

# Automation of Liquid-Liquid Extractions in a 96-Well Format Using the Packard MultiPROBE™

**#3415**

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## **ABSTRACT**

**Purpose:** To assess the feasibility of automating liquid-liquid extractions using a Packard MultiPROBE.

**Methods:** Two manual liquid-liquid extractions were transferred to the 96-well format using the Packard MultiPROBE. To test the general utility of this approach, assays based on extraction solvents more dense (theophylline) and less dense (naproxen) than water were chosen. After a single extraction and evaporation, the samples were reconstituted and injected onto an HPLC system using a 96-well autosampler.

**Results:** Both compounds yielded successfully validated methods. The resulting assays were sensitive, selective, accurate and precise. Inter-assay precision and accuracy for theophylline ranged from 3.1% to 7.5%, and - 0.3% to 1.3% respectively. Inter-assay precision and accuracy for naproxen ranged from 3.7% to 10.7%, and - 5.3% to 7.2% respectively.

**Conclusions:** General strategies for automating liquid-liquid extractions have been achieved using the 96-well format. The resulting assays drastically reduce sample preparation time (at least 3-fold faster when compared to manual extraction), decrease material and labor costs and increase throughput in clinical studies.

## **PURPOSE - Designing the Feasibility and Ease of Automation**

- Two commonly available drugs (naproxen and theophylline) used to test feasibility and ease of automating liquid-liquid extraction
- Test general utility of approach in using solvents more and less dense than water
- Decrease the cumbersome nature of traditional liquid-liquid extractions
- Automation will help reduce sample preparation time, decrease material and labor costs and increase throughput in clinical studies

## **METHOD - Validation Scheme**

- Fresh Calibration Line
- QCs at the limits of detection (n=6)
- 3 day Inter-assay precision and accuracy

## General Assay Procedure-Naproxen

- Load Samples (calibrators, QCs, unknowns) onto 96-well plate
- Add buffer, ISTD, and extraction solvent to each well
- Vortex plate, centrifuge and aspirate lower aqueous layer to waste
- Evaporate the organic and reconstitute before injecting onto HPLC system using a 96-well autosampler

### Calibration Standard Statistics - Naproxen

Nominal Concentration	100	50.0	10.0	5.00	1.00	0.250	0.100
Average Concentration	98.2	52.3	9.83	4.85	1.01	0.255	0.0993
Standard Deviation	2.73	3.13	0.257	0.130	0.0504	0.0118	0.00713
Precision (%)	2.8%	6.0%	2.6%	2.7%	5.0%	4.6%	7.2%
Accuracy (%)	98.2%	104.6%	98.3%	97.0%	100.7%	101.8%	99.3%
N	6	6	6	6	6	6	6

Batch														
N23K	101	94.6	53.5	52.2	9.37	10.0	4.87	4.64	1.07	0.981	0.256	0.260	0.102	0.0955
N24K	97.1	96.3	55.0	52.8	9.72	10.0	4.83	4.90	0.956	1.05	0.239	0.251	0.0902	0.111
N25K	98.4	102	54.0	46.2	9.98	9.84	4.82	5.04	0.953	1.03	0.274	0.248	0.0970	0.0998

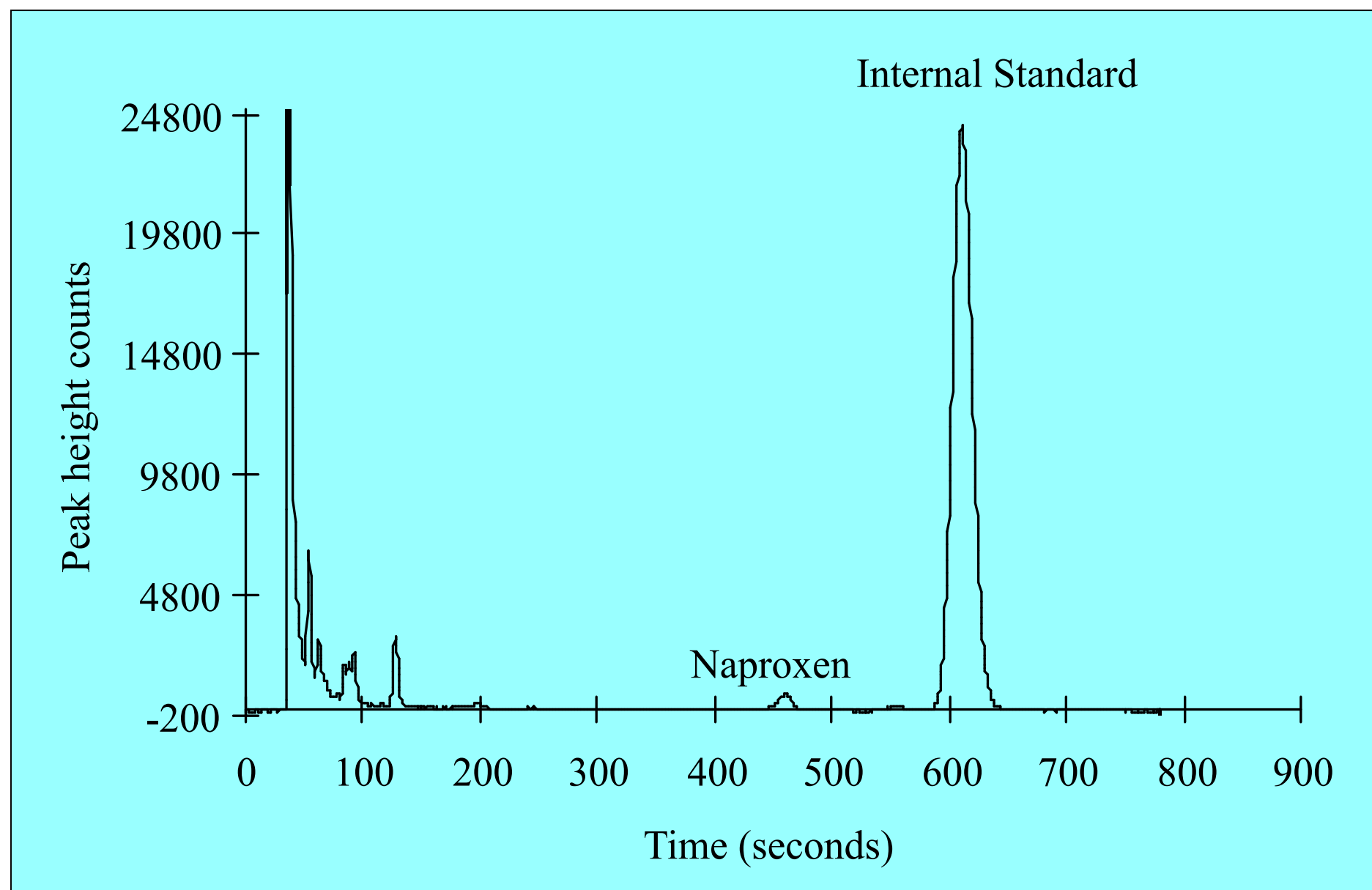
### Inter-Assay Quality Control Sample Statistics - Naproxen

Nominal Concentration	100	8.00	0.100
Average Concentration	94.7	7.96	0.107
Standard Deviation	3.51	0.487	0.0115
Precision (%)	3.7%	6.1%	10.7%
Accuracy (%)	94.7%	99.5%	107.3%
N	18	18	16

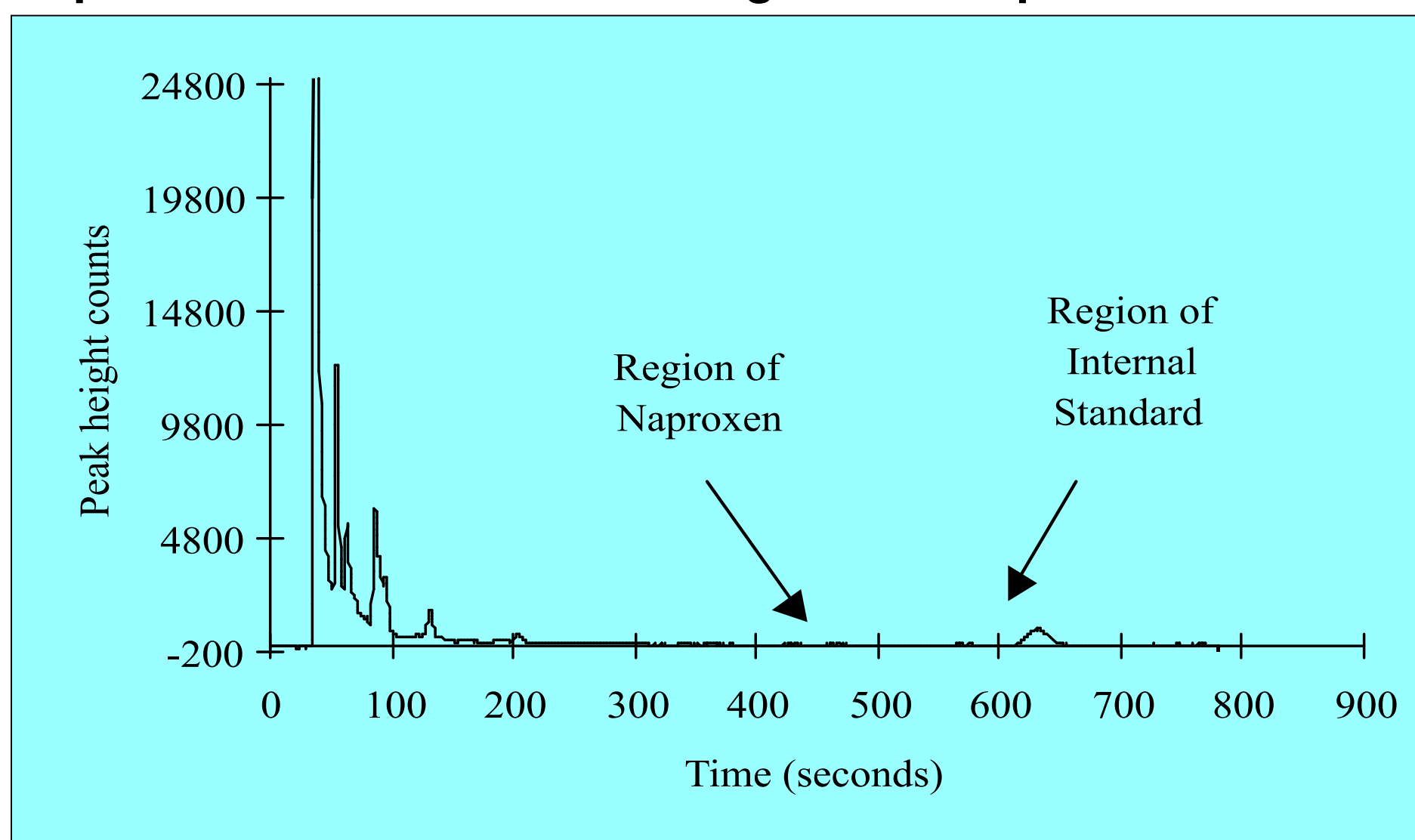
  

Batch			
N23K	94.4	7.41	0.0991
	101	7.75	0.107
	91.3	8.77	0.0962
	98.2	7.73	0.0999
	95.8	7.40	0.0944
N24K	93.7	8.04	0.110
	93.1	8.59	0.103
	89.0	7.54	0.131
	98.6	7.74	0.108
	90.3	7.79	0.0934
N25K	92.7	7.75	0.114
	95.9	8.75	B
	99.5	7.51	0.127
	95.9	7.60	0.106
	96.6	7.72	0.124
	95.5	8.80	0.104
	88.8	8.45	0.0999
	93.8	7.96	B

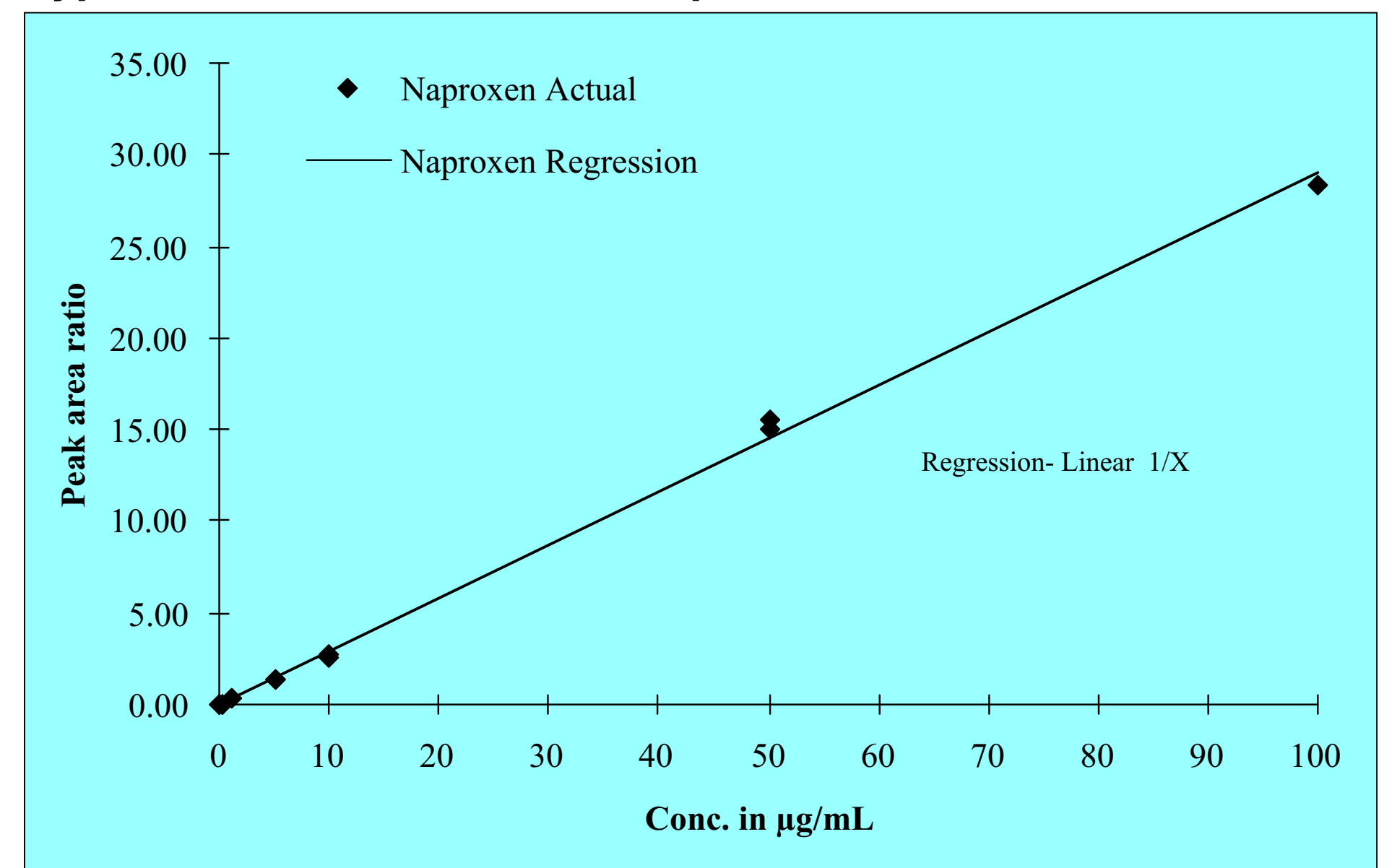
### Representative Low Calibrator Chromatogram for Naproxen



### Representative Blank Chromatogram for Naproxen



### Typical Calibration Line for Naproxen



## General Assay Procedure- Theophylline

- Load Samples (calibrators, QCs, unknowns) onto 96-well plate
- Add buffer, ISTD, and extraction solvent to each well
- Vortex plate, centrifuge and aspirate lower organic layer to another 96-well plate
- Evaporate the organic and reconstitute before injecting onto HPLC system using a 96-well autosampler

### Calibration Standard Statistics-Theophylline

Nominal Concentration	20.0	16.0	10.0	5.00	3.00	1.00	0.750	0.500	0.300
Average Concentration	19.7	16.1	10.2	5.05	2.86	1.01	0.754	0.506	0.298
Standard Deviation	0.831	0.389	0.211	0.294	0.291	0.0515	0.0182	0.0174	0.0114
Precision (%)	4.2%	2.4%	2.1%	5.8%	10.2%	5.1%	2.4%	3.4%	3.8%
Accuracy (%)	98.7%	100.8%	102.1%	101.0%	95.4%	100.8%	100.6%	101.1%	99.5%
N	6	6	6	6	6	6	6	6	5

Batch	20.1	19.7	16.0	16.1	10.2	10.1	5.11	4.57	2.80	3.11	1.05	1.05	0.743	0.756	0.513	0.488	0.294	0.294	
C09L																			
C10L	20.2	19.9	15.6	16.1	10.1	9.90	5.09	4.86	3.13	3.08	1.02	1.05	0.743	0.733	0.492	0.491	0.286	0.301	
C11L	20.4	18.1	16.8	16.2	10.3	10.5	5.34	5.34	2.42	2.65	0.942	0.945	0.781	0.770	0.526	0.524	F	0.317	

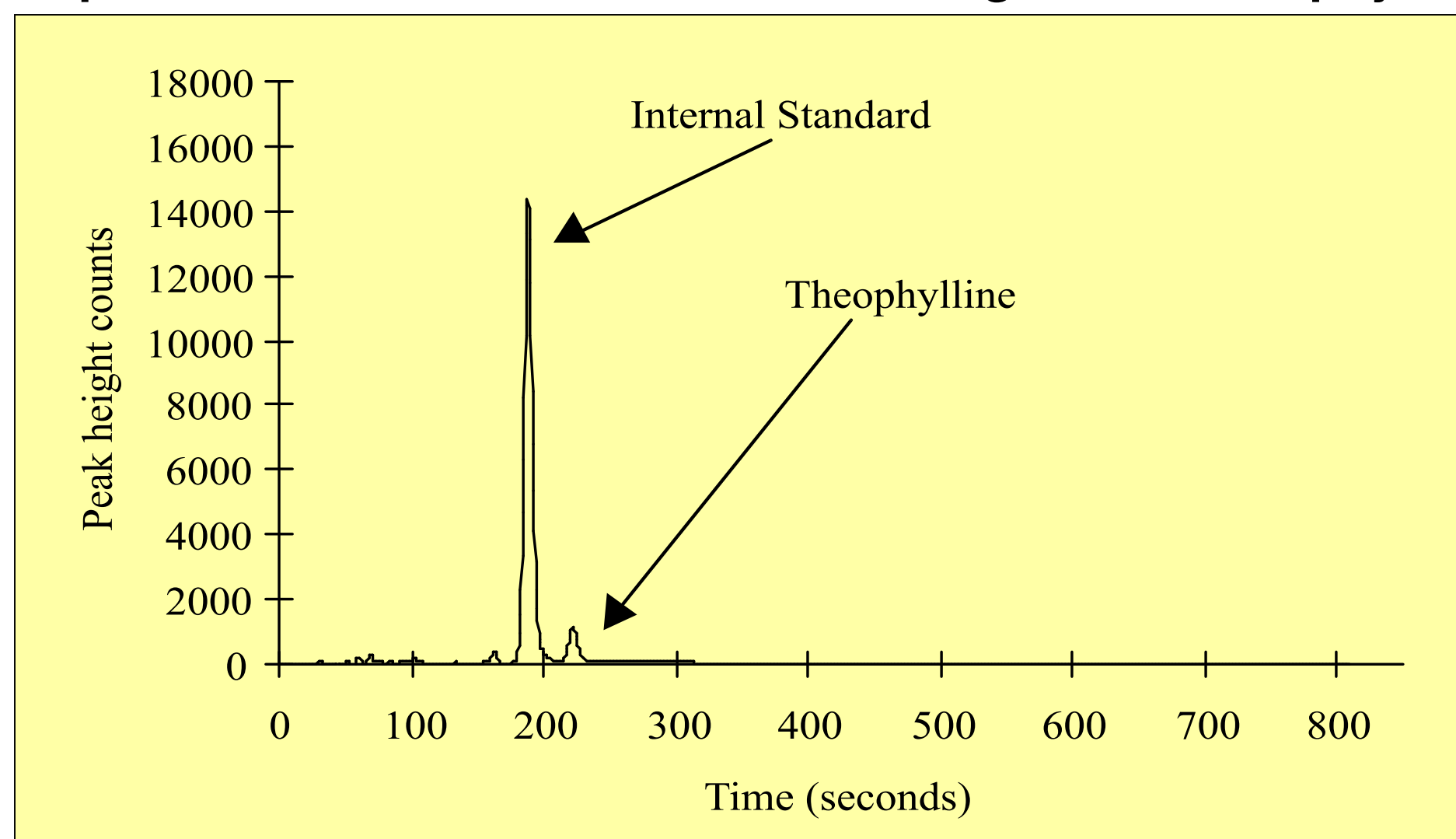
### Inter-Assay Quality Control Sample Statistics - Theophylline

Nominal Concentration	20.0	10.0	0.300
Average Concentration	20.3	9.92	0.299
Standard Deviation	0.721	0.308	0.0223
Precision (%)	3.6%	3.1%	7.5%
Accuracy (%)	101.3%	99.2%	99.7%
N	18	18	18

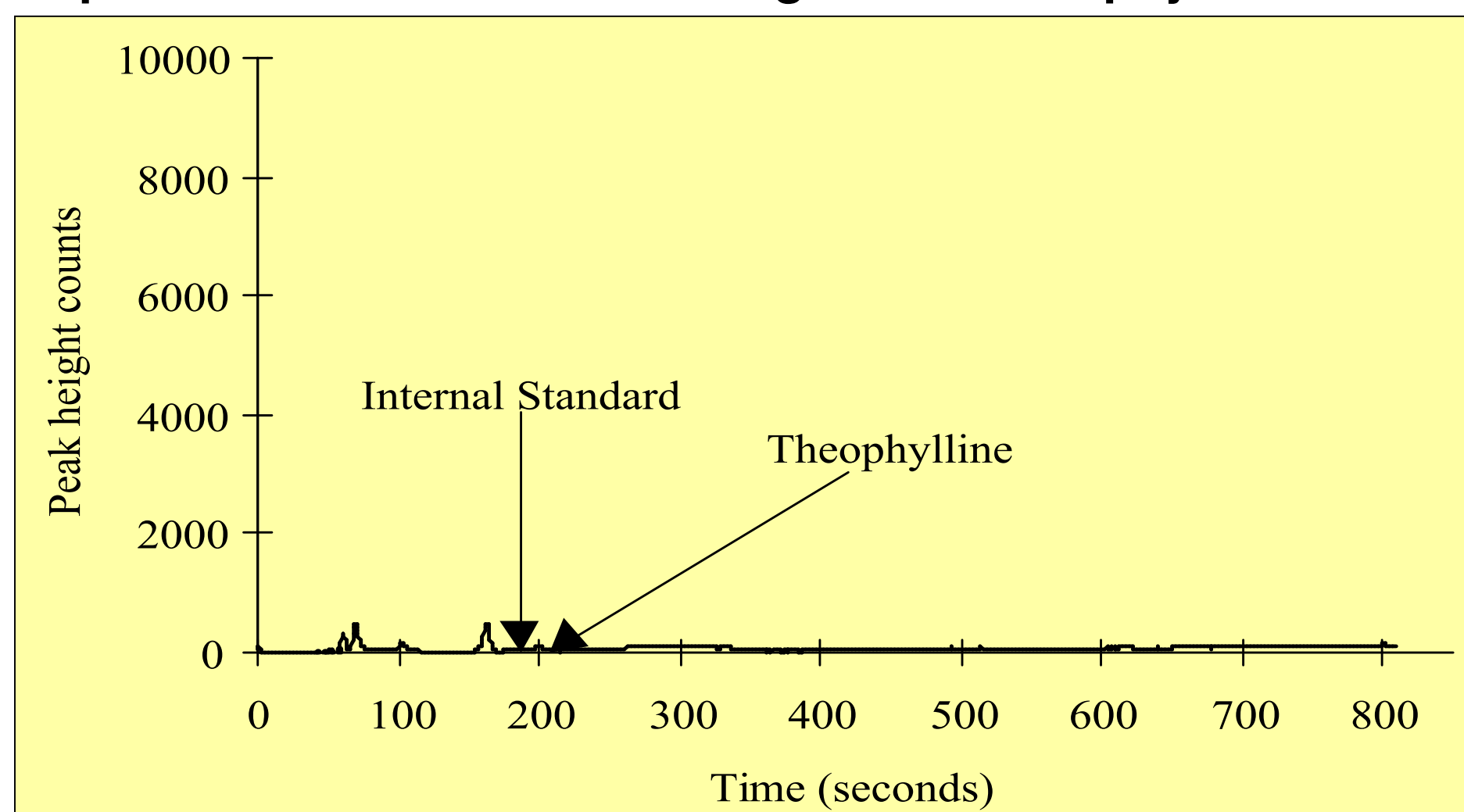
  

Batch	20.1	10.1	0.290
C09L	20.0	9.28	0.289
	20.5	9.97	0.292
	20.7	9.82	0.287
	21.1	10.2	0.284
	20.0	9.81	0.284
	19.7	10.2	0.286
C10L	19.2	9.69	0.295
	19.8	9.42	0.290
	19.5	9.52	0.282
	19.8	9.89	0.276
	20.4	9.75	0.285
	19.7	10.2	0.286
C11L	21.8	10.3	0.369
	21.0	10.5	0.314
	20.6	9.91	0.318
	20.5	9.95	0.315
	21.1	10.2	0.321
	19.1	10.2	0.309

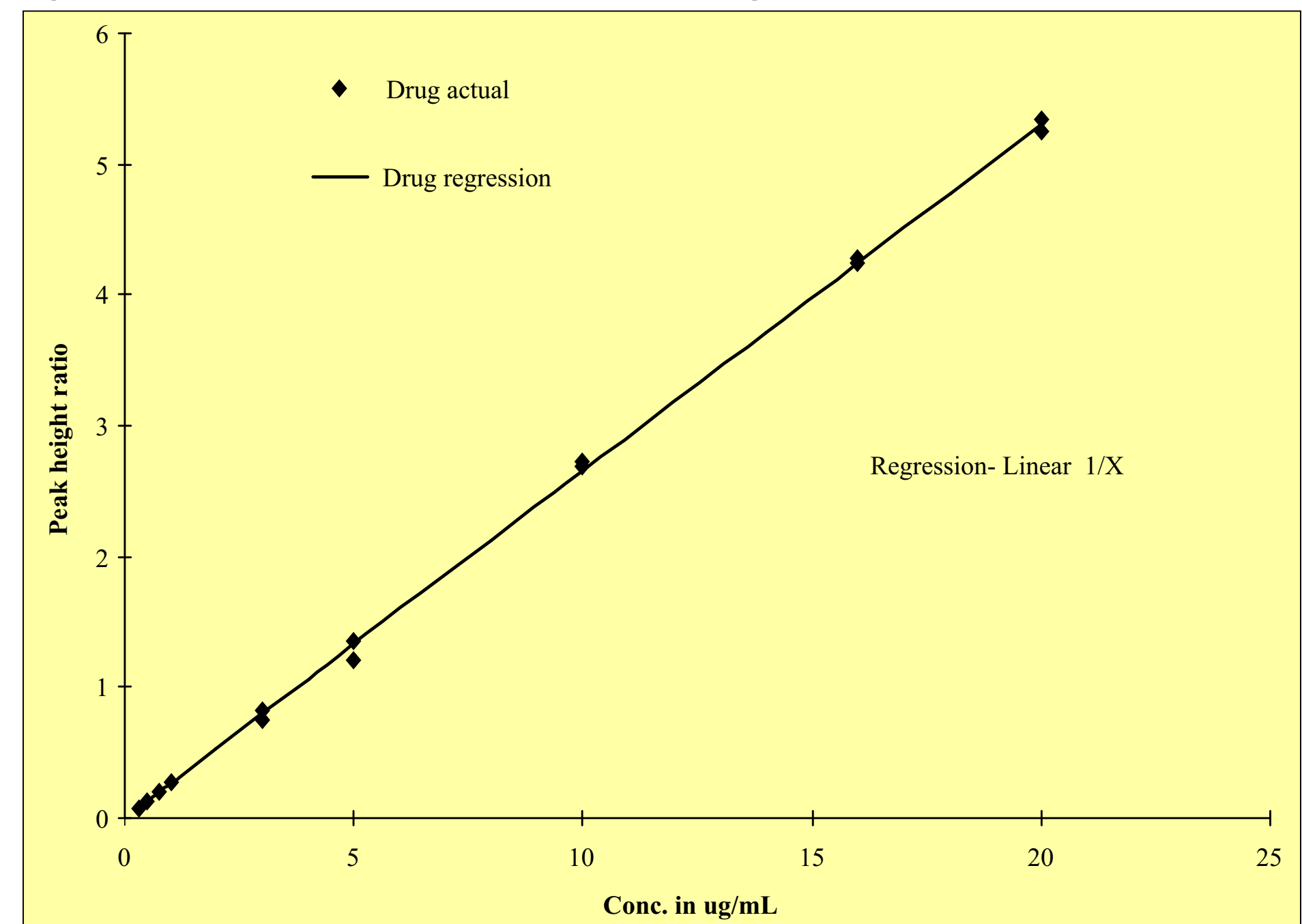
### Representative Low Calibrator Chromatogram for Theophylline



### Representative Blank Chromatogram for Theophylline



### Typical Calibration Line for Theophylline



## Conclusions

- Faster - At least 3 - fold faster when compared to manual extraction
- Analyst time used more effectively
- Increased throughput of clinical samples
- Traditionally liquid-liquid extractions associated with being cumbersome, automation reduces many repetitive activities
- Decreased material and labor costs
- Proves the utility of this approach for solvents that are either more or less dense than water