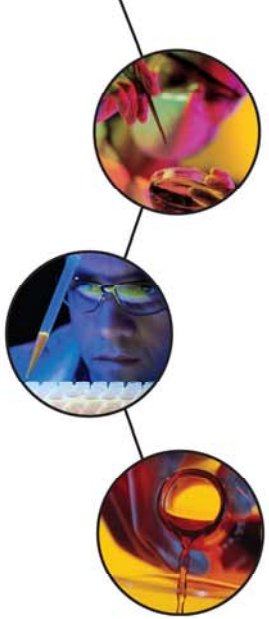

Revisiting Traditional Liquid-Liquid Extraction Techniques using SLE

Abram N. Brubaker, PhD
MPI Research
Mattawan, MI

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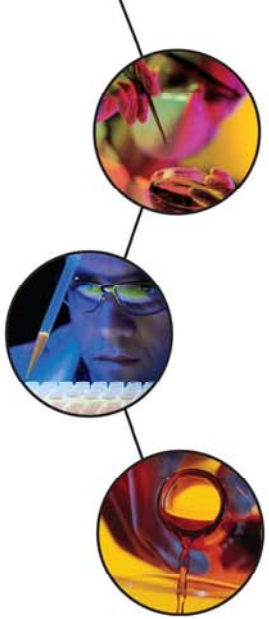

Method Development Approach at MPI Research

- Select mixed mode SPE based on functional groups present on molecule
  - For basic amine containing molecules select MCX
  - For molecules possessing a carboxyl group select MAX
  - Easier to debug a failed extraction: pH control problem most common cause
- Select SLE for the following reasons
  - Small molecular weight compound with good organic solvent solubility properties; for acidic, basic, or neutral compounds
  - SLE is preferred over HLB: partitioning differences between analyte and analog IS are minimized
  - Mixed mode or HLB extraction recoveries are extremely low due to strong interaction of analyte with the polymeric stationary phase
  - KISS principal: analysts prefer the simplicity of SLE vs SPE (automation)

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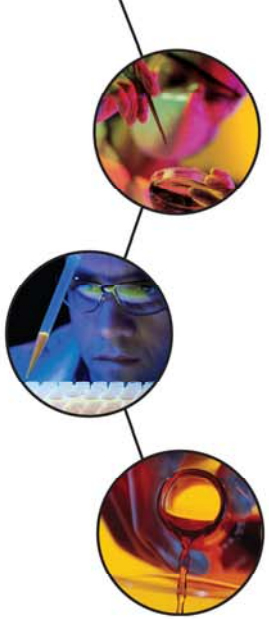
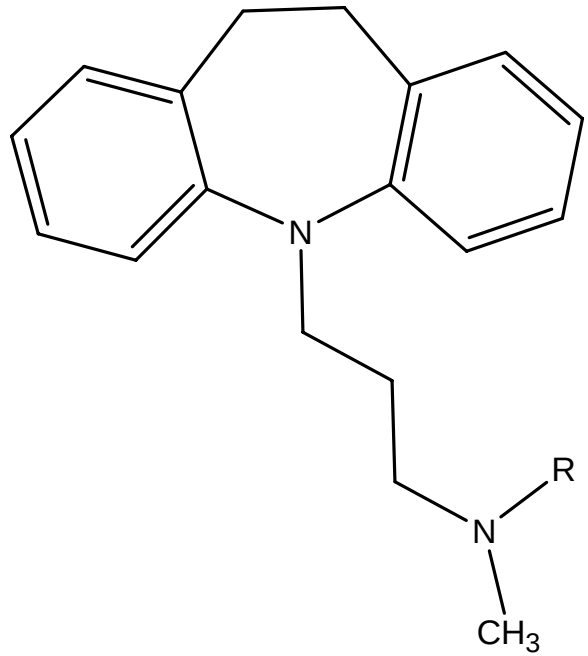


Using Ion Pairing/Back-extraction with SLE

- SLE selected because previously developed using traditional LLE
- SLE selected over SPE due to milder conditions during organic solvent removal step: time is money
- Literature example using SLE by Biotage
- Method was unsatisfactory because of analyte loss during organic solvent evaporation step
- Encountered similar problems during initial LLE



Desipramine R = H

Imipramine R = CH_3

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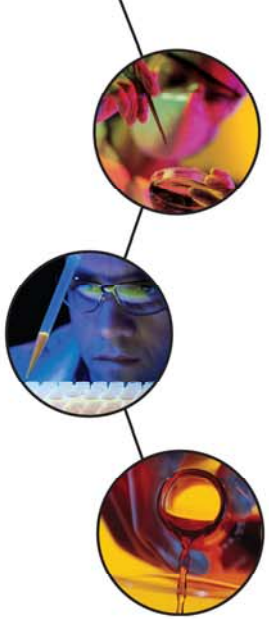
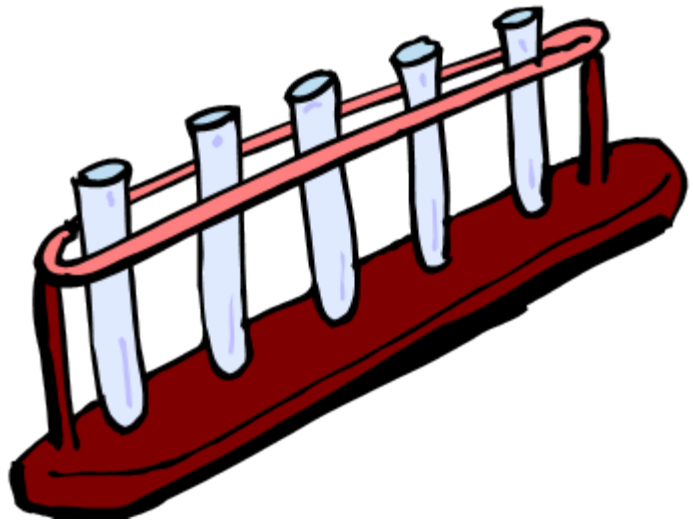


LLE Method

- Add plasma sample to tube
- Add ion pairing agent ($ArCH_2COOH$ in phosphate buffer pH 7
- Add ethyl acetate
- Mix and centrifuge
- Transfer organic layer to a tube containing 5% sodium bicarbonate to back extract the ion pairing agent
- Mix and centrifuge
- Transfer organic to another tube and remove volatiles under stream of nitrogen



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Adapt the LLE Method to SLE

- In a 96-well plate mix
  - 50 µL of plasma sample
  - 50 µL internal standard solution
  - 100 µL Ion pairing agent in phosphate buffer pH 7
- Mix and transfer to SLE plate
- Add ethyl acetate and collect organic solvent in receiver plate
- Add aqueous citric acid to receiver plate and mix well to back extract analytes
- Transfer an aliquot of lower layer to injection plate for analysis by LCMSMS

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	Desipramine							Imipramine					
	QC Low 0.75 ng/mL	Rec. Std.	QC Mid 10 ng/mL	Rec. Std.	QC High 85 ng/mL	Rec. Std.		QC Low 7.5 ng/mL	Rec. Std.	QC Mid 100 ng/mL	Rec. Std.	QC High 850 ng/mL	Rec. Std.
	5536	11878	84460	173112	562849	1259870		24766	49340	380323	707766	2477890	4462700
	4795	10867	81355	165873	616081	1216290		24913	47254	367971	688349	2669870	4427430
	5160	11086	84862	168410	555441	1212690		25554	46467	400756	691914	2419370	4393350
	6310	11126	82562	166537	584384	1238570		27532	47370	384957	677355	2528930	4489890
	6050	11476	76994	161214	646029	1216430		28456	45815	354697	652094	2691750	4406710
	6032	10802	79504	154080	625255	1217780		27367	46508	361997	628213	2648550	4402550
Mean	5647	11206	81623	164871	598340	1226938		26431	47126	375117	674282	2572727	4430438
SD	587	406	3014	6543	36354	18604		1551	1226	16861	29169	112945	38212
%RSD	10.4	3.6	3.7	4.0	6.1	1.5		5.9	2.6	4.5	4.3	4.4	0.9
%REC	50		50		49			56		56		58	

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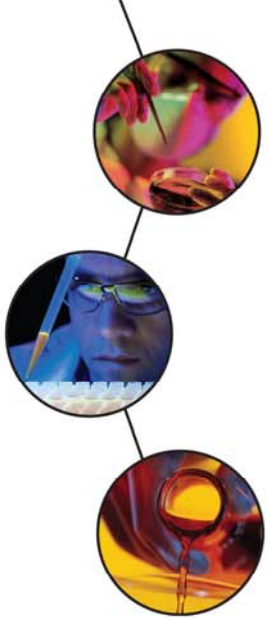
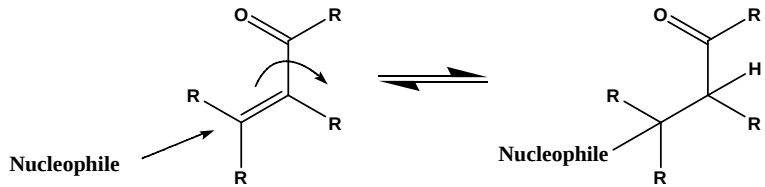


SLE for Whole Blood Assay of an Unstable Compound

- Compound undergoes rapid degradation in plasma via 1,4-addition reaction
- Surprisingly stable in whole blood – stable for several weeks stored at 4º C
- Developed a LLE extraction but converted to an SLE method for automation
- Mixed whole blood, internal standard solution, and acetone
- Samples did not clog the frit even though significant residue collected on frit
- Recovery was low but greater than a protein precipitation method



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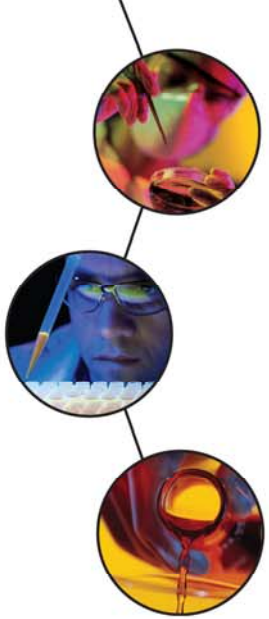

SLE Procedure in Whole Blood

- To a 96-well plate add
  - 100 µL of whole blood
  - 20 µL of internal standard solution
  - 300 µL of acetone
- Vortex, centrifuge, and transfer 200 µL to SLE plate well and load sample onto bed
- Extract analytes by addition of 1 mL of MTBE
- Evaporate volatiles under stream of nirtrogen and reconstitute sample for analysis by LC-MS/MS

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	QC Low	Normalized Response	Rec. Std.		QC Mid	Normalized Response	Rec. Std.		QC High	Normalized Response	Rec. Std.
	6759	0.027	57387		151950	0.287	619462		1487230	2.93	3717320
	6196	0.024	60583		112824	0.299	525874		1666630	3.02	3555570
	9432	0.026	61213		114643	0.307	332017		1931190	3.02	3649060
	10582	0.026	57796		134994	0.302	356053		1600150	2.98	4350980
	10415	0.025	51942		123065	0.313	362181		1546860	3.00	4537660
	11688	0.027	48775		135261	0.309	523673		1696260	3.21	4872520
Mean	9179	0.026	56283		128790	0.303	453210		1654720	3.02	4113852
SD	2219	0.001	4930		14863	0.009	118573		155543	0.10	547059
%RSD	24.2	4.3	8.8		11.5	3.0	26.2		9.4	3.2	13.3
%REC	16				28				40		

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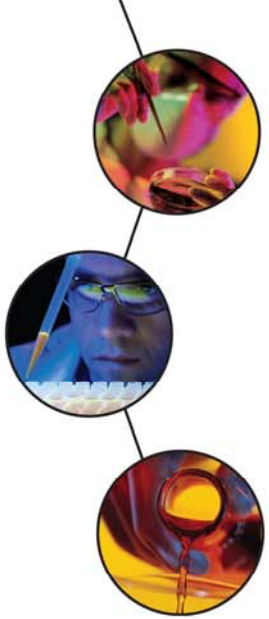
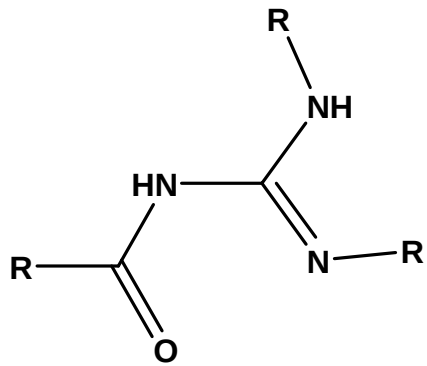


Using SLE when SPE Failed for a Complex Molecule

- Very polar molecule containing seven nitrogens – three basic nitrogens pKa 3 – 9
- Two aromatic rings give some lipophilic character to the molecule
- Recovery from HLB or MCX polymer based beds was very poor <50%
- Could not elute the compound even under drastic conditions – polar aprotic solvents and high concentration buffers



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SLE Method using Ion-Paired Extraction

- To a 96-well plate add
  - 50 µL plasma sample
  - 50 µL of internal standard working solution
  - 100 µL of 0.5% $ArCH_2COOH$ in phosphate buffer pH 7
- Mix and transfer entire volume to SLE plate
- Extract analytes with 2 x 500 µL of MTBE
- Remove volatiles under a stream of nitrogen
- Reconstitute dried residue and analyze by LC-MS/MS

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	QC Low 6 ng/mL	Low Rec. Std.	QC Mid 60 ng/mL	Mid Rec. Std.	QC High 800 ng/mL	High Rec. Std.
	26969	27579	271169	244481	3069180	3577920
	25926	27908	266247	255005	3218410	3483490
	26389	24971	268216	248272	3025840	3760070
	25887	27680	267646	257881	3068660	3744700
	28366	27573	261687	249272	3094740	3516140
	25418	28586	261662	253818	3179210	3600270
Mean	26493	27383	266105	251455	3109340	3613765
SD	1057	1241	3788	4960	73799	115342
%RSD	4.0	4.5	1.4	2.0	2.4	3.2
%REC	97		106		86	
n	6	6	6	6	6	6

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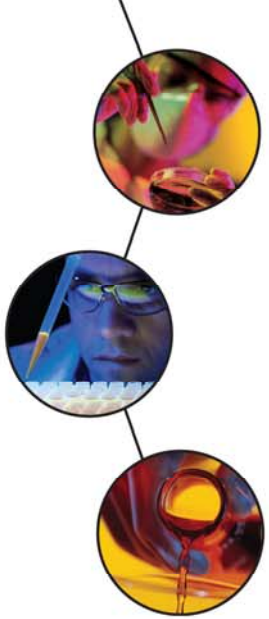

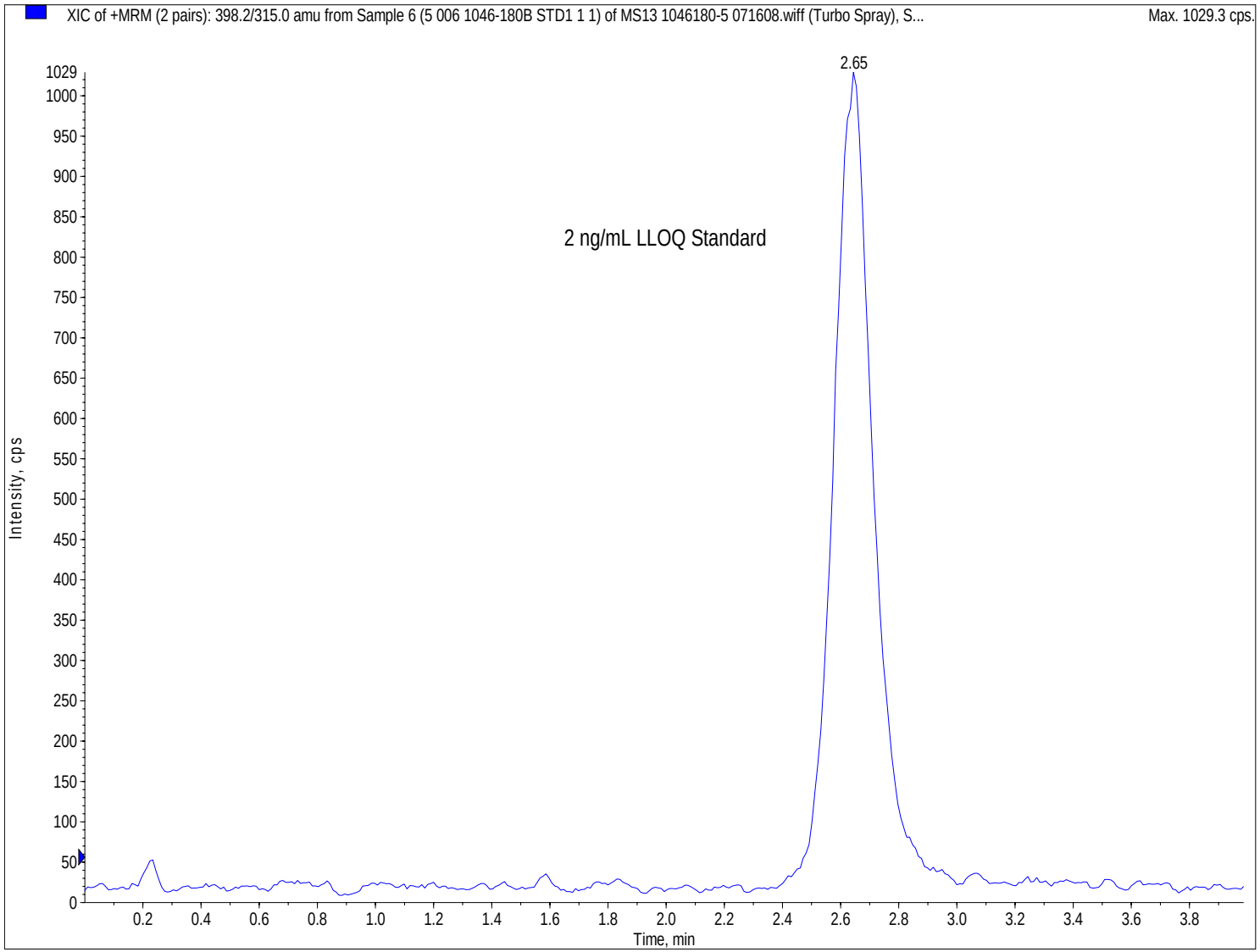

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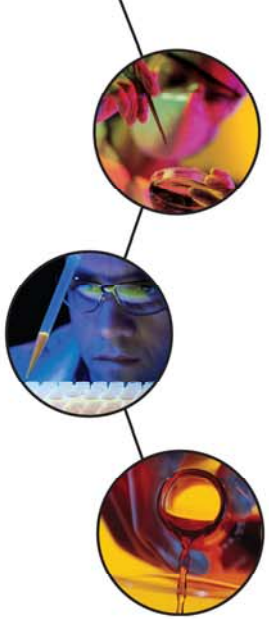
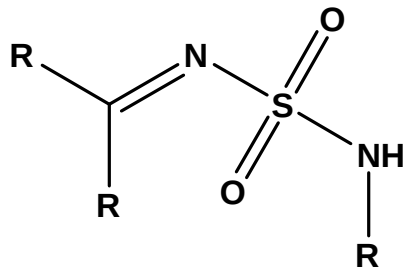
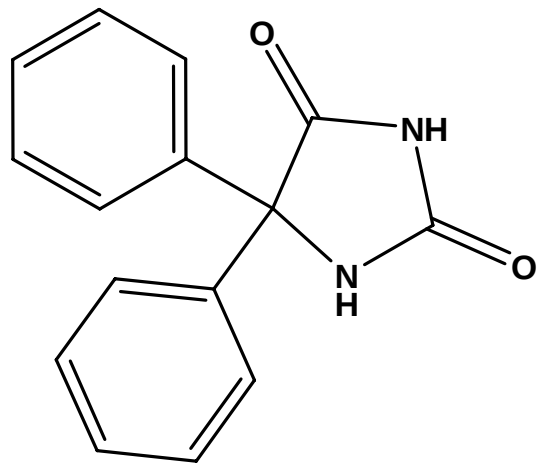


Using SLE to Develop a Mixed Mode SPE Method

- Previous method development project using an anion exchange SPE plate failed with a phenytoin type analogue
- New compound possesses active moiety similar to saccharin and should ionize at pH 4 to 5
- Chose SLE to evaluate extraction from aqueous standards to avoid secondary “chromatographic” behavioral properties of molecule and evaluate true partition coefficient properties
- Evaluated recovery of analyte at acidic, neutral, and basic pH using SLE





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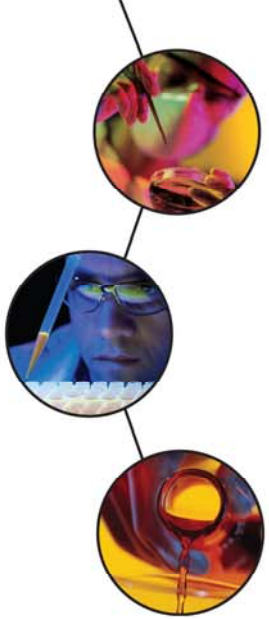

Method:

1) 100 uL of plasma (100 ng/mL analyte and IS) was diluted 1:1 with loading solvent (total volume = 200 uL). The entire sample was loaded onto the SLE+ plate.

2) Solution was allowed to flow into the bed using minimal vaccum and allowed to equilibrate for at least 5 min.

3) Water immiscible solvent was added to the bed and allowed to flow through. After the wells appeared dry, the solvent was allowed to equilibrate for an additional 2 min before vacuum was applied.

4) Samples were dried under a stream of nitrogen (~45 C) and reconstituted in 20:80 (ACN:H_2O, v/v)

Acidic loading solvent = 0.1% Formic acid in Water

Neutral loading solvent = Water

Basic loading solvent = 0.5% Ammonium Hydroxide in Water

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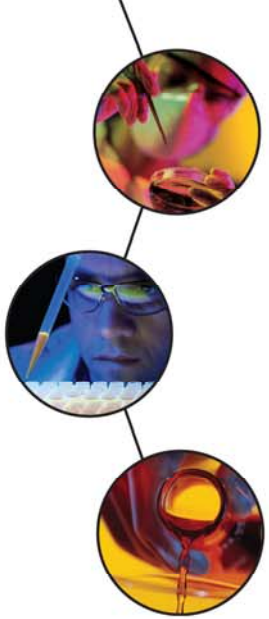

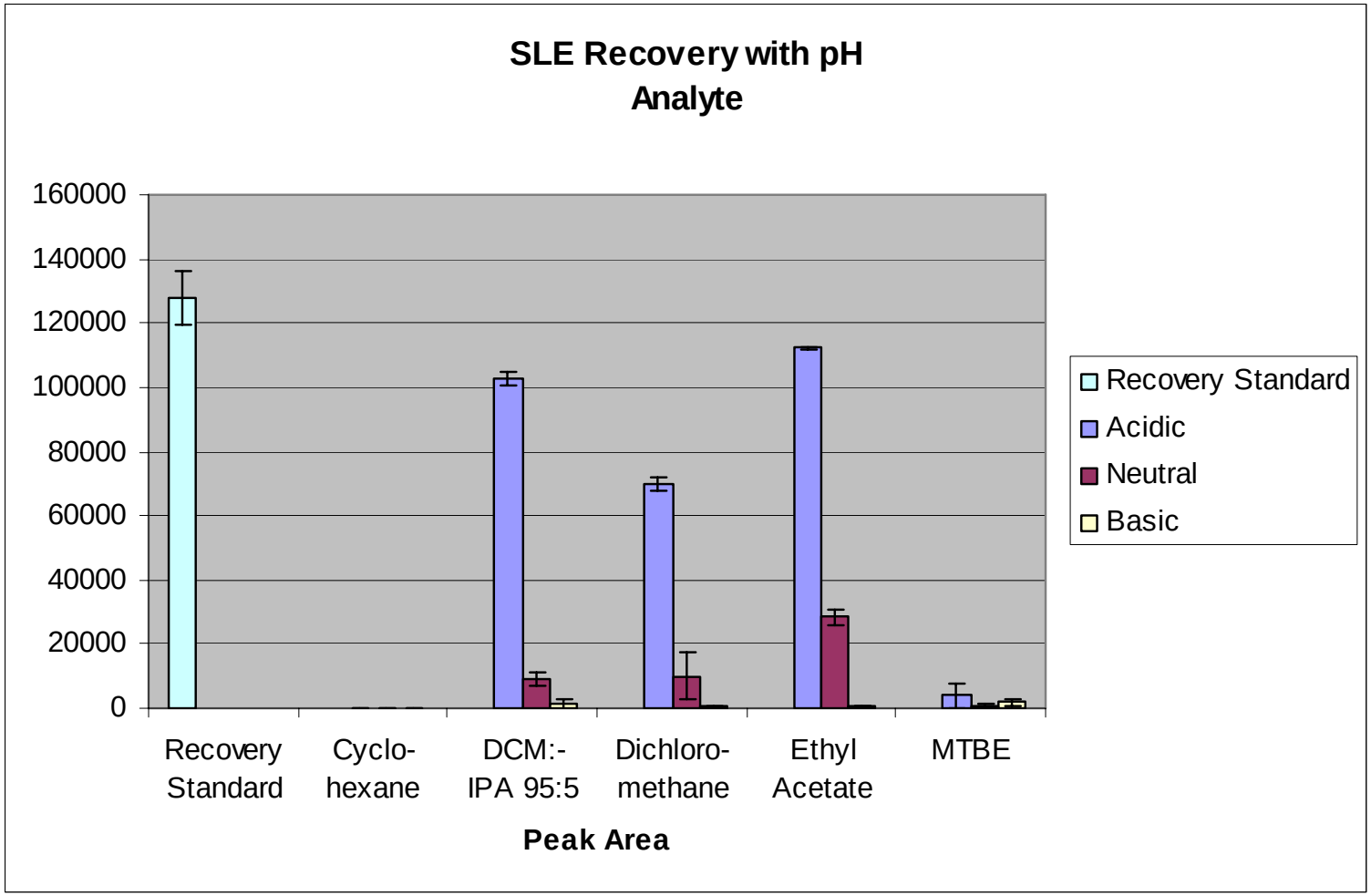

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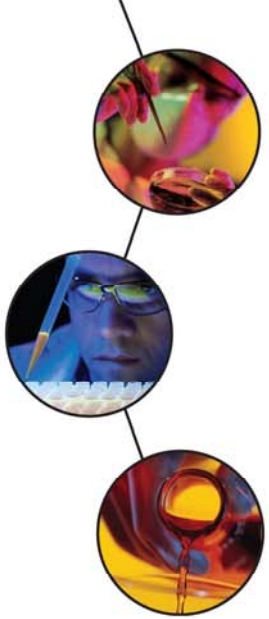

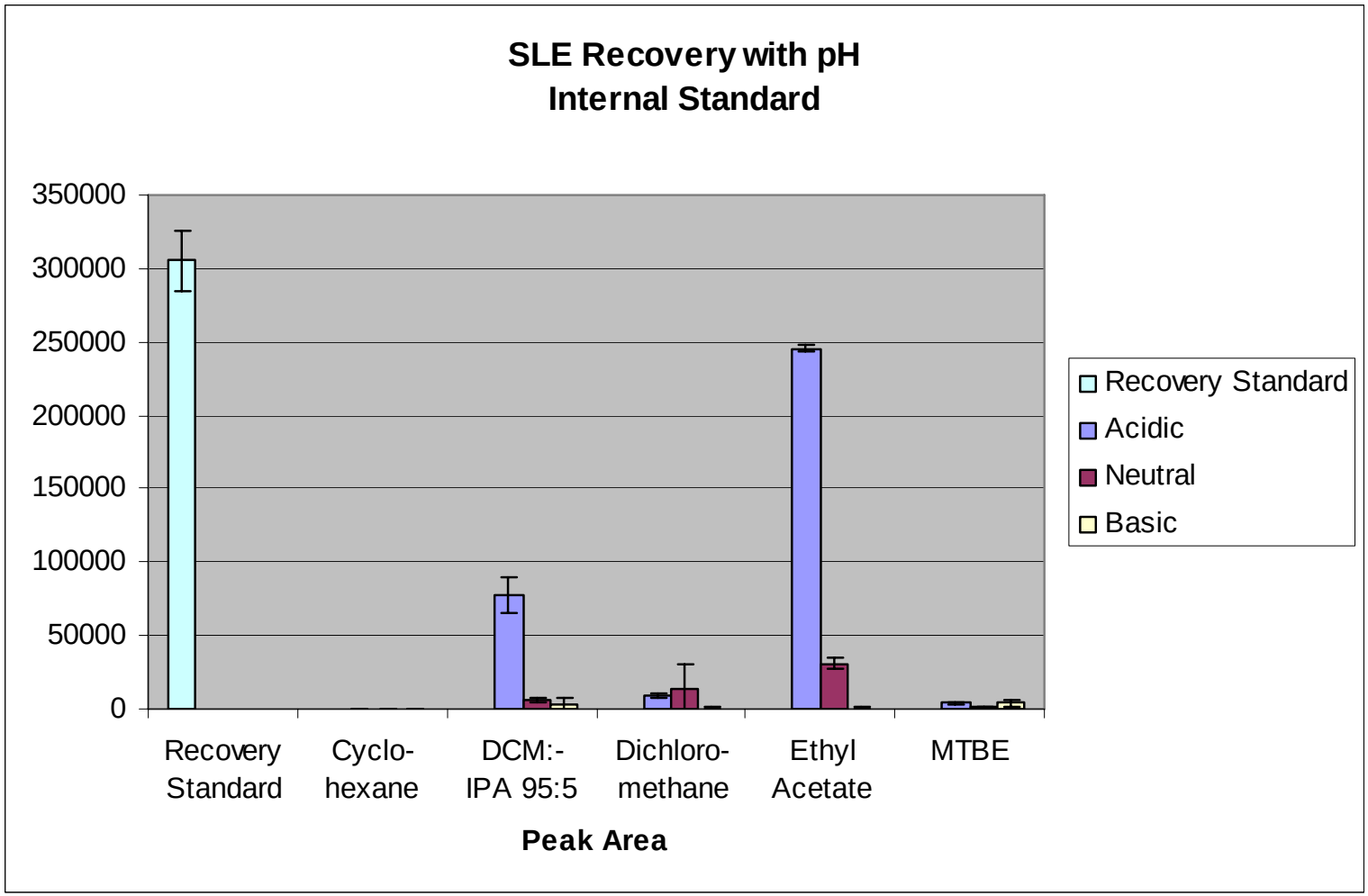

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