

# **Quantitation of Pamoic Acid in Human, Rat, and Canine Plasma**

**#2496**

*N. Simmons*

*Bioanalytical Systems, Inc., 2701 Kent Avenue, West Lafayette, IN, 47906*

## **ABSTRACT**

**Purpose:** To develop a method for the quantitation of pamoic acid in human, rat and canine plasma.

**Methods:** Pamoic acid is an aromatic dicarboxylic acid used as an adjuvant to prolong therapeutic action. Pamoic acid and internal standard are extracted from plasma by protein precipitation. The supernatant is evaporated to dryness, reconstituted in a buffer, and then loaded onto SPE cartridges. Extracts are injected onto an HPLC system utilizing a YMC Basic column with a buffer/ion pair reagent/acetonitrile mobile phase.

**Results:** The three different matrices yielded successfully validated methods. The resulting assays were sensitive, selective, accurate and precise. Inter-assay precision and accuracy for pamoic acid ranged from: 5.3% to 8.5% and -9.6% to -6.2%, respectively (rat plasma) and 7.8% to 11.4% and -1.4% to 0.3%, respectively (human plasma). The method was subsequently cross-validated into canine plasma.

**Conclusions:** This assay serves as a specific and sensitive analytical method to aid in pharmacokinetic measurements of pamoic acid in three different matrices.

## **PURPOSE**

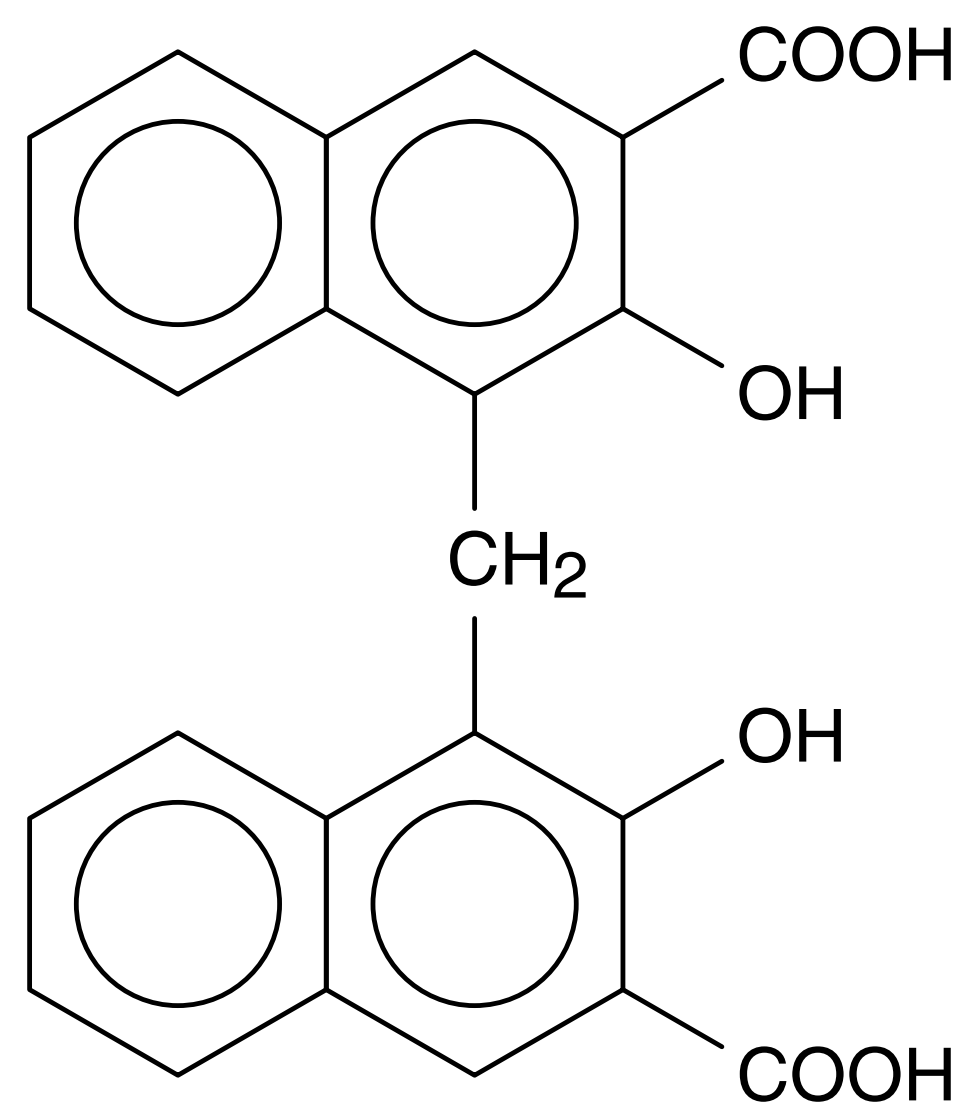
- To develop a method for the quantitation of pamoic acid in human, rat, and canine plasma
- Extraction and chromatographic conditions will be based on previous method for pamoic acid in dog and rat serum\*
- Achieve a lower limit of quantitation of 2 ng/mL for human and canine plasma and 10 ng/mL for rat plasma
- Maximize the extraction efficiency to hit the required lower limits

## **METHOD - Validation Scheme**

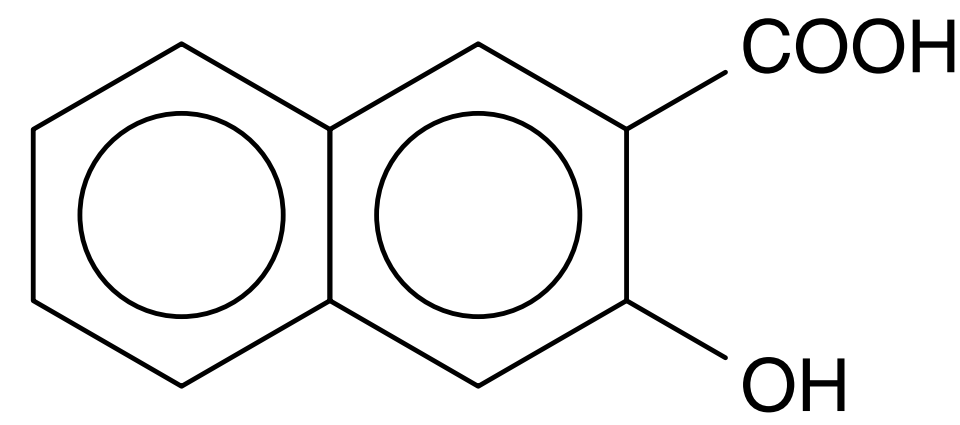
- Fresh Calibration Line
- QCs at the Limits of Quantitation (n = 6)
- 3-Day Inter-Assay Precision and Accuracy (Human and Rat Plasma)
- 1-Day Cross-validation (Canine Plasma)

\*Jorgensen, Martin. "Quantitative determination of pamoic acid in dog and rat serum by automated ion-pair solid-phase extraction and reversed-phase high-performance liquid chromatography"; J. Chrom. B, 716 (1998), 315-323.

# Chemical Structures



Pamoic Acid



Internal Standard

## General Assay Procedure

- Precipitate proteins with acetonitrile
- Transfer supernatant, evaporate acetonitrile, and reconstitute residue in pH 6 phosphate buffer
- Extract samples using C18 SPE cartridges
- Evaporate solvent and reconstitute before injecting on HPLC system equipped with fluorescence detector

## Assay Specifics

Sample volume: 0.5 mL

Column: YMC Basic 150 x 4.6 mm, 5  $\mu$ m (and guard)

Mobile phase: 52% acetonitrile / 48% phosphate buffer (10 mM), cetyltrimethylammonium hydrogensulfate (5 mM)

Flow rate: 0.8 mL/min

Fluorescence detection:  $\lambda_{ex} = 240$  nm,  $\lambda_{em} = 523$  nm

Injection volume: 35  $\mu$ L

## Calibration Standard Statistics

Human Plasma									
Nominal Concentration	200	150	100	75.0	40.0	20.0	8.00	5.00	2.00
Average Concentration	200	146	105	75.1	39.6	19.7	7.96	5.09	1.99
Standard Deviation	4.25	7.50	3.43	4.35	1.13	1.79	0.502	0.413	0.119
Precision (%)	2.1%	5.1%	3.3%	5.8%	2.8%	9.1%	6.3%	8.1%	6.0%
Accuracy (%)	-0.2%	-2.7%	4.6%	0.2%	-1.0%	-1.7%	-0.5%	1.8%	-0.5%
N	6	6	6	6	6	6	6	6	5

Batch	201	202	135	145	103	109	71.4	82.8	39.6	39.6	19.0	21.7	7.81	7.84	4.88	4.83	1.95	2.11
H08E	201	202	135	145	103	109	71.4	82.8	39.6	39.6	19.0	21.7	7.81	7.84	4.88	4.83	1.95	2.11
H09E	194	194	149	150	106	105	75.7	75.6	39.8	39.0	21.6	17.9	7.83	7.32	5.24	5.31	1.85	2.12
H10E	203	203	141	156	106	98.5	74.8	70.6	41.5	38.1	17.6	20.3	8.10	8.84	4.55	5.71	D	1.92

Canine Plasma									
Nominal Concentration	200	150	100	75.0	40.0	20.0	8.00	5.00	2.00
Average Concentration	191	148	114	74.1	37.1	20.2	8.01	4.88	2.02
Standard Deviation	11.9	9.17	5.80	6.89	2.92	1.24	0.588		
Precision (%)	6.0%	6.1%	5.7%	8.9%	7.6%	6.2%	5.8%		
Accuracy (%)	-4.6%	-1.2%	14.3%	-1.2%	-7.2%	1.2%	0.1%	-2.4%	1.0%
N	2	2	2	2	2	2	2	2	2

Batch	191	191	149	148	123	106	71.5	76.7	38.6	35.7	19.5	21.0	7.80	8.22	4.97	4.79	1.96	2.08
H11E	191	191	149	148	123	106	71.5	76.7	38.6	35.7	19.5	21.0	7.80	8.22	4.97	4.79	1.96	2.08

Rat Plasma							
Nominal Concentration	200	150	100	75.0	40.0	20.0	10.0
Average Concentration	199	149	101	77.6	38.7	20.0	10.0
Standard Deviation	11.9	9.17	5.80	6.89	2.92	1.24	0.588
Precision (%)	6.0%	6.1%	5.7%	8.9%	7.6%	6.2%	5.8%
Accuracy (%)	-0.7%	-0.5%	1.0%	3.4%	-3.3%	-0.2%	0.5%
N	6	6	6	6	6	6	6

Batch	194	191	147	164	93.5	99.3	85.0	83.8	39.3	33.3	19.8	20.2	9.19	11.0
K02W	194	191	147	164	93.5	99.3	85.0	83.8	39.3	33.3	19.8	20.2	9.19	11.0
K03W	213	193	141	157	98.0	101	70.6	76.5	41.5	40.6	18.0	21.9	10.0	9.94
K04W	214	187	142	145	110	104	80.9	68.4	37.9	39.6	19.7	20.1	9.94	10.2

## Inter-Assay Quality Control Sample Statistics

Human Plasma			
Nominal Concentration	200	100	2.00
Average Concentration	199	100	1.97
Standard Deviation	22.6	7.82	0.154
Precision (%)	11.4%	7.8%	7.8%
Accuracy (%)	-0.6%	0.3%	-1.4%
N	18	18	18

Batch	218	100	2.03
H08E	218	100	2.03
	199	100	2.07
	152	102	1.91
	187	95.5	1.82
	202	98.2	1.82
	203	83.2	2.00

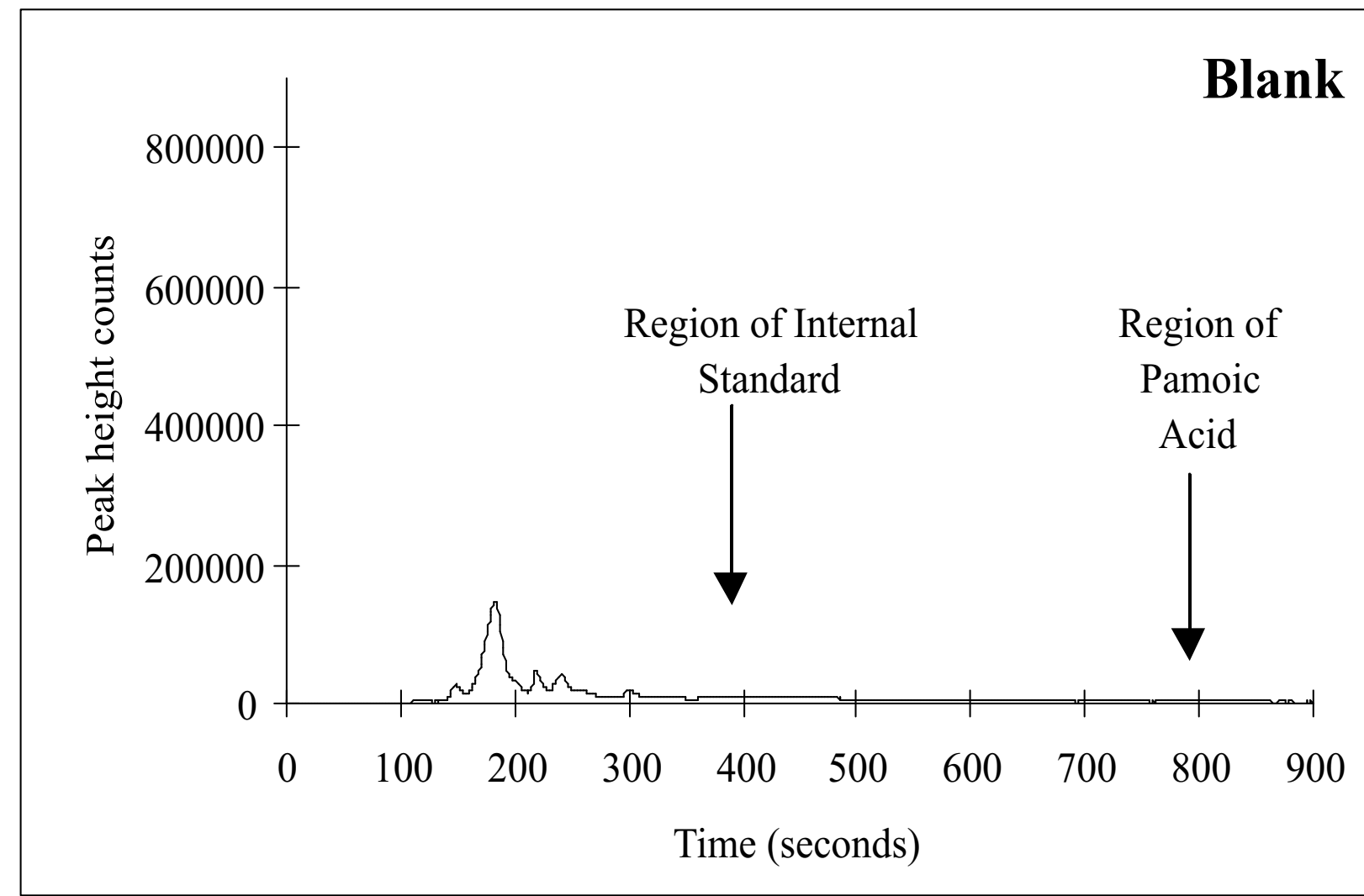
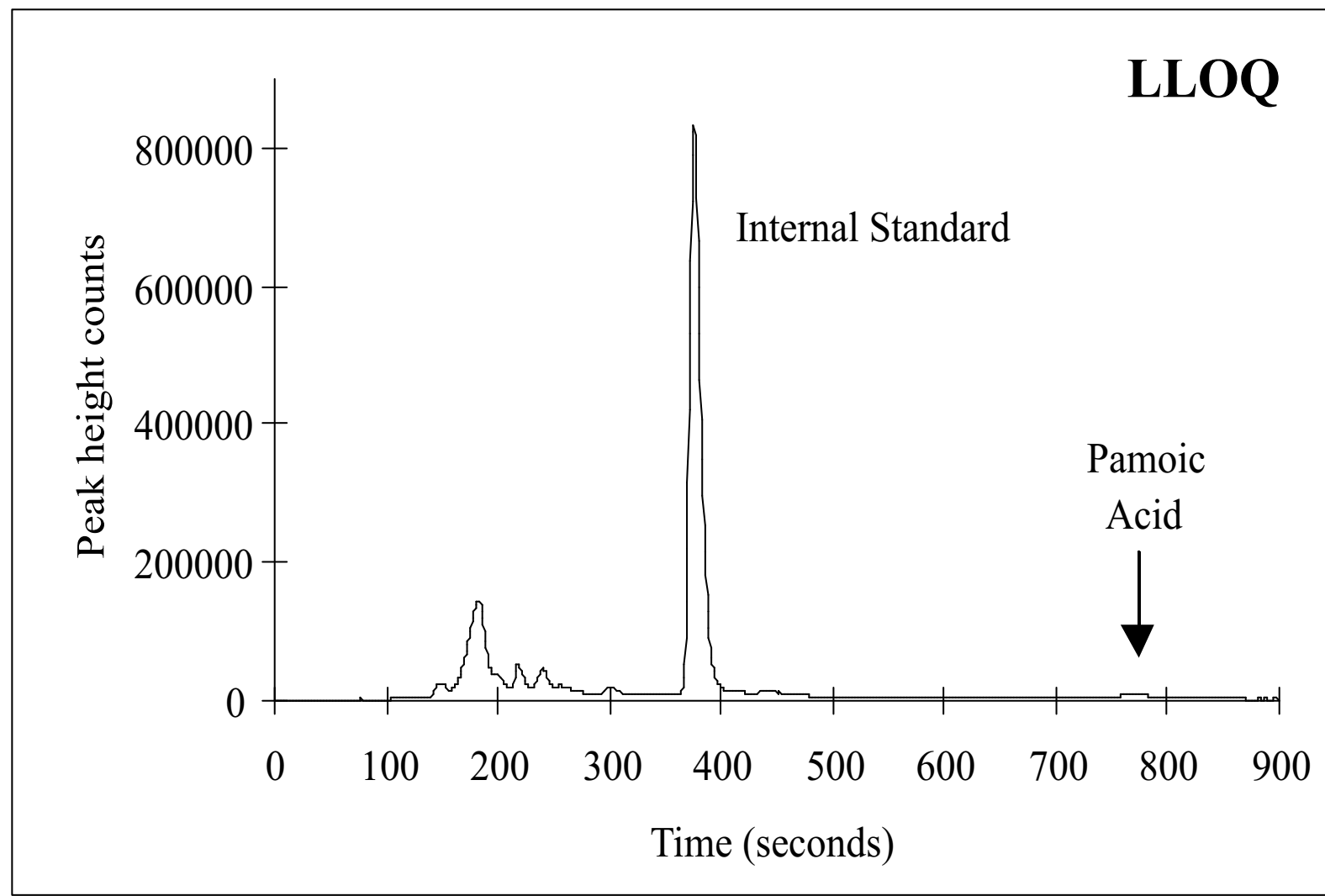
Batch	256	117	2.17
H09E	256	117	2.17
	206	111	1.79
	188	106	1.79
	229	91.8	1.84
	213	104	2.03
	173	109	1.81
H10E	205	105	2.01
	191	99.2	2.23
	201	100	1.86
	185	94.7	1.91
	181	95.5	2.20
	190	93.4	2.20

Rat Plasma			
Nominal Concentration	200	100	10.0
Average Concentration	188	90.7	9.04
Standard Deviation	10.3	4.83	0.772
Precision (%)	5.5%	5.3%	8.5%
Accuracy (%)	-6.2%	-9.3%	-9.6%
N	18	18	18

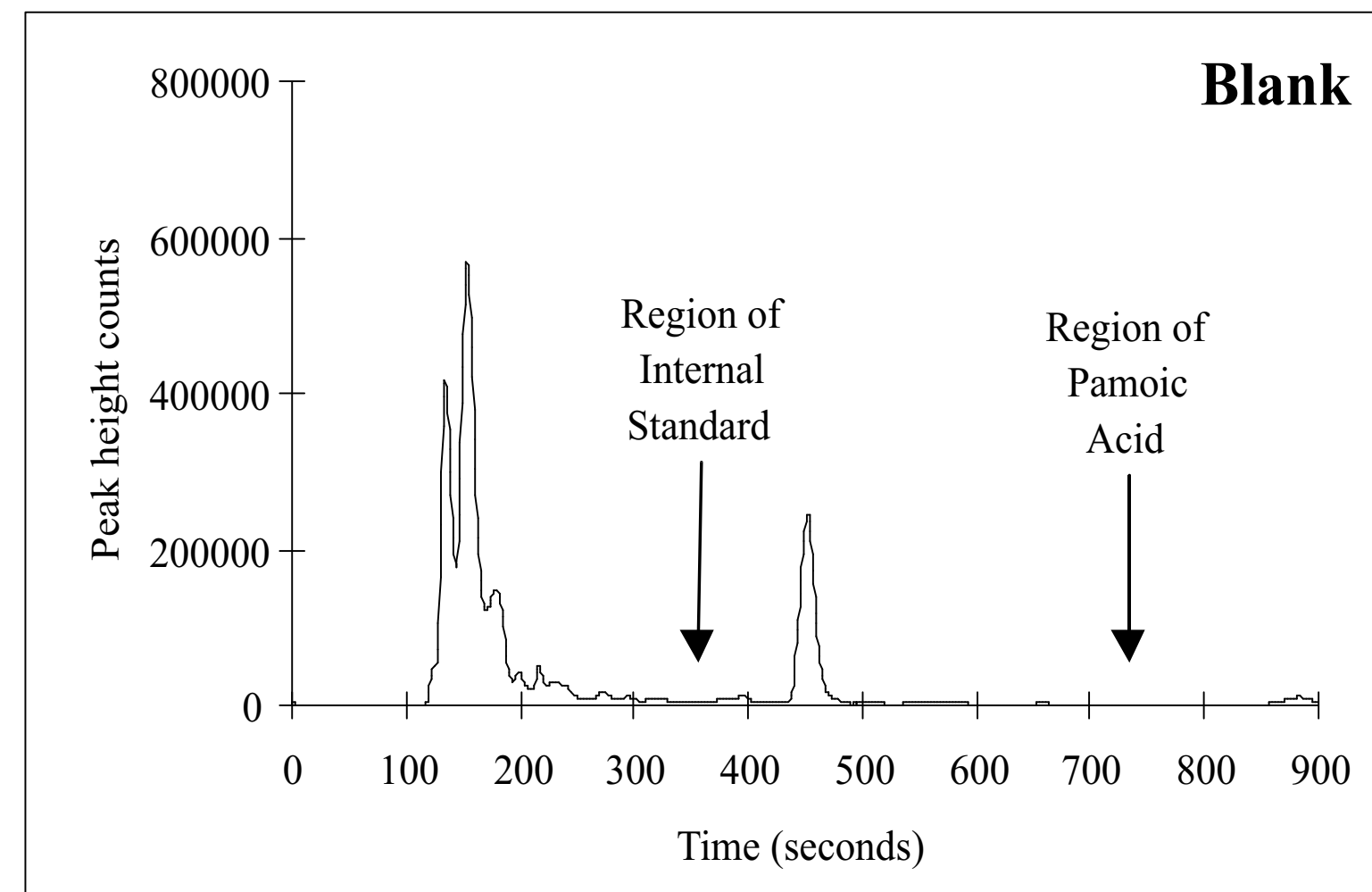
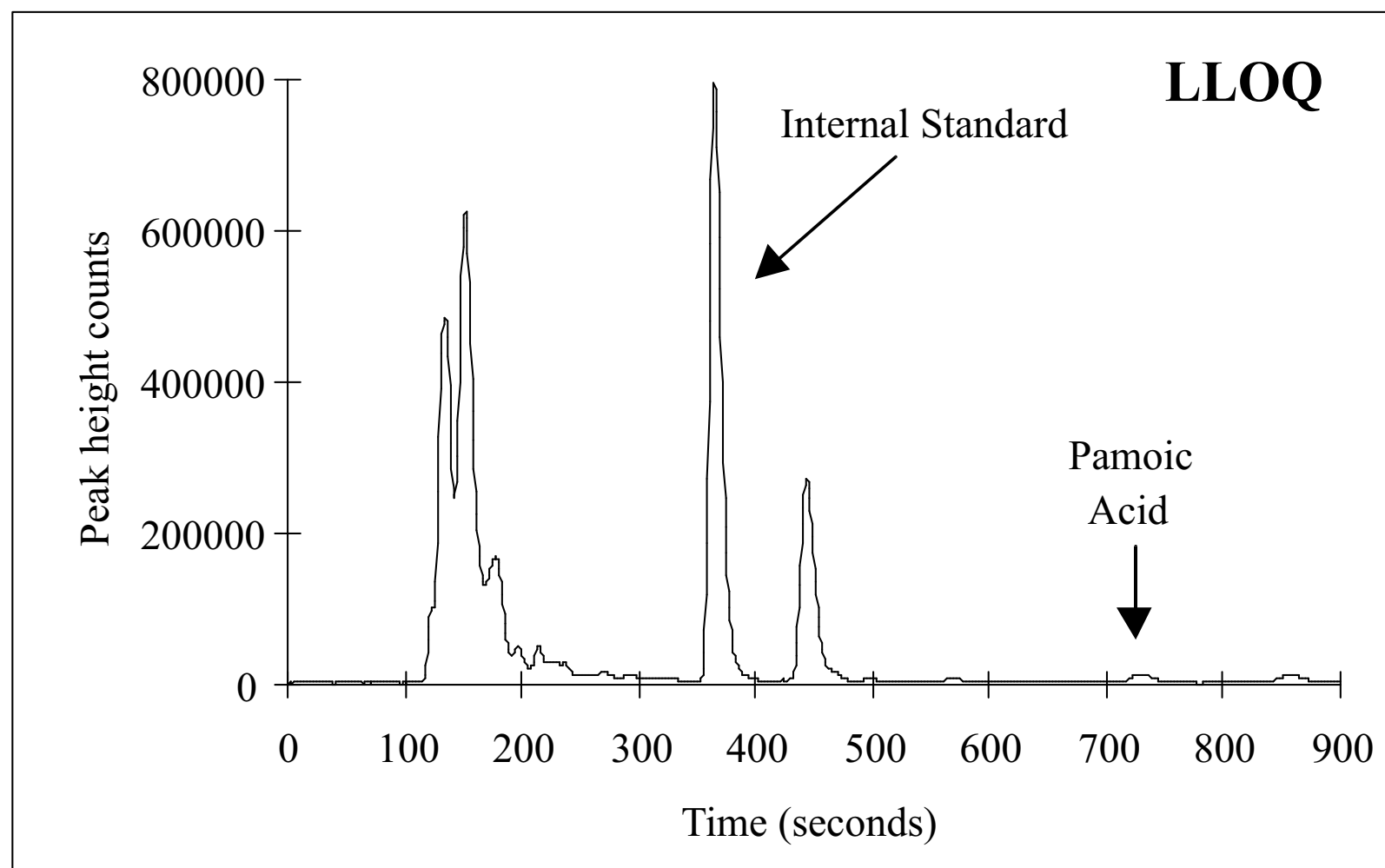
Batch	181	78.7	9.00
K02W	181	78.7	9.00
	196	89.0	8.79
	175	90.7	8.47
	170	87.3	8.60
	189	87.9	8.17
	180	88.5	8.10

Batch	188	92.4	7.76
K03W	188	92.4	7.76
	200	87.0	9.03
	194	87.4	8.26
	180	88.7	9.10
	180	89.0	9.01
	184	92.5	8.92
K04W	183	97.7	9.57
	186	95.7	10.0
	185	93.3	9.93
	191	93.3	10.6
	213	100	9.42
	200	94.3	10.0

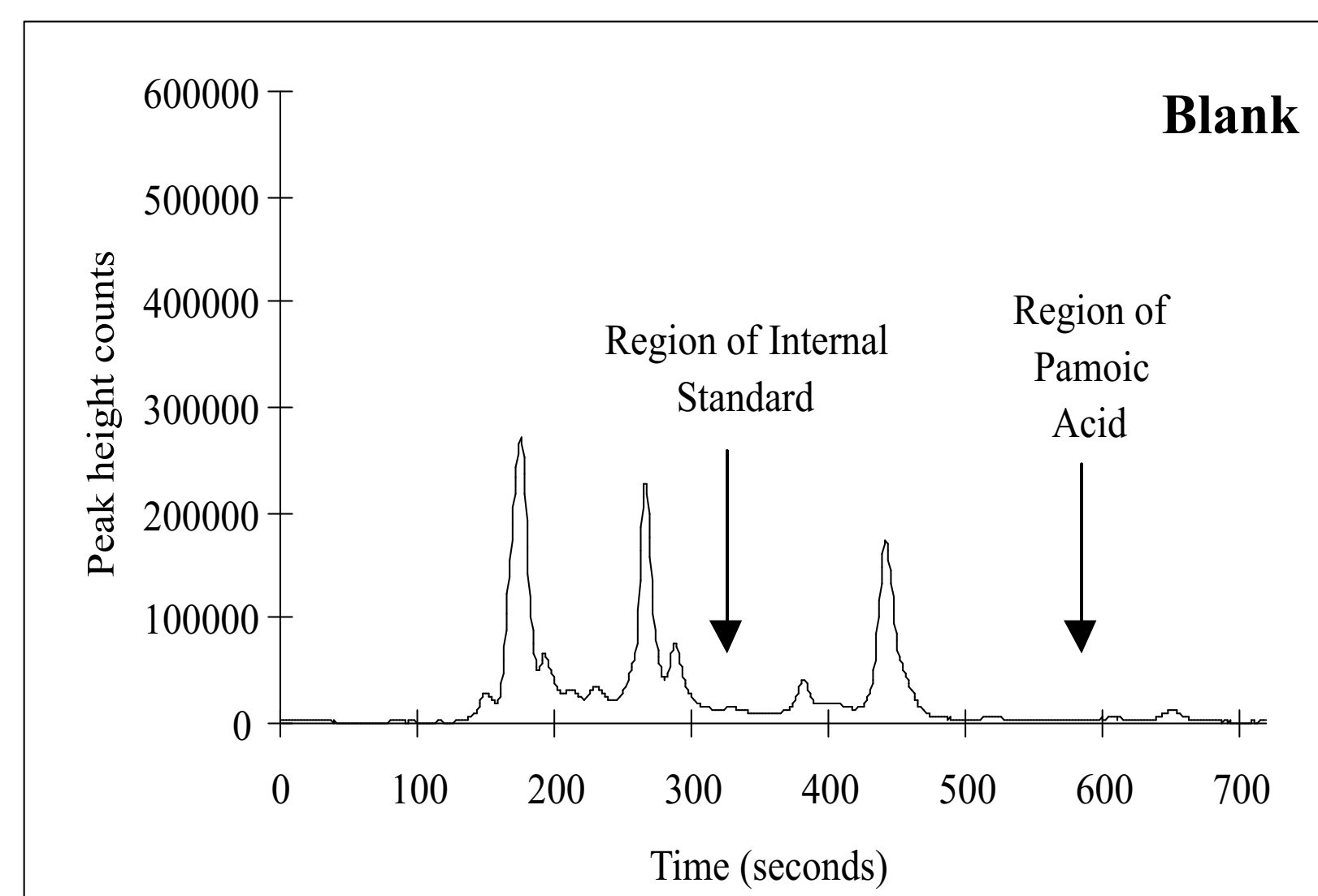
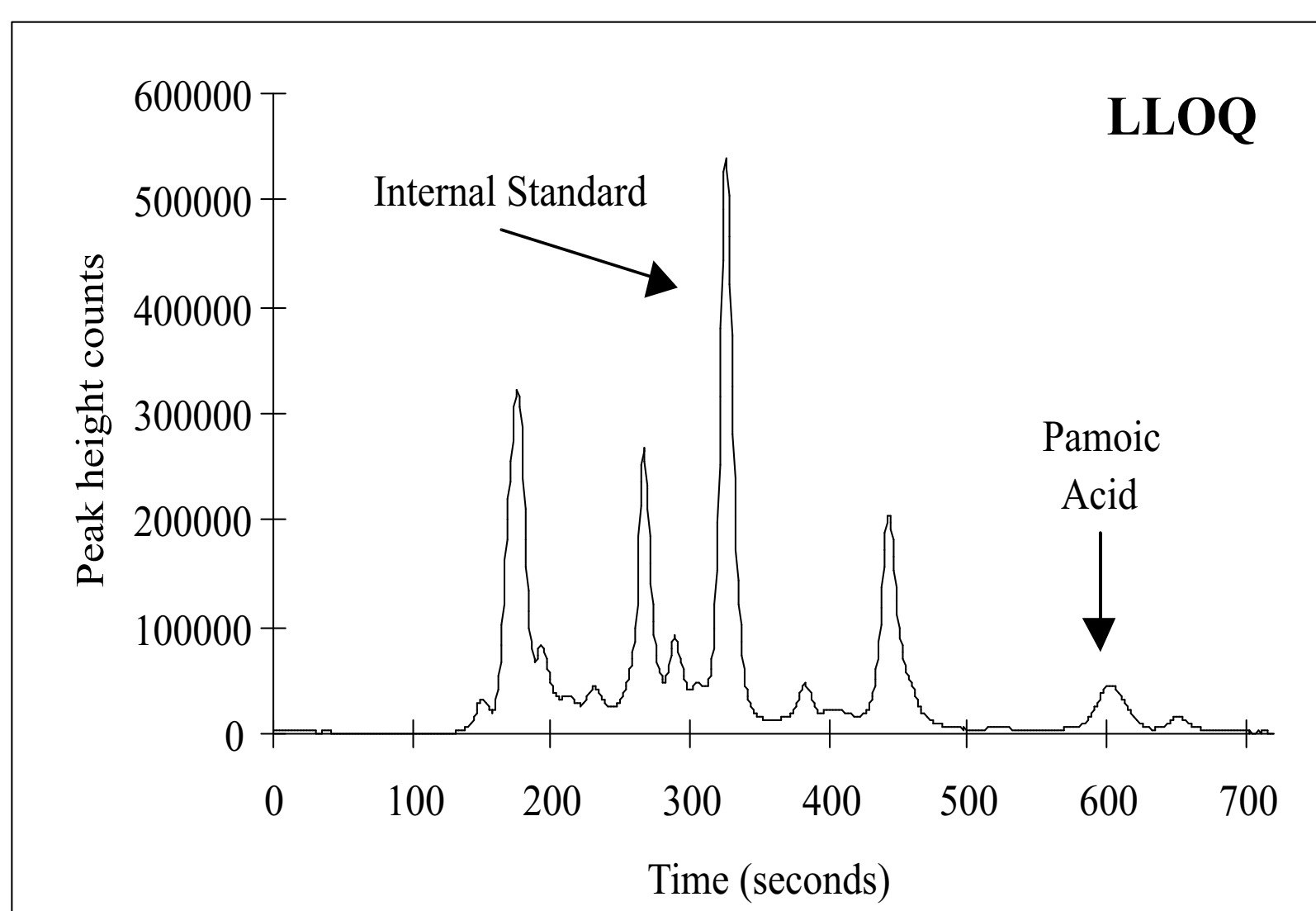
## Representative Human Plasma Chromatograms



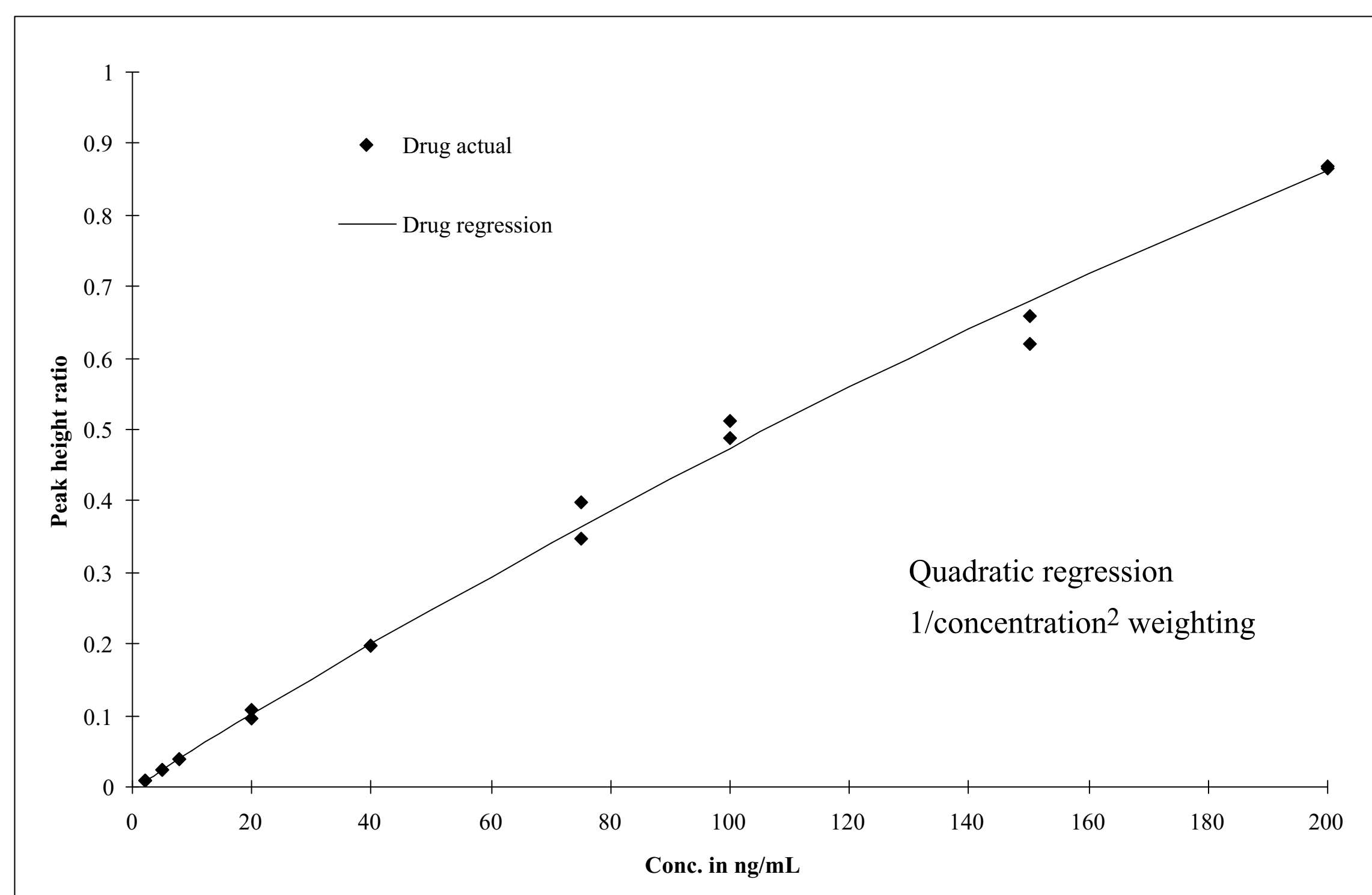
## Representative Dog Plasma Chromatograms



## Representative Rat Plasma Chromatograms



## Typical Calibration Line



## CONCLUSIONS

- All three matrices validated with extraction efficiencies >95%
- Selectivity/specificity: One method for human, canine, and rat plasma
- Ruggedness: >5 months of preclinical sample analysis