Development and optimization of a new method to determine antidepressants in oral fluid by microextraction by packed sorbent and analysis by GC-MS/MS

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Objective

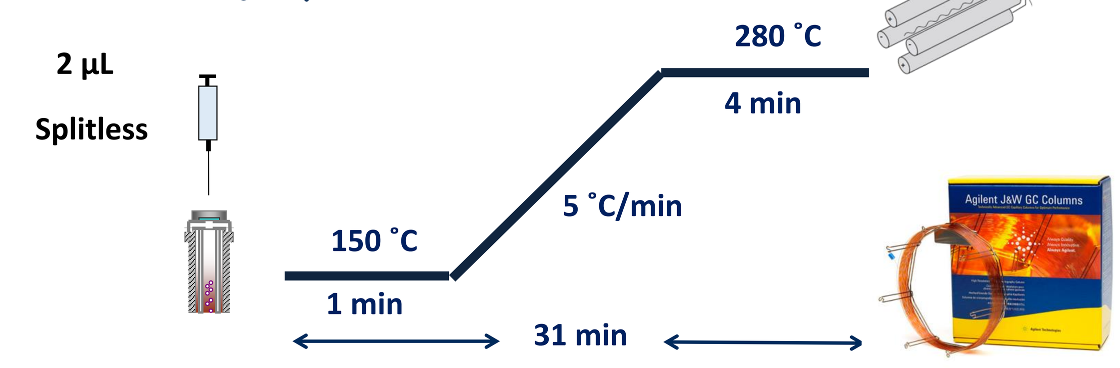
This work aims to develop a methodology for the detection of antidepressants (fluoxetine (FLX), norfluoxetine (NFLX), venlafaxine (VLX), *O*-desmethylvenlafaxine (DVLX), citalopram (CIT), sertraline (SRT), and paroxetine (PXT)) in 250 µL of oral fluid samples by microextraction by packed sorbent (MEPS) and analysis by gas chromatography coupled with tandem mass spectrometry (GC-MS/MS).

Introduction

Between 2013 and 2016, the consumption of antidepressants doubled in Portugal, with around 30 million packages of medication for depression, anxiety and other mental health problems dispensed annually. This phenomenon has even given rise to several alerts, which was highlighted in the 2017 report of the National Program for Mental Health, as Portugal has one of the highest rates of mental illness in Europe[1]. Therapeutic drug monitoring is well established for a small number of drugs, namely for those where a direct relationship between concentration and pharmacological effect at the site of action exists, which in turn is predictably reflected in the response. Drug concentrations in the various biological fluids are used in conjunction with other clinical observation measures to assess the patient's condition, and further support the individualization of therapy. Within this group of drugs targeted for monitoring are antidepressants. In recent years, oral fluid has gained more and more importance in the field of therapeutic drug monitoring, as a non-invasive and painless alternative to traditionally sampled specimens (e.g. blood, plasma). MEPS is a miniaturized solid-phase extraction (SPE) and therefore has a great advantage in the reduced operating volumes and consequently lower amounts of sample and organic solvents are required. As a result, MEPS decreases the time required for extraction, clean-up and concentration of analytes.

Methodology

Chromatographic conditions:



GC system HP 7890A GC equipped with a 7000B MS/MS detector (Agilent technologies)
HP-5MS (30 m x 0.250 mm x 0.25 µm) J&W Scientific (Folsom)

- Injector's temperature: 250 ˚C;
- Detector's temperature: 280 ˚C;
- Helium flow: 0.8 mL/min;
- IE: 70 eV.

Table 1. Retention times, selected transitions and collision energy for the identification of antidepressants

Analytes	Retention time (min)	Quantifying transition (m/z)	Qualifying transition (m/z)	Collision energy (eV)
FLX	14.39	261.2 - 219.1	381.6 - 116.1	20 (20)
VLX	16.06	134.1 - 119.1	178.8 - 134.1	10 (20)
DVLX	17.08	191.7 - 177.1	391.0 - 273.3	10 (15)
NFLX	17.50	319.2 - 215.1	319.2 - 86.1	5 (15)
PTP*	21.08	190.4 - 189.1	-	20
CIT	21.47	237.2 - 220.2	237.2 - 208.2	20 (20)
SRT	23.48	346.8 - 189.2	346.8 - 73.2	5 (20)
PXT	24.88	250.1 - 154.2	250.1 - 140.2	20 (15)

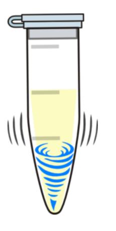
*PTP: Protriptyline - Internal standard

Table 2. MRM response and cross-contribution of quantifying transitions

	FLX	VLX	DVLX	NFLX	PTP*	CIT	SRT	PXT
FLX	100	-	-	0.015	-	0.003	-	-
VLX	-	100	-	-	-	0.002	-	-
DVLX	-	0.225	100	-	-	-	-	-
NFLX	-	0.008	0.052	100	-	-	-	-
PTP*	-	-	-	-	100	-	-	-
CIT	-	-	-	0.001	-	100	-	-
SRT	-	-	-	-	0.059	0.090	100	-
PXT	-	0.003	-	-	-	0.006	-	100

*Internal standard. Underlined antidepressants were injected isolated, and bold antidepressants report the contribution (%) when underlined antidepressants were injected.

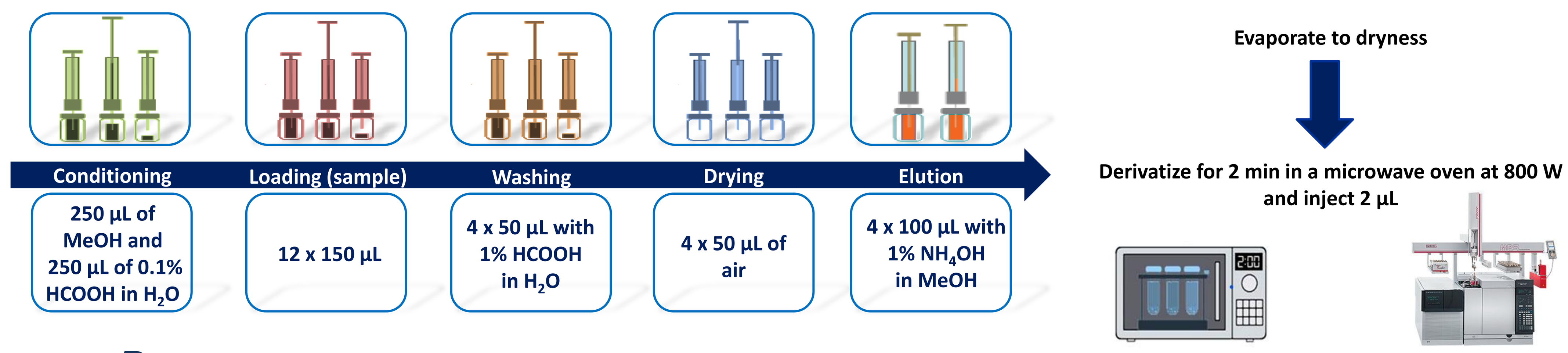
Sample treatment:



Mix 250 µL of oral fluid with 500 µL of acetonitrile; centrifuge, decant and evaporate
+
Dissolve with 1 mL of 25 mM phosphate buffer (pH 2.5); add the internal standard and homogenize

Extraction procedure:

250 µL syringe; M_1 mixed mode cartridge (4 mg; 80% C_8 and 20% SCX) (SGE Analytical Science)



Results

Design of Experiments:

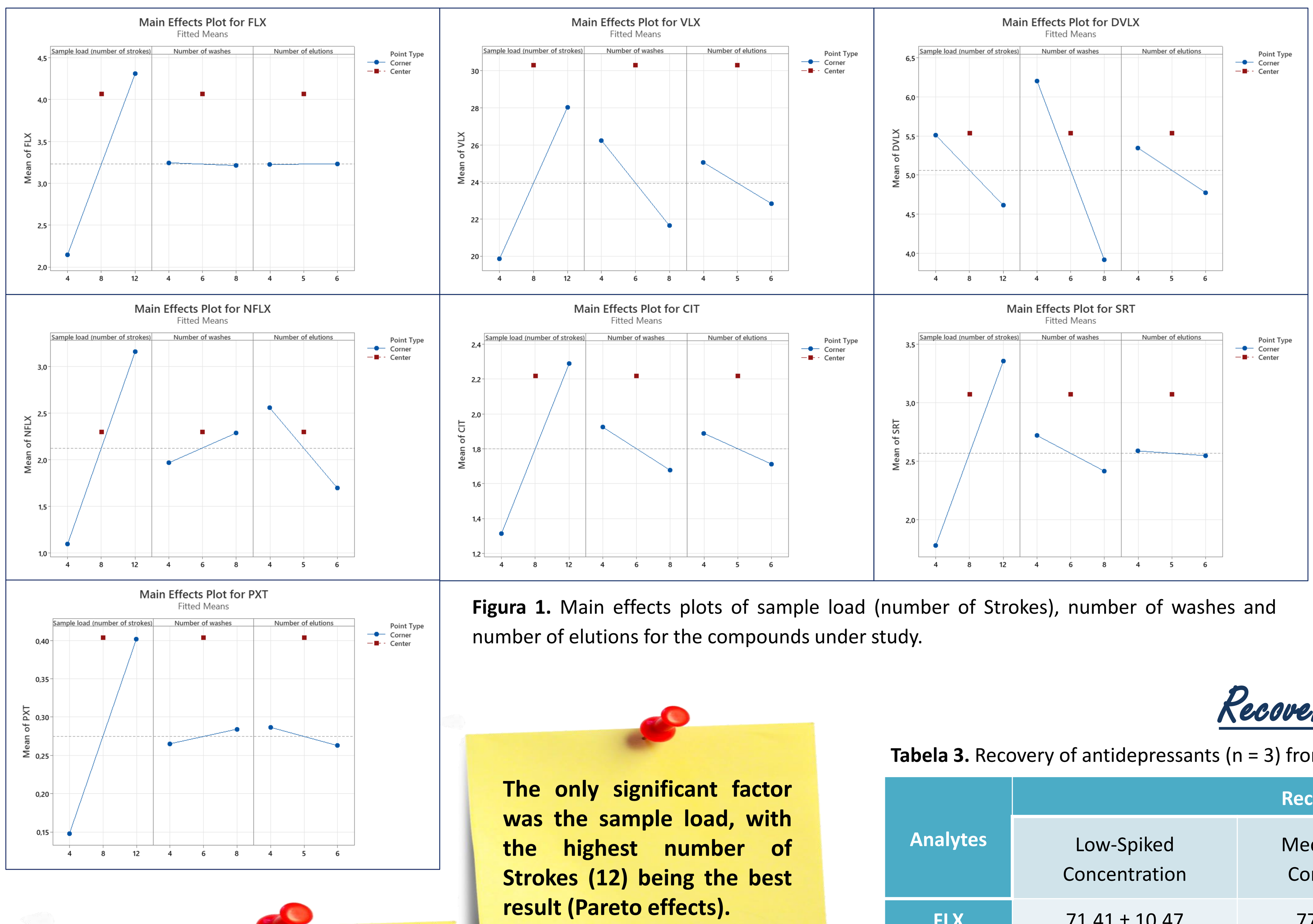


Figura 1. Main effects plots of sample load (number of Strokes), number of washes and number of elutions for the compounds under study.

The only significant factor was the sample load, with the highest number of Strokes (12) being the best result (Pareto effects).

Regarding the other two factors, the response optimizer showed that the best results were the lowest number of washes (4) and the lowest number of elutions (4).

Evaluation of the number of Strokes:

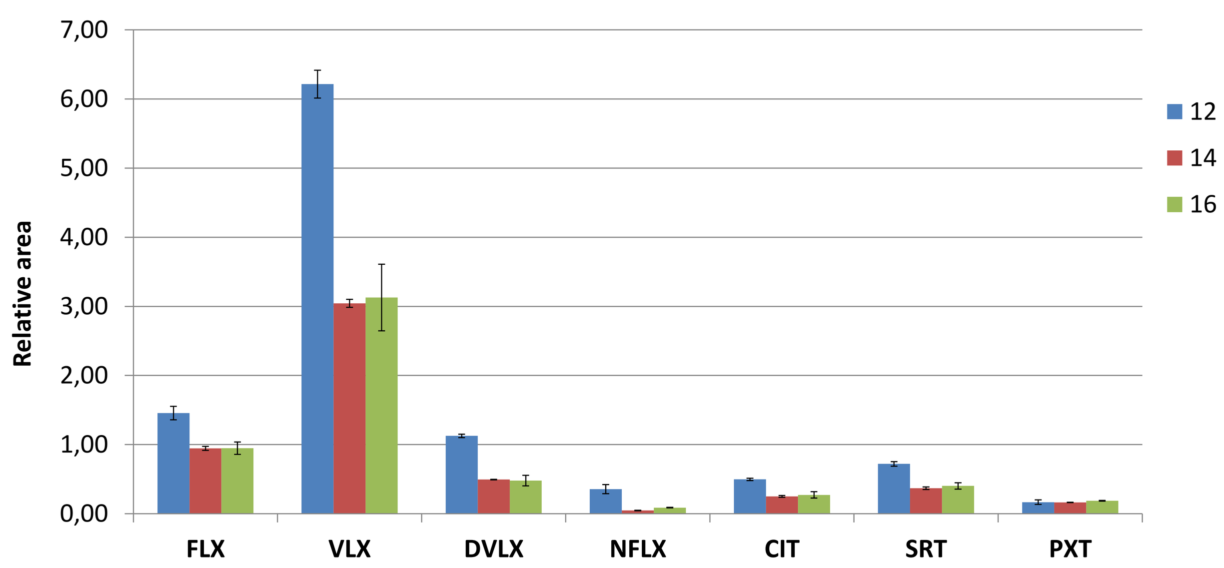


Figura 2. Effect of the different number of strokes to be implemented in the extraction process.

Significant differences were observed between the number of Strokes 12 and the remaining numbers of Strokes for the antidepressants FLX, VLX, DVLX, NFLX, CIT and SRT (Fisher's test).

Recoveries:

Tabela 3. Recovery of antidepressants (n = 3) from the oral fluid samples

Analytes	Recoveries[a] (%)		
	Low-Spiked Concentration	Medium-Spiked Concentration	High-Spiked Concentration
FLX	71.41 ± 10.47	77.67 ± 4.17	83.40 ± 3.87
VLX	69.69 ± 11.82	57.90 ± 4.76	72.79 ± 7.71
DVLX	17.29 ± 3.30	11.93 ± 1.38	15.75 ± 2.12
NFLX	47.41 ± 2.47	42.14 ± 0.58	58.24 ± 7.92
CIT	86.47 ± 13.68	83.49 ± 5.02	92.90 ± 5.99
SRT	82.33 ± 14.02	86.42 ± 8.68	92.39 ± 1.58
PXT	86.89 ± 11.85	52.21 ± 7.34	71.89 ± 10.60

[a]Mean values ± standard deviation

Sensitivity:

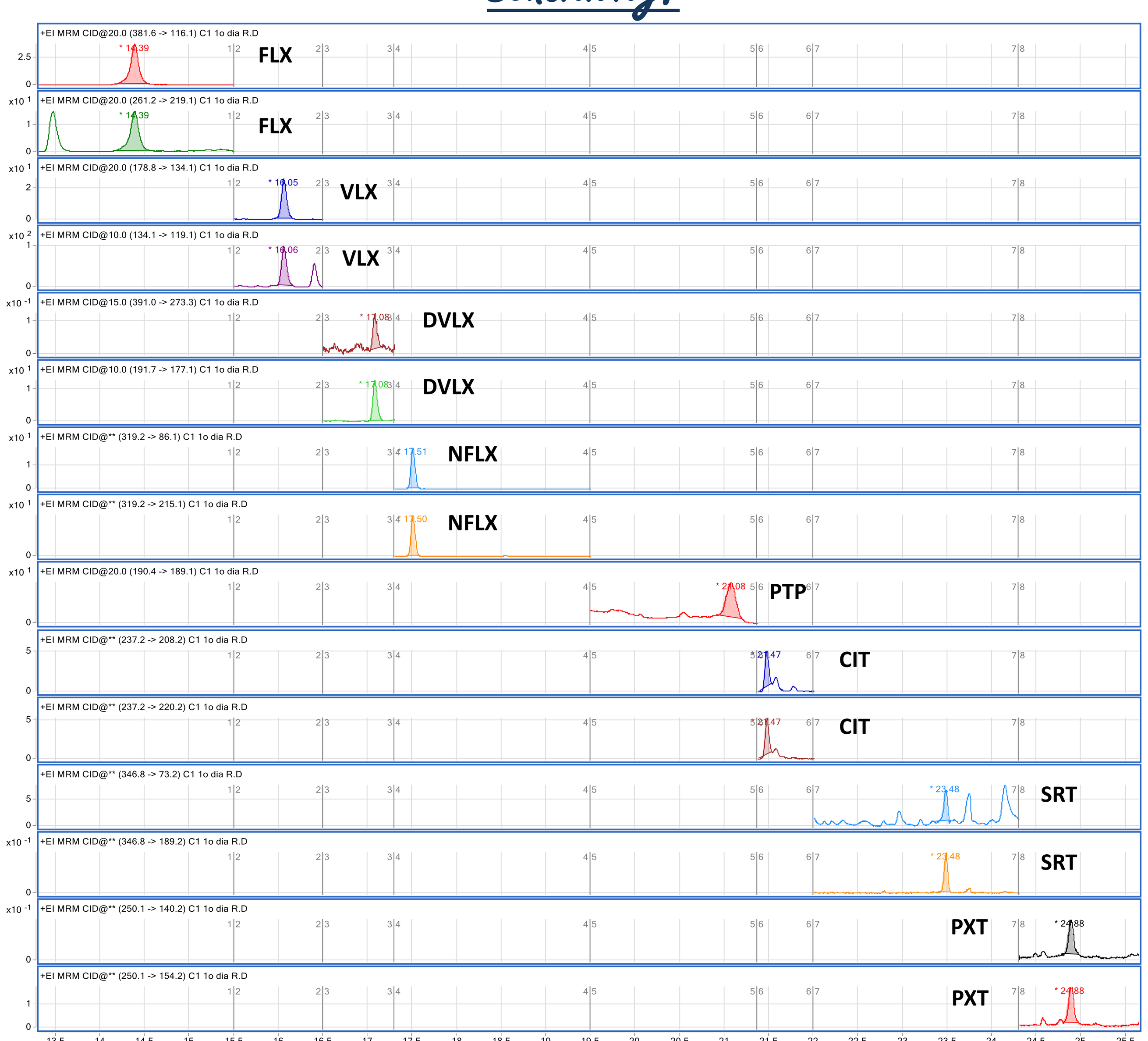


Figura 3. Chromatogram of selected fragments obtained after extraction of the oral fluid samples spiked at the detection limit.

Conclusions

- ✓ This is the first work that uses MEPS as sample preparation technique for the determination of antidepressants and metabolites in oral fluid samples (250 µL).
- ✓ The simple extraction procedure proved to be efficient, requiring only 250 µL of biological sample, making it an excellent alternative for the determination of these compounds in routine clinical and forensic toxicology laboratories and for therapeutic monitoring purposes.

References

1. S. Soares, M. Barroso, E. Gallardo, "A review of current bioanalytical approaches in sample pretreatment techniques for the determination of antidepressants in biological specimens" Reviews in Analytical Chemistry, 40 (2021) 12-32. https://doi.org/10.1515/revac-2021-0124

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