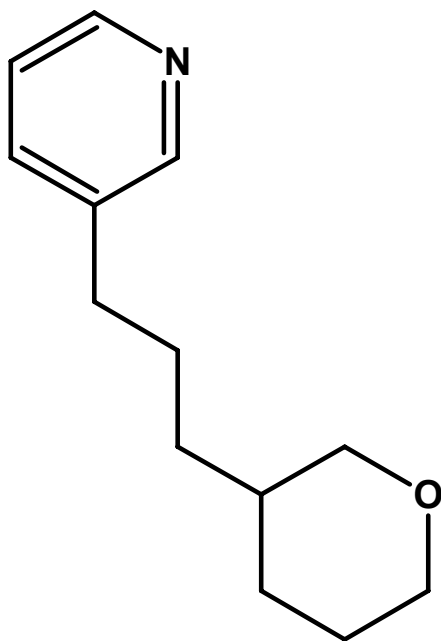
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# pKa, LogD and tR

## Reversed phase HPLC:

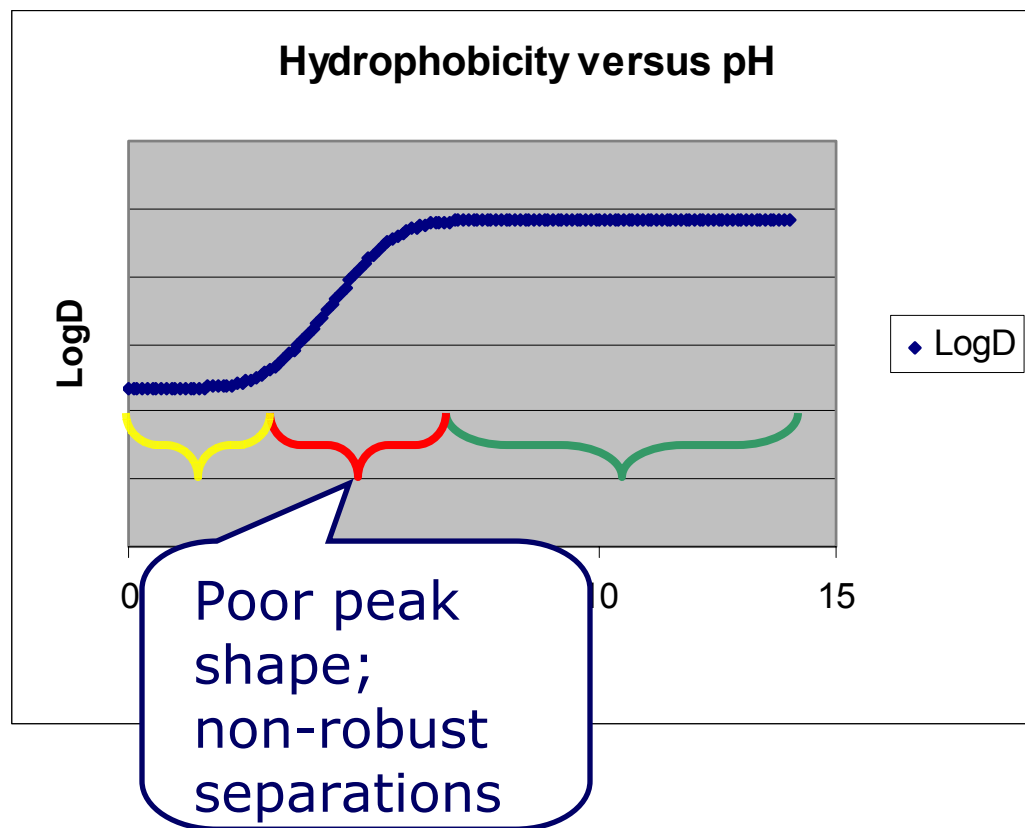
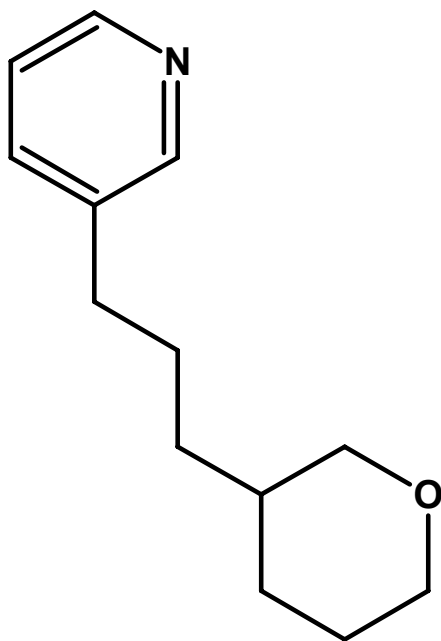


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# pKa, LogD and tR

## Reversed phase HPLC:

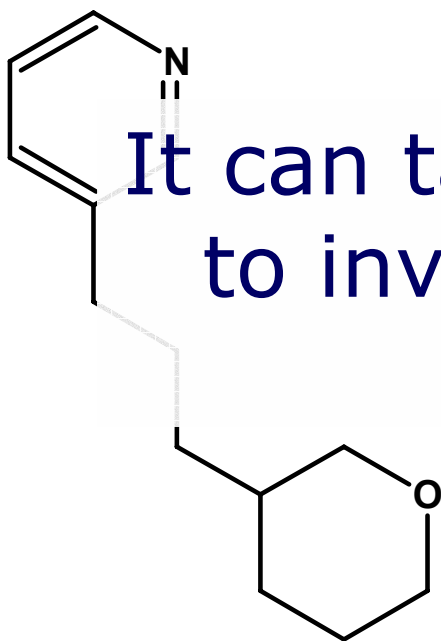


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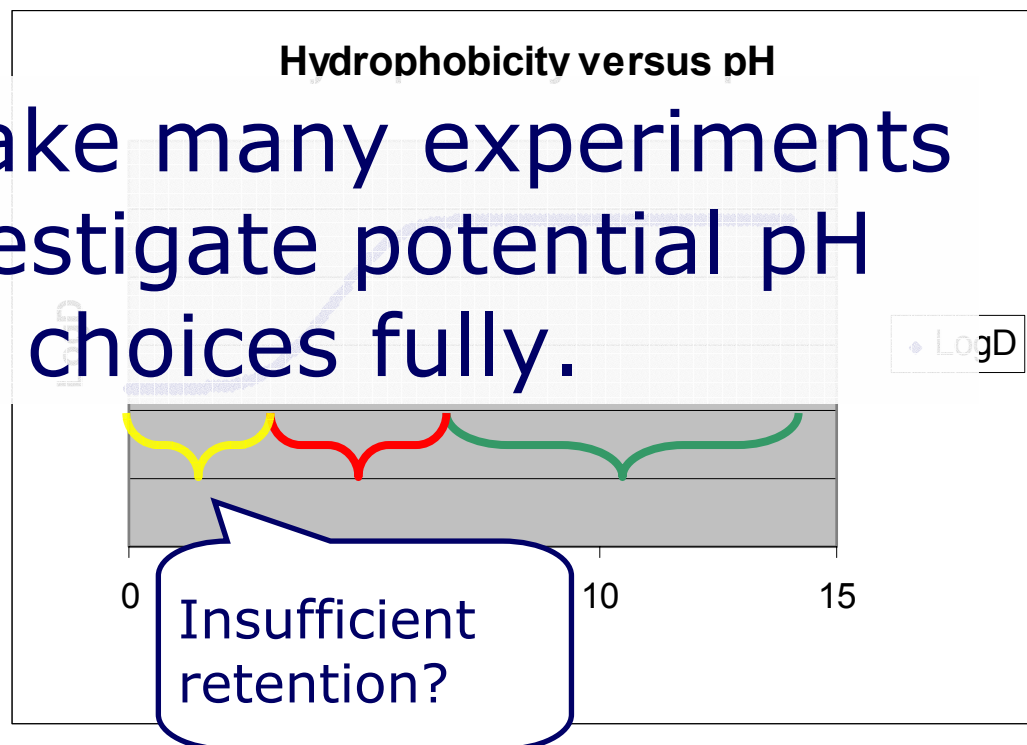
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# pKa, LogD and tR

## Reversed phase HPLC:



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



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# 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

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# pKa Prediction

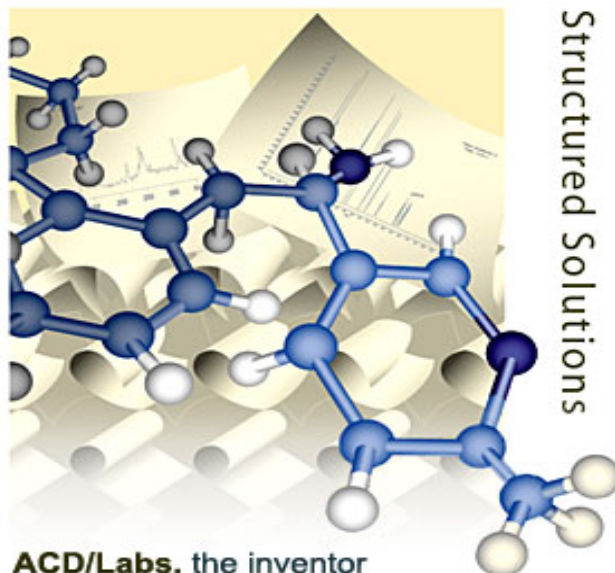
- The value of pKa prediction is underestimated
- A great deal of time is spent optimizing pH with little regard for the pKas of interest.

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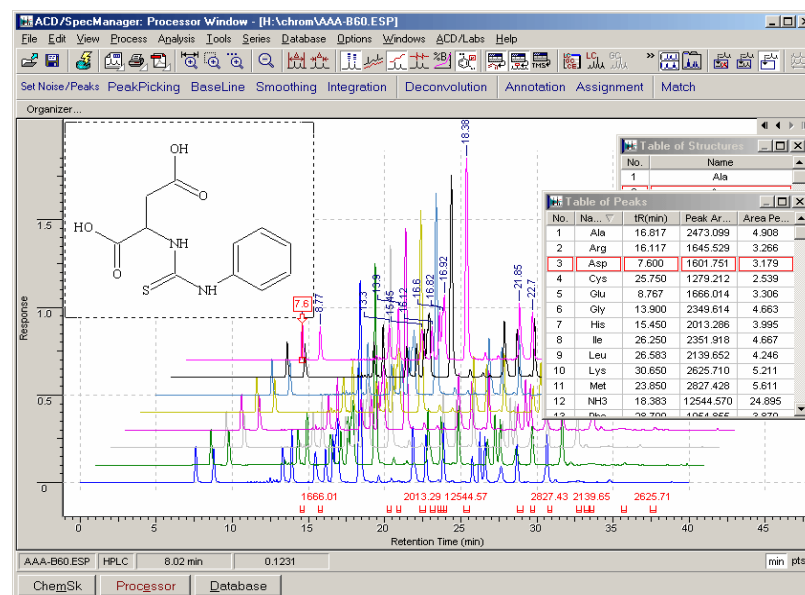


# Switching gears...



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

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# 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 😊

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# 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

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# What are other obstacles that chromatographers face?

- ④ Limited resources – columns, solvents, detectors, time
- ④ Problems retrieving and sifting through historical data
- ④ Compounds behave differently depending on pH.

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# 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

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# 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

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# 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.

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# The Value of Application Databasing

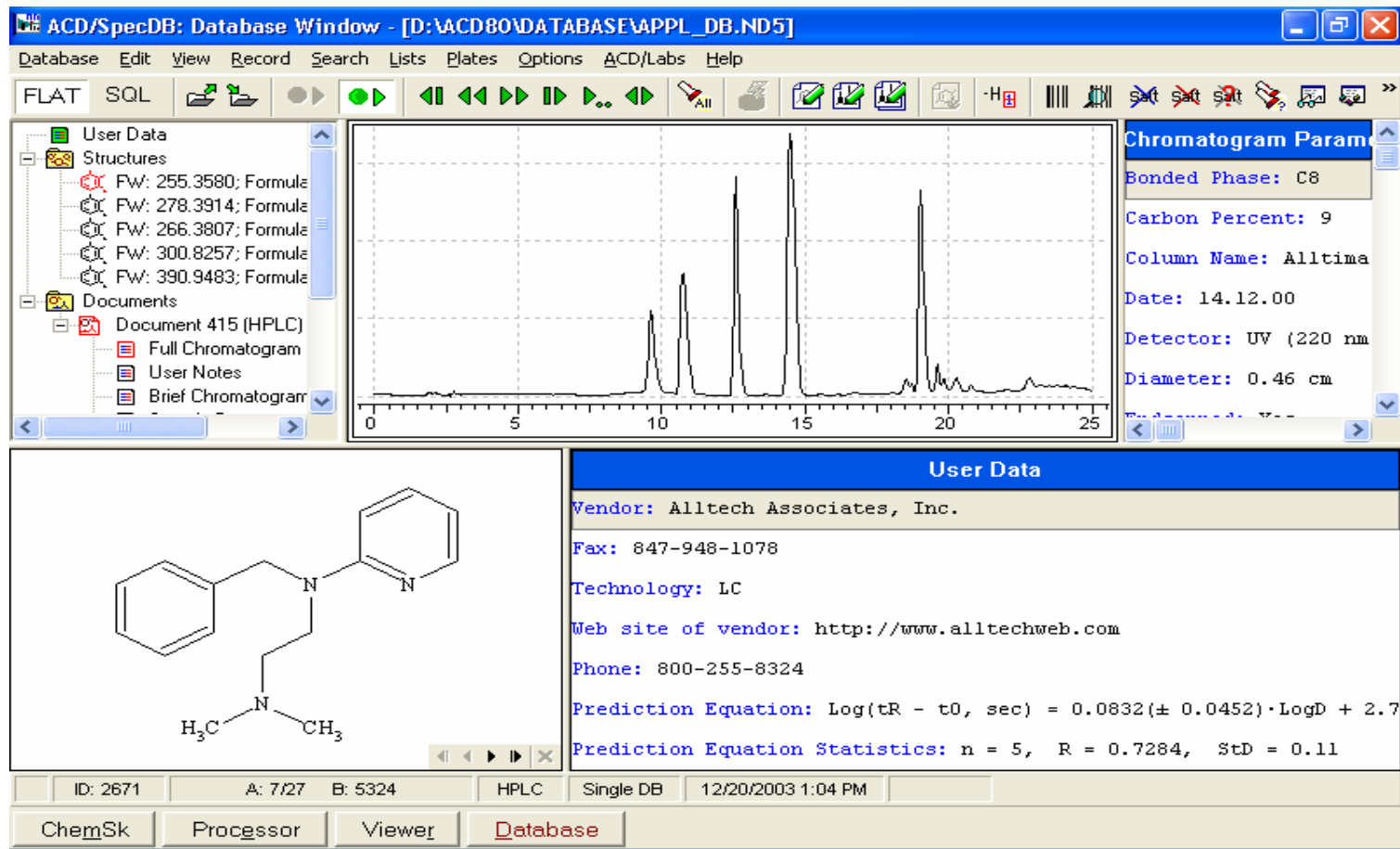
- Storage of organizational chromatographic information
  - Find developed methods:
    - Avoid redoing the same work
    - Basis for new compound development
  - Structure similarity search is a primary tool.
- Access to commercially available applications

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# Search the database

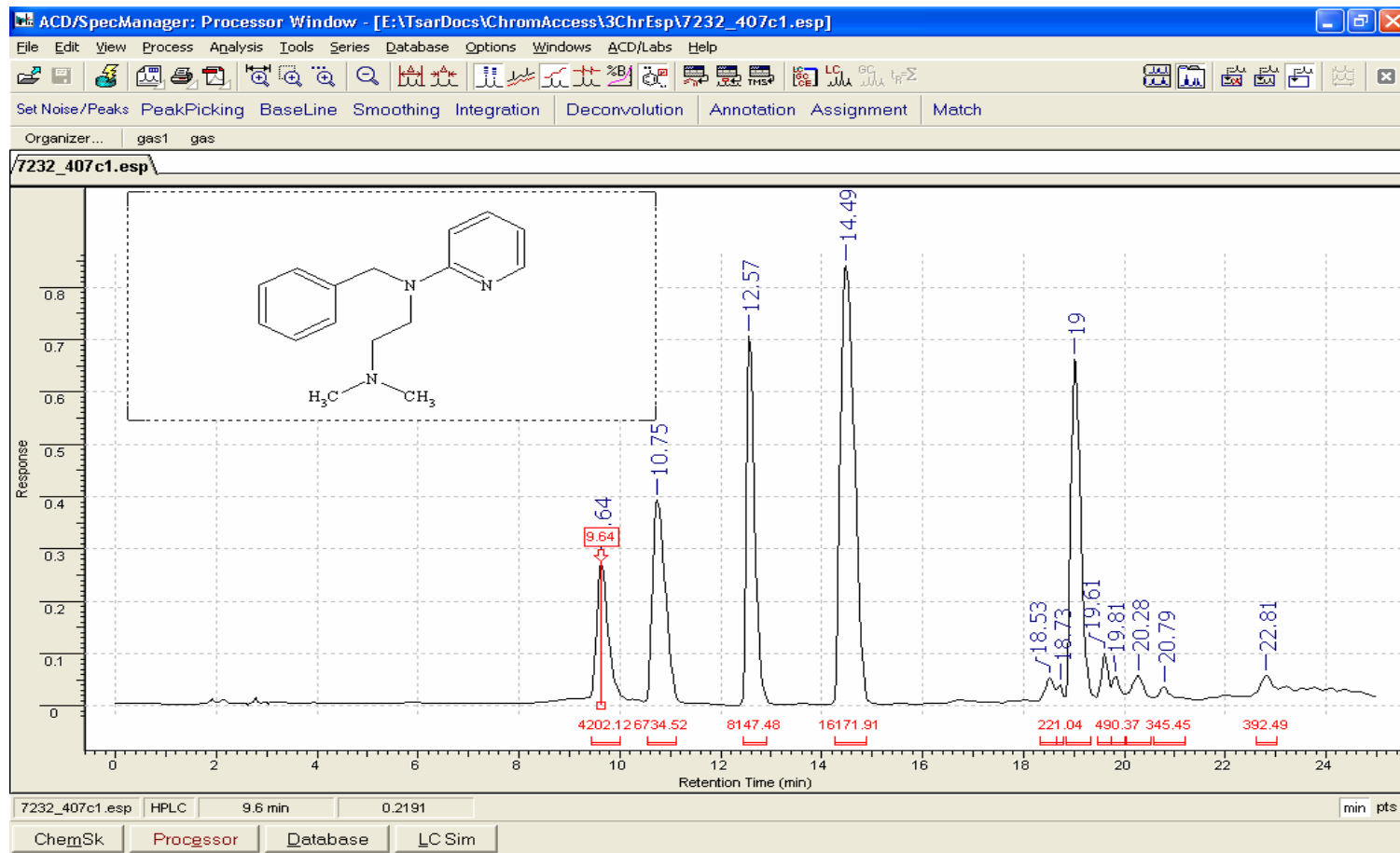


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# Find a suitable starting point



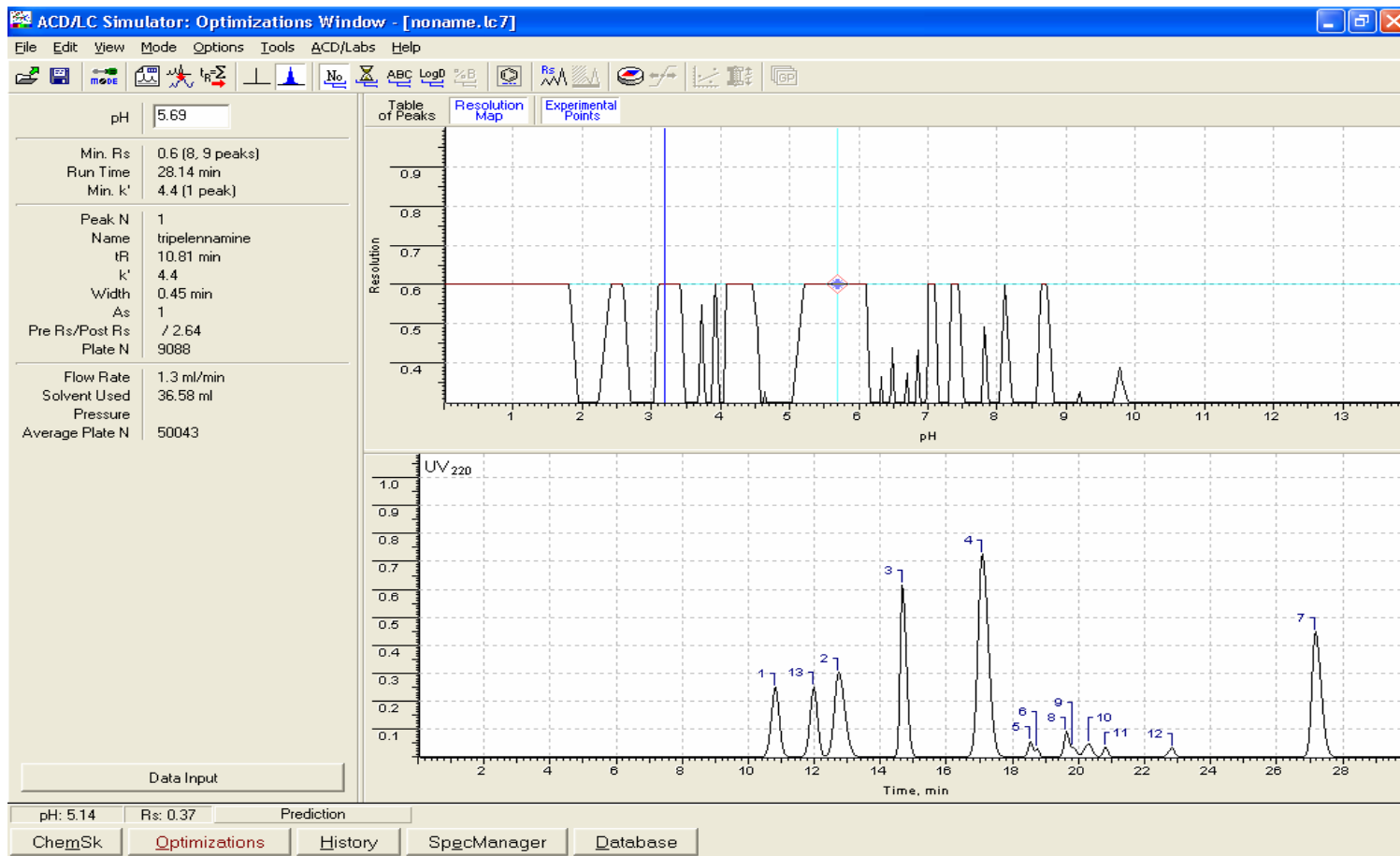
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# Predict retention times



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# Predict pKas

ACD/LC Simulator: History Window - [temporary storage]

History View Edit Record Search Lists Options ACD/Labs Help

LogP LogD Sol pKa LC

**pKa Results**

Diss. Atom	Acidic/Basic	Value	Error
22	MB	6.22	0.70
24	MA	3.46	0.10
23	B	2.10	0.50

pKa calculated from ACD/LC Simulator

**Solubility Results**

pH	Sol, mg/mL	Flags	%
Solubility in Pure Water: Not Calculated			
Intrinsic Solubility: Not Calculated			

**LogD Results**

pH	LogD
Solubility in Pure Water: Not Calculated	
Intrinsic Solubility: Not Calculated	

**Single-valued Properties**

Name	Value	Error
Molar Refractivity, cm <sup>3</sup>	105.94	0.30
Molar Volume, cm <sup>3</sup>	314.24	3.00
Parachor, cm <sup>3</sup>	838.89	6.00
Index of Refraction	1.59	0.02
Surface Tension, dyne	50.79	3.00
Density, g/cm <sup>3</sup>	1.24	0.06
Wizability, 10e-24 cI	42.00	0.50
Boiling Point, °C	542.08	45.00

ID: 6 A: 6/6 B: 6 5/19/2004 11:18 PM

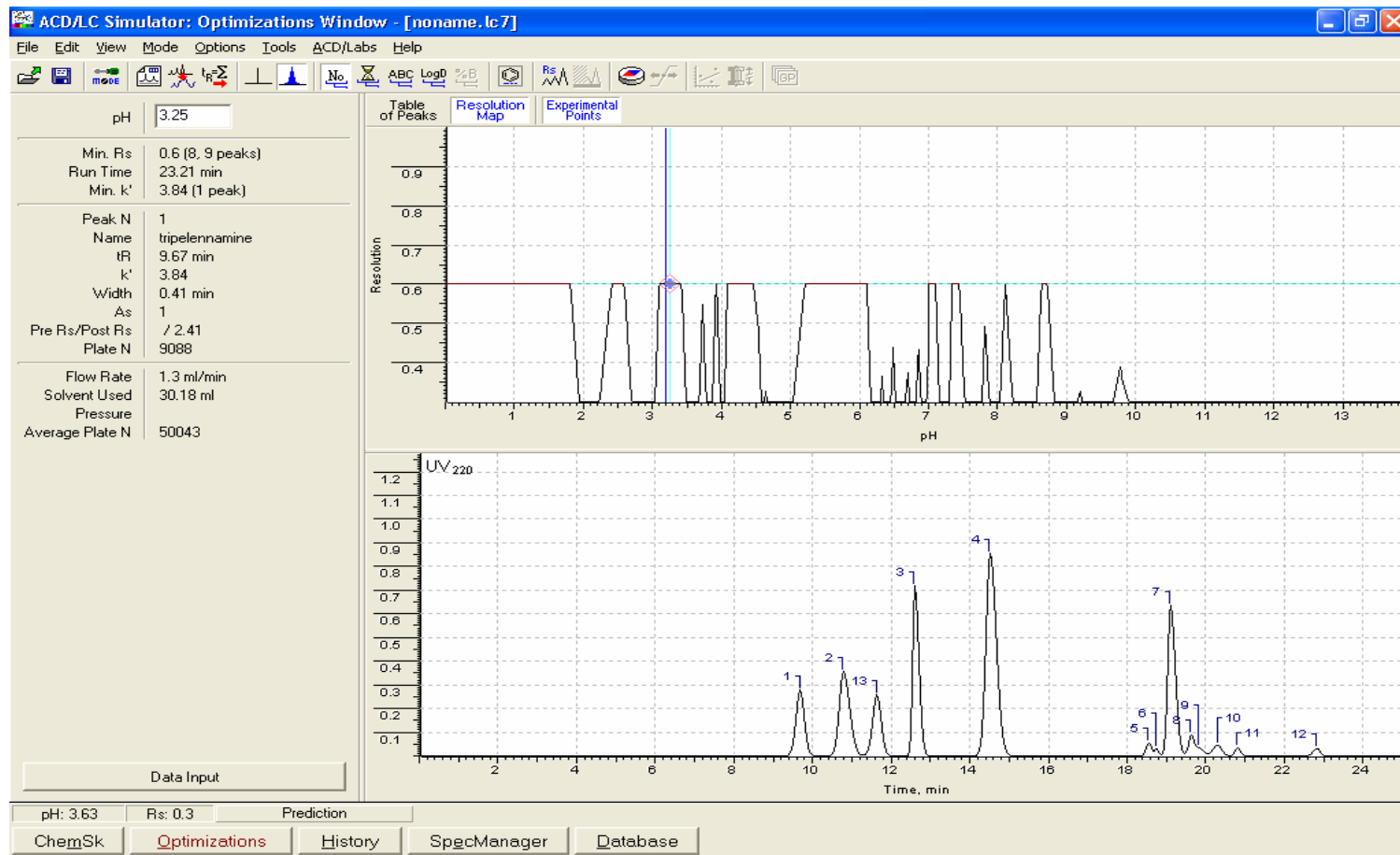
ChemSk Calculate All Optimizations History

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# To determine optimal pH

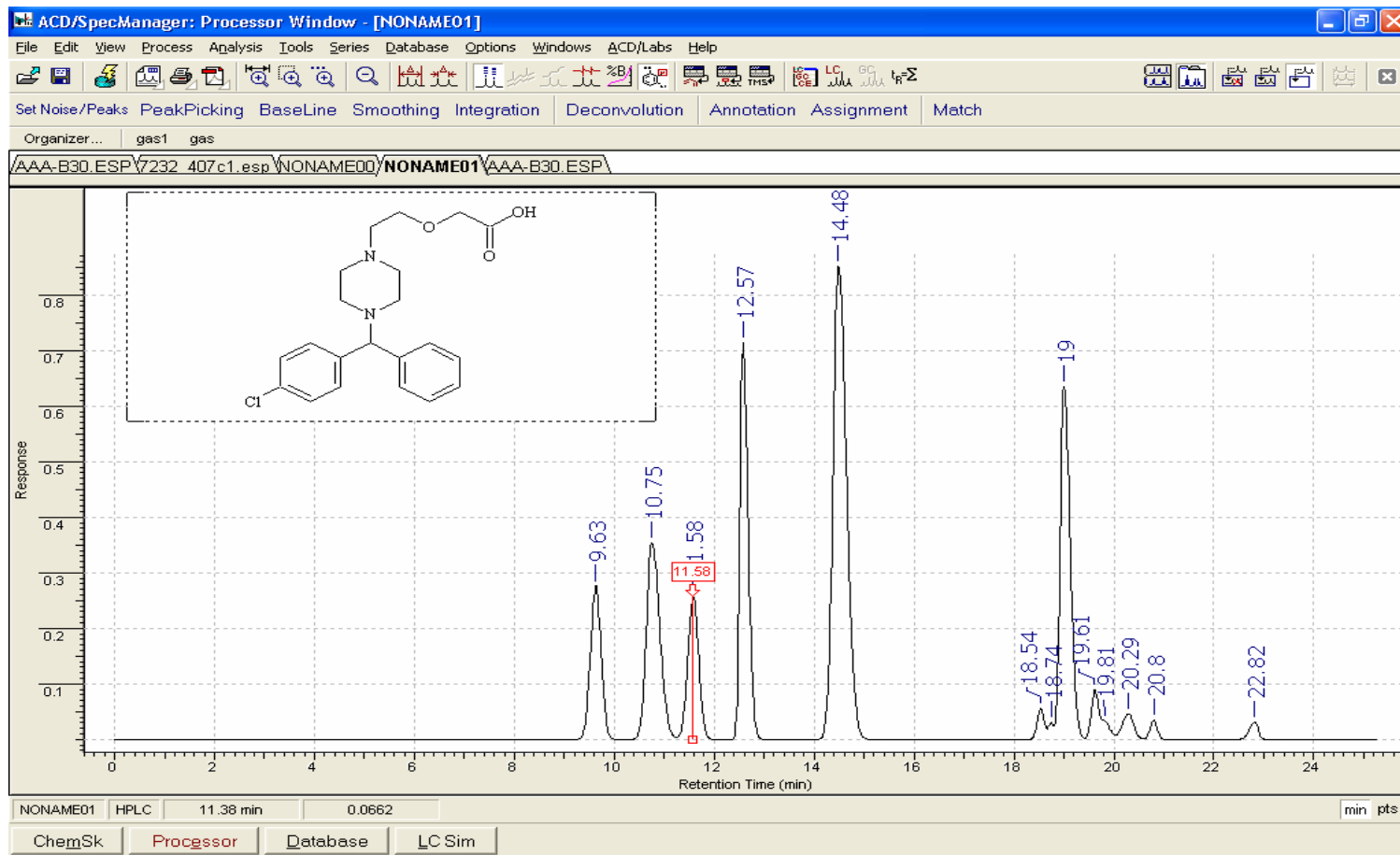


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# Database your new method!



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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”

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# Why ACD/LC Simulator?

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

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# 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

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# Thanks for your attention!

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