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FloMass Drugs of Abuse in Urine

Reagents for 100 assays

Instruction Manual

REF **EUM02100**

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

CE

PROVIDE PERSONAL SOLUTIONS

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1 INTRODUCTION

1.1 IVD SYMBOLS

IVD	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
LOT	Batch code / Code du lot / Chargenbezeichnung / Código de lote / Codice del lotto / Código do lote / Número do lote / Lotnummer
PN	Packing number / Numéro d'emballage / Packnummer / Número de envase / Numero confezioni / Número de embalagem / Número de embalagem / Emballagenummer
REF	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

4-ANPP: 4-Aminophenyl-1-phenethylpiperidine
6-MAM: 6-MonoAcetylMorphine
BEG: Benzoylecgonine
CAD: Collision Gas Pressure
CCL2: Collision Cell Lens 2
CE: Collision energy
CLSI: Clinical and Laboratory Standards Institute
CUR: Curtain Gas
CV: Coefficient of Variation
CXP: Collision Exit Potential
DP: Desolvation Potential
ED: Entrance Deflector
EDDP: 2-Ethyliden-1,5-Dimethyl-3,3-Diiphenylpyrrolidine
EME: Ecgonine Methyl Esther
EP: Entrance Potential
ESI: Electrospray Ionization
EV: Entrance Voltage
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
M3G: Morphine 3-Glucoronate
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
MRM: Multiple Reactions Monitoring
PP: Polypropylene
Q1: Quadrupole 1
Q3: Quadrupole 3
RT: Retention Time
S/N: Signal/Noise ratio
TEM: Source temperature
THC-COOH: 11-Nor-9-carboxylic-Δ9-Tetrahydrocannabinol Acid

1.3 CLINICAL APPLICATION

FloMass Drugs of Abuse in Urine is an in vitro diagnostic kit intended for the quantitative and simultaneous determination of drugs of abuse in human urine 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
Morphine 3-Glucuronate
Codeine
Dihydrocodeine
6-MAM
THC-COOH
Methadone
EDDP
Buprenorphine
Norbuprenorphine
Ketamine
Norketamine
4-ANPP
LSD

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 [1-3].

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 [4-6].

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).
Sample preparation provides a first step in which the pH of the urinary matrix is adjusted with a buffer solution. Internal Standard are added to treated urine (Table 2), followed by the extraction of the analytes by precipitation. For glucuronated substances (Morphine, THC-COOH, Codeine, Buprenorphine, Norbuprenorphine) an enzymatic hydrolysis step is required to obtain the drugs in their free form before proceeding to the precipitation step. Finally, sample is diluted and analyzed by HPLC-MS/MS technique.

ANALYTE	INTERNAL STANDARD
Amphetamine	Amphtamina $^{2}H_{11}$
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 $^{2}H_{3}$
Morphine 3- Glucuronate	Morphine $^{2}H_{3}$
Codeine	Codeine $^{2}H_{6}$
Dihydrocodeine	Dihydrocodeine $^{2}H_{6}$
6-MAM	6-MAM $^{2}H_{3}$
THC-COOH	THC-COOH $^{2}H_{9}$
Methadone	Methadone $^{2}H_{3}$
EDDP	EDDP $^{2}H_{3}$
Buprenorphine	Buprenorphine $^{2}H_{4}$
Norbuprenorphine	Norbuprenorphine $^{2}H_{3}$
Ketamine	Ketamine $^{2}H_{4}$
Norketamine	Norketamine $^{2}H_{4}$
4-ANPP	Methamphetamine $^{2}H_{5}$
LSD	LSD $^{2}H_{3}$

Table 2: Analytes measured by kit EUM02100 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 in to 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 COMPONENTS AND ACCESSORIES

3.1 KIT 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
EUM02021	Buffer Solution	6.5 mL	Room temperature*
EUM02022	Precipitant Solution	32 mL	Room temperature
EUM02023	Hydrolysis Solution	1.0 mL	2-8°C
EUM02025	Diluting Solution	12 mL	Room temperature
EUM02031	Internal Standard Mix	2 x 0.8 mL	-20°C

Table 3: Components, description, quantity and storage of kit EUM02100

*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
EUM02042	6-Levels Calibrators, lyophil.	3 x 6 x 0.6 mL	-20°C
EUM02055	2-Levels Control, lyophil.	3 x 2 x 0.6 mL	-20°C
EUM02052	FloMix DOA Control	2 x 1.0 mL	-20°C

CATALOG NUMBER	DESCRIPTION	QUANTITY	STORAGE
EUM00C02	Chromatographic Column	1 pc	Room Temperature
EUM00A04	Precolumn	4 pcs	Room Temperature
EUM00A05	Holder + precolumn	1 pc	Room Temperature

Table 4: Accessories, description, quantity and storage of kit EUM02100

3.3 CONTROLS AND CALIBRATION OF THE ANALYTICAL SYSTEM

Calibration should be done using 6-Levels Calibrators (EUM02042) containing the analytes. Calibrators should follow patient samples preparation without hydrolysis (Paragraph 7.1.1 or 7.2.1).
A new calibration series should be prepared for each analytical run.
BSN supplies quality control sets at two different concentration levels (EUM02055). Urinary matrix Quality Lyophilized Controls are useful to verify the accuracy and precision of the analytical procedures.
For analyte concentrations, stability and accessories preparation, refer to package leaflet.

3.4 CHROMATOGRAPHIC SYSTEM

The kit has been validated using an 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.
The kit EUM02100 can be used with two different chromatographic procedures, called “PROCEDURE A” and “PROCEDURE B”.
PROCEDURE A 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).

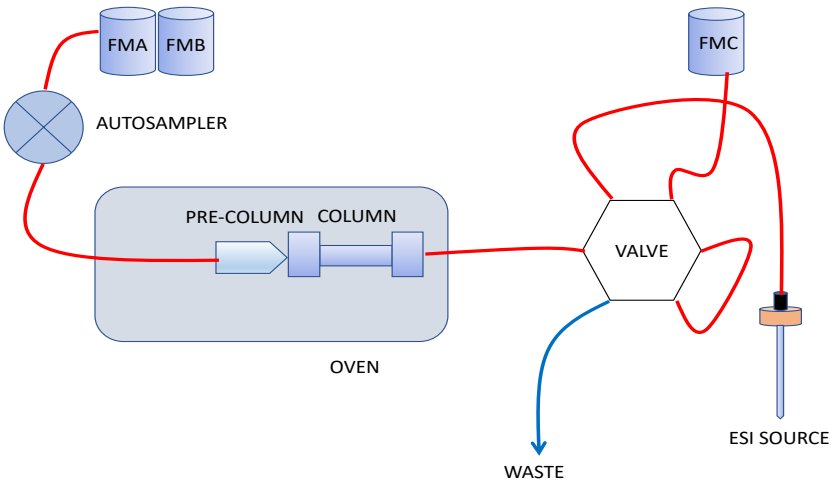

Figure 1: Plumbing configuration PROCEDURE A

"PROCEDURE B" involves the use of 2 mobile phases (A and B) and therefore just the binary pump is sufficient (see Figure 2).

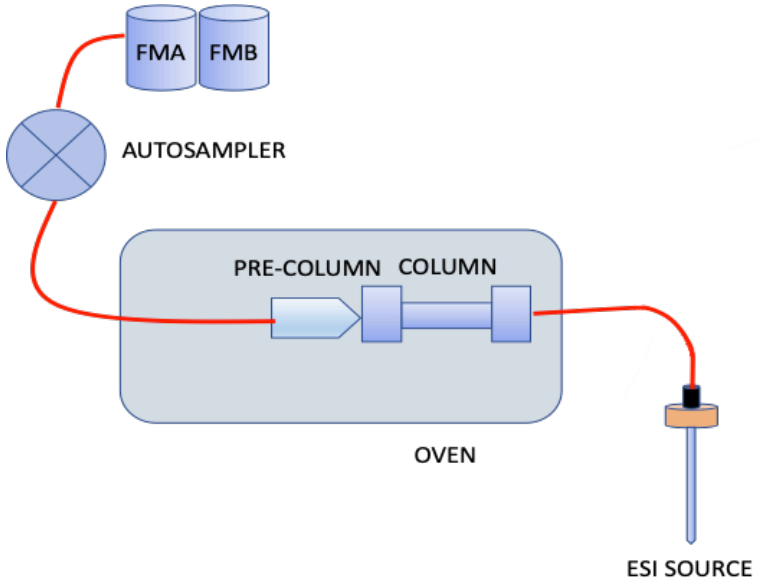

Figure 2: Plumbing configuration PROCEDURE B

In case of adoption of the "PROCEDURE B" DO NOT USE the Mobile Phase C (code EUM02013) and the Diluent Solution (code EUM02025).
For sample preparation relating to the two different applicable PROCEDURES, see chapter 7.

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.

4.1 REQUIRED HPLC MODULES

1. Binary pump able to support a backpressure of 400 bar or more
2. Additional pump (Only for PROCEDURE A)
3. 6-port switching valve (Only for PROCEDURE A)
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. Chemical hood
7. Thermoblock

5 HPLC-MS/MS SYSTEM CONDITIONS

5.1 CHROMATOGRAFIC GRADIENT FOR PROCEDURE A

Ionization: ESI positive mode, except for THC-COOH analyzed in negative mode
MS/MS: specific MRM
Injection volume: 15 µL (variable according to instrumental sensitivity)
Running time: 12 min
Column heater: 40°C

Chromatographic gradient

TIME (min)	%MPA	%MPB	MS Valve	FMC 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 EUM02100 PROCEDURE A

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 0.29 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 chromatograms PROCEDURE A

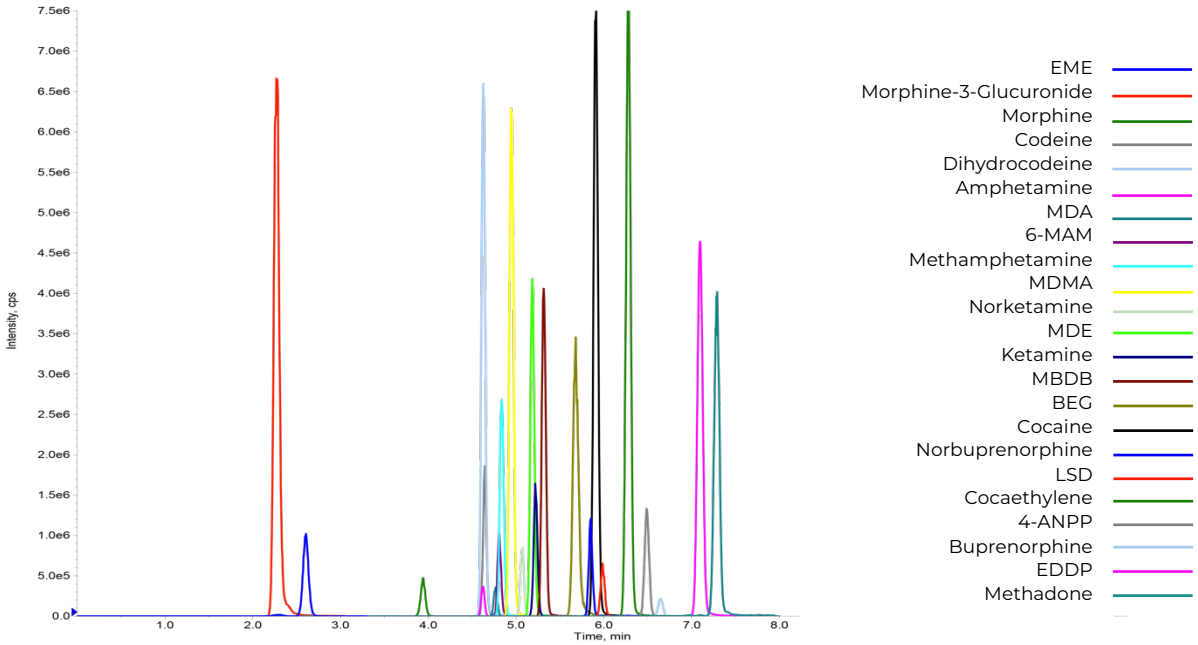

Figure 3: Example of chromatogram identified using kit EUM02100 – ESI Positive Mode

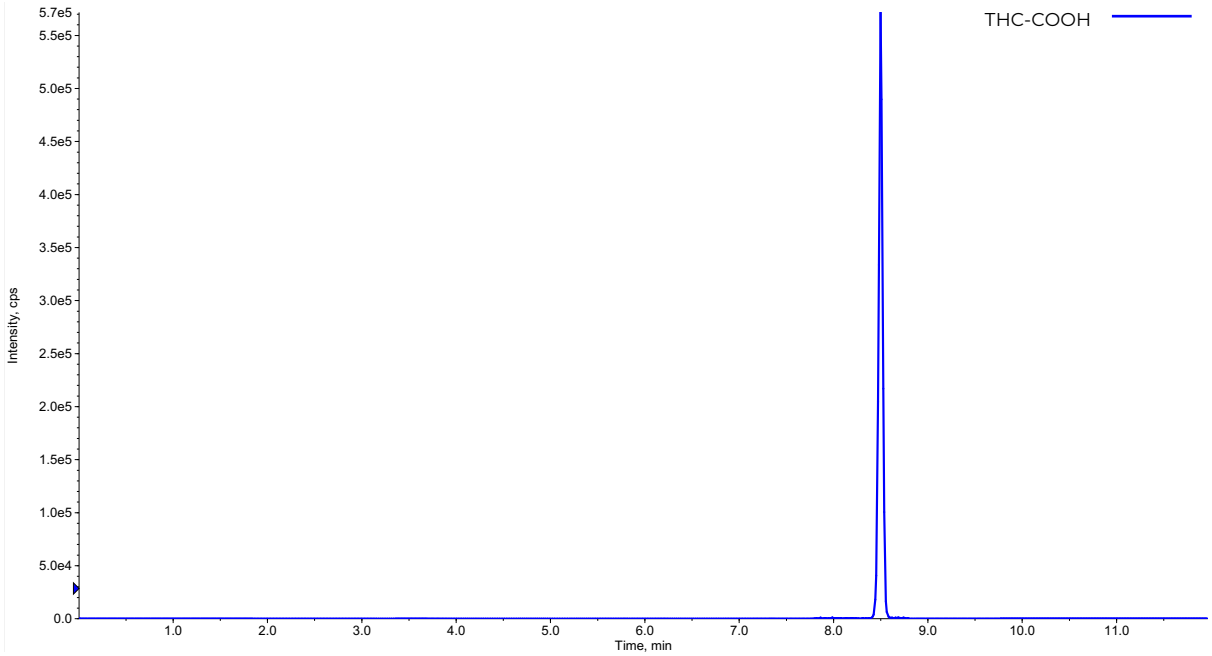

Figure 4: Example of chromatograms identified using kit EUM02100 – ESI Negative Mode

5.2 CHROMATOGRAFIC GRADIENT FOR PROCEDURE B

Ionization: ESI positive mode, except for THC-COOH analyzed in negative mode
MS/MS: specific MRM
Injection volume: 15 µL (variable according to instrumental sensitivity)
Running time: 15 min
Column heater: 31 °C

TIME (min)	%MFA	%MFB	FL0W (mL/min)
0.0	92	8	0.29
1.0	85	15	0.29
2.0	80	20	0.29
4.0	60	40	0.29
7.0	50	50	0.29
8.0	35	65	0.29
9.5	10	90	0.29
10.5	10	90	0.29
12.0	60	40	0.29
13.0	92	8	0.29
15.0	Stop	Stop	Stop

Table 6: Chromatographic gradient of kit EUM02100 PROCEDURE B

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 0.29 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 chromatograms PROCEDURE B

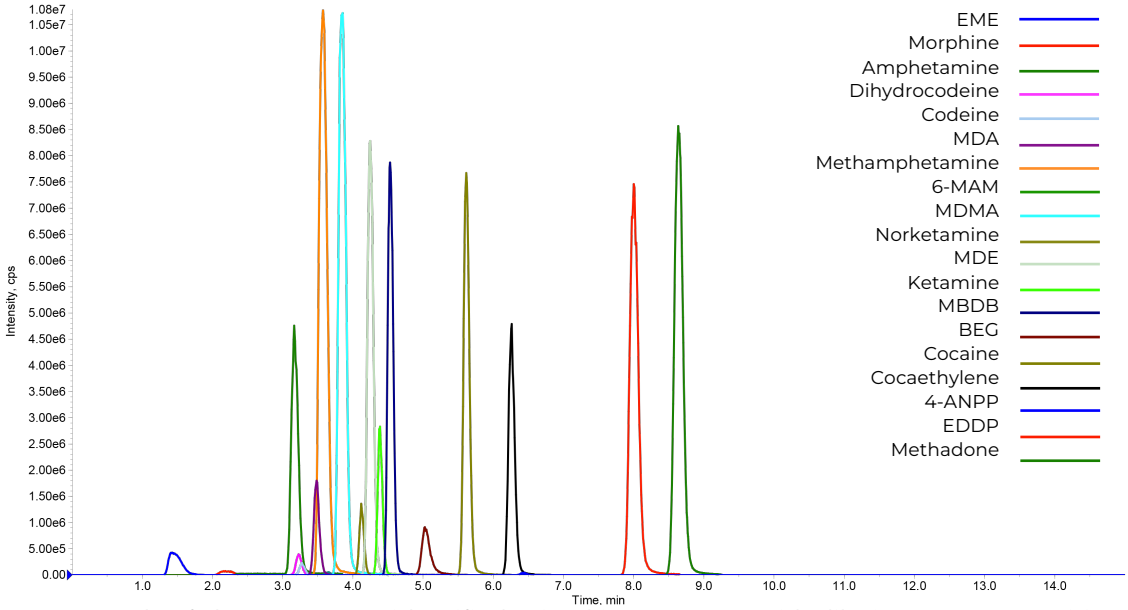

Figure 5: Example of chromatogram identified using kit EUM02100 with dilution 1:10 – ESI Positive Mode

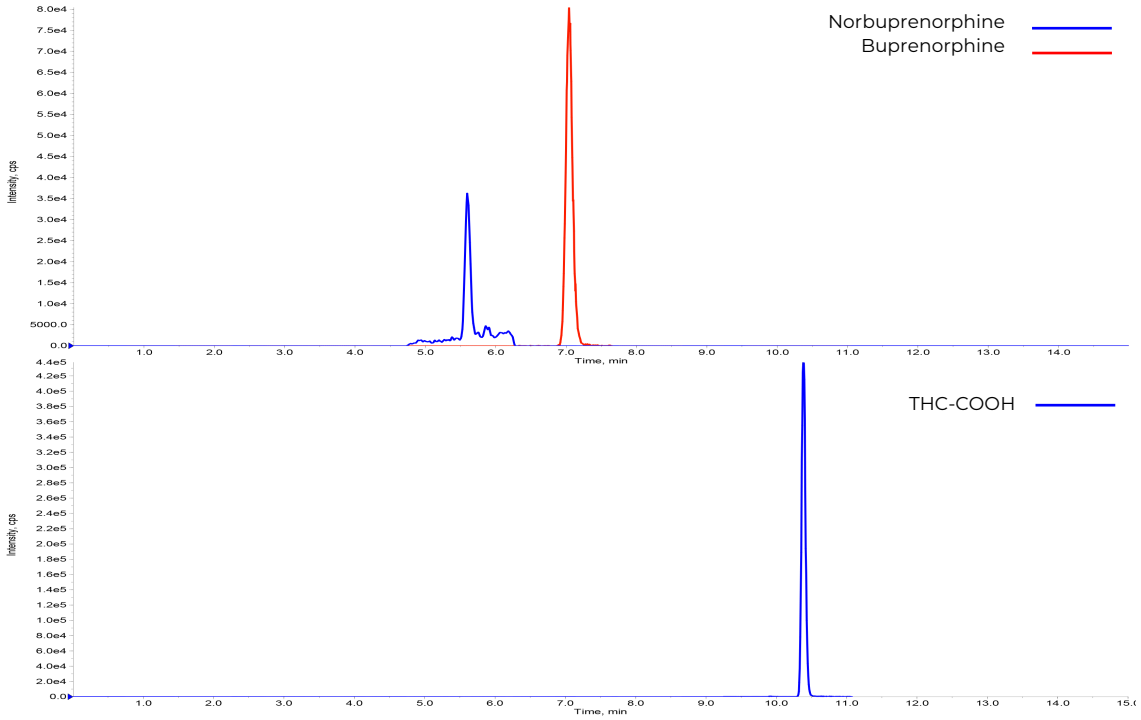

Figure 6: Example of chromatogram identified using kit EUM02100 with dilution 1:2 – ESI Positive and Negative Mode

6 SOURCE PARAMETERS AND TRANSITIONS

6.1 SOURCE PARAMETERS

Source parameters used in MS the Method of EUM02100 kit with a Sciex series 4500 QTrap mass spectrometer are shown below.

Curtain Gas (CUR): 30 psi
Collision Gas Pressure (CAD): Medium
Ion Spray Voltage (IS): 5000 V (MRM+) / -4500 V (MRM-)
Temperature (TEM): 500°C
Gas 1 (GS1): 55 psi
Gas 2 (GS2): 60 psi

6.2 TRANSITIONS PROCEDURE A

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 7. ESI positive mode, except for THC-COOH analyzed in negative mode.

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
Amphetamine 1	4.5	136.1	119.1	45	10	11	12
Amphetamine 2	4.5	136.1	91.1	45	10	24	10
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	119.2	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	213.1	179.2	45	10	25	8
Cocaine 1	5.7	304.2	182.2	50	10	30	14
Cocaine 2	5.7	304.2	77.0	50	10	77	6
Cocaine IS	5.7	307.1	185.2	50	10	30	14

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
BEG 1	5.3	290.1	168.2	40	10	30	15
BEG 2	5.3	290.1	82.0	40	10	40	12
BEG IS	5.3	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	286.2	165.1	15	10	47	14
Morphine IS	3.9	289.1	152.2	15	10	73	12
Morphine-3-Glucuronate 1	2.5	462.3	286.2	70	10	40	14
Morphine-3-Glucuronate 2	2.5	462.3	201.1	70	10	55	10
Codeine 1	4.4	300.1	152.1	60	10	84	12
Codeine 2	4.4	300.1	165.0	60	10	45	12
Codeine IS	4.4	306.1	152.1	60	10	84	12
Dihydrocodeine 1	4.4	302.1	199.2	60	10	42	12
Dihydrocodeine 2	4.4	302.1	128.2	60	10	83	12
Dihydrocodeine IS	4.4	308.1	202.0	60	10	42	12
6-MAM 1	4.6	328.1	165.1	90	10	50	14
6-MAM 2	4.6	328.1	211.2	90	10	35	14
6-MAM IS	4.6	331.1	165.1	90	10	50	14
THC-COOH 1	8.5	343.1	299.2	-90	-10	-29	-15
THC-COOH 2	8.5	343.1	245.2	-90	-10	-38	-15
THC-COOH IS	8.5	352.1	308.2	-90	-10	-29	-15
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	281.2	234.3	70	10	40	14
Buprenorphine 1	6.5	468.3	414.1	80	10	45	20
Buprenorphine 2	6.5	468.3	396.1	80	10	50	20
Buprenorphine IS	6.5	472.3	400.1	80	10	45	20
Norbuprenorphine 1	5.7	414.0	414.0	80	10	40	6
Norbuprenorphine 2	5.7	414.0	83.2	80	10	70	6
Norbuprenorphine IS	5.7	417.4	417.4	80	10	40	10

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
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
Norketamine 1	4.9	224.0	125.0	30	10	35	10
Norketamine 2	4.9	224.0	179.1	30	10	25	10
Norketamine IS	4.9	228.0	129.0	30	10	35	10
4-ANPP 1	6.4	281.0	188.1	40	10	25	10
4-ANPP 2	6.4	281.0	105.0	40	10	45	10
LSD 1	5.8	324.3	223.2	90	10	32	10
LSD 2	5.8	324.3	208.2	90	10	40	10
LSD IS	5.8	327.3	226.2	90	10	32	10

Table 7: Detected transitions, retention times and potentials using HPLC Shimadzu + Sciex mass spectrometer

6.3 TRANSITIONS PROCEDURE B

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 8. ESI positive mode, except for THC-COOH analyzed in negative mode.

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
Amphetamine 1	3.2	136.1	119.1	45	10	11	12
Amphetamine 2	3.2	136.1	91.1	45	10	24	10
Methamphetamine 1	3.6	150.2	91.1	30	10	26	10
Methamphetamine 2	3.6	150.2	119.2	30	10	14	12
Methamphetamine IS	3.6	155.2	92.0	30	10	26	10
MDA 1	3.5	180.1	133.1	30	10	22	12
MDA 2	3.5	180.1	163.1	30	10	25	12
MDE 1	4.2	208.2	163.1	30	10	25	12
MDE 2	4.2	208.2	105.0	30	10	30	12
MDMA 1	3.8	194.1	163.1	30	10	16	14
MDMA 2	3.8	194.1	105.1	30	10	32	12
MBDB 1	4.4	208.2	177.1	45	10	15	8
MBDB 2	4.4	208.2	135.2	45	10	25	8
Cocaine 1	5.2	304.2	182.2	50	10	30	14
Cocaine 2	5.2	304.2	77.0	50	10	77	6
Cocaine IS	5.2	307.1	185.2	50	10	30	14
BEG 1	4.9	290.1	168.2	40	10	30	15
BEG 2	4.9	290.1	82.0	40	10	40	12

ANALYTE	TR	Q1	Q3	DP	EP	CE	CXP
BEG IS	4.9	293.1	171.1	40	10	30	15
EME 1	1.5	200.1	82.0	40	10	35	6
EME 2	1.5	200.1	150.0	40	10	28	12
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
Morphine 1	2.1	286.1	152.2	15	10	73	12
Morphine 2	2.1	286.2	165.1	15	10	47	14
Morphine IS	2.1	289.1	152.2	15	10	73	12
Codeine 1	3.1	300.1	152.1	60	10	84	12
Codeine 2	3.1	300.1	165.0	60	10	45	12
Codeine IS	3.1	306.1	152.1	60	10	84	12
Dihydrocodeine 1	3.0	302.1	199.2	60	10	42	12
Dihydrocodeine 2	3.0	302.1	128.2	60	10	83	12
6-MAM 1	3.6	328.1	165.1	90	10	50	14
6-MAM 2	3.6	328.1	211.2	90	10	35	14
THC-COOH 1	11.3	343.1	299.2	-90	-10	-29	-15
THC-COOH 2	11.3	343.1	245.2	-90	-10	-38	-15
THC-COOH IS	11.3	352.1	308.2	-90	-10	-29	-15
Methadone 1	8.2	310.2	265.2	40	10	19	20
Methadone 2	8.2	310.2	105.2	40	10	38	12
EDDP 1	7.7	278.2	234.3	70	10	40	14
EDDP 2	7.7	278.2	249.3	70	10	30	14
EDDP IS	7.7	283.2	234.3	70	10	40	14
Buprenorphine 1	6.7	468.3	414.1	80	10	45	20
Buprenorphine 2	6.7	468.3	396.1	80	10	50	20
Norbuprenorphine 1	5.7	414.0	414.0	80	10	40	6
Norbuprenorphine 2	5.7	414.0	83.2	80	10	70	6
Norbuprenorphine IS	5.2	417.4	417.4	80	10	40	10
Ketamine 1	4.4	238.2	125.0	40	10	37	12
Ketamine 2	4.4	238.2	179.1	40	10	23	12
Norketamine 1	4.9	224.0	125.0	30	10	35	10
Norketamine 2	4.9	224.0	179.1	30	10	25	10
4-ANPP 1	6.4	281.0	188.1	40	10	25	10
4-ANPP 2	6.4	281.0	105.0	40	10	45	10

Table 8: Detected transitions, retention times and potentials using HPLC Shimadzu + Sciex mass spectrometer

7 SAMPLE PREPARATION

7.1 PROCEDURE A

7.1.1 SAMPLE PREPARATION (CALIBRATOR/CONTROL) WITHOUT HYDROLYSIS

1. Prepare a mix composed of 50 µL of Buffer Solution (EUM02021) + 10 µL of Internal Standards Mix (EUM02031) for sample
2. Pipette 250 µL of urine. Add 60 µL of the mix prepared at step 1 of the procedure and 15 µL of Buffer Solution (EUM02021)
3. Vortex for 10 sec
4. Add 300 µL of Precipitant Solution (EUM02022)
5. Vortex for 10 sec and centrifuge at 12000 rpm for 5 min
6. Transfer the supernatant into another vial and dilute it with Diluent Solution (EUM02025) with 1:2 ratio.
7. Transfer into an autosampler vial with plastic adapter and analyze with HPLC-MS/MS technique

7.1.2 SAMPLE PREPARATION (CALIBRATOR/CONTROL) WITH HYDROLYSIS

1. Prepare a mix composed of 50 µL of Buffer Solution (EUM02021) + 10 µL of Internal Standards Mix (EUM02031) per sample
2. Pipette 250 µL of urine. Add 60 µL of the mix prepared at step 1 of the procedure and 15 µL of Hydrolysis Solution (EUM02023)*
3. Vortex for 10 sec
4. Incubate in thermoblock at 65°C for 4 hours
5. Add 300 µL of Precipitant Solution (EUM02022)
6. Vortex for 10 sec and centrifuge at 12000 rpm for 5 min
7. Transfer the supernatant into another vial and dilute it with Diluent Solution (EUM02025) with 1:2 ratio
8. Transfer into an autosampler plastic vial with plastic adapter and analyze with HPLC-MS/MS technique

* Solution EUM02023 is highly viscous, it is recommended to pay great attention on pipetting it.

7.2 PROCEDURE B

7.2.1 SAMPLE PREPARATION (CALIBRATOR/CONTROL) WITHOUT HYDROLYSIS

1. Prepare a mix composed of 50 µL of Buffer Solution (EUM02021) + 10 µL of Internal Standards Mix (EUM02031) per sample
2. Pipette 250 µL of urine. Add 60 µL of the mix prepared at step 1 of the procedure and 15 µL of Buffer Solution (EUM02021)
3. Vortex for 10 sec
4. Add 300 µL of Precipitant Solution (EUM02022)
5. Vortex for 10 sec and centrifuge at 12000 rpm for 5 min
6. Transfer the supernatant into another vial and dilute it with Mobile Phase A (EUM02011) depending on the instrumental sensitivity
7. Minimum dilution for Opiates, Amphetamines, Ketamine, Cocaine and its metabolites, Methadone and its metabolite is 1:10 (20 µL of supernatant + 180 µL of MPA) variable according to instrumental sensitivity
8. Minimum dilution for Cannabinoids, Buprenorphine and Norbuprenorphine is 1:2 (100 µL of supernatant + 100 µL of MPA)
9. Transfer into an autosampler vial with plastic adapter and analyze with HPLC-MS/MS technique

7.2.2 SAMPLE PREPARATION (CALIBRATOR/CONTROL) WITH HYDROLYSIS

1. Prepare a mix composed of 50 µL of Buffer Solution (EUM02021) + 10 µL of Internal Standards Mix (EUM02031) per sample
2. Pipette 250 µL of urine. Add 60 µL of the mixture prepared at step 1 and 15 µL of Hydrolysis Solution (EUM02023)*
3. Vortex for 10 sec
4. Incubate in thermoblock at 65°C for 4 hours
5. Add 300 µL of Precipitant Solution (EUM02022)
6. Vortex for 10 sec and centrifuge at 12000 rpm for 5 min
7. Transfer the supernatant into another vial and dilute it with Mobile Phase A (EUM02011) depending on the instrumental sensitivity
8. Minimum dilution for Opiates, Amphetamines, Ketamine, Cocaine and its metabolites, Methadone and its metabolite is 1:10 (20 µL supernatant + 180 µL MPA) variable according to instrumental sensitivity
9. Minimum dilution for Cannabinoids, Buprenorphine and Norbuprenorphine is 1:2 (100 µL supernatant + 100 µL MPA)
10. Transfer into an autosampler plastic vial with plastic adapter and analyze with HPLC-MS/MS technique

* Solution EUM02023 is highly viscous, it is recommended to pay great attention on pipetting it.

8 COLLECTION AND STORAGE OF THE SAMPLES

This kit is intended for the analysis of human urine samples collected following standard methods such as those described in document GP16-A3 of the Clinical and Laboratory Standards Institute (CLSI) [7].

Stability of the samples: although drugs stability is affected by substance chemical structure, physical-chemical properties, collecting methods, storage and additive addition, drugs of abuse detected by urine dosage keep their own values ±5% for 12 months if stored in glass vials at -20°C [8-11].

9 VALIDATION DATA

Validation data have been obtained with a HPLC-MS/MS system consisting of a HPLC Shimadzu Nexera coupled with a Sciex 6500 mass spectrometer.
Refer to Paragraph 4.2 for materials and instruments used in 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 Table 9 (PROCEDURE A) and 10 (PROCEDURE B).

ANALYTE	LLOD (ng/mL)	LLOQ (ng/mL)	LINEARITY (ng/mL)
Amphetamine	1,13	3,77	3,77 – 3552
Methamphetamine	1,07	3,55	3,55 – 3412
MDA	0,925	3,08	3,08– 3360
MDE	0,910	3,03	3,03– 1630
MDMA	0,775	2,58	2,58– 1746
MBDB	0,656	2,19	2,19– 759
Cocaine	0,856	2,85	2,85– 3012
BEG	0,765	2,55	2,55– 1506
EME	0,766	2,55	2,55– 2220
Coca Ethylene	0,688	2,29	2,29– 2332
Morphine	0,782	2,61	2,61– 3952

ANALYTE	LLOD (ng/mL)	LLOQ (ng/mL)	LINEARITY (ng/mL)
Morphine-3-Glucuronate	0,649	2,16	2.16 – 900
Codeine	0,986	3,29	3,29– 851
Dihydrocodeine	0,992	3,31	3,31– 782
6-MAM	0,159	0,531	0,531– 278
Methadone	0,569	1,90	1,90– 759
EDDP	0,646	2,15	2,15– 814
Buprenorphine	0,083	0,278	0,278– 355
Norbuprenorphine	0,061	0,205	0,205– 327
Ketamine	0,695	2,32	2,32– 825
Norketamine	0,645	2,15	2,15– 1868
4-ANPP	0,012	0,040	0,040– 33.0
LSD	0,002	0,008	0,008– 33.3
THC-COOH	0,172	0,572	0,572– 864

Table 9: LLOD, LLOQ and linearity PROCEDURE A

ANALYTE	LLOD (ng/mL)	LOQ (ng/mL)	LINEARITY (ng/mL)
Amphetamine	2.57	8.58	8.58 – 600
Methamphetamine	0.250	0.83	0.830 – 600
MDA	1.74	5.81	5.81 – 600
MDE	0.170	0.55	0.550 – 600
MDMA	0.300	1.00	1.00 – 600
Cocaine	0.080	0.26	0.260 – 300
BEG	0.120	0.40	0.400 – 300
EME	1.39	4.64	4.64 – 300
Morphine	0.710	2.36	2.36 – 300
Codeine	0.210	0.71	0.710 – 300
Dihydrocodeine	0.160	0.54	0.500 – 300
6-MAM	0.360	1.20	1.20 – 300
Methadone	0.220	0.72	0.720 – 300
EDDP	0.070	0.24	0.240 – 300
Buprenorphine	0.060	0.22	0.220 – 30.0
Norbuprenorphine	0.300	1.01	1.01 – 60.0
Ketamine	0.220	0.73	0.730 – 660
THC-COOH	0.530	1.78	1.78 – 60.0

Table 10: LLOD, LLOQ and linearity PROCEDURE B

9.2 RECOVERY

Increasing amount of standard has been added to 3 real human urine pools in order to evaluate the analytical recovery characteristics. Three different levels of enriched urine were obtained (low, medium and high) to be compared wih the non-enriched matrix.

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

Average recovery range of acceptability = ±20%, all results obtained are higher than the above-mentioned value.
Considering all the analytes of the panel, average recovery rate is 91 – 113% for PROCEDURE A and 98-103% for PROCEDURE B.

9.3 PRECISION

Average concentration values (ng/mL) measured in 3 pools (low, medium and high) are reported in Table 11 (PROCEDURE A) and 12 (PROCEDURE B).
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) of 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 (ng/mL)			INTRA CV%			INTER CV%			TOTAL CV%		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Amphetamine	133	267	640	2.3%	1.1%	1.7%	5.2%	4.1%	4.5%	5.6%	4.3%	4.8%
Methamphetamina	133	267	640	3.9%	2.7%	2.6%	5.6%	3.5%	4.5%	6.8%	4.4%	5.2%
MDA	133	267	640	3.1%	2.6%	3.4%	4.6%	4.4%	4.3%	5.6%	5.1%	5.5%
MDE	133	267	640	2.6%	1.5%	2.4%	3.6%	3.0%	3.9%	4.4%	3.4%	4.6%
MDMA	133	267	640	2.9%	2.1%	1.9%	3.8%	3.9%	3.8%	4.9%	4.9%	4.9%
MBDB	133	267	640	3.0%	1.9%	1.3%	4.7%	4.7%	5.6%	5.6%	5.1%	5.8%
Cocaine	33.0	67.0	160	2.6%	2.8%	1.2%	4.9%	3.2%	3.3%	5.5%	4.3%	3.5%
BEG	33.0	67.0	160	5.4%	2.5%	2.6%	10.8%	4.5%	3.8%	12.1%	5.2%	4.6%

ANALYTE	AVER. CONC (ng/mL)			INTRA CV%			INTER CV%			TOTAL CV%		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
EME	33.0	67.0	160	2.5%	1.8%	1.5%	6.1%	4.6%	4.2%	6.6%	4.9%	4.4%
Cocaethylene	33.0	67.0	160	2.5%	3.6%	1.4%	7.5%	3.6%	3.5%	7.9%	5.1%	3.8%
Morphine	33.0	67.0	160	2.4%	0.7%	1.5%	4.3%	2.5%	3.7%	5.0%	2.5%	4.0%
Codeine	33.0	67.0	160	2.9%	1.9%	2.7%	4.4%	3.4%	4.2%	5.3%	3.9%	5.0%
Dihydrocodeine	67.0	160	33.0	2.9%	1.9%	1.7%	5.6%	4.9%	5.5%	6.3%	5.3%	5.8%
6-MAM	7.00	13.0	32.0	5.7%	4.4%	2.3%	7.1%	4.6%	4.8%	9.1%	6.4%	5.3%
THC-COOH	10.0	20.0	48.0	3.3%	1.3%	1.9%	4.8%	4.7%	4.7%	5.9%	4.9%	5.1%
Methadone	67.0	133	320	2.8%	1.8%	2.1%	7.4%	4.1%	5.1%	7.9%	4.4%	5.6%
EDDP	67.0	133	320	2.3%	1.9%	1.1%	5.5%	2.5%	4.5%	5.9%	3.1%	4.7%
Buprenorphine	3.00	7.00	16.0	4.9%	4.6%	4.3%	10%	6.6%	5.9%	11.1%	8.1%	7.3%
Norbuprenorphine	3.00	7.00	16.0	5.5%	4.6%	3.9%	8.3%	7.7%	5.7%	10.0%	9.0%	6.9%
Ketamine	33.0	67.0	160	3.1%	1.9%	2.4%	6.1%	4.7%	5.2%	6.8%	5.1%	5.8%
Norketamine	33.0	67.0	160	2.7%	2.9%	2.7%	7.7%	5.8%	6.4%	8.2%	6.4%	7.0%
4-ANPP	0.500	1.00	3.00	2.4%	3.5%	2.1%	4.9%	3.7%	4.9%	5.5%	5.1%	5.3%
LSD	0.833	1.67	4.00	2.7%	4.4%	6.1%	6.3%	5.1%	4.9%	6.9%	6.7%	7.8%

Table 11: Intra-assay, inter-assay and total precision PROCEDURE A

ANALYTE	AVER.CONC (ng/mL)			INTRA CV%			INTER CV%			TOTAL CV%		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Amphetamine	10.5	70.0	409	12.9%	1.83%	6.50%	13.0%	4.15%	4.13%	18.3%	4.54%	7.70%
Methamphetamine	22.2	110	625	12.4%	8.26%	7.88%	9.97%	4.21%	3.14%	15.9%	9.27%	8.48%
MDA	23.2	124	713	18.6%	15.1%	13.2%	13.8%	5.32%	4.62%	23.2%	16.0%	14.0%
MDE	19.3	103	603	8.95%	3.76%	8.85%	15.2%	4.25%	3.60%	17.7%	5.67%	9.55%
MDMA	21.3	108	640	10.1%	7.86%	7.62%	9.37%	4.35%	3.53%	13.8%	8.98%	8.40%
Cocaine	9.91	49.6	301	8.32%	4.88%	9.87%	13.6%	4.32%	7.37%	15.9%	6.52%	12.3%
BEG	10.8	47.2	290	7.78%	4.35%	9.23%	16.1%	6.04%	7.18%	17.9%	7.44%	11.7%
EME	10.1	52.6	303	15.6%	4.64%	7.11%	19.9%	7.01%	10.3%	25.3%	8.41%	12.5%
Morphine	10.1	51.1	311	4.77%	5.90%	4.30%	18.8%	3.52%	7.42%	19.4%	6.87%	8.58%
Codeine	8.78	48.2	283	7.45%	4.46%	4.50%	7.33%	4.34%	3.31%	10.5%	6.22%	5.59%
Dihydrocodeine	11.3	52.7	313	5.74%	4.86%	3.50%	10.7%	2.12%	1.26%	12.1%	5.30%	3.72%
6-MAM	9.92	53.4	309	8.92%	8.71%	6.52%	13.8%	6.19%	4.93%	16.4%	10.7%	8.17%
Methadone	11.4	49.8	302	6.86%	6.75%	7.41%	14.9%	6.89%	8.34%	16.4%	9.65%	11.2%
EDDP	10.7	46.6	282	2.86%	9.28%	6.20%	12.1%	6.74%	7.62%	12.5%	11.5%	9.82%
Buprenorphine	1.12	5.25	31.6	10.1%	4.21%	6.66%	14.3%	7.91%	5.71%	17.5%	8.96%	8.77%
Norbuprenorphine	1.63	8.30	44.3	7.86%	9.01%	1.81%	18.6%	5.38%	3.53%	20.2%	10.5%	3.97%
Ketamine	16.9	99.5	577	8.58%	3.82%	8.18%	12.1%	5.27%	4.07%	14.8%	6.51%	9.14%

ANALYTE	AVER.CONC (ng/mL)			INTRA CV%			INTER CV%			TOTAL CV%		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
THC-COOH	1.94	8.51	53.6	7.20%	4.19%	0.58%	17.1%	4.17%	10.35%	18.6%	5.91%	10.4%

Table 12: Intra-assay, inter-assay and total precision PROCEDURE B

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 Urine	EUM02100	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
Buffer Solution	EUM02021	CE-IVD marked medical device according to Annex III
Precipitant Solution	EUM02022	CE-IVD marked medical device according to Annex III
Hydrolysis Solution	EUM02023	CE-IVD marked medical device according to Annex III
Diluent Solution	EUM02025	CE-IVD marked medical device according to Annex III
Internal Standards Mix for Drugs of Abuse in Urine	EUM02031	CE-IVD marked medical device according to Annex III
6-Levels Calibrators, lyophil.	EUM02042	CE-IVD marked medical device according to Annex III
2-Levels Controls, lyophil.	EUM02055	CE-IVD marked medical device according to Annex III
FloMix DOA	EUM02052	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), 15 april 2022 Director