FloMass Drugs of Abuse in Hairs

Reagents for 100 assays

Instruction Manual

REF **EUM20100**

IVD **For *in vitro* diagnostic use**

CE

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Document Version: 1
Date of revision: 8.01.2024
File name: M-EUM20100 (eng)

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1 INTRODUCTION

1.1 IVD SYMBOLS

	In vitro diagnostic medical device / Dispositif médical de diagnostique en vitro/In-Vitro-Diagnostikum / Producto sanitario para diagnóstico in vitro / Dispositivo medico-diagnostico in vitro / Dispositivo médico para in til in vitro diagnostik
	Batch code / Code du lot / Chargenbezeichnung / Código de lote / Codice del lotto / Código do lote / Número do lote / Lotnummer
	Packing number / Numéro d'emballage / Packnummer / Número de envase / Numero confezioni / Número de embalagem / Número de embalagem / Emballagenummer
	Catalog number / Référence du catalogue / Bestellnummer / Número de catálogo / Numero di catalogo / Referência de catálogo / Código / Katalognummer
	Use by / Utiliser jusqu'au / Verwendbar bis / Fecha de caducidad / Utilizzare entro / Prazo de validade / Data limite de utilização / Holdbar til
	Temperature limitation / Limites de température / Temperaturbegrenzung / Limite de temperatura / Limiti di temperatura / Limites de temperatura / Limite de temperatura / Temperaturbegrænsning
	Add liquid / Ajout de liquide / Flüssigkeit zugeben / Añadir líquido / Aggiungi liquido / Adicionar líquido / Adicionar líquido / Tilføj væske
	Store in the dark / Conserver à l'abri de la lumière / Dunkel aufbewahren / Almacenar en ambiente oscuro / Conservare al buio / Armazenar no escuro / Guardar longe da luz / Opbevares mørkt
	Contains sufficient for <n> tests / Contenu suffisant pour "n" tests / Inhalt ausreichend für <n> Prüfungen / Contenido suficiente para <n> ensayos /Contenuto sufficiente per "n" saggi / Conteúdo suficiente para "n" ensaios / Conteúdo suficiente para <n> testes / Indeholder tilstrækkeligt til "n" test
	Consult instructions for use / Consulter les instructions d'utilisation / Gebrauchsanweisung beachten / Consulte las instrucciones de uso / Consultare le istruzioni per l'uso / Consulte as instruções de utilização / Consultar Instruções de uso / Se brugsanvisning
	Manufacturer / Fabricant / Hersteller / Fabricante / Fabbricante / Fabricante / Fabricado por / Producent
	This way up / Haut / Diese Seite oben / Este lado arriba / Questo lato in alto / Este lado para cima / Este lado para cima / Denne side op
	Recyclable / Recyclable / Recyclebar / Reciclable / Riciclabile / Reciclável / Reciclável / Genanvendeligt
	Brittle / Fragile / Zerbrechilich / Fragile / Fragil / Skrøbelig

1.2 ABBREVIATIONS

11-OH-THC: 11-Hydroxy-Tetrahydrocannabinol
4-ANPP: 4-Aminophenyl-1-phenethylpiperidine
6-MAM: 6-MonoAcetylMorphine
BEG: Benzoylecgonine
CAD: Collision Gas Pressure
CE: Collision energy
CLSI: Clinical and Laboratory Standards Institute
CUR: Curtain Gas
CV: Coefficient of Variation
CXP: Collision Exit Potential
DP: Desolvation Potential
EDDP: 2-Ethyliden-1,5-Dimethyl-3,3-Diiphenylpyrrolidine
EME: Ecgonine Methyl Esther
EP: Entrance Potential
ESI: Electrospray Ionization
GS1: Gas 1
GS2: Gas 2
HPLC-MS/MS: High Performance Liquid chromatography–tandem mass spectrometry
IS: Ion Spray Voltage
LLOD: Lower Limit of Detection
LLOQ: Lower Limit di Quantification
M/Z: Mass/Charge ratio
MBDB: 3,4-metilendiossi-N-metil-a-etilfeniletilammina
MDA: 3,4-methylenedioxy-N-amphetamine
MDE: 3,4-methylenedioxy-N-ethyl amphetamine
MDMA: 3,4-methylendioxy methamphetamine
MPA: Mobile Phase A
MPB: Mobile Phase B
MPB: Mobile Phase C
MRM: Multiple Reactions Monitoring
PP: Polypropylene
Q1: Quadrupole 1
Q3: Quadrupole 3
RT: Retention Time
S/N: Signal/Noise ratio
TEM: Source temperature
THC: Tetrahydrocannabinol

1.3 CLINICAL APPLICATION

FloMass Drugs of Abuse in Hairs is an in vitro diagnostic kit intended for the quantitative and simultaneous determination of drugs of abuse in human hair samples (Table 1) using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).

ANALYTE
Amphetamine
Metamphetamine
MDA
MDE
MDMA
MBDB
Cocaine
BEG
EME
Coca ethylene
Morphine
Codeine
Dihydrocodeine
6-MAM
THC
11-OH-THC
Methadone
EDDP
Buprenorphine
Norbuprenorphine
Ketamine

Table 1: Analytes measured by FloMass Drugs of Abuse in Urine

The terms “psychoactive substance” or “drug” identify any substance able to change the mood, perception of reality or behavior of the person who assume the substance. There are several ways to assume drugs (orally, intravenously, inhalation) and it’s possible to detect substances in many tissues and biological fluids. Rapid screening tests with immunochromatographic and immunochemical methods are used for the determination of drug of abuse assumption. They are based on antigen-antibody interaction. They are neither specific nor sensitive and they are able only to identify family drug. These tests can suffer from interferences that can lead to a false positive test result.

The analysis in hairs can also provide information on the intake of these substances over time as they are deposited within the keratin matrix and are very stable in it. High performance liquid chromatography method coupled with tandem mass spectrometry (HPLC-MS/MS) present

significant advantages compared to with immunometric techniques as greater specificity, lower limits of detection and the possibility of giving results in terms of quality and quantity [1-7].

2 PRINCIPLE OF THE METHOD

The kit is intended for the quantitative and simultaneous determination of drugs of abuse using high performance liquid chromatography technique coupled with tandem mass spectrometry (HPLC-MS/MS).
The preparation of the sample involves a first phase of washing hairs, followed by a shredding performed by hand or with a ball mill and finally the extraction of the analyte from the hair matrix. During extraction Internal Standard marked with stable isotopes are added to treated urine (Table 2). Finally, sample is diluted and analyzed by HPLC-MS/MS technique.

ANALYTE	INTERNAL STANDARD
Amphetamine	Amphetamine $^{2}H_{11}$
Methamphetamine	Methamphetamine $^{2}H_{5}$
MDA	MDA $^{2}H_{5}$
MDE	MDE $^{2}H_{5}$
MDMA	MDMA $^{2}H_{5}$
MBDB	MBDB $^{2}H_{5}$
Cocaine	Cocaine $^{2}H_{3}$
BEG	BEG $^{2}H_{3}$
EME	EME $^{2}H_{3}$
Coca ethylene	Coca ethylene $^{2}H_{3}$
Morphine	Morphine $^{2}H_{3}$
Codeine	Codeine $^{2}H_{6}$
Dihydrocodeine	Dihydrocodeine $^{2}H_{6}$
6-MAM	6-MAM $^{2}H_{3}$
THC	THC $^{2}H_{3}$
11-OH-THC	11-OH-THC $^{2}H_{3}$
Methadone	Methadone $^{2}H_{3}$
EDDP	EDDP $^{2}H_{3}$
Buprenorphine	Buprenorphine $^{2}H_{4}$
Norbuprenorphine	Norbuprenorphine $^{2}H_{3}$
Ketamine	Ketamine $^{2}H_{4}$

Table 2: Analytes measured by kit EUM20100 and related internal standards

Once extracted, analytes are chromatographically separated by a specific reverse phase column. Subsequently, they enter in ESI source where they are transferred to the gas phase and ionized.

Then ions enter in the triple quadrupole mass spectrometer, where they are measured in MRM mode.
Thus, only selected ions with defined mass/charge ratio (m/z) are isolated in the first quadrupole and subsequently transferred into the collision cell where they are fragmented by impact with an inert gas (nitrogen or argon). Among the fragments, only those with defined m/z ratio are isolated in the third quadrupole for subsequent detection.
Measurement in MRM mode with HPLC separation ensure high selective and sensitive analyte identification and quantification [3-9].

3 COMPONENTS AND ACCESSORIES

3.1 KITS CONTENTS

Components for sample preparation included in the kit are shown in Table 3.

CATALOG NUMBER	DESCRIPTION	QUANTITY	STORAGE
EUM02011	Mobile Phase A	600 mL	Room temperature *
EUM02012	Mobile Phase B	500 mL	Room temperature
EUM02013	Mobile Phase C	500 mL	Room temperature
EUM20021	Wash Solution	440 mL	Room temperature
EUM20022	Reconstituting Solution	440 mL	Room temperature
EUM20023	Extraction Solution	45 mL	Room temperature
EUM20024	Diluting solution	20 mL	Room temperature
EUM20031	Internal Standard Mix	4.5mL	-20°C

Table 3: Components, description, quantity and storage of kit EUM20100

*After opening store MPA at 2-8°C.

The kit consists of reagents for 100 assays.
The expiry date of the intact kit is shown on external product label. Follow storage conditions given on the product label of each component of the kit and keep it away from light and/or heat.

3.2 KIT SUPPORT ACCESSORIES

CATALOG NUMBER	DESCRIPTION	QUANTITY	STORAGE
EUM20041	6-Levels Calibrators	3 x 6 x 0.4 mL	-20°C
EUM20051	2-Levels Control	3 x 2 x 0.4 mL	-20°C
EUM00C02	Chromatographic Column	1 pcs	Room Temperature
EUM00A04	Precolumn	4 pcs	Room Temperature
EUM00A05	Holder + precolumn	1 pc	Room Temperature

Table 4: Accessories, description, quantity and storage of kit EUM20100

3.3 CONTROLS AND CALIBRATION OF THE ANALYTICAL SYSTEM

Calibration should be done using 6-Levels Calibrators (EUM20041) containing the analytes. Calibrators should follow patient samples preparation starting at step 8 of the procedure (Chapter 7). A new calibration series should be prepared for each analytical run.

BSN supplies quality control sets at two different concentration levels (EUM20051). Controls from extracted hairs are useful to verify the accuracy and precision of analytical procedures and to determine the analysis in the matrix. Controls must be prepared following the sample preparation from step 8 (Chapter 7).

For analytes concentrations, stability and accessories preparation, refer to package leaflets.

3.4 CHROMATOGRAPHIC SYSTEM

The kit has been validated using analytical column (EUM00C02) coupled to the precolumn (EUM00A04) and its holder (EUM00A05).

Stress tests on column showed that it is possible to carry out approximately 200-250 analysis in matrix with a single precolumn. It is recommended to perform some blank injections before each analytical run and verify the backpressure values.

Procedure involves the use of 3 mobile phases (A, B and C) and therefore, beside the binary pump, an additional isocratic pump and a 6-port switching valve are needed (see Figure 1).

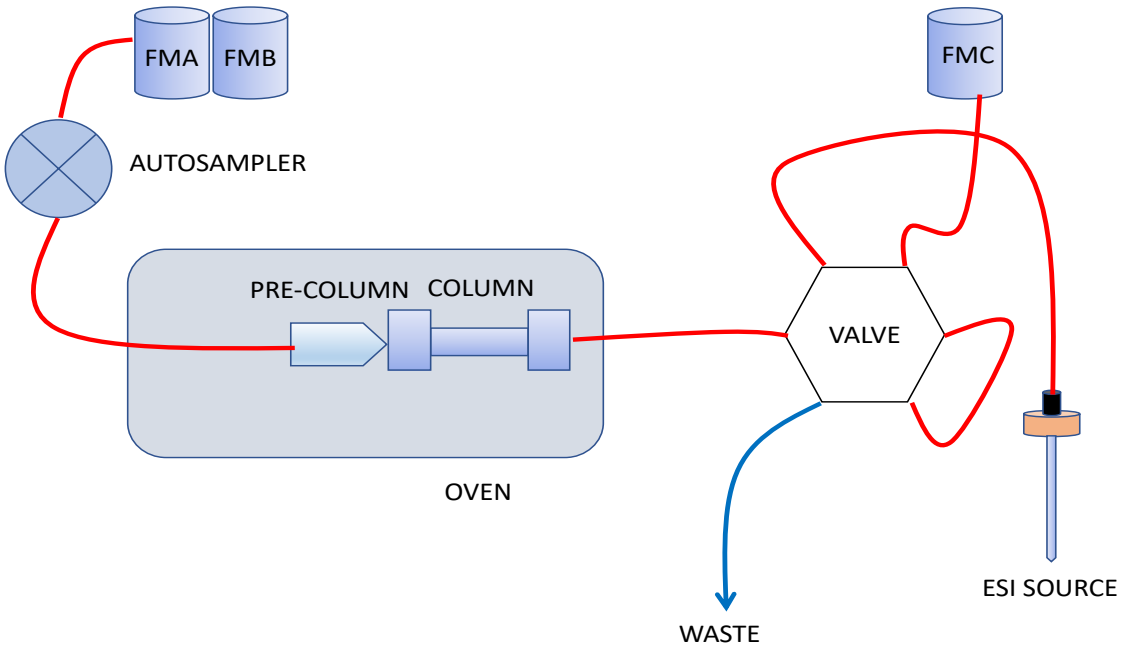

Figure 1: Plumbing configuration

4 REQUIRED INSTRUMENTS

The method requires a HPLC system with tandem mass spectrometer and dedicated software. Triple quadrupole mass spectrometer should be medium or medium-high level. (A high-level instrument is required for Norbuprenorphine).

4.1 REQUIRED HPLC MODULES

1. Binary pump able to support a backpressure of 400 bar or more
2. Additional pump
3. 6-port switching valve
4. Autosampler with cooling function (10°C)
5. Column Heater (40°C)
6. Degasser to module 1 and 2

4.2 REQUIRED EQUIPMENT AND MATERIALS FOR SAMPLE PREPARATION

1. Centrifuge (10000-13000 rpm) for 1.5- or 2-mL vials
2. Vortex for vials
3. Pipettes and tips
4. 1.5- or 2-mL PP vials
5. Autosampler vials with plastic adapter for 200 µL
6. Thermoblock at 45°C
7. Ball mill, 7-mL tubes and balls for ball mill
8. Nitrogen evaporator
9. Thermostated ultrasonic batth
10. Chemical hood

5 HPLC-MS/MS SYSTEM CONDITIONS

Ionization: ESI positive mode
MS/MS: specific MRM
Injection volume: 15 µL (variable according to instrumental sensitivity)
Running time: 12 min
Column heater: 40°C

Cromatographic gradient

TIME (min)	%MPA	%MPB	MS Valve	MPC Flow (mL/min)	Total Flow (mL/min)
0.00	95	5	MS	0.05	0.30
0.30			Waste	0.30	0.30
1.00	95	5			0.30
2.10			MS	0.30	0.30
2.15				0.05	0.30
8.00	2	98			0.30
9.50	2	98			0.30
9.60	100	0			0.30
9.65					0.30
9.70					0.40
11.95	95	5			0.40
12.00					Stop

Table 5: Chromatographic gradient of kit EUM20100

Column conditioning: column should be conditioned for 5 min at the chromatographic condition initial. Then run 3 blank samples (MPA only) using the gradient as above.

Backpressure: at a flow rate of 0.3 mL/min, chromatographic system backpressure should not exceed 450 bar.

Column storage: in order to preserve the column once detached from instrument, it is necessary to leave it in the initial conditions of the chromatographic gradient and insert it in the suitable package closing firmly with caps.

Example of chromatogram

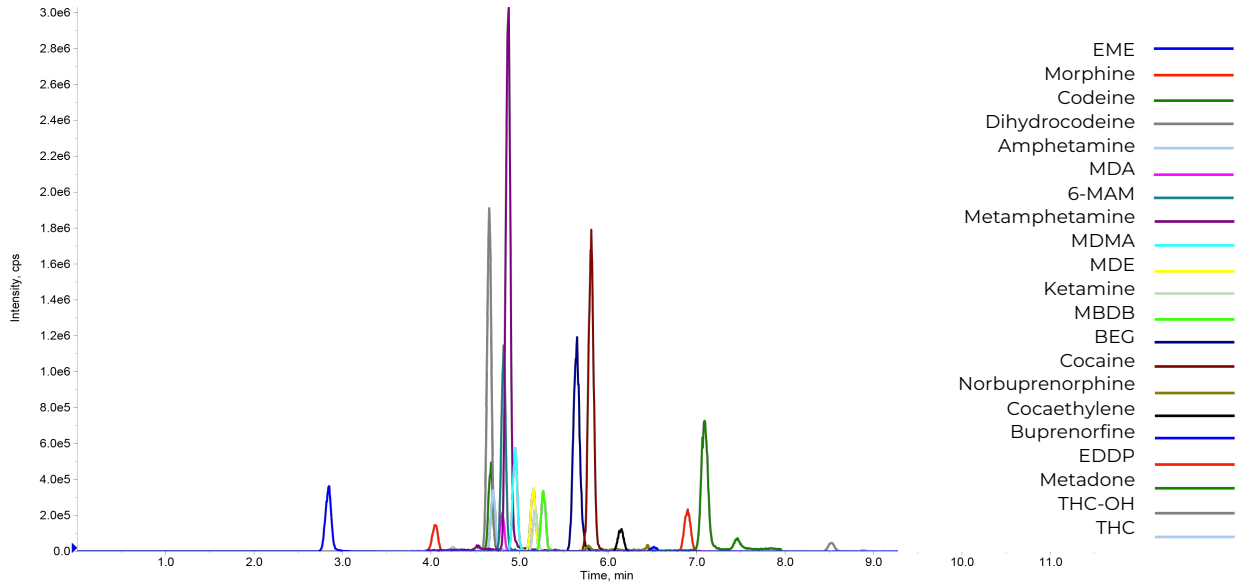

Figure 2: Example of chromatogram identified using kit EUM20100

6 SOURCE PARAMETERS AND TRANSITIONS

6.1 SOURCE PARAMETERS

Source parameters used in MS the Method of EUM20100 with a Sciex series X500 QTrap mass spectrometer are shown below.

Curtain Gas (CUR): 30 psi
Collision Gas Pressure (CAD): Medium
Ion Spray Voltage (IS): 5000 V (MRM+)
Temperature (TEM): 500°C
Gas 1 (GS1): 55 psi
Gas 2 (GS2): 60 psi

6.2 TRANSITIONS

Monitored mass transitions and the MS parameters for each analyte using HPLC Shimadzu Nexera combined with the Sciex series X500 QTrap mass spectrometer are shown in Table 6. ESI positive mode.

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
Amphetamine 1	4.5	136.1	91.1	45	10	23	10
Amphetamine 2	4.5	136.1	119.1	45	10	11	12
Amphetamine IS	4.5	147.1	130.0	45	10	11	12
Methamphetamine 1	4.7	150.2	91.1	30	10	26	10
Methamphetamine 2	4.7	150.2	119.1	30	10	14	12
Methamphetamine IS	4.7	155.2	92.0	30	10	26	10
MDA 1	4.6	180.1	133.1	30	10	22	12
MDA 2	4.6	180.1	163.1	30	10	25	12
MDA IS	4.6	185.1	138.1	30	10	22	12
MDE 1	5.0	208.2	163.1	30	10	25	12
MDE 2	5.0	208.2	105.0	30	10	30	12
MDE IS	5.0	213.2	163.1	30	10	25	12
MDMA 1	4.8	194.1	163.1	30	10	16	14
MDMA 2	4.8	194.1	105.1	30	10	32	12
MDMA IS	4.8	199.1	165.1	30	10	16	14
MBDB 1	5.1	208.2	177.1	45	10	15	8
MBDB 2	5.1	208.2	135.2	45	10	25	8
MBDB IS	5.1	213.2	179.1	45	10	15	8
Cocaine 1	5.7	304.2	182.2	50	10	30	14

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
Cocaine 2	5.7	304.2	77.0	50	10	77	6
Cocaine IS	5.7	307.3	185.1	50	10	30	14
BEG 1	5.4	290.1	168.2	40	10	30	15
BEG 2	5.4	290.1	82.0	40	10	40	12
BEG IS	5.4	293.1	171.1	40	10	30	15
EME 1	2.6	200.1	82.0	40	10	35	6
EME 2	2.6	200.1	150.0	40	10	28	12
EME IS	2.6	203.1	85.0	40	10	35	6
Coca ethylene 1	6.1	318.3	196,1	70	10	30	10
Coca ethylene 2	6.1	318.3	82,1	70	10	45	10
Coca ethylene IS	6.1	321.3	199,1	70	10	30	10
Morphine 1	3.9	286.1	152.2	15	10	73	12
Morphine 2	3.9	286.1	165.1	15	10	47	14
Morphine IS	3.9	289.1	152.1	15	10	73	12
Codeine 1	4.5	300.1	152.1	60	10	84	12
Codeine 2	4.5	300.1	165.0	60	10	45	12
Codeine IS	4.5	306.1	152.1	60	10	84	12
Dihydrocodeine 1	4.5	302.1	199.2	60	10	42	12
Dihydrocodeine 2	4.5	302.1	128.2	60	10	83	12
Dihydrocodeine IS	4.5	308.1	202.0	60	10	42	12
6-MAM 1	4.7	328.1	165.1	90	10	50	14
6-MAM 2	4.7	328.1	211.2	90	10	35	14
6-MAM IS	4.7	331.1	165.1	90	10	50	14
THC 1	9.5	315.1	193.1	50	10	31	10
THC 2	9.5	315.1	123.0	50	10	45	10
THC IS	9.5	318.3	196.1	50	10	31	10
11-OH-THC 1	8.1	331.1	193.1	60	10	35	10
11-OH-THC 2	8.1	331.1	201.1	60	10	34	10
11-OH-THC IS	8.1	334.2	196.1	60	10	35	10
Methadone 1	7.2	310.2	265.2	40	10	19	20
Methadone 2	7.2	310.2	105.2	40	10	38	12
Methadone IS	7.2	313.3	268.2	40	10	19	20
EDDP 1	7.0	278.2	234.3	70	10	40	14
EDDP 2	7.0	278.2	249.3	70	10	30	14
EDDP IS	7.0	281.1	234.3	70	10	40	14
Buprenorphine 1	6.6	468.3	414.1	80	10	45	20
Buprenorphine 2	6.6	468.3	396.1	80	10	50	20
Buprenorphine IS	6.6	472.1	400.1	80	10	50	20

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
Norbuprenorphine 1	5.7	414.0	414.0	80	10	40	10
Norbuprenorphine 2	5.7	414.0	83.2	80	10	70	10
Norbuprenorphine IS	5.7	417.0	417.0	80	10	40	10
Ketamine 1	5.0	238.2	125.0	40	10	37	12
Ketamine 2	5.0	238.2	179.1	40	10	23	12
Ketamine IS	5.0	242.2	183.0	40	10	23	12

Table 6: Detected transitions, retention times and potentials using HPLC Shimadzu + Sciex mass spectrometer

7 SAMPLE PREPARATION

Calibrators and controls follow the same samples preparation starting from step 8.

7.1 SAMPLE PREPARATION

1. Bring a sample strand of at least 50 mg, segmenting them as little as possible, into a 7 mL mill tube
2. Add 4 mL of Wash Solution (EUM20021), vortex for 1 min, remove the supernatant
3. Add 4 mL of Reconstituting Solution (EUM20022), vortex for 1 min, remove the supernatant
4. Remove the solvent residue by leaving the open tube in the dry bath at 45°C for 10 min
5. Add 20 steel balls in each tube and grind the entire quantity using the mill with the following operating conditions: rate = 5.3 m/s, cycle time = 3 min, nr cyles = 3, waiting time among two cycles = 20 sec
6. After removing steel balls, weigh exactly about 20 mg in vials
7. Add 400 µL of Extraction Solution (EUM20023)
8. Vortex for 30 sec
9. Incubate over night at 60°C
10. Add 40 µL of Internal Standard Mix (EUM20031) *
11. Vortex for 30 sec
12. Place in the ultrasonic bath at no more than 60°C for 2.5 h
13. After centrifuging at 12000 rpm for 5 min, add 20 µL of supernatant in a different tube
14. Add 180 µL of Extracting Solution (EUM20024)
15. Inject in the HPLC-MS/MS system.

* For calibrators and controls add the Internal Standard Mix directly in their vials.

8 COLLECTION AND STORAGE OF SAMPLES

The kit is intended for the analysis of human hairs samples collected with standard methods, such as those described in Guidelines of Italian NHS [10].

Stability of the samples: The removal of the hair matrix is not invasive, but it is essential to follow the Standard Operating Procedures throughout the process from collection, storage to transport to the toxicology laboratory that will carry out the analysis. Transport and storage do not require special precautions, they are normally carried out at room temperature and in the dark. Under these conditions, the concentrations of the drugs of abuse in question remain stable for up to 3 months [1].

9 VALIDATION DATA

Validation data have been obtained with an HPLC-MS/MS system consisting of a HPLC Shimadzu Nexera coupled to a Sciex 6500 QTrap triple quadrupole mass spectrometer.
Refer to Paragraph 4.2 for the materials and equipment used in the sample preparation.

9.1 LINEARITY, DETECTION LIMITS AND QUANTIFICATION

A linear regression analysis of real values concentration has been completed to evaluate linearity of calibration curve for each analytic session.
Linearity range of acceptability corresponds to $R^2 \geq 0.98$. All values obtained are higher than the above-mentioned value.
Detection limit (LLOD) and quantification limit (LLOQ), which concentration provide a peak with S/N>3 and S/N>10 respectively, are reported in the table below (Table 7).

ANALYTE	LINEARITA' (pg/mg)	LLOD (pg/mg)	LLOQ (pg/mg)
Amphetamine	5.21 – 10000	1.56	5.21
Methamphetamine	1.75 – 10000	0.525	1.75
MDA	2.02 – 10000	0.605	2.02
MDE	3.35 – 10000	1.01	3.35
MDMA	0.621 – 10000	0.186	0.621
MBDB	0.646 – 10000	0.194	0.646
Cocaine	0.604 – 25000	0.181	0.604
BEG	0.161 – 2500	0.048	0.161
EME	0.094 – 2500	0.028	0.094
Cocaethylene	0.228 – 2500	0.068	0.228
Morphine	0.682 – 20000	0.204	0.682
Codeine	0.794 – 20000	0.238	0.794

ANALYTE	LINEARITA' (pg/mg)	LLOD (pg/mg)	LLOQ (pg/mg)
Dihydrocodeine	0.750 – 20000	0.225	0.750
6-MAM	1.40 – 20000	0.420	1.40
THC	0.581 – 2500	0.174	0.581
THC-OH	1.57 – 2500	0.473	1.57
Methadone	3.20 – 10000	0.961	3.20
EDDP	0.218 – 2500	0.065	0.218
Buprenorphine	0.419 – 1000	0.126	0.419
Norbuprenorphine	1.51 – 1000	0.454	1.51
Ketamine	1.96 – 10000	0.588	1.96

Table 7: LLOD, LLOQ and linearity

9.2 RECOVERY

Increasing amount of standard has been added to 3 blank extracted matrix pools to evaluate the analytical recovery characteristics. Three different levels of enriched urine (low, medium and high level) have been obtained.

Recovery = (Measured quantity on enriched matrix - Measured quantity on non-enriched matrix) / Added quantity

Average recovery range of acceptability = ±20%, all the values obtained are higher than the above-mentioned value.

ANALYTE	AVERAGE RECOVERY (%)	MIN RECOVERY (%)	MAX RECOVERY (%)
Amphetamine	102.2	86.3	112.3
Methamphetamine	111.9	107.2	115.3
MDA	109.5	103.9	116.8
MDE	106.2	90.6	119.2
MDMA	109.2	106.4	112.9
MBDB	110.6	101.5	119.9
Cocaine	109.7	105.1	112.4
BEG	109.5	105.2	114.7
EME	109.8	107.1	115.3
Coca Ethylene	110.8	107.5	114.4
Morphine	110.1	106.2	115.6
Codeine	108.2	105.7	111.1
Dihydrocodeine	107.3	97.8	111.6
6-MAM	110.8	107.4	115.6
THC	110	107.7	113.2
THC-OH	108.9	105.4	116.4

ANALYTE	AVERAGE RECOVERY (%)	MIN RECOVERY (%)	MAX RECOVERY (%)
Methadone	109.6	103.4	114.2
EDDP	109.5	107.1	115.2
Buprenorphine	108.9	101.5	118.7
Norbuprenorphine	108.4	92.4	122.1
Ketamine	109.5	102.1	116

Table 8: Average, minimum and maximum recovery values

9.3 PRECISION

Average concentration values (pg/mg) measured in the 3 pools enriched with increasing concentrations of analytes (medium and high level) are reported in Table 9.
Precision has been evaluated as intra-assay, inter-assay and total coefficient of variation.

Intra-assay precision has been determined assaying 10 replicates (n=10) of each sample.
Inter-assay precision has been determined assaying 3 repetitions in 8 analytical series (n=24) for each sample.

Total CV% = $(CV\%Intra^2 + CV\%Inter^2)^{1/2}$

Range of acceptability used for each variation coefficient are reported below.
Range of acceptability CV% Intra-assay = 10%
Range of acceptability CV% Inter-assay = 20%
Range of acceptability CV% Total = 20%

Obtained results respect the imposed ranges of acceptability.

ANALYTE	AVER. CONC (pg/mg)			INTRA CV%			INTER CV%			TOTAL CV%		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Amphetamine	174	853	1652	5.1%	2.6%	1.5%	12.7%	11.8%	9.9%	14%	12%	10%
Methamphetamina	99.8	182	890	1.2%	1.7%	2.4%	3.3%	2.7%	3.2%	4%	3%	4%
MDA	184	891	1743	6.0%	4.5%	3.3%	6.5%	4.2%	4.5%	9%	6%	6%
MDE	182	896	1734	4.5%	1.8%	2.3%	9.7%	8.3%	5.8%	11%	8%	6%
MDMA	178	876	1742	3.0%	2.2%	3.3%	4.0%	3.7%	3.7%	5%	4%	5%
MBDB	183	927	1754	4.1%	1.9%	3.8%	5.3%	4.4%	4.2%	7%	5%	6%
Cocaine	451	2194	4295	2.8%	1.0%	3.0%	1.6%	1.4%	1.6%	3%	2%	3%
BEG	44.6	219	439	1.7%	2.0%	2.1%	2.1%	1.5%	3.7%	3%	3%	4%
EME	43.7	217	427	1.0%	0.5%	0.8%	2.1%	1.4%	2.4%	2%	1%	3%
Cocaethylene	45	216	430	2.5%	2.9%	1.8%	2.5%	2.2%	3.2%	4%	4%	4%
Morphine	364	1744	3452	2.1%	1.9%	1.4%	3.6%	1.8%	2.2%	4%	3%	3%
Codeine	356	1714	3385	3.4%	2.5%	3.5%	3.9%	3.1%	4.0%	5%	4%	5%

ANALYTE	AVER. CONC (pg/mg)			INTRA CV%			INTER CV%			TOTAL CV%		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Dihydrocodeine	365	1758	3444	1.8%	1.5%	1.8%	3.7%	4.4%	3.9%	4%	5%	4%
6-MAM	368	1775	3510	2.3%	1.4%	1.9%	3.5%	2.3%	2.5%	4%	3%	3%
THC	44.6	220	432	1.8%	1.2%	0.8%	2.0%	1.3%	1.1%	3%	2%	1%
THC-OH	44.5	216	427	3.8%	2.6%	3.0%	6.1%	3.9%	2.7%	7%	5%	4%
Methadone	184	888	1739	2.0%	1.4%	0.8%	7.2%	1.9%	2.3%	7%	2%	2%
EDDP	44.5	220	435	1.7%	1.3%	1.7%	2.2%	1.6%	2.3%	3%	2%	3%
Buprenorphine	16.3	86	172	8.4%	6.7%	4.5%	8.2%	7.4%	7.1%	12%	10%	8%
Norbuprenorphine	17.4	78.9	156	9.9%	8.0%	8.2%	7.3%	9.0%	9.0%	12%	12%	12%
Ketamine	177	828	1622	8.8%	3.6%	2.1%	7.0%	5.6%	4.8%	11%	7%	5%

Table 9: Intra-assay, inter-assay and total precision

10 GENERAL LIMITATIONS

- Kit must be used with the calibrators and the internal standard indicated in the kit instructions. The use of other standards or materials with this kit has not been validated.
- The use of different mobile phases, solutions or reagents other than those indicated in Paragraph 3.1 “KIT CONTENTS” has not been validated.
- This kit has been validated with configuration described in Chapter 9 “VALIDATION DATA”.
  The use of other triple quadrupole system, HPLC system and columns, which may require further development of the method, has not been validated.
- Do not use the kit after expiry date of its components.

11 REFERENCES

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ANNEX 1: EC DECLARATION OF CONFORMITY

B.S.N. srl as Manufacturer and the only responsible for in-vitro diagnostic medical devices placed on the market under his own name, declares that these products meet all the provisions of the Legislative Decree n. 332 of the 8th September 2000, directive of in vitro diagnostic medical device 98/79/EC (in particular with regard to annex I) and subsequent amendments and additions. According to point 9 of Legislative Decree 332/2000 and subsequent amendments, the in vitro diagnostic medical device belongs to the fourth category of devices, that is GENERIC IN VITRO MEDICAL-DIAGNOSTIC DEVICES.

COMPONENT	CODE	CERTIFICATION
FloMass Drugs of Abuse in Hair Matrix	EUM20100	CE-IVD marked medical device according to Annex III
Mobile Phase A	EUM02011	CE-IVD marked medical device according to Annex III
Mobile Phase B	EUM02012	CE-IVD marked medical device according to Annex III
Mobile Phase C	EUM02013	CE-IVD marked medical device according to Annex III
Washing Solution	EUM20021	CE-IVD marked medical device according to Annex III
Reconditioning Solution	EUM20022	CE-IVD marked medical device according to Annex III
Extracting Solution	EUM20023	CE-IVD marked medical device according to Annex III
Diluting Solution	EUM20024	CE-IVD marked medical device according to Annex III
Internal Standard Mix	EUM20031	CE-IVD marked medical device according to Annex III
Calibrators in Hair Matrix	EUM20041	CE-IVD marked medical device according to Annex III
Controls in Hair Matrix	EUM20051	CE-IVD marked medical device according to Annex III
Chromatographic Column	EUM00C02	CE-IVD marked medical device according to Annex III
Precolumns	EUM00A04	CE-IVD marked medical device according to Annex III
Holder + Precolumn	EUM00A05	CE-IVD marked medical device according to Annex III

Quality assurance system complying following directives:

- ✓ UNI CEI EN ISO 13485:2016
- ✓ UNI EN ISO 9001:2015

This declaration becomes invalid if modifications are introduced without B.S.N. Srl consent.
It is declared that the product is placed on the market in non-sterile package.
It is declared that B.S.N. Srl will keep all documents referred to the Annex III of the European Directive 98/79/EC at the disposal of the competent authorities for a 5-year period from the last date of production of the kit.
After the placing on the market of the products in question, it is declared that the Manufacturer has notified the competent authority of the application of post-market surveillance as requested from the European Directive 98/79/CE.
This declaration is valid five years from the date of issue.

Castelleone (CR), 13 May 2022 Director