

Supplementary Table S1: Response Factor (RF) Determination for Anandamide (AEA) and its Deuterated Analog, Ethanolamide-d₈ (AEA-d₈) in Canine Plasma Using UPLC-MS/MS

Concentration (ng/mL)	Mean Response		Response Factor (RF)
	AEA	AEA-d ₈	
0.78	1,639	1,705	1.04
1.56	3,202	3,108	0.97
3.13	5,857	5,934	1.01
6.25	13,056	12,492	0.96
12.5	24,842	23,700	0.95
25	47,146	45,189	0.96
50	92,857	91,440	0.98
100	168,750	182,429	1.08
200	343,108	358,011	1.04
Pooled RF			1.00
Pooled SD			0.046
Pooled RSD (%)			4.57

Legend: Due to the presence of endogenous anandamide (AEA), the response factor (RF) of different concentrations of AEA and its deuterated analog, ethanolamide-d₈ (AEA-d₈), was determined to ensure accurate quantification of AEA from canine plasma. Stock solutions of AEA and AEA-d₈ were prepared in methanol and serially diluted with acetonitrile to generate working standard solutions from 19.53 to 5000 ng/mL. Concentrations similar to the standard curve were prepared by serially diluting 10 µL of the working standards into blank canine plasma to obtain final concentrations of 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, and 200 ng/mL. The RF was determined by taking the Area-AEA-d₈/Area-AEA determined by ultra-high-performance liquid chromatography-tandem electrospray positive-ionization mass spectrometry (UHPLC-MS/MS) in mixed reaction monitoring (MRM) mode. The RF range and pooled values were determined, and the suitability of AEA-d₈ as a surrogate for determining AEA concentrations in canine plasma was demonstrated. The standard deviation (SD) and relative standard deviation (RSD) were determined.