DETERMINATION OF KETAMINE AND NORKETAMINE IN HAIR SAMPLES USING MEPS AS SAMPLE CLEAN-UP

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Introduction

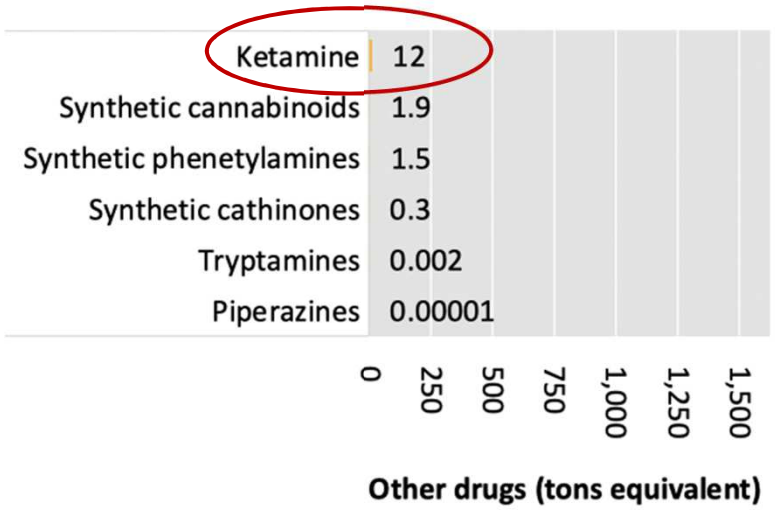

Fig. 1 – Global quantities of seized drugs, by drugs, 2019

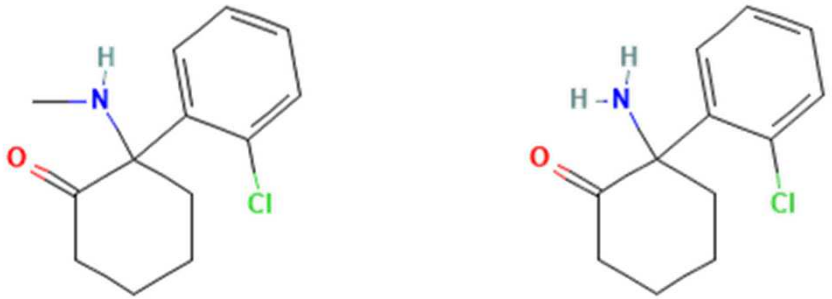

Fig. 2- Chemical structure of Ketamine (K) (left) and Norketamine (NK) (right)

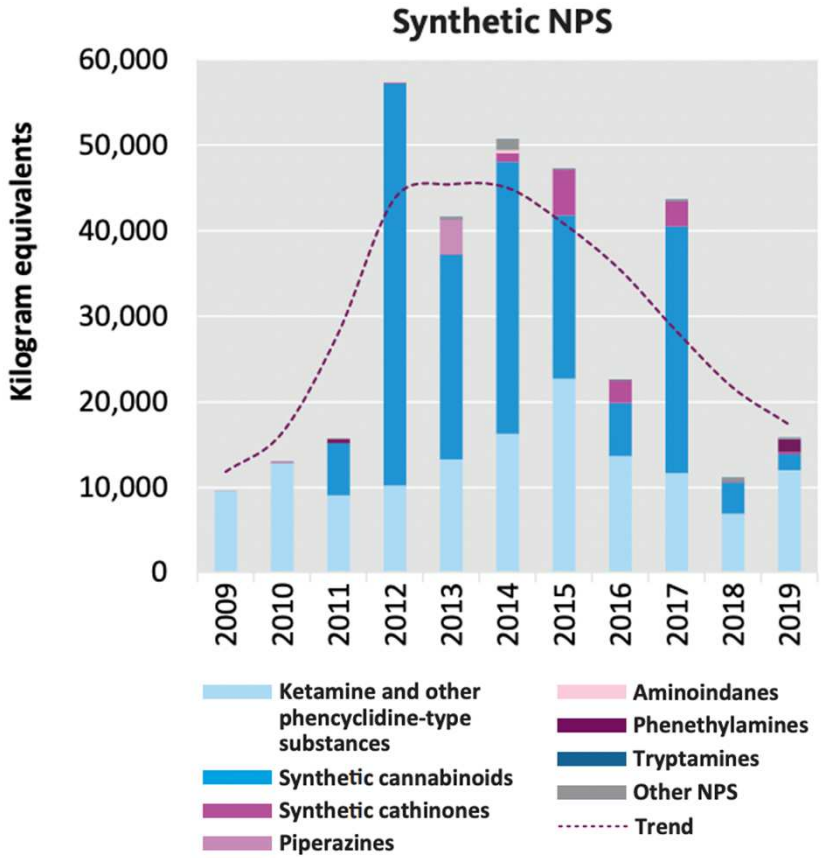

Fig. 3 - Global quantities of new psychoactive substances seized, 2009–2019

- **K is one of the most seized NPS, worldwide from 2009 – 2019;**
- **Mostly in Eastern countries.**

Data available in: United Nations Office on Drugs and Crime, World Drug Report 2021

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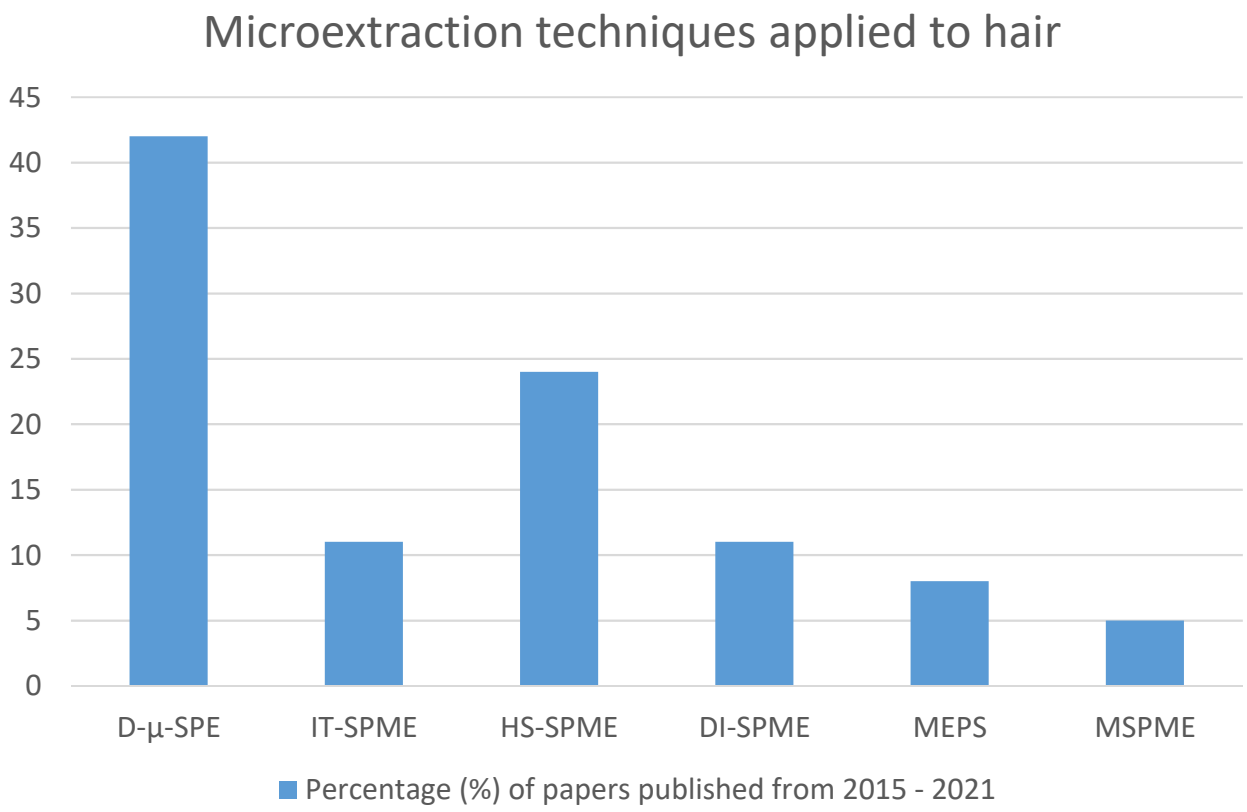
Introduction
Microextraction by packed sorbent vs. SPE

	SPE	MEPS	SPME
Sorbent amount	50 – 2000 mg	2 – 4 mg	thickness 150 µm
Time	15 min	1 min	20 – 40 min
Recovery	good	good	low
Sensitivity	good	good	low
Cartridge re-use	1 extraction	100 extractions	50 extractions

Source: Ciric *et al.*, G. A. (2019) in *Chemia Naissensis*, *1*(1), 93-123.

Introduction – Microextraction by packed sorbent



Source: Rosado T. *et al*. in Analytica Chimica Acta (2021) 1185:338792

Articles with microextractions applied to hair to determine K and/or NK (2004 – 2022)	Cleanup
Gentili *et al*. in Journal of Chromatography B (2004) 801(2):289-96	HS-SPME
Merola *et al.* in Analytical and Bioanalytical Chemistry (2010) 397, 2987–2995	HS-SPME
Meng *et al*. in Forensic Sciences Research (2021) 6(3), 273-280	DI-SPME

- ✓ **Minimization /elimination of toxic organic solvents;**
- ✓ **User-friendly and fast;**

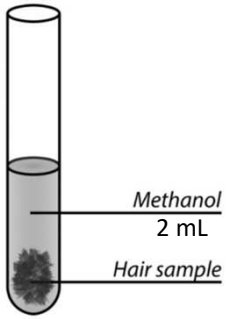
Aims

Optimization and validation of a MEPS clean-up procedure to determine K and its metabolite NK in hair samples by GC-EI-MS/MS

Collection → Sample preparation → Extraction → Cleanup → Analysis

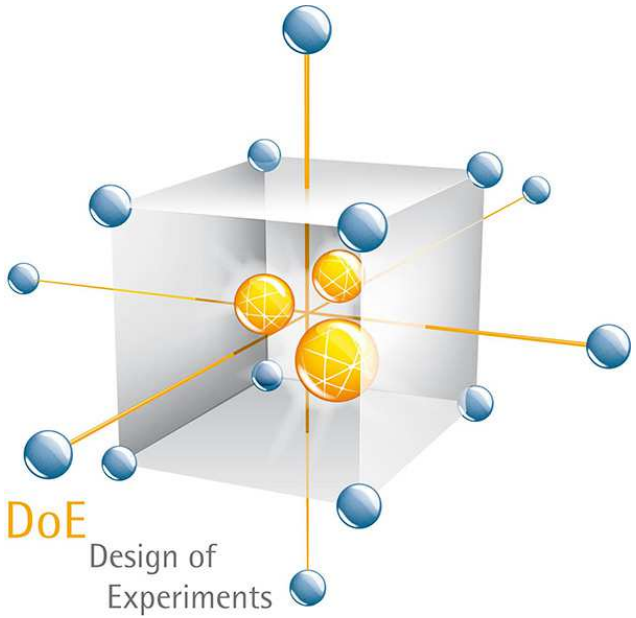
Washing and cutting

50 mg



MEPS

Task 1: Design of Experiments -Optimization of the MEPS procedure



Two-level full factorial design with three factors (2^3) and a central point (n = 3)

Sample draw-eject cycles **(5 to 15 × 150 µL);**
Volume of washing solvents **(1 to 3 × 50 µL);**
Elution cycles **(1 to 3 × 100 µL).**

Blank hair samples spiked at 10 ng/mg

OPTIMIZATION

FINAL CONDITIONS

Sample draw-eject cycles **(10× 150 µL);**
Volume of washing solvents **(1 × 50 µL);**
Elution cycles **(1 × 100 µL).**

Materials and methods

M1 sorbent
(4 mg; 80% C8 and 20% SCX)

50 mg of Hair

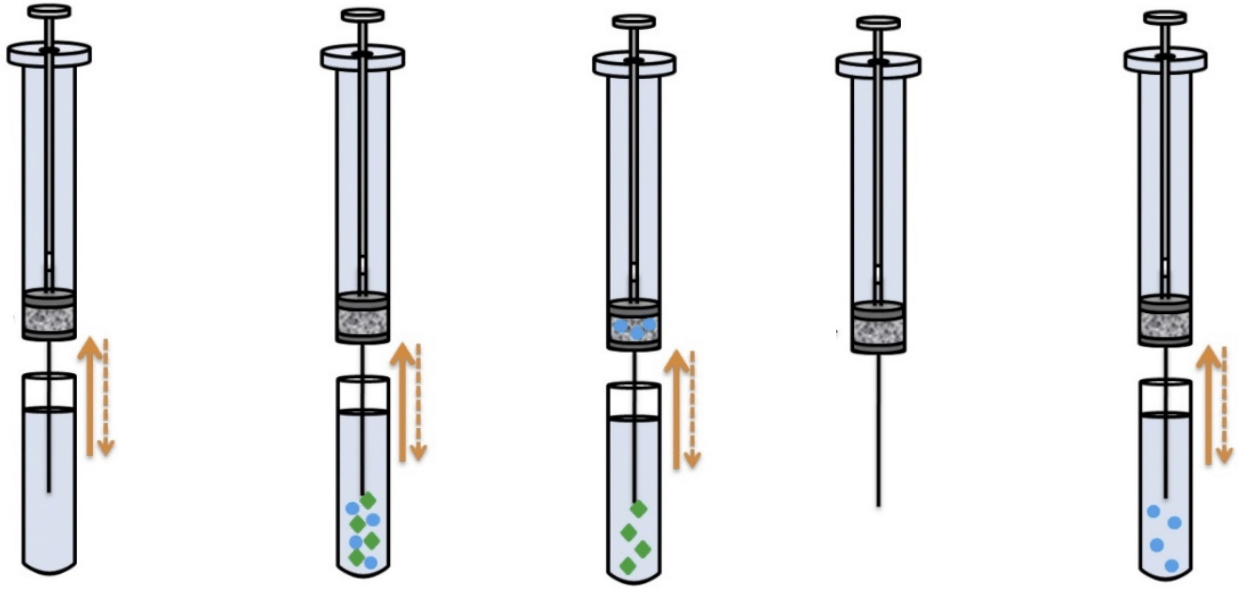
2 mL of Methanol

65ºC; overnight

3500 RPM; 15 min.

Evaporation

500 µL Phosphate buffer



CONDITIONING
Methanol (5 x 250 µL)
H_2O (4 x 250 µL)

SAMPLING
10 x 150 µL

WASHING
Acetic acid
0.1 % (50 µL)
Methanol 10 %
(50 µL)

DRY
1 x 50 µL
air

ELUTION
3 % NH_4OH in
methanol
(1 x 100 µL)

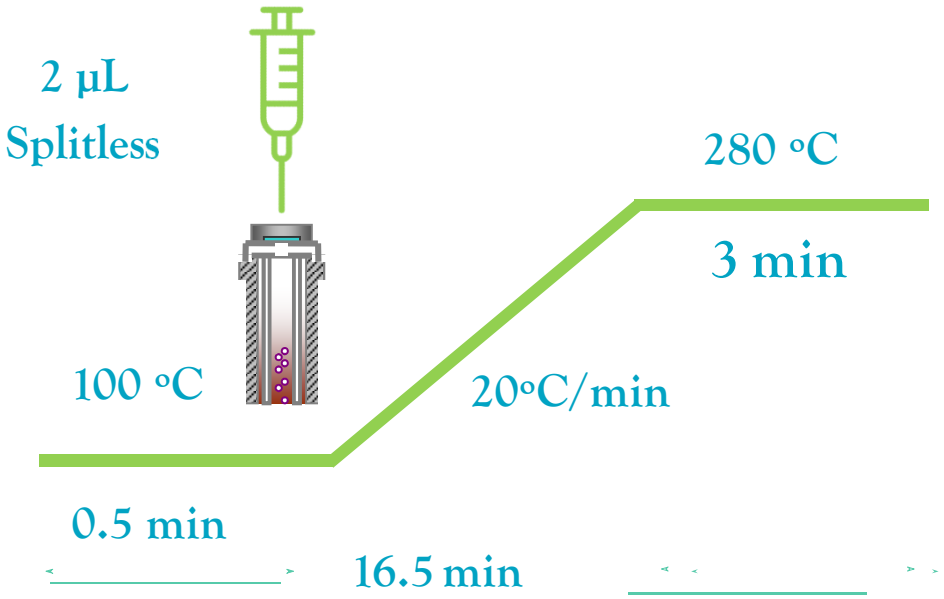
Evaporated to dryness;
Reconstituted in 50 µL methanol

SORBENT RECONSTITUTION
Methanol (5 x 250 µL)
H_2O (4 x 250 µL)

MEPS image adapted from Casado *et al. in* Molecules **2020** 25(3), 702

Materials and methods

Inlet Temperature : 250 °C
Ion Source Temperature: 280 °C
Helium flow: 0.8 mL/min
IE: 70 eV



Compound	RT (min)	Transitions (m/z)	Colision Energy (eV)	Dwell time (µs)
K	8.36	181.0 – 151.0* 181.0 – 116.1	10	50
K-d4	8.35	183.7 – 155.0	5	
NK	8.18	194.4 – 131.0 194.4 – 166.1	10	
NK-d4	8.17	169.8 – 135.2	10	

GC HP 7890A GC coupled to 7000B MS/MS detector

Capillary column: 30 m x 0.25 mm I.D., 0.25-µm film thickness with 5% phenylmethyl siloxane (HP-5 MS m I.D.)

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Task 2: Method Validation

Scientific Working Group for SWGTOX Forensic Toxicology

- ✓ **Selectivity**
- ✓ **Linearity**
- ✓ **LOD and LLOQ**
- ✓ **Intra-day, Inter-day and intermediate precision and accuracy**
- ✓ **Recovery**

ICH harmonisation for better health

SOCIETY OF HAIR TESTING

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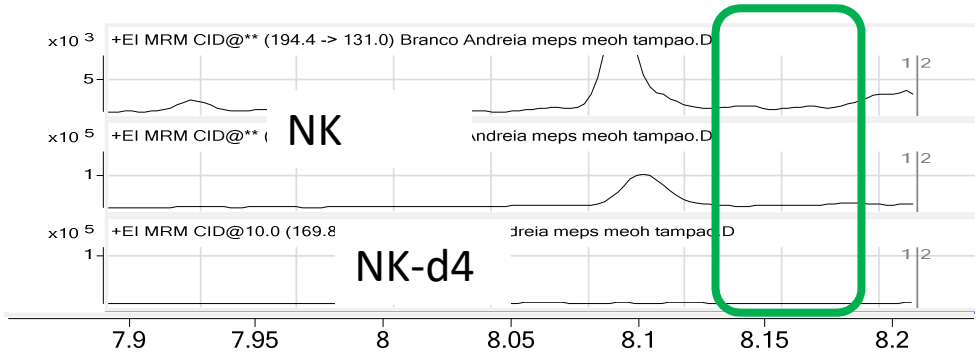
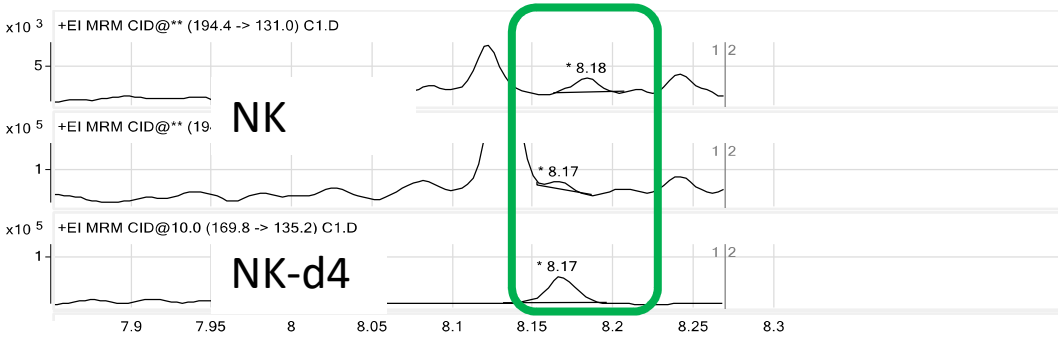
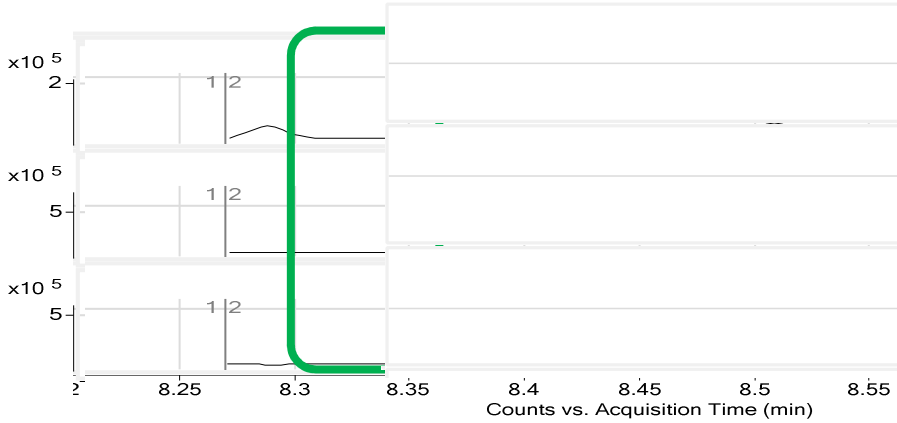
Results: Selectivity

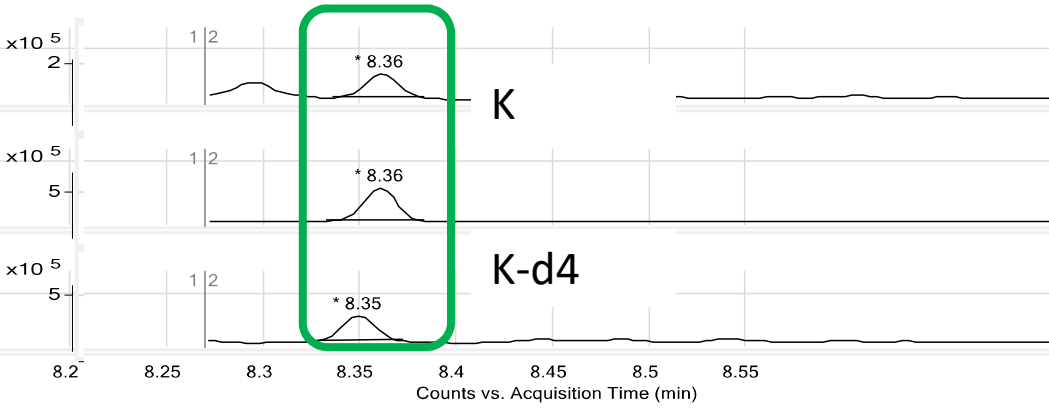
Blank Hair Sample



Hair Sample Spiked at LLOQ







Results: Calibration curves and limits

Linearity data (n = 3)

Analyte	Weight	Linear range (ng/mg)	Linearity*		R^{2*}	LLOQ (ng/mg)	LOD (ng/mg)
			Slope	Intercept			
KET	1/x	0.05 - 10	2.937 ± 0.541	0.354 ± 0.069	0.997 ± 0.002	0.05	0.01
NK			0.677 ± 0.156	-0.018 ± 0.006	1.007 ± 0.001		0.05

* Mean values ± standard deviation

- ✓ LLOQ measured with a relative standard deviation (RSD) ≤ 20% and a relative error (RE) within ± 20% of the nominal concentration;
- ✓ LOD with S/N of at least 3 and retention time within ± 0.2 min of the average retention time of the calibrator (n = 3).

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Results: Precision and Accuracy

Inter-day (n = 3), intra-day (n = 5) and intermediate (n = 12) precision and accuracy

Analyte	Concentration (ng/mg)	Inter-day*		Intra-day*		Intermediate*	
		CV (%)	Bias	CV (%)	Bias	CV (%)	Bias
K	0.05	5.8	110.7 ± 5.2	12.9	98.3 ± 13.1	11.4	101.7 ± 11.2
	0.1	4.9	101.5 ± 4.8				
	0.2	8.7	105.8 ± 8.2	4.4	92.3 ± 4.7	7.4	99.7 ± 7.4
	0.5	2.4	90.1 ± 2.6				
	1	3.8	90.1 ± 4.2	4.1	87.58 ± 4.6	6.0	90.0 ± 6.6
	5	0.9	102.8 ± 0.9				
	10	3.7	106.7 ± 3.5	4.7	110.2 ± 4.2	9.0	107.9 ± 8.2
Analyte	Concentration (ng/mg)	Inter-day*		Intra-day*		Intermediate*	
		CV (%)	Bias	CV (%)	Bias	CV (%)	Bias
NK	0.05	10.0	91.8 ± 10.8	4.4	91.7 ± 4.8	10.4	99.8 ± 10.3
	0.1	8.6	102.2 ± 8.4				
	0.2	2.9	107.8 ± 2.7	9.6	106.1 ± 9.0	11.9	103.3 ± 11.4
	0.5	4.9	94.51 ± 5.2				
	1	7.5	104.6 ± 7.2	7.1	109.8 ± 6.4	8.4	106.0 ± 7.9
	5	1.4	99.9 ± 1.4				
	10	2.2	98.1 ± 2.3	11.6	100.1 ± 11.6	8.0	98.9 ± 8.0

CVs lower than 13 %

Accuracy from 88 to 110 %

ues ± standard deviation

Results: Recovery

Recovery (%)* (n = 3)				
Analyte	Concentration (ng/mg)			
	0.05	0.2	1	10
K	39.06 ± 5.31	46.98 ± 1.19	51.47 ± 4.14	60.63 ± 3.33
NK	42.8 ± 1.92	38.09 ± 2.55	32.19 ± 4.34	43.32 ± 5.00

* Mean values ± standard deviation

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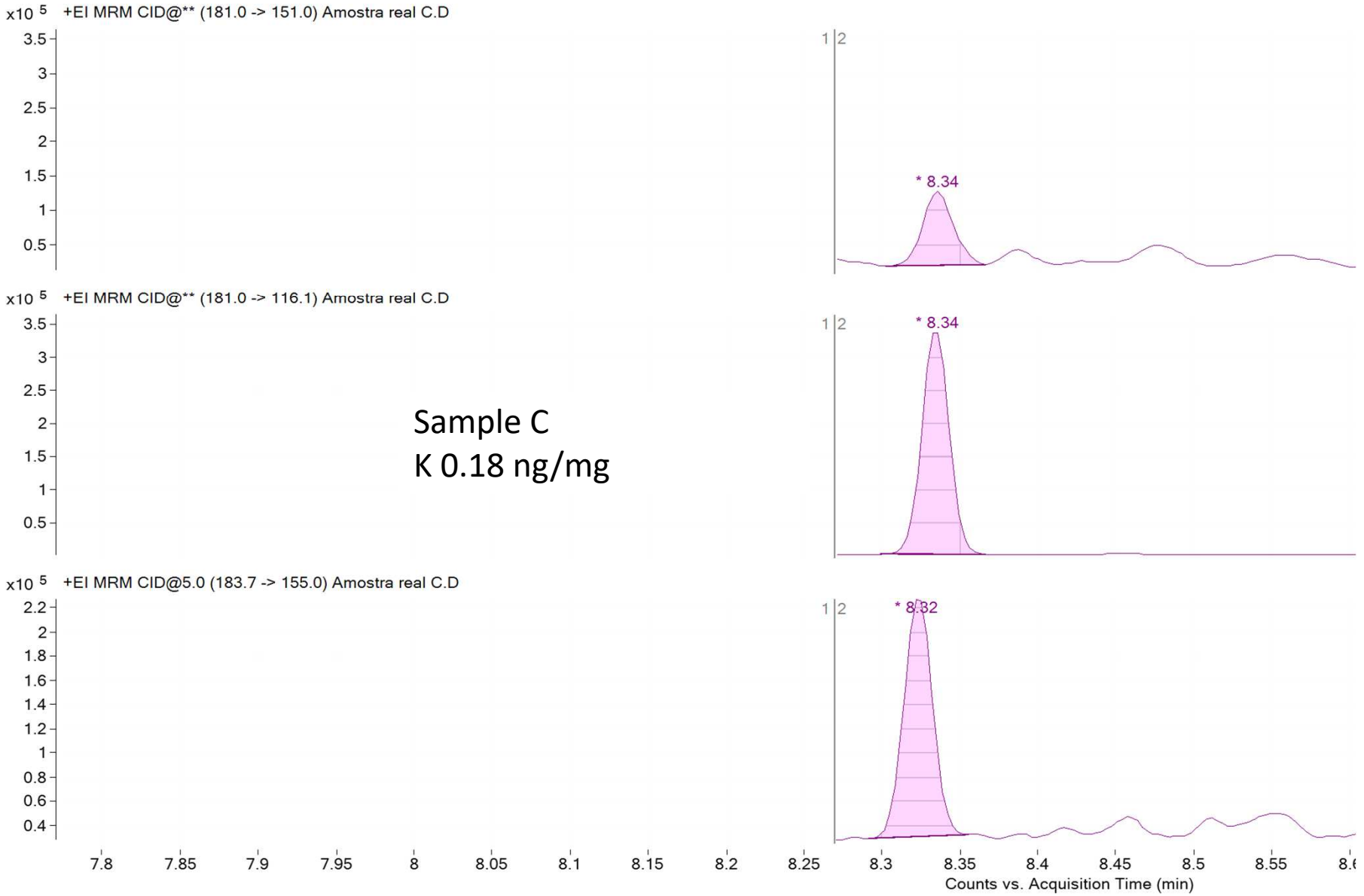
Method applicability

Concentration (ng/mg)				
This work	**Sample A**	**Sample B**	**Sample C**	**Sample D**
K	**< LOD**	**< LLOQ**	**0.178**	**0.098**
NK	**< LOD**	**< LOD**	**< LOD**	**< LOD**
SPE (*Barreto et. al 2016*)				
K	**< LOD**	**< LLOQ**	**0.186**	**0.082**
NK	**< LOD**	**< LOD**	**< LOD**	**< LOD**

Concentration (ng/mg)				
	Barreto et al. 2016 (single dose)	*Salomone et al. 2015 (review of case reports)*	*Salomone et al. 2015 (review of case reports)*	*Favretto et al. 2013*
K	0.06-0.11	0.46-28.15	0.11-11.4	1.6
NK	<LLOQ (0.04)	0.02-5.28	0.02-0.71	0.2

Method applicability



Sample C
K 0.18 ng/mg

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Method comparison with other miniaturized techniques (2004 – 2022)

Analyte	Hair (mg)	Extraction	Cleanup	Linear range (ng/mg)	LOQ and LOD (ng/mg)	Recovery (%)	Detection mode	Reference
K	20 mg	Acid hydrolysis (200 µL HCl, 1M)	HS-SPME	1.77 - 20	1.77; 0.59	97.00	GC-(EI)-MS	Gentili *et al.* in Journal of Chromatography B (2004) 801(2):289-96
K	10 mg	Acid hydrolysis (200 µL HCl, 1 M)	HS-SPME; Derivatization (MSTFA)	0.18 – 5	0.18; 0.06	13.9	GC-(EI)-MS	Merola *et al.* in Analytical and Bioanalytical Chemistry (2010) 397, 2987–2995
K, NK	20 mg	High-speed griding (oscillation with 70 Hz for 2 min at 4 ºC)	DI-SPME	0.1 – 10	K and NK 0.1; 0.067	93.3 – 95.2 (K); 90.1 – 95.8 (NK)	LC-(ESI)-MS/MS	Meng *et al.* in Forensic Sciences Research (2021) 6(3), 273-280
K and NK	50 mg	Methanolic extraction (2 mL; overnight 65 ºC)	MEPS (M1 cartridge)	0.05 - 10	K 0.05; 0.01 NK 0.05; 0.05	39.1 – 60.6 (K); 32.2 – 43.3 (NK)	GC-(EI)-MS/MS	This work

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Conclusions

- ✓ Successful **optimization** and **full validation** of the analytical method to determine K and NK in hair samples using **MEPS** as clean-up procedure and **GC-EI-MS/MS**;
- ✓ Method linear between **0.05 – 10 ng/mg** for both **K and NK**;
- ✓ Satisfactory and acceptable precision and accuracy;
- ✓ The combination of this microextraction technique for clean-up with tandem mass spectrometry proved to be a **simple and rapid procedure**;
- ✓ **Sensitive**, **selective**, **precise** and **accurate** method, that can be applied to routine analysis.