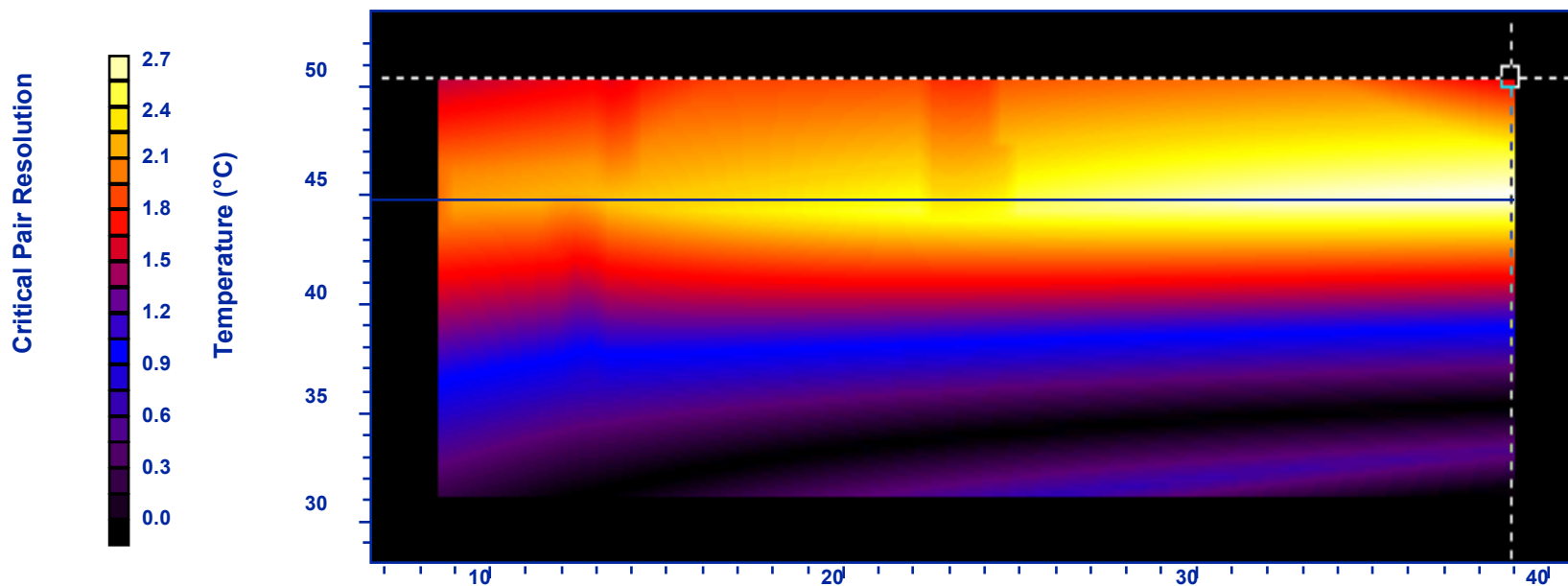




Approaches for HPLC Method Development and Optimization for Late Stage Pharmaceuticals



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CoSMoS 2005



Methods Development Strategies

- ◆ **Reviewing LC methods development strategies for assay and purity evaluation for late stage compounds**
- ◆ **Method Development for Full Development**
 - **Drivers**
 - **Strategies / Considerations**
 - **Inputs / Outputs**
- ◆ **Two approaches (Case 1 and 2)**
- ◆ **Other considerations**
- ◆ **Conclusions**





Method Development Considerations

Molecular Structure

- PKa
- Functional Groups
- Hydrophobicity
- Chirality
- Counterion

Synthetic Route

- (Especially last 2 steps)
- Process Intermediates
- Reaction By-Products
- Reagents, Catalysts, etc
- Isomers

Sample Availability

- API
- Stressed
- Precursors
- Isomers
- Rxn By-Products

Method Development: Some Factors to Consider

Methods Screen

- RPLC Starting Point
- Purity Assessment

Detection Methodology

- UV, EC, Conductivity, RI, LS, MS, Fluorescence,

Secondary Purity Evaluation Technique

- TLC, Normal Phase HPLC, CE, GC ...

Method Development Goals

In Full development we have a good understanding of the impurities and degradation products. However, during development the synthetic route of the API and the final dosage form may be modified or changed.

- ◆ **Our goal is to deliver HPLC methods for assay and purity**
- ◆ **The outcome would be suitable methods for transfer to the manufacturing site**





Method Development Process

- ◆ **Step-wise development progression with four key steps:**
 1. **Determine the Key Predictive Sample Set (KPSS)**
 2. **Perform a HPLC Screen**
 3. **Utilize DryLab chromatography software**
 4. **Perform Method Optimization Experiments**
- ◆ **Systematic and Practical**
- ◆ **Suitable for Pharmaceutical Compounds intended for advanced human clinical trials**
- ◆ ***Provides a suitable method development summary package***
- ◆ ***Well documented***



KPSS - Define the universe

- ◆ **Essential Peak set or Key Predictive Sample Set = Defined from the start**
- ◆ **Includes impurities from stability samples, degradation samples, representative drug substance lots, authentic or pure impurities and mother liquors**
- ◆ **Worksheet includes rationale, identification references, purposeful degradation information, stability indications**
- ◆ **Focuses method development on the most critical impurities**
 - **If KPSS is set *too wide* then it may be unnecessary complex possibly resulting in longer development time and needless separations**
 - **If KPSS is set *too narrow*, the result is an unsuitable method for analysis, as key components may not be separated and quantitated**
- ◆ **KPSS document can be easily updated based on changing needs**



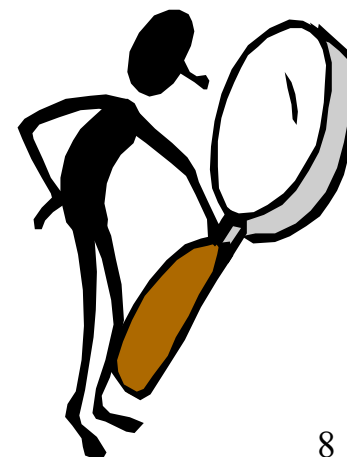
KPSS Worksheet - Case 1

Identity	Authentic Material Available?	Also in Purp Deg?	Grows on Stability?	Status	Rationale
PRI #1	Yes	No	No	Must	Commercial Route Precursor
PRI #2	Yes	No	No	Must	Commercial Route Precursor
PRI/Degradant	Yes	Yes	Yes	Must	Commercial Route Precursor
PRI#3	Yes	No	No	Must	Commercial Route Raw material
p-t sulfonic acid	Yes	No	No	Want	Previous Route counterion of isolated intermediate
PRI #4	Yes	No	No	Want	Commercial Route PRI
Diastereomers	Yes	No	No	Must	Potential PRI
Deg #1	No	Yes	Yes	Must	Known Base Degradant



Step 2 - HPLC Screen

- ◆ Determine effects of changing HPLC parameters on the separation of the Key Predictive Sample Set
- ◆ Important to recognize which parameters will provide the most potential for selectivity changes
- ◆ A well-planned HPLC screen can be written that effectively reduces the number of parameters investigated and the number of HPLC experiments
 - Consider everything you know about the chromatography and impurities (and this is quite a bit)





Screening Conditions

Mobile Phase Components

Mobile Phase	pH = 2	pH = 7
A	60:40 - 0.2% H ₃ PO ₄ buffer: Acetonitrile	60:40 - 0.2% H ₃ PO ₄ buffer pH=7 w/ NH ₄ OH:Acetonitrile
B	10:90 - 0.2% H ₃ PO ₄ buffer: Acetonitrile	30:70 - 0.2% H ₃ PO ₄ buffer pH=7 w/ NH ₄ OH:Acetonitrile

HPLC Conditions

Column Temp: 30C

Flow Rate: 1.0 mL/min

Detection: UV @210 nm and 252 nm
(Also PDA 200-400 nm)

Columns to Be Screened (4.6 x 150mm, 5µm)

1. Zorbax SB-Phenyl
2. Symmetry C8
3. Waters RP Shield C18

Gradient Parameters

20 minute gradient:

<u>Time</u>	<u>%A</u>	<u>%B</u>
Initial	100	0
20 min	0	100
30 min	0	100
35 min	100	0
40 min	100	0

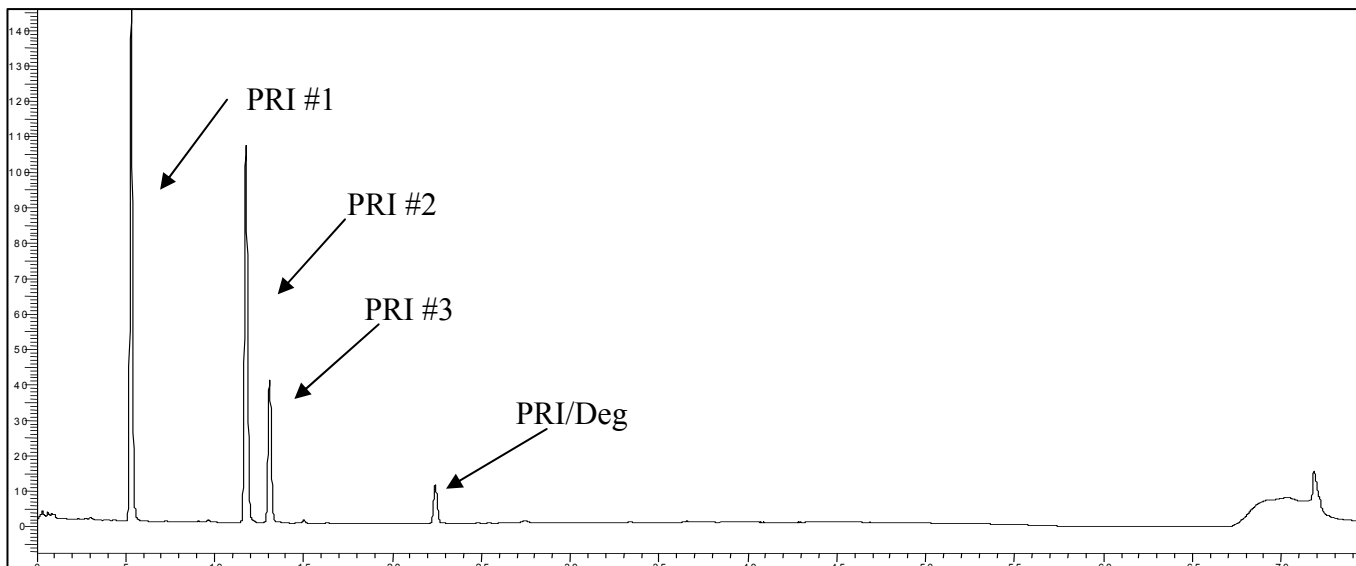
60 minute gradient:

<u>Time</u>	<u>%A</u>	<u>%B</u>
Initial	100	0
60min	0	100
65 min	0	100
70 min	100	0
75 min	100	0



Several ways to track peaks during method development

1. **Area % - preferred method if authentic standards are available**
2. **Multi-wavelength and UV spectrum data through a PDA detector**
3. **Mass Identification - LCMS**



An example of peak tracking by area % of the impurity

- ◆ **The HPLC Screen is the most time consuming (in laboratory) section of the process**
- ◆ **Creates large amounts of data sets**
- ◆ **Beneficial to organize data in tables which track retention time shifts between columns and gradients (done by hand!)**
- ◆ **Data in this format are easily imported into DryLab**

	<i>20 Minute Gradient</i>		<i>60 Minute Gradient</i>	
	rt	RRT	rt	RRT
p-t sulfonic acid	5.51	0.32	5.34	0.15
PRI#1	4.67	0.27	5.31	0.15
PRI#2	8.21	0.48	11.77	0.32
PRI#3	8.76	0.51	13.10	0.36
PRI/degradant	9.73	0.57	16.08	0.44
CP-550,654	12.70	0.74	22.42	0.62
Deg #1	16.22	0.94	33.49	0.91
PRI #4	16.45	0.96	34.20	0.94
Parent	17.27	1.00	36.63	1.00
Diastereomers	17.53	1.02	36.97	1.01
PRI #5	20.59	1.20	48.15	1.32

An example HPLC screen result table

HPLC Screen - Preliminary Assessment

- ◆ **An initial assessment of the screening data was performed to determine what screening parameters yielded the most selectivity for the KPSS**
 - **A pH effect was quickly ruled out as a development option – didn't offer anything for retaining polars**
 - **Waters Symmetry Shield RP18 exhibited the best selectivity**
 - **Polar embedded phase provided more retention of the polar impurities**
 - **Showed selectivity towards two process-related impurities not resolved under prior methodology**
- ◆ **Next step was to import data into DryLab**

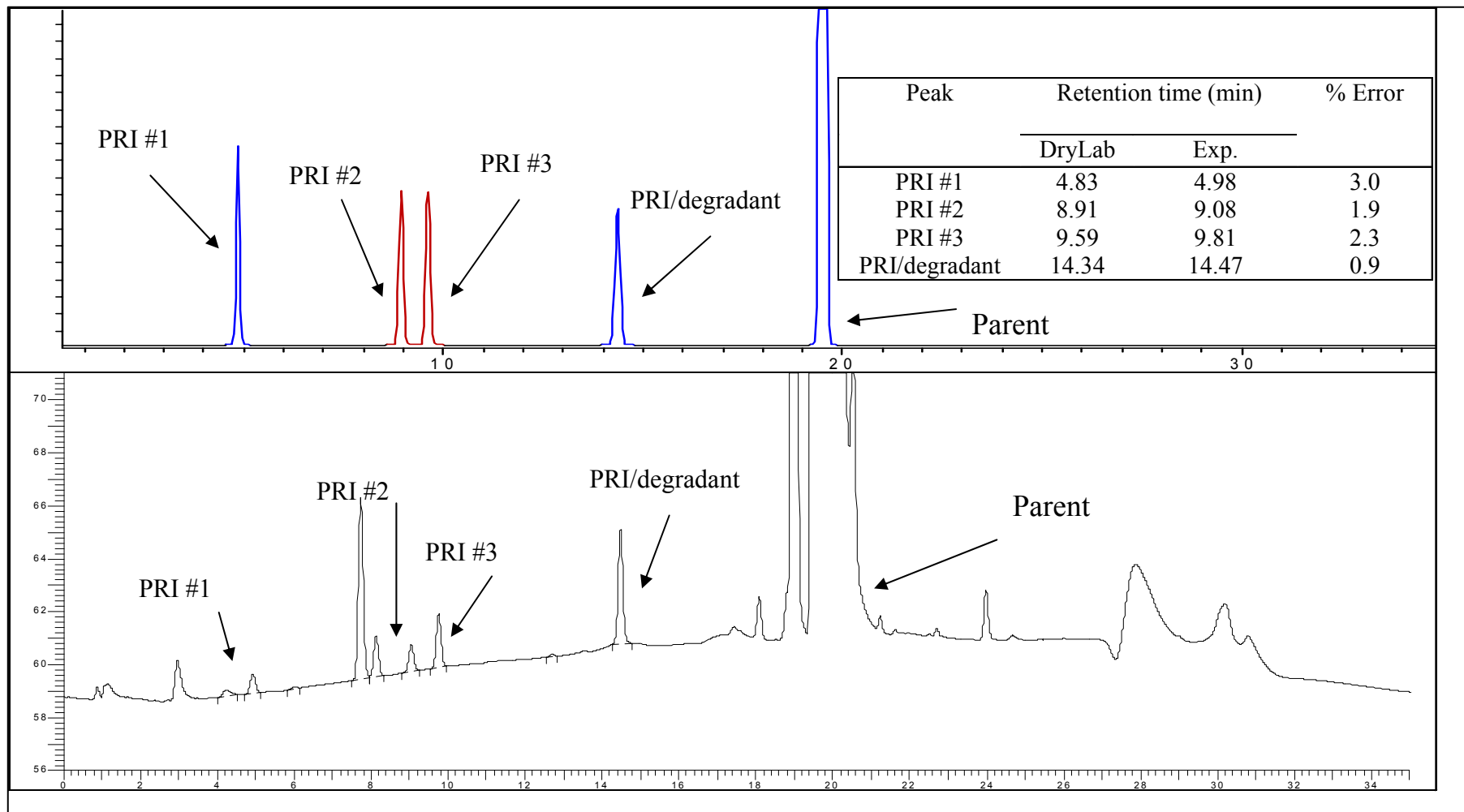


DryLab Analysis (Part 1)

- ◆ **DryLab software was utilized for modeling potential methods by predicting peak retention times and resolution**
- ◆ **Showed the diastereomers could not be separated with a resolution ≥ 2.0 using ACN as the organic modifier – further optimization would be needed for Method 2**
- ◆ **Method 1 was predicted without further gradient optimization. Further development included modifying the sample dissolving solvent, injection volume and sample load.**
- ◆ **DryLab was able to predict the retention times of these peaks within tenths of a minute of the actual retention time**



Accuracy of DryLab



Optimization Experiments

- ◆ Further Optimization was required for resolution of diastereomers in the assay and purity method for torcetrapib
- ◆ The HPLC screen showed that mobile phase pH and stationary phase did provide the needed selectivity
- ◆ Column temperature was not expected to affect the separation
- ◆ **Method screening technology**
 - **COMET screen demonstrated THF provide selectivity for diastereomers,**
- ◆ A modified HPLC Screen was re-run using THF as the organic modifier



Conclusions -Case 1

- ◆ **The process works**
 - **We utilized the strongest predictive capability from DRYLAB (gradients).**
- ◆ **Methods were validated based on ICH guidelines.**
- ◆ **Successful transfers to a manufacturing facility and a contract research organization demonstrated the ruggedness of the methods.**
- ◆ **In addition, a suitable method development package is available that includes:**
 - **KPSS documentation**
 - **HPLC screening protocols**
 - **DryLab predictions and results.**
- ◆ **This information will make it easier for analysts to interpret the method development data at a future date, and it provides a good foundation for future development work if required.**



DoE Modeling for Method Development - Case 2

- ◆ **Limited column screening data from previous work in early development using Luna phenyl-Hexyl column. This method could not be transferred to stability contract laboratories.**
- ◆ **The method was originally developed as a DS/DP method, KPSS included impurities for both.**
- ◆ **It was developed based on phenyl attributes of the API, understanding the pi interaction between stationary phase and drug substance.**
 - **Phenyl stationary phase provides greater retention of API and impurities.**



Old Method - Conditions

- ◆ Zorbax SB-Phenyl 5 μ m, 250x4.6mm ID
- ◆ Column Temp = 40°C
- ◆ Aqueous Buffer = 0.3% H₃PO₄ in Water
 - System 1 – Gradient from 5-20% acetonitrile
(65 minutes)
 - System 2 – Gradient from 25-50% acetonitrile
(55 minutes)
- ◆ Detection – 210nm
- ◆ Injection Volume – 30 μ L
- ◆ Flow Rate – 1.0 mL/min



Reasons for Change

- ◆ **At the request of the customer and with agreement of our laboratory, it was suggested to limit the number of chromatography conditions**
 - **Started with 2 purity gradient methods (2 hr total runtime)**
 - **Potency – 10 minute isocratic with TEA**
 - **Ideal to be able to run purity and potency with one method.**
- ◆ **Analysis time would be more desirable if total runtime could be <1 hour per sample.**





First Method Development

- ◆ **Initial column screen using long (45min) and short (25min) methanol gradients**
 - **Acidic pH (0.1% formic acid) - Devosil TMS, SB-CN, YMC ProPack C18, Intersil C8, Xterra- C18 MS, Hydrosphere C18, Xterra Phenyl, Devosil Phenyl, SB-Phenyl, Atlantis dC18**
 - **Neutral pH (15mM Ammonium Acetate pH=6.5) - Xterra- C18 MS, Hydrosphere C18, Xterra Phenyl, Devosil Phenyl, Atlantis dC18**
 - **Basic pH (15mM Ammonium Acetate pH=10.5)- Xterra- C18 MS, Xterra Phenyl**
- ◆ **Column Screen directed us to a neutral pH using the Atlantis dC18 column.**
- ◆ **Drylab predictions were used**

Road Block with Method Development

- ◆ **Before the validation began, it was observed that there was extreme column to column variation.**
 - **After final method optimization was completed, new Atlantis dC18 columns increased in Tailing factor >4.0 with NMT 200 injections. This was not seen in initial method development**
- **Decision was made to stop further work on the Atlantis dC18 column and take a look at ion pair chromatography or revisit original column screen.**
 - **Ion pair methodology was evaluated with OSA and DSA and three C18 column. Promising methodology was seen using OSA with Zorbax SB-C18.**



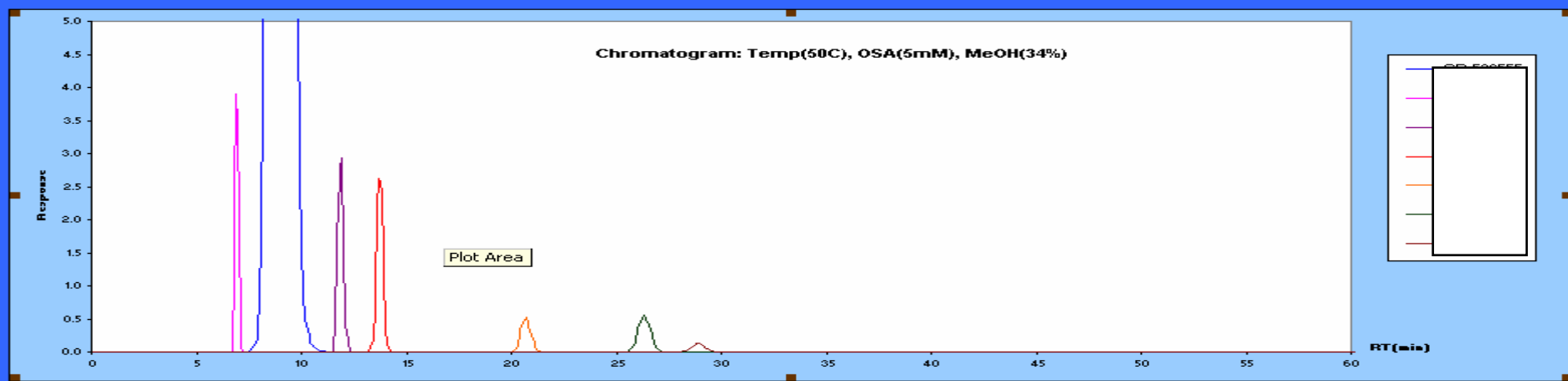
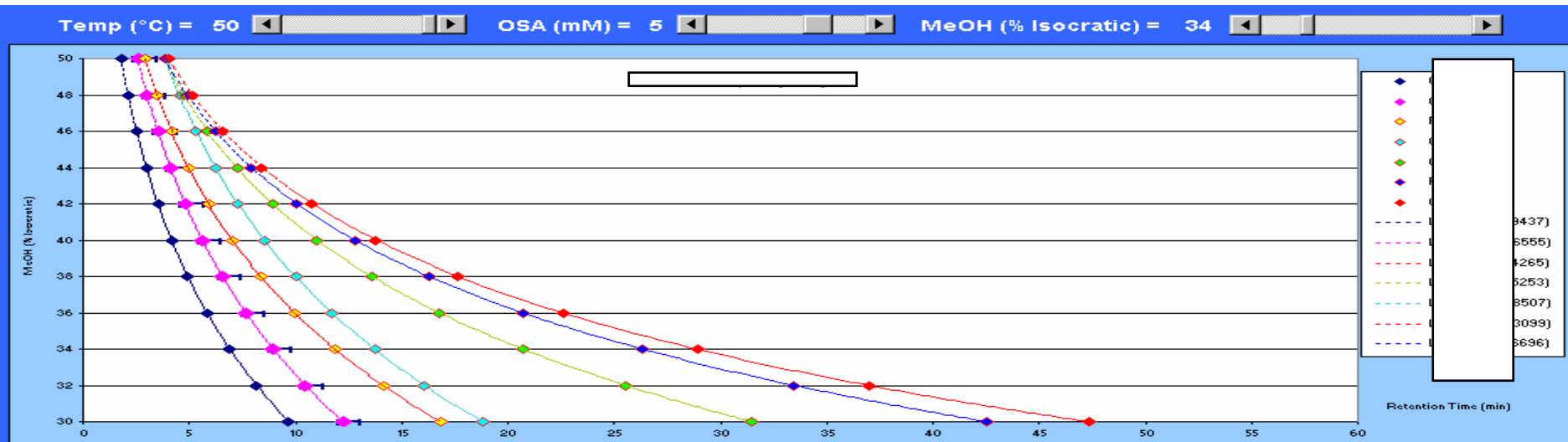


Based on DoE Modeling

- ◆ **A factorial design was used to evaluate the OSA and DSA method conditions.**
 - **Three C18 columns were used**
 - Nova-Pak C18, Symmetry C18 and Zorbax SB-C18
 - **Zorbax SB-C18 with OSA provided the best resolution between impurities on the initial screen.**
 - Factorial designs were set up to evaluate mM OSA concentration, Temperature, % H₃PO₄ and % Methanol.
 - **GAP**
 - No software to visualize results
 - No software to optimize this problem
 - **Examine in Design Expert (DOE software)**
 - Generate mathematical models
 - Builds in method robustness
 - **Represent data in Excel for the chromatographer**



Trajectory -Methanol verses time





Trajectory Plot Example



Microsoft Excel
Worksheet



Other Considerations (Temperature)



Temperature and HPLC Performance

- Temperature control used to stabilize system
- Temperature important in ion-pairing chromatography
- Temperature can be used to improve column selectivity (especially for isomers)
- High temperature and temperature gradients have recently received attention in LC separations
- **In late stage method development we have a good understanding of the properties of compounds in KPSS**

Temperature Study

- ◆ 8 compounds with varying polarity were evaluated by HPLC @ 10, 30, and 60 °C
- ◆ The compounds were then evaluated by NMR @ 10, 30, and 50 °C



Structures of Test Compounds



N-methylphenethylamine



N,N-Dimethylbenzylamine



2-phenylacetamide



1-phenylbutane



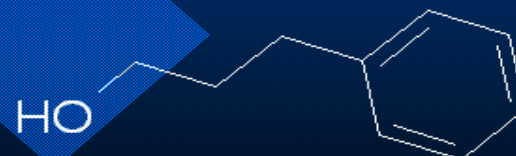
2-phenylethyl methyl ether



Phenylacetic acid



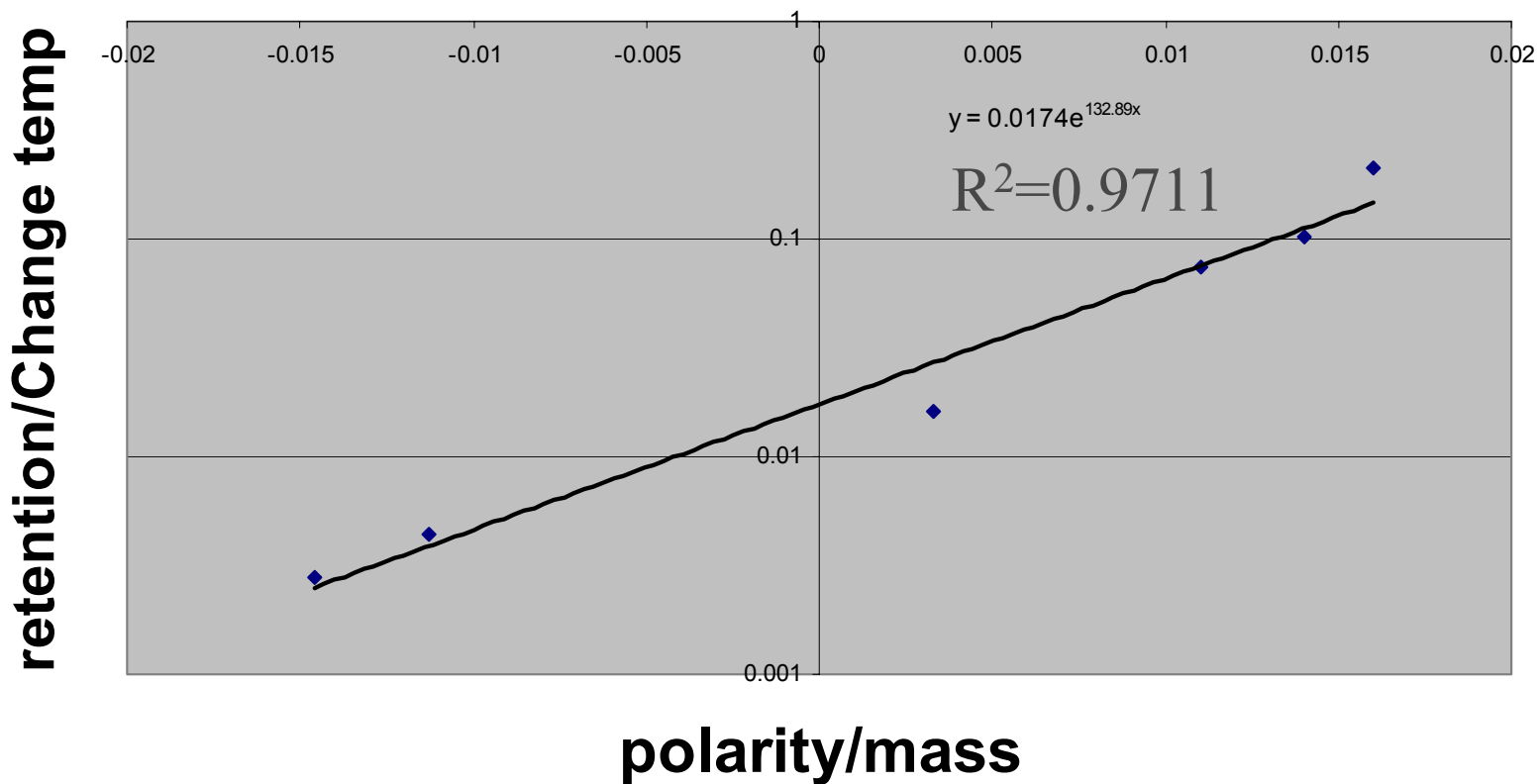
Hydrocinnamaldehyde



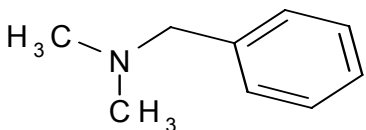
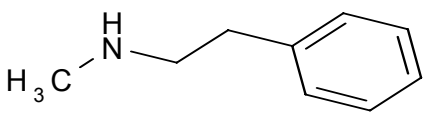
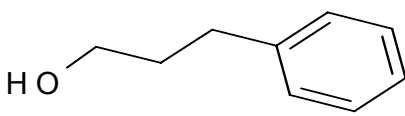
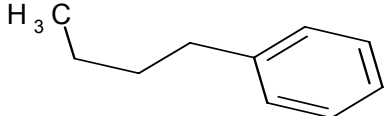
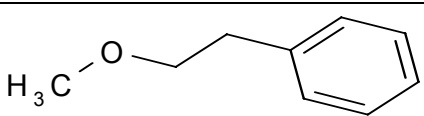
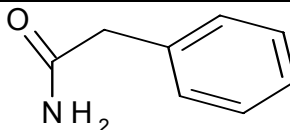
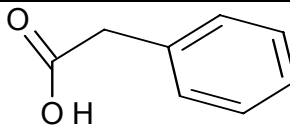
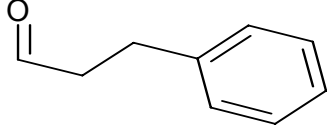
3-phenyl-1-propanol



Relation Between Retention Time and Change in Temperature (35°C-60°C)



NMR Proton shift versus Temperature

Structure	Run	H1	H2	H3	H4	H5	H6
 <chem>CN(C)Cc1ccccc1</chem>	10	7.31	3.38	2.16	2.15		
	30	7.31	3.40	2.18	2.18		
	50	7.32	3.41	2.19	2.19		
 <chem>CNCc1ccccc1</chem>	10	7.23	2.73	2.73	2.32	1.76	
	30	7.25	2.76	2.76	2.36	1.43	
	50	7.24	2.76	2.76	2.36	1.59	
 <chem>OCCCCc1ccccc1</chem>	10	7.23	2.65	1.78	3.51	2.87	2.40
	30	7.23	2.67	1.80	3.53	2.71	2.28
	50	7.24	2.68	1.82	3.54	2.57	2.17
 <chem>CCCCc1ccccc1</chem>	10	7.21	2.60	1.57	1.34	0.92	
	30	7.21	2.61	1.59	1.35	0.93	
	50	7.22	2.63	1.61	1.37	0.94	
 <chem>COCc1ccccc1</chem>	10	7.26	2.85	3.57	3.29		
	30	7.26	2.86	3.59	3.30		
	50	7.27	2.86	3.60	3.31		
 <chem>NC(=O)Cc1ccccc1</chem>	10	7.28	3.45	6.20	5.69		
	30	7.29	3.46	6.12	5.62		
	50	7.30	3.47	6.05	5.57		
 <chem>OC(=O)Cc1ccccc1</chem>	10	7.27	3.60	2.30			
	30	7.28	3.61	2.21			
	50	7.29	3.61	2.13			
 <chem>O=CCCC1=CC=CC=C1</chem>	10	7.23	2.90	2.75	9.73		
	30	7.24	2.92	2.75	9.74		
	50	7.24	2.93	2.75	9.75		



Summary for Temperature Study

- **A linear relationship between a substance's retention time and its polarity/mass over a temperature range was noted**
- **Temperature can be a useful property for increasing selectivity of for polar compounds**
- **NMR proton shift was noted for polar compounds with changing temperature**

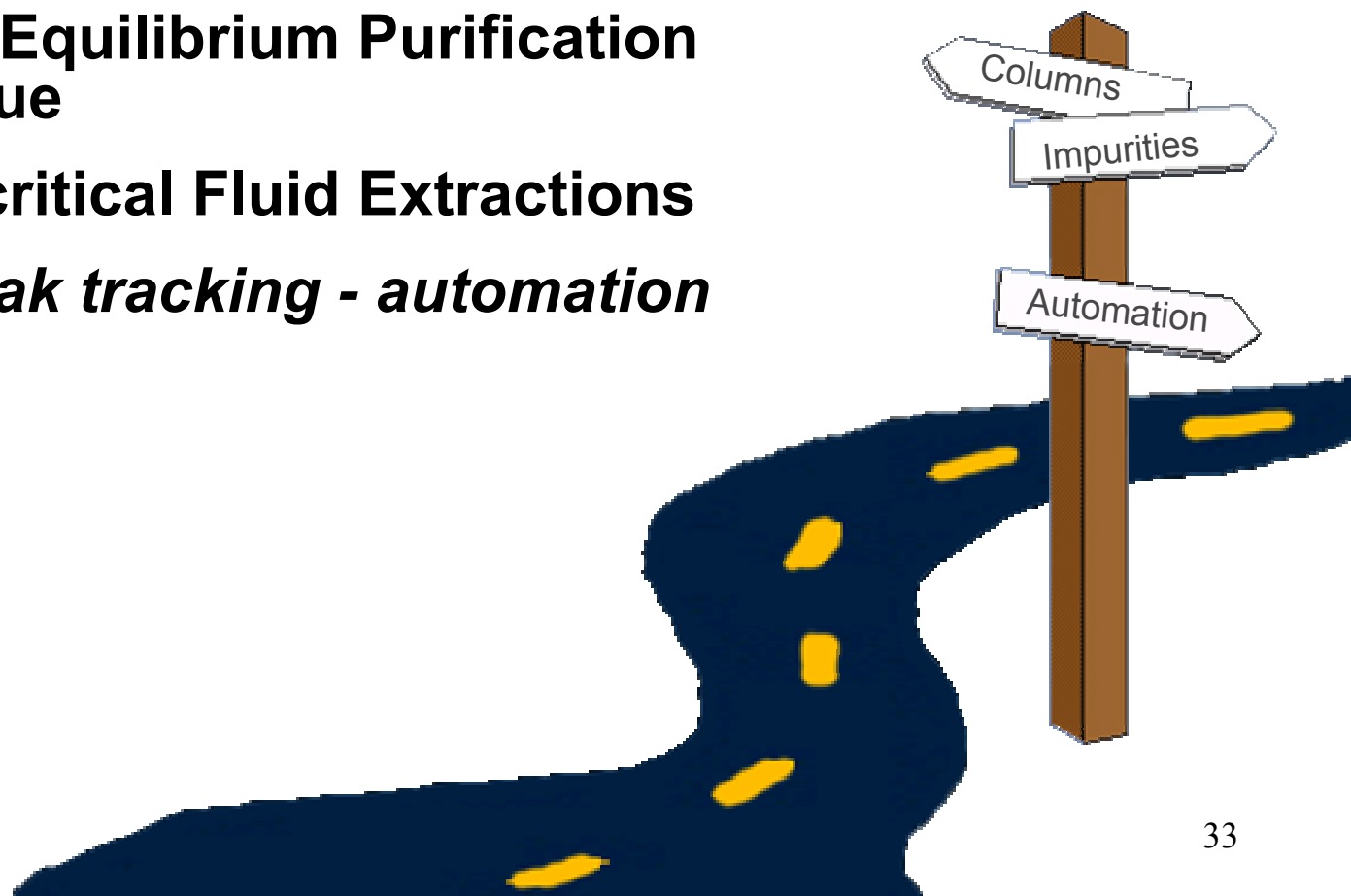


Methods Development Conclusions

- ◆ **Strategies are in place for methods development**
- ◆ **Extensive use of degradation studies**
 - Kinetics, ID of impurities, mass balance, documentation.
- ◆ **Increased levels of documentation (KPSS, protocols etc.)**
- ◆ **Rely on DRYLAB and then when it fails in-house DOEs.**
- ◆ **To increase selectivity for polar components temperature can be a useful parameter**



- ◆ **Improve Peak Tracking Process**
 - ◆ **Impurity Isolation**
 - ◆ **Phase Equilibrium Purification Technique**
 - ◆ **Supercritical Fluid Extractions**
 - ◆ ***LC-MS peak tracking - automation***



Acknowledgements

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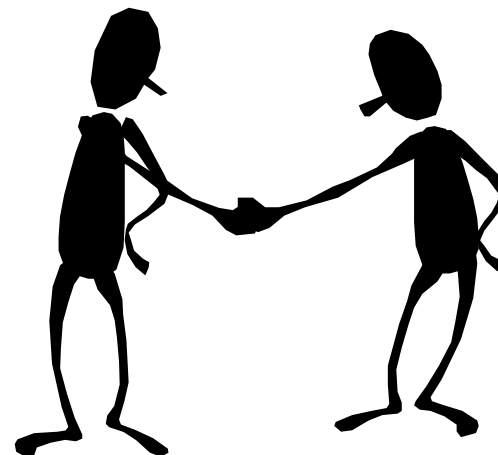
John Salisbury

DoE Modeling

Tim Graul

Mark Hardink

Jim Morgado





Additional Slides



Prediction Strategies

★ Single Isocratic

- ★ Critical Pair Resolution > 2.0 (Main band > 3.0 either side)
- ★ k' earliest eluter > 1.5
- ★ Run Time < 60 minutes

★ Single Gradient

- ★ Critical Pair Resolution > 2.0 (Main band > 3.0 either side)
- ★ R_t earliest eluter > 5.0 minutes
- ★ Gradient Slope < 1.5% Organic/min
- ★ Starting %Organic > 5.0%
- ★ Gradient Time < 50 minutes

★ Gradient Purity & Quick Potency Combination

- ★ Above Gradient criteria met
- ★ Isocratic Potency Method with Run Time < 10 minutes

★ Two Isocratic (More Polar/Less Polar)

- ★ Above Single Isocratic Criteria Met
- ★ Run Time < 30 minutes desirable
- ★ Marker Peak usually main band (present & resolved in both methods)
- ★ Marker Peak - latest eluter in MP method and earliest eluter in LP method