eXtreme|FV® Extraction for the Detection of 11 Antidepressants in Oral Fluid Samples

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Introduction

The use of oral fluid has recently become more prevalent in drug testing laboratories. While a urine sample provides a longer detection window and more comprehensive analysis of drug use, oral fluid is a viable option for testing[1,2]. The benefits of using oral fluid include the ability to detect recent drug use, ease of collection, and the collection process can be observed to prevent adulteration of the sample[3]. Many laboratories currently use solid phase extraction techniques to detect drugs and metabolites in oral fluid, however this extraction technique is laborious, expensive, and time consuming. A new, efficient technique has been introduced which optimizes the extraction process by reducing waste and amount of time spent extracting samples. Thomson eXtreme™ Filter Vials provide a simple and efficient extraction technique that has demonstrated adequate analyte recovery, reduced matrix interferences and the elimination of solvent waste and other consumables. This project specifically explores the efficacy of these vials in extracting a wide range of antidepressants in oral fluid specimens.

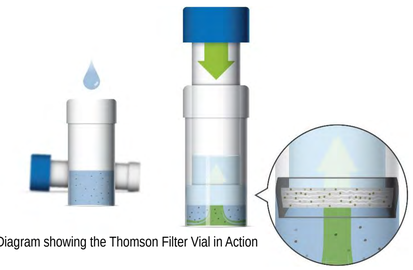


Diagram showing the Thomson Filter Vial in Action

Methods

1. Oral fluid sample collected using OraSure Intercept I2he collection device
   i. Place swab under tongue and hold until tab changes from white to blue (approximately 3-4 minutes and for a maximum of 15 mins)
   ii. Slide swab into collection vial and screw on cap
2. Break tab on end of collection vial, and place into test tube
3. Centrifuge samples to pull diluted sample into test tube
4. Aliquot 100 µL sample, calibrator or control into eXtreme™ filter vials shells
5. Add 100 µL of mobile phase to vial shell
6. Add 20 µL of internal standard to vial shell
7. Place plunger filter into vial shell and press slowly and firmly until cap is secured in place
8. Vortex samples and inspect samples to assure no bubbles are present
9. Place sample onto instrumentation to be analyzed via LC/MS/MS

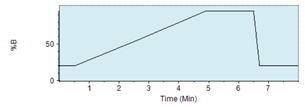
Results

Samples were analyzed using a Shimadzu Liquid Chromatograph and Sciex Triple Quad Mass Spectrometer. The developed method injects 12 µL of sample onto a Kinetex® Biphenyl LC column. The sample is chromatographically separated at a flow rate of 0.7 mL/min using the following gradient:

Table 1: LC-MS/MS gradient

Time (min)	Mobile Phase B (%)
0	20
0.5	20
2.6	55
4.9	95
6	95
6.2	20
7	20
8	Stop

Figure 1: LC-MS/MS gradient



Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in Methanol

Adequate chromatographic separation of all tested analytes was achieved while still attaining optimal sensitivity.

Table 2: Antidepressants analyzed and respective retention times

Analyte	Retention Time
Citalopram	4.80
Amitriptyline	5.40
Nortriptyline	5.40
Fluoxetine	5.00
Norfluoxetine	5.00
Sertraline	5.60
Norsertraline	5.60
Venlafaxine	4.50
Desvenlafaxine	3.60
Trazodone	4.90
mCPP	4.10

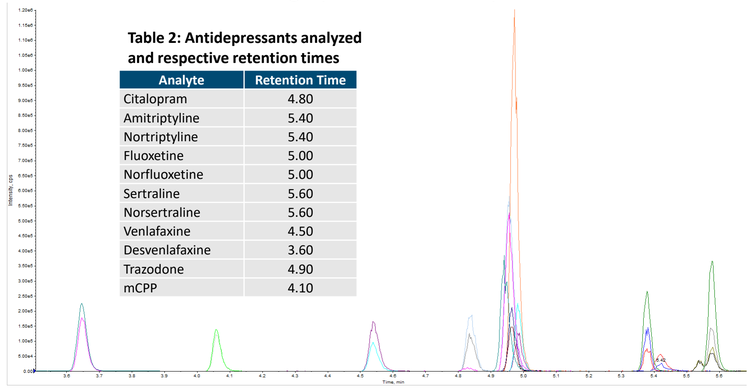


Figure 2: Chromatogram of antidepressant calibrator

Calibration range was established between 5 ng/mL to 200 ng/mL for each analyte. Controls sufficiently passed quantitatively and qualitatively within established ranges of targeted values (15 and 150 ng/mL respectively). To obtain the undiluted concentration of analyte in the sample, values were multiplied by a factor of three.

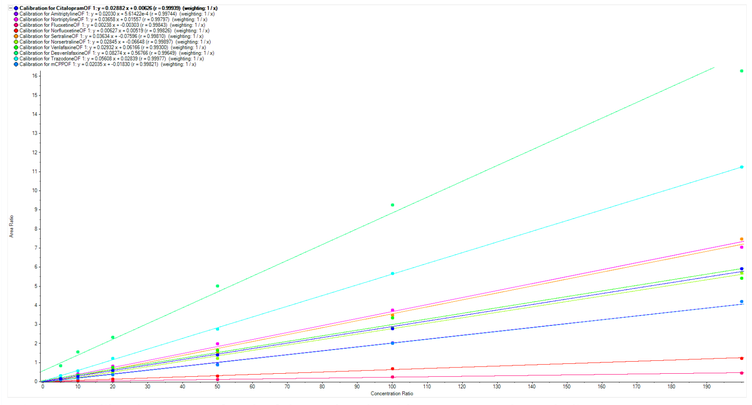


Figure 3: Calibration curves of each of the analyzed antidepressants and metabolites

Results

Of the 115 samples analyzed, 50 were positive for antidepressants and their metabolites. These results were consistent with the provided medication lists. Samples were also simultaneously analyzed for opioids, benzodiazepines, barbiturates and drugs of abuse.

Table 3: Antidepressant positive samples

Analyte/Metabolite	# of Positives
Citalopram	8
Trazodone	8
mCPP	5
Sertraline	3
Norsertaline	4
Venlafaxine	7
Desvenlafaxine	7
Amitriptyline	2
Nortriptyline	1
Fluoxetine	2
Norfluoxetine	1

Figure 4: Result classification

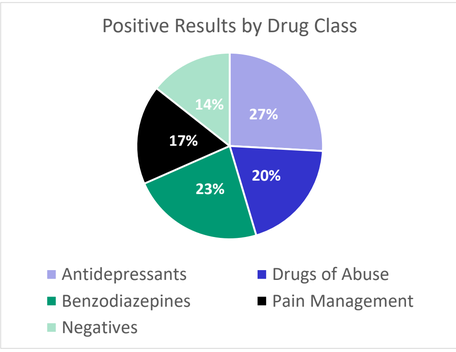


Table 4: Average analyte concentrations of samples analyzed

Parent	Average Concentration	Metabolite	Average Concentration
Citalopram	997	N/A	
Trazodone	73*	mCPP	32*
Sertraline	24	Norsertraline	28
Venlafaxine	1424	Desvenlafaxine	369
Amitriptyline	22	Nortriptyline	290 (n=1)
Fluoxetine	267	Norfluoxetine	103 (n=1)

*Outlier removed from Average calculation

Conclusion

The developed method utilizing the eXtreme ™ filter vials proved successful in extracting and detecting antidepressants and metabolites present in oral fluid with a high level of sensitivity and accuracy. A simple, rapid, and accurate comprehensive method was developed for the detection of 48 drugs in oral fluid samples. Citalopram, venlafaxine and desvenlafaxine showed high average concentrations in samples. With the exception of desvenlafaxine, metabolite positives were not without the presence of parent drug (this is likely a result of desvenlafaxine being available as a prescription medication). While urine is considered a better comprehensive test to detect drug use over time, in the pain management field, oral fluid is a viable alternative for drug detection in patients that have difficulty voiding or when adulteration may be suspected[2]. However, in this particular application, limitations include narrower detection windows and detection issues with analytes such as benzodiazepines that have low drug concentrations in oral fluid.

References

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[2]Casolin, A. Comparison of Urine and Oral Fluid for Workplace Drug Testing. J Anal Toxicol. 2016 Sept;40(7):479-485.
[3]Cone, E.J.; Presley, L.; Lehrer, M.; Seiter, W.; Smith, M.; Kardos, K.W.; Fritch, D.; Salamone, S.; Niedbala, R.S. Oral fluid testing for drugs of abuse: positive prevalence rates by Intercept immunoassay screening and GC-MS-MS confirmation and suggested cutoff concentrations. J Anal Toxicol. 2002 Nov-Dec;26(8):541-6.