

Poster Reprint

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# Screening, Identification, and Quantitation of Drugs of Abuse in Human Whole Blood by LC/Q-TOF and LC/MS/MS

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## Introduction

In forensic toxicology, the demand for fast and reliable for identification and accurate quantitation. screening and quantitative determination of drugs of abuse (DoA) and misused prescription-type drugs in biological specimens is steadily increasing, due to the increasing number of drugs of abuse, as well as samples submitted for analysis.

LC/MS technology, including LC/Q-TOF and LC/TQ, have been applied as promising techniques, having been increasingly used in forensic toxicology for a wide range of biological samples. Easier sample preparation without derivatization, and shorter analysis time are the major advantages that make them widely accepted.

This study investigated the complete workflow for over 100 drugs of abuse and misused prescription drugs subject to screening, identification, and quantitation in human whole blood. Samples were prepared using in-cartridge protein precipitation (PPT) followed by Captiva EMR-Lipid cartridge cleanup, and then analyzed on LC/Q-TOF for screening and identification, or on LC/TQ

## Experimental

### LC conditions (Agilent 1290 Infinity II UHPLC)

Columns	Agilent ZORBAX Eclipse Plus C18, 2.1 x 100 mm, 1.8 $\mu$ m column (p/n 959758-902) Agilent ZORBAX Eclipse Plus C18 guard, 2.1 x 5 mm, 1.8 $\mu$ m (p/n 821725-901)		
Flow Rate	0.4 mL/min		
Column Temp.	40 °C	Injection volume	5 $\mu$ L
Mobile Phase	A: 10 mM ammonium formate in water, 0.125% FA B: 10 mM ammonium formate in 95:5 ACN/water, 0.125%FA		
Needle Wash	1:1:1:1 ACN/MeOH/IPA/water, 0.2% formic acid		
Gradient	Time (min)	%B	Flow (mL/min)
	0	10	0.4
	0.5	10	0.4
	8.0	80	0.4
	8.01	100	0.5
Stop Time	11 min	Post time	2 min

### TQ conditions (Agilent 6490 LC/MS system)

Gas Temperature	220 °C	Gas Flow	18 L/min
Nebulizer	22 psi		
Sheath Gas Temp	400 °C	Sheath Gas Flow	12 L/min
Capillary Voltage	3500 V	Nozzle Voltage	0 V
iFunnel Parameters	High-pressure RF: 120 V (POS), 110 V (NEG) Low-pressure RF: 60 V (POS), 60 V (NEG)		
Polarity	POS & NEG	Acquisition	dMRM

### Q-TOF conditions (Agilent 6546A LC/Q-TOF system)

Drying Gas Temp.	250 °C	Drying Gas Flow	13 L/min
Nebulizer	30 psi	Skimmer	65 V
Sheath Gas Temp.	375 °C	Sheath Gas Flow	12 L/min
Nozzle voltage	0 V (POS) 2000 V (NEG)	Capillary voltage	3500 V (POS) 5000 V (NEG)
Octopole RF	750 V	Fragmentor	125 V
Acquisition mode	Hi-Res mode, 10GHz		

### Human whole blood sample preparation workflow

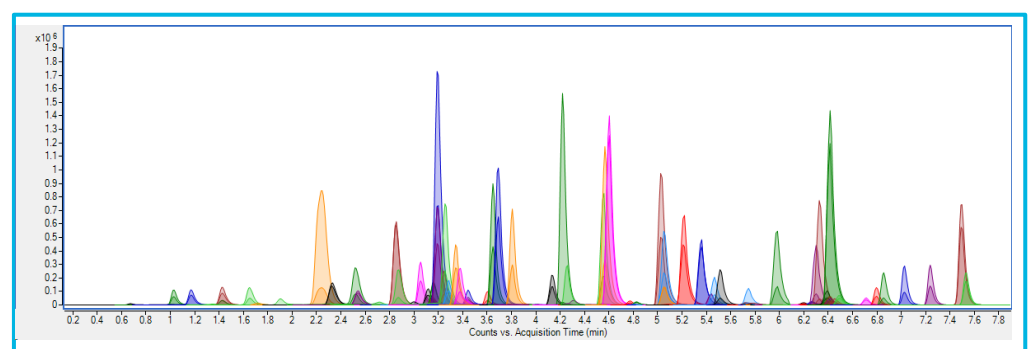
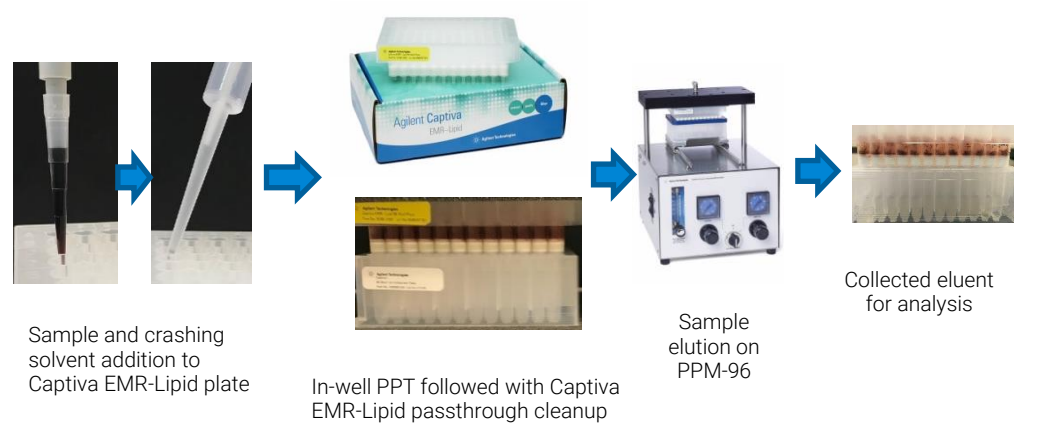


Figure 1. LC/TQ chromatogram (dMRM) for human whole blood samples fortified at 1 ng/mL of DoA.



## Results and Discussion

### Selected analyte identification parameters on LC/Q-TOF

Compound Name	Formula	Adduct	RT, min	RT Diff., 10 ng/mL	Target Pseudo molecular Ion	Accurate Fragment 1	Accurate Fragment 2	Accurate Fragment 3	Accurate Fragment 4	Precursor Mass Accuracy (ppm), 10 ng/mL	Number of Verified Ions, 10 ng/mL
2-Hydroxyethylflurazepam	C <sub>17</sub> H <sub>14</sub> ClFN <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	5.61	0.004	333.08006	211.07918	109.04481	140.02567	140.02567	1.15	5
6-Acetylmorphine	C <sub>19</sub> H <sub>21</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	2.42	0.008	328.15433	165.06988	211.07536	181.06479	58.06513	0.70	3
7-Aminoclonazepam	C <sub>15</sub> H <sub>12</sub> ClN <sub>3</sub> O	[M+H] <sup>+</sup>	3.51	0.008	286.07417	121.07603	250.09749	222.10257	94.06513	0.36	3
Acepromazine	C <sub>19</sub> H <sub>22</sub> N <sub>2</sub> OS	[M+H] <sup>+</sup>	4.99	0.012	327.15256	58.06513	86.09643	222.09134	254.06341	1.30	4
Alprazolam	C <sub>17</sub> H <sub>13</sub> ClN <sub>4</sub>	[M+H] <sup>+</sup>	5.62	0.001	309.09015	205.07603	281.07143	240.04488	219.09168	0.07	4
Amobarbital <sup>2</sup>	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	[M-H] <sup>-</sup>	5.13	0.006	225.12447	41.99854	182.11865	68.99820	85.00435	-1.90	1
Amphetamine	C <sub>9</sub> H <sub>13</sub> N	[M+H] <sup>+</sup>	2.32	0.007	136.11208	91.05423	65.03858	119.08553	63.02293	0.98	3
Atenolol	C <sub>14</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	1.18	0.003	267.17032	145.06479	56.04948	190.08626	74.06004	0.32	2
Benzoylcegonine	C <sub>16</sub> H <sub>19</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	2.97	0.005	290.13868	105.03349	168.10191	77.03858	82.06513	0.53	3
Butabarbital <sup>2</sup>	C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	[M-H] <sup>-</sup>	4.35	0.009	211.10882	41.99854	168.10300	85.00435	124.11317	-0.26	1
Butalbital <sup>2</sup>	C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	[M-H] <sup>-</sup>	4.61	0.014	223.10882	41.99854	180.10300	85.00435	136.11317	1.96	1
Carbamazepine	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O	[M+H] <sup>+</sup>	5.10	0.002	237.10224	194.09643	193.08860	179.07295	192.08078	1.39	5
Carisoprodol	C <sub>12</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	5.58	0.004	261.18088	55.05423	62.02366	176.12812	97.10118	0.51	5
Chlorpheniramine	C <sub>16</sub> H <sub>19</sub> ClN <sub>2</sub>	[M+H] <sup>+</sup>	4.29	0.010	275.13095	230.07310	167.07295	201.03398	118.06513	0.36	4
Chlorpromazine	C <sub>17</sub> H <sub>19</sub> ClN <sub>2</sub> S	[M+H] <sup>+</sup>	5.70	0.002	319.10302	58.06513	86.09643	214.04180	246.01387	1.64	5
cis-Tramadol	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	3.54	0.002	264.19581	58.06513	246.18524			-0.01	3
Clobazam	C <sub>16</sub> H <sub>13</sub> ClN <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	6.05	0.004	301.07383	224.09441	259.06327	105.03349	153.02092	0.28	4
Clonazepam	C <sub>15</sub> H <sub>10</sub> ClN <sub>3</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	5.51	0.003	316.04835	214.04180	241.05270	270.05544	207.09166	-0.19	5
Clozapine	C <sub>18</sub> H <sub>19</sub> ClN <sub>4</sub>	[M+H] <sup>+</sup>	4.65	0.003	327.13710	270.07925	192.06820	227.03705	84.08078	0.79	4
Cocaethylene	C <sub>18</sub> H <sub>23</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	4.26	0.006	318.16998	82.06513	196.13321	105.03349	91.05423	1.33	4
Cocaine	C <sub>17</sub> H <sub>21</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	3.79	0.003	304.15433	82.06513	182.11756	105.03349	77.03858	0.48	3
Codeine	C <sub>18</sub> H <sub>21</sub> NO <sub>3</sub>	[M+H] <sup>+</sup>	1.89	0.011	300.15942	165.06988	153.06988	199.07536	181.06479	0.56	1
Cyclobenzaprine	C <sub>20</sub> H <sub>21</sub> N	[M+H] <sup>+</sup>	5.30	0.008	276.17468	215.08553	216.09335	231.11683	58.06513	0.58	3
Demoxepam	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	4.90	0.001	287.05818	241.02999	207.06835	77.03858	123.99485	-0.51	3
Desalkylflurazepam	C <sub>15</sub> H <sub>10</sub> ClFN <sub>2</sub> O	[M+H] <sup>+</sup>	5.82	0.005	289.05385	140.02567	226.09008	165.02092	214.04180	0.64	5
Dextromethorphan	C <sub>18</sub> H <sub>25</sub> NO	[M+H] <sup>+</sup>	4.57	0.006	272.20089	171.08044	147.08044	213.12739	173.09609	1.20	5
Diethylpropion	C <sub>13</sub> H <sub>19</sub> NO	[M+H] <sup>+</sup>	2.79	0.007	206.15394	105.06988	77.03858	100.11208	79.05423	0.22	3
Dihydrocodeine	C <sub>18</sub> H <sub>23</sub> NO <sub>3</sub>	[M+H] <sup>+</sup>	1.81	0.054	302.17507	199.07536	171.08044	201.09101	183.08044	-1.32	3
Diphenhydramine	C <sub>17</sub> H <sub>21</sub> NO	[M+H] <sup>+</sup>	4.72	0.001	256.16959	167.08553	152.06205	165.06988	166.07770	0.07	5
Dothiepin	C <sub>19</sub> H <sub>21</sub> NS	[M+H] <sup>+</sup>	5.18	0.002	296.14675	203.08553	221.04195	223.05760	217.10118	0.08	5
Doxylamine	C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O	[M+H] <sup>+</sup>	3.51	0.009	271.18049	167.07295	182.09643	90.09134	72.08078	0.52	5
Ecgonine methyl ester	C <sub>10</sub> H <sub>17</sub> NO <sub>3</sub>	[M+H] <sup>+</sup>	0.66	0.013	200.12812	82.06513	182.11756	68.04948	91.05423	-2.34	1
EDDP	C <sub>20</sub> H <sub>23</sub> N	[M+H] <sup>+</sup>	5.10	0.003	278.19033	234.12773	186.12773	219.10425	249.15120	1.34	5
Ephedrine	C <sub>10</sub> H <sub>15</sub> NO	[M+H] <sup>+</sup>	1.79	0.019	166.12264	148.11208	91.05423	115.05423	133.08860	0.08	5
Fentanyl <sup>9</sup>	C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O	[M+H] <sup>+</sup>	4.63	0.001	337.22744	105.06988	188.14338	216.13829	132.08078	0.98	3
Fluoxetine	C <sub>17</sub> H <sub>15</sub> F <sub>3</sub> NO	[M+H] <sup>+</sup>	5.59	0.004	310.14133	44.04948	148.11208			1.27	2
Fluvoxamine	C <sub>15</sub> H <sub>21</sub> F <sub>3</sub> N <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	5.33	0.007	319.16279	71.05028	258.11003	200.06816	55.05537	0.25	4
Hydrocodone	C <sub>18</sub> H <sub>21</sub> NO <sub>3</sub>	[M+H] <sup>+</sup>	2.57	0.001	300.15942	199.07536	171.08044	141.06988	181.06479	0.20	3
Hydromorphone	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>	[M+H] <sup>+</sup>	1.15	0.000	286.14377	185.05971	157.06479	153.06988	181.06479	0.70	2
Hydroxyzine	C <sub>21</sub> H <sub>27</sub> ClN <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	5.36	0.001	375.18338	166.07770	201.04655	165.06988	173.12845	0.00	5
Ketamine	C <sub>13</sub> H <sub>16</sub> ClNO	[M+H] <sup>+</sup>	3.14	0.007	238.09932	125.01525	179.06221	207.05712	220.08875	1.34	3
Lorazepam	C <sub>15</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	5.49	0.003	321.01921	275.01373	229.05270	303.00865	163.00527	0.87	3
MDA	C <sub>10</sub> H <sub>13</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	2.44	0.006	180.10191	105.06988	163.07536	77.03858	135.04406	0.52	4
MDMA	C <sub>11</sub> H <sub>15</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	2.65	0.005	194.11756	105.06988	163.07536	77.03858	135.04406	0.44	5
Meperidine	C <sub>15</sub> H <sub>21</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	3.85	0.012	248.16451	70.06513	220.13321	174.12773	91.05423	0.95	4
Meprobamate	C <sub>9</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	3.99	0.006	219.13393	55.05423	158.11756	97.10118	69.06988	2.43	3
Methadone	C <sub>21</sub> H <sub>27</sub> NO	[M+H] <sup>+</sup>	5.56	0.005	310.21654	105.03349	265.15869	77.03858	91.05423	0.38	5
Methamphetamine	C <sub>10</sub> H <sub>15</sub> N	[M+H] <sup>+</sup>	2.59	0.004	150.12773	91.05423	119.08553	65.03858	63.02293	0.85	5
Midazolam	C <sub>18</sub> H <sub>13</sub> ClFN <sub>3</sub>	[M+H] <sup>+</sup>	4.84	0.004	326.08548	291.11663	249.08225	223.07918	209.06353	1.12	5
Morphine	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>	[M+H] <sup>+</sup>	0.94	0.018	286.14377	165.06988	153.06988	157.06479	181.06479	2.67	3
Naloxone	C <sub>19</sub> H <sub>21</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	1.78	0.022	328.15433	310.14377	212.07061	253.10973	268.13321	0.36	5
N-desmethyl-cis-tramadol	C <sub>15</sub> H <sub>23</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	3.57	0.002	250.18016	58.06513	232.16959			0.63	1
Nitrazepam	C <sub>15</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	5.36	0.002	282.08732	180.08078	207.09168	236.0944	190.06513	-1.02	5
Norbuprenorphine	C <sub>25</sub> H <sub>35</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	4.11	0.005	414.26389	101.09609	83.08553	57.06988	187.07536	-0.66	3
Norfentanyl	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O	[M+H] <sup>+</sup>	3.16	0.006	233.16484	84.08078	55.05423	56.04948	94.06513	0.78	3
Norpropoxyphene	C <sub>21</sub> H <sub>27</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	5.38	0.010	326.21146	44.04948	91.05423			0.61	2
Nortriptyline	C <sub>19</sub> H <sub>21</sub> N	[M+H] <sup>+</sup>	5.36	0.003	264.17468	91.05423	105.06988	233.1325	117.06988	-0.37	4
Oxazepam	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	5.35	0.003	287.05818	104.04948	241.05270	269.04762	163.00527	-2.18	4
Oxycodone	C <sub>18</sub> H <sub>21</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	2.36	0.009	316.15433	241.10973	298.14377	212.10699	226.08626	0.75	5
Oxymorphone	C <sub>17</sub> H <sub>19</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	1.02	0.005	302.13868	227.09408	284.12812	198.09134	199.09649	1.11	4
Paroxetine	C <sub>19</sub> H <sub>20</sub> FNO <sub>3</sub>	[M+H] <sup>+</sup>	5.08	0.003	330.15000	70.06513	192.11830	135.06046	109.04481	0.86	3
PCP	C <sub>17</sub> H <sub>25</sub> N	[M+H] <sup>+</sup>	4.45	0.006	244.20598	86.09643	91.05423	159.11683	81.06988	-0.10	4
Phendimetrazine	C <sub>12</sub> H <sub>17</sub> NO	[M+H] <sup>+</sup>	2.54	0.017	192.13829	91.05423	115.05423	144.08078	146.09643	0.14	4
Phentermine	C <sub>10</sub> H <sub>15</sub> N	[M+H] <sup>+</sup>	2.84	0.007	150.12773	91.05423	65.03858	133.10118	105.06988	1.00	4
Phenylpropanolamine	C <sub>9</sub> H <sub>13</sub> NO	[M+H] <sup>+</sup>	1.42	0.031	152.10699	91.05423	117.06988	134.09643	115.05423	-0.38	2
Prednisone	C <sub>21</sub> H <sub>26</sub> O <sub>5</sub>	[M+H] <sup>+</sup>	4.68	0.004	359.18530	147.08044	237.12739	171.08044	341.17474	0.32	2
Proadifen	C <sub>23</sub> H <sub>31</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	6.33	0.004	354.24276	91.05423	209.13248	105.06988	167.08553	-0.02	3
Promethazine	C <sub>17</sub> H <sub>20</sub> N <sub>2</sub> S	[M+H] <sup>+</sup>	5.03	0.004	285.14200	86.09643	198.03720	71.07295	56.04948	0.26	4
Propoxyphene	C <sub>22</sub> H <sub>29</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	5.49	0.007	340.22711	58.06513	266.19033	91.05423	143.08553	0.64	3
Quetiapine	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> S	[M+H] <sup>+</sup>	4.76	0.001	384.17402	221.10733	253.07940	210.03720	247.12298	0.22	5
Ritalinic acid	C <sub>13</sub> H <sub>17</sub> NO <sub>2</sub>	[M+H] <sup>+</sup>	2.82	0.000	220.13321	84.08078	56.04948	85.08860	91.05423	0.27	2
Sertraline	C <sub>17</sub> H <sub>17</sub> Cl <sub>2</sub> N	[M+H] <sup>+</sup>	5.67	0.006	306.08108	158.97628	275.03888	129.06988	122.99960	-0.70	5
Strychnine	C <sub>21</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	2.87	0.000	335.17540	184.07569	156.08078	264.10191	222.09134	0.13	3
Temazepam	C <sub>16</sub> H <sub>13</sub> ClN <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	5.95	0.002	301.07383	255.06835	283.06327	177.02092	193.08860	0.41	5
Verapamil	C <sub>27</sub> H <sub>38</sub> N <sub>2</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	5.47	0.002	455.29043	165.09101	150.06753	303.20671	105.06988	0.85	4
Zolpidem	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O	[M+H] <sup>+</sup>	4.03	0.006	308.17574	235.12298	263.11789	236.13080	92.04948	1.32	5

### Targets Recovery

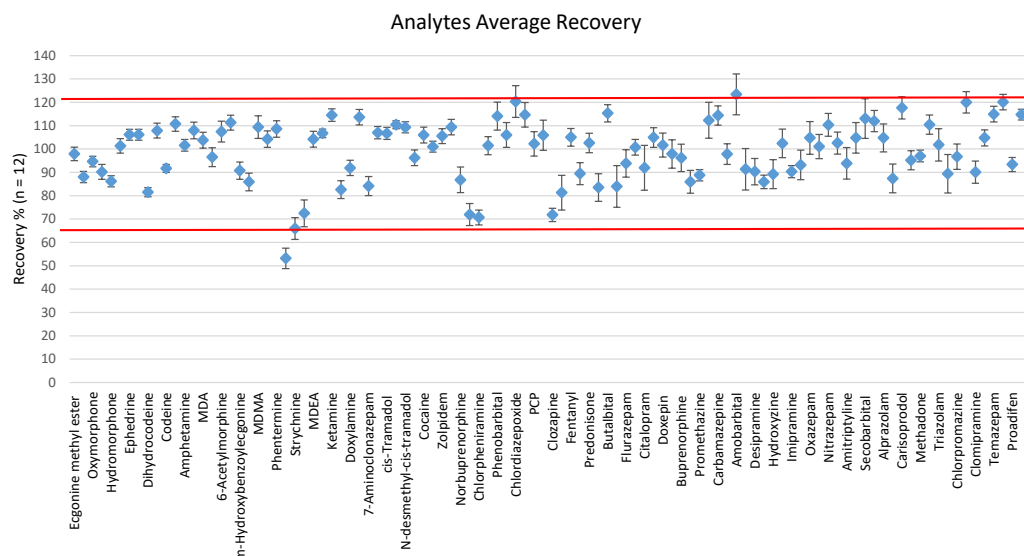


Figure 2. Analytes average recovery for 102 analytes in human whole blood samples fortified at 10 ng/mL and 50 ng/mL.

### Targets Matrix Effect

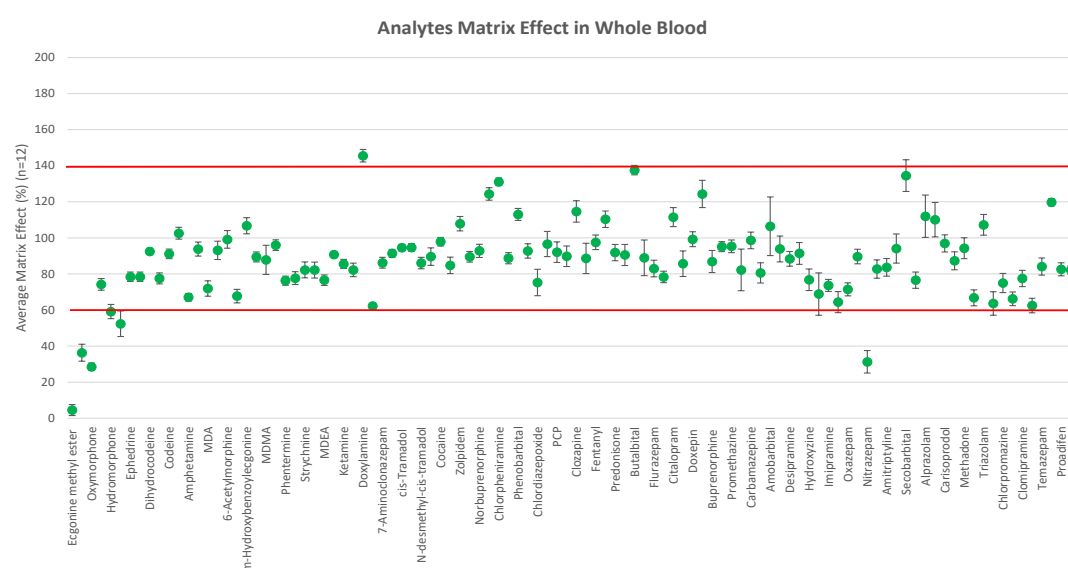
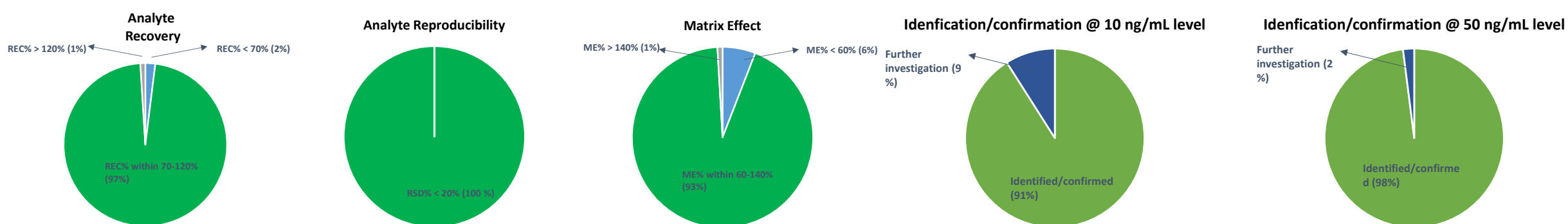


Figure 3. Analytes average matrix effect for 102 analytes in human whole blood samples fortified at 10 ng/mL and 50 ng/mL.

Statistical results for quantitative analysis on LC/TQ (left) and screening analysis on LC/Q-TOF (right).



### Conclusions

- An end-to-end workflow, from sample preparation, instrument analysis to data processing, was developed and verified.
- Over 100 drugs of abuse and medicinal drugs in human whole blood screening,
- Screening and identification on LC/Q-TOF
- Quantitation on LC/TQ.
- In-situ PPT followed by Captiva EMR–Lipid cleanup.

### References

Agilent application note 5994-2830EN,  
 Agilent application note 5991-9251EN,  
 Agilent application note 5991-9222EN

<https://explore.agilent.com/asms>

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