Development of a General Solvents Method for DMSO Soluble Compounds

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Outline

- Goals of Solvent Work
- Method Development
- Method Validation
- Method Use
- Solvent Control Strategy
- Reference Standards

Goals of General Solvent work

- Develop chromatographic conditions
  - Resolve all solvents of interest to process development
  - Minimize run time
  - Similar to USP/PhEur tests
  - Obtain accurate results
    - Quantitative
    - Limit test
  - Assure sensitivity
  - ☺ **No additional method development when applying method to new matrix**
  - Use for all steps to monitor/control solvents

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Solvent list

	USP<467> or *toxicology Target, %	Solvent Class
Acetone (Actn)	*0.45	3
Acetonitrile (ACN)	0.041	2
Anisole	0.5	3
Benzene	0.0002	1
1-Butanol (1-BuOH)	*0.05	3
2-Butanol (2-BuOH)	0.5	3
Cyclohexane (cyclo)	0.388	2
1,2-Dichloroethane	0.0005	1
Diethyl ether	*0.1	3
Dimethylformamide (DMF)	*0.04	2
DMSO	0.5	3
Ethanol (EtOH)	0.5	3
Ethyl Acetate (EtOAc)	*0.45	3
Ethyl benzene (etbenz)	0.0369	2
Heptane (Hept)	*0.2	3
Hexane	0.029	2
Isobutyl alcohol (2Me-1-PrOH)	0.5	3

	USP<467> or *toxicology Target, %	Solvent Class
Isopropyl acetate (IPAC)	0.5	3
Methanol (MeOH)	*0.25	2
t-Methyl butyl ether (MTBE)	0.5	3
Methyl ethyl ketone (MEK)	*0.3	3
Methyl isobutylketone (MIBK)	0.5	3
Methylene Chloride (MeCl2)	0.06	2
N-methylmorpholine	0.1	n/c
1-Pentanol (amyl alcohol)	0.5	3
n-Propanol (n-PrOH)	0.5	3
2-Propanol (IPA)	0.5	3
Pyridine	0.02	2
Sulfolane	0.016	2
Tetrahydrofuran (THF)	0.072	2
Toluene	0.089	2
Triethylamine	0.1	n/c
m-Xylene	0.1302	2
o-Xylene	0.0195	2
p-Xylene	0.0304	2

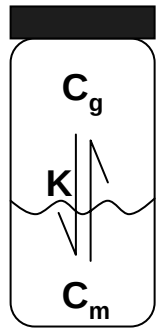
Method Development

- Temperature program
  - ProEzGC ™
    - Computer based simulation software for gas chromatography
    - Based on 2 sets of lab runs
  - Use same conditions for Direct Injection techniques
- Headspace conditions
  - Evaluate vial conditions
    - Temperature
    - Time
    - Shake
- Sample solvent/concentration
  - Target 100 mg/mL to assure sensitivity
  - More compounds soluble in DMSO

Principle of Head Space GC

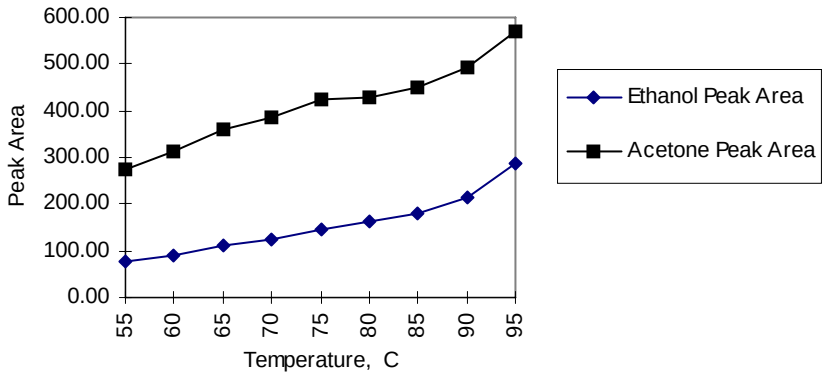
- HS Vial
  - Equilibrate at a given temperature
  - Volatile solvents partition between the gas headspace and the sample
  - Partition coefficient, K, is related to the degree of solubility of the analyte in the matrix or solution Cm and the tendency of the analyte to go to the gaseous phase, Cg.

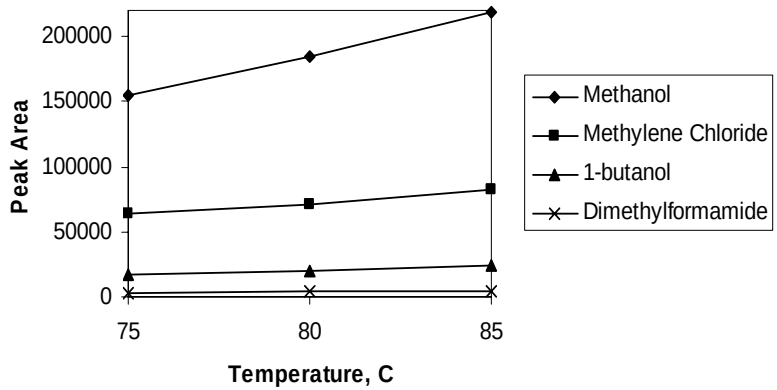
$$K = C_m/C_g$$



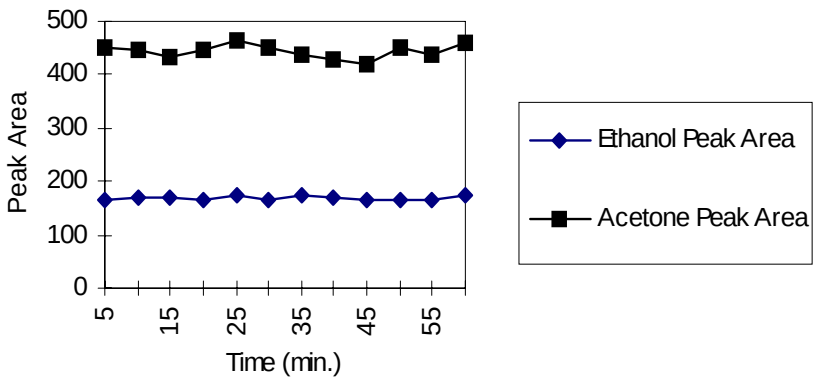
Vial Parameters

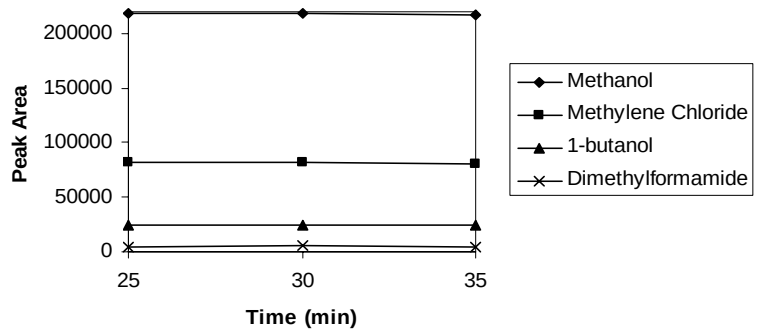
- Equilibration Temperature





- Equilibration Time at 85˚C





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Standard Evaluation

Evaluation of standard solvent mixture as compared to individual solvent standards for response. (Solvent partitioning is not significantly affected by presence of other solvents in the standard mix)

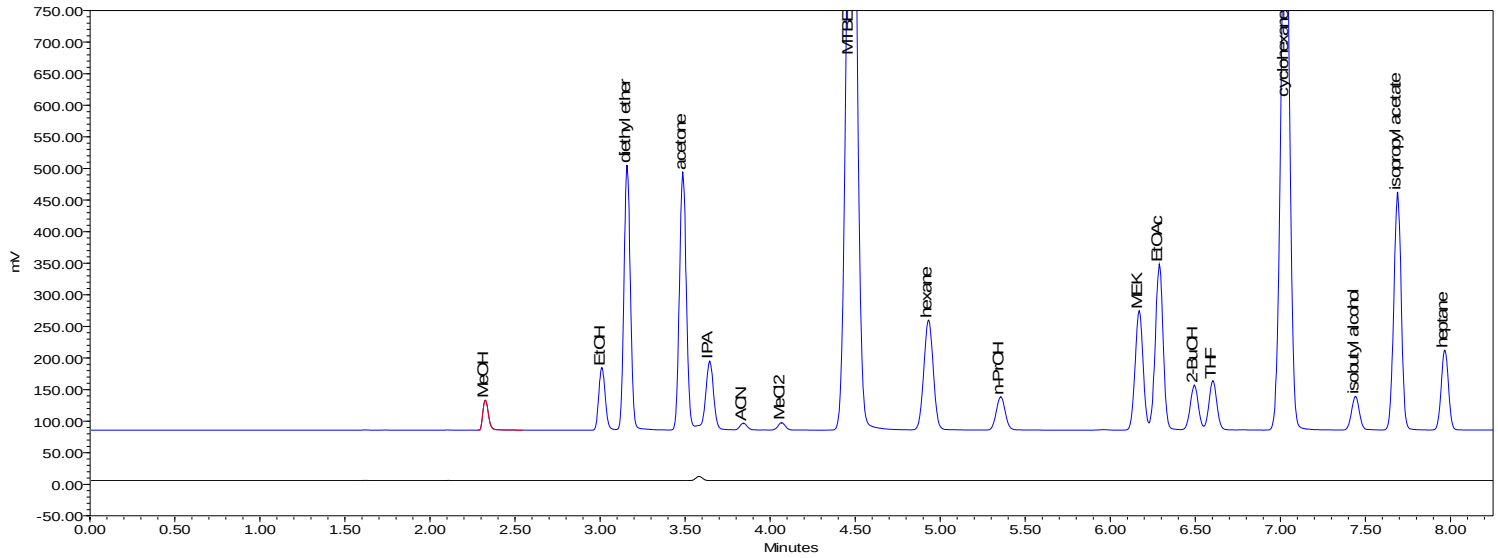
	Area of std alone : Area of std in mixture ratio, %
Acetone (Actn)	99.6
Acetonitrile (ACN)	93.3
Anisole	100.7
1-Butanol (1-BuOH)	102
2-Butanol (2-BuOH)	109.2
Cyclohexane (cyclo)	94.5
Diethyl ether	90.4
Ethanol (EtOH)	109.3
Ethyl Acetate (EtOAc)	102.1
Ethyl benzene (etbenz)	104.6
Heptane (Hept)	84.7
Hexane	72.9
Isobutyl alcohol (2Me-1-PrOH)	104.3
Isopropyl acetate (IPAC)	103.7

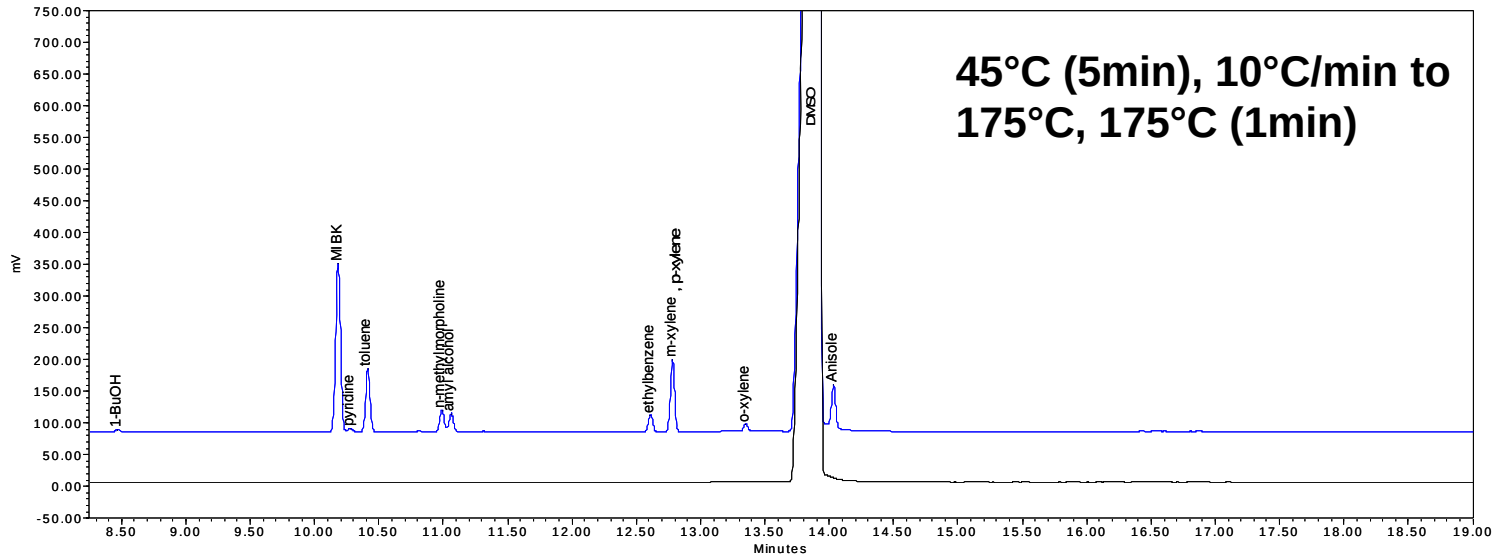
	Area of std alone : Area of std in mixture ratio, %
Methanol (MeOH)	102.4
t-Methyl butyl ether (MTBE)	90.7
Methyl ethyl ketone (MEK)	104.7
Methylene Chloride (MeCl2)	84.2
N-methylmorpholine	109.6
1-Pentanol (amyl alcohol)	103.9
n-Propanol (n-PrOH)	105.2
2-Propanol (IPA)	88.1
Tetrahydrofuran (THF)	101.9
Toluene	108.3
m-Xylene	93.1
o-Xylene	109.8
p-Xylene	93.1

The Method GC Conditions

Column	6% polycyanopropylphenylsiloxane-94% polydimethylsiloxane , for example an Agilent DB-624 or equivalent
Column Dimensions	30 m x 0.32 mm i.d., 1.8 µm film thickness
Carrier Gas	Helium
Flow Rate	Approximately 2.1 mL/min
Injector Liner	Direct Inlet Liner, 2mm ID, quartz, deactivated
Detection	Flame Ionization (FID)
Injector	140ºC
Detector Temperature	250°C
Head Pressure	Approximately 10 psi
Split Ratio	**Approximately 10:1**
Initial Temperature	**45°C**
Initial Time	**5 min.**
Rate	**10°C/min.**
Final Temperature	**175°C**
Final Temperature Time	**1 min.**
GC Run Time	**19 minutes**

The Method: Chromatography





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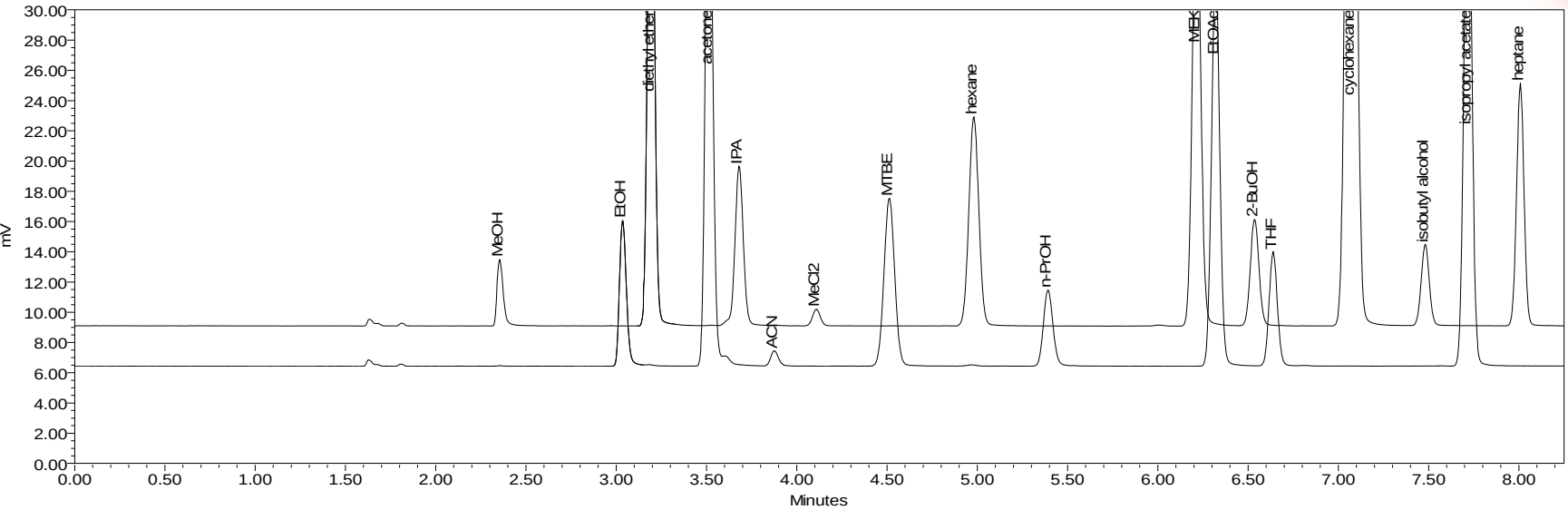
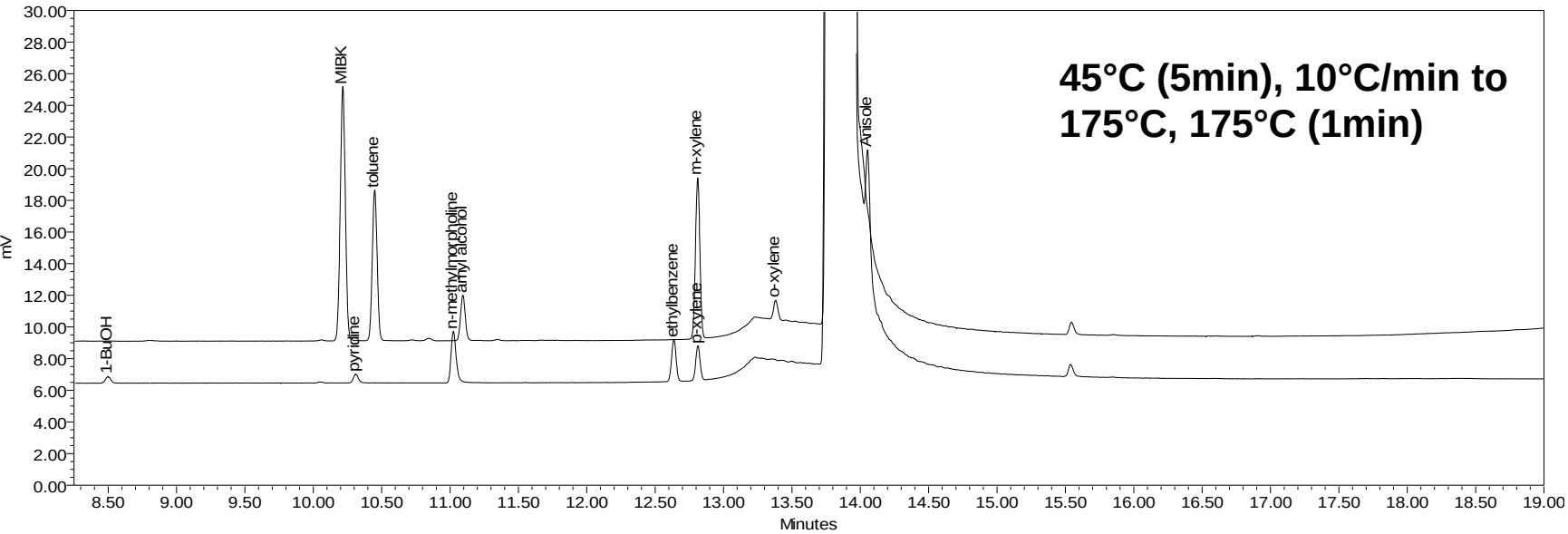
The Method: Chromatography-10% target concentration





45°C (5min), 10°C/min to 175°C, 175°C (1min)

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The Method

- Head Space Conditions

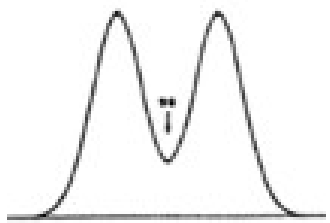
Oven Temperature	**85°C**
Loop Temperature	**95°C**
Transfer Line Temp.	**130°C**
GC cycle time	**Approximately 25 minutes (adjust as necessary for system cool down and equilibration)**
Vial Equilibration Time	**30 minutes**
Pressurize Time	0.5 minutes
Loop Fill Time	0.2 minutes
Loop Equilibration Time	0.1 minutes
Inject Time	1.0 minutes
Injection Loop	1 mL
Vial Pressurization	~ 2.5 psi
Shake Velocity	Low

The Method-Standard Addition

- Sample information
  - Prepare 4 samples to be 100mg/mL when diluted
  - Dilute one with DMSO
  - Dilute one with standards at the QL
  - Dilute one with standards at target
  - Dilute one with standards at 2x target

The Method

- System Suitability
  - 3 injections of standard at the target
    - RSD_{area} <20%
    - $R_{S\ \text{closest peaks}}$ >1.0



Rs = 1.0*

  - Use the sample spiked with the QL standard to verify S/N >10

*Figure from: Dolan, J.W.; Snyder, L.R. “Troubleshooting LC Systems”, 1989, Humana Press, NJ

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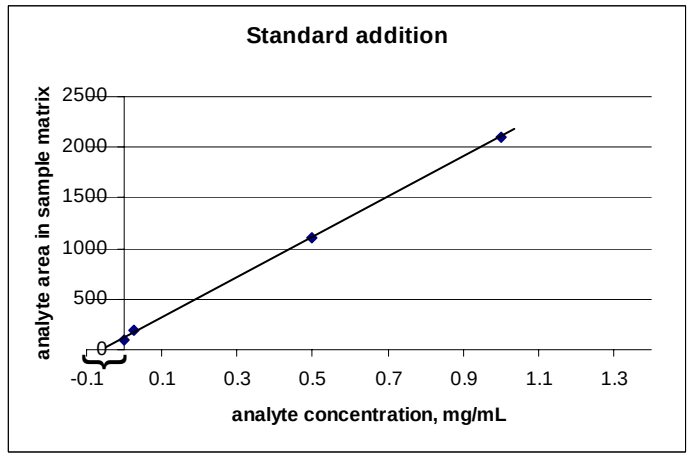
The Method

Standard Addition Calculations

- Calibration curve (area vs. conc.) for each solvent
  - Using: unspiked, QL, target, 2 x target



$$\text{solvent conc, mg/mL} = \frac{\text{y - intercept}}{\text{slope}}$$

$$\text{solvent conc, \%} = \frac{\text{solvent conc, mg/mL}}{\text{100 mg/mL}} \text{ x 100 (\%)}$$

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Method Validation

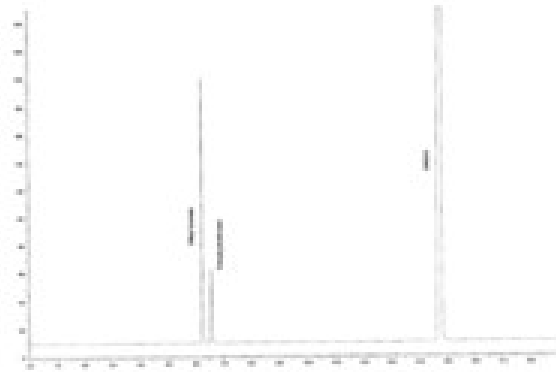
- Evaluation of standards
  - Specificity
    - Absence of background interference
    - Identifiable peaks, Rs≥1.0
  - Linearity
    - Range evaluated: 10-200% of target limit
    - Number levels: 6
    - Number of replicates/level: 3
    - Results: range for r = 0.998 to 1.000

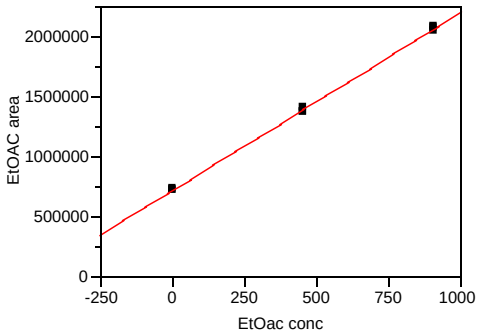
Method Validation, con't

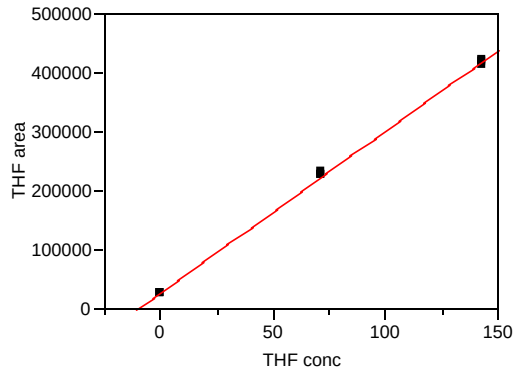
- Repeatability
  - Range evaluated: 10-200% of target limit
  - Number levels: 6
  - Number of replicates/level: 3
  - Results, range for %RSD: 0.07% - 3.67%
- QL
  - QL is defined as 10% of target limit (lowest point evaluated)
  - At the QL, S/N $\geq$ 10

Example Compound A

- Two solvents potentially present
  - EtOAc, THF
- Performed test 3 times







	Replicate1	Replicate2	Replicate3
Slope	1485.617	1449.996	1510.728
y-Intercept	723935.3	724115.1	706393
Result, %	0.49	0.50	0.47

	Replicate1	Replicate2	Replicate3
Slope	2743.09	2705.384	2763.106
y-Intercept	28368.37	27718.82	26692.16
Result, ppm	103	102	97

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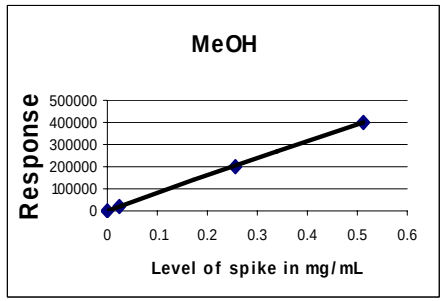
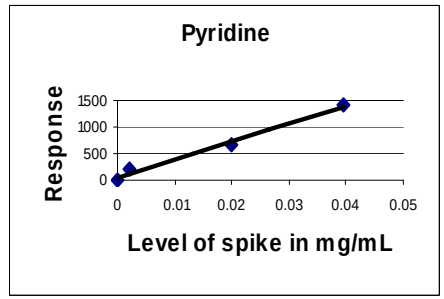
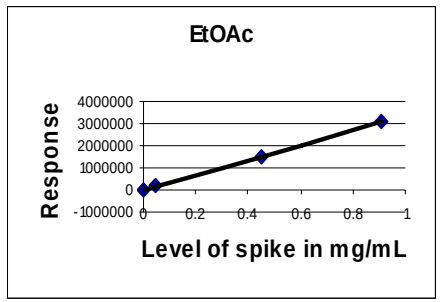
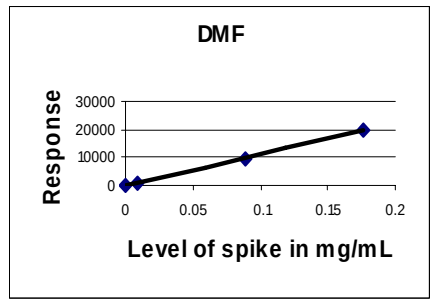
Example Compound B

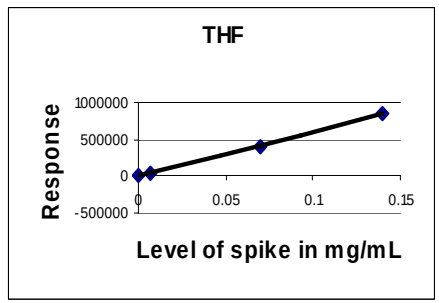
- Five solvents potentially present
- No analyte detected in unspiked sample
- S/N>10 for sample spiked at 10% target











Analyte	Slope	Intercept	ppm Result
MeOH	776869	2514	<250
EtOAc	3387238	-9552	<450
THF	6068225	-3358	<72
pyridine	33835	50	<20
DMF	110231	56	<40

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Comparison to compendia

	PhEur	USP	Lilly general
sample solvent	**water/DMF/DMI/other demonstrated suitable**	**water/DMF**	**DMSO**
concentration, mg/mL	**10**	**10**	**100**
standard solvent	DMSO then water	DMSO then water/water	DMSO
vial equilibration Temp, °C	80/105/80/as demonstrated suitable*	80/105/80/as demonstrated suitable^	85
vial equilibration time, min	**60/45/45/as demonstrated suitable**	**60/45/45/as demonstrated suitable**	**30**
transfer line Temp, °C	85/110/105/as demonstrated suitable	85/110/105/as demonstrated suitable	130
Carrier gas	Nitrogen or Helium	Nitrogen or Helium	Helium
Pressurization time, sec	30	30	30
Injection volume, mL	1	1	1
column phase	6% polycyanopropylphenylsiloxane : 94% polydimethylsiloxane	6% polycyanopropylphenylsiloxane : 94% polydimethylsiloxane	6% polycyanopropylphenylsiloxane : 94% polydimethylsiloxane
column dimensions	30m x 0.32mm (or 0.53mm) x 1.8um (or 3um)	30m x 0.32mm (or 0.53mm) x 1.8um (or 3um)	30m x 0.32mm x 1.8um
split ration	**5:1**	**5:1**	**10:1**
linear velocity cm/s	~35	~35	~35
detector	FID (/MS/ECD)	FID	FID
detector temp, °C	250	250	250
injection temperature, °C	140	140	140
Temperature program	**40°C (20min), 10°C/min to 240°C, 240°C (20min)**	**40°C (20min), 10°C/min to 240°C, 240°C (20min)**	**45°C (5min), 10°C/min to 175°C, 175°C (1min)**
Run time	**60 min**	**60 min**	**19 min**
standards	**all class 1 and 2 solvents and relevant class 3 solvents**	**class 1 and 2 stds- USP RS mix (class 3 by LOD/valid procedure)**	**all relevant solvents**
System suitability	**S/N(1,1,1 trichloroethane) ≥ 5 Rs (ACN,MeCl2) ≥ 1.0, chromatography resembles Figure**	**S/N(1,1,1 trichloroethane) ≥ 5 S/N(class 1 solvents) ≥ 3 Rs (ACN,MeCl2) ≥ 1.0,**	**RSD of smallest peak ≤ 20% (n=3) Rs(closest peaks) ≥ 1.0 S/N(smallest pk in detectability sample) ≥ 10**
	no pk for class 1 or 2 solvent in sample: pass	**sample peak < class 1 or 2 std pk: pass**	
	pk for class 1 or 2 present in sample, confirm with PEG column	**pk for class 1 or 2 present in sample, confirm with PEG column**	
		stds spiked individually into samples	**stds combined**
result format	**pass / fail limit test**	**qualitative/semi-quantitative**	**quantitative test**
	uses matrix	**assumes no matrix effect**	**uses matrix**

DMI- 1,3-dimethyl-2-imidazolidinone used for DMF determinations

*the temperature depends on the sample solvent

^no conditions described to determine when to use what parameters

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Advantages

- Standard addition eliminates need to validate every matrix individually
  - Validation exists every time method is run
- Demonstrating method suitability every time
- No need for additional comparison of results to compendial methods
- No change in GC setup required
  - Efficiency gain
- Overall control strategy for process
  - Starting materials
  - Intermediates

Issues

- Sample Solubility
  - Some initial solubility information is needed
    - If not DMSO soluble then determine what is a good solvent
    - Use similar chromatographic conditions
- DMSO quality
  - Preferred supplier that is reliable
- Reactivity of some samples with DMSO
- Not enough sensitivity for every analyte at 1/10 target
  - benzene and 1,2 DCE
    - Use a limit test method with same chromatographic conditions
- Also issue with high boiling point solvents

Additional topics

- Routine GC testing may not be required
  - No testing
  - Skip testing
- Determine long-term strategy for solvent reference standards...some options
  - Prepare by analyst
  - Prepare by vendor (Restek, Supelco, etc)
  - Maintained by corporate ref std group
  - Maintained from USP

Reference Standard Recommendations

- Maintained standard locally in laboratory
- Utilize external vendor for tailored solvent mixtures
  - Fully characterized standards with certificate of analysis to assure correct standard potency
  - No shipping issues
  - Standard material will be available when needed

Thank You

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