

Reduced Drying Times Enable Rapid Paper Spray Analysis of Whole Blood for Clinical Toxicology

Stefania Boccuzzi¹, David Cowan¹, Paul I Dargan^{2,3}, Edward Goucher⁴, and Vincenzo Abbate^{1*}.

¹Department of Analytical, Environmental & Forensic Sciences, Faculty of Life Sciences & Medicine, King's College London, London, UK

²Clinical Toxicology, Faculty of Life Sciences and Medicine, King's College London, London, UK

³Clinical Toxicology, Guy's and St Thomas' NHS Foundation Trust and King's Health Partners, London, UK

⁴Thermo Fisher Scientific, San Jose, CA, USA

*Corresponding author.

Table S1. Compound specific mass spectrometry settings for SRM acquisitions. Quantifier ion transitions are denoted by *

Compound	Precursor (m/z)	Product (m/z)	Ion ratios (%)	Collision Energy (eV)	RF Lens (V)
Alprazolam	309.0	*165.0	100	32	85
Alprazolam	309.0	205.0	75	45	85
Alprazolam	309.0	274.0	40	26	85
Alprazolam	309.0	281.0	14	27	85
Alprazolam-d5	314.0	*165.0	100	31	85
Alprazolam-d5	314.0	210.0	74	44	85
Alprazolam-d5	314.0	279.0	41	27	85
Alprazolam-d5	314.0	286.0	13	28	85
BZE	290.0	*105.0	100	31	61
BZE	290.0	119.0	8	29	61
BZE	290.0	122.0	4	30	61
BZE	290.0	168.0	27	20	61
BZE-d3	293.0	*105.0	100	30	62
BZE-d3	293.0	153.0	5	24	62
BZE-d3	293.0	171.0	6	19	62
BZE-d3	293.0	275.0	29	17	62
Cocaine	304.0	*82.0	100	31	64
Cocaine	304.0	105.0	13	32	64
Cocaine	304.0	150.0	8	26	64
Cocaine	304.0	182.0	24	20	64
Cocaine-d3	307.0	*85.0	100	32	65
Cocaine-d3	307.0	105.0	13	33	65
Cocaine-d3	307.0	119.0	7	31	65
Cocaine-d3	307.0	185.0	25	20	65
Diazepam	285.0	*154.0	100	28	91
Diazepam	285.0	193.0	63	33	91
Diazepam	285.0	222.0	41	28	91
Diazepam	285.0	257.0	98	23	91
Diazepam-d5	290.0	*154.0	100	29	85
Diazepam-d5	290.0	198.0	67	33	85
Diazepam-d5	290.0	227.0	43	28	85
Diazepam-d5	290.0	262.0	89	23	85
EDDP	278.0	*186.0	100	35	82
EDDP	278.0	219.0	24	43	82
EDDP	278.0	234.0	36	31	82
EDDP	278.0	249.0	22	25	82
EDDP-d3	281.0	*189.0	100	38	78
EDDP-d3	281.0	219.0	23	45	78
EDDP-d3	281.0	234.0	36	32	78
EDDP-d3	281.0	249.0	21	25	78
Gabapentin	172.0	*95.1	100	25	40
Gabapentin	172.0	119.1	45	20	40
Gabapentin	172.0	137.1	22	18	40
Gabapentin	172.0	154.1	13	15	40
Gabapentin-d4	176.0	*97.1	100	25	40
Gabapentin-d4	176.0	122.1	20	22	40

Gabapentin-d4	176.0	139.1	17	20	40
Gabapentin-d4	176.0	158.1	4	15	40
Ketamine	238.0	*125.0	100	27	40
Ketamine	238.0	179.0	52	18	40
Ketamine	238.0	207.1	38	15	40
Ketamine	238.0	220.1	34	15	40
Ketamine-d4	242.0	*129.0	100	28	50
Ketamine-d4	242.0	183.1	59	18	50
Ketamine-d4	242.0	211.1	39	15	50
Ketamine-d4	242.0	224.1	34	16	50
Methadone	310.0	*105.0	100	29	50
Methadone	310.0	219.0	8	25	50
Methadone	310.0	223.0	8	22	50
Methadone	310.0	265.0	35	15	50
Methadone-d3	313.0	*105.0	100	29	55
Methadone-d3	313.0	220.0	6	25	55
Methadone-d3	313.0	226.0	8	22	55
Methadone-d3	313.0	268.0	37	16	55
Pregabalin	160.0	*83.0	100	16	32
Pregabalin	160.0	97.0	67	15	32
Pregabalin	160.0	125.0	34	13	32
Pregabalin	160.0	142.0	85	12	32
Pregabalin-d6	166.0	*103.0	100	17	37
Pregabalin-d6	166.0	130.0	23	17	37
Pregabalin-d6	166.0	131.0	15	15	37
Pregabalin-d6	166.0	148.0	24	13	37
Zopiclone	389.0	*112.0	100	55	50
Zopiclone	389.0	217.1	50	32	50
Zopiclone	389.0	245.0	39	17	50
Zopiclone	389.0	345.1	33	10	50
Zopiclone-d4	393.2	*112.0	100	55	40
Zopiclone-d4	393.2	217.0	52	32	40
Zopiclone-d4	393.2	245.0	43	20	40
Zopiclone-d4	393.2	349.1	36	10	40

Table S2. The compounds within the QCs used for interference studies and their respective concentrations.

<i>Analyte</i>	<i>Concentration (ng/mL)</i>
3-methyl-fentanyl	10
6-MAM	250
Adrafinil	500
AICAR	100
Altizide	1000
Amfepramone	500
Amphetaminil	500
Andarine	10
Bendroflumethiazide	1000
Benfluorex	500
Benzphetamine	500
Benzthiazide	1000
Benzylpiperazine	500
Butizide	1000
Carphedon	500
Cathine	500
Clobenzorex	500
Clomiphene	200
Cropropamide	500
Crotethamide	500
Cyclopenthiazide	1000
Cyclothiazide	1000
Dexamethasone	600
Diacetolol	500
Dimethylamphetamine	500
Dobutamine	500
Efaproxiral	100
Ephedrine	500
Epitizide	1000
Eplerenone	1000
Etamivan	500
Ethylphenylbutylamine	500
Etilamphetamine	500
Etilefrine	500
Exemestane metabolite	200
Famprofazone	500
Fenbutrazate	500
Fencamfamin	500
Fencamine	500
Fenetylline	500
Fenfluramine	500
Fenproporex	500
Flunisolid	300
Fulvestrant	200
Furfenorex	500
Heptaminol	500
Hydroxybromantan	500
Isometheptene	500
Letrozole metab.	200
MDMA	500
Mefenorex	500
Mephentermine	500
Methamphetamine	500
Methoxyphenamine	500
Methylephedrine	500
Methylhexanamine	500
Methylphenidate	500
Metolazone	1000
Nadolol	500

Nikethamide	500
Norfenfluramine	500
Ostarine	10
Oxilofrine	500
Pemoline	500
Pentetrazol	500
Phenmetrazine	500
Phenpromethamine	500
Phentermine	500
Pholedrine	500
Piretanide	1000
p-OH amphetamine.	500
Polythiazide	1000
Prenylamine	500
Probenecid	1000
Prolintane	500
Propylhexedrine	500
Salmeterol	100
Sibutramine	500
Strychnine	500
Trichlormethiazide	1000
Tuaminoheptane	500

Table S3. Between-run method validation parameters for whole blood spot analysis.								
Compound	Bias (%)			Precision (% CV)			R ²	LOD (ng/mL)
	QC Low	QC Med	QC High	QC Low	QC Med	QC High		
Alprazolam	1	-4	17	1	3	3	0.9892	11.4
BZE	16	2	21	1	3	4	0.9854	29.9
Cocaine	17	3	19	1	3	4	0.9843	29.5
Diazepam	17	2	16	1	3	4	0.9845	26.0
EDDP	13	1	14	2	3	3	0.9908	5.7
Gabapentin	7	-1	25	1	3	4	0.9686	518
Ketamine	17	3	18	1	3	4	0.9838	32.3
Methadone	3	-5	16	1	3	3	0.9894	12.3
Pregabalin	9	0	28	2	4	4	0.9684	469
Zopiclone	-2	-5	17	1	3	4	0.9882	12.3

Table S4. Bias and precision (% CV) of pseudo-unknown QC samples across 10, 20, 40, and 60 minutes for all analytes.								
Analyte	Bias (%)				Precision (% CV)			
	60 min	40 min	20 min	10 min	60 min	40 min	20 min	10 min
Alprazolam	3	7	8	7	0	0	1	0
BZE	8	13	12	11	0	0	0	1
Cocaine	5	9	9	9	0	0	0	0
Diazepam	11	13	12	12	1	0	1	1
EDDP	-9	-5	-6	-5	1	1	0	0
Gabapentin	3	30	30	8	0	1	0	0
Ketamine	5	9	10	11	0	0	0	1
Methadone	-1	4	3	3	0	0	0	1
Pregabalin	4	8	9	13	1	1	1	1
Zopiclone	-4	-6	-2	0	1	1	0	1

Table S5. SST results for propranolol (100 ng/mL, n = 3) at m/z 260.1 → 116.0 and m/z 260.1 → 183.0 across all drying intervals. Reference (day zero) values represent mean propranolol responses obtained during initial instrument runs and are used to monitor long-term performance.								
Drying time	Precursor (m/z)	Product (m/z)	Mean propranolol area (n = 3)	Reference propranolol area ^s	% of reference	Target ion ratio (%)	Observed ion ratio (%)	SST within acceptability criteria?
60 min	260.1	116.0	4.15 × 10 ⁷	2.50 × 10 ⁷	166	90 - 110	100	Yes
	260.1	183.0	2.77 × 10 ⁷	1.70 × 10 ⁷	163	60 - 80	68	Yes
40 min	260.1	116.0	4.21 × 10 ⁷	2.50 × 10 ⁷	169	90 - 110	100	Yes
	260.1	183.0	2.89 × 10 ⁷	1.70 × 10 ⁷	170	60 - 80	69	Yes
20 min	260.1	116.0	3.31 × 10 ⁷	2.50 × 10 ⁷	132	90 - 110	100	Yes
	260.1	183.0	2.25 × 10 ⁷	1.70 × 10 ⁷	132	60 - 80	68	Yes
10 min	260.1	116.0	3.45 × 10 ⁷	2.50 × 10 ⁷	138	90 - 110	100	Yes
	260.1	183.0	2.36 × 10 ⁷	1.70 × 10 ⁷	139	60 - 80	68	Yes