

## Supplementary Material

### Placental passage of protopine in an *ex vivo* human perfusion system

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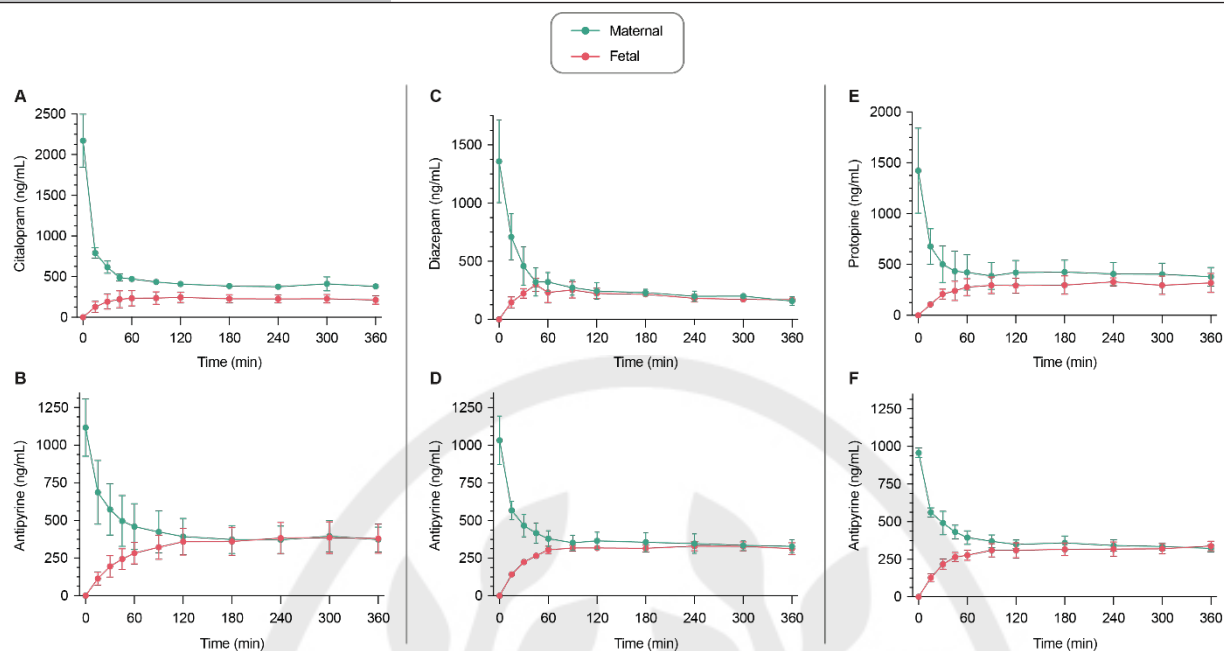
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**Fig. 1S.** *Ex vivo* human placental perfusion profiles of citalopram (A), diazepam (C), and protopine (E) with corresponding connectivity control (antipyrine) transfers (B, D and F, respectively). Concentrations are expressed as absolute concentrations in ng/mL (● maternal and ● fetal). All values are expressed as mean  $\pm$  standard deviation (SD) of 3 independent experiments.

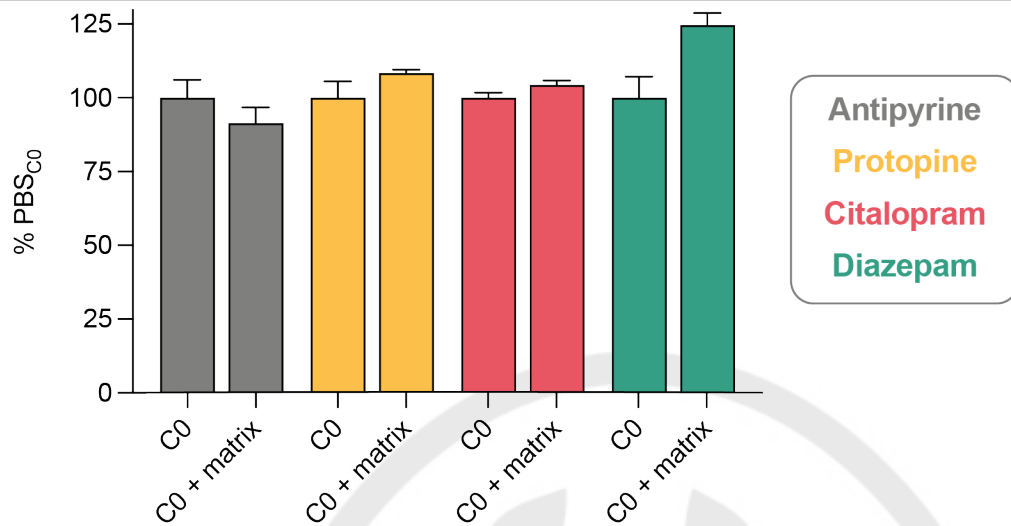
**Table 1S.** Recovery of study compounds after 360 min of *ex vivo* human placental perfusion in relative amounts (% found)  $\pm$  standard deviation (SD) in different compartments.

	Adherence (% lost)	M perfusate (% found)	F perfusate (% found)	Sampling (% found)	Final recovery* (% found)
Antipyrine	8.2 $\pm$ 4.3	22.0 $\pm$ 4.2	19.8 $\pm$ 3.1	21.2 $\pm$ 1.5	71.2 $\pm$ 7.2
Protopine	19.5 $\pm$ 8.3	23.1 $\pm$ 4.8	14.0 $\pm$ 3.7	14.8 $\pm$ 1.5	71.4 $\pm$ 8.6
Citalopram	42.7 $\pm$ 11.3	11.4 $\pm$ 1.5	6.4 $\pm$ 1.5	11.4 $\pm$ 2.9	71.9 $\pm$ 5.2
Diazepam	44.4 $\pm$ 5.6	7.9 $\pm$ 2.2	7.9 $\pm$ 2.3	13.4 $\pm$ 1.3	73.6 $\pm$ 4.8

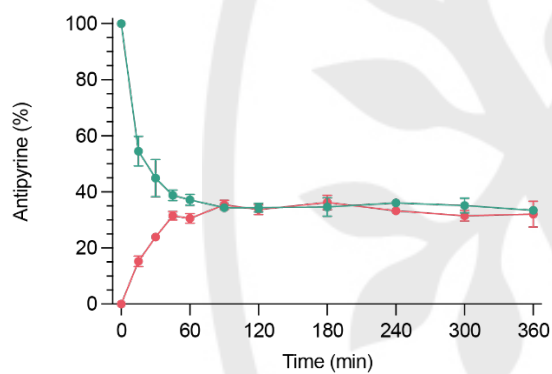
M = maternal; F = fetal; \* sum of compound present in fetal and maternal perfusates at the end of a perfusion, the amount sampled during the perfusion (sampling from fetal and maternal perfusates), and adherence of compounds by binding to the perfusion model, determined by the system adherence test (empty perfusion).

**Table 2S.** Fraction unbound ( $f_{u, \text{hom}}$ ) of study compounds to the placental homogenate determined by rapid equilibrium membrane dialysis. The homogenates (from 3 different donors) were each spiked with the compounds and added to the donor chamber. The receiver chamber consisted of blank buffer (PBS). Samples were collected after equilibration (240 min at 37°C, 600 rpm) and were analyzed by U(H)PLC-MS/MS. A dilution factor of 4 was applied to calculate the undiluted fraction unbound.

	Fraction unbound ( $f_{u, \text{hom}}$ )
Antipyrine	1.00
Protopine	0.48 $\pm$ 0.04
Citalopram	0.21 $\pm$ 0.01
Diazepam	0.09 $\pm$ 0.007



**Fig. 2S.** Matrix effects by the homogenate on the substances antipyrine, protopine, citalopram, and diazepam. The compounds were spiked into PBS and then diluted with either an equal volume of PBS (C0) or an equal volume of tissue homogenate (C0 + matrix). The samples were processed for LC-MS analysis within 1-3 min after the dilution to avoid interference of any stability issues. All values are expressed as mean  $\pm$  standard deviation (SD) of 3 to 4 independent experiments.

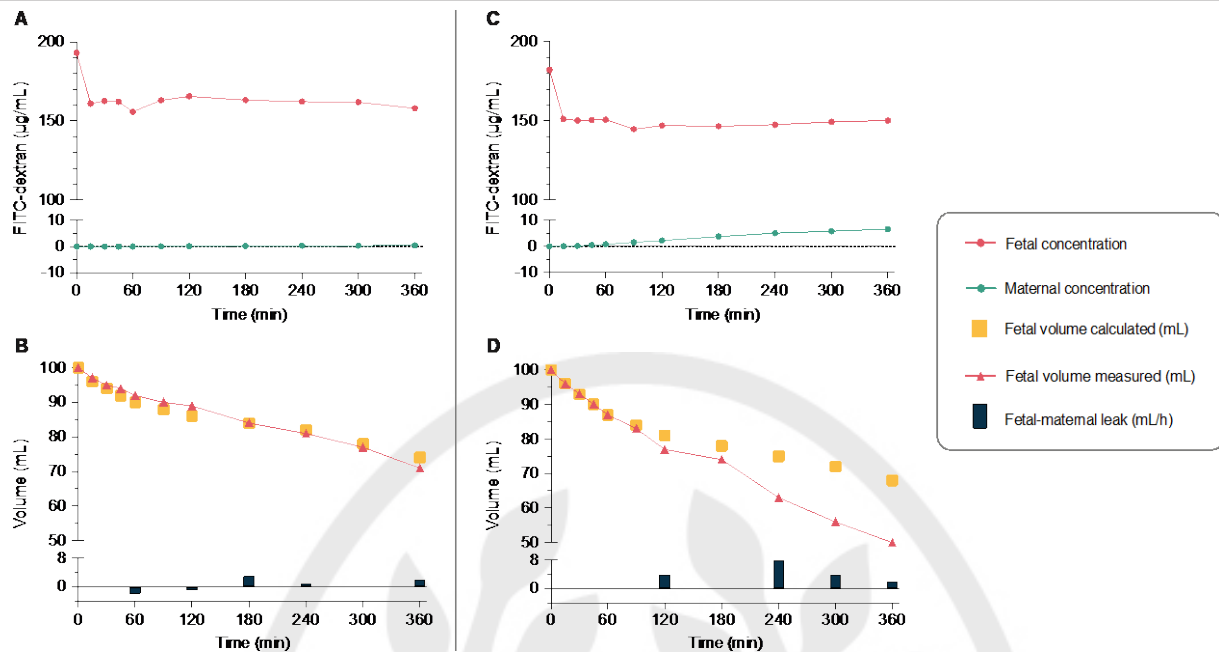


**Fig. 3S.** Perfusion profile of antipyrine from control perfusions. Concentrations are expressed as a percentage (%) of initial analyzed concentration in the maternal sample ( $\bullet$  maternal and  $\bullet$  fetal). All values are expressed as mean  $\pm$  standard deviation (SD) of at least 3 independent experiments (except timepoint 360 min in which only 2 values are included).

**Table 3S.** Characteristics of placentae used, and data from individual perfusions with all study compounds (antipyrine, n=3; protopine, n=4; citalopram, n=4; and diazepam, n=5).

	Antipyrine			Protopine				Citalopram				Diazepam				
Experiment number	1	2	3	1	1	1	1	1	2	3	4	1	2	3	4	5
Placenta weight (g)	690	680	580	716	555	555	555	660	720	720	480	520	500	580	480	690
Cotyledon weight (g)	30.95	23.26	6.15	17.13	22.27	22.27	22.27	24.56	45.93	12.50	12.95	12.92	7.32	18.17	15.8	17.18
Volume loss* (M, mL)	-4	6	11	-7	4	4	4	1	-5	-11	14	-6	-2	0	0	-1
Volume loss* (F, mL)	8	10	4	6	3	3	3	5	8	4	9	4	4	0	11	3
Preparation time <sup>°</sup> (min)	42	14	29	17	22	22	22	25	18	13	30	15	15	15	15	18
Cannulation time <sup>§</sup> (min)	16	15	30	30	13	13	13	35	40	19	19	27	21	18	22	23
Open pre-phase (min)	20	20	20	20	20	20	20	22	20	20	20	21	20	20	22	13
Close pre-phase (min)	20	20	20	23.5	21	21	21	22.5	20	20	20	25	24	26	24	43
Perfusion time (min)	300	360	360	240	360	360	360	360	360	300	360	360	360	360	360	360

\* Total volume loss at the end of the perfusion time; M = maternal; F = fetal; <sup>°</sup> time from birth to begin of cannulation; <sup>§</sup> time from cannulation to begin of open pre-phase.



**Fig. 4S.** Establishment and validation of the human *ex vivo* placental perfusion model. Assessment of the suitability of fluorescein isothiocyanate (FITC)-dextran (40 kDa) as a fetal capillary integrity marker when added to the fetal circuit at a concentration of 200 µg/mL. **(A)** A representative perfusion profile without a transfer of fluorescence (FITC-dextran) from the fetal to the maternal side occurs when the following two conditions were met: **(B)** the measured fetal volume corresponded to the calculated volume and a fetal-maternal (FM) leak of 4 mL/h was not exceeded. **(C)** A representative profile with a FITC-dextran transfer into the maternal circuit, if these conditions were violated: **(D)** the course of measured fetal volume in the corresponding perfusion deviated from the calculated volume, indicative of FM leaks (e.g. 8 mL/h at 240 min). A tracking of fetal and maternal volumes was therefore considered an adequate indicator for FM leaks, and no FITC-dextran was added in the subsequent placental perfusions.

**Table 4S.** U(H)PLC gradients, flow rate, and internal standards for the analysis of antipyrine, protopine, verapamil, diazepam, and citalopram. The mobile phase consisted of A (water with 5% MeCN and 0.1% formic acid) and B (MeCN with 0.1% formic acid). In the case of diazepam the mobile phase A contained only water and 0.1% formic acid.

<b>Antipyrine</b>					
	<b>Time (min)</b>	<b>%A</b>	<b>%B</b>	<b>Flow rate (mL/min)</b>	<b>Internal standard</b>
	0.00	100.0	0.0		
	0.50	100.0	0.0		
	3.00	37.89	62.11		
	3.01	0.0	100.0	0.4	Antipyrine-d3
	4.00	0.0	100.0		
	4.01	100.0	0.0		
	5.00	100.0	0.0		
<b>Protopine</b>					
	<b>Time (min)</b>	<b>%A</b>	<b>%B</b>	<b>Flow rate (mL/min)</b>	<b>Internal standard</b>
	0.00	100.0	0.0		
	1.00	100.0	0.0		
	3.00	0.0	100.0		
	4.00	0.0	100.0	0.5	Verapamil
	4.01	100.0	0.0		
	5.00	100.0	0.0		
<b>Diazepam</b>					
	<b>Time (min)</b>	<b>%A</b>	<b>%B</b>	<b>Flow rate (mL/min)</b>	<b>Internal standard</b>
	0.00	98.0	2.0		
	0.50	98.0	2.0		
	4.00	10.0	90.0		
	4.50	0.0	100.0	0.4	Diazepam-d5
	5.00	0.0	100.0		
	5.01	98.0	2.0		
	6.00	98.0	2.0		
<b>Citalopram</b>					
	<b>Time (min)</b>	<b>%A</b>	<b>%B</b>	<b>Flow rate (mL/min)</b>	<b>Internal standard</b>
	0.00	100.0	0.0		
	1.00	100.0	0.0		
	3.00	0.0	100.0		
	4.00	0.0	100.0	0.4	Citalopram-d4
	4.10	100.0	0.0		
	5.00	100.0	0.0		

**Table 5S.** MRM transitions and internal standards for the analysis of antipyrine, protopine, verapamil, diazepam, and citalopram. Gradients as in Table 4S.

Compound	Range (ng/mL)	MRM transitions	Internal standard	Ionization
Antipyrine	5 - 500	189.1 > 104.0 189.1 > 56.1	Antipyrine-d3	ESI <sup>+</sup>
Protopine	5 - 250	354.14 > 189.1 354.14 > 149.0	Verapamil*	ESI <sup>+</sup>
Diazepam	10 - 1000	285.08 > 193.1 285.08 > 154.0	Diazepam-d5	ESI <sup>+</sup>
Citalopram	10 - 1000	324.9 > 109.1 324.9 > 262.1	Citalopram-d4	ESI <sup>+</sup>

\* MRM transition 455.29 > 77.1

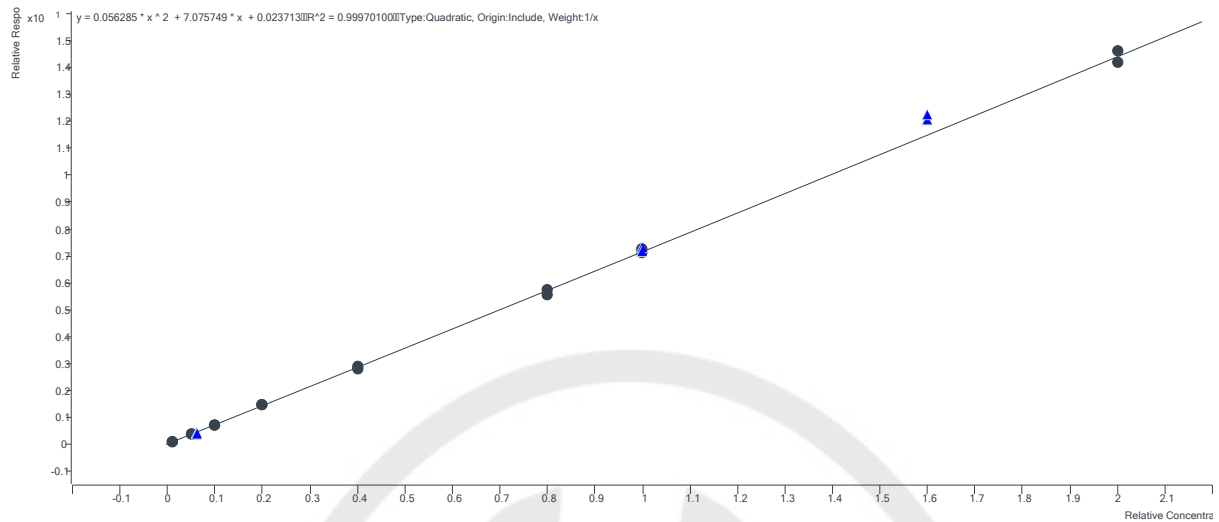


Fig. 5S. Calibration curve of antipyrine (calibrators are shown as dots and quality controls [QCs] are shown as triangles).

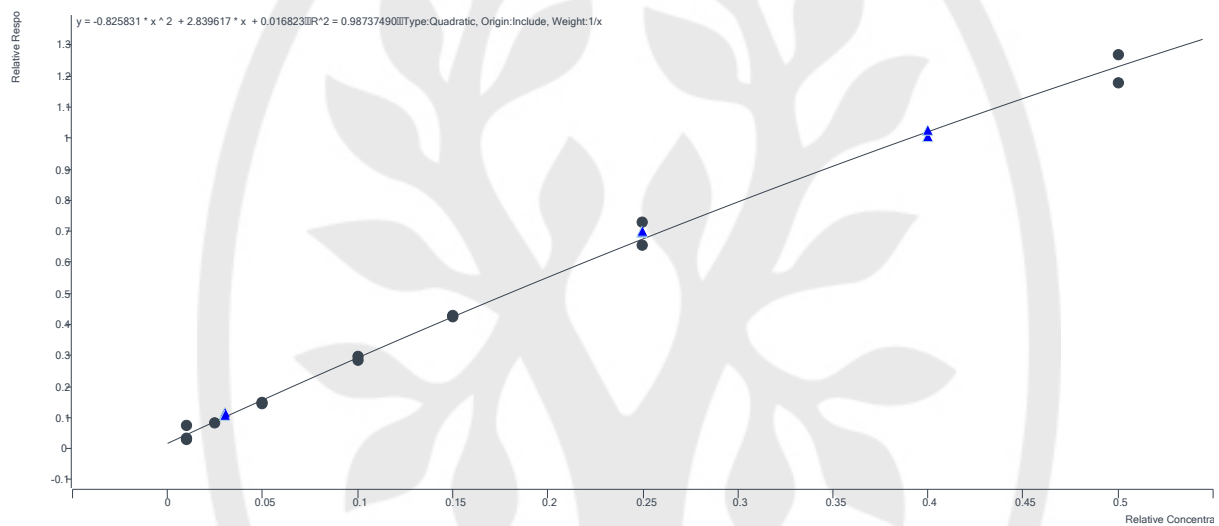


Fig. 6S. Calibration curve of protopine (calibrators are shown as dots and quality controls [QCs] are shown as triangles).

Coefficient of Determination:  $R^2 = 0.998447$   
 Calibration curve:  $2.47105e-007 \cdot x^2 + 0.00518175 \cdot x + 0.000974941$   
 Response type: Internal Std ( Ref 1 ), Area \* ( IS Conc. / IS Area )  
 Curve type: 2nd Order, Origin: Include, Weighting: 1/x, Axis trans: None

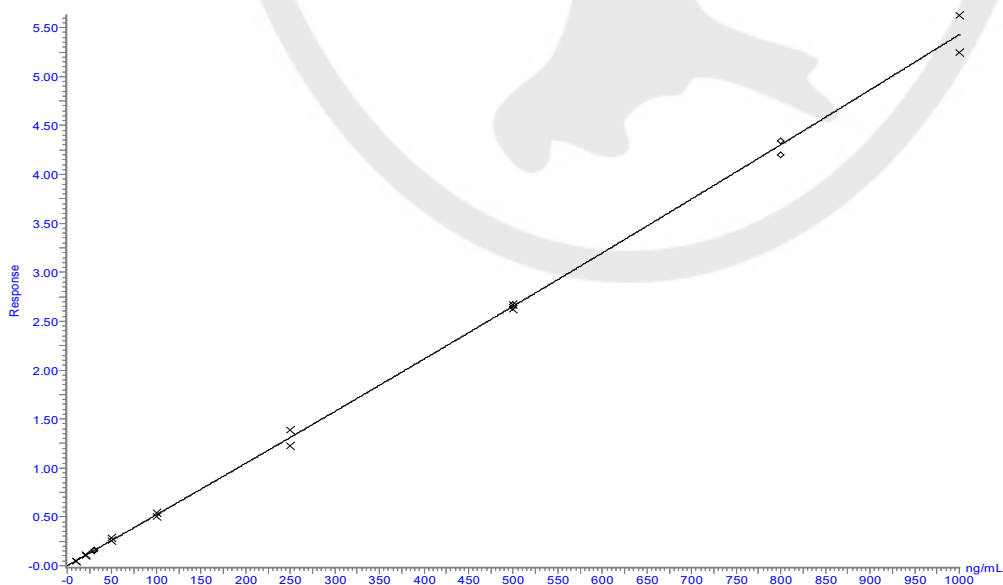
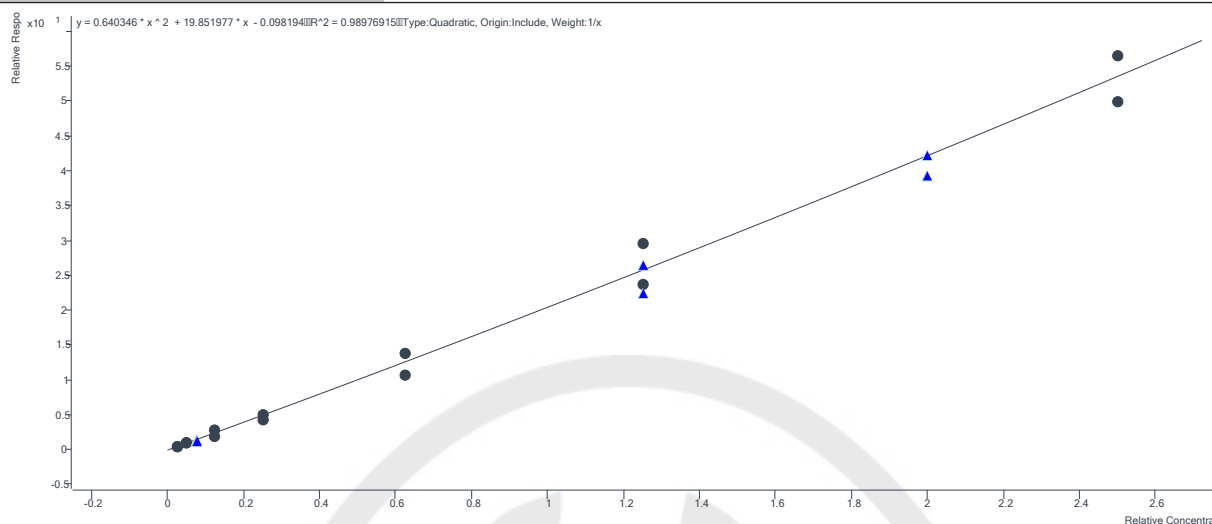


Fig. 7S. Calibration curve of citalopram (calibrators are shown as x and quality controls [QCs] are shown as diamond).



**Fig. 8S.** Calibration curve of diazepam (calibrators are shown as dots and quality controls [QCs] are shown as triangles).

**Table 6S.** Calibrators and calibration curve parameters for the determination of antipyrine.

Response:  $A \times Conc^2 + B \times Conc + C$ , 1/X weighting, Quadratic regression, included origin (n=40).

	Concentration (ng/mL)							Regression parameters			
	5	25	50	100	200	400	500	A	B	C	R <sup>2</sup>
Mean	5.019	25.361	50.383	99.622	198.024	394.796	509.635	-8.02E-02	2.270	0.008	0.998
SD	0.398	1.386	2.803	2.936	6.501	12.441	17.382	1.75E-01			
CV%	7.937	5.467	5.563	2.947	3.283	3.151	3.151				
RE%	0.380	1.445	0.767	-0.378	-0.988	-1.301	1.927				

**Table 7S.** Calibrators and calibration curve parameters for the determination of protopine.

Response:  $A \times Conc^2 + B \times Conc + C$ , 1/X weighting, Quadratic regression, included origin (n=12).

	Concentration (ng/mL)							Regression parameters			
	5	12.5	25	50	75	125	250	A	B	C	R <sup>2</sup>
Mean	4.900	12.600	24.800	51.700	76.200	120.300	253.000	-1.98E-00	3.760	-0.007	0.996
SD	0.295	0.765	2.058	3.856	4.854	6.182	15.707	2.39E-00			
CV%	6.030	6.070	8.300	7.450	6.400	5.140	6.210				
RE%	-2.280	0.785	-0.980	3.400	1.579	-3.740	1.194				

**Table 8S.** Calibrators and calibration curve parameters for the determination of citalopram.

Response:  $A \times Conc^2 + B \times Conc + C$ , 1/X weighting, Quadratic regression, included origin (n=12).

	Concentration (ng/mL)							Regression parameters			
	10	20	50	100	250	500	1000	A	B	C	R <sup>2</sup>
Mean	10.300	19.400	51.000	102.200	253.100	490.600	1001.500	-1.43E-06	0.020	0.043	0.997
SD	0.815	1.207	3.875	6.408	12.177	31.771	52.529	1.31E-06			
CV%	7.900	6.220	7.600	6.270	4.800	6.480	5.250				
RE%	3.180	-2.955	1.980	2.180	1.250	-1.870	0.146				



**Table 9S.** Calibrators and calibration curve parameters for the determination of diazepam.  
Response:  $A \times Conc^2 + B \times Conc + C$ , 1/X weighting, Quadratic regression, included origin (n=12).

	Concentration (ng/mL)							Regression parameters			
	10	20	50	100	250	500	1000	A	B	C	R <sup>2</sup>
Mean	10.300	19.600	49.000	101.700	251.600	498.500	986.200	3.83E-02	0.761	-0.021	0.994
SD	0.910	1.386	4.380	6.962	20.408	41.574	58.331	9.39E-02			
CV%	8.800	7.050	8.900	6.840	8.100	8.340	5.910				
RE%	3.330	-1.794	-1.940	1.730	0.639	-0.290	-1.380				

**Table 10S.** Quality control samples of antipyrine (n=40).

	QCL 15	QCM 250	QCH 400
Mean	16.100	239.400	380.500
SD	1.680	50.420	76.350
CV%	10.400	21.070	20.070
RE%	7.600	-4.250	-4.865

**Table 11S.** Quality control samples of protopine (n=12).

	QCL 15	QCM 125	QCH 200
Mean	16.900	125.900	202.800
SD	0.260	12.360	23.420
CV%	1.500	9.820	11.550
RE%	12.990	0.690	1.385

**Table 12S.** Quality control samples of citalopram (n=12).

	QCL 30	QCM 500	QCH 800
Mean	33.025	520.560	835.480
SD	0.860	16.730	50.480
CV%	2.700	3.240	6.030
RE%	8.670	3.360	4.663

**Table 13S.** Quality control samples of diazepam (n=12).

	QCL 30	QCM 500	QCH 800
Mean	30.600	504.400	801.200
SD	2.760	27.350	36.840
CV%	9.000	5.420	4.600
RE%	1.900	0.890	0.147

Table 14S. Carry-over assessment for antipyrine.

Run	Replicate	Peak area response (cts)				Individual carry-over (%)		Mean carry-over (%)		
		Blank sample		LLOQ		Analyte	IS	Analyte	IS	
		Analyte	IS	Analyte	IS					
1	1	0.00	0.00	15483.14	222133.29	0.00	0.00	4.00	0.04	
	2	1054.29	141.84	13171.49	183540.99	8.00	0.08			
2	1	900.18	0.00	11057.83	158834.00	8.14	0.00	7.69	0.04	
	2	824.46	121.97	11378.04	160274.60	7.25	0.08			
3	1	776.10	126.21	6320.00	137976.60	12.28	0.09	8.86	0.17	
	2	387.52	366.51	7125.06	152498.84	5.44	0.24			
4	1	108.88	292.98	8766.76	171126.49	1.24	0.17	1.58	0.18	
	2	169.18	345.30	8826.13	180080.12	1.92	0.19			
5	1	0.00	0.00	10196.77	199575.63	0.00	0.00	2.21	0.00	
	2	389.88	0.00	8809.64	216615.27	4.43	0.00			
6	1	2631.78	336.44	17131.43	143976.90	15.36	0.23	16.24	0.53	
	2	3260.05	1623.08	19055.93	195630.14	17.11	0.83			
7	1	*5588.23108212824	355.29	12784.71	124385.47	-	0.29	6.31	0.16	
	2	998.41	50.59	15824.74	155791.82	6.31	0.03			
8	1	634.72	1613.31	12325.89	131973.29	5.15	1.22	5.20	1.17	
	2	712.30	1524.55	13553.97	136836.17	5.26	1.11			
9	1	414.42	19.14	8142.70	75724.15	5.09	0.03	8.58	0.03	
	2	1862.45	61.24	15422.24	149839.59	12.08	0.04			
10	1	1071.57	68.63	10048.99	105887.43	10.66	0.06	9.79	0.05	
	2	1011.62	53.25	11353.70	120649.40	8.91	0.04			
11	1	2564.87	71.50	16715.36	142553.79	15.34	0.05	15.73	0.05	
	2	2916.05	80.08	18096.49	149729.07	16.11	0.05			
12	1	1020.98	85.83	14592.02	140330.04	7.00	0.06	8.20	0.05	
	2	1336.30	63.12	14218.52	142716.85	9.40	0.04			
13	1	837.07	66.31	12497.91	131122.86	6.70	0.05	8.49	0.05	
	2	2948.15	65.65	28692.43	167114.61	10.28	0.04			
14	1	1232.57	284.99	16362.89	149697.19	7.53	0.19	7.53	0.19	
	2	*10334.6123602373	*89.7065169210691	18322.91	166673.99	-	-			
15	1	2132.27	99.25	12386.00	112776.64	17.22	0.09	14.56	0.08	
	2	1528.94	87.59	12836.22	108165.64	11.91	0.08			
16	1	0.00	0.00	17787.36	172245.67	0.00	0.00	4.84	0.00	
	2	877.25	0.00	9056.74	90599.25	9.69	0.00			
17	1	177.39	114.45	1912.78	39997.00	9.27	0.29	8.00	0.19	
	2	165.10	46.87	2458.12	52289.93	6.72	0.09			
18	1	113.69	41.44	1859.26	39040.54	6.11	0.11	7.46	0.12	
	2	177.74	58.88	2020.34	42213.96	8.80	0.14			
19	1	415.72	151.04	2147.98	52787.39	19.35	0.29	16.71	0.23	
	2	423.71	146.43	3012.98	84257.39	14.06	0.17			
20	1	334.18	12.76	3290.16	84747.64	10.16	0.02	9.90	0.02	
	2	298.87	25.83	3097.33	86228.19	9.65	0.03			
*Cal 1 outside acceptance criteria, carry-over not calculated.								<b>Mean carry-over</b>	8.19	0.16

**Table 15S.** Carry-over assessment for protopine.

Run	Replicate	Peak area response (cts)				Individual carry-over (%)		Mean carry-over (%)	
		Blank sample		LLOQ		Analyte	IS	Analyte	IS
		Analyte	IS	Analyte	IS				
1	1	359.33	209.10	8285.35	97552.87	4.34	0.21	4.20	0.31
	2	326.79	461.11	8031.53	111516.56	4.07	0.41		
2	1	1213.96	117.56	4957.28	73555.43	24.49	0.16	22.73	0.15
	2	1138.38	110.52	5426.01	79165.82	20.98	0.14		
3	1	91.71	201.65	8069.87	123427.64	1.14	0.16	1.13	0.14
	2	83.68	138.95	7451.73	129270.72	1.12	0.11		
4	1	200.34	133.66	9992.82	168839.27	2.00	0.08	3.15	0.10
	2	583.21	268.32	13547.80	210399.04	4.30	0.13		
5	1	254.09	705.94	9336.76	165637.38	2.72	0.43	1.90	0.28
	2	133.64	261.65	12376.77	185863.41	1.08	0.14		
6	1	150.42	81.08	9258.74	173662.54	1.62	0.05	2.39	0.07
	2	316.08	178.34	10013.84	200257.87	3.16	0.09		
							<b>Mean carry-over</b>	5.919	0.176

**Table 16S.** Carry-over assessment for citalopram.

Run	Replicate	Peak area response (cts)				Individual carry-over (%)		Mean carry-over (%)	
		Blank sample		LLOQ		Analyte	IS	Analyte	IS
		Analyte	IS	Analyte	IS				
1	1	12143.52	130.52	334833.44	23864.26	3.63	0.55	2.72	0.31
	2	8979.30	13.29	495520.31	20404.75	1.81	0.07		
2	1	261.93	13.56	6005.38	22682.23	4.36	0.06	3.80	0.06
	2	194.69	13.45	6002.61	22931.61	3.24	0.06		
3	1	280.06	6.42	7185.11	19199.77	3.90	0.03	3.63	0.02
	2	273.09	1.74	8126.70	26066.70	3.36	0.01		
4	1	247.25	6.52	7132.82	20700.86	3.47	0.03	3.42	0.05
	2	248.09	14.25	7371.08	24053.68	3.37	0.06		
5	1	1358.55	230.73	3639.41	59973.48	37.33	0.38	32.77	0.35
	2	1156.00	191.78	4097.65	61940.79	28.21	0.31		
6	1	1028.20	174.39	3601.83	59565.51	28.55	0.29	26.42	0.28
	2	898.50	162.33	3698.60	60280.36	24.29	0.27		
							<b>Mean carry-over</b>	12.126	0.176

**Table 17S.** Carry-over assessment for diazepam.

Run	Replicate	Peak area response (cts)				Individual carry-over (%)		Mean carry-over (%)	
		Blank sample		LLOQ		Analyte	IS	Analyte	IS
		Analyte	IS	Analyte	IS				
1	1	699.34	27.05	21728.50	68480.63	3.22	0.04	2.93	0.08
	2	644.56	77.08	24476.12	67962.90	2.63	0.11		
2	1	1642.00	72.00	42957.00	168257.00	3.82	0.04	4.32	0.05
	2	1173.00	56.00	24311.00	95654.00	4.82	0.06		
3	1	894.09	34.42	25148.81	104117.53	3.56	0.03	9.10	0.06
	2	2419.19	46.12	16526.26	52667.72	14.64	0.09		
4	1	778.72	64.97	20966.67	209367.04	3.71	0.03	2.89	0.07
	2	356.70	167.37	17301.90	141594.28	2.06	0.12		
5	1	438.00	20.00	29823.00	103765.00	1.47	0.02	1.36	0.02
	2	335.00	9.00	26665.00	79608.00	1.26	0.01		
6	1	137.00	5.00	25805.00	68408.00	0.53	0.01	0.93	0.01
	2	671.00	18.00	50813.00	109412.00	1.32	0.02		
							<b>Mean carry-over</b>	3.587	0.048