

DMD # 77883

Title: Elucidation of the impact of P-glycoprotein and Breast Cancer Resistance Protein on the brain distribution of catechol-*O*-methyltransferase inhibitors

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Supplemental Table 1. Chromatographic conditions and validation parameters of HPLC-DAD techniques applied for the quantification of reference and test compounds in samples from *in vitro* and *in vivo* assays.

	Mobile phase	Flow rate (mL/min)	Detection wavelength (nm)	Injection volume (μ L)	Calibration range (μ M)	Coefficient of determination (r^2)	LLOQ (μ M)	Precision (% CV)	Accuracy (% Bias)
Reference compounds									
Carbamazepine	H ₂ O/MeOH/ACN (60:30:10, v/v/v)	1.0	235	20	0.25 – 12	0.999	0.25	≤ 2.6	-2.8 to 3.5
Propranolol	H ₂ O/ACN/TCA (70:30:0.03, v/v/v; pH 2.5-2.6)		235		0.035 – 2	0.999	0.035	≤ 3.2	0.1 to 0.9
Trazodone	50 mM phosphate buffer/ACN/MeOH/TEA (70:25:5:0.3; v/v/v/v; pH 3.5)		249		0.1 – 4	0.996	0.1	≤ 0.6	-9.0 to -0.7
Atenolol	H ₂ O/MeOH/TEA (88:12:0.05, v/v/v; pH 3.0)		223	50	0.03 – 3	0.999	0.03	≤ 0.9	0.02 to 6.2
Cimetidine	H ₂ O/MeOH/TEA (86:14:0.05, v/v/v; pH 3.0)		220		0.01 – 0.4	0.999	0.01	≤ 2.6	-12.1 to -2.7

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Quinidine	H ₂ O with 0.1% formic acid pH 3.0/ACN/MeOH (84:10:6, v/v/v)		249		0.02 – 1	0.999	0.02	≤ 4.6	–3.5 to 0.6
Sulfasalazine	50 mM phosphate buffer pH 2.5/ACN (70:30, v/v)		360		0.01 – 0.4	0.999	0.01	≤ 4.1	–4.0 to –2.3
Test compounds									
BIA 9-1059	50 mM phosphate buffer pH 2.45/ACN (63:37, v/v)		274		0.01 – 0.4	0.999	0.01	≤ 8.5	–5.9 to –0.7
Entacapone	50 mM phosphate buffer pH 2.45/ACN (63:37, v/v)		305		0.014 – 0.7	0.999	0.014	≤ 6.4	–1.2 to 6.1
Opicapone			273	50	0.013 – 0.4	0.999	0.013	≤ 7.8	–7.4 to 1.5
BIA 9-1079	50 mM phosphate buffer pH 2.45/ACN (50:50, v/v)		281		0.04 – 2	0.999	0.04	≤ 4.3	–9.4 to –0.7
Nebicapone	50 mM phosphate buffer pH 2.45/ACN (50:50, v/v)		276		0.02 – 0.1	0.999	0.02	≤ 8.6	–6.1 to 3.8
Tolcapone			270	0.8	0.018 – 1	0.999	0.018	≤ 8.8	–2.6 to –2.3
					(µg/mL)			(µg/mL)	
BIA 9-1079	Plasma	A: 50 mM phosphate buffer pH 2.45	281		0.04 – 6	0.998	0.04	≤ 12.1	–7.0 to 1.1
	Brain	B: ACN [33-60% (0-8 min); 60% (8-9 min); 60-33% (9-10 min); 33% (10-14 min)]		20	0.02 – 4	0.996	0.02	≤ 7.7	–3.9 to 5.6
Tolcapone	Plasma		270		0.04 – 6	0.998	0.04	≤ 10.7	–6.1 to 9.3
	Brain				0.02 – 4	0.997	0.02	≤ 4.6	–5.7 to 2.0

ACN, acetonitrile; Bias, deviation from nominal value; CV, coefficient of variation; LLOQ, lower limit of quantification ; MeOH, methanol; TCA, trichloroacetic acid; TEA, triethylamine.

Supplemental Table 2. Stability (values in percentage) of COMT inhibitors in HBSS with 10mM HEPES pH 7.4 under cellular assay conditions (37° C for 120 min).

	Nominal concentration		Stability/reference analyte concentrations (%)			
		(μ M)	30 min	60 min	90 min	120 min
BIA 9-1059	QC1	0.03	103.5 (2.20)	101.0 (2.43)	102.9 (1.22)	102.8 (3.94)
	QC3	0.35	100.0 (0.58)	100.8 (1.88)	101.6 (0.61)	100.4 (2.10)
BIA 9-1079	QC1	0.1	89.1 (2.26)	86.6 (4.97)	76.7 (2.67)	72.7 (6.14)
	QC3	1.6	96.8 (1.93)	95.4 (1.03)	93.1 (9.05)	96.3 (3.80)
Entacapone	QC1	0.04	100.6 (0.75)	99.3 (3.13)	100.2 (1.37)	99.9 (1.81)
	QC3	0.6	101.1 (1.57)	99.3 (2.60)	99.9 (1.15)	100.2 (1.42)
Nebicapone	QC1	0.06	95.0 (8.79)	96.4 (7.36)	95.7 (8.93)	95.6 (7.43)
	QC3	0.8	99.6 (1.08)	98.8 (0.60)	96.6 (1.16)	95.0 (1.73)
Opicapone	QC1	0.04	100.0 (5.65)	103.0 (4.73)	100.3 (1.61)	102.0 (7.63)
	QC3	0.32	97.7 (1.94)	97.5 (1.28)	95.4 (5.64)	96.2 (2.07)
Tolcapone	QC1	0.05	97.3 (2.85)	95.9 (1.41)	96.0 (1.51)	95.9 (3.60)
	QC3	0.8	101.9 (4.11)	99.2 (3.68)	98.4 (4.55)	96.4 (3.56)

Results are expressed as mean (SD) ($n = 3$).