Comparison and Evaluation of Recombinant β-Glucuronidase Enzymes for Urine Drug Testing

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Disclosures

- The following companies have donated or significantly discounted products for use in this research
  - IMCS
  - Kura Biotec
  - MilliporeSigma
  - Phenomenex
  - Restek Corporation
- No conflicts of interest

Overview

- NWPL Background
  - Medium throughput laboratory
  - Current incubation time: 2 hours
  - New enzymes purport complete hydrolysis in as little as 15 minutes
- Objective
  - Reduce incubation time to 30-60 minutes
  - Determine overall value of selected protocol
    - Enzyme cost
    - Hydrolysis time
    - Sample preparation steps
    - Labor
    - Ruggedness (column lifetime)
  - Hydrolysis threshold of 80% used for "completion"

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    - Ruggedness (column lifetime)
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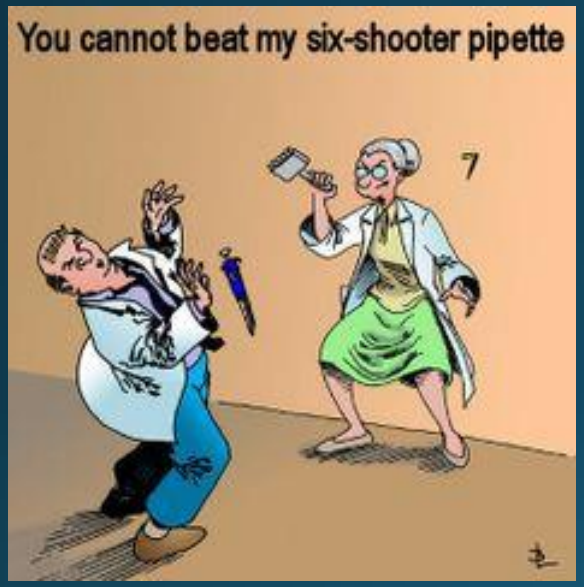
Workflow: Hydrolysis time 30-60 min

Workflow: Hydrolysis time 15 min



Too much time to do nothing, not enough time to do anything!

Experimental: Sample Analysis

- Sample Analysis
  - LC-MS/MS
  - Shimadzu Prominence-AB SCIEX QTRAP® 4500
- Evaluation Criteria
  - Hydrolysis efficiency: >80% glucuronide cleavage
  - Ruggedness
    - Protein concentration (Bradford Assay)
    - Sample clean-up
    - Column degradation
  - Overall value

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    - Sample clean-up
    - Column degradation
  - **Overall value**

Value

- Data quality
- Material costs
  - Column
  - Enzyme
- Labor
  - Sample preparation steps
  - Total time

Experimental: Sample Preparation

- Five Enzymes
  - BG100® (Kura Biotec)
  - BGTurbo™ (Kura Biotec)
  - IMCSzyme® (IMCS)
  - IMCSzyme XL (IMCS)
  - β-Glucuronide Glucuronosohydrate (Millipore Sigma)
- Samples were prepared according to manufacturers' recommended guidelines
  - Slight modifications for workflow efficiency
  - Main modification – incubation temperature
    - Ranges were 50-70°C
    - 60°C used for research

Experimental: Sample Preparation

- Sample preparation
  - "Dilute and shoot"
  - 50 µL urine
  - Enzymes
    - 15 µL BG100 (>100,000 units/mL)
    - 15 µL BGTurbo (>200,000 units/mL)
    - 15 µL IMCSzyme (>50,000 units/mL)
    - 15 µL IMCSzyme XL (>100,000 units/mL)
    - 15 µL Sigma (100,000-200,000 units/mL)
    - Note: User has ability to increase amount enzyme added to decrease hydrolysis times
  - Hydrolysis solution diluted approximately 4x prior to analysis
  - Urine sample diluted approximately 12x for analysis.

Hydrolysis Efficiency

- Fifteen glucuronidated drug standards were utilized to determine hydrolysis efficiency
- Drugs from several classes were studied
  - Benzodiazepines
  - Opiates
  - Opioids
  - Tricyclic antidepressants
  - THC
- Time points (min): 0 (w/ and w/o enzyme), 15, 30, 45, 60, and 75

Hydrolysis Efficiency: Drug Standards

- Mix 1
  - Codeine 6β glucuronide: 20000 ng/mL
  - Morphine 3β glucuronide: 20000 ng/mL
  - Oxymorphone 3β glucuronide: 20000 ng/mL
  - Oxazepam glucuronide: 10000 ng/mL
- Mix 2
  - Dihydrocodeine 6β glucuronide: 20000 ng/mL
  - Morphine 6β glucuronide: 20000 ng/mL
  - Tapentadol glucuronide: 10000 ng/mL
  - Lorazepam glucuronide: 10000 ng/mL
  - Temazepam glucuronide: 10000 ng/mL

Hydrolysis Efficiency: Drug Standards

- Mix 3
  - Amitriptyline glucuronide: 5000 ng/mL
  - 6β Naltrexol 3β-D glucuronide: 5000 ng/mL
  - Naltrexone 3β-D glucuronide: 5000 ng/mL
  - Buprenorphine glucuronide: 2500 ng/mL
  - Norbuprenorphine glucuronide: 2500 ng/mL
  - 11-Nor-Δ9-THC-carboxylic acid glucuronide: 2500 ng/mL

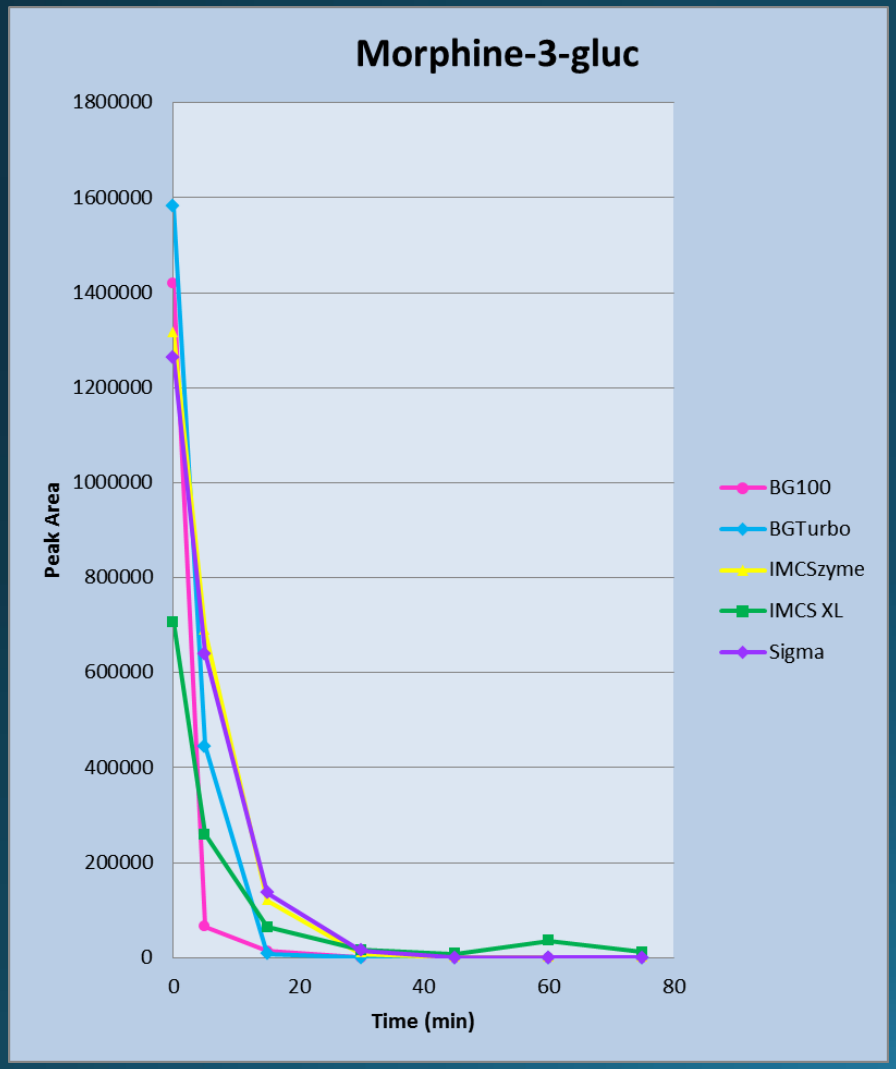
Hydrolysis Results: Summary

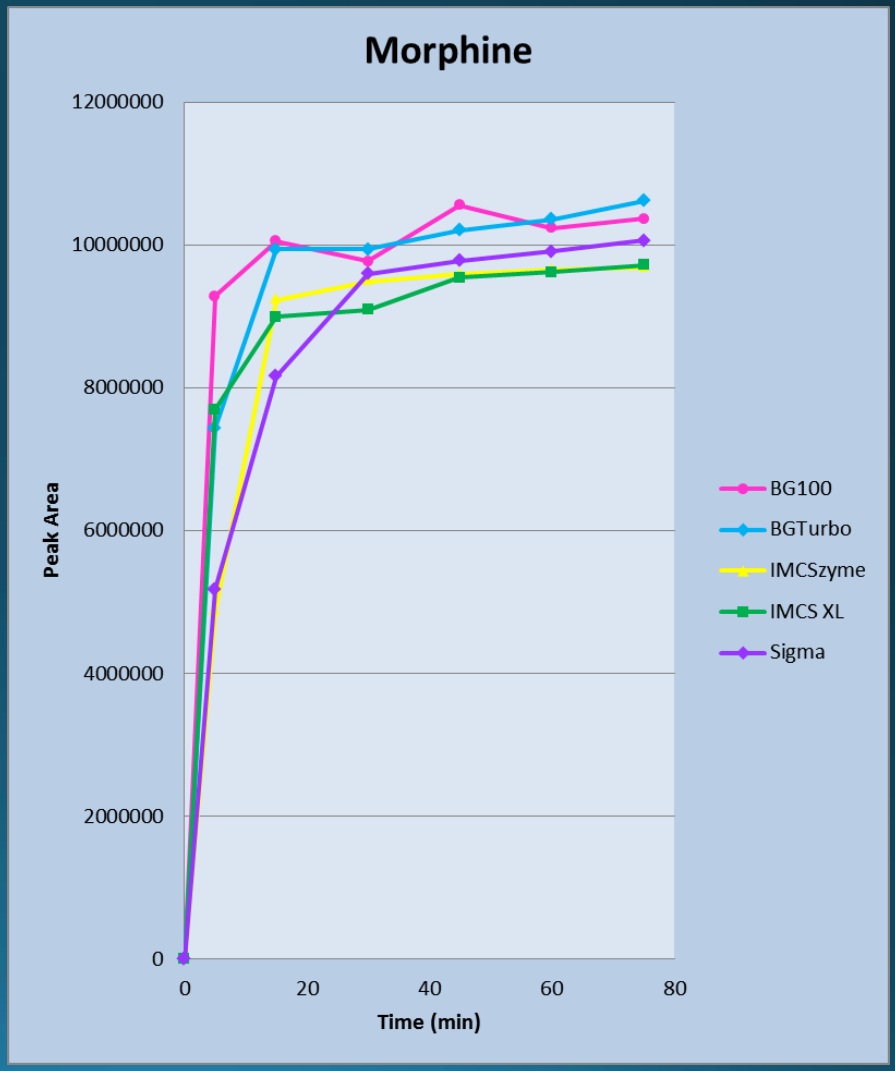
	BG100	BGTurbo	IMCSzyme	IMCS XL	Sigma
Morphine 3	<15 min	<15 min	<15 min	<15 min	<15 min
Buprenorphine	<15 min	<15 min	<15 min	<15 min	<15 min
Norbuprenorphine	<15 min	<15 min	<15 min	<15 min	<15 min
6-B-Naltrexol	<15 min	<15 min	<15 min	<15 min	<15 min
Naltrexone	<15 min	<15 min	<15 min	<15 min	<15 min
Lorazepam	<15 min	<15 min	<15 min	<15 min	<15 min
Oxazepam	<15 min	<15 min	<15 min	<15 min	<15 min
Temazepam	<15 min	<15 min	<15 min	<15 min	<15 min
THC-COOH	<15 min	<15 min	<15 min	<15 min	<15 min
Oxymorphone	<15 min	15 min	30 min	30 min	15 min
Tapentadol	~40 min	<15 min	15 min	30 min	45%
Morphine 6	45 min	45 min	~40 min	30 min	45%
Codeine 6	60 min	30 min	~55 min	~50 min	30%
Amitriptyline	40%	<15 min	<15 min	<15 min	<10%
Dihydrocodeine	60%	<15 min	75%	~40 min	<10%

Hydrolysis graphs

- Two replicate injections per enzyme and time point
- ***Raw area counts*** are graphed (did not run calibration for glucuronidated compounds)
- Mean of the two injections is graphed
- Two "To" time points
  - Without enzyme – graphed at 0 minues
  - With enzyme – graphed at 5 minutes

Morphine-3-Glucuronide Hydrolysis





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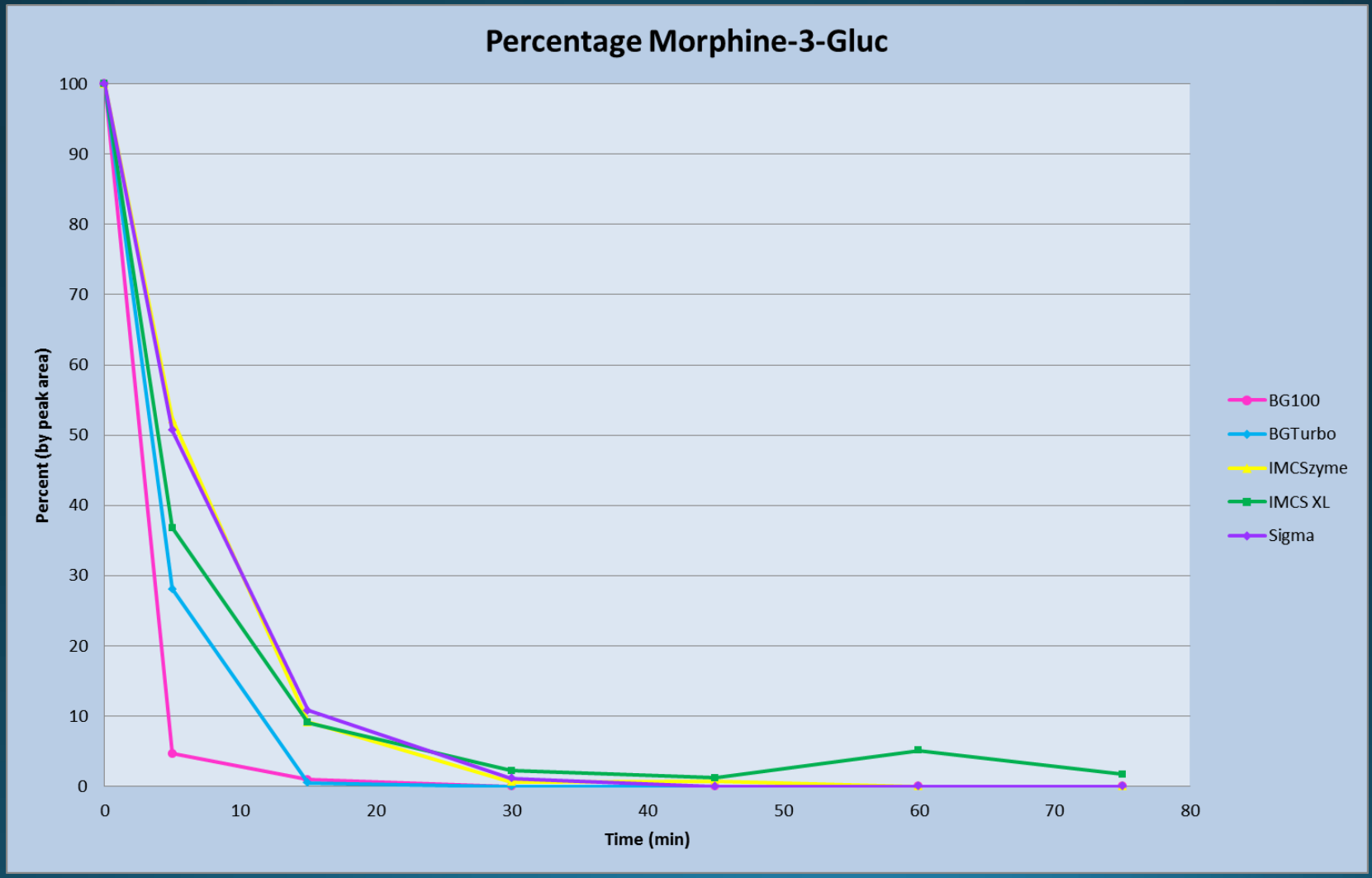
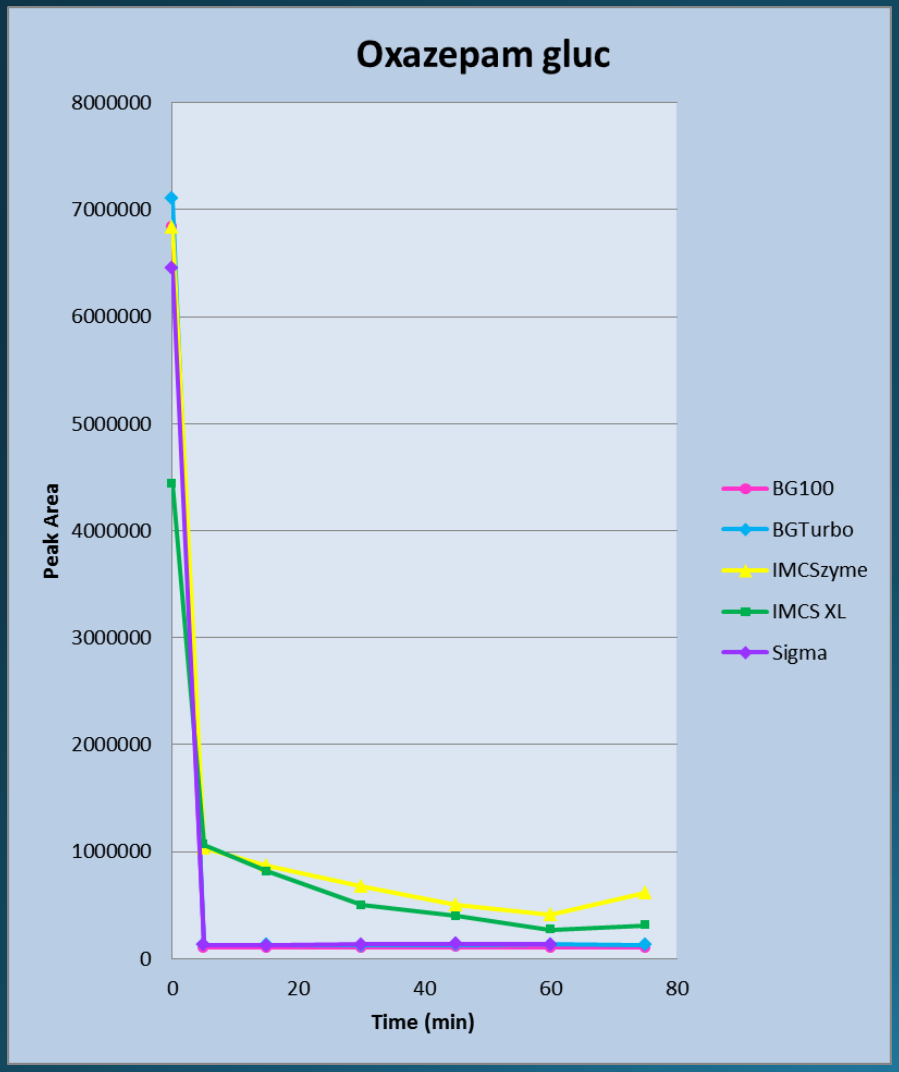
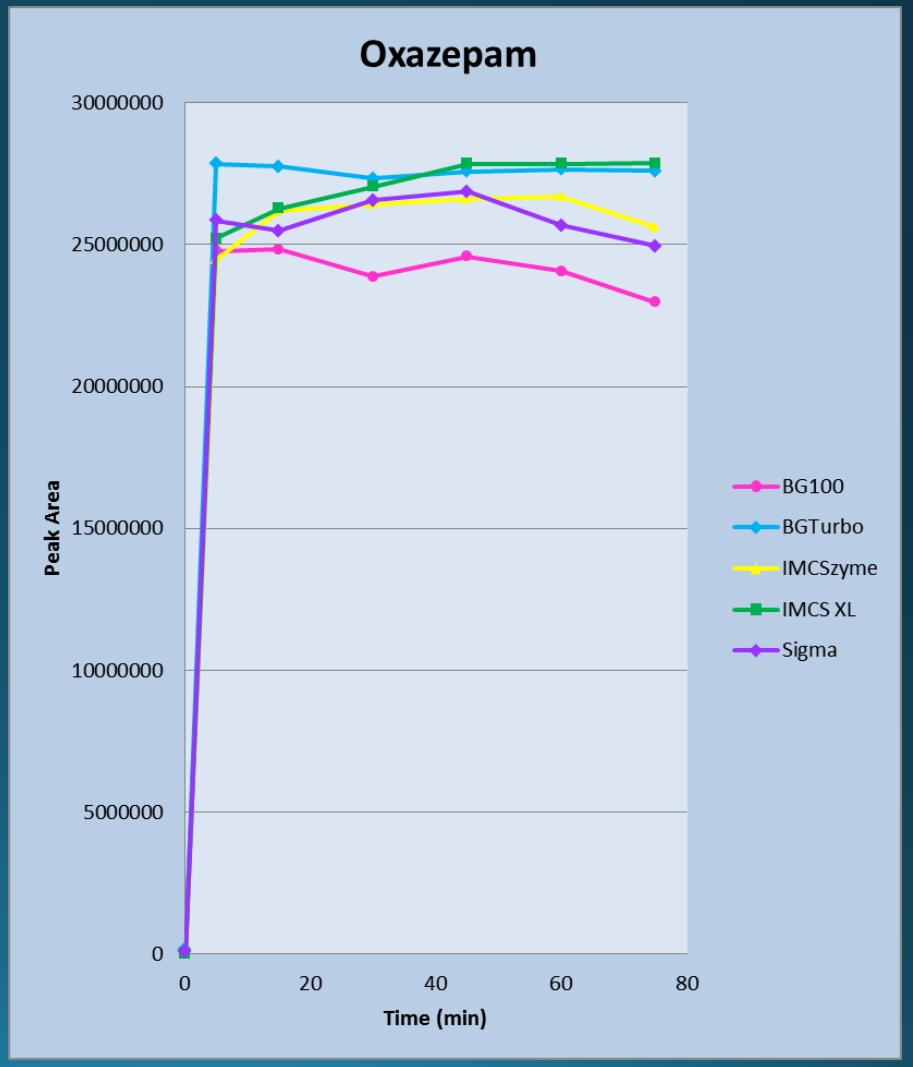


Oxazepam Hydrolysis





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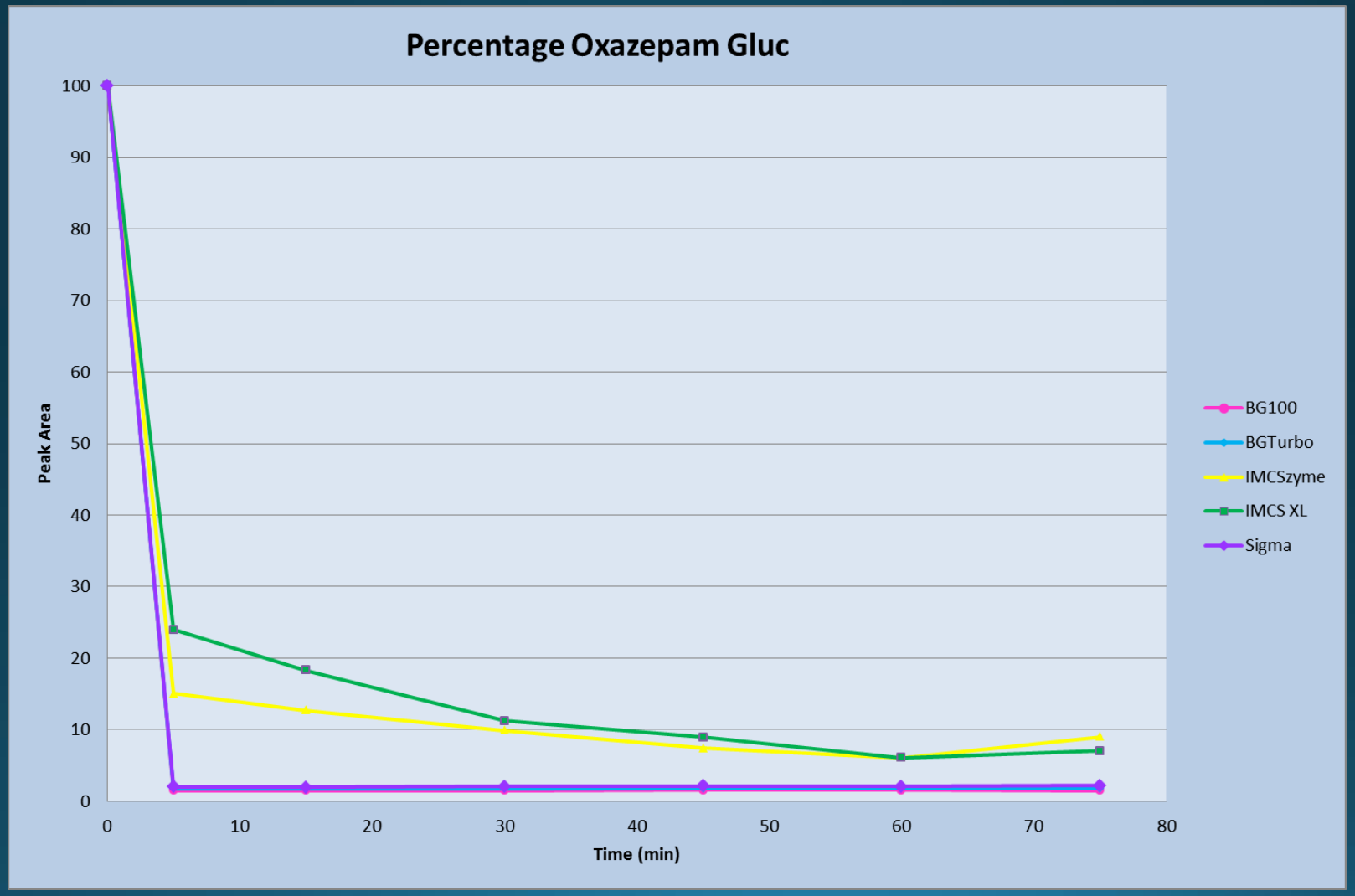
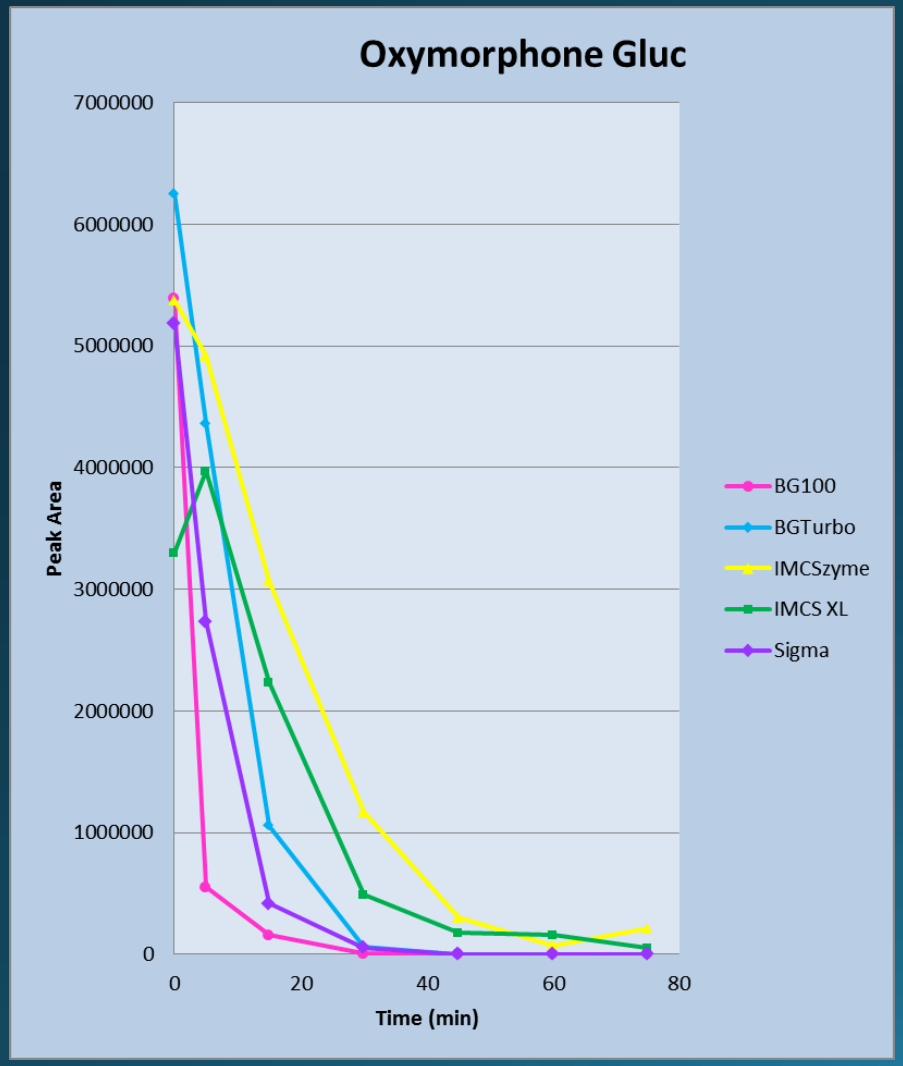
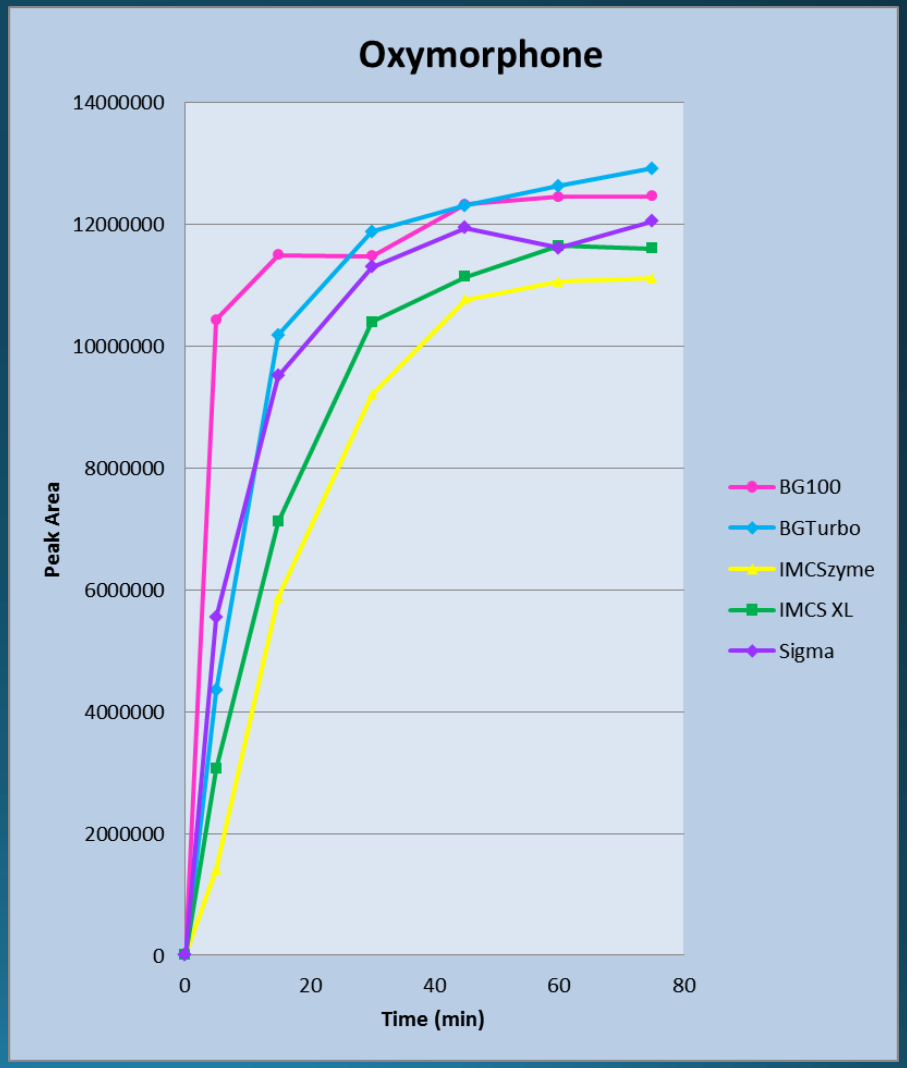


Oxymorphone Hydrolysis





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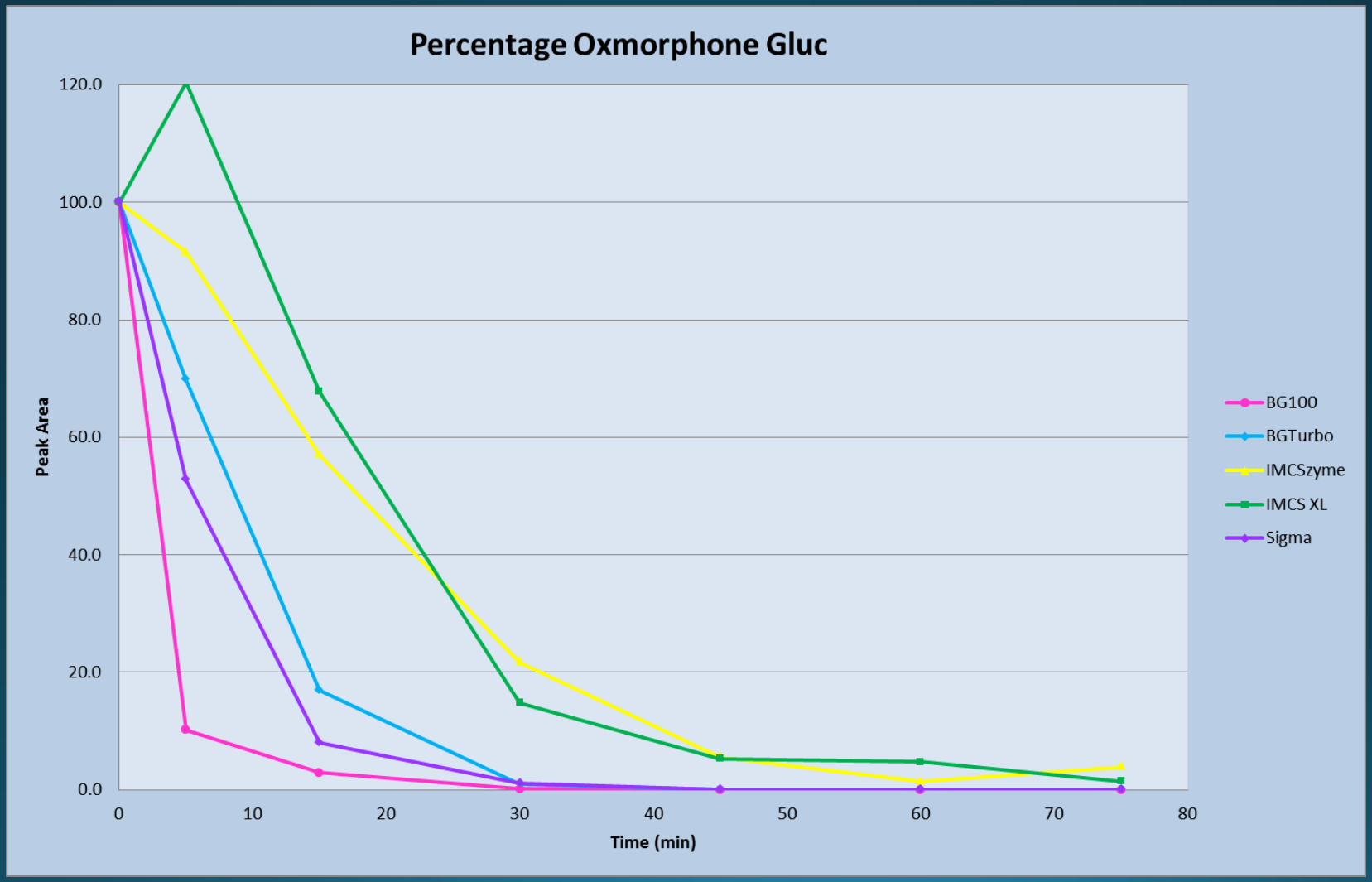
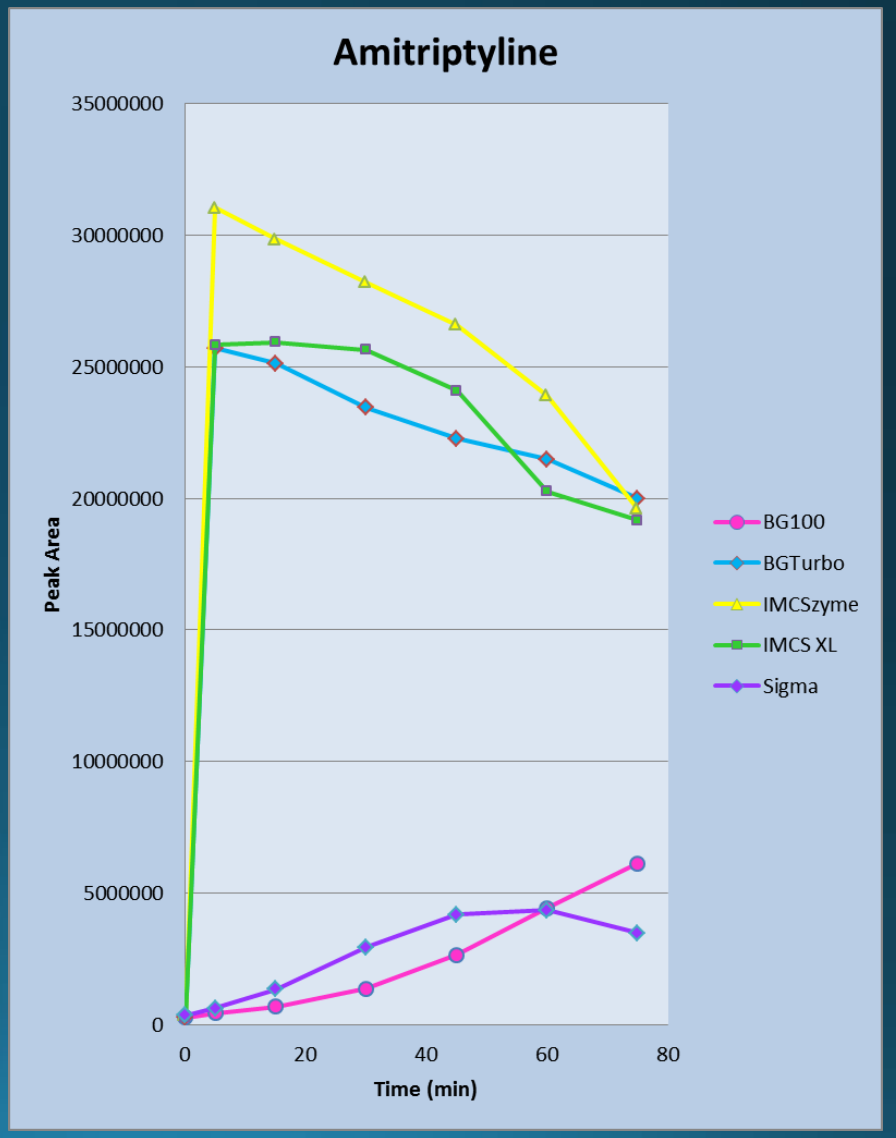


Amitriptyline Hydrolysis



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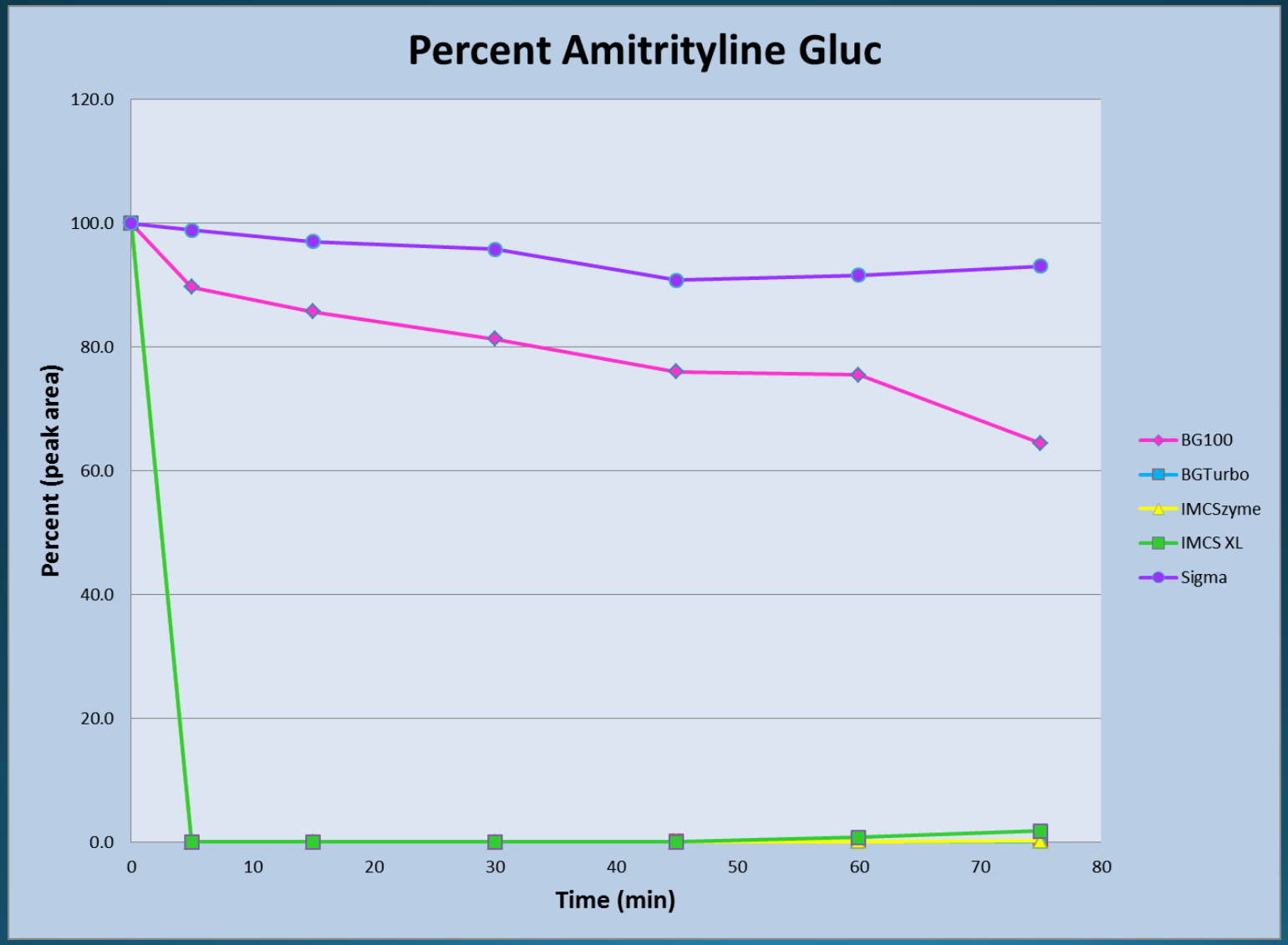
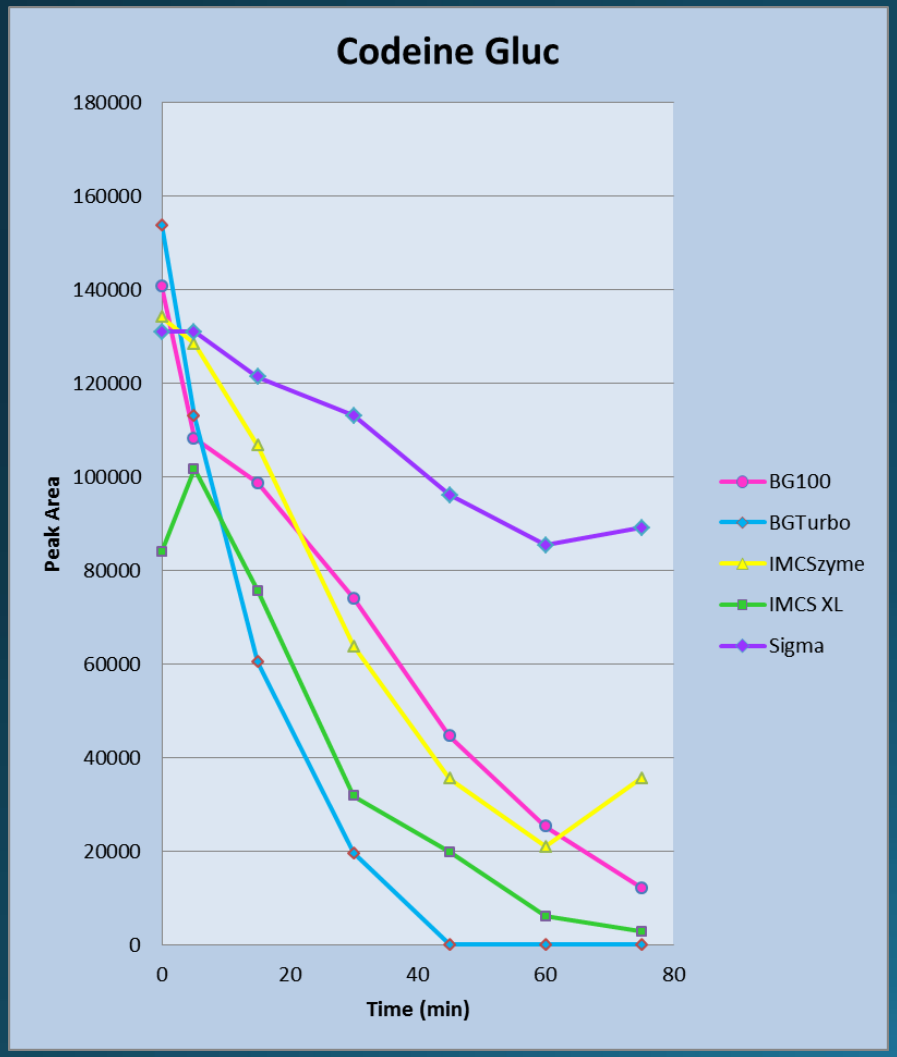
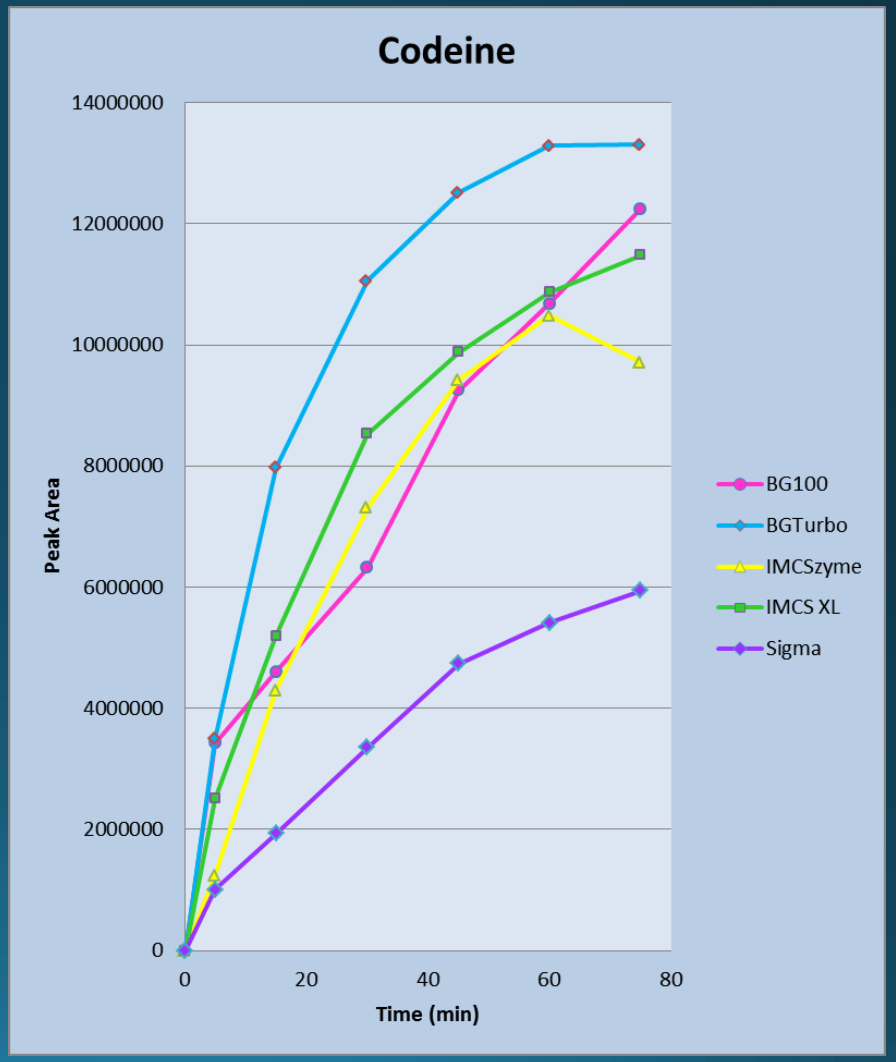


Codeine 6β Glucuronide Hydrolysis





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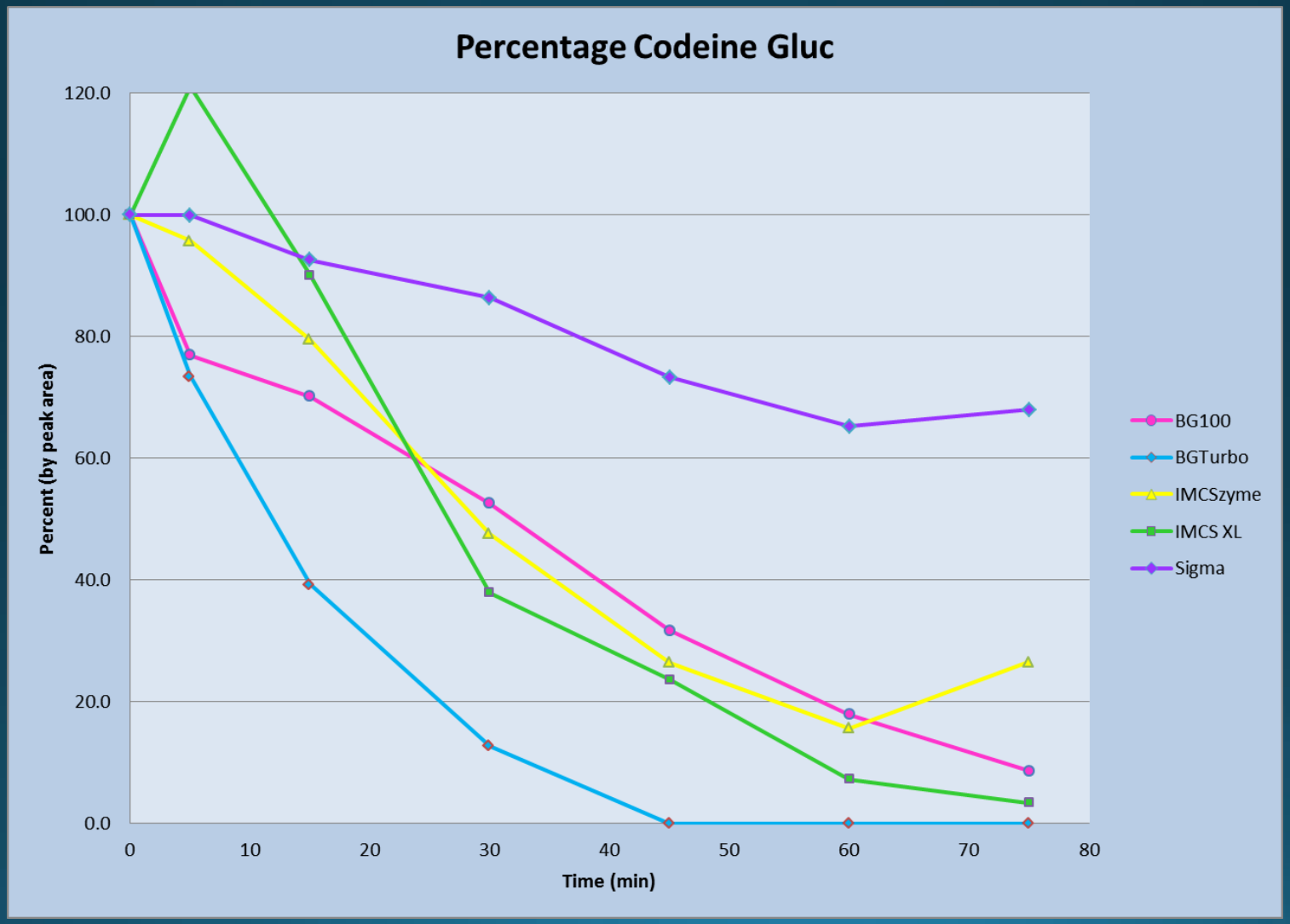
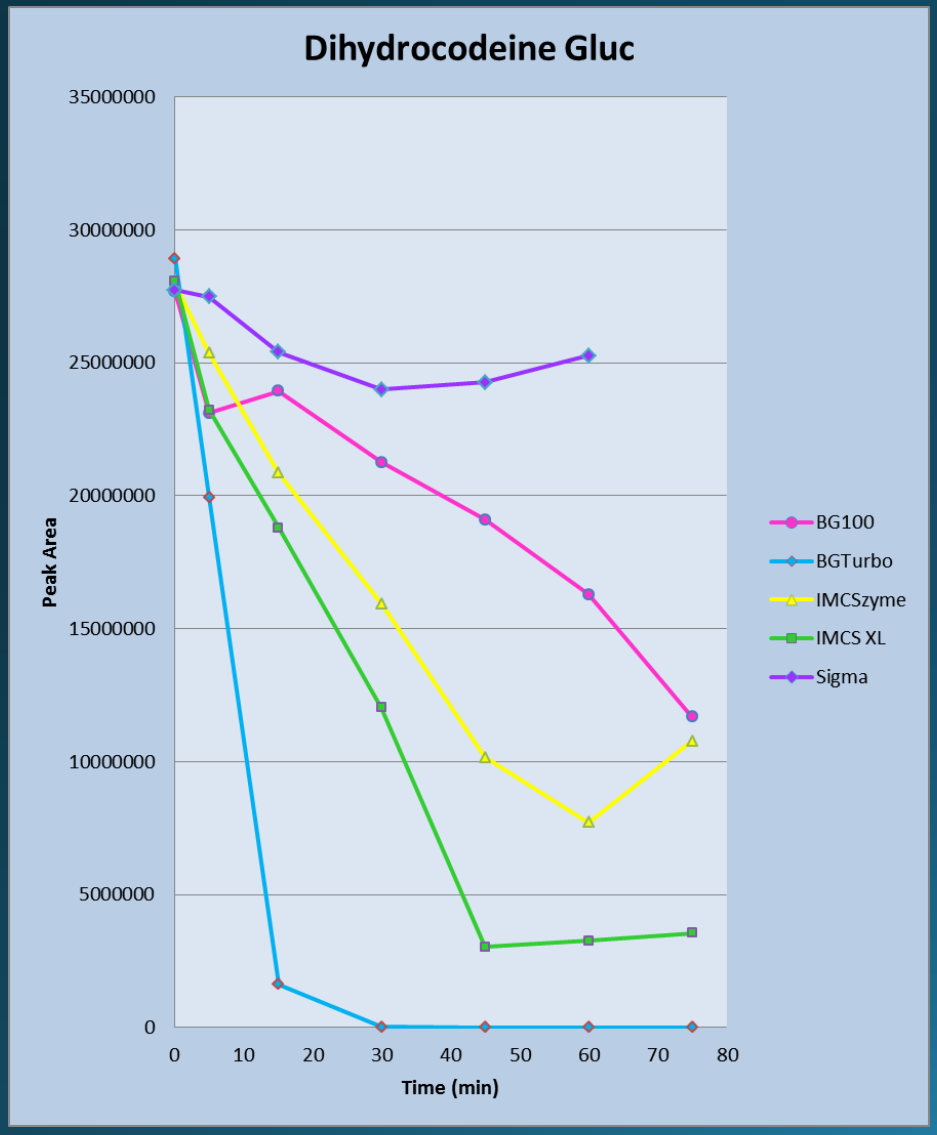
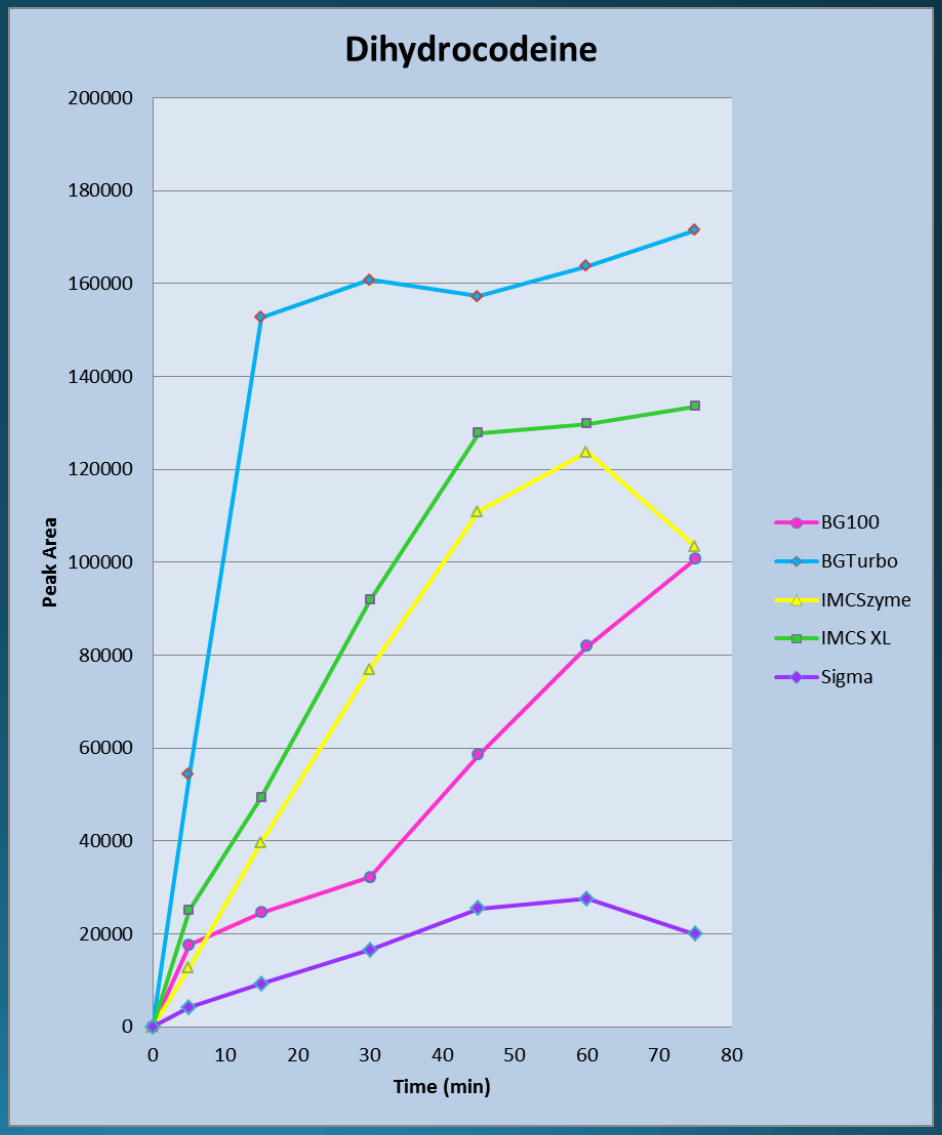


Dihydrocodeine 6β Glucuronide Hydrolysis





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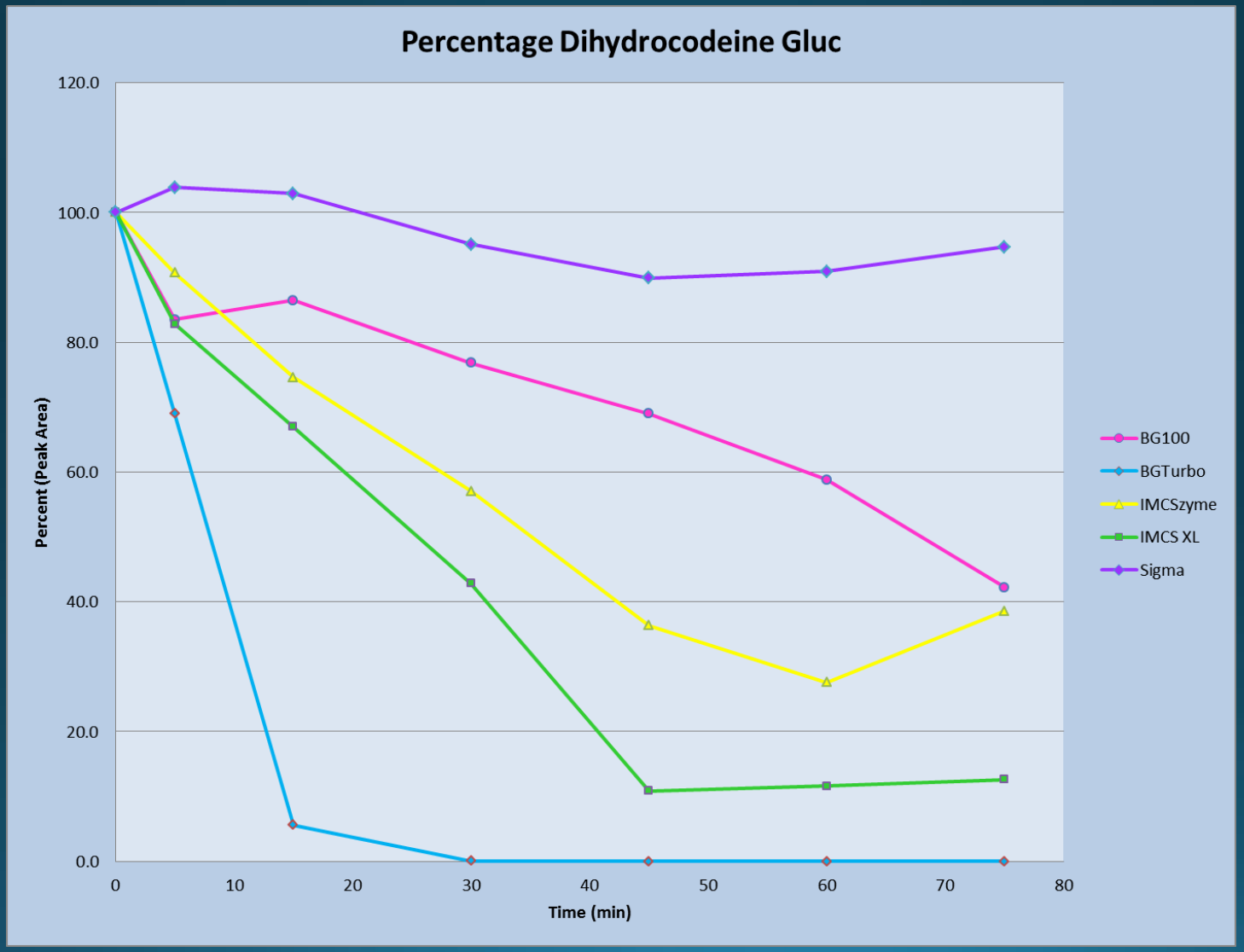
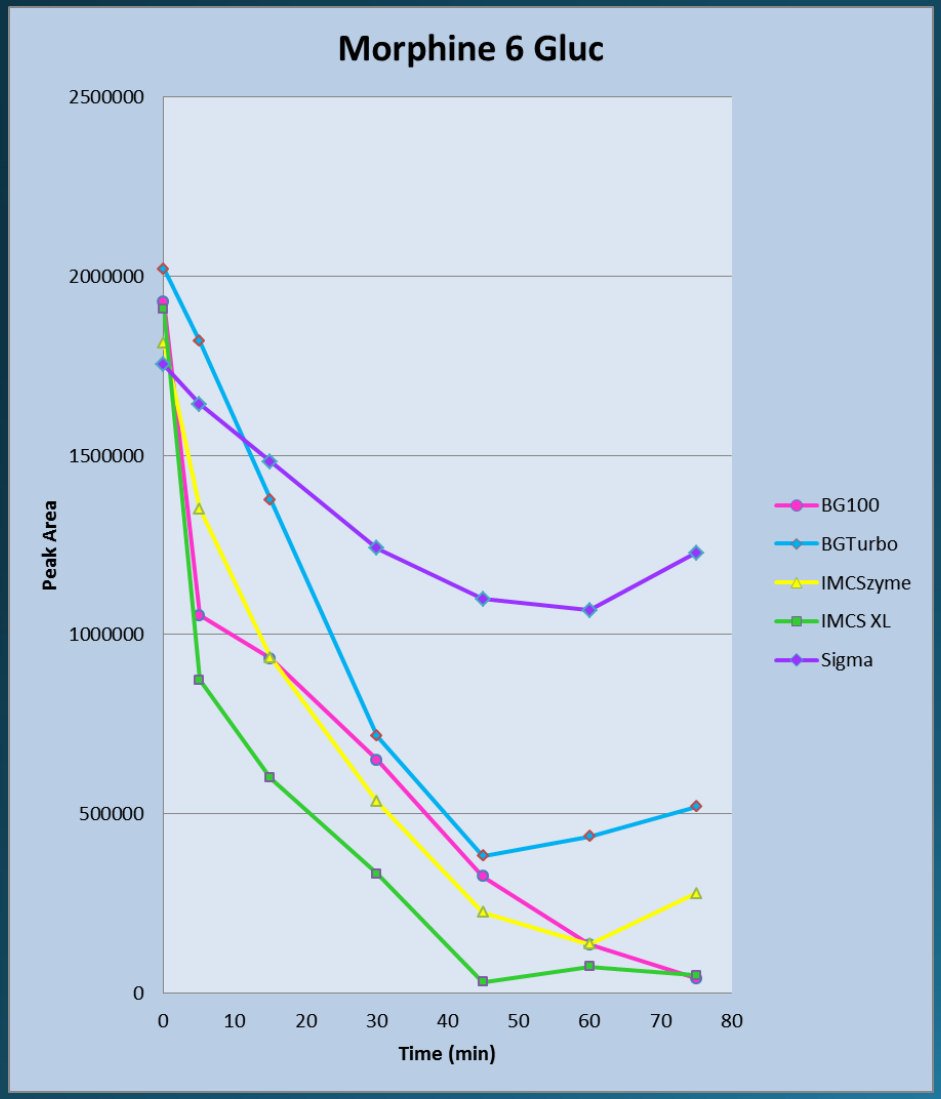
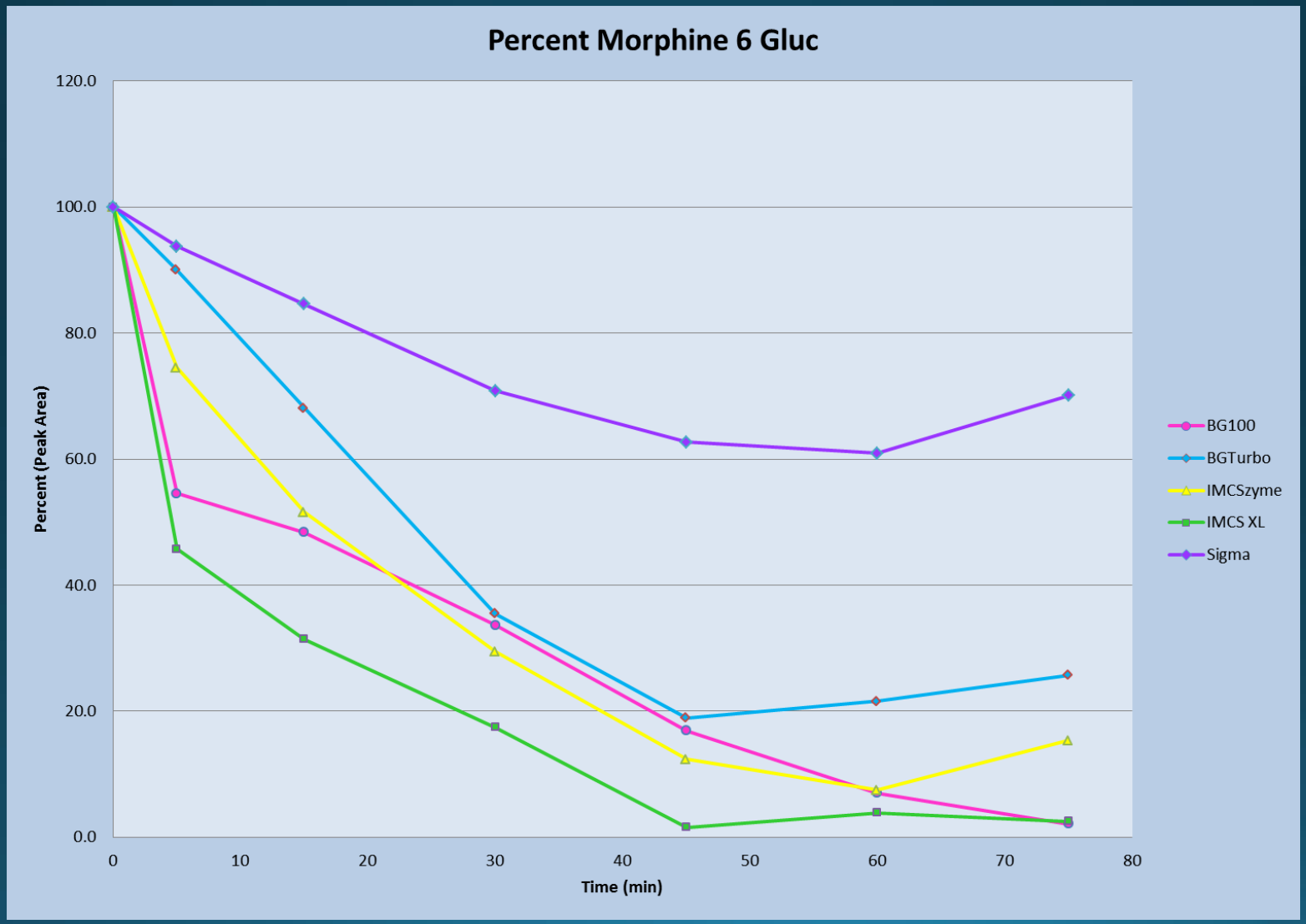


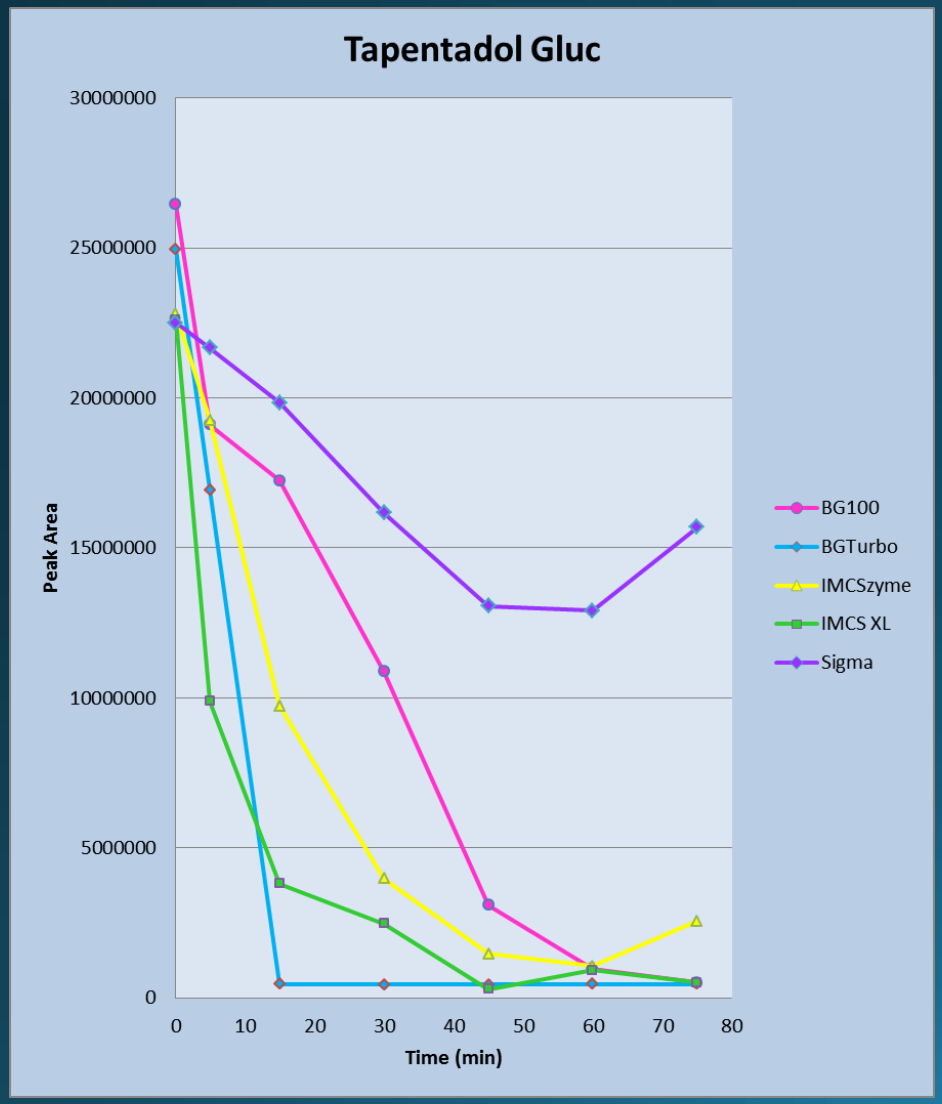
Morphine 6β Glucuronide Hydrolysis

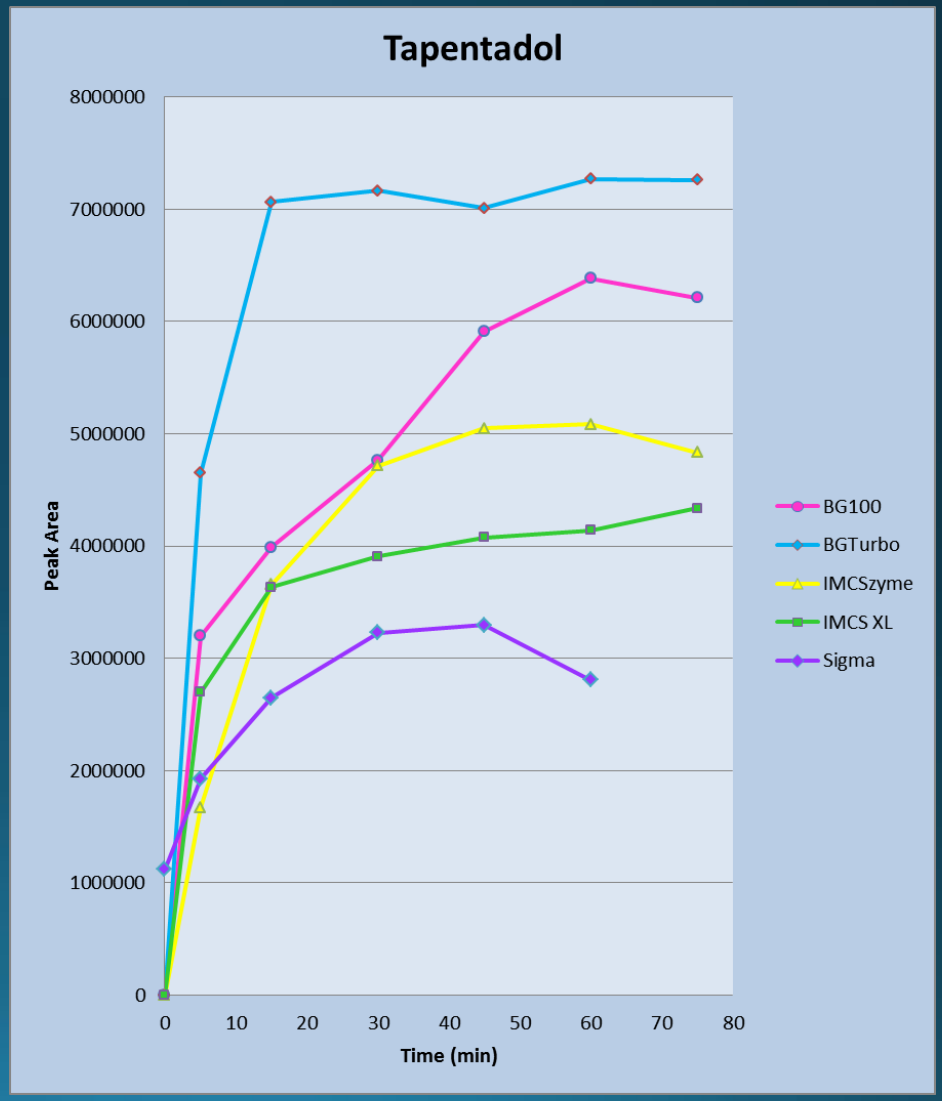


Morphine 6β Glucuronide Hydrolysis



Tapentadol Hydrolysis





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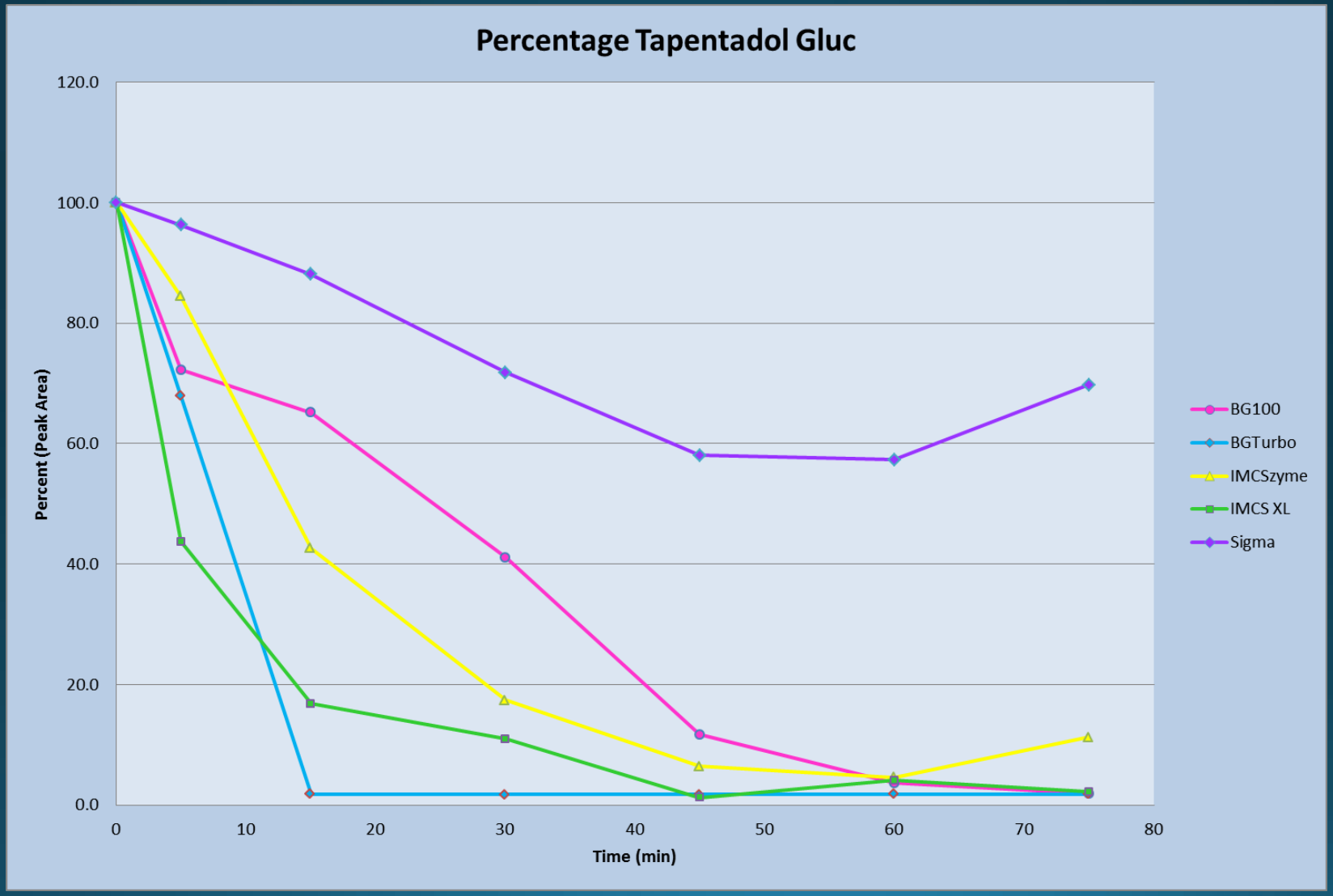

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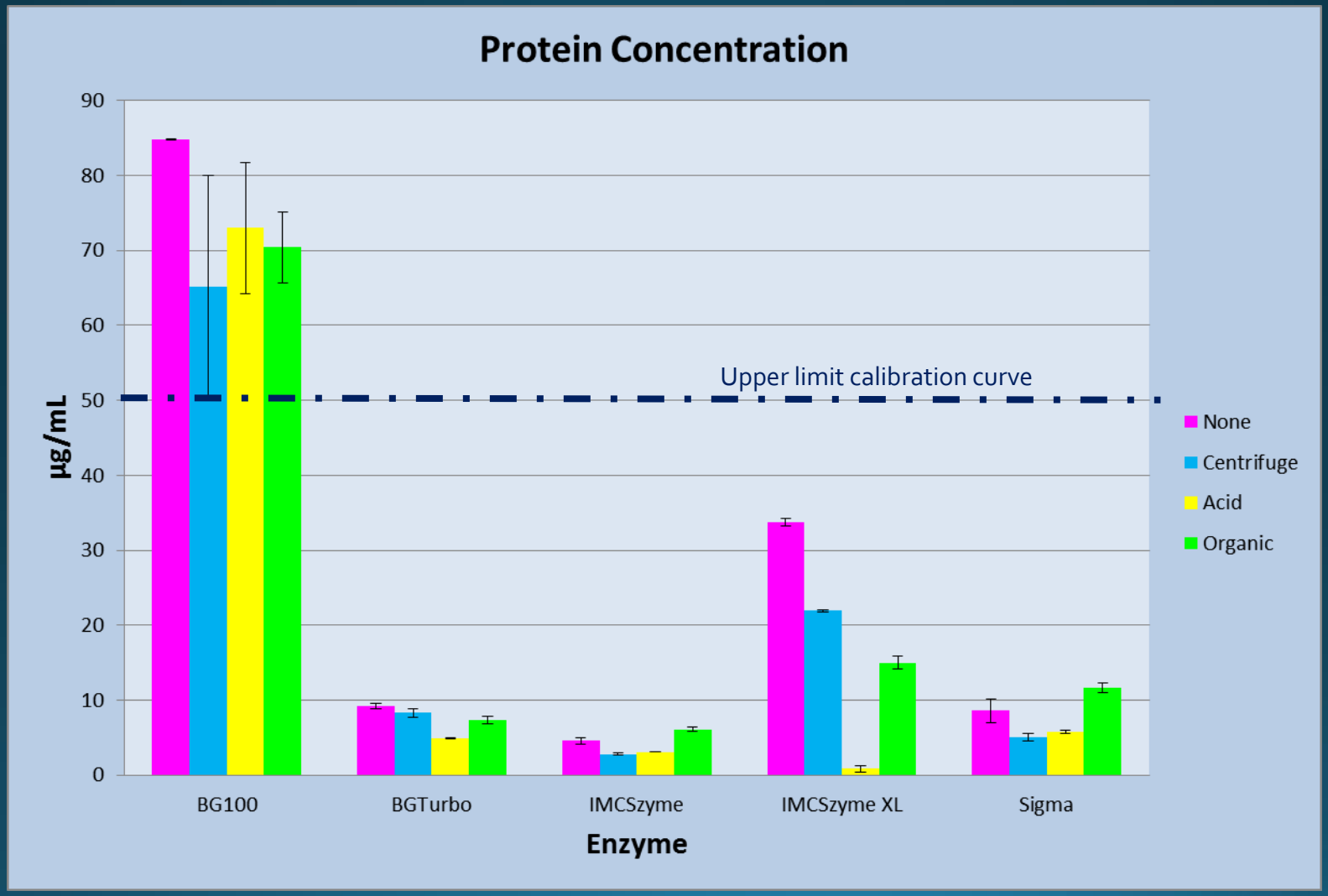
  - Lorazepam glucuronide: 10000 ng/mL
  - Temazepam glucuronide: 10000 ng/mL

Protein Concentration

- Bradford Assay
- Commercial blank urine (UTAK) used as matrix
- Four sample preparation protocols
  - None
  - Centrifuge only
  - 5 mM AmmFor with 0.1% formic acid precipitation + centrifuge
  - ACN organic precipitation + centrifuge

Protein Concentration



Ruggedness

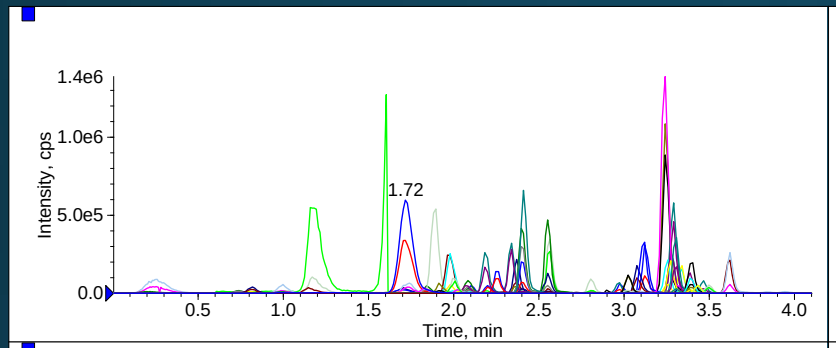
- Approximately 2000 injections of mid-level QC
- Parameters evaluated
  - Peak area
  - Retention time (shift)
  - Peak shape/width
  - Accuracy of measured concentration
- Results
  - BG100 showed most degradation
    - RT Shift
    - Peak width increase
    - No significant affect on accuracy or detection
  - Other enzymes showed no significant affect on chromatographic data across ~2000 injections

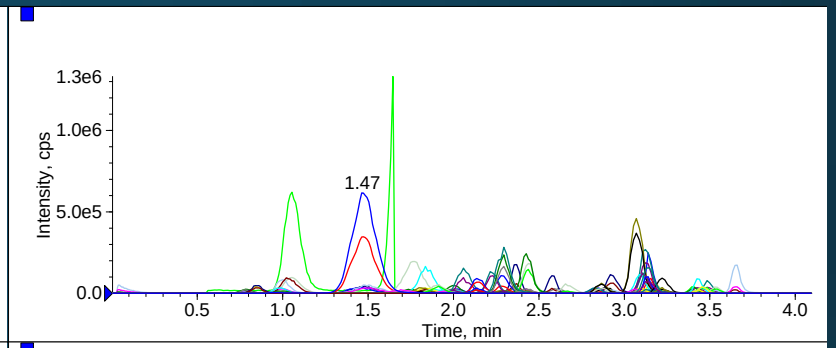
Ruggedness: BG100 - Chromatograms

Injection 1

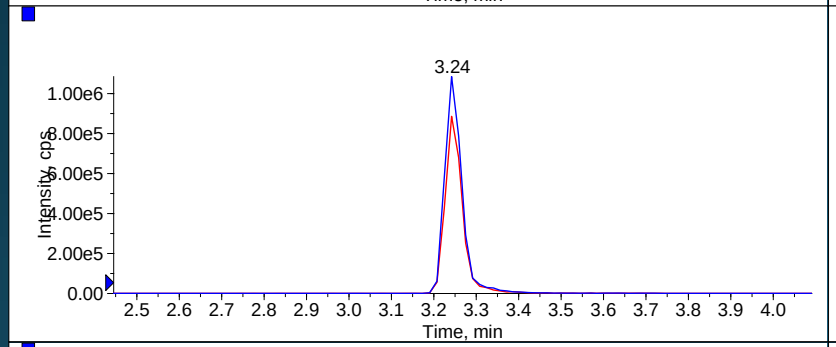
Injection ~1600

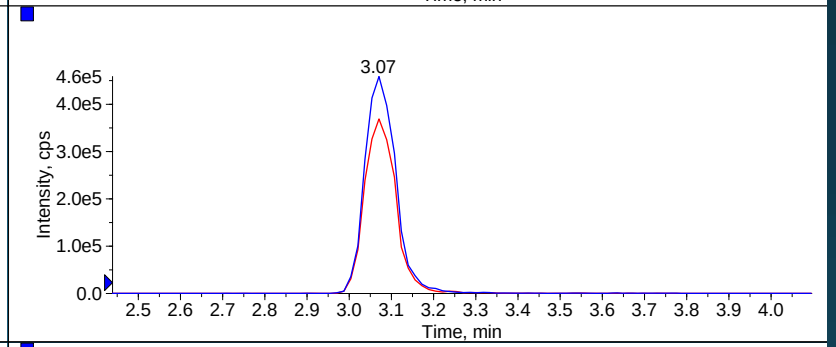
All



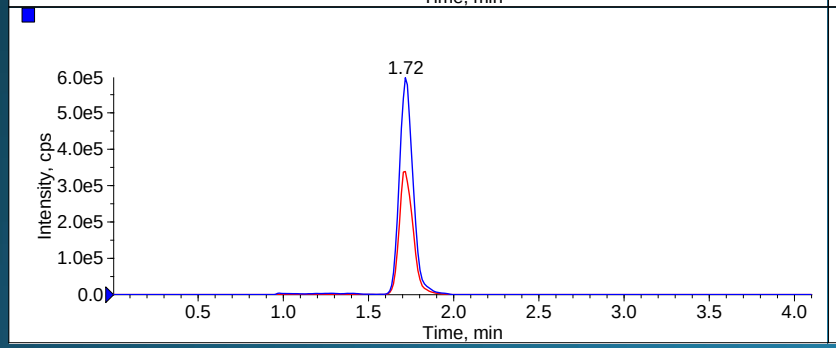


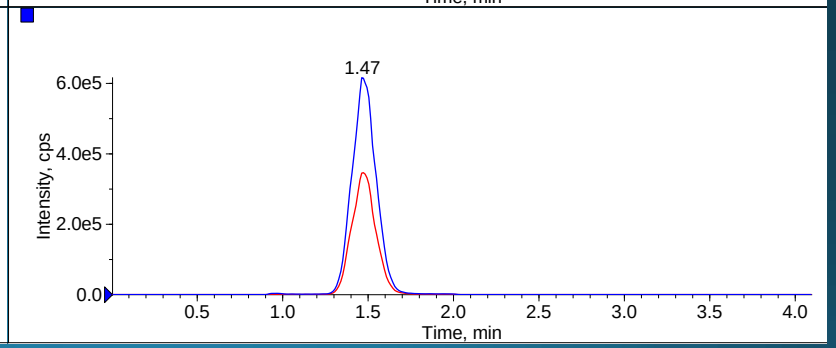
EDDP



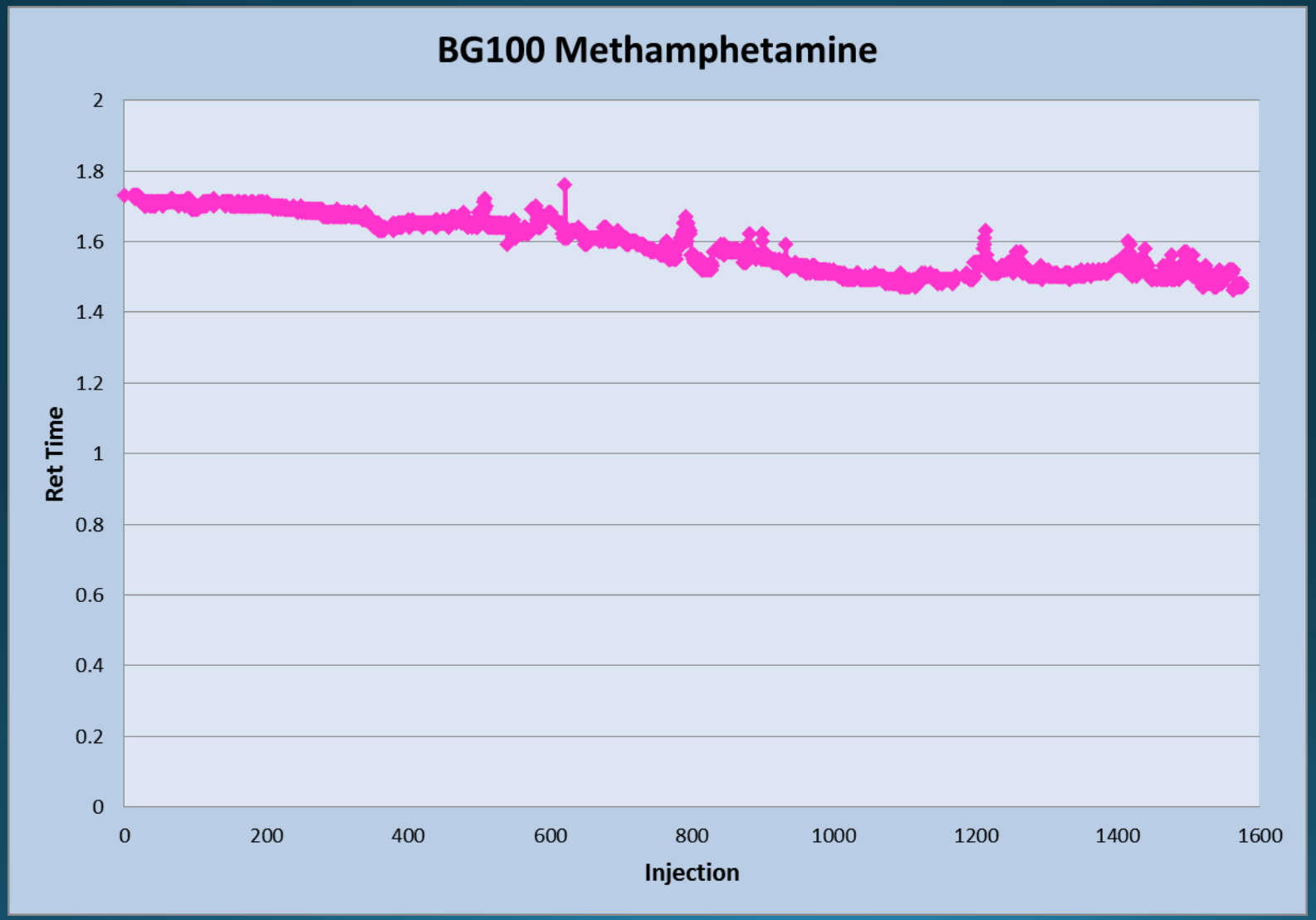


mAMP

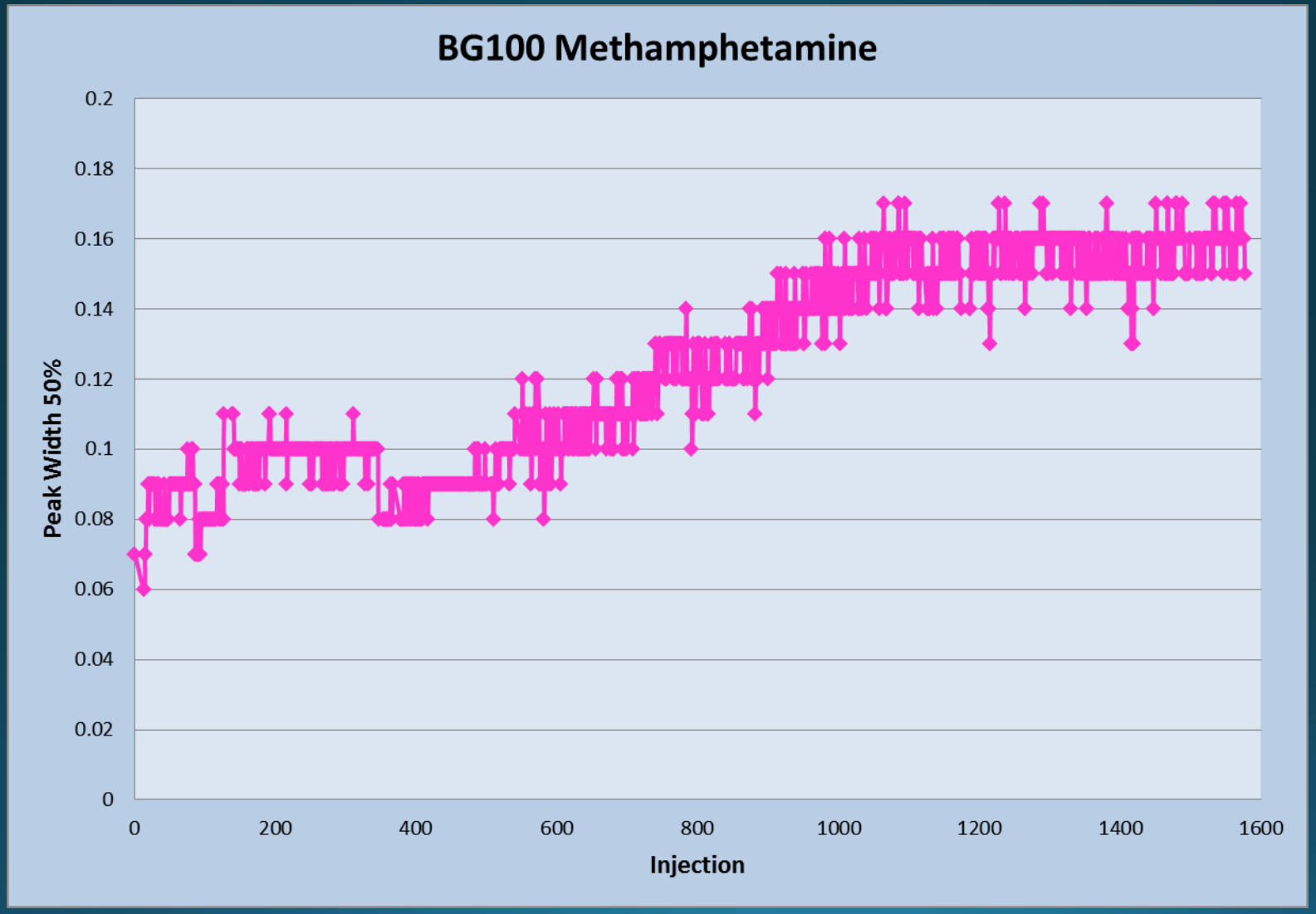




Ruggedness: BG100 – Retention Time



Ruggedness: BG100 – Peak Width



Value: Cost

Enzyme	List Price (USD)	Size (mL)	$/mL	$/sample*
BG100	$205.00	10	$20.50	$0.32
	$410.00	25	$16.40	$0.25
BG Turbo	$200.00	5	$40.00	$0.62
	$300.00	10	$30.00	$0.46
	$490.00	20	$24.50	$0.38
IMCSzyme	$99.00	5	$19.80	$0.30
	$189.00	10	$18.90	$0.29
	$899.00	50	$17.98	$0.28
IMCSzyme XL	$99.00	5	$19.80	$0.30
	$189.00	10	$18.90	$0.29
	$899.00	50	$17.98	$0.28
Sigma	$138.00	10	$13.80	$0.21
	$321.00	25	$12.84	$0.20
	$610.00	50	$12.20	$0.19

*based on 15 µL/sample

Conclusions: Summary Table

Enzyme	Hydrolysis Time (min)	Hydrolysis Coverage	Ruggedness	Enzyme Cost (USD)*
BG100	45-60	+++	+	$0.25-$0.32
BG Turbo	15-30	++++	++++	$0.38-$0.62
IMCSzyme	45-60	++++	++++	$0.28-$0.30
IMCSzyme XL	30-45	++++	++++	$0.28-$0.30
Sigma	30-45	++	+++	$0.19-$0.21

***based on 15 µL/sample**

Conclusions

- Hydrolysis times
  - BGTurbo: 15 minutes
  - IMCSzyme XL: 15-30 minutes
  - IMCSzyme: 45 minutes
  - BG100 and Sigma: Insufficient hydrolysis for TCAs, some opiates --- Good enough?
  - Can increase amount of enzyme used to decrease times
- Column ruggedness
  - BG100 showed some degradation over 1600 injections
  - All other enzymes appear relatively equivalent with no noticeable change in data quality across 2000+ injections
- Enzyme cost per sample (based on list price)
  - Sigma<BG100≈IMCSzyme=IMCSzyme XL<BGTurbo

Acknowledgments

- NWPL
  - Scott Cole, Lab Manager
  - L. Vanimireddy, Ph.D., Lab Director
- IMCS
  - Claire Collins, Director Sales and Marketing
  - Nikki Sitasuwan, Ph.D., Research Scientist
- Kura Biotec
  - Manual Rozas, CEO
  - Yves-Vincent Duperron, Director Global Sales and Marketing
- MilliporeSigma
  - Caryn Kiesow, Product Specialist
  - John Cooper, Product Marketing Manager
  - Hillel Brandes, Analytical Technology Specialist
- Phenomenex
  - Haroon Rehmani, Technical Consultant
- Restek
  - Eisho Beythaji, Sales Representative

Thank you for your attention