Challenges of Peak Tracking in Information-Rich HPLC Experiments

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Answers That Matter.

Method Development Strategy

Design space approach

- structured data generation across columns, aqueous buffers, and organic solvents leading to predictive retention models
- prospective design of analytical methods made possible using the retention models and an understanding of the specific separation requirements

Benefits

- identification of primary and orthogonal HPLC separation conditions
- efficient and consistent method development process
- high success rate for separations of <10-15 components

Column Screening Experiments

Column, Aqueous Phase	Organic Modifier	Gradient Range (%)	Gradient Times (min)
Zorbax SB-C8, 0.1% formic acid in water	ACN	4 – 77	9.5, 38.1
	MeOH	5 – 95	9.5, 38.1
Ace Phenyl, 0.1% formic acid in water	ACN	4 – 77	9.5, 38.1
	MeOH	5 – 95	9.5, 38.1
Zorbax Bonus RP, 0.1% formic acid in water	ACN	4 – 77	9.5, 38.1
	MeOH	5 – 95	9.5, 38.1
XBridge C18, 0.1% NH_4OH in water	ACN	4 – 77	9.5, 38.1
	MeOH	5 – 95	9.5, 38.1

Data Acquisition

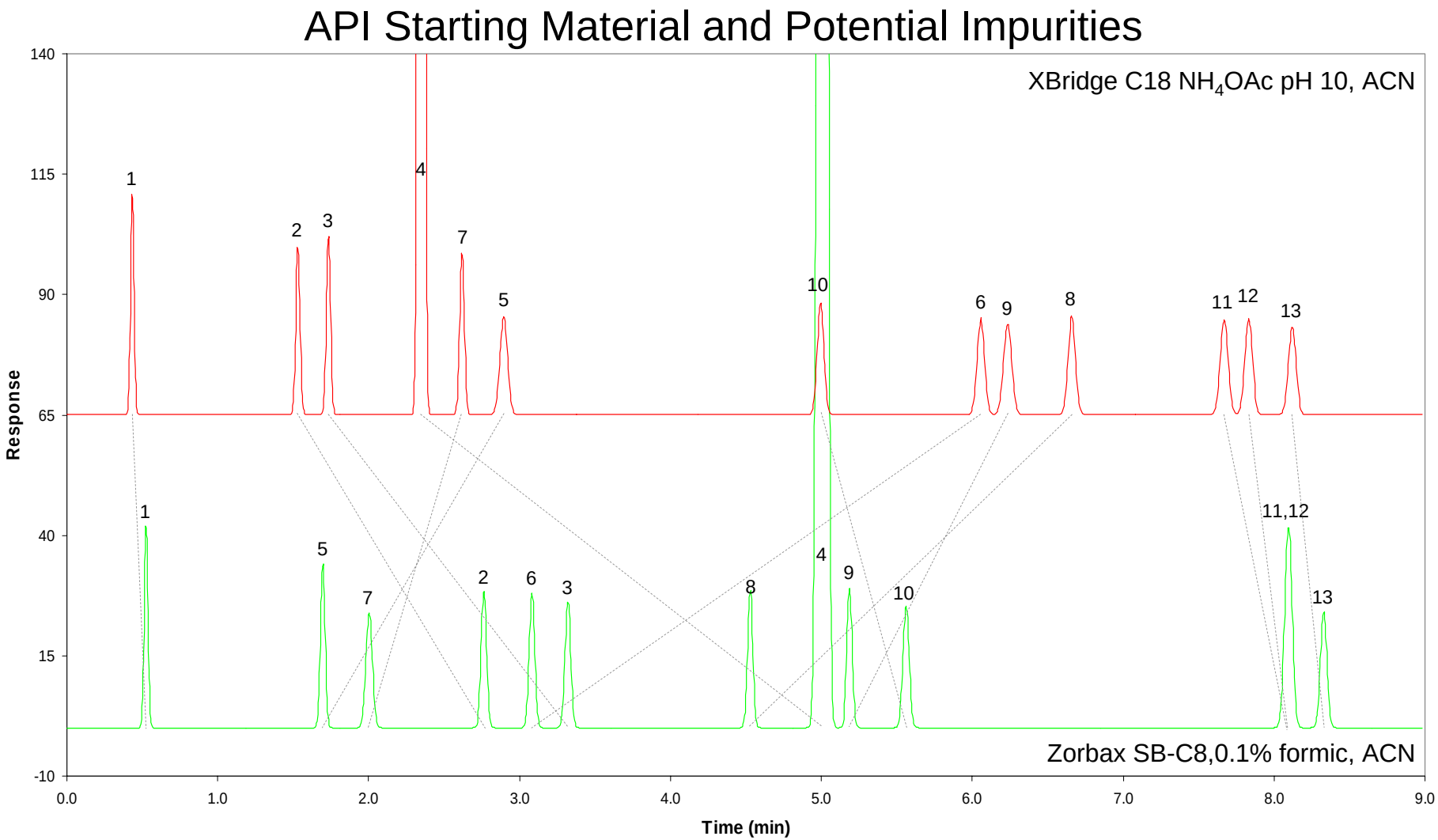
Experimental

- 8 sets of chromatographic conditions
- 2 run times (17.8, 46.4 min) per condition
- X individual compound and analyte mixture solution injections per run time (median=13)
- 1 single channel UV detector

Cost/Benefit

- total instrument time (8 x 1.1hr x 13) = 111hr
- total injections = 208
- data manipulation can be time consuming
- peak tracking is relatively simple and reliable

Typical Results



High Throughput Screening Protocol

Experimental

- 1 analyte mixture solution per project
- 8 sets of chromatographic conditions
- 1 gradient time (9.5min) per condition
- PDA and MS detection

Strategy

- detect and match peaks in MS signals
- detect and match peaks in UV signals
- reconcile component peak data across detector signals
- assign identity based on component spectra
- report retention time, peak width, and asymmetry of each component in each experiment

Method Development Suite for LC/MS

Signal Processing

- MS_MAP provides for very sensitive peak detection even when components are significantly overlapped with intelligent clustering of individual mass chromatograms and automated interpretation of component spectra
- UV_MAP reliably detects and matches components when they are partially separated

Additional Benefits

- cycle time for data analysis reduced from >1 week to <1 day
- software provides a platform for paperless flow of information from raw data files

Potential Failure Modes

Nature of component mixtures

- drug substance, starting materials, intermediates, synthetic impurities, and degradation products
- approximately 70% of projects require tracking structural isomers or stereo-isomers
- median number of components per project is 12

Sources of ambiguity

- detection sensitivity
- response changes with experimental conditions
- multiple components with similar spectra
- significant component overlap

Detection Sensitivity

MS Signal

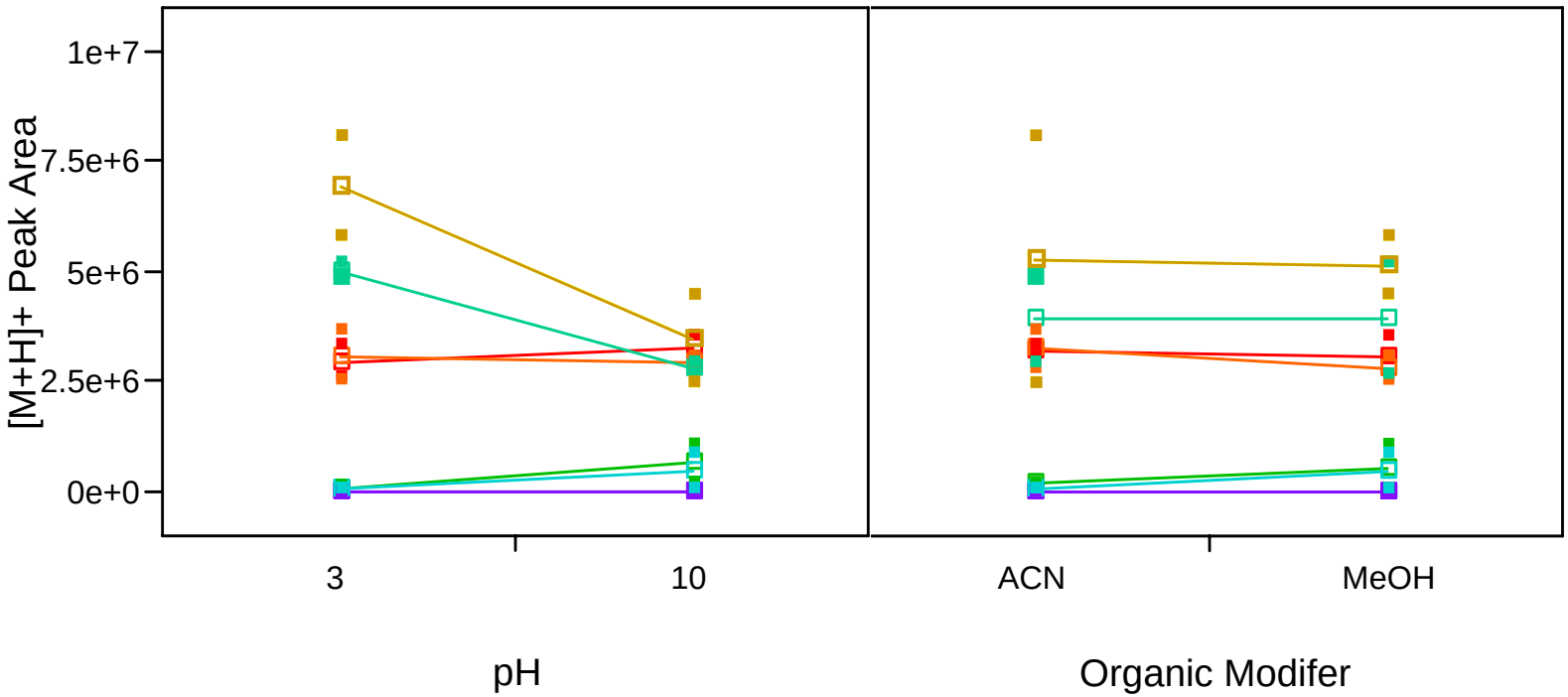
- ionization efficiencies achieved in an electrospray source can vary dramatically among components
- about 20% of analytes provide insufficient response using ESI

UV Signal

- >95% of compounds of interest have adequate UV response
- authentic samples of most components are available for use in analyte mixture preparation

MS Response Changes

- response trends with pH are compound specific



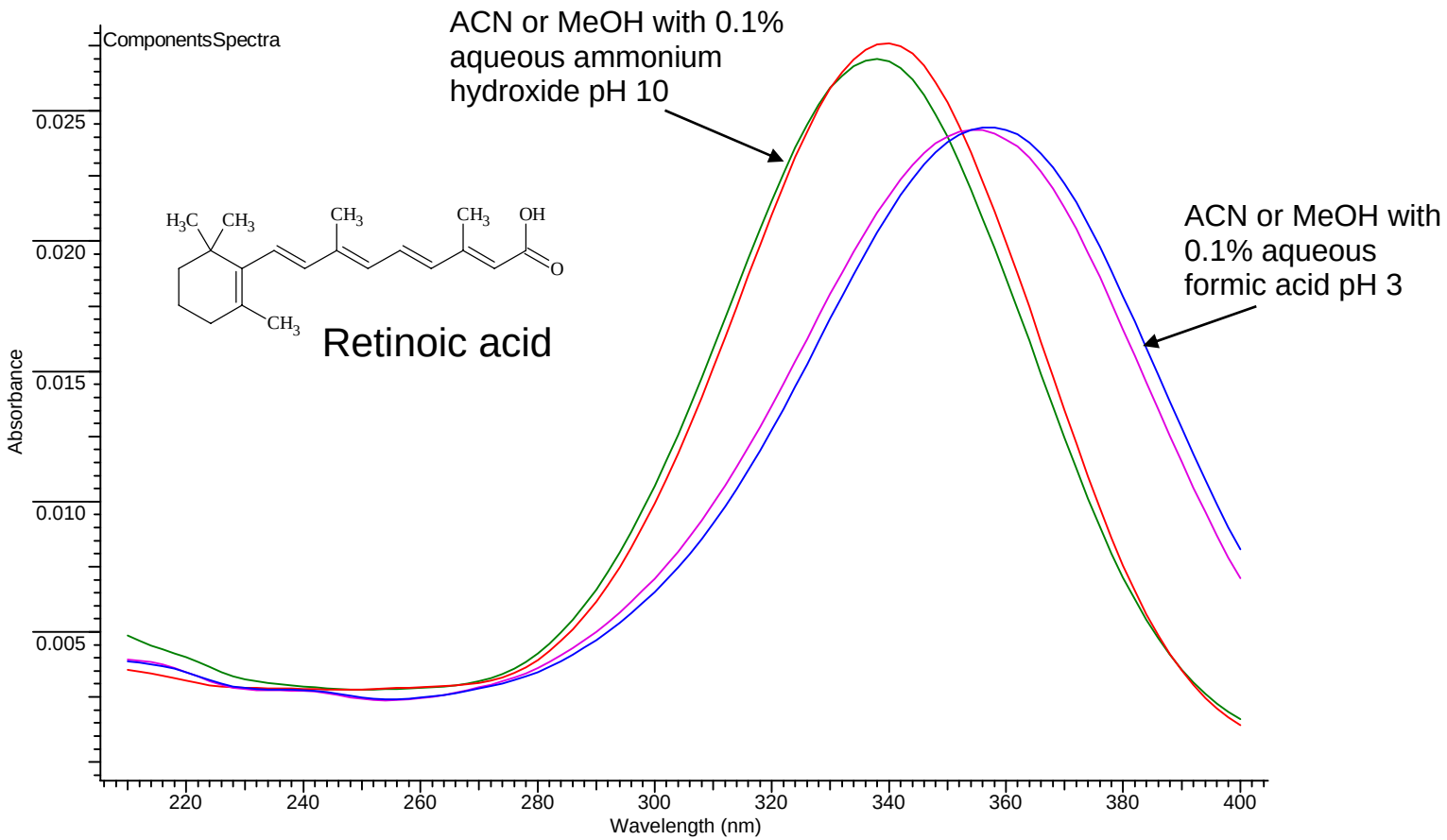
Chromatography:

Zorbax SB-C8 with 0.1% formic acid, ACN or MeOH with ESI+ detection

XBridge C18 with 0.1% NH_4OH, ACN or MeOH with ESI+ detection

UV Response Changes

- spectral shifts with pH are compound specific



MS Spectral Similarity

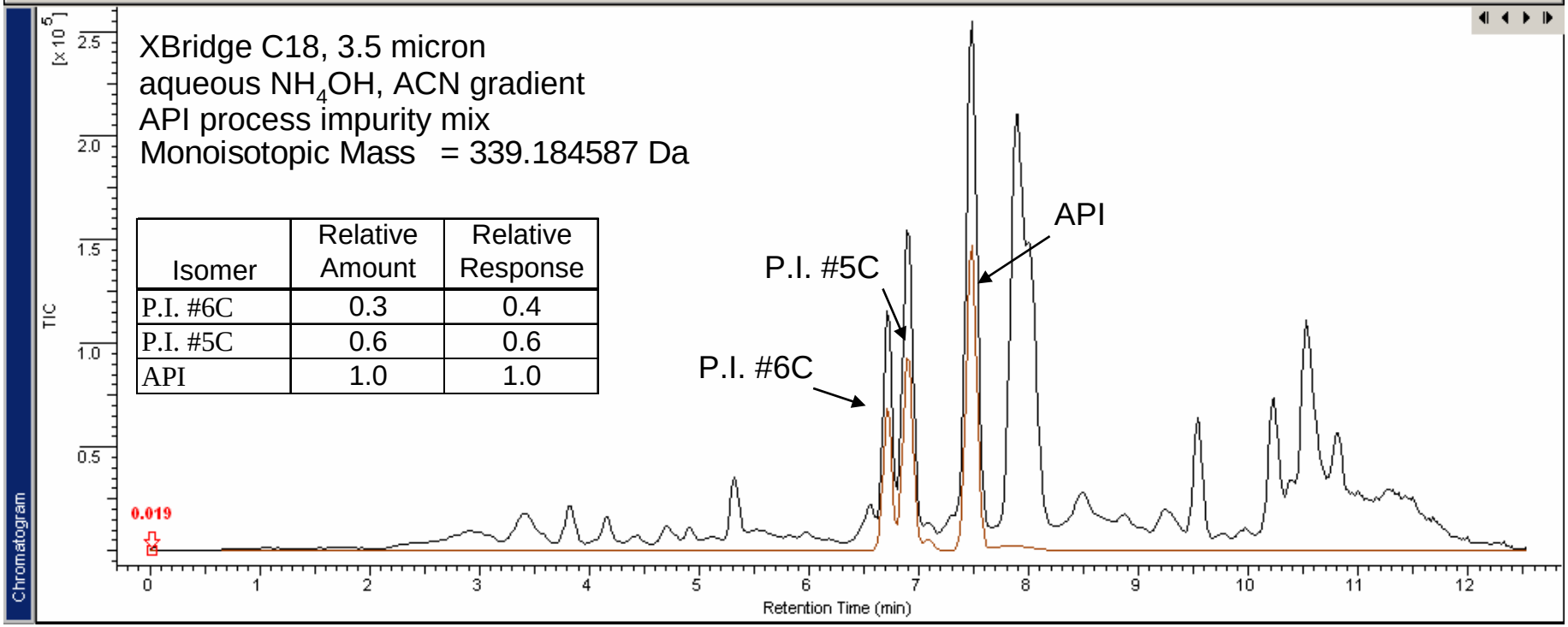
Table of Mass C...

	Number of maxima	m/z	Compon...	Notation	tR (min)	Area (counts)	Height (counts)	Color	Carbon	A+2	12C/13C
☐	1	338.099	76		8.031	13009.816	1741.699		13C		
☑	5	340.064	53	[M+H]+	6.895	570842.000	93050.422		12C	Br 0 Cl 0 S 0	Pass
☑	5	340.064	71	[M+H]+	7.848	51657.965	2384.017		12C	Br 0 Cl 0 S 0	Pass
☑	5	340.064	51	[M+H]+	6.711	370389.250	68765.672		12C	Br 0 Cl 0 S 1	Pass
☑	5	340.064	62	[M+H]+	7.481	918597.000	146973.313		12C	Br 0 Cl 0 S 0	Pass
☑	5	340.064	57	[M+H]+	7.078	29597.457	5562.550		12C	Br 0 Cl 0 S 0	Pass

msd1.MS msd1.MS msd1.MS

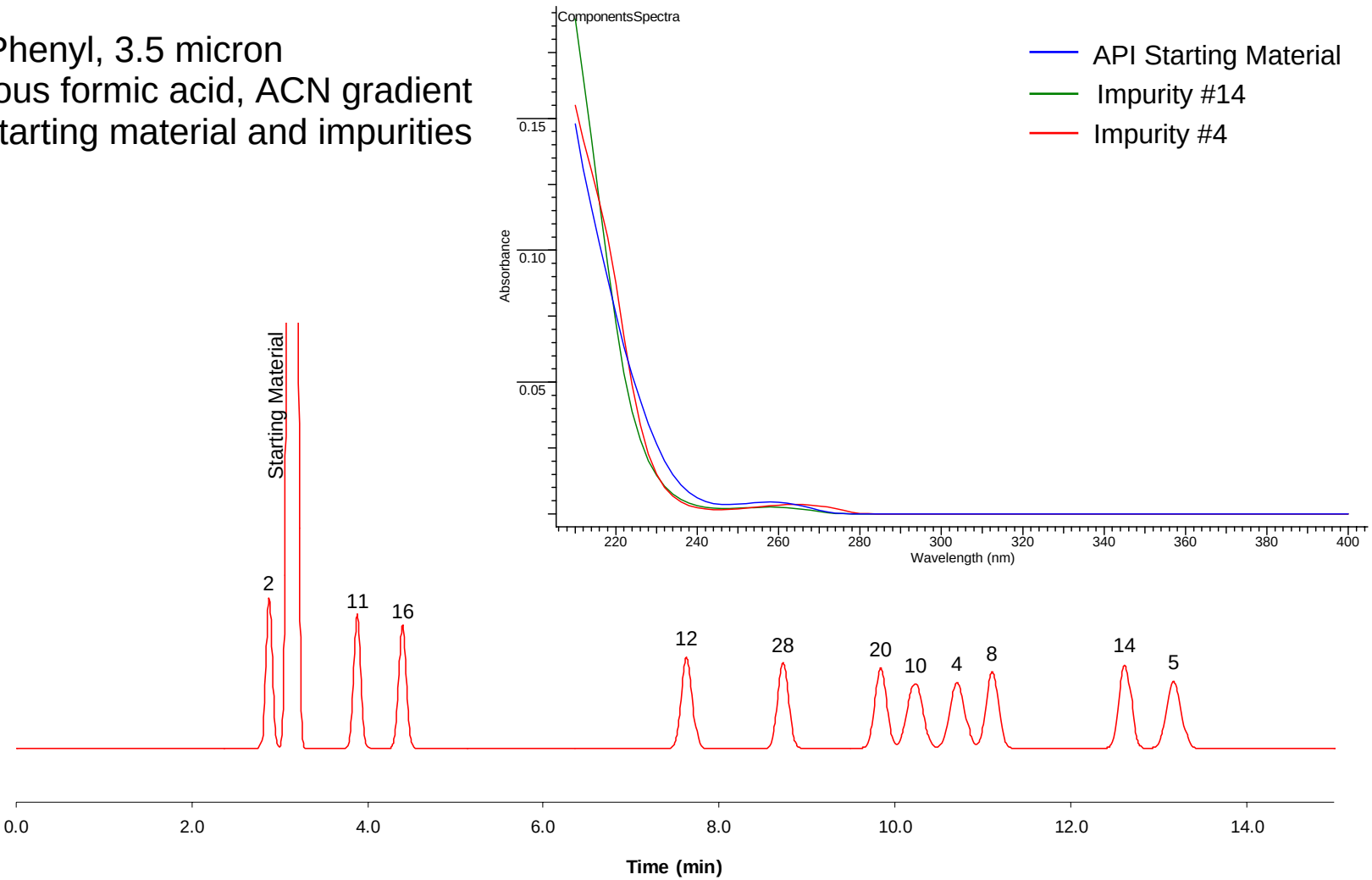
XBridge C18, 3.5 micron
aqueous NH_4OH, ACN gradient
API process impurity mix
Monoisotopic Mass = 339.184587 Da

Isomer	Relative Amount	Relative Response
P.I. #6C	0.3	0.4
P.I. #5C	0.6	0.6
API	1.0	1.0

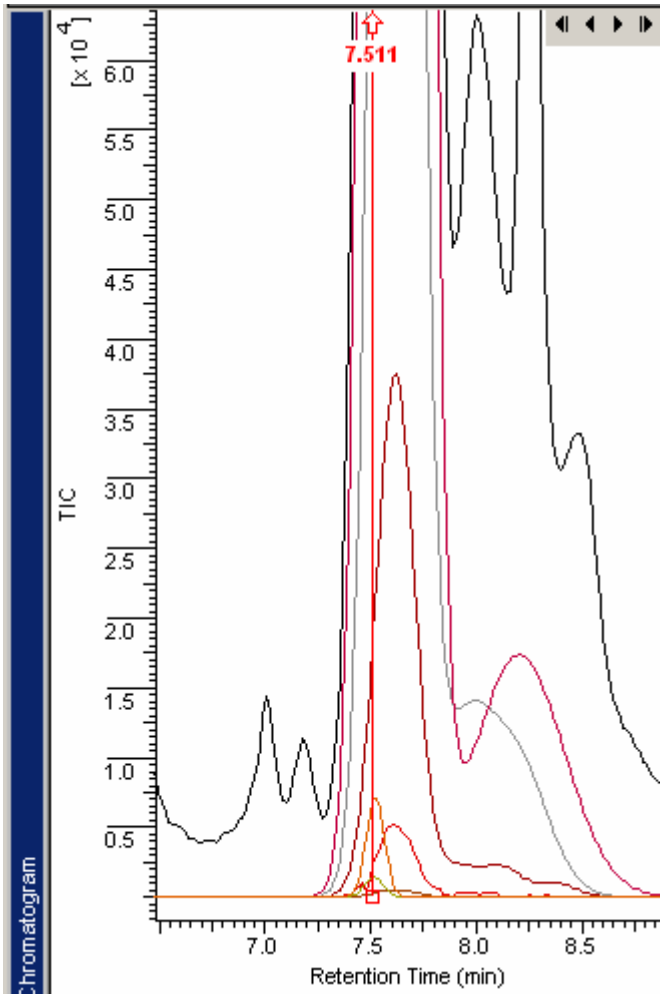


UV Spectral Similarity

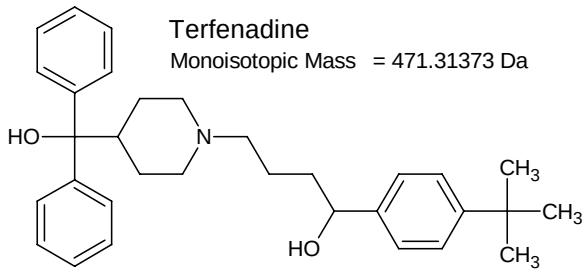
Ace Phenyl, 3.5 micron
aqueous formic acid, ACN gradient
API starting material and impurities



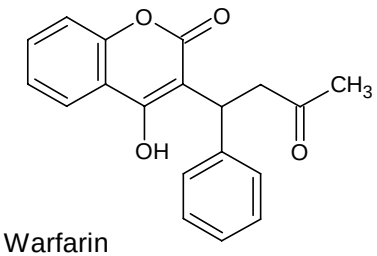
Peak Overlap in MS Signal



Zorbax SB-C8, 3.5 micron
0.1% formic acid, ACN gradient
response ratio = 200:1
critical pair R_s = 0.39



Terfenadine
Monoisotopic Mass = 471.31373 Da

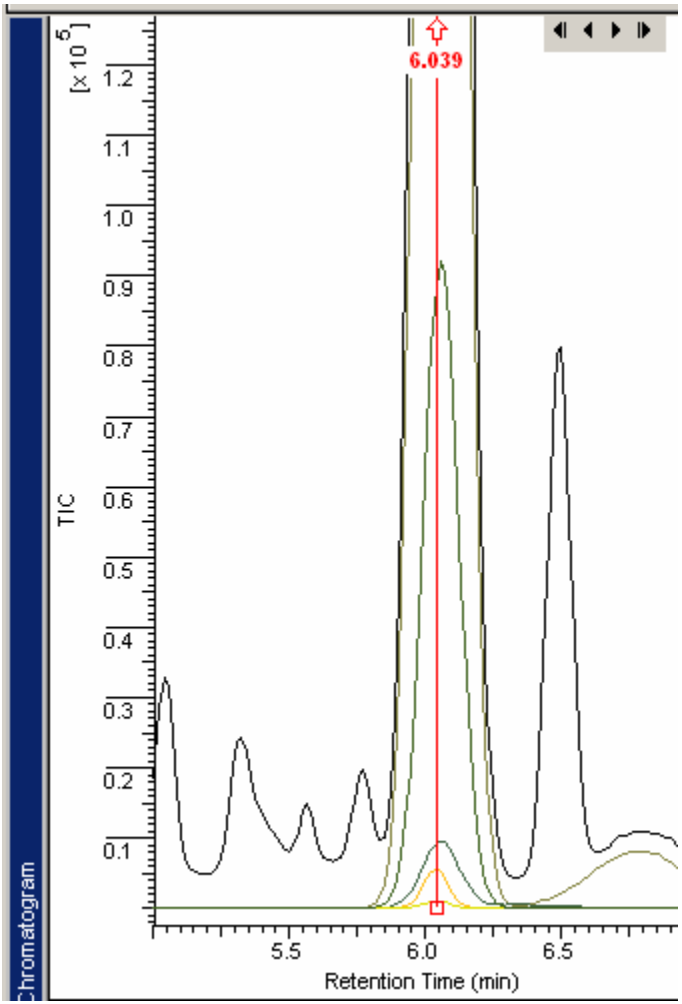


Warfarin
Monoisotopic Mass = 308.104859 Da

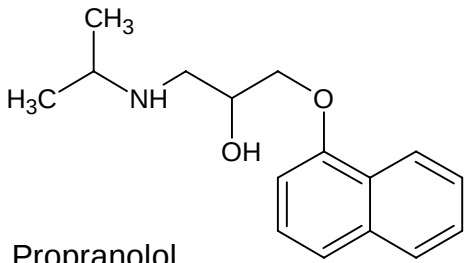
	m/z	Compon...	Notation	tR ...	Color	Carbon	12C/13C
☑	310.004	62		7.511		13C	
☑	309.060	62	[M+H]+	7.527		12C	Pass
☑	472.269	63	[M+H]+	7.622		12C	Pass
☑	476.007	63		7.622		12C+4	
☑	474.245	63		7.622		12C+2	
☑	473.148	63		7.622		13C	
☑	475.026	63		7.622		12C+3	

grams

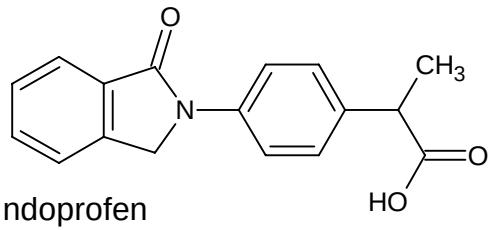
Peak Overlap in MS Signal



Zorbax SB-C8, 3.5 micron
0.1% formic acid, MeOH gradient
response ratio = 130:1
critical pair R_s = 0.07



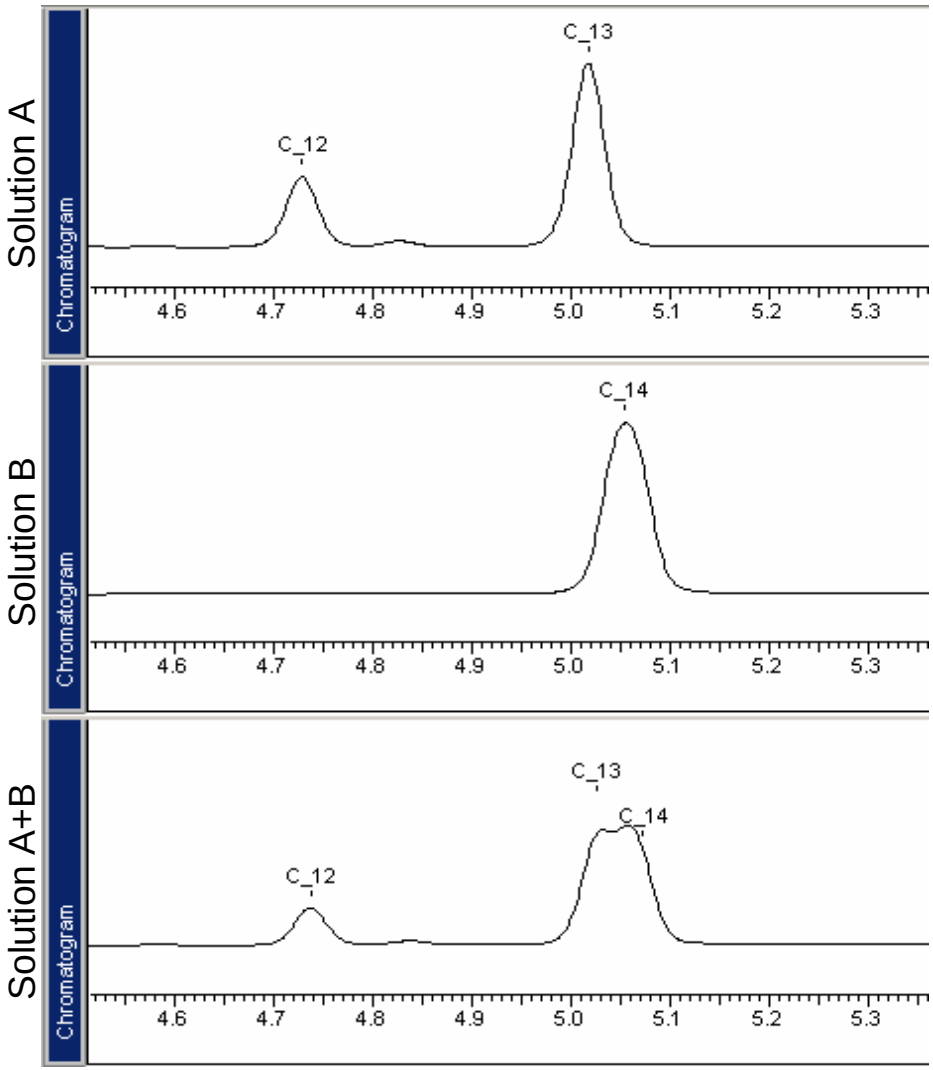
Propranolol
Monoisotopic Mass = 259.157229 Da



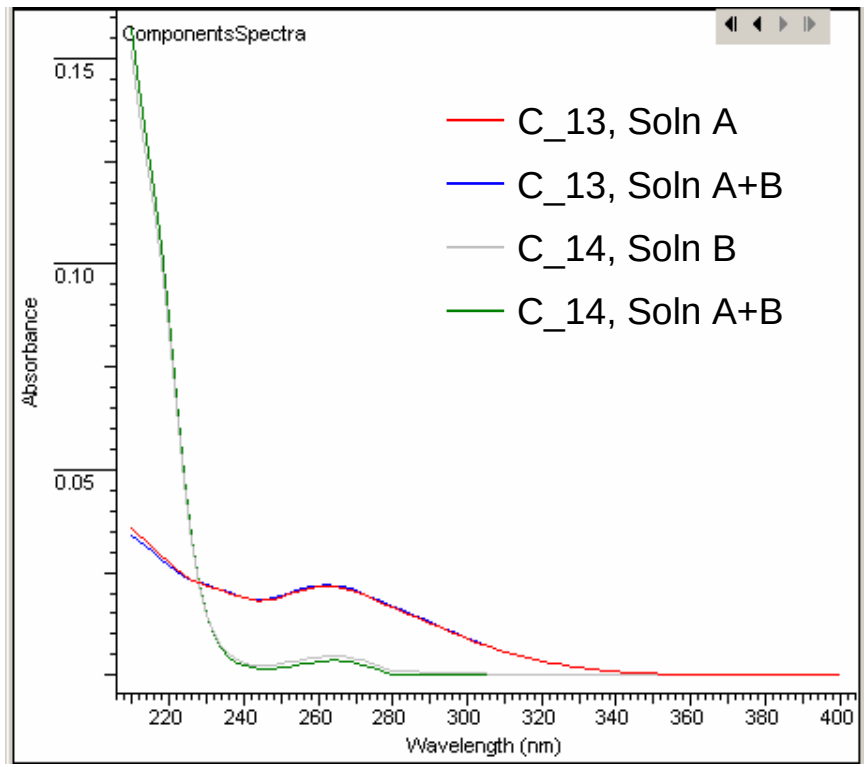
Indoprofen
Monoisotopic Mass = 281.105193 Da

	m/z	Compon...	Notation	tR (min)	Color	Carbon	12C/13C
☑	260.104	73	[M+H]+	6.055		12C	Pass
☑	261.094	73		6.055		13C	
☑	262.083	73		6.055		12C+2	
☑	282.058	72	[M+H]+	6.039		12C	Pass
☑	283.006	72		6.039		13C	

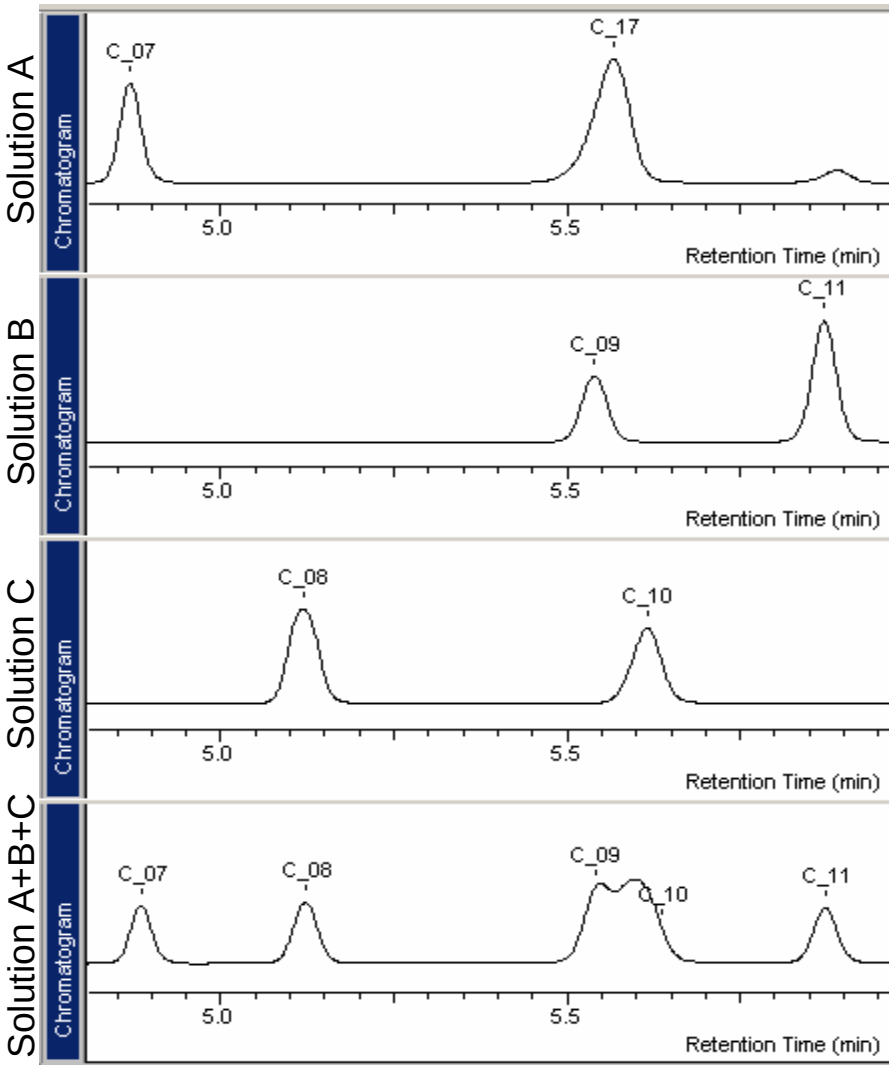
Peak Overlap in UV Signal



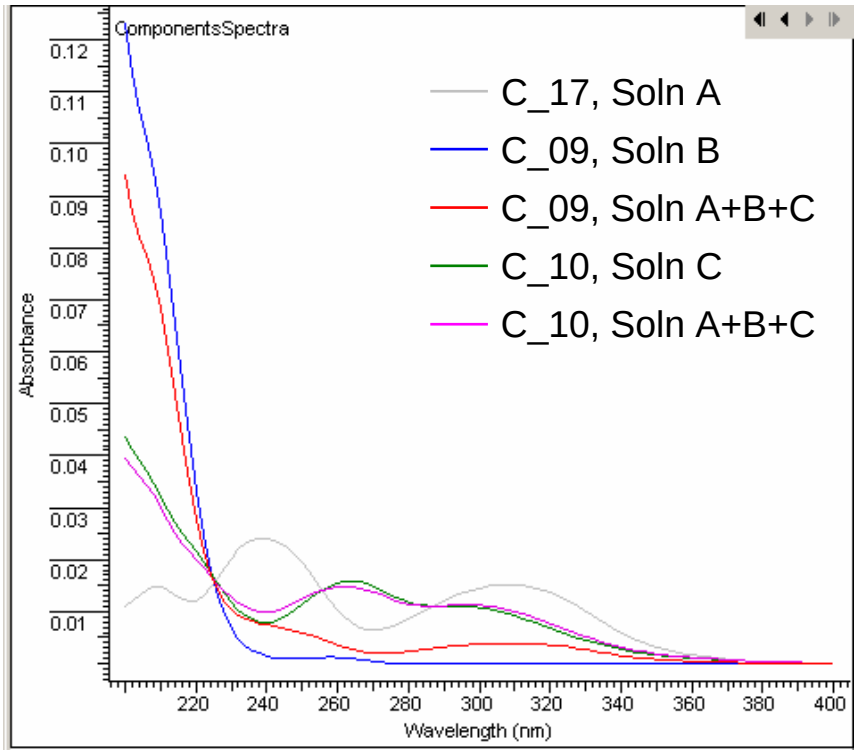
Atlantis dC18, 3 micron
0.1% formic acid, ACN gradient
relative amount = 1:1
critical pair R_s = 0.7



Peak Overlap in UV Signal



Zorbax SB-C8, 3.5 micron
0.1% formic acid, ACN gradient
relative amount: 1.8 : 1 : 2.1
critical pair R_s : C_09/C_17=0.4
C_17/C_10=0.5
C_09/C_10=1.0



Ensuring Component Identity

Lack of adequate MS detection sensitivity

- rely on UV signal for tracking components across experiments
- need additional data to identify components based on UV spectra

Multiple components with similar spectra

- MS and UV signals will not provide the required specificity
- control relative amounts of components in analyte mixture solution

Mitigation Plan

- collect MS and UV spectra at low and high pH for each component of interest individually
- use information to define relative amounts of components in analyte mixture solution
- cost: typically adds about 8 hours of instrument time and 2 hours of data processing time to screening protocol

Conclusions

- A high throughput approach to screening columns, aqueous buffers, and organic solvents can dramatically reduce cycle time
- ACD/Labs Method Development Suite for LC/MS is well-suited for processing MS and UV signals and managing project information
- Components of interest in pharmaceutical separations can have similar structures which lead to identical UV spectra and in the case of isomers, identical MS spectra
- Collecting MS and UV spectral and response data at low and high pH for each component of interest provides additional information needed to resolve ambiguities in assigning identity of components in complex mixtures

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