



# Trace Analysis of Impurities with Potential Genotoxicity in Pharmaceutical Samples

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# Big effort in monitoring impurities

- The majority of impurities may be monitored by the conventional LC/UV purity method according to ICH guidelines for reporting, setting specifications and identification.
- Some impurities may require more sensitive methods, including ones with suspected potential for genotoxicity.
  - Residual catalysts (such as TPP/TPPO)
  - Sulfonic or phosphonic esters (MSA, BSA, p-TSA, etc.)
  - Alkyl halides (e.g. synthetic precursors)
  - Compounds with other structural alerts
    - Epoxides, aldehydes, nitro, azo, etc.
  - Leachables & Extractables





# The pressure is on! – Challenges

- Development of sensitive analytical methods for monitoring and process understanding
- How low should we go?
  - Not specific/clear guidelines from ICH
  - Big strain on analytical labs to develop high sensitivity methods early in the development process
    - Complicated by changing processes/formulations





# European Medicines Agency (EMA)

- **Published guideline on limits for genotoxic impurities**
  - [www.emea.eu.int](http://www.emea.eu.int)
  - In effect: January 2007 for marketing applications
  - Emphasizes need for understanding chemistry
  - Use of state-of-the-art analytical techniques
  - Two classes based on threshold mechanism
    - Sufficient exp evidence of threshold – ICH Q3C steps
    - No sufficient evidence – TTC Approach
- **TTC Approach – Risk Based Approach**
  - Conservative – 1.5 µg/person/day lifelong exposure baseline
  - Extreme LOQ requirements on methods
  - Should be re-evaluated as new data is available
  - Allows for proposals based on dose, exposure, risk/benefits





# Further Publications

- **PhRMA proposal – “Staged” TTC Approach**
  - Regulatory Toxicology and Pharmacology 44(2006) 198-211
  - Authors from numerous pharmaceutical companies
  - “ALARP” Principle
  - Takes into account: phase of development, duration of exposure and feasibility of sensitive analytical methods besides safety and risk/benefits
  - Divides potential impurities into classes (1 through 5)
  - Proposes monitoring at levels between 1.5 and 120  $\mu\text{g}/\text{day}$ 
    - Alleviates burden of early analytical methods





# Methods

Methods should be reliable, fast and simple for the support of release activities and in-process control

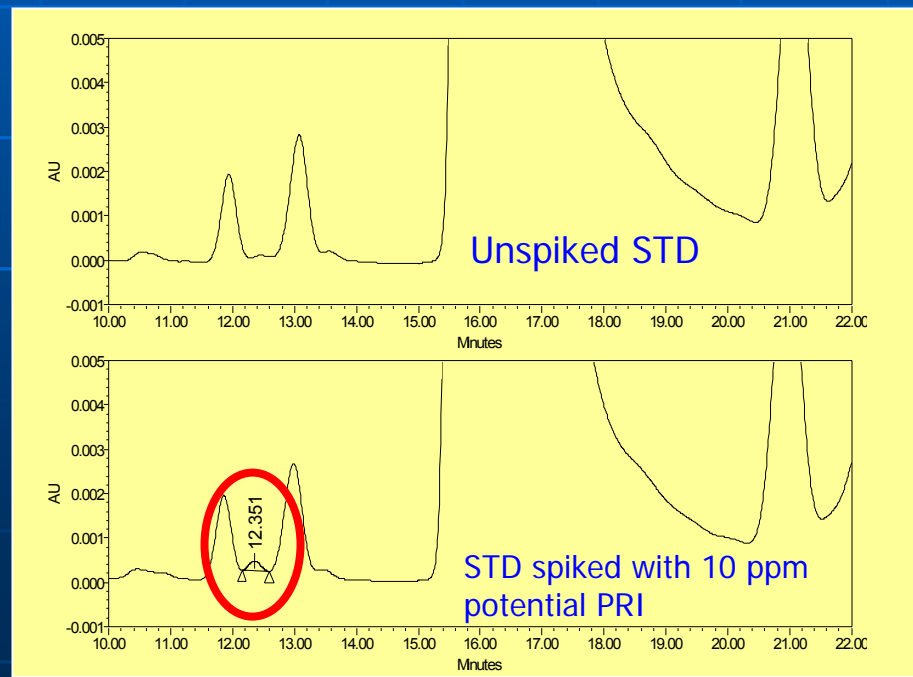
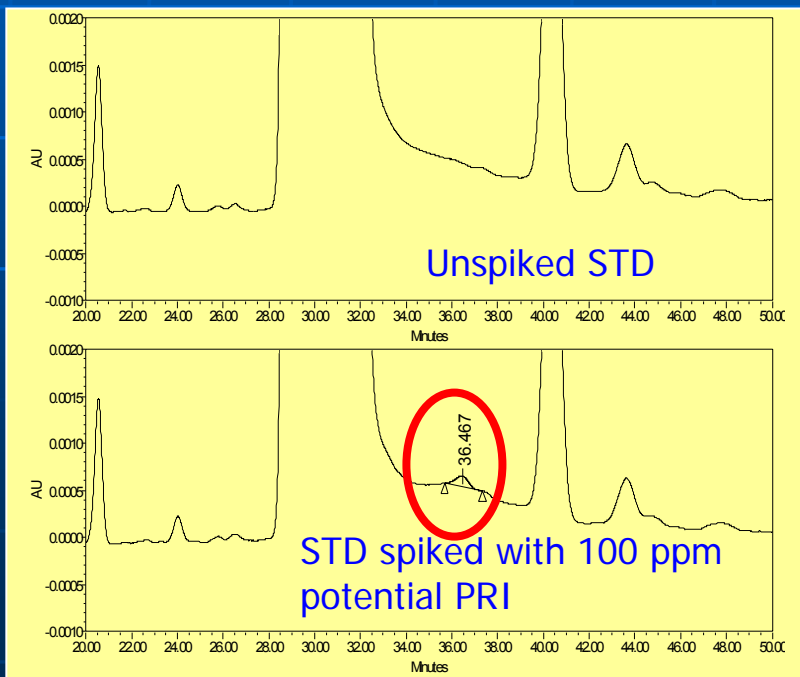
- **GC (headspace or direct injection)**
  - Modified residual solvents method
- **LC with conventional UV detection**
  - No additional training, minimal method development, familiar
  - High-temperature LC benefits
- **LC with MS-MS detection (or alternate ECD?)**
  - Sensitive, almost no sample preparation
- **Use of sample enrichment techniques**
  - Fast, sensitive, simple





# LC/UV Methods

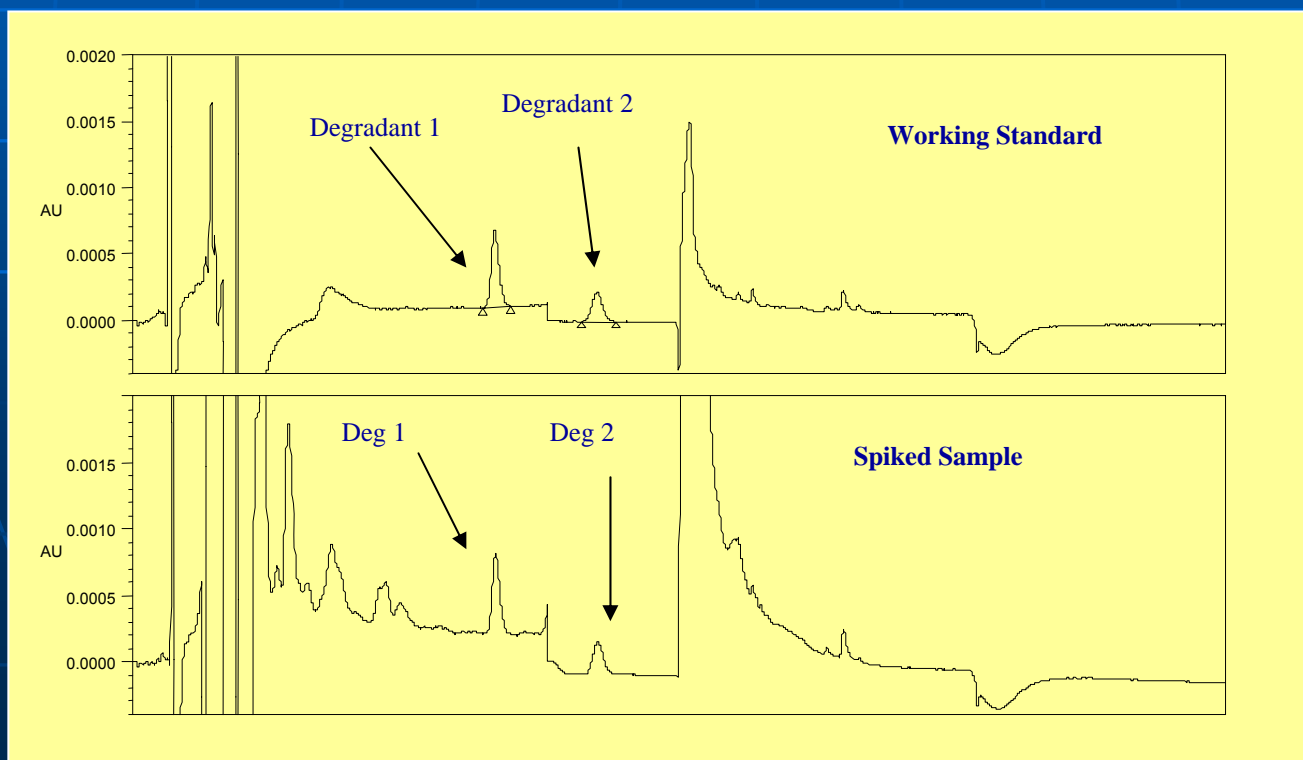
- Some necessitate alternate method (smaller/more polar)
- Very small LOQ relative to the API
  - Overloading of columns, tailing, interferences, etc.
- Need strong chromophore for sensitive detection
- Tweaking - HT-LC, wavelength change





# LC/UV – “Success” Story

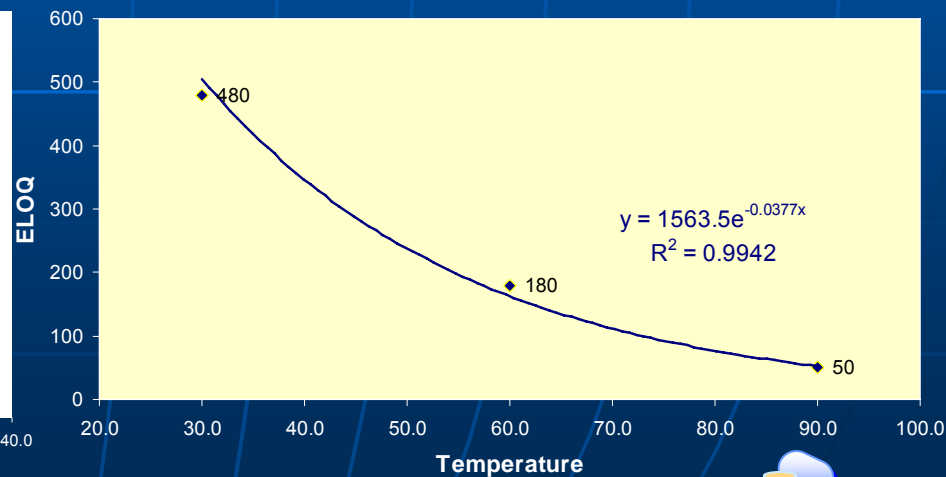
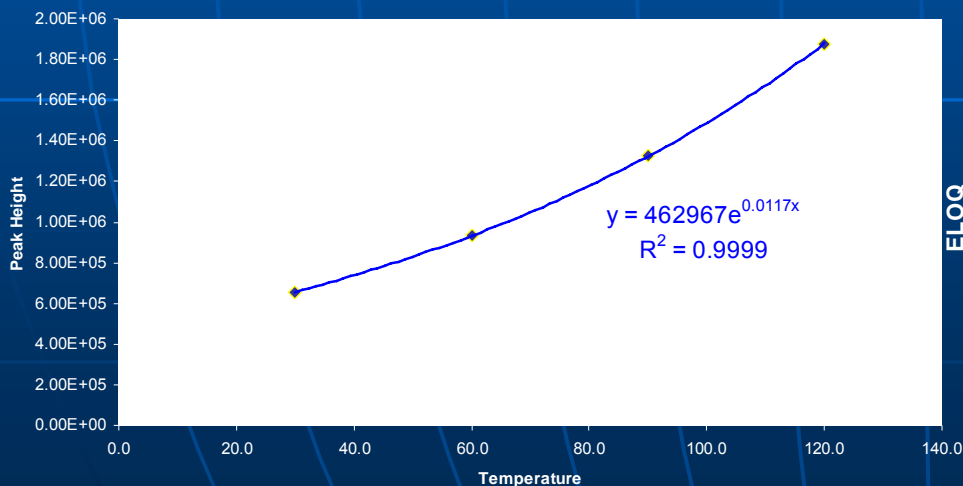
- Successful for monitoring desired degradation products (primary amines) during development
  - Dual wavelength detector (PDA not acceptable)
  - Wavelength switch (or dual  $\lambda$ ) for increased sensitivity
  - Optimized sample preparation / injection
  - LOQ of 80 ppm for 0.1 mg tablet – consistent “staged TTC”





# High – Temperature LC

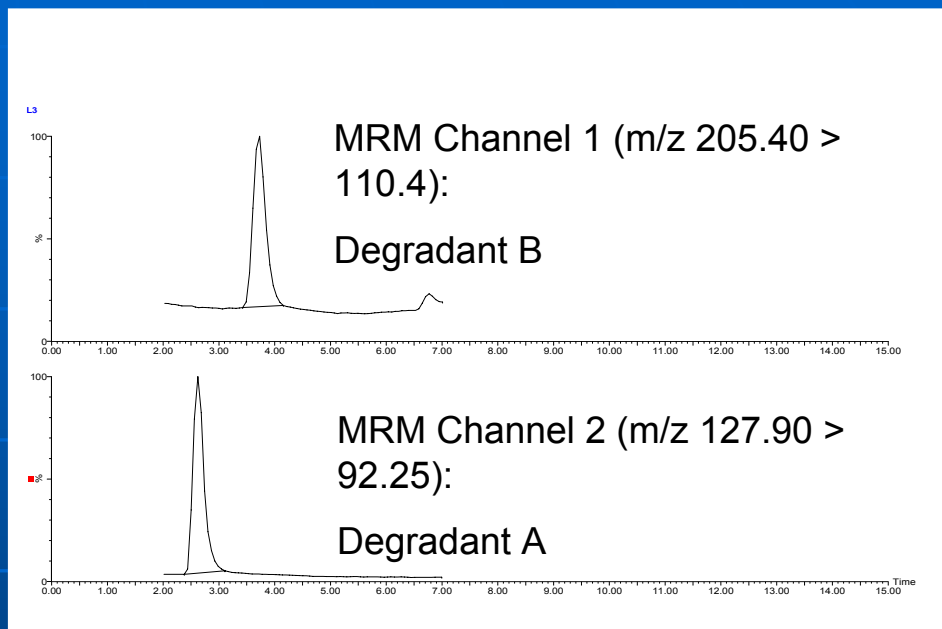
- Selerity Technologies - Polaratherm Series 9000
- Any chromatographic instrument
- Columns: Blaze, Zirchrom, Zorbax, Hypercarb
- Mobile Phases: Low pH, mostly MS compatible





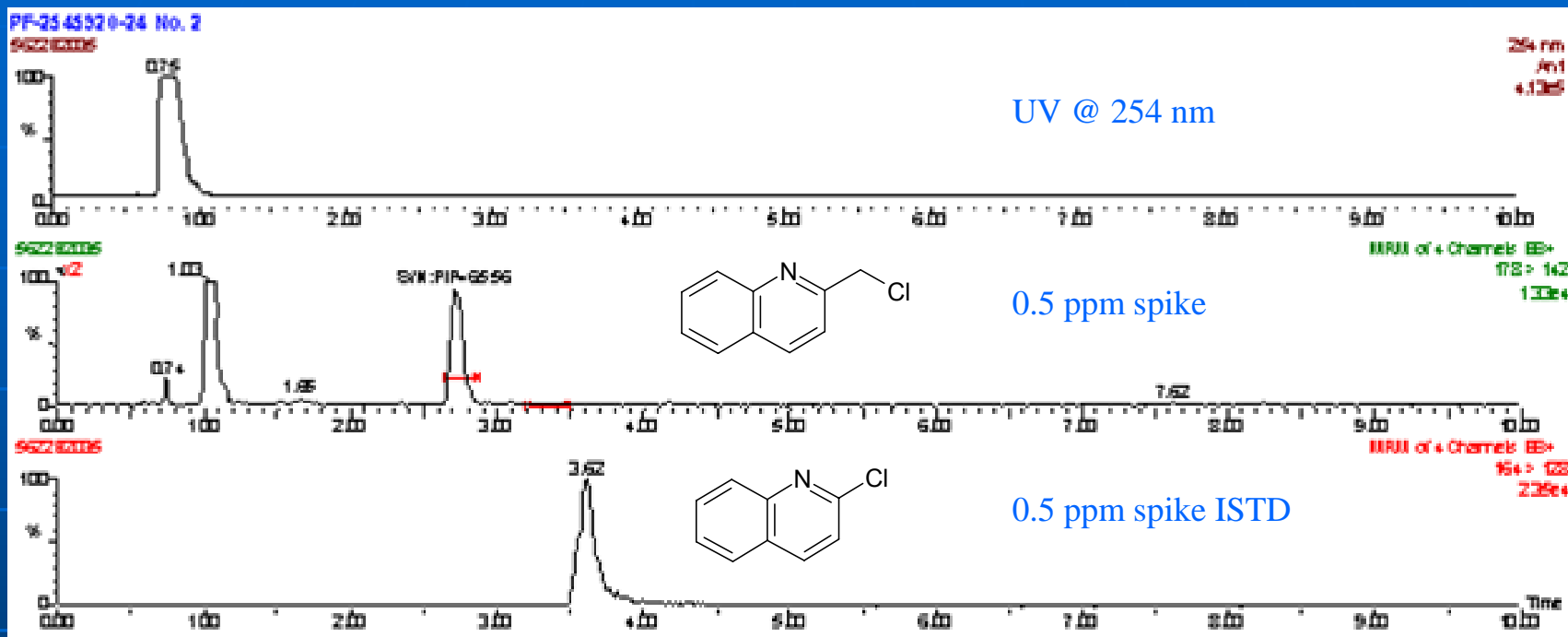
# LC with alternate detection – MS/MS

- Two degradants of concern
- Low-dose compound (0.1 mg)
  - Process understanding phase
- Benefits
  - Selectivity → Sensitivity & Easier Sample Preparation
  - Based on existing method?
- Specialized/Expensive
  - Trained scientists – Matrix effects (suppression, adducts)
  - Use of internal standards
  - Difficult to transfer – PU Phase





# Another example – LC/MS/MS





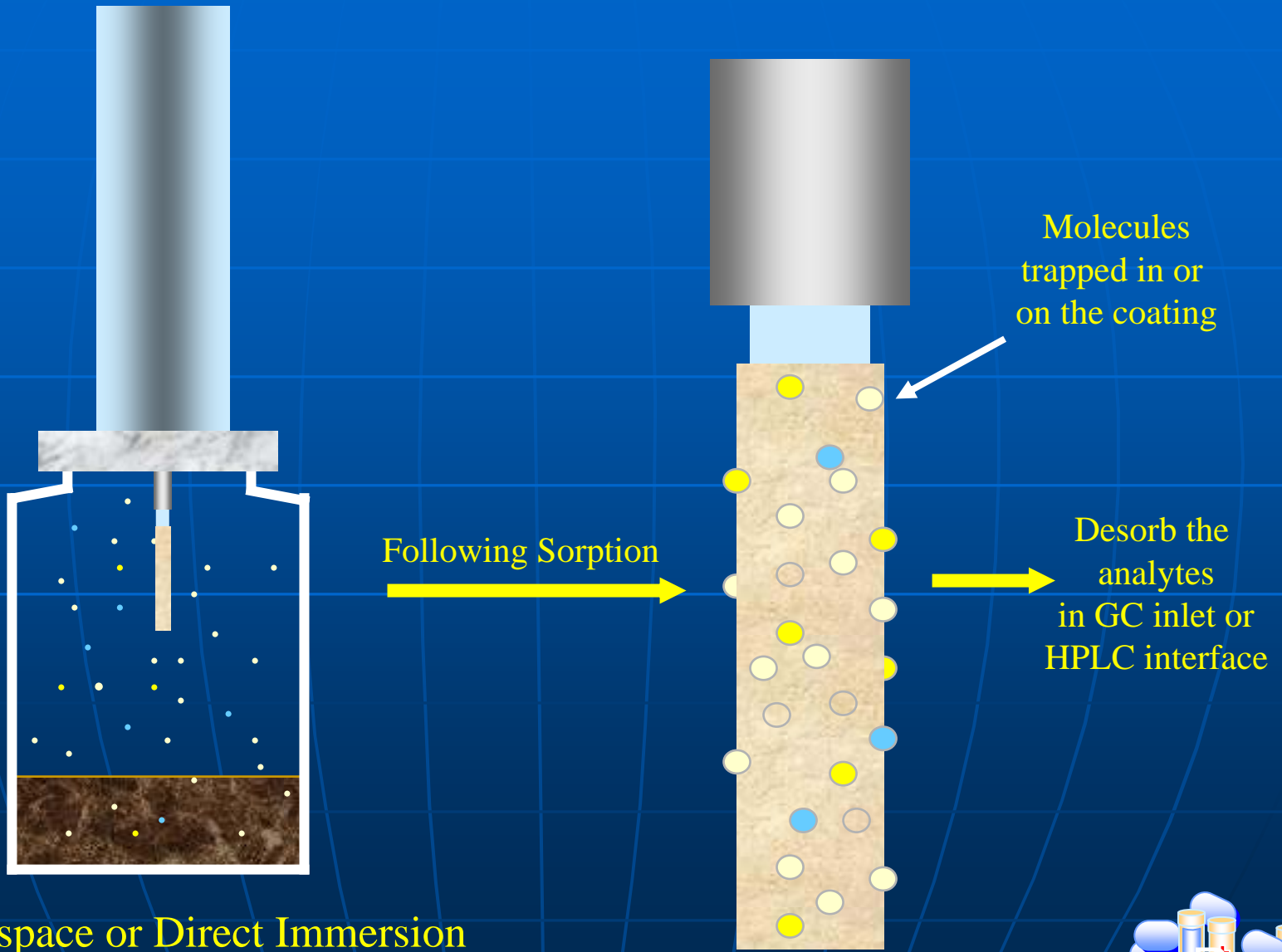
# Sample Enrichment - Microextraction

- Sample preparation using small volumes or no organic solvents – no need for further concentration
- Fast, simple, some of them disposable, cost-effective
- Unfortunately, most of the pharmaceutical applications reported are in the pharmacokinetics and drug metabolism arena
  - Hesitation for validated or GMP analysis (familiarity, precision, robustness, recovery, etc.)
- **Some examples:**
  - SPME and in-tube SPME
  - MEPS
  - SPE in pipette tips
  - Supported liquid membranes
  - LPME
  - SBSE
  - SDME
  - HPLC on a chip





# Solid-Phase Microextraction (SPME)

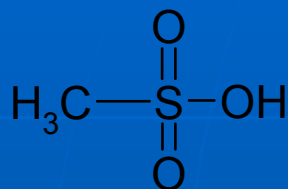


Headspace or Direct Immersion

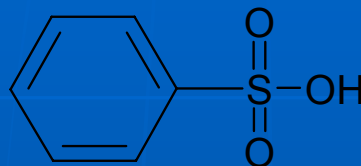




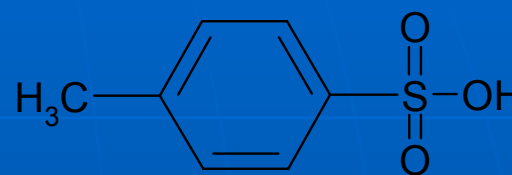
# Solid-Phase Microextraction (SPME)



Methanesulfonic Acid



Benzenesulfonic Acid



p-Toluenesulfonic Acid

- Chromatographic method, fibers, extraction time, ionic strength, pH
  - PDMS/DVB fiber for 30 min under constant agitation
  - pH or ionic strength did not make a difference in extraction efficiency
- Limit test → 5 ppm in API or 50 ppb solution
- Validation requirements:
  - Repeatability < 10%
  - Similar response with/without API (within 10%)
  - Linearity 80%-120% to assess saturation of fiber





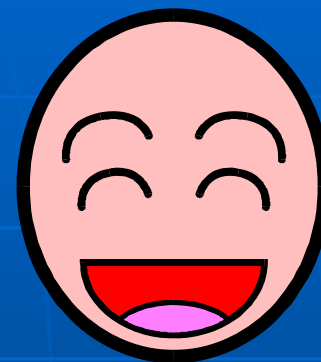
# SPME Method Validation

## Reproducibility

MMS (5.5%), EMS (3.0%)

MBS (3.5%), EBS(1.5%)

Mp-TS (3.7%), Ep-TS (1.7%)



## Response Bias

MMS (99.7%), EMS (104.0%)

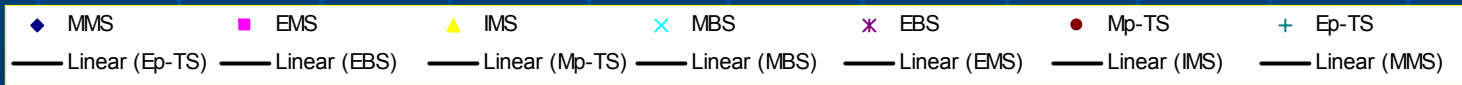
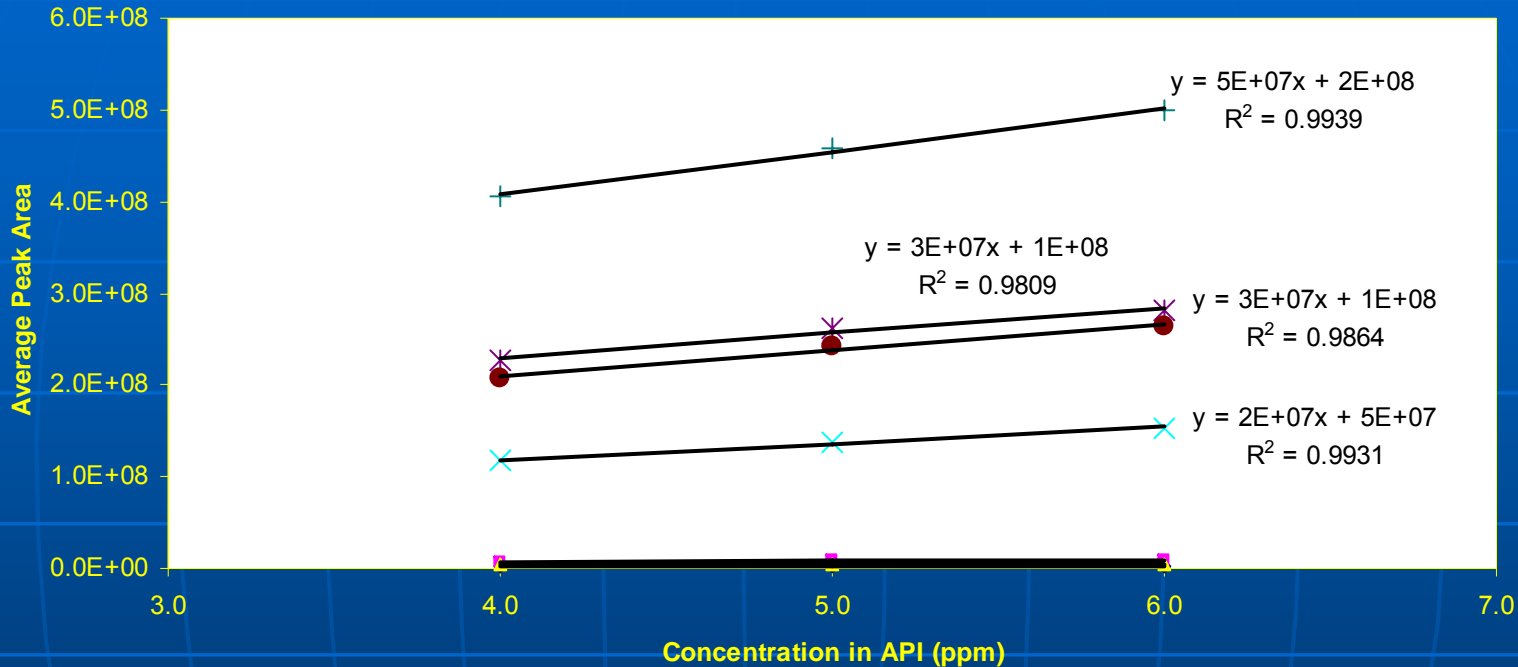
MBS (97.2%), EBS (93.1%)

Mp-TS (92.0%), Ep-TS (93.1%)





# Differentiating 4, 5 and 6 ppm... Is it possible?



## Linearity Results:

MMS (>0.99, >0.99)

MBS (0.99, 0.90)

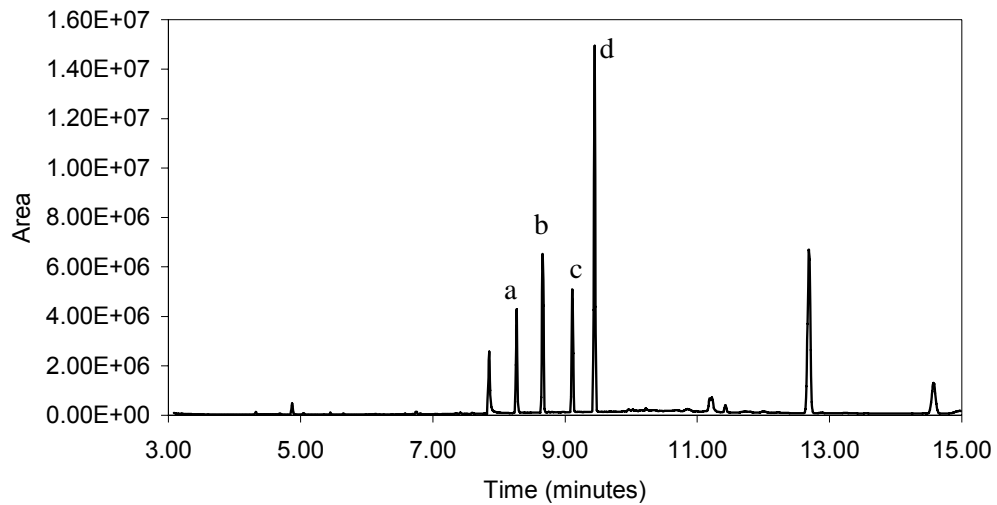
Mp-TS (0.99, 0.91)

EMS (0.99, 0.99)

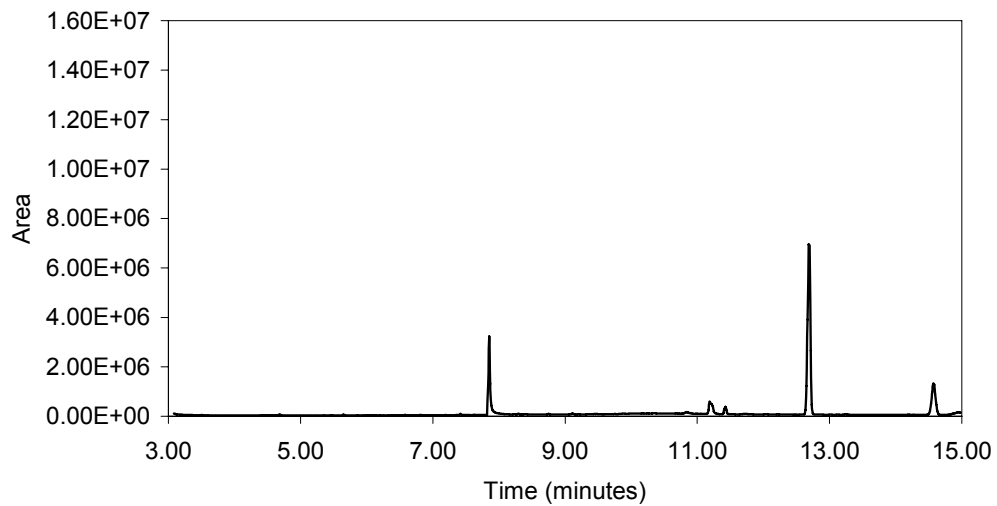
EBS (0.98, 0.94)

Ep-TS (0.99, 0.99)



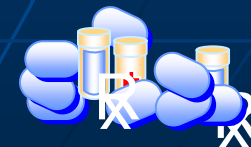


(a)



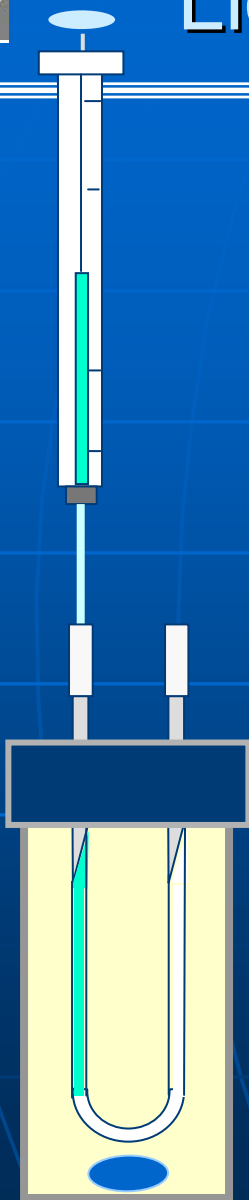
(b)

Application to API  
samples  
(5 ppm spike)





# Liquid-Phase Microextraction (LPME)

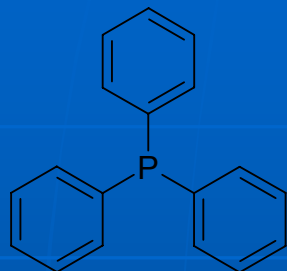


A 100  $\mu\text{L}$  syringe is used to fill the hollow fiber with fresh extracting solvent. The extraction device will hold approximately 40  $\mu\text{L}$ .

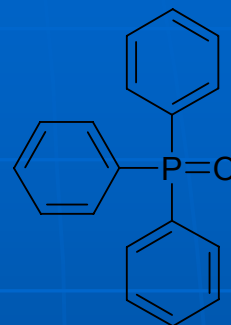
While stirring, the system is allowed to equilibrate. After a suitable amount of time, a syringe is used to withdraw the extracting solvent from the fiber.



# LPME - Optimization



Triphenylphosphine



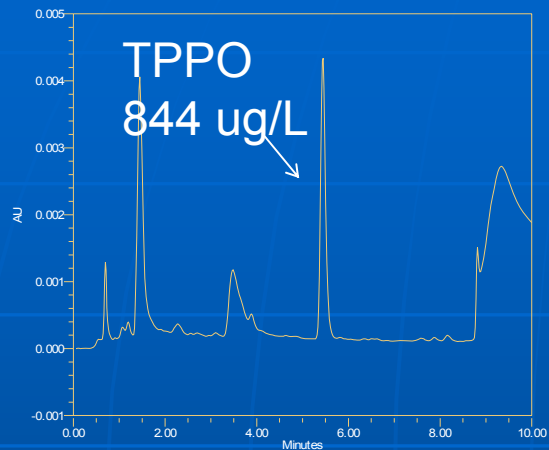
Triphenylphosphine Oxide

- Optimize: LC/UV method, extracting solvent, sample dissolving solvent and extraction time
- The objective is to develop a quantitative assay for TPP/TPPO in API samples with at least 5 ppm LOQ.
- The method will be validated testing precision, accuracy and linearity.

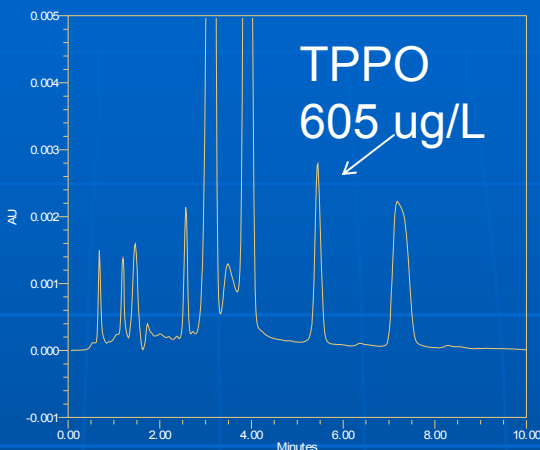




# LPME – Extracting Solvent



1-Octanol extract

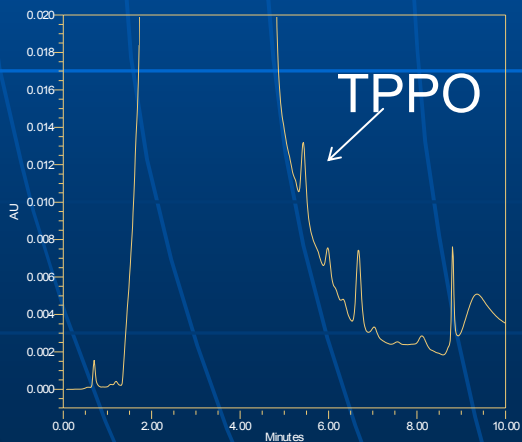


Toluene extract

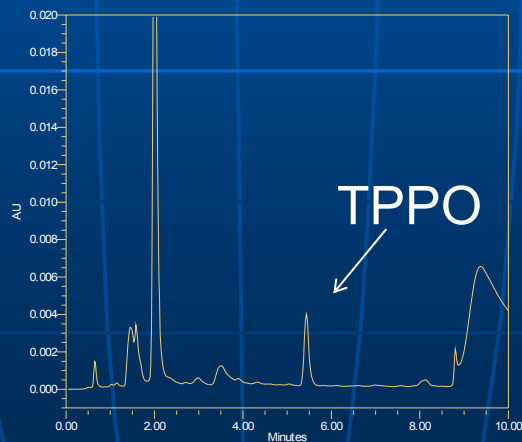
## The choice of solvents:

- ✓ API solubility
- ✓ Solubility in water
- ✓ Volatility
- ✓ Elimination of interferences

# LPME – Dissolving Solvent



Extract of sample in pH 3 buffer



Extract of sample in 0.1 N HCl





# LPME Method – Validation

## Extraction conditions

- Extraction solvent: 1-Octanol
- Sample solvent: 0.1 N aq. HCl
- Sample size: ca. 22 mg
- Extraction time: 60 minutes
- Stirring rate: 800 rpm
- Hollow fiber length: 10 cm

## HPLC Conditions

- Column: ACE 3 C8 column (4.6 x 150 mm, 3  $\mu$ m particle size)
- Mobile phase: Component A: phosphate buffer (pH 3)  
Component B: acetonitrile
- Gradient program:
  - Initial 55/45 (v/v) A/B for 10 min.
  - Linear gradient to 20/80 (v/v) A/B over 5 minutes
  - Hold 20/80 (v/v) A/B for 5 minutes
  - Linear gradient to 55/45 (v/v) A/B over 5 minutes
- Flow rate: 1.0 mL/min
- Detection: UV at 210 nm.
- Injection volume: 20  $\mu$ L

- Precision: without API – 8.6% for a 7.5  $\mu$ g/L spike  
with 1500 mg/L API – 8.5% for a 7.5  $\mu$ g/L spike
- Linearity: Five points 2.7 – 16.1  $\mu$ g/L range  $R^2$  0.97 and 0.99





# LPME Method – Accuracy

## Comparison of Intended and Measured TPPO Concentrations

Intent (ug/L)	Measured (ug/L)	% of Intent
2.69	2.78	103
8.06	8.70	108
10.7	10.9	101
13.4	14.6	109
16.1	15.8	98

The average % of Intent is 104%  
(%RSD = 5)



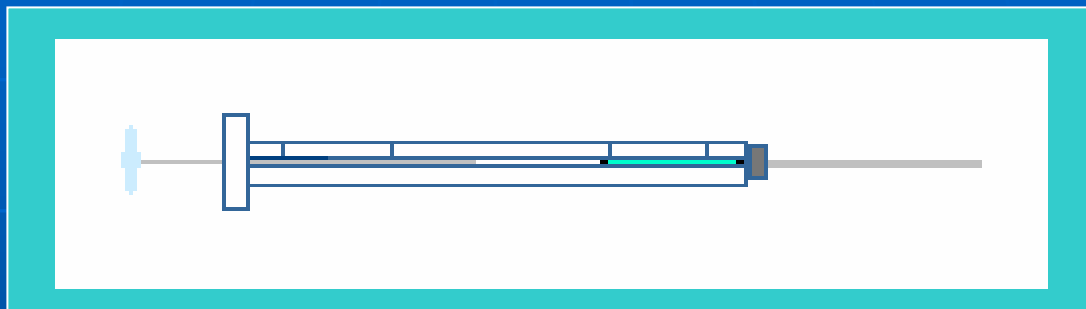


# LPME Method for TPPO - Applications

API	pKa	TPPO Added ug/L	TPPO Measured ug/L	% of Intent
1	7.2	3.74	3.71	99
		7.47	7.70	103
		11.2	11.8	105
2	6.2, 9.3	3.74	3.59	96
		7.47	8.17	109
		11.2	10.2	91
3	9.5	3.74	4.01	107
		7.47	7.23	97
		11.2	11.4	102



# Microextraction in a packed syringe (MEPS)

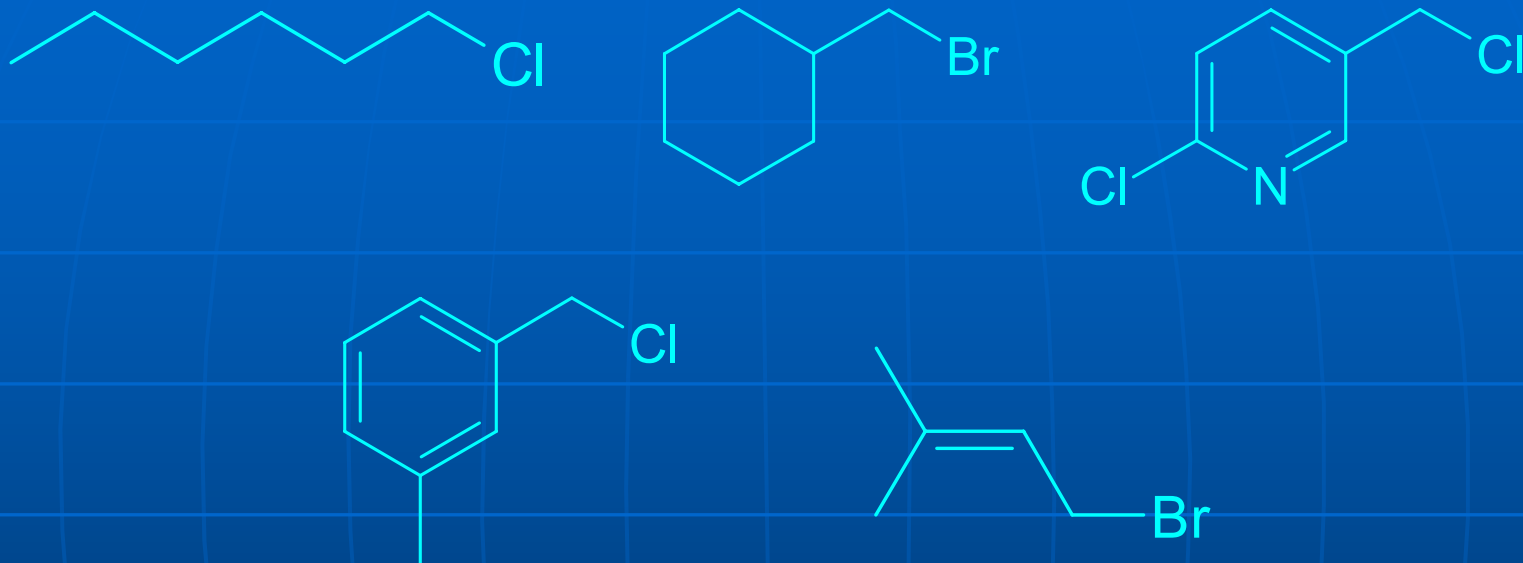


- A conventional glass syringe (e.g. 250  $\mu\text{L}$ ) is packed with a few milligrams of sorbent material
- A small volume of sample is pumped through
- A few  $\mu\text{L}$  of eluting solvent used to desorb compounds straight into the analytical system
- Microscale version of solid phase extraction
- Easily automated





# MEPS for the analysis of alkyl halides



Primary objective: To develop a limit test (with the potential for quantitation) to the 10 ppm level for the analysis of alkyl halides in API solutions. This method should be simple, reliable and appropriate for synthesis support (automated?).





# Summary and Conclusions

- Low-level impurity analysis poses additional challenges (but interesting!!) to the pharmaceutical analytical chemist.
- Many techniques are viable options, however new equipment and training might be necessary.
  - Need to aid in general awareness and familiarization
- Advances in techniques such as MS and extraction have minimized concerns and have opened the door to new opportunities!!
- Keep in mind that a lot of these might be only used for process understanding in a QbD world.



Thank you...  
Questions?

