Electronic Supplementary Material (ESI) for Analyst.

Supplementary materials

Analysis of illicit pills and drug of abuse in urine samples by 3D-printed open port probe hyphenated to differential mobility spectrometry -mass spectrometry

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Figure S1. Configuration of OPP to ESI

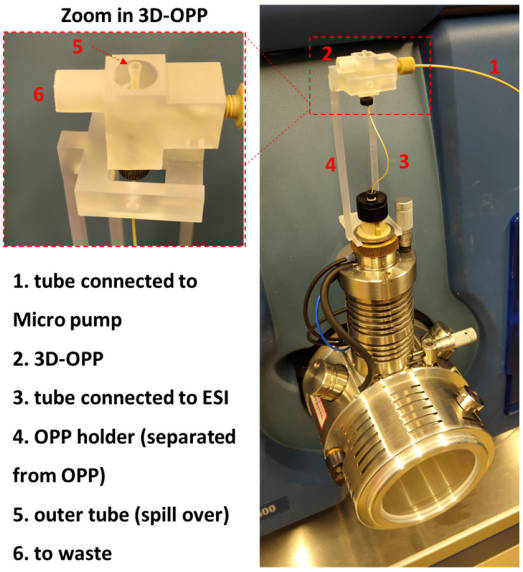


Figure S2. Auto sample delivery with the liquid handler

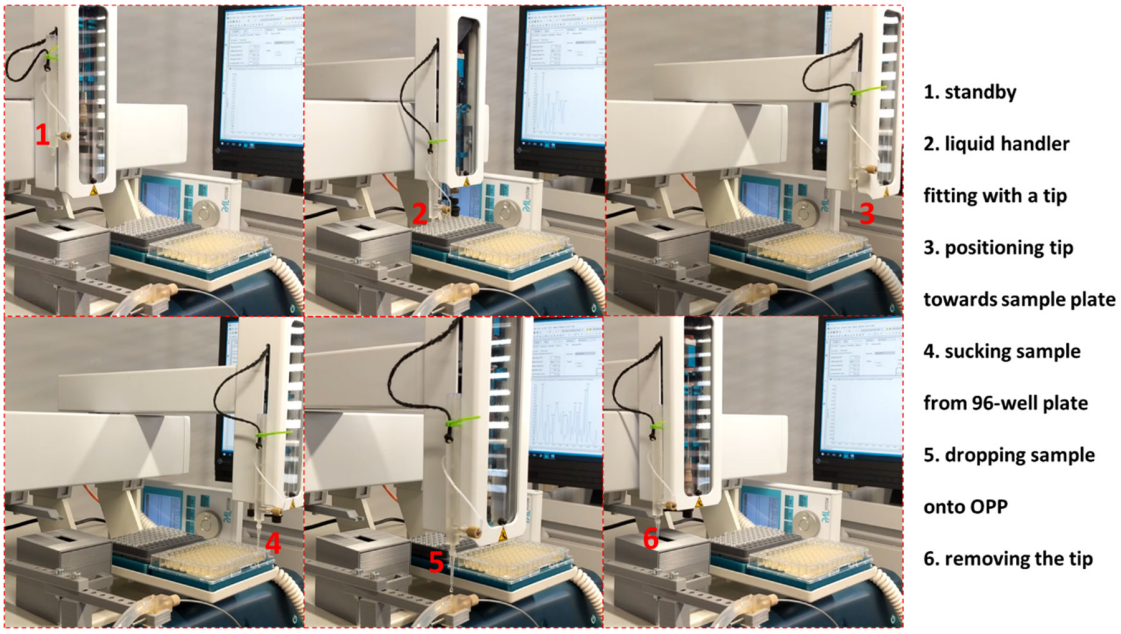


Figure S3. Design of new 3DP-OPP holder mounted directly on TIS.

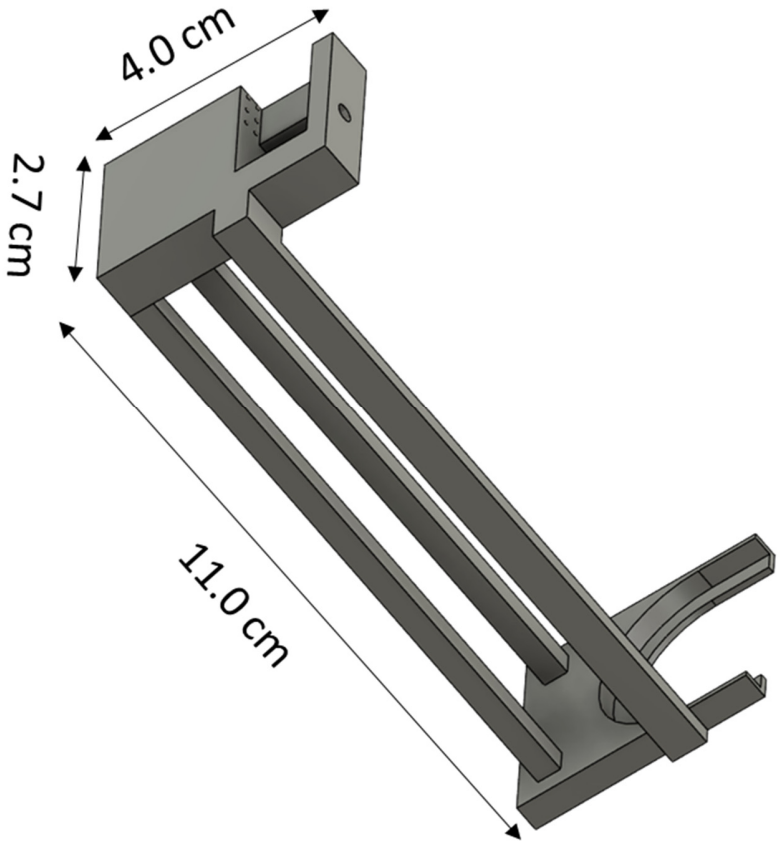


Figure S4. FDM 3D printed updated waste box.

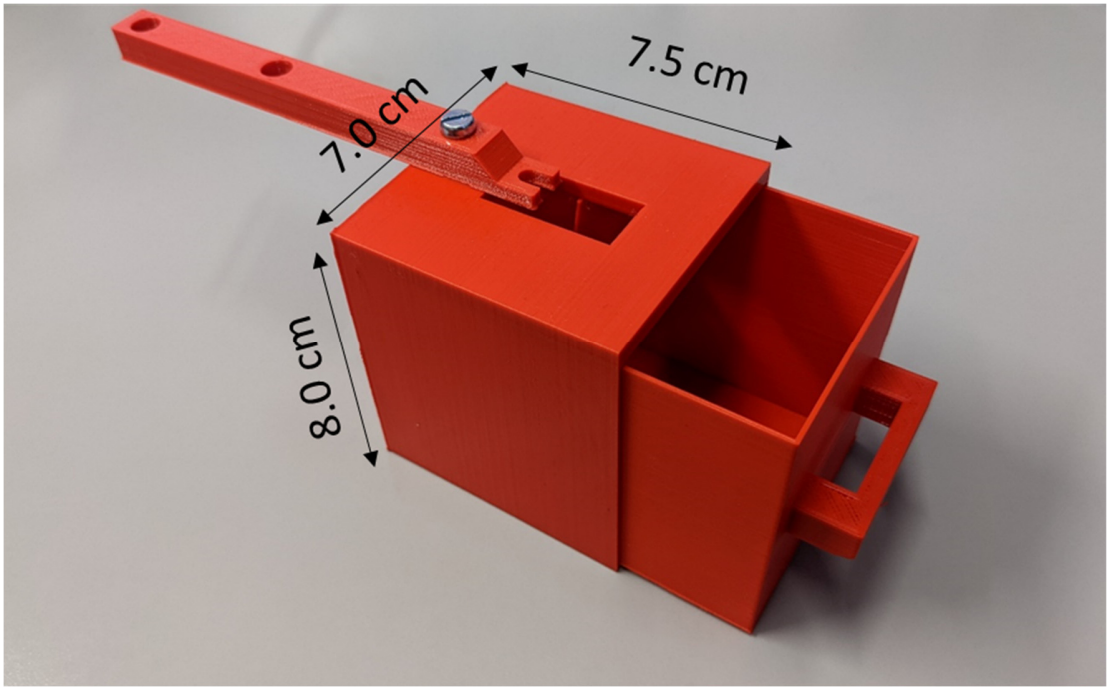


Table S1. Weblinks to unmodified 3D models of utilized tools.

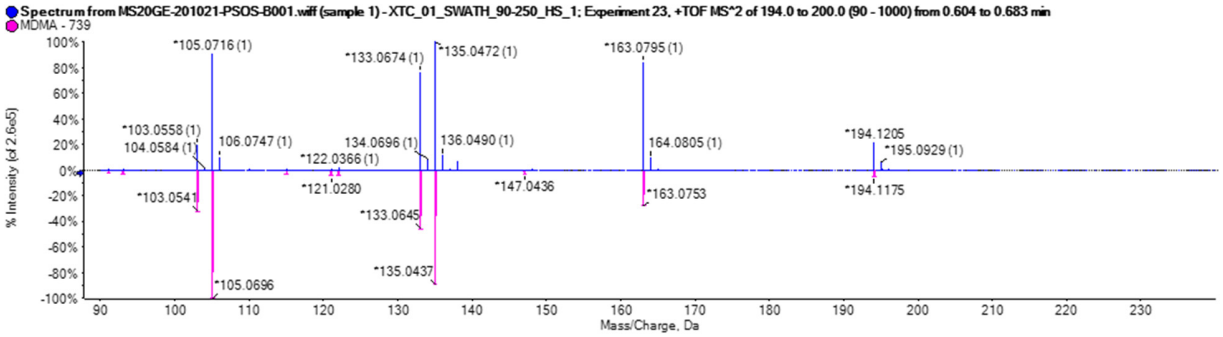
96-wells plate adapter	https://ars.els-cdn.com/content/image/1-s2.0-S0039914020301855-mmc3.zip
3DP-OPP model	https://ars.els-cdn.com/content/image/1-s2.0-S0039914020301855-mmc4.zip
pipette tips rack adapter	https://ars.els-cdn.com/content/image/1-s2.0-S0039914020301855-mmc5.zip
Pipette tool for PAL sampler	https://ars.els-cdn.com/content/image/1-s2.0-S0039914020301855-mmc6.zip

Table S2. Accurate mass of known drugs used for screening of analytes ecstasy pills

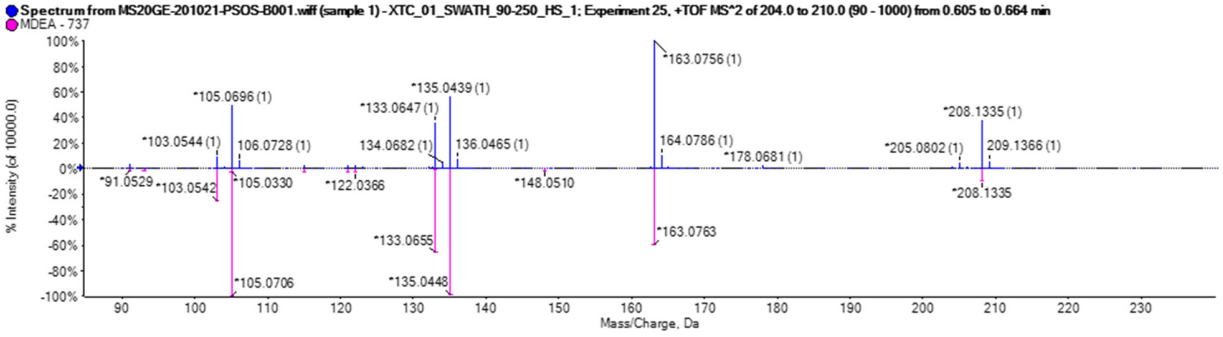
Analyte	formula	theoretical mass $[M+H]^+$ [*m/z*]
MDMA	$C_{11}H_{15}NO_2$	194.1176
MDEA	$C_{12}H_{17}NO_2$	208.1332
caffeine	$C_8H_{10}N_4O_2$	195.0877
amphetamine	$C_9H_{13}N$	136.1121
cocaine	$C_{17}H_{21}NO_4$	304.1543
ketamine	$C_{13}H_{16}ClNO$	238.0993
MDHOET	$C_{12}H_{17}NO_3$	224.1281
2DPMP	$C_{18}H_{21}N$	252.1747
DPIA	$C_{18}H_{23}N$	254.1903

Figure S5. Experimental (blue) and theoretical (pink) MS/MS spectra of the compounds identified with HRMS forensic library.

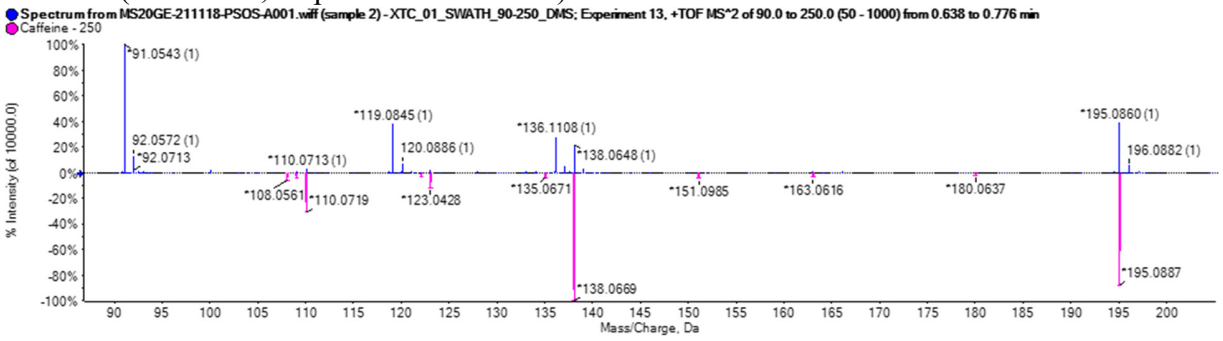
MDMA



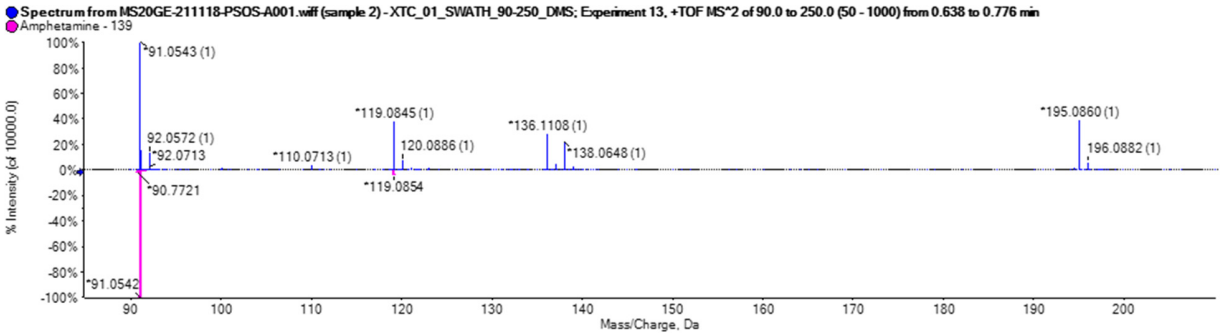
MDEA



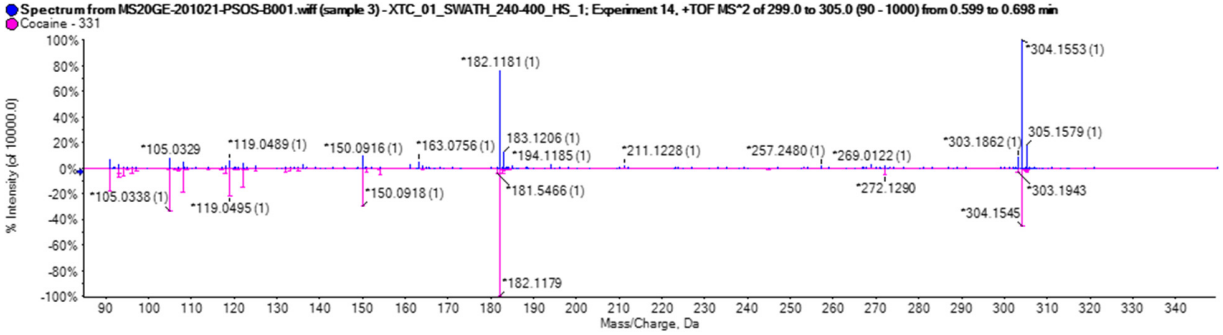
Caffeine (with DMS, amphetamine coeluted)



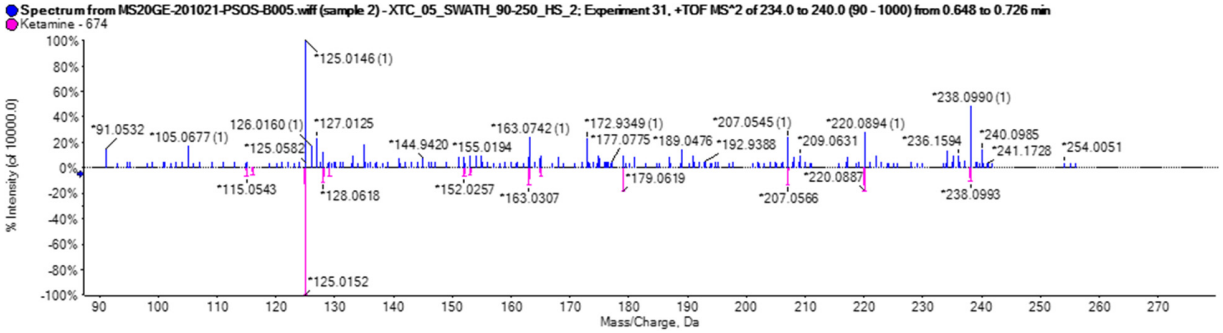
Amphetamine (with DMS, caffeine coeluted)



Cocaine



Ketamine



2DPMP

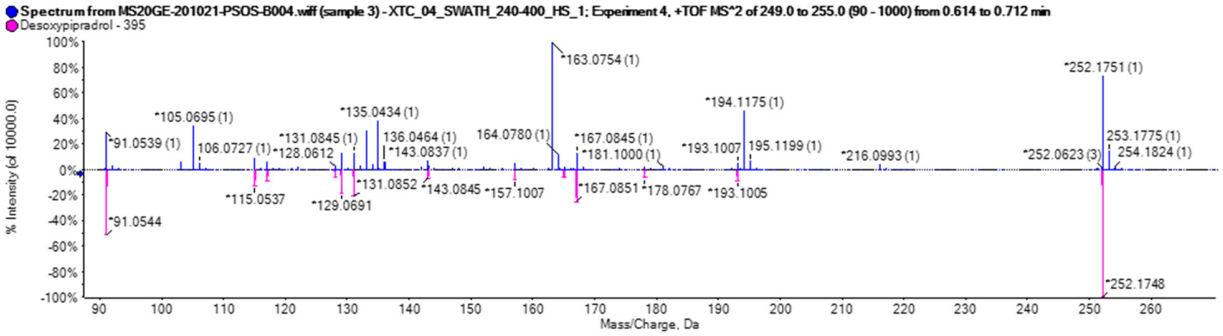
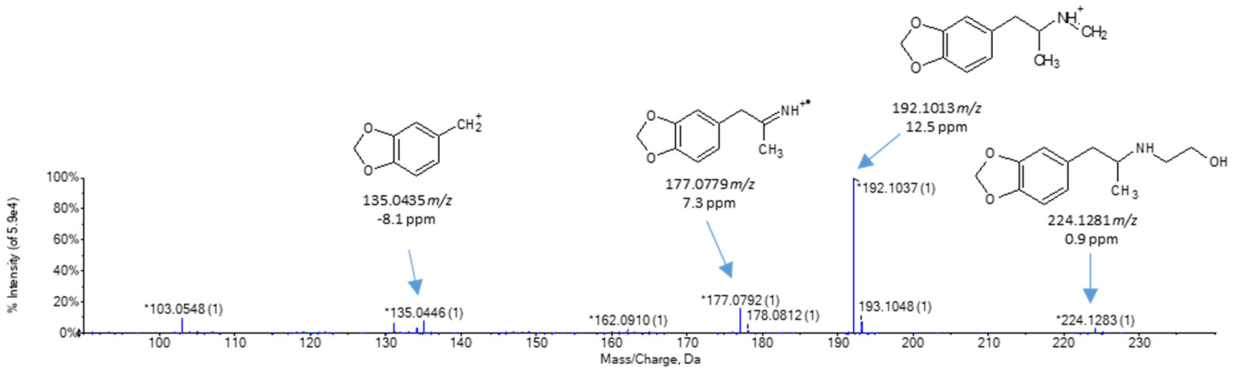


Figure S6. MS/MS spectra of compounds tentatively identified using accurate mass and fragments assignment.

MDHOET



DPIA [di(β-phenylisopropyl)amine]

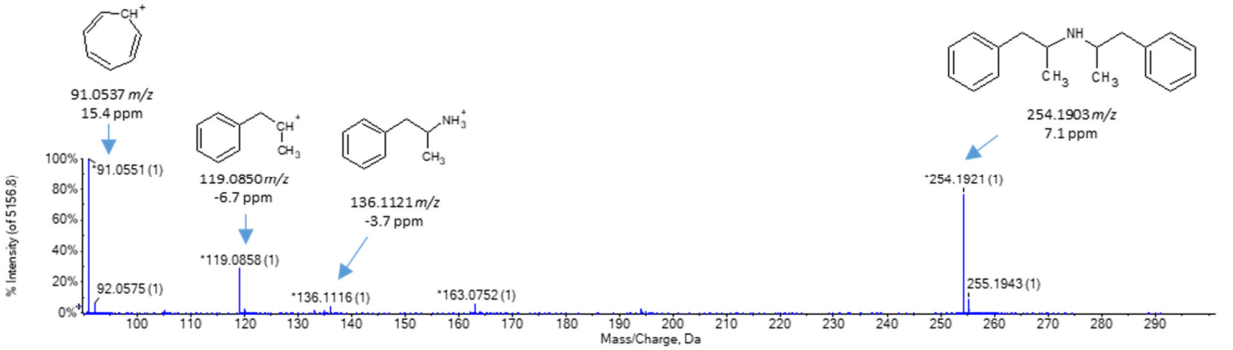


Figure S7. Ionograms obtained during CoV value ramp for tramadol and its O- and N-desmethylmetabolites at 100 ng/mL. Acetonitrile was used as modifier and the separation voltage (SV) was of 2600 V.

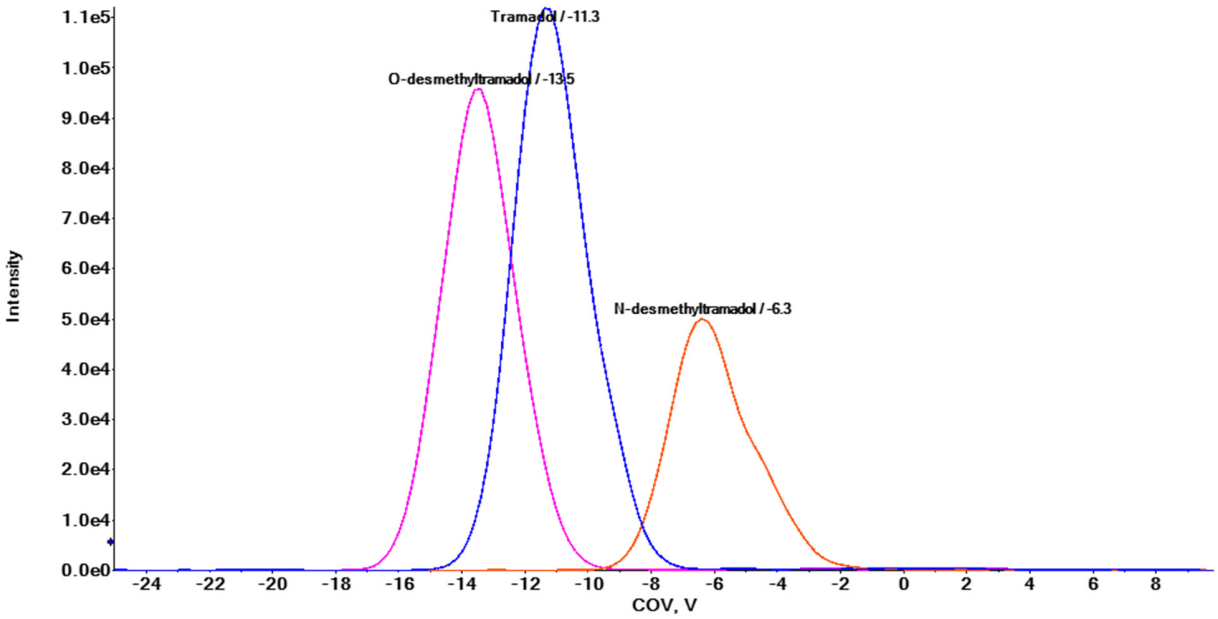


Table S3. Optimized SRM transitions used for compounds quantified in urine.

Name	Q1	Q3	Dwell [ms]	CE [V]	CoV [V]	CXP [V]	DMO [V]
Cocaine-d3	*m/z* 307.3	*m/z* 185.1	50	27	-9.1	16	-20
Benzoylecgonine	*m/z* 290.2	*m/z* 168.2	100	29	-10.6	20	-30
Norcocaine	*m/z* 290.2	*m/z* 136.3	100	33	-3.7	16	-10
Ecgonine	*m/z* 200	*m/z*	50	25	-18.5	24	20
Cocaine	*m/z*	*m/z*	50	27	-9.1	16	-20
Cocaethylene	*m/z* 318.1	*m/z* 196	50	29	-8	24	20
Tramadol-d3	*m/z* 267.1	*m/z* 58	100	57	-11.2	12	20
Tramadol	*m/z*	*m/z* 58	100	57	-11.2	12	20
O-desmethyltramadol	*m/z* 250	*m/z* 58	100	45	-13.5	14	20
N-desmethyltramadol	*m/z* 250	*m/z* 44	100	49	-6	4	10

Table S4. Accuracy and precision for all quantified compounds in urine QC samples (3 runs, n = 5).

compound	QC sample	Nominal concentration	Within-run Accuracy (%; 3 runs)			Between runs Accuracy (%)	Within-run Precision (%CV; 3 runs)			Between runs Precision (%CV)
benzoylecgonine	QCLLOQ	25	94.84	90.73	102.49	96.01	8.04	6.99	3.33	6.21
	QCL	75	104.89	92.45	102.52	99.95	3.1	3.09	3.18	6.62
	QCM	400	100.27	98.22	105.53	101.34	1.19	3.22	4.37	3.72
	QCH	2000	101.3	100.63	108.24	103.40	2.56	4.98	1.82	4.07
norcocaine	QCLLOQ	25	98.17	89.71	88.2	92.03	3.97	10.04	5.36	5.83
	QCL	75	111.85	92.04	100.87	101.59	2.04	3.59	1.1	9.77
	QCM	400	101.78	99.84	98.71	100.10	4.13	3.93	2.05	1.55
	QCH	2000	105.69	102.61	107.44	105.25	4.77	5.41	6.96	2.33
methylecgonine	QCLLOQ	10	91.17	89.87	93.31	91.45	6.53	2.25	7.4	1.90
	QCL	30	108.2	88.51	101.79	99.50	2.75	3.68	2.18	10.09
	QCM	200	100.69	95.96	104.55	100.40	6.39	10.19	2.7	4.29
	QCH	800	109.17	99.68	98.48	102.45	2.8	1.55	6.01	5.69
cocaine	QCLLOQ	10	100.61	99.41	112.96	104.34	7.02	1.83	2.51	7.21
	QCL	30	103.8	104.59	109.31	105.90	1.71	3.81	3.27	2.81
	QCM	200	98.57	98.57	100.05	99.05	3.88	3.84	3.15	0.87
	QCH	800	101.32	100.88	109.88	104.03	1.76	7.66	3.42	4.89
cocaethylene	QCLLOQ	10	104.66	100.66	109.29	104.90	2.87	2.46	3.55	4.10

	QCL	30	103.42	109.3	103.16	105.29	2.14	4.27	2.65	3.30
	QCM	200	99.41	99.87	95.95	98.40	2.31	3.94	4.1	2.17
	QCH	800	107.52	103.37	106.89	105.92	5.26	4.28	3.12	2.11
tramadol	QCLLOQ	10	104.75	106.77	93.86	101.82	2.35	4.05	2.35	6.84
	QCL	30	107.55	103.22	105.78	105.52	4.88	5.31	3.57	2.06
	QCM	200	100.95	98.28	97.93	99.07	3.32	1.81	6.64	1.66
	QCH	800	103.98	103	104.04	103.68	4.99	4.33	3.51	0.57
O-desmethyltramadol	QCLLOQ	10	105.92	115.9	107.78	109.87	5.99	7.35	4.83	6.69
	QCL	30	109.78	109.78	109.52	109.69	6.81	2.75	0.12	2.51
	QCM	200	103.85	100.37	99.29	101.17	3.42	1.29	2.35	3.21
	QCH	800	110.15	105.34	105.51	107	5.56	2.97	2.55	1.88
N-desmethyltramadol	QCLLOQ	10	107.49	101.12	97.27	99.19	4.37	8.21	2.52	2.73
	QCL	30	107.03	102.02	112.5	107.27	5.4	5.89	2.89	6.90
	QCM	200	100.37	101.55	100.26	100.90	2.99	1.23	2.89	0.91
	QCH	800	103.99	107.46	106.15	106.81	2.47	2.71	3.69	0.87

Table S5. Detailed calibration curve data for all compounds quantified in urine.

Compound	Run	Calibration curve	Weighting factor	Correlation coefficie
Benzoylecgonine	1	y = 6.19212e^{-4}x +		0.99854
	2	y = 5.26125e^{-4}x + 0.00116	$1/x^2$	0.99051
	3	y = 3.59698e^{-4}x +		0.99698
Norcocaine	1	y = 8.24169e^{-4}x + 0.00159		0.99789
	2	y = 4.85772e^{-4}x + 0.00148	$1/x^2$	0.99297
	3	y = 4.85896e^{-4}x + 0.00117		0.99704
Methylecgonine	1	y = 0.00234x + 0.00347		0.99763
	2	y = 0.00186x + 0.00284	1/x	0.99931
	3	y = 0.00121x + 0.00311		0.99851
Cocaine	1	y = 0.01067x + 0.00380		0.99891
	2	y = 0.01051x + 0.00276	$1/x^2$	0.99653
	3	y = 0.00695x + 0.00352		0.99824
Cocaethylene	1	y = 0.00896x + 0.00134		0.99887
	2	y = 0.00788x + 0.00254	$1/x^2$	0.99629
	3	y = 0.00561x + 0.00228		0.99773
Tramadol	1	y = 0.01181x + 0.00554		0.99842
	2	y = 0.00907 + 0.00895	$1/x^2$	0.99722
	3	y = 0.00701 + 0.00937		0.99923
O-desmethyltramadol	1	y = 0.00566x + 0.00159		0.99813
	2	y = 0.00482x + 0.00143	$1/x^2$	0.99782
	3	y = 0.00352x + 9.26258e^{-}		0.99940
N-desmethyltramadol	1	y = 0.00329x + 1.57573e^{-}		0.99933
	2	y = 0.00174x + 8.23949e^{-}	$1/x^2$	0.99368
	3	y = 0.00198x + 4.07439e^{-}		0.99961

Table S6. Back calculated accuracy values for standards of benzoylecgonine, norcocaine and methylecgonine in urine (3 runs, n=1).

Nominal concentration (ng/ml)	Accuracy(%;3runs)								
	benzoylecgonine			norcocaine			methylecgonine		
25	95.65	83.44	104.67	100.88	83.78	85.62	92.48	91.91	89.67
50	94.07	97.92	90.71	97.18	94.87	96.14	89.49	86.73	88.09
100	101.95	85.84	93.08	96.85	89.50	96.87	98.00	90.17	88.74
250	97.33	99.66	97.75	91.97	100.52	100.26	91.37	91.13	100.70
500	96.05	127.24	93.14	98.57	120.01	102.28	85.73	97.27	110.81
1000	104.98	110.34	111.75	100.02	113.74	110.36	96.79	104.13	104.78
2500	109.97	95.56	108.90	114.52	97.57	108.46	105.37	100.52	96.65

Table S7. Back calculated accuracy values for standards cocaine and cocaethylene in urine (3 runs, n=1).

Nominal concentration (ng/ml)	Accuracy(%;3runs)					
	cocaine			cocaethylene		
10	91.23	90.43	97.15	96.58	88.5	96.57
25	106.71	88.58	96.31	92.54	87.6	88.45
50	100.45	94.90	91.43	101.30	104.	97.16
100	104.87	103.51	101.71	104.39	97.6	97.35
250	98.85	103.77	96.74	103.56	103.	109.90
500	97.52	113.69	107.93	95.53	113.	103.65
1000	100.36	105.13	108.72	106.10	105.	106.91

Table S8. Back calculated accuracy values for standards tramadol, O-desmethyltramadol and N-desmethyltramadol in urine (3 runs, n=1).

Nominal concentration (ng/ml)	Accuracy(%;3runs)								
	Tramadol			O-desmethyltramadol			N-desmethyltramadol		
10	25	87.85	96.54	90.62	88.78	106.60	97.52	80.07	99.72
25	50	95.40	94.37	97.33	92.76	96.97	97.22	92.18	94.59
50	100	100.58	103.97	106.83	108.60	100.45	107.27	114.50	99.73
100	250	98.68	98.27	103.60	100.07	99.91	100.46	101.85	98.43
250	500	96.96	106.17	96.89	99.61	102.45	96.49	93.18	102.95
500	1000	109.88	98.49	95.75	104.31	98.23	101.04	107.15	101.08
1000	25	110.64	102.20	108.97	105.8	95.39	105.9	111.08	103.50

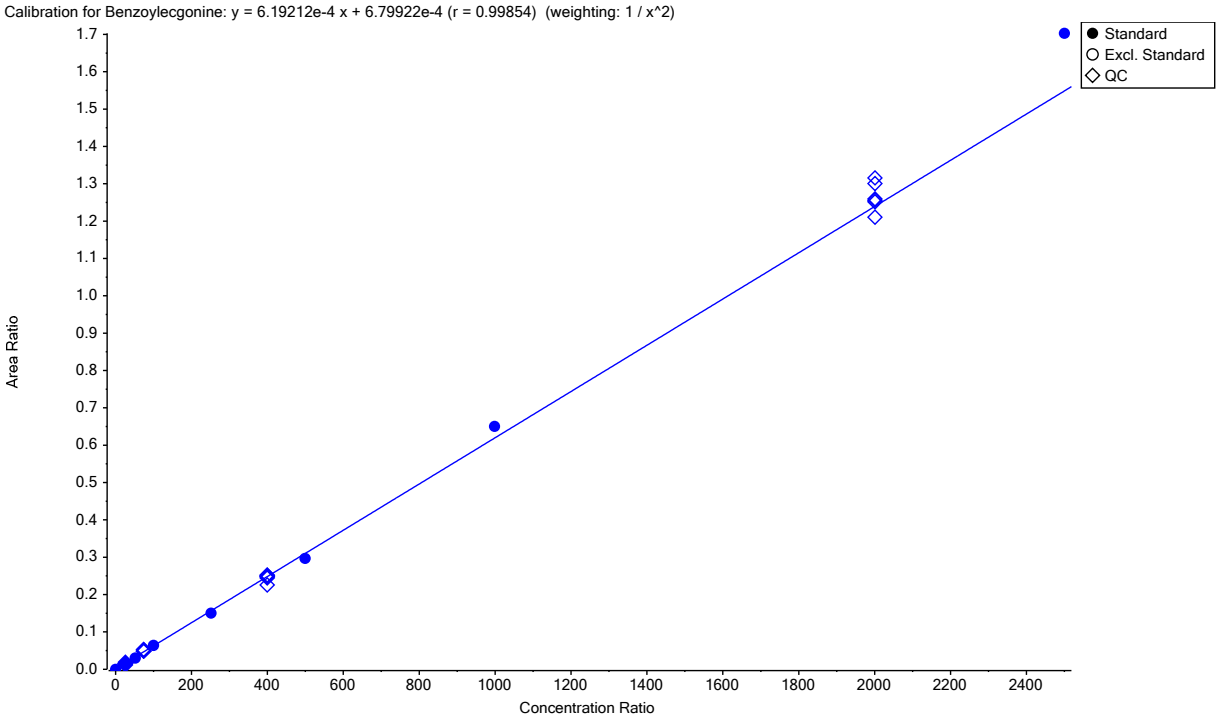
Table S9. Accuracy and precision data obtained for diluted QC (5000 pg/µl) at 10x and 100x dilution factors (n=5).

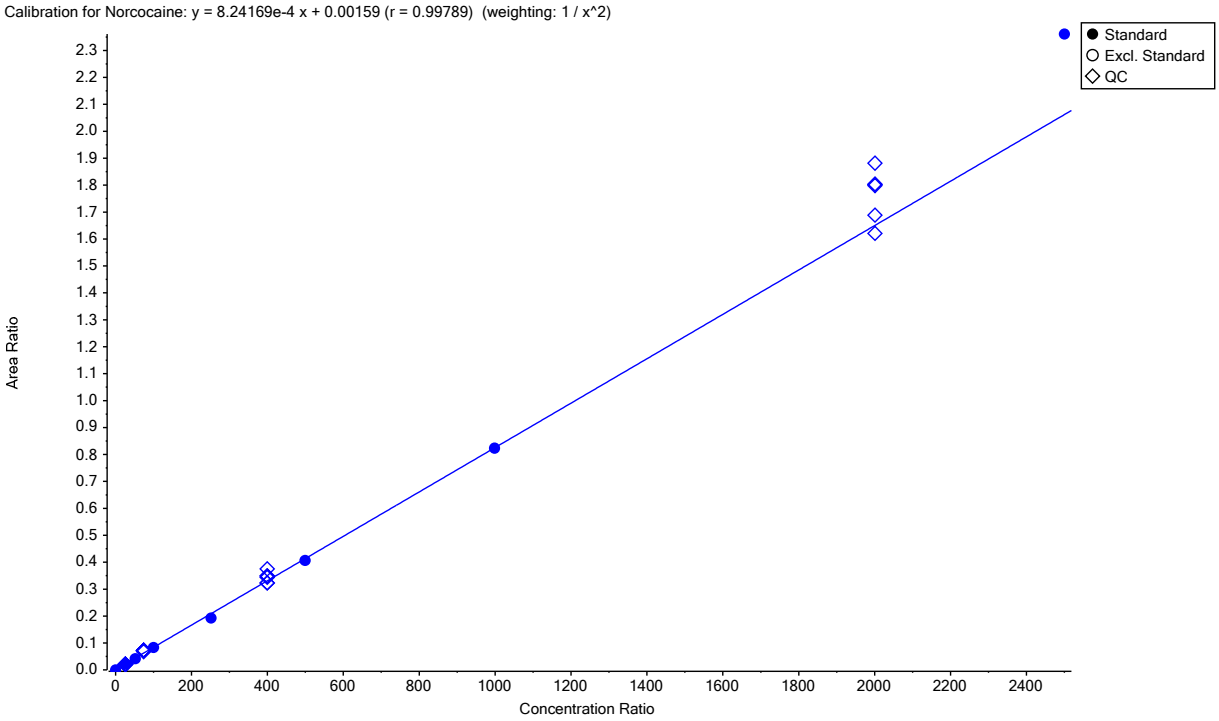
Compound	dilution factor	Accuracy (%)	Precision (%CV)
benzoylecgonine	10	1.97	95.76
	100	4.59	104.28
norcocaine	10	6.36	100.06
	100	4.09	105.15
methylecgonine	10	2.34	96.28
	100	3.58	98.65
cocaine	10	6.52	101.35
	100	2.04	105.44
cocaethylene	10	2.3	101.41
	100	3.16	102.77
tramadol	10	2.81	103.32
	100	2.14	111.32
O-desmethyltramadol	10	4.74	102.59
	100	1.41	105.42
N-desmethyltramadol	10	2.76	103.89
	100	5.09	105.75

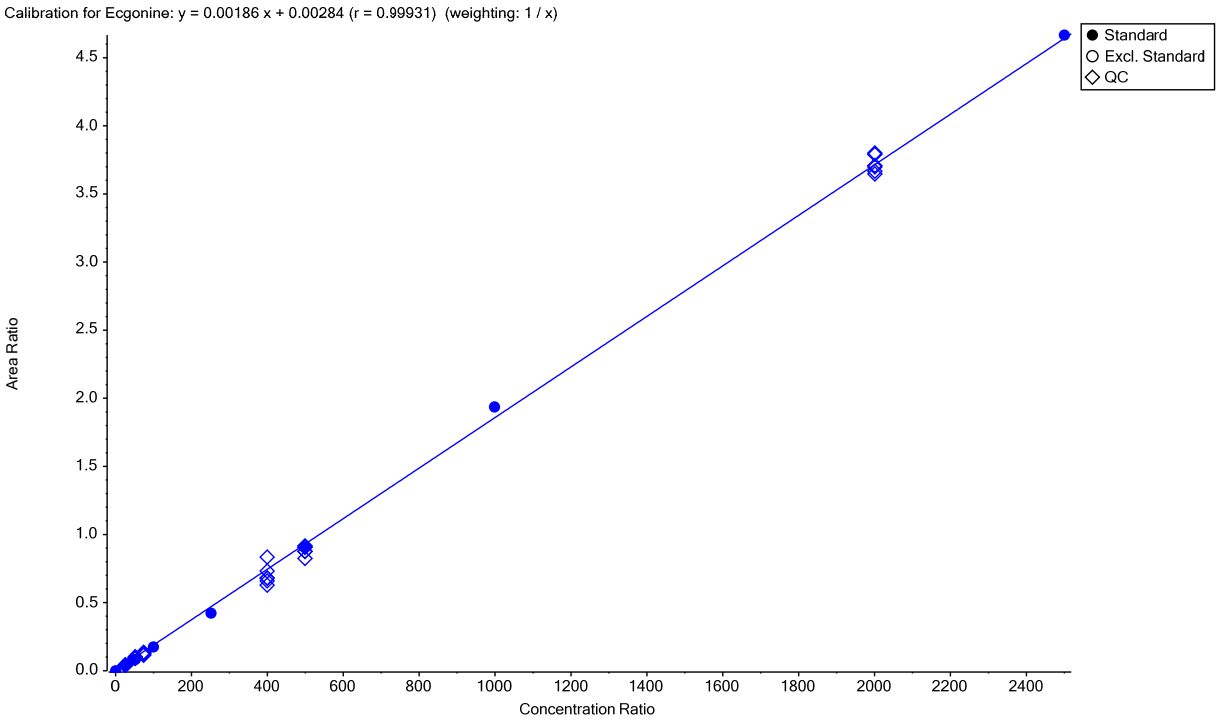
Table S10. Carry-over data obtained by injecting blank sample directly after highest calibration point and comparing the response to the lowest calibration point.

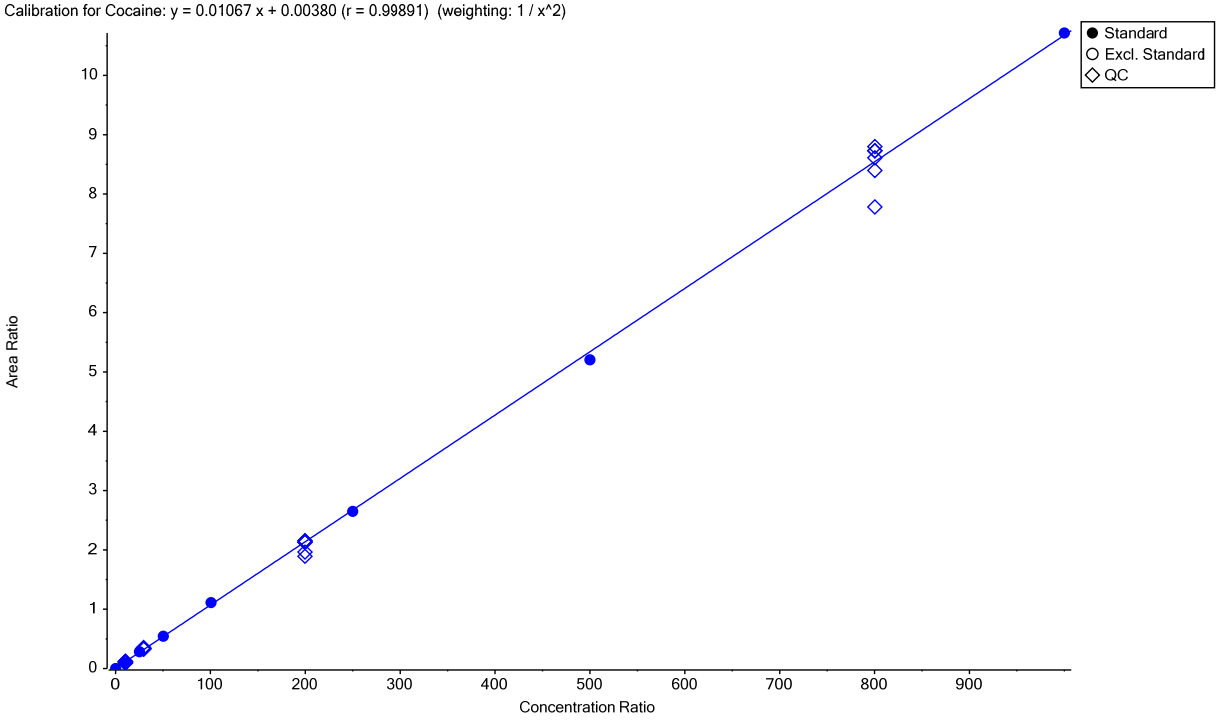
Compound	sample	response (area)	Carry-over ($Area_{blank}/Area_{LLOQ}$ as %)
benzoylecgonine	LLOQ	7.96E+03	12.48%
	blank	9.93E+02	
norcocaine	LLOQ	9.26E+03	15.86%
	blank	1.47E+03	
methylecgonine	LLOQ	3.26E+04	8.03%
	blank	2.62E+03	
cocaine	LLOQ	5.45E+04	9.96%
	blank	5.43E+03	
cocaethylene	LLOQ	4.73E+04	8.28%
	blank	3.92E+03	
tramadol	LLOQ	1.40E+04	5.52%
	blank	7.75E+02	
O-desmethyltramadol	LLOQ	6.37E+03	6.84%
	blank	4.36E+02	
N-desmethyltramadol	LLOQ	3.88E+03	6.88%
	blank	2.67E+02	

Figure S8. Representative calibration curves for all analytes in urine.

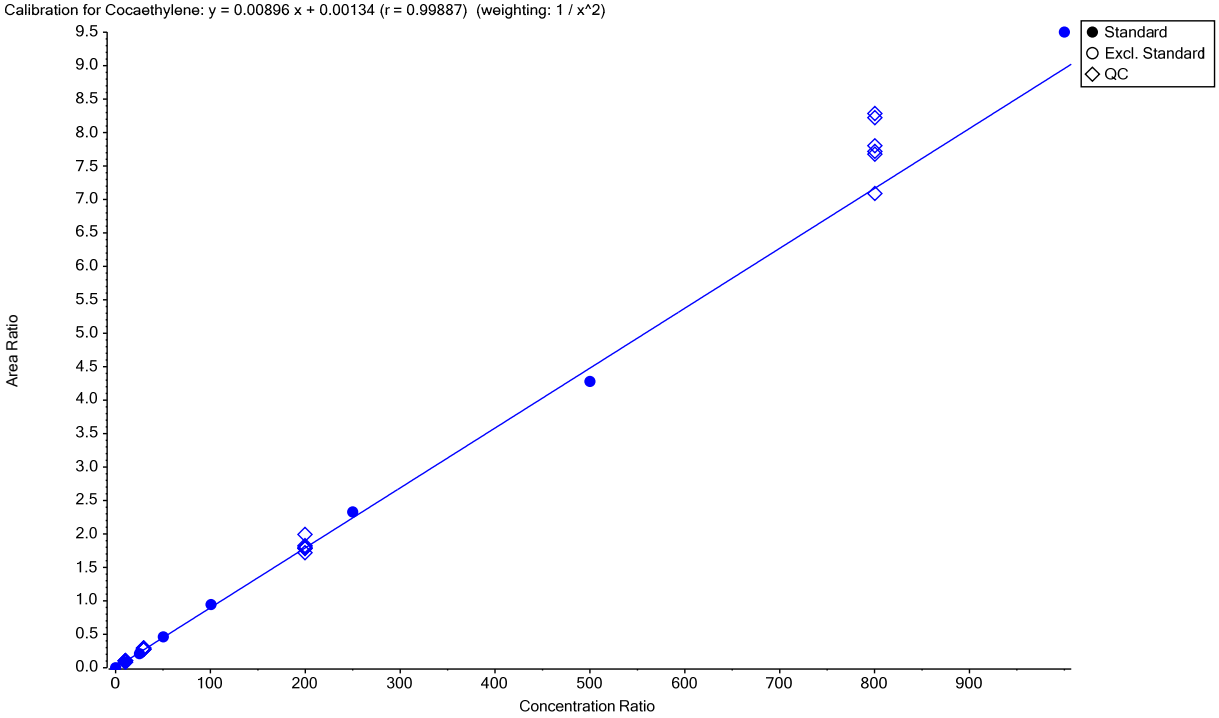




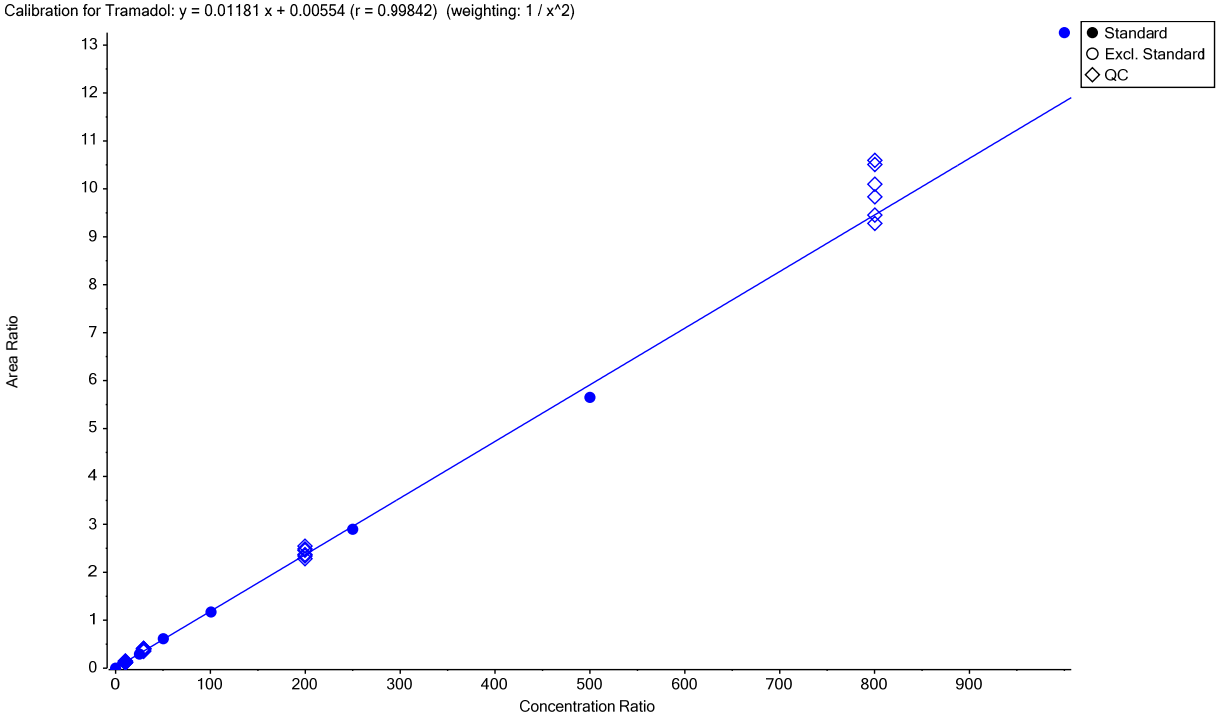

Calibration for Ecgonine: y = 0.00186 x + 0.00284 (r = 0.99931) (weighting: 1 / x)
Standard
Excl. Standard
QC
Area Ratio
Concentration Ratio
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400


Calibration for Cocaine: y = 0.01067 x + 0.00380 (r = 0.99891) (weighting: 1 / x^2)
Standard
Excl. Standard
QC
Area Ratio
Concentration Ratio
0
1
2
3
4
5
6
7
8
9
10
0
100
200
300
400
500
600
700
800
900

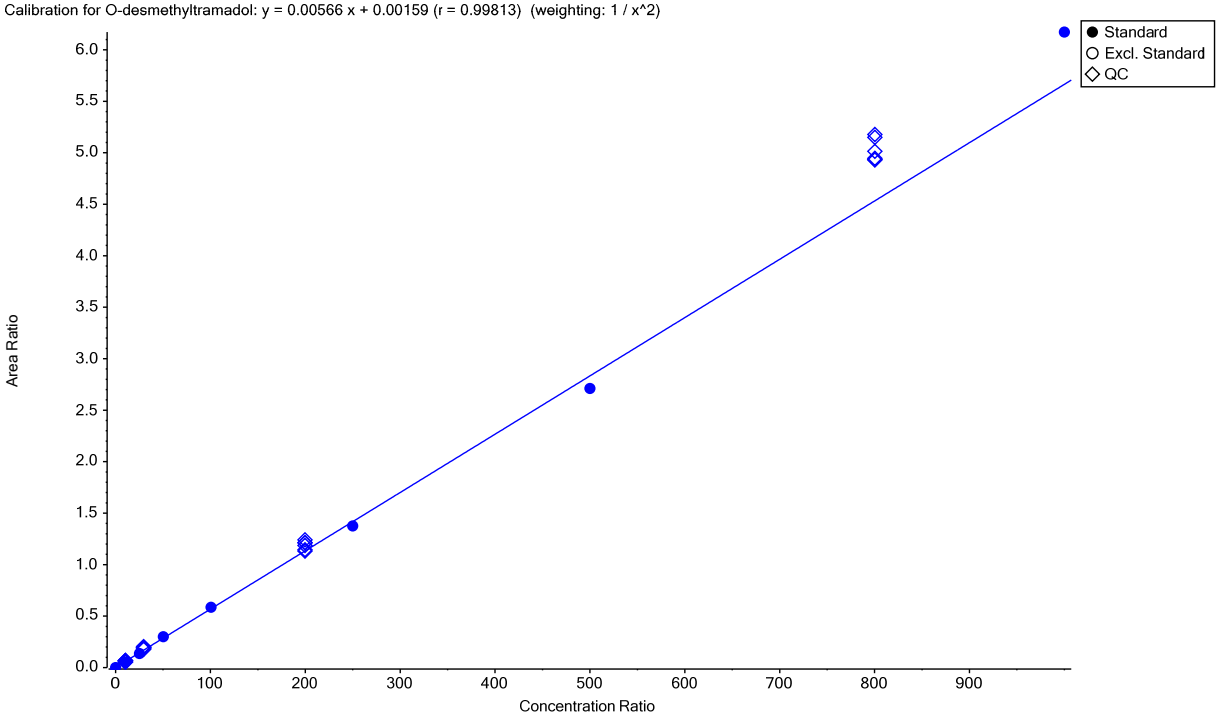
Calibration for Cocaethylene: y = 0.00896 x + 0.00134 (r = 0.99887) (weighting: 1 / x^2)



Calibration for Tramadol: y = 0.01181 x + 0.00554 (r = 0.99842) (weighting: 1 / x^2)



Calibration for O-desmethyltramadol: y = 0.00566 x + 0.00159 (r = 0.99813) (weighting: 1 / x^2)



Calibration for N-desmethyltramadol: y = 0.00329 x + 1.57573e-4 (r = 0.99933) (weighting: 1 / x^2)