

Fast LC-MS/MS confirmation assay for the determination of 17 drugs of abuse in oral fluid

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INTRODUCTION

Road checks carried out by the police contemplate the collection of a salivary sample, which is analyzed with first level screening methods. These semi-quantitative results must be confirmed with confirmatory techniques, such as liquid chromatography combined with mass spectrometry. The aim of this study was to validate a method for the quantitative analysis of the major substances of abuse on a salivary sample with LC-MS/MS systems.

METHODS

The developed and validated method allows the quantification of **17 drugs of abuse (DOA): cocaine, benzoylecgonine, cocaethylene, THC-Δ9, buprenorphine, 6-monoacetylmorphine, morphine, codeine, methadone, EDDP, amphetamine, methamphetamine, MDMA, MDA, MDE, ketamine, MBDB**. The analytical procedure consists of a dilution of saliva sample with 15 internal standards (drug of abuse labeled with stable isotopes). After the centrifugation, the supernatant is directly injected in LC-MS/MS system. Multiparametric and multi levels calibrators and controls are supplied with the kit to ensure a stable calibration curve in the suitable range of interest for each molecule.

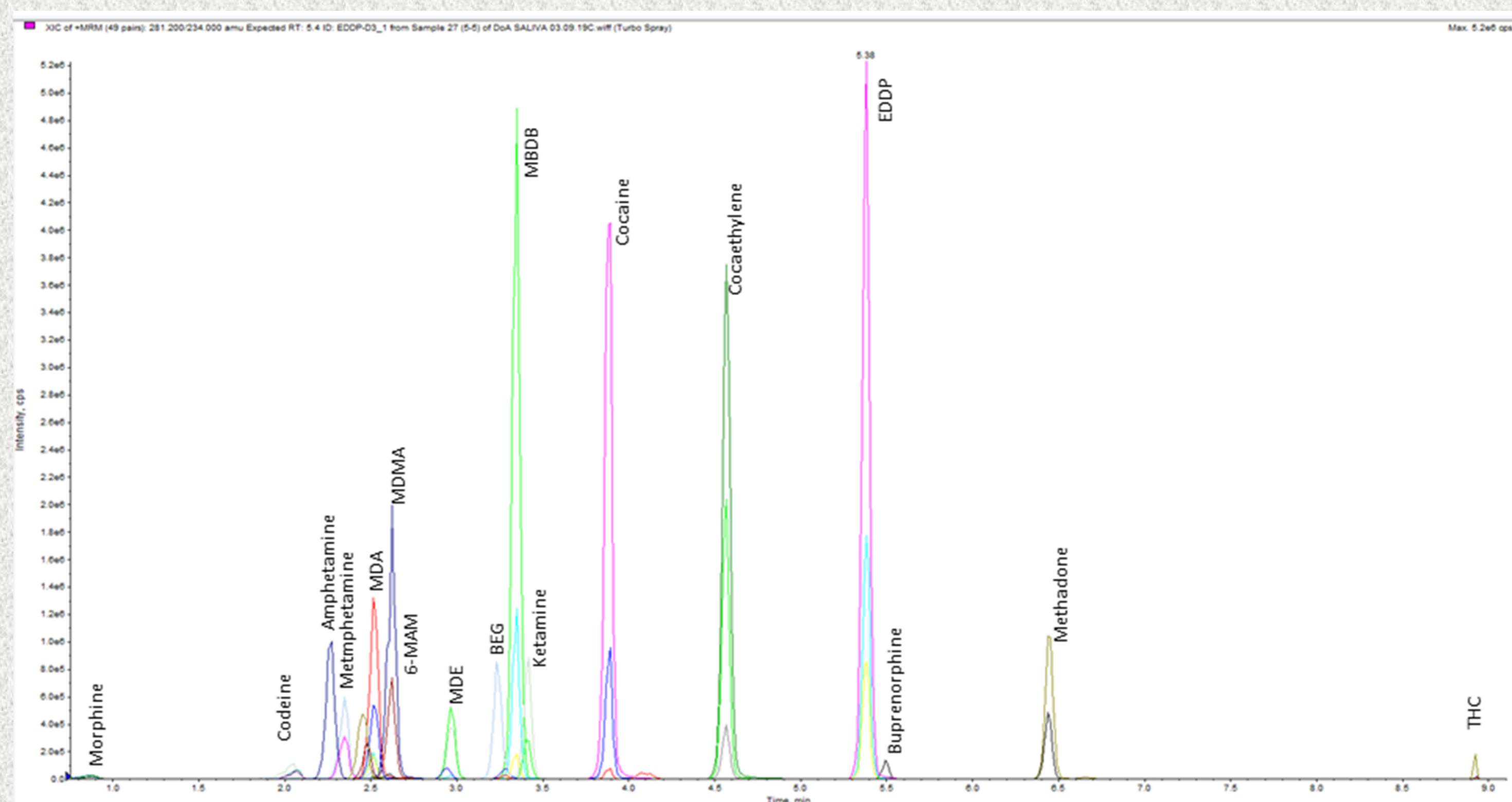
RESULTS

An LC-MS/MS method for determination of 17 different drugs of abuse, in saliva samples, was successfully developed and validated according to FDA guidelines for bioanalytical method validation. The method was considered to be selective: analyzing 6 blank samples there were no unexpected endogenous interferences >20% of LLOQ for all drugs, nor >5% of the IS signals. Recovery from collection devices was comprised between 91 and 110%. No matrix effect was found. The method was linear for all drugs with $R^2 \geq 0.991$ in all cases. Intra-assay (CVs variable from 1.04 to 5.93%) and inter-assay (CVs variable from 3.37 to 10.14%) reproducibility analyses demonstrated accuracy and precision within acceptance criteria. Carry-over and interferences were negligible. Lower limits of quantitation are below the limits defined by international guidelines (European Guidelines for Workplace in Oral Fluid, Version 2.0, by European Workplace Drug Testing Society, 2015) and Italian national laws. Run time is 10 minutes. Samples were tested for stability and resulted stable at least six month at -20°C and one week at +4°C.

CONCLUSIONS

The research use only (RUO) version of this new kit (from Eureka Lab Division) for the determination of drugs of abuse in saliva has been successfully validated according to FDA guidelines in a collaborative study and is now becoming applied to real samples. This kit, thanks to its easy of use and its performances in terms of accuracy and precision could become a reference standard for the laboratories involved in toxicology and confirmation tests for drug of abuse.

Drug of Abuse	calibration curves		CV% intra-assay (n=5)		CV% inter-assay (n=5)		accuracy intra-assay (n=5, bias %)		accuracy inter-assay (n=5, bias %)	
	R ²	ng/mL	L-QC	H-QC	L-QC	H-QC	L-QC	H-QC	L-QC	H-QC
cocaine	0.995	0,5 - 50	3.45	2.25	9.37	7.56	14.29	6.66	14.29	8.78
6 MAM	0.997	0,5 - 50	8.3	2.91	8.14	4.33	8.57	5.01	12.60	6.18
amphetamine	0.994	5 - 250	3.65	2.10	8.25	3.37	5.66	8.91	5.66	8.91
buprenorphine	0.997	0,5 - 50	5.86	5.34	10.06	7.00	5.18	5.38	10.77	4.97
MDA	0.998	5 - 250	5.93	3.44	6.23	3.98	8.73	4.87	9.74	5.13
MDE	0.998	5 - 250	4.17	5.74	5.60	7.67	6.81	2.55	9.69	4.11
MDMA	0.993	5 - 250	3.16	3.49	9.72	9.06	9.06	5.02	11.44	8.14
metadone	0.993	5 - 250	1.71	2.48	4.11	4.31	5.58	3.46	7.01	4.77
metamphetamine	0.994	5 - 250	1.59	2.96	6.52	6.67	8.88	7.53	8.89	9.71
morfine	0.993	5 - 250	5.93	5.15	9.34	8.21	12.68	5.48	12.64	9.62
MBDB	0.993	5 - 250	3.01	1.04	6.71	4.95	9.12	9.63	11.13	11.53
ketamine	0.993	5 - 250	4.50	4.70	5.29	6.51	9.44	9.67	10.11	11.17
THC-Δ9	0.995	0,5 - 50	4.96	4.27	8.41	6.50	7.08	5.88	8.60	8.36
BEG	0.995	0,5 - 50	2.70	4.00	9.15	6.28	13.21	6.10	13.40	8.81
codeine	0.994	5 - 250	1.93	1.32	7.03	6.04	1.94	1.05	8.79	7.32
cocaethylene	0.991	5 - 250	4.80	3.91	10.14	6.34	6.11	2.37	10.76	6.67
EDDP	0.996	5 - 250	2.64	3.35	6.07	8.02	2.43	2.87	9.33	6.31



Chromatograms of 17 DoA in saliva and 15 IS labeled with stable isotopes: high level calibrator C6 showing 50 ng/mL 6 MAM, cocaine, BEG, THC, buprenorphine and 250 ng/mL of morphine, codeine, MDMA, amphetamine, metamphetamine, MDE, MDA, MBDB, ketamine, methadone, EDDP, cocaethylene.