SPEctra™: A High Sensitivity Magnetic Beads-Based Extraction Kit and Method for Fully Automated Oral Fluid Toxicology

Introduction

CURA's new Xpeedy® SPEctra™ magnetic beads solid phase extraction (mSPE) kit marks a major advancement in oral fluid (OF) drug of abuse (DOA) toxicology. Designed for use with automated magnetic beads processors and liquid handlers, the SPEctra™ kit enables efficient extraction of a wide range of drugs from OF for LC-MS/MS analysis. While OF offers significant advantages over urine in toxicological testing, it requires specialized collection/storage devices like Quantisal™ and complex processing techniques such as solid phase extraction (SPE). Though effective, traditional SPE methods are labor-intensive, difficult to automate, and unsuitable for high-throughput laboratories, posing a major barrier to widespread adoption of OF testing. SPEctra™ addresses these limitations by combining high sensitivity with streamlined workflows, enabling fewer steps and full compatibility with automation platforms. This innovation makes it possible for laboratories to transition to oral fluid testing without sacrificing throughput or analytical performance.

In this study, the recovery rates, matrix effects, and limits of quantification (LOQ) for OF samples using the SPEctra™ kit are evaluated, demonstrating its robustness and reliability for routine DOA testing.

Figure 1. SPEctra™ Extraction Kit and contents

Materials and Methods

The SPEctra™ extraction kit, comprising mixed mode cation exchange magnetic beads in sealed 96 deep well plates (DWP), loading buffer, 3 wash buffers, elution buffer and reconstitution buffer is manufactured by CURA Diagnostics Inc. (Woburn, MA). The testing samples contain synthetic OF (Utak) fortified at various concentrations using reference standards from Cerilliant (Texas) and Quantisal™ buffer (Abbot Laboratories Inc.).

Sample preparation workflow is summarized and presented in Figure 2. Briefly, Oral Fluid samples are transferred to and mixed with loading buffer in a 96 DWP by the Xpeedy® A20 Liquid Handler (CURA Diagnostics Inc.). The prepared plate is loaded onto a Thermo KingFisher Flex™ where automated magnetic solid phase extraction (mSPE) is performed using the reagents provided in the SPEctra™ kit. Following extraction, the eluted solution is evaporated and subsequently reconstituted using the reconstitution buffer included in the kit.

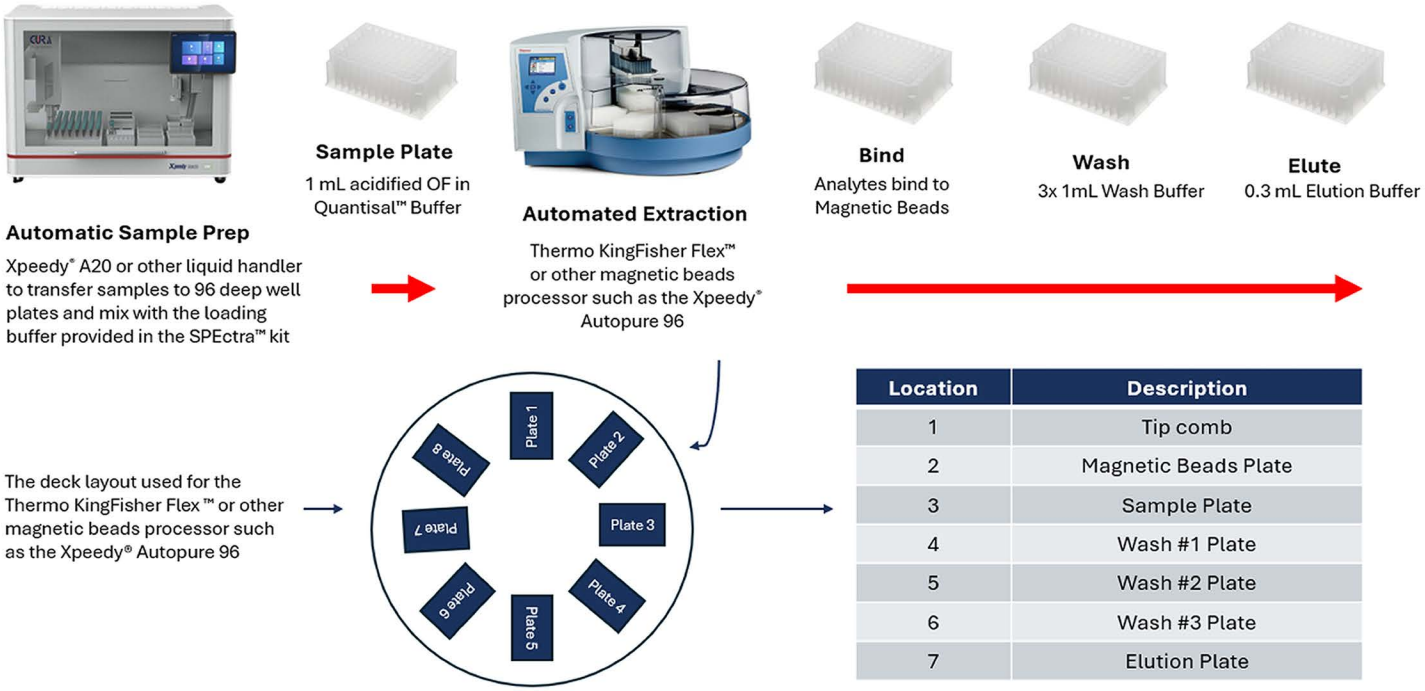
Analysis

The reconstituted samples are analyzed on a Shimadzu 8060 LC-MS/MS system (Kyoto, Japan) with a Shimadzu Prominence HPLC system. The analytical LC column is Raptor Biphenyl, 2.7 µm, 50 x 3.0 mm HPLC Column (Restek). Injection volume is 4 µL, and the mobile phases used are 0.1% formic acid in water (Mobile Phase A) and methanol (Mobile Phase B).

Recovery and matrix effects are determined for each analyte at the lowest level of quality control (QC) according to the validation method proposed by Matuszewski et al.[1]

[1] B. K. Matuszewski, M. L. Constanzer, and C. M. Chavez-Eng, Analytical Chemistry 2003 75 (13), 3019-3030, DOI: 10.1021/ac020361s

Phone +1(339) 999-2959
Email info@cura-dx.com
Website www.cura-dx.com
Address 400 West Cummings Park, Suite 5600 Woburn MA 01801



Location	Description
1	Tip comb
2	Magnetic Beads Plate
3	Sample Plate
4	Wash #1 Plate
5	Wash #2 Plate
6	Wash #3 Plate
7	Elution Plate

Figure 2. The SPEctra™ kit workflow integrated with the Xpeedy® A20 and Kingfisher Flex™. Similar workflows can be achieved using other magnetic beads processor such as Xpeedy® Autopure 96.

Three sets, A, B and C, were prepared. In set A, synthetic blank matrix is fortified with low QC prior to mSPE and internal standard (IS) after mSPE. In set B, synthetic blank matrix is fortified with both low QC and IS after mSPE. In set C, elution solvent is fortified with low QC and IS. Each sample in sets A and B are analyzed on the Shimadzu 8060 with 5 replicates, while those in set C are done with 4 replicates. Recovery rate for each target is calculated by dividing the analyte mean peak ratios of set A by those of set B and multiplying by 100%. The matrix effect is calculated by dividing the analyte mean peak area ratios of set B by those of set C and multiplying by 100% then subtracting by 100% for the bias. The acceptable matrix effect range is between -30% and 30%. Moreover, the comparison of coefficients of variation (CV) between SPEctra™ and manual SPE is performed with 5 replicates at concentrations of 10xLOQ.

Results and Discussion

The recovery rates and matrix effects (%) for all analytes are presented in Figure 3 and Figure 4, respectively. Recovery rates ranged from 15.27% to 91.92%, reflecting the challenge of designing a universal extraction method for a broad spectrum of analytes. Despite lower recoveries observed for certain compounds, signal strength remained sufficient to achieve the desired LOQs in oral fluid, as detailed in Table 1.

Matrix effects spanned –28.65% to 18.83%, falling within the acceptable range of ±30% and indicating excellent analytical robustness. The LOQs obtained using the SPEctra™ kit along with their corresponding CVs are shown in Table 1, demonstrating the kit's capability to meet clinically relevant cutoff levels.

Finally, Table 2 compares precision between the SPEctra™ method and manual SPE at 10×LOQ for 54 of the 56 analytes. SPEctra™ consistently outperforms manual SPE, achieving a maximum CV of 7.9% to manual SPE's 19.1%, underscoring its reproducibility and suitability for high-throughput clinical toxicology.

Conclusion

These results demonstrate the SPEctra™ kit's capability to efficiently extract clinically relevant analytes with full integration into automated LC-MS workflows, achieving some of the lowest cutoff levels in the industry. Compared to traditional SPE, SPEctra™ halves the processing time for a 96-sample batch while maintaining equal or superior precision. As a best-in-class extraction solution for oral fluid testing, SPEctra™ enables fully automated sample preparation, making oral fluid analysis accessible and scalable for both low and high-throughput laboratories.

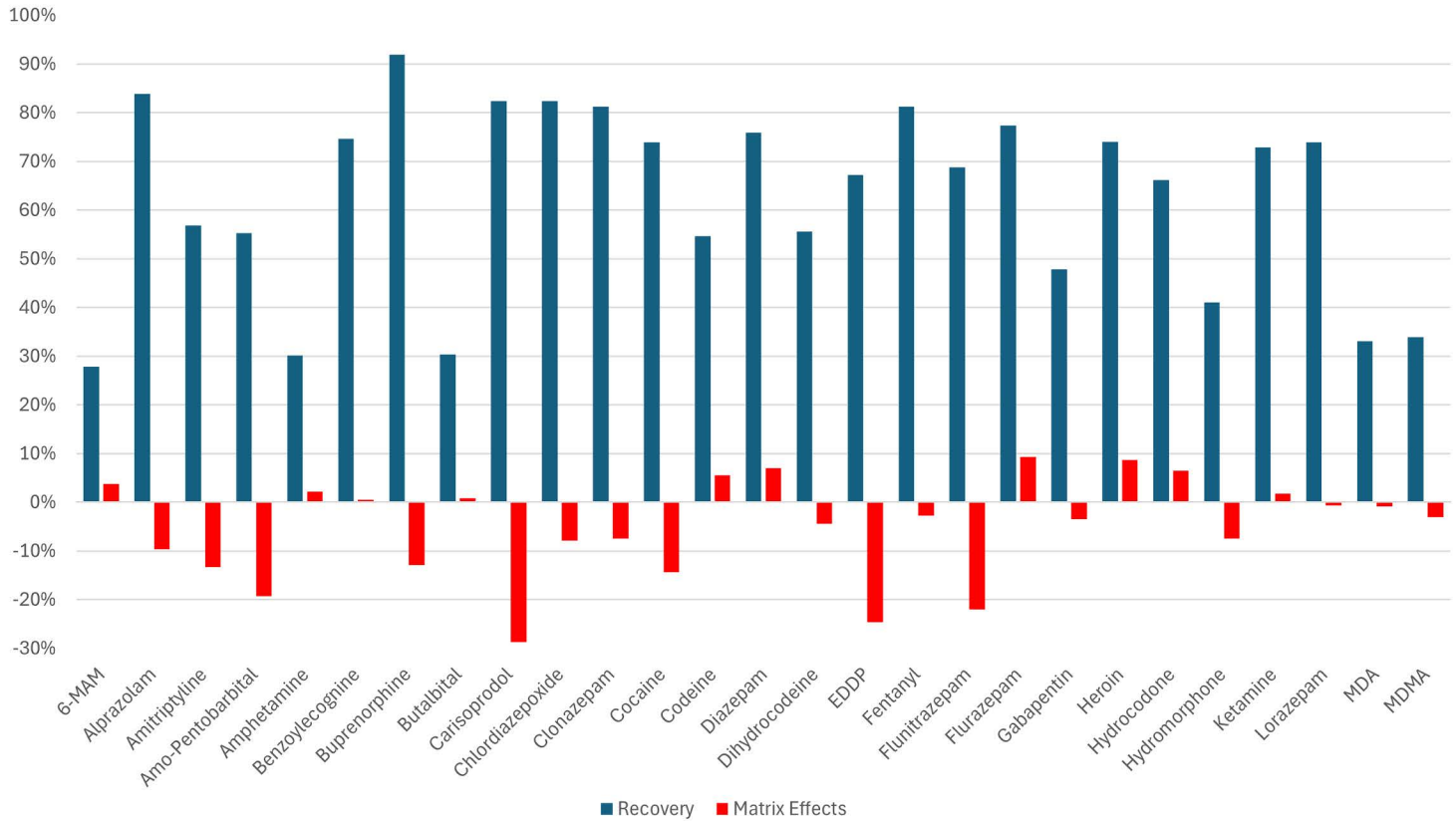


Figure 3. Target Drug Recovery and Matrix Effects 1

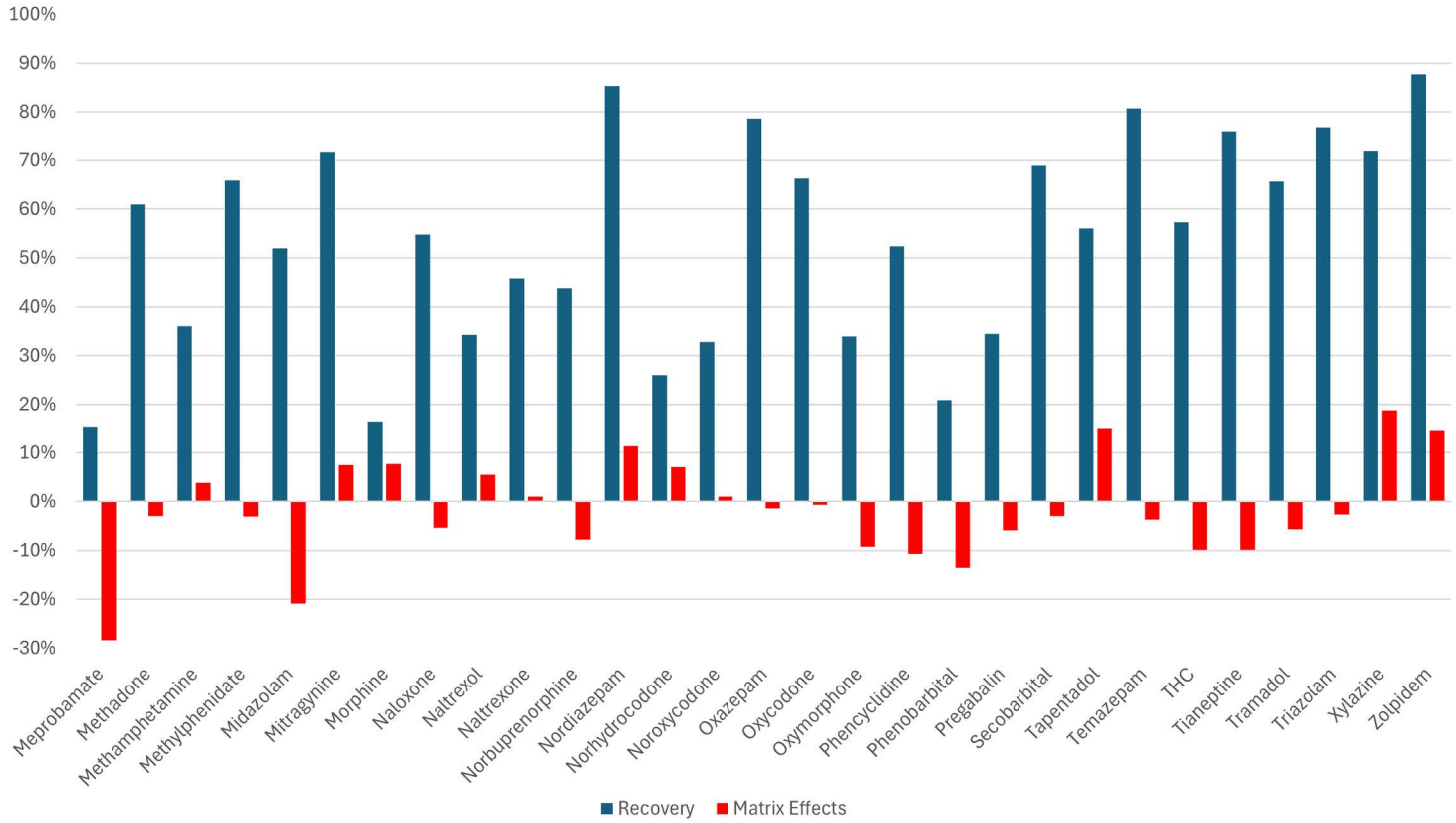


Figure 4. Target Drug Recovery and Matrix Effects 2

Analyte	LOQ (ng/mL)	Mean (ng/mL)	CV (%)
6-MAM	1	0.9	7.8
Alprazolam	0.5	0.5	8.4
Amitriptyline	10	10.0	3.2
Amo-Pentobarbital	10	9.5	10.1
Amphetamine	10	9.9	2.1
Benzoylecognine	5	4.9	2.7
Buprenorphine	0.1	0.1	11.3
Butalbital	10	10.6	10.3
Carisoprodol	2.5	2.6	6.6
Chlordiazepoxide	0.5	0.4	8.4
Clonazepam	0.5	0.6	5.0
Cocaine	5	5.3	3.2
Codeine	2.5	2.4	7.0
Diazepam	0.5	0.5	4.5
Dihydrocodeine	2.5	2.5	7.9
EDDP	5	5.4	2.4
Fentanyl	0.1	0.1	7.1
Flunitrazepam	0.5	0.5	8.1
Flurazepam	0.5	0.5	4.8
Gabapentin	10	9.7	3.4
Heroin	1	1.0	7.8
Hydrocodone	2.5	2.4	4.5
Hydromorphone	2.5	2.3	6.2
Ketamine	10	11.8	6.9
Lorazepam	0.5	0.5	5.4
MDA	10	9.7	5.6
MDMA	10	8.0	7.3
Meprobamate	2.5	2.7	7.1
Methadone	5	4.7	2.4
Methamphetamine	10	7.7	7.6
Methylphenidate	1	1.0	3.1
Midazolam	0.5	0.5	6.0
Mitragynine	1	1.0	5.4
Morphine	2.5	2.5	5.5
Naloxone	0.25	0.2	9.6
Naltrexol	0.25	0.3	4.0
Naltrexone	0.25	0.3	11.9
Norpbuprenorphine	0.5	0.5	7.4
Nordiazepam	0.5	0.5	5.5
Norhydrocodone	2.5	2.3	5.8
Noroxycodone	2.5	2.3	6.6
Oxazepam	0.5	0.5	6.5
Oxycodone	5	5.1	4.6
Oxymorphone	5	4.5	5.4
Phencyclidine	10	7.9	7.2
Phenobarbital	10	10.0	13.6
Pregabalin	10	9.4	4.4
Secobarbital	10	9.9	7.5
Tapentadol	5	2.5	4.2
Temazepam	0.5	4.9	2.2
THC	2.5	0.5	2.7
Tianeptine	10	2.1	4.5
Tramadol	5	5.1	2.8
Triazolam	0.5	0.5	5.0
Xylazine	1	0.8	7.2
Zolpidem	5	3.9	4.3

Table 1: SPEctra™ LOQ analysis. 20 replicates were analyzed. (Acceptance Criteria: CV% <20%)

Analyte	SPEctra™ CV (%)	SPE CV (%)
6-MAM	0.6	2.1
Alprazolam	2.1	0.8
Amitriptyline	1.5	5.5
Amo-Pentobarbital	5.3	13.7
Amphetamine	2.1	6.0
Benzoylecognine	1.6	1.8
Buprenorphine	7.9	3.6
Butalbital	4.2	18.2
Carisoprodol	3.8	5.6
Chlordiazepoxide	3.5	4.5
Clonazepam	3.4	2.3
Cocaine	6.7	4.2
Codeine	1.8	3.9
Diazepam	7.1	5.4
Dihydrocodeine	5.4	2.5
EDDP	1.7	3.0
Fentanyl	1.7	5.7
Flunitrazepam	2.5	6.9
Flurazepam	3.6	3.2
Gabapentin	2.1	19.1
Heroin	3.2	1.8
Hydrocodone	1.5	3.6
Hydromorphone	3.2	3.6
Ketamine	4.9	5.0
Lorazepam	2.3	5.1
MDA	1.9	2.7
MDMA	4.3	8.9
Meprobamate	3.8	6.5
Methadone	1.3	4.2
Methamphetamine	0.5	6.6
Methylphenidate	1.5	3.9
Midazolam	1.2	2.8
Mitragynine	1.4	4.1
Morphine	5.4	5.7
Naloxone	3.7	7.7
Naltrexol	3.6	3.7
Naltrexone	2.1	2.2
Norpbuprenorphine	5.0	9.3
Nordiazepam	5.8	4.4
Norhydrocodone	1.6	2.0
Noroxycodone	4.0	2.1
Oxazepam	4.9	4.7
Oxycodone	3.1	1.4
Oxymorphone	3.1	4.7
Phencyclidine	1.9	2.0
Phenobarbital	2.4	9.9
Pregabalin	2.2	17.6
Secobarbital	6.3	13.4
Tapentadol	3.5	5.2
Temazepam	3.4	4.9
THC	4.2	2.8
Tianeptine	N/A	N/A
Tramadol	1.2	1.8
Triazolam	5.8	3.7
Xylazine	N/A	N/A
Zolpidem	1.9	5.6

Table 2: Head-to-head comparison of SPEctra™ and manual SPE precision at 10 x LOQ