Electronic Supplementary Material (ESI) for Analytical Methods.

Appendix:

Table A1
Reported methods for analysis of methadone enantiomers in biological samples.

Method	Sample type/ size	Sample preparation	Linear range for single enantiomer (μg mL^{-1})	LOQ for single enantiomer (μg mL^{-1})	Reference
HPLC/UV	Human serum/1 mL	LLE	0.0034-0.204	0.0017	1
HPLC/UV	Serum/1 mL	SPE/LLE	0.025-1.000	0.025	2
HPLC/UV	Plasma/2 mL	LLE	0.0025-0.025 and 0.025-0.400	0.0025	3
HPLC/UV	Plasma/1 mL	LLE	0.015-0.600	0.015	4
	Urine		0.039-3.900	0.039	
LC/MS	Plasma/1 mL	LLE	0.005-1.000	0.005	5
HPLC/MS	Saliva/500 μL	-	0.005-0.600	0.005	6
CE/MS	Plasma/200 μL	PP	0.250-0.500	0.250	7
	Plasma/1 mL	LLE	0.0005-0.175	0.0005	
HPLC/UV	Plasma/1 mL	LLE	0.025-1.000	0.010	8
	Urine /1 mL				
HPLC/UV	Serum/100 μL	SPE	0.200-5.000	-	9
	Urine/ 200 μL				
HPLC/MS	Serum/5 mL	SPE	0.025-0.750	5	10
	Plasma/5 mL				
LC-MS/MS	Plasma	PP	0.0125-1.000	0.0125	11
LC/MS	Whole blood/0.6 g	SPE	0.500-2.100	0.200	12
CE/DAD	Oral fluid	LLE	0.0081–0.625	0.0024	13
CE/DAD	Serum	LLE	0.0025-0.500	-	14
	Urine		0.010-2.500		
CE/UV	Urine	SPE	1.500-26.700	-	15

LLE, liquid-liquid extraction; SPE, solid phase extraction; PP, protein precipitation.

Table A2

Assay precision and accuracy of EBC samples spiked with methadone.

Analyte	Enantiomer 1			Enantiomer 2		
Nominal concentration (μg mL^{-1})	0.62	1.25	2.50	0.62	1.25	2.50
Inter-day assay (n=5)[a] concentration (μg mL^{-1})	0.63	1.10	2.84	0.63	1.11	2.56
	0.63	1.11	2.47	0.63	1.11	2.62
	0.63	1.07	2.39	0.63	1.11	2.24
	0.64	1.05	2.39	0.62	1.07	2.40
	0.63	1.06	2.62	0.62	1.07	2.39
Average	0.63	1.08	2.54	0.63	1.09	2.44
[b]SD	0.00	0.03	0.19	0.01	0.02	0.15
[c]RSD	0.73	2.61	7.50	0.98	2.06	6.18
[d]RE%	2.30	-13.79	1.75	1.01	-12.65	-2.30
Intra-day assay (n=5)[e] concentration (μg mL^{-1})	0.67	1.19	2.53	0.62	1.14	2.44
	0.63	1.32	2.50	0.62	1.20	2.70
	0.60	1.39	2.48	0.59	1.25	2.20
	0.82	1.11	2.19	0.76	1.25	2.89
	0.75	1.14	2.00	0.64	1.45	2.90
Average	0.69	1.23	2.34	065	1.26	2.63
SD	0.09	0.12	0.23	0.07	0.12	0.30
RSD	13.11	9.59	10.00	10.26	9.26	11.52
RE%	11.97	-1.71	-6.28	4.14	0.62	5.03

[a]Number of replicates; [b]Standard deviation; [c]Relative standard deviation, [d]RE (relative error)%= 100 × ((Found value-Nominal value)/ Nominal value); [e]Number of days.

Table A3

Recoveries for analysis of methadone in spiked EBC.

Analyte	Nominal concentration (μg mL^{-1})	Mean found concentration (μg mL^{-1}; n = 5)	Mean recovery (%)
Enantiomer 1	0.62	0.63	102.3
	1.25	1.08	86.1
	2.50	2.54	101.7
Enantiomer 2	0.62	0.63	100.9
	1.25	1.09	87.3
	2.50	2.44	97.6

Table A4

Results of stability tests for determination of methadone enantiomers in EBC samples.

Analyte	Concentration added (µg mL^{-1})	Freeze-thaw stability		Room temperature stability	
		Concentration found (µg mL^{-1}; n=3)	Accuracy (%RE)	Concentration found (µg mL^{-1}; n=3)	Accuracy (%RE)[a]
Enantiomer 1	0.62	0.62	-0.27	0.60	-3.77
	1.25	1.11	-11.48	1.12	-10.16
	2.50	2.60	4.14	2.62	4.74
Enantiomer 2	0.62	0.63	1.54	0.63	1.54
	1.25	1.12	-10.76	1.11	-10.64
	2.50	2.57	2.80	2.53	1.00

[a]RE (relative error)%= 100 × ((Found value-Nominal value)/ Nominal.

Table A5

Results of the evaluation of method robustness in three different levels.

Analyte	Level	Nominal concentration (µg mL^{-1})	Mean found concentration (µg mL^{-1})	Accuracy (RE%)[a]
Enantiomer 1	1	2.50	2.75	10.13
	2	2.50	2.71	8.33
	3	2.50	2.80	11.93
Enantiomer 2	1	2.50	2.78	11.17
	2	2.50	2.72	8.77
	3	2.50	2.74	9.67

1: Buffer pH = 2.4, Buffer concentration: 148 mM, Temprature:14°C.

2: Buffer pH = 2.5, Buffer concentration: 150 mM, Temprature:15°C.

3: Buffer pH = 2.6, Buffer concentration: 152 mM, Temprature:16 °C.

[a]RE (relative error)%= 100 × ((Found value-Nominal value)/ Nominal.

Figure A1. A real photograph of the EBC collection setup.

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