GERSTEL Application Note No. **192**, *2017*

Drugs of Abuse in Oral Fluids: Automated SPE Extraction and LC/MS/MS Determination using a Robotic Autosampler

Fred D. Foster[1], John R. Stuff[1], Jacqueline A. Whitecavage[1], Edward A. Pfannkoch[1], Mark Hayward[2]

1. GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD, 21090, USA
2. ITSP Solutions, Inc., 10 South Carolina St, Hartwell, GA 30643, USA

Keywords

Forensics, Sample Preparation, LC/MS/MS, High Throughput Lab Automation, SPE

Abstract

Solid phase extraction (SPE) is one of the sample preparation methods most widely used by chromatographers, as can be seen from the large number of SPE methods found in the literature. Typically, a liquid sample is passed across an adsorbent bed to retain and concentrate target analytes and eliminate interference from the sample matrix. Alternatively, the adsorbent can be used to retain interferences while allowing the target analytes to pass through. Manual SPE cartridge formats can vary from disks to individual cartridges with various volumes of sorbent to 96-well plates. However, solid phase extraction can be tedious and time consuming when performed manually and there is increasing demand for automation of SPE methods.

The MPS robotic^{pro} is a highly efficient LC or GC autosampler with extended robotic functionality. The MPS robotic^{pro} provides reliable processing of complex tasks including automation of SPE procedures. Syringe holders and syringes are integrated in special syringe modules, which can be exchanged automatically within a running sequence for maximum flexibility. The system is controlled in a simple and efficient manner using the proven GERSTEL MAESTRO software. In this study, we show that an existing SPE method using ITSP cartridges [1] can be automated using the MPS robotic^{pro} autosampler under MAESTRO control. An example of a solid phase extraction method illustrating the determination of drugs of abuse in oral fluid is shown.

Introduction

Drug testing based on the analysis of oral fluid samples is increasingly used due to sample collection advantages over other biological matrices and due to the early detection window it offers compared with other approaches. The collection of oral fluid is noninvasive and can be performed under directly observed conditions. The simplicity of collecting an oral fluid sample also reduces the likelihood of test sample adulteration or sample swapping helping to ensure the validity of the results. Some collection devices, including the Quantisal devices [2] used within this study, have an indicator to ensure an exact volume of oral fluid is collected. Following the collection step, the devices are subsequently placed in storage tubes containing stabilization buffer, ensuring that the sample can be shipped or stored for subsequent analysis without analyte degradation.

While the buffer helps to stabilize analytes present in the oral fluid sample, it may also cause analyte suppression effects in the LC/MS/MS analysis leading

to inaccurate results. Prior to analysis, oral fluid samples are typically extracted using SPE to eliminate possible matrix suppression effects and to ensure that the required limits of detection can be met for analytes present at low concentrations in the buffer-diluted matrix. ITSP Solutions, Inc. manufactures miniaturized SPE cartridges that are highly suitable for automation. The MPS robotic^{pro} offers highly accurate and precise control of the flow rate and volume of solutions used in the SPE process to clean, pre-concentrate, and elute drugs of abuse from oral fluid samples prior to analysis. An existing manual SPE method using ITSP cartridges was automated using the MPS robotic^{pro}. Data obtained through this study demonstrate the successful coupling of the MPS robotic^{pro} to an Agilent 6470 LC/MS/MS system providing a completely automated solution for the extraction and analysis of drugs of abuse in oral fluid samples.

EXPERIMENTAL

Materials. All stock solutions for the compounds listed in Table 1 were purchased from Cerilliant. An intermediate analyte stock solution was prepared by combining the analyte stock solutions with Acetonitrile, at appropriate concentrations, to evaluate the different drug classes.

Deuterated analogues, d_3-Morphine, d_5-Fentanyl, d_5-Nordiazepam, d_5-Propoxyphene, d_7-Carisoprodol, d_6-Amphetamine, d_4-Ketamine, d_4-Meperidine, d_4-7-Aminoclonazepam, d_5-PCP, d_9-Methadone, d_4-Bupreonorphine, d_3-Norbuprenorphine, d_3-Tramadol-C13, d_4-Clonazepam, d_5-Oxazepam, d_5-Estazolam, d_5-α-Hydroxyalprazolam, d_5-MDA, d_5-MDMA, d_5-Methamphetamine, d_3-Amitriptyline, and d_3-Cocaine were purchased from Cerilliant. A working internal standard stock solution containing the deuterated internal standards was prepared at a concentration of 1000 ng/mL and used as internal standards for the drug classes being evaluated. Table 1 shows which deuterated internal standard was used with each respective analyte during quantitation.

Table 1. Mass spectrometer acquisition parameters.

Compound Name	Precursor Ion [m/z]	Product Ion [m/z]		Fragmentor Voltage [V]		Collision Energy [V]		Ret. Time [min]	High Std Conc. [ng/mL]	Limit of Quant. [ng/mL]
6-Monoacetylmorphine[1]	328.2	165.1	58.1	158	158	41	29	1.92	100	1
7-Aminoclonazepam[2]	286.1	222.1	121.1	138	138	25	33	2.49	500	5
α-Hydroxyalprazolam[6]	325.1	297	216	150	150	28	41	3.68	200	2
Alprazolam[3]	309.1	281	205	179	179	25	49	3.82	400	4
Amitriptyline[4]	278.2	233	117	130	130	15	20	3.7	250	2.5
Benzoylecgonine[7]	290.1	168.1	105	118	118	17	29	2.34	250	2.5
Bromazepam[6]	316	209	182.1	150	150	25	37	3.44	400	4
Buprenorphine[8]	468.3	396.2	55.1	200	200	41	60	3.41	100	1
Carisoprodol[9]	261.2	176.1	97.1	80	80	1	10	3.76	500	5
cis-Tramadol[23]	264.2	58.1	42.1	107	107	16	108	2.52	250	2.5
Clobazam[6]	301.1	259	244.1	138	138	17	33	3.73	400	4
Clonazepam[10]	316.1	270.1	214	158	158	25	41	3.56	400	4
Cocaine[7]	304.2	182.1	77	138	138	17	61	2.54	250	2.5
Codeine[1]	300.2	165.1	128	158	158	45	60	1.76	500	5
d_3-Amitriptyline	281.3	233.1	117.1	150	150	20	25	3.7	-	-
d_3-cis-Tramadol-13C	268.2	58.1	-	102	-	16	-	2.51	-	-
d_3-Cocaine	307.2	85.1	77	133	133	32	64	2.54	-	-
d_3-Morphine	289	165.1	152	153	153	40	68	1.2	-	-
d_3-Norbuprenorphine	417.3	152	55.1	190	190	124	76	2.97	-	-
d_4-7-Aminoclonazepam	290.1	198.1	-	140	-	35	-	2.47	-	-
d_4-Buprenorphine	472.3	400.2	59.1	210	210	44	60	3.38	-	-
d_4-Clonazepam	320.1	218	154	144	144	40	84	3.55	-	-

Table 1 (cont.). Mass spectrometer acquisition parameters.

Compound Name	Precursor Ion [m/z]	Product Ion [m/z]		Fragmentor Voltage [V]		Collision Energy [V]		Ret. Time [min]	High Std Conc. [ng/mL]	Limit of Quant. [ng/mL]
d_4-Ketamine	242.1	129	119	102	102	32	68	2.37	-	-
d_4-Merperidine	252.2	105	42.1	138	138	48	64	2.68	-	-
d_5-α-Hydroxyalprazolam	330.1	302.1	210.1	179	179	28	52	3.51	-	-
d_6-Amphetamine	141.1	124.1	93.1	70	70	5	33	2.01	-	-
d_5-Estazolam	300	272	-	140	-	25	-	3.69	-	-
d_5-Fentanyl	342.3	188.1	105.1	92	92	24	44	3.01	-	-
d_5-MDA	185.1	168.1	110.1	61	61	10	21	2.04	-	-
d_5-MDMA	199.2	165.1	107.1	92	92	8	24	2.07	-	-
d_5-Methamphetamine	155.2	121.1	92	82	82	8	20	2.07	-	-
d_5-Nordiazepam	276.1	213.1	140.1	61	61	28	28	4.02	-	-
d_5-Oxazepam	292.1	246.1	109	123	123	24	40	3.77	-	-
d_5-PCP	249.3	96.1	86.1	97	97	36	8	2.95	-	-
d_5-Propoxyphene	345.3	271.2	58.1	117	117	4	16	3.58	-	-
d_7-Carisoprodol	268.2	183.2	165.2	81	81	0	4	3.74	-	-
d_9-Methadone	319.3	268.1	-	118	-	12	-	3.65	-	-
d-Amphetamine	136.1	119.1	91	66	66	5	17	2.03	1000	10
Diazepam[11]	285.1	257.1	154	169	169	25	25	4.15	400	4
EDDP[12]	278.2	234.1	219.1	158	158	33	45	3.18	500	5
Estazolam[13]	295.1	267.1	205.1	159	159	21	45	3.7	400	4
Fentanyl[14]	337.2	188.1	105.1	143	143	21	41	3.02	10	0.1
Flunitrazepam[13]	314.1	268.1	239.1	153	153	25	37	3.61	400	4
Flurazepam[6]	388.2	317.1	315	158	158	17	21	3.22	400	4
Hydrocodone[1]	300.2	199	128	159	159	29	65	1.9	500	5
Hydromorphone[1]	286.2	185	157	159	159	29	45	1.42	500	5
Ketamine[15]	238.1	220.1	125	105	105	11	11	2.38	1000	10
Lorazepam[9]	321	275	194	102	102	21	49	3.78	400	4
MDA[16]	180.1	163	135	61	61	5	17	2.04	1000	10
MDEA[16]	208.1	163	135	107	107	9	21	2.22	1000	10
MDMA[17]	194.1	163	105	97	97	9	25	2.08	1000	10
Meperidine[18]	248.2	220.1	174.1	128	128	21	17	2.69	500	5
Meprobamate[12]	219.1	158	97	65	65	1	7	2.97	500	5
Methadone[19]	310.2	265.1	105	112	112	9	29	3.67	500	5
Methamphetamine[20]	150.1	119	91	92	92	5	17	2.09	1000	10
Methylphenidate[2]	234.1	84.1	56.1	112	112	21	53	2.59	500	5
Midazolam[13]	326.1	291.1	249.1	170	170	29	41	3.51	400	4
Morphine[1]	286.2	165.1	152	158	158	41	60	1.2	500	5
Nitrazepam[13]	282.1	236.1	180	148	148	25	41	3.55	400	4
Norbuprenorphine[21]	414.3	187.1	83.1	205	205	41	57	2.98	100	1
Nordiazepam[11]	271.1	165	140	138	138	25	29	4.03	400	4
Norfentanyl[14]	233.2	84.1	55.1	112	112	16	40	2.58	10	0.1
Norketamine[15]	224	207	125	92	92	8	24	2.4	1000	10
Normeperidine[18]	234.2	160.1	56.1	138	138	12	20	2.75	500	5

Table 1 (cont.). Mass spectrometer acquisition parameters.

Compound Name	Precursor Ion [m/z]	Product Ion [m/z]		Fragmentor Voltage [V]		Collision Energy [V]		Ret. Time [min]	High Std Conc. [ng/mL]	Limit of Quant. [ng/mL]
Norpropoxyphene[8]	308.2	100.1	44.1	107	107	8	20	3.41	1000	10
o-Desmethyltramadol[5]	250.1	58.1	42.1	97	97	16	104	2.06	250	2.5
Oxazepam[3]	287.1	269	241	133	133	20	21	3.78	400	4
Oxycodone[1]	316.2	298.1	241.1	143	143	17	29	1.84	500	5
Oxymorphone[1]	302.1	227.1	198.1	133	133	28	48	1.29	500	5
PCP[12]	244.2	91	86.1	86	86	41	9	2.96	50	0.5
Propoxyphene[22]	340.2	266.2	58.1	92	92	5	25	3.6	1000	10
Temazepam[11]	301.1	255.1	177	117	117	29	45	3.9	400	4
Triazolam[13]	343.1	308.1	239	160	160	30	50	3.79	400	4

1 d_3-Morphine
2 d_4-7-Aminoclonazepam
3 d_5-Oxazepam
4 d_3-Amitriptyline
5 d_5-Amphetamine
6 d_5-α-Hydroxyalprazolam
7 d_3-Cocaine
8 d_4-Buprenorphine
9 d_7-Carisoprodol
10 d_4-Clonazepam
11 d_5-Nordiazepam
12 d_5-PCP
13 d_5-Estazolam
14 d_5-Fentayl
15 d_4-Ketamine
16 d_5-MDA
17 d_5-MDMA
18 d_4-Merperidine
19 d_9-Methadone
20 d_5-Methamphetamine
21 d_3-Norbuprenorphine
22 d_5-Propoxyphene
23 d_3-Tramadol-C13

Oral fluid matrix was collected from a male volunteer using Quantisal Saliva Collection Devices, (cat.#QS-0025) received from Immunalysis. High concentration calibration standard and intermediate QC oral fluid samples were prepared by making appropriate dilutions of the combined intermediate analyte stock solution using analyte free oral fluid to give the concentrations in saliva listed in Table 1. Calibration standards were then prepared using a dilution ratio strategy from the high concentration sample of 1:2:2:2.5:2:5. The QC samples were prepared using a dilution ratio strategy from the high concentration sample of 1.33:3.33:3. Table 1 lists the concentrations for the highest calibration standard and the limits of quantitation found during analyses.

All other reagents and solvents used were reagent grade.

Instrumentation. All automated SPE PrepSequences were performed using a MultiPurpose Sampler (MPS robotic^{pro}) equipped with GERSTEL ITSP Option as shown in Figure 1. All analyses were performed using an Agilent 1290 HPLC with a Poroshell 120 C18 column (3.0 x 50 mm, 2.7 µm) and an Agilent 6470 Triple Quadrupole Mass Spectrometer with jet stream electrospray source. The GERSTEL MPS robotic^{pro} was configured with LCMS Tool, a Universal Syringe Module (USM) with a 100 µL syringe, and a USM with a 1000 µL syringe. Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 2 µL stainless steel sample loop.

Figure 1. GERSTEL MultiPurpose Sampler (MPS robotic^{pro}) with GERSTEL ITSP Option.

The automated SPE extraction used for this method consisted of the following steps:

Automated SPE Prep Sequence:

1. The user transfers 1.5 mL of oral fluid sample into a 2 mL vial.
2. Working internal standard (15 µL) is added to the sample and the sample is mixed.
3. Conditioning buffer (150 µL) (20 % ammonium acetate in water (w/w)) is added to the sample and the sample is mixed.
4. The ITSP SPE cartridge (UCT C18 endcapped, 10 mg) is washed using 100 µL of wash solvent (0.2 % ammonium acetate in water) followed by 100 µL of the conditioning buffer.

5. The oral fluid sample (1 mL) is added to the ITSP SPE cartridge at a flow rate of 5 µL/sec.
6. The ITSP SPE cartridge is washed using 100 µL of the conditioning buffer.
7. The analytes are then eluted from the ITSP SPE cartridge using 2 x 75 µL aliquots of the buffered elution solvent (0.2 % ammonium acetate in methanol) and then diluted (1:2) using the wash solvent to ensure good chromatography and separation of isobaric compounds.

Analysis conditions LC.

Pump:	gradient (600 bar), flowrate = 0.5 mL/min	
Mobile Phase:	A - 5 mM ammonium formate with 0.05 % formic acid	
	B- 0.05 % formic acid in methanol	
Gradient:	Initial	5 % B
	0.5 min	5 % B
	1.5 min	30 % B
	3.5 min	70 % B
	4.5 min	95 % B
	6.49 min	95 % B
	6.5 min	5 % B
Run time:	6.5 minutes	
Injection volume:	2.0 µL (loop over-fill technique)	
Column temperature:	55°C	

Analysis conditions MS.

Operation:	electrospray positive mode
Gas temperature:	350°C
Gas flow (N_2):	5 L/min
Nebulizer pressure:	35 psi
Sheath gas heater:	250°C
Sheath gas flow (N_2):	11 L/min
Capillary voltage:	4000 V
delta EMV:	+ 500 V
delta RT (min):	0.5 min

The mass spectrometer acquisition parameters are shown in Table 1 with qualifier ions. A retention time window value of 0.5 minute was used for each positive ion transition being monitored over the course of the dynamic MRM experiment.

Results and Discussion

Figure 2 shows representative mass chromatograms for all drugs of abuse, along with their respective qualifier ion transitions, from an extracted low QC sample.

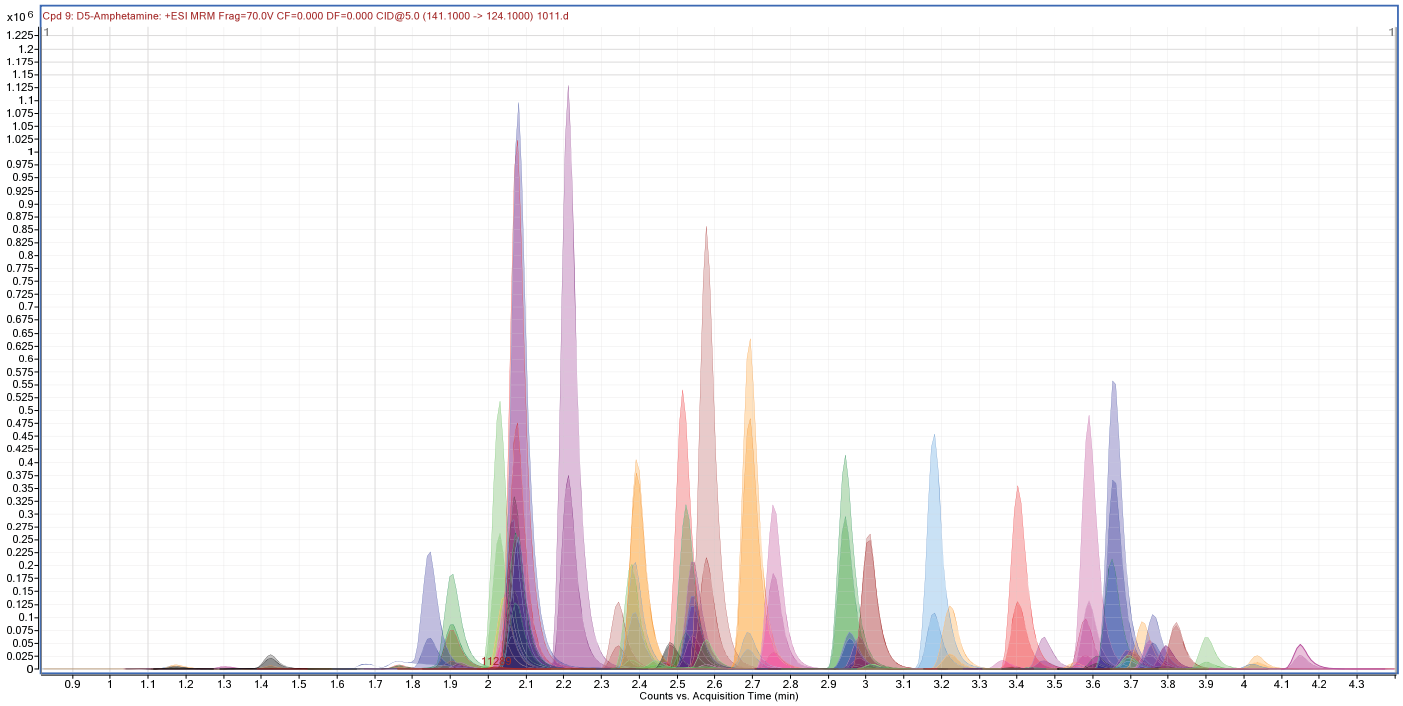
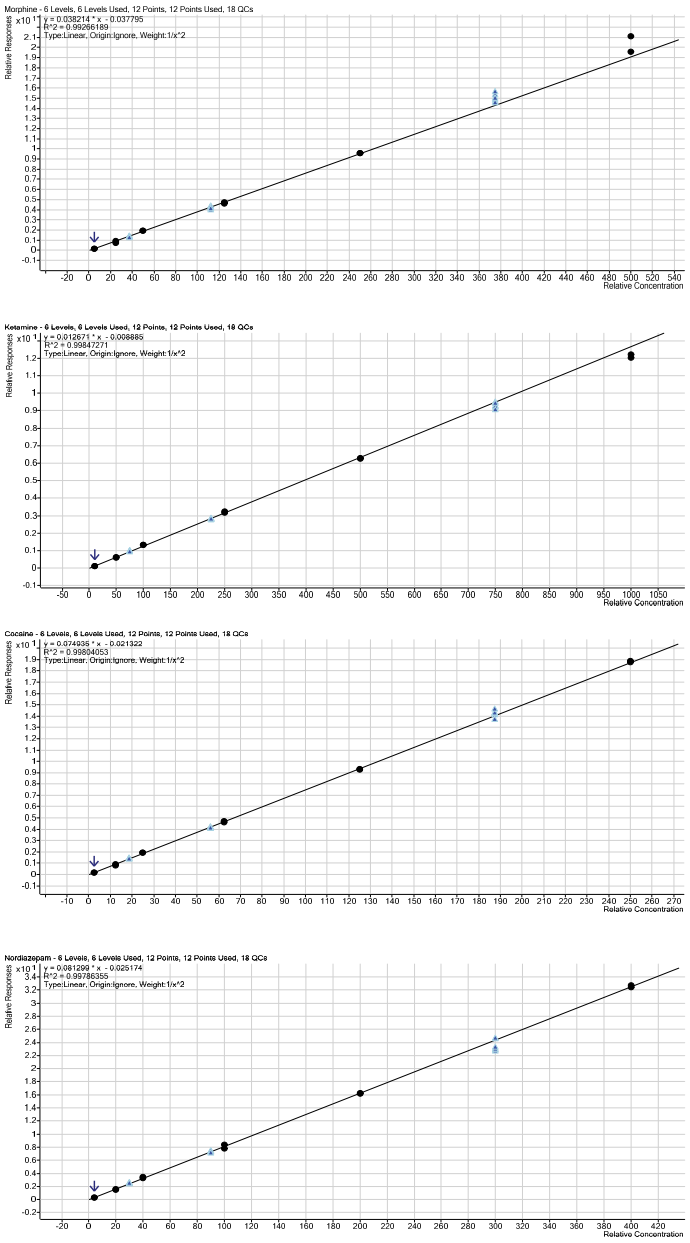


Figure 2. Representative mass chromatograms for low QC sample.

The lower limits of quantitation of this method are shown in Table 1. Representative calibration curves are shown in Figures 3 a-d. Regression analysis for all drugs of abuse analyzed within this method resulted in R^2 values of 0.99 or greater.



Figures 3 a-d. Representative calibration curves: Morphine, Ketamine, Cocaine, and Nordiazepam.

The accuracy and precision of the method was measured for all determined drugs of abuse using QC samples at high, middle, and low concentrations. Table 2 shows the resulting accuracy and precision data for all drug compounds. Accuracy data averaged 101 % (range: 86.3 % - 113 %) and precision data averaged 5.97 % RSD (range: 0.840 % -16.7 %) for all drugs of abuse analyzed.

Table 2. QC samples accuracy and precision table.

Compound	QC Level	Exp. Conc. [ng/mL]	Ave. Conc. [ng/mL]	Ave. Prec. [%]	Ave. Acc. [%]
Morphine	low	37.50	37.33	4.22	99.6
	middle	112.50	112.13	2.83	99.7
	high	375.00	394.87	2.93	105
Oxymorphone	low	37.50	38.15	10.6	102
	middle	112.50	107.87	8.14	95.9
	high	375.00	402.38	2.66	107
Hydromorphone	low	37.50	36.62	7.47	97.7
	middle	112.50	112.91	10.2	100
	high	375.00	383.91	4.31	102
Oxycodone	low	37.50	35.98	10.9	95.9
	middle	112.50	109.95	11.5	97.7
	high	375.00	397.98	6.17	106
Codeine	low	37.50	36.77	6.13	98.1
	middle	112.50	112.30	10.7	99.8
	high	375.00	400.84	6.74	107
Hydrocodone	low	37.50	36.52	7.57	97.4
	middle	112.50	110.58	12.1	98.3
	high	375.00	399.43	7.99	107
6-Monoacetylmorphine	low	7.50	7.07	12.6	94.2
	middle	22.50	21.46	10.4	95.4
	high	75.00	77.98	7.53	104
d-Amphetamine	low	75.00	77.62	5.25	103
	middle	225.00	222.67	6.83	99.0
	high	750.00	736.09	2.81	98.1
MDA	low	75.00	74.51	7.04	99.3
	middle	225.00	228.47	3.80	102
	high	750.00	777.13	1.52	104
o-Desmethyl-cis-Tramadol	low	18.75	17.70	10.7	94.4
	middle	56.25	52.76	5.86	93.8
	high	187.50	189.41	3.27	101
MDMA	low	75.00	79.81	2.07	106
	middle	225.00	230.92	1.40	103
	high	750.00	688.05	2.75	91.7
Methamphetamine	low	75.00	80.14	0.95	107
	middle	225.00	231.32	1.83	103
	high	750.00	685.97	2.58	91.5
MDEA	low	75.00	74.03	16.7	98.7
	middle	225.00	232.82	9.27	103
	high	750.00	674.94	5.25	90.0
Benzoylecgonine	low	18.75	19.64	9.01	105
	middle	56.25	57.09	8.14	101
	high	187.50	174.56	6.44	93.1
Ketamine	low	75.00	79.04	1.84	105
	middle	225.00	224.85	1.00	99.9
	high	750.00	735.79	1.68	98.1

Table 2 (cont.). QC samples accuracy and precision table.

Compound	QC Level	Exp. Conc. [ng/mL]	Ave. Conc. [ng/mL]	Ave. Prec. [%]	Ave. Acc. [%]
Norketamine	low	75.00	80.74	2.77	108
	middle	225.00	220.51	3.10	98.0
	high	750.00	679.57	6.54	90.6
7-Aminoclonazepam	low	37.50	41.07	5.17	110
	middle	112.50	112.97	7.10	100
	high	375.00	360.04	1.53	96.0
cis-Tramadol	low	18.75	19.09	3.32	102
	middle	56.25	55.52	1.94	98.7
	high	187.50	187.94	1.66	100
Cocaine	low	18.75	19.83	1.42	106
	middle	56.25	55.93	0.839	99.4
	high	187.50	190.39	2.07	102
Methylphenidate	low	37.50	36.18	12.5	96.5
	middle	112.50	116.27	4.01	103
	high	375.00	323.44	6.25	86.3
nor-Fentanyl	low	0.75	0.79	7.57	105
	middle	2.25	2.30	11.6	102
	high	7.50	7.40	3.50	98.6
Meperidine	low	37.50	40.36	2.10	108
	middle	112.50	114.59	1.08	102
	high	375.00	338.37	3.96	90.2
Normeperedine	low	37.50	39.11	3.27	104
	middle	112.50	108.38	6.36	96.3
	high	375.00	401.52	3.71	107
PCP	low	3.75	3.91	3.26	104
	middle	11.25	11.05	4.58	98.2
	high	37.50	36.29	2.44	96.8
Norbuprenorphine	low	7.50	7.93	9.19	106
	middle	22.50	21.77	3.76	96.8
	high	75.00	82.16	3.12	110
Meprobamate	low	37.50	42.32	8.04	113
	middle	112.50	112.85	9.57	100
	high	375.00	383.05	5.44	102
Fentanyl	low	0.75	0.80	6.74	107
	middle	2.25	2.26	4.73	101
	high	7.50	7.58	2.67	101
EDDP	low	37.50	42.24	4.43	113
	middle	112.50	111.29	8.59	98.9
	high	375.00	336.16	6.54	89.6
Flurazepam	low	30.00	27.88	13.2	92.9
	middle	90.00	88.73	11.2	98.6
	high	300.00	324.10	7.52	108
Buprenorphine	low	7.50	8.03	2.63	107
	middle	22.50	22.93	5.02	102
	high	75.00	73.33	2.93	97.8
Norpropoxyphene	low	75.00	80.56	11.5	107
	middle	225.00	240.51	6.41	107
	high	750.00	818.57	4.99	109

Compound	QC Level	Exp. Conc. [ng/mL]	Ave. Conc. [ng/mL]	Ave. Prec. [%]	Ave. Acc. [%]
Bromazepam	low	30.00	30.43	13.1	101
	middle	90.00	87.70	7.60	97.4
	high	300.00	264.41	3.73	88.1
Midazolam	low	30.00	32.15	6.99	107
	middle	90.00	88.32	10.4	98.1
	high	300.00	287.62	5.63	95.9
Nitrazepam	low	30.00	29.50	6.55	98.3
	middle	90.00	86.22	4.57	95.8
	high	300.00	322.20	7.84	107
Clonazepam	low	30.00	29.98	9.95	99.9
	middle	90.00	90.16	5.26	100
	high	300.00	314.85	9.65	105
Propoxyphene	low	75.00	81.95	2.02	109
	middle	225.00	228.13	1.40	101
	high	750.00	757.73	1.99	101
Flunitrazepam	low	30.00	32.87	8.52	110
	middle	90.00	90.22	9.27	100
	high	300.00	280.19	5.23	93.4
Methadone	low	37.50	39.83	2.59	106
	middle	112.50	114.20	1.72	102
	high	375.00	387.75	1.18	103
α-Hydroxyalprazolam	low	15.00	14.40	7.82	96.0
	middle	45.00	43.78	10.7	97.3
	high	150.00	160.12	2.69	107
Amitriptyline	low	18.75	19.65	3.57	105
	middle	56.25	57.02	2.34	101
	high	187.50	194.99	3.17	104
Estazolam	low	30.00	29.47	5.25	98.2
	middle	90.00	85.43	5.94	94.9
	high	300.00	310.11	5.52	103
Clobazam	low	30.00	29.60	9.79	98.7
	middle	90.00	90.88	8.70	101
	high	300.00	288.37	7.24	96.1
Carisoprodol	low	37.50	38.24	2.07	102
	middle	112.50	111.42	2.29	99.0
	high	375.00	380.03	2.32	101
Lorazepam	low	30.00	29.26	12.8	97.5
	middle	90.00	88.37	10.1	98.2
	high	300.00	320.44	8.28	107
Oxazepam	low	30.00	30.64	11.7	102
	middle	90.00	88.44	3.83	98.3
	high	300.00	297.64	4.96	99.2
Triazolam	low	30.00	30.96	12.6	103
	middle	90.00	88.26	4.83	98.1
	high	300.00	298.83	8.66	99.6
Alprazolam	low	30.00	31.91	6.84	106
	middle	90.00	88.32	3.72	98.1
	high	300.00	326.19	12.4	109

Table 2 (cont.). QC samples accuracy and precision table.

Compound	QC Level	Exp. Conc. [ng/mL]	Ave. Conc. [ng/mL]	Ave. Prec. [%]	Ave. Acc. [%]
Temazepam	low	30.00	31.32	6.14	104
	middle	90.00	88.48	4.64	98.3
	high	300.00	282.74	15.0	94.2
Nordiazepam	low	30.00	32.08	2.52	107
	middle	90.00	90.99	1.73	101
	high	300.00	290.32	3.62	96.8
Diazepam	low	30.00	32.09	3.74	107
	middle	90.00	92.49	8.51	103
	high	300.00	325.81	11.2	109

The LCMS Tool eliminates sample-to-sample carryover. This is especially important given today's extremely sensitive LC/MS/MS systems. The method was evaluated for any potential carryover by extracting and analyzing a high concentration standard followed by the injection and analysis of a solvent blank and comparing the results of the blank to that of an extracted low standard. Figure 4 shows a comparison of an extracted low standard (blue TIC) to that of the solvent blank (orange TIC). In the solvent blank trace, no response was found at the retention times of any of the analytes in the low standard.

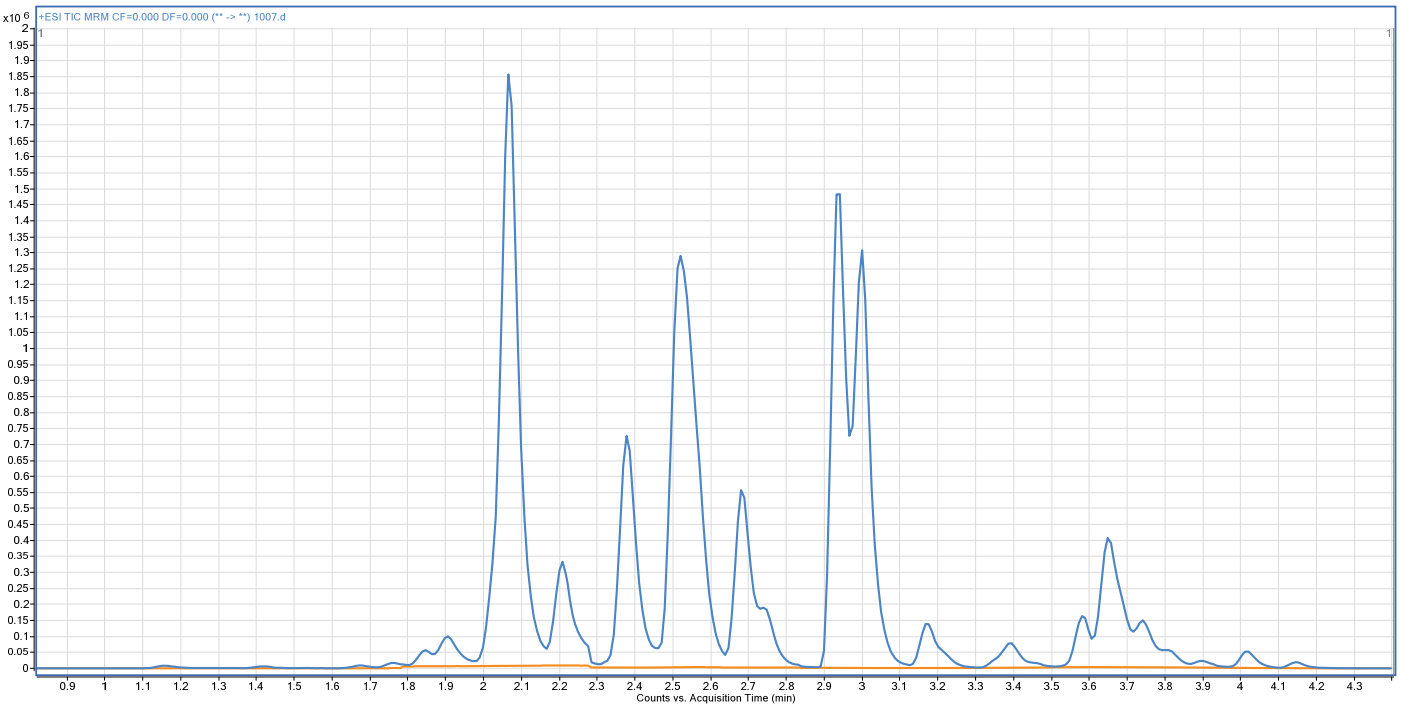


Figure 4. Overlay mass chromatogram results from carryover evaluation.

Conclusions

As a result of this study, we were able to show:

- Over 50 drugs of abuse and internal standards can be successfully extracted from oral fluid samples using an automated SPE procedure coupled to LC/MS/MS analysis using the Agilent 6470 Triple Quadrupole Mass Spectrometer.
- This method proved to be readily automated using the GERSTEL MultiPurpose Sampler (MPS robotic^{pro}).
- Linear calibration curves resulting in R^2 values 0.99 or greater were achiived for the determined drugs of abuse.
- The SPE-LC/MS/MS method proved to be accurate and precise. Accuracy data averaged 101 % (range: 86.3 % - 113 %) and precision data averaged 5.97 % RSD (range: 0.840 % -16.7 %) for all determined drugs of abuse.
- Evaluation of the method showed no detectable carryover following injection of an extracted high standard.

References

[1] "Drugs of abuse measured in urine or oral fluid for forensic and medical purposes." *ITSP Solutions, Inc. Application Note,* accessed April 2017.

[2] Quantisal oral fluid collection device (2017, January) *Immunalysis Product Catalog*, Jan 2017, retrieved from https://immunalysis.com/products/oral-fluid/quantisal/.

GERSTEL AppNote

GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany
+49 (0) 208 - 7 65 03-0
+49 (0) 208 - 7 65 03 33
gerstel@gerstel.com
www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.
701 Digital Drive, Suite J
Linthicum, MD 21090
USA
+1 (410) 247 5885
+1 (410) 247 5887
sales@gerstelus.com
www.gerstelus.com

GERSTEL AG
Wassergrabe 27
CH-6210 Sursee
Switzerland
+41 (41) 9 21 97 23
+41 (41) 9 21 97 25
swiss@ch.gerstel.com
www.gerstel.ch

GERSTEL K.K.
1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan
+81 3 5731 5321
+81 3 5731 5322
info@gerstel.co.jp
www.gerstel.co.jp

GERSTEL LLP
Level 25, North Tower
One Raffles Quay
Singapore 048583
+65 6622 5486
+65 6622 5999
SEA@gerstel.com
www.gerstel.com

GERSTEL Brasil
Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil
+55 (11)5665-8931
+55 (11)5666-9084
gerstel-brasil@gerstel.com
www.gerstel.com.br

CERTIFIED QM-SYSTEM * * * ISO 9001 * ISO 9001:2008 CERTIFICATE NO. 0086 D

ECOPROFIT®
Awarded for the active pursuit of environmental sustainability