Psychotropic Medication 3 in serum

Antidepressants

4090 P PS3

Instructions for use, LC-MS/MS assay

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Contents

1. General Information

1.1 Information for the Device

4090 P PS3 - Psychotropic Medication 3 reagent set
UDI-DI: 08720514312810

For information on the individual components of this set, refer to chapter 2 of these instructions for use.

1.2 Intended Purpose

1.2.1 Measurand

Citalopram
Cipramil

Norcitalopram
Lexapro
Cipralex

Fluoxetine
Prozac

Norfluoxetine

Fluvoxamine
Fevarin

Mirtazapine

Normirtazapine

Paroxetine
Seroxat

Sertraline
Zoloft

Norsertraline

Venlafaxine
Effexor

Norvenlafaxine

1.2.2 Function

The function of this device is to aid in the Therapeutic Drug Monitoring (TDM)of several psychotropic medications, refer to paragraph 1.2.1, by the assessment of medicine levels by determination of these levels of psychotropic medication in serum, performed by automated quantitative LC-MS/MS assay technology.

1.2.3 Specific Information indented to be provided

Levels of specified medication in patients.

1.2.4 Required Specimen

Human serum.

1.2.4.1 Conditions for collection, handling and preparation of specimen

Serum tubes are suitable for collecting specimen.
Sample stability is 3 days at 2-8 °C and at least a month at -20 °C[1].

1.2.5 Testing Population

Patients known or suspected to be using one of the measured psychotropic medications specified under paragraph 1.2.1.

1.3 Intended User

Laboratory Professional Use

1.4 Test Principle

In this analytical method, 12 compounds including some metabolites are determined from human serum by UHPLC LC-MS/MS. Prior to the LC-MS/MS analysis, a sample clean-up is performed to remove the sample matrix and to spike the samples with the internal standard. The prepared samples are injected into the UHPLC system and, after separation by chromatography on an analytical Biphenyl column, the compounds are ionized by electrospray ionization (ESI) and detected by LC-MS/MS.

[1] https://tdm-monografie.org/monografieen/tdm-monografieen/

1.5 Clinical Background

Therapeutic Drug Monitoring (TDM) is based on the assumption that there is a relationship between the blood concentration and clinical effect (therapeutic improvement and adverse effects). It also assumes there is a concentration range of the drug which is characterized by maximal effectiveness and maximal safety, the "therapeutic window"[2]. The Diagnotix kit for measuring antidepressants includes fluoxetine (including the metabolite norfluoxetine), fluvoxamine, mirtazapine (including the metabolite normirtazapine), paroxetine, (es)citalopram (including the metabolite norcitalopram), sertraline (including the metabolite norsertraline) and venlafaxine (including the metabolite norvenlafaxine).

The antidepressants are used for the treatment of moderate to major depressive illness, obsessive-compulsive disorder, bulimia nervosa, panic disorders, anxiety disorders, and posttraumatic stress disorder[3,4,5,6,6,7,9,8]. The antidepressant drugs have a pronounced interindividual pharmacokinetic variability and a narrow therapeutic window. Studies on the relation between blood concentrations and clinical improvement have supported this relation for the antidepressant drugs. TDM of fluvoxamine, mirtazapine, escitalopram, sertraline, and venlafaxine is therefore recommended in the consensus guidelines for TDM in Psychiatry[2]. TDM will increase the probability of response in non-responders. At subtherapeutic drug concentrations, there is a risk of poor response and at supratherapeutic drug concentrations, there is an increased risk of intolerance or intoxication. TDM of citalopram is strongly recommended in the consensus guidelines for TDM in Psychiatry[2]. At drug concentrations within the reported therapeutic reference range, the highest probability of response or remission can be expected. At subtherapeutic drug concentrations in blood, the response rate is similar to placebo under acute treatment, and there is a risk of relapse under chronic treatment. At supratherapeutic drug concentrations in blood, there is an increased risk of adverse drug reactions or outright toxicity. The guidelines further state that TDM of fluoxetine and paroxetine is useful for special indications or problem solving. TDM can be used to control whether drug concentrations are in accordance with the dose-related reference range. Clinical improvement can be attained by dose increase in non-responders who display low drug concentrations[2].

[2] Hiemke et al. AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2017. Pharmacopsychiatry 2018;51:9-62.
[3] Summary of Product Characteristics Fluoxetine. Available via: https://www.ema.europa.eu.
[4] Summary of Product Characteristics Fluvoxamine. Available via: https://www.ema.europa.eu.
[5] Summary of Product Characteristics Mirtazapine. Available via: https://www.ema.europa.eu.
[6] Summary of Product Characteristics Paroxetine. Available via: https://www.ema.europa.eu.
[7] Summary of Product Characteristics Citalopram. Available via: https://www.ema.europa.eu.
[8] Summary of Product Characteristics Escitalopram. Available via: https://www.ema.europa.eu. [9] Summary of Product Characteristics Sertraline. Available via: https://www.ema.europa.eu.
[10] Summary of Product Characteristics Venlafaxine. Available via: https://www.ema.europa.eu.

TDM of the antidepressant drugs is recommended for dose titration at the start of the treatment and for special indications, such as in patients with therapeutic failure, adverse events, drugdrug interactions, relevant comorbidities such as elderly patients and patients with altered hepatic and/or renal clearance, patients who start or stop smoking, patients with altered CYP2D6, CYP1A2, CYP3A4, CYP2C9, or CYP2C19 metabolic activity, and if nonadherence is suspected[2,11,12,13,14,15,16]. Reference concentrations are based on literature and an overview of target concentrations can be found in several articles and in the consensus guidelines for TDM in Psychiatry[2,11,9,10,11,12]. Concentrations of (es)citalopram, paroxetine, fluoxetine, sertraline, and venlafaxine in blood were also shown to correlate well with serotonin transporter occupancy[1314,22,15].

Furthermore, TDM-guided dosing of citalopram has the potential to be cost effective by reducing the length of hospitalization and TDM of citalopram, paroxetine and sertraline influences clinical dosing strategies and reduces drug costs in depressed elderly patients[16,17].

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[11] Gerstenberg et al. Relationship between clinical effects of fluvoxamine and the steady-state plasma concentrations of fluvoxamine and its major metabolite fluvoxamino acid in Japanese depressed patients. Psychopharmacology (Berl). 2003;167(4):443-8

[12] Katoh et al. Effects of cigarette smoking and cytochrome P450 2D6 genotype on fluvoxamine concentration in plasma of Japanese patients. Biol Pharm Bull. 2010;33(2):285-8.

[13] Lind et al. Steady-state concentrations of mirtazapine, N-desmethylmirtazapine, 8-hydroxymirtazapine and their enantiomers in relation to cytochrome P450 2D6 genotype, age and smoking behavior. Clin Pharmacokinet. 2009;48(1):63-70.

[14] Chang et al. Impact of cytochrome P450 2C19 polymorphisms on citalopram/escitalopram exposure: systematic review and meta-analysis. Clin Pharmacokinet. 2014;53(9):801-11.

[15] Gjestad et al. Effect of proton pump inhibitors on the serum concentrations of the selective serotonin reuptake inhibitors citalopram, escitalopram, and sertraline. Ther Drug Monit. 2015;37(1):90-7.

[16] Wenzel-Seifert et al. Influence of concomitant medications on the total clearance and the risk for supra-therapeutic plasma concentrations of Citalopram. A population-based cohort study. Pharmacopsychiatry. 2014;47(7):239-44.

[17] Tomita et al. Therapeutic reference range for plasma concentrations of paroxetine in patients with major depressive disorders. Ther Drug Monit. 2014;36(4):480-5.

[18] Ostad Haji et al. Association between citalopram serum levels and clinical improvement of patients with major depression. J Clin Psychopharmacol. 2011;31(3):281-6.

[19] Grasmäder et al. Relationship between mirtazapine dose, plasma concentration, response, and side effects in clinical practice. Pharmacopsychiatry. 2005;38(3):113-7

[20] Gex-Fabry et al. Time course of clinical response to venlafaxine: relevance of plasma level and chirality. Eur J Clin Pharmacol. 2004;59(12):883-91

[21] Meyer. Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. J Psychiatry Neurosci. 2007;32(2):86-102.

[22] Meyer et al. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. Am J Psychiatry. 2004;161(5):826-35.

[23] Gründer et al. Therapeutic plasma concentrations of antidepressants and antipsychotics: lessons from PET imaging. Pharmacopsychiatry. 2011;44(6):236-48,

[24] Ostad Haji et al. Potential cost-effectiveness of therapeutic drug monitoring for depressed patients treated with citalopram. Ther Drug Monit. 2013;35(3):396-401.

[25] Lundmark et al. Therapeutic drug monitoring of selective serotonin reuptake inhibitors influences clinical dosing strategies and reduces drug costs in depressed elderly patients. Acta Psychiatr Scand. 2000;101(5):354-9.

Besides TDM, measuring blood concentrations of the antidepressant drugs is helpful in the management of an intoxication with one of these drugs. Measuring blood concentrations will help to identify intoxications and guide clinical patient management[18,19,28]. In such situations, the Diagnotix kit could provide quick measurement of blood concentrations to guide the treatment of intoxications with fluoxetine, fluvoxamine, mirtazapine, paroxetine, (es)citalopram, sertraline, and venlafaxine.

1.6 Notice Regarding Serious Incidents

Following (EU) 2017/746 Annex I, Chapter III, 20.4.1 af), any serious incident that has occurred in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

[26] Borys et al. Acute fluoxetine overdose: a report of 234 cases. Am J Emerg Med. 1992;10(2):115-20.
[27] Kirkton et al. Therapeutic and toxic concentrations of mirtazapine. J Anal Toxicol. 2006;30(9):687-91.
[28] Jimmink et al. Clinical toxicology of citalopram after acute intoxication with the sole drug or in combination with other drugs: overview of 26 cases. Ther Drug Monit. 2008;30(3):365-71.

2. Components and Accessories

2.1 Description of Components

All components are for LC-MS/MS use only, components also may contain other ingredients than those listed as active ingredients below which might influence the measurement. All declared stabilities are only valid in case of no bacterial contamination.

2.1.1 Calibrators and Controls

4091 CAL P PS3 | Psychotropic Medication 3 Calibrator Set
UDI-DI: 8720514311554

A six-point lyophilized serum calibrator at clinically relevant levels, refer to the value data sheet provided with each set for specific values per production batch.

4092 CON P PS3 | Psychotropic Medication 3 Control Set
UDI-DI: 8720514311561
4101 P PS3 | Psychotropic Medication 3 Control I
UDI-DI: 8720514311646
4102 P PS3 | Psychotropic Medication 3 Control II
UDI-DI: 8720514311653
4103 P PS3 | Psychotropic Medication 3 Control III
UDI-DI: 8720514311660

Three levels of lyophilized serum controls at clinically relevant levels for quality control purposes, refer to the value data sheet provided with each set for specific values per production batch.

2.1.1.1 Handling

Reconstitute the calibrators and controls as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Psychotropic Medication 3 Calibrator Set and Controls with exactly 500 µl distilled or deionised water using a volumetric pipette.
3. Re-place the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Swirl the vial carefully, do not shake. Avoid foaming.
7. Use the preparation as a patient sample.

2.1.1.2 Stability and Storage

The stability of the calibrators and controls are:

Before reconstitution: 2 - 8 °C Until expiry date printed on the product label
Use immediately after reconstitution, do not store.

The declared stated stabilities are only valid in case of no bacterial contamination.

2.1.1.1 Metrological Traceability

Metrological traceability is established by comparing each batch to the highest available order of reference material, as well as the last batch produced before the current batch.

For Psychotropic Medication 3 the highest available order of reference material has been established to be the material from Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology, which is a section of the reference laboratory network of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML).

Refer to the Metrological Traceability report for this specific set for more information.

2.1.2 Internal Standard

4099 P PS3 | Psychotropic Medication 3 Internal Standard
UDI-DI: 8720514311639

A lyophilized deuterated version of the measurands. Used to identify and correct potential deviating values as well as sample specific interferences, due to errors or varying circumstances in sample preparation or within the LC-MS/MS.

Active ingredient(s):

Citalopram-D6, Venlafaxine-D6, Norvenlafaxine-D6, Fluoxetine-D5, Norfluoxetine-D5, Sertraline-D3, Norcitalopram-D3, Fluvoxamine-D3, Mirtazapine-D3, Normirtazapine-D6, Paroxetine-D6, Norsertraline 13C6

2.1.2.1 Handling

Reconstitute the internal standard as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Psychotropic Medication 3 Internal Standard with exactly 2.5 ml distilled or deionised water using a volumetric pipette.
3. Re-place the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Swirl the vial carefully, do not shake. Avoid foaming.

2.1.2.2 Stability and Storage

The stability of the internal standard is:

Before reconstitution: 2 - 8 °C Until expiry date printed on the product label
Use immediately after reconstitution, do not store.

The declared stated stabilities are only valid in case of no bacterial contamination.

2.1.3 Deproteinization Solution

4100 P PS3 | Psychotropic Medication 3 Deproteinization Solution
UDI-DI: 8720514312223

A solution provided to deproteinize the sample in order to remove any interfering substances from the matrix.

Active ingredient(s):	Methanol	25 - <50%
	Acetonitrile	10 - <25%

2.1.3.1 Handling

The Reagent is liquid and ready for use.

2.1.3.2 Storage and Stability

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C.

The declared stated stabilities are only valid in case of no bacterial contamination.

2.1.4 Mobile Phases

4104 P PS3 | Psychotropic Medication 3 Mobile Phase I
UDI-DI: 8720514311677
4105 P PS3 | Psychotropic Medication 3 Mobile Phase II
UDI-DI: 8720514311684

Two mobile phases are added to carry the sample through the LC-MS. Different ratios of the mobile phases will allow different components to eluate from the column at differing speeds.

Active ingredient(s):

Mobile Phase I:	Acetonitrile	2.5 - <10%
Mobile Phase II:	Acetonitrile	75%-<100%

2.1.4.1 Handling

The Reagents are liquid and ready for use.

2.1.4.2 Stability and storage

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC.

2.1.5 Autosampler Washing Solution

4106 P PS3 | Psychotropic Medication 3 Autosampler Washing Solution UDI-DI: 8720514311691

A solution used to clean the LC-MS system after use, specifically designed to remove residue from testing the measurand.

Active ingredient(s): Acetonitrile 50% - <75%

2.1.5.1 Handling

The Reagent is liquid and ready for use.

2.1.5.2 Stability and storage

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC.

2.2 List of components provided

4090 KIT M PS3 - Complete Kit for Psychotropic Medication 3 in whole blood

Contents (for 300 assays):

Psychotropic Medication 3 Calibrator Set (Calibrator 1 – 6)	4091 CAL P PS3	6 x 2 x 500 µl
Psychotropic Medication 3 Internal Standard	4099 P PS3	6 x 2.5 ml
Psychotropic Medication 3 Deproteinization Solution	4100 P PS3	3 x 40 ml
Psychotropic Medication 3 Mobile Phase I	4104 P PS3	2 x 250 ml
Psychotropic Medication 3 Mobile Phase II	4105 P PS3	2 x 250 ml
Psychotropic Medication 3 Autosampler washing solution	4106 P PS3	1 x 500 ml
Psychotropic Medication 3 Manual		

2.3 Separately available materials and components

Psychotropic Medication 3 Calibrator Set (Calibrator 1 – 6)	4091 CAL P PS3	6 x 2 x 500 µl
Psychotropic Medication 3 Internal standard	4099.10 P PS3	10 x 2,5 ml
Psychotropic Medication 3 Deproteinization Solution	4100 P PS3	40 ml
Psychotropic Medication 3 Mobile Phase I	4104 P PS3	250 ml
Psychotropic Medication 3 Mobile Phase II	4105 P PS3	250 ml
Psychotropic Medication 3 Autosampler washing solution	4106 P PS3	500 ml

Analytical column Kinetex Biphenyl 2.7 µm, 150 x 2.1 mm	00F-4622-AN	1 pc
Psychotropic Medication 3 Control I	4101.10 P PS3	10 x 500 µl
Psychotropic Medication 3 Control II	4102 .10P PS3	10 x 500 µl
Psychotropic Medication 3 Control III	4103.10 P PS3	10 x 500 µl

Psychotropic Medication 3 Control Set	4092 CON P PS3	3 x 3 x 500 µl

3. Warnings, precautions, measures and limitations of use

3.1 General

The device and its components must only be used in line with the intended purpose by the intended user as stated in chapter 1. Due to their nature, most reagents of this device contain or are largely composed of hazardous substances. Please refer to the Safety Data Sheets (SDS) for each of the components for specific hazards and measures to be taken.

Used components should be discarded and are not suitable for re-use.

3.1.1 Potentially infectious material

The human serum used for manufacturing calibrators and controls was tested for the following infectious markers and found negative: HIV1/2-, HBV- and HCV-antibodies, Hepatitis B-surface antigen, HIV1- and HCV-RNA, HBV-DNA (NAT). Nevertheless, the serum controls should be considered as potentially infectious and treated with appropriate care.

3.2 Interferences & Limitations

Visual evidence of lipemia, homolysis, or icterus (hyperbilirubinemia) and/or older age of the specimen may affect the performance of the device.

3.3 CMR substances

No CMR substances are used in the manufacturing of this kit or its components.

3.4 Disposal

For the safe disposal of the components of this kit, please refer to the safety data sheet of the component in question.

4. Assay procedure

4.1 Settings and procedure

4.1.1 Required instruments and LC modules

Using this test kit requires a UHPLC system with tandem mass spectrometer (LC-MS/MS) with the following modules:

- Autosampler
- UHPLC gradient pump
- Column heater
- Degasser

4.2 The analytical system

4.2.1 Preparation of the analytical system

- Flush the LC system excluding the column.
- Set the UHPLC pump at a flow rate of 1 ml/min and flush the system for 10 minutes with Mobile Phase I and II (50 : 50).
- Connect the column with the column heater.
  (see arrow marking on the column)

After flushing the system, the equilibration is performed as follows:

- Set the UHPLC pump to a flow rate of 0.4 ml/min.
- Set the column heater to 40°C.
- Equilibrate the column for 15 minutes with Mobile Phase I.
- Start the program for the gradient and equilibrate for another 10 minutes.

4.2.2 Starting the analytical system

- Equilibrate the system.
- Check the temperature of the column.
- Initialize the injector.
- Start the programme on the LC-MS/MS system.

4.3 LC-MS/MS Parameters and Condition

4.3.1 LC Parameters

UHPLC pump	Flow rate 0.4 ml/min
Mobile Phases I and II	Close the bottles to avoid alteration of RT's through evaporation of the mobile phases
Column	The column is installed in the column heater at 30°C. For the complete UHPLC system the backpressure should not exceed 1000 bar. 1 bar = 14.5 PSI

4.3.2 Autosampler Conditions

Injection volume:	2 - 20 µL
Sample temperature:	10 °C
Runtime:	7 min
Column temperature:	30 °C ± 2 °C alarm
Needle wash:	Pre-injection wash: 0 sec. Post-injection wash: 12 sec.
Seal Wash:	10:90 ACN:H_2O
Wash Solvent:	Auto sampler Washing Solution; 50:50 H_2O:ACN
Collision gas:	Argon

4.3.3 Gradient

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.4	95	5	Initial
1.4	0.4	70	30	6
2.6	0.4	50	50	6
3.1	0.4	0	100	6
3.5	0.4	0	100	6
5	0.4	95	5	11
6	0.4	95	5	11
7	0.4	95	5	11

Please note that the gradient is dependent on the analyser used. End users will need to define the optimal gradient for the analyser in use.

4.3.4 MS Conditions (e.g. Waters Xevo TQS-Micro)

MS System:	(Waters Xevo TQS Micro)
Ion mode:	Electrospray
Capillary voltage:	2.5 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	350°C
Desolvation gas flow:	550 L/hr
Detection mode:	ESI
Dwell time:	Auto

Collision gas: Argon

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Citalopram	325.1	108.95	0.002	20	25
	325	262.1	0.002	20	16

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norcitalopram	311	109	0.002	20	25
	311	261.7	0.002	20	16

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Fluoxetine	310.15	44.25	0.002	15	32
	310.15	148.1	0.002	15	9

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norfluoxetine	296.2	134.1	0.002	40	6
	296.2	30.30	0.002	40	4

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Fluvoxamine	319.2	71.15	0.002	30	15
	319.2	87.2	0.002	30	15

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Mirtazapine	266.3	195.2	0.002	50	30
	266.3	72.2	0.002	50	15

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Normirtazapine	252.1	208.9	0.002	40	20
	252.1	195.1	0.002	50	15

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Paroxetine	330.2	192	0.002	40	16
	330.2	70	0.002	40	16

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Sertraline	306.1	158.9	0.002	20	25
	306.1	275	0.002	20	12

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norsertraline	274.9	158.85	0.002	35	18
	274.9	90.9	0.002	30	12

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Venlafaxine	278.2	58	0.002	20	18
	278.2	121	0.002	20	25

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norvenlafaxine	264.1	246.1	0.002	20	11
	264.1	58	0.002	20	15

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Citalopram-D6	331.1	108.95	0.002	20	25
	331.1	262.1	0.002	20	18

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norcitalopram-D3	314	109	0.002	20	25
	314	261.7	0.002	20	16

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Fluoxetine-D5	315.15	44.25	0.002	15	32
	315.15	153.1	0.002	15	9

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norfluoxetine-D5	301.2	139.1	0.002	40	6

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Fluvoxamine-D3	322.2	74.15	0.002	30	15
	322.2	90.2	0.002	30	15

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Mirtazapine-D3	269.3	195.2	0.002	50	30
	269.3	75.2	0.002	50	15

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Normirtazapine-D6	258.1	197.05	0.002	50	15
	258.1	213.2	0.002	40	20

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Paroxetine-D6	336.2	198	0.002	40	16
	336.2	76	0.002	40	16

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Sertraline-D3	309.1	278	0.002	20	12
	309.1	158.9	0.002	20	25

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Venlafaxine-D6	284.2	64	0.002	20	18
	284.2	121	0.002	20	25

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norvenlafaxine-D6	270.1	64	0.002	20	15
	270.1	132.9	0.002	20	25

Substance	Precursor	Product	Dwell (s)	Cone (V)	Collision (V)
Norsertraline 13C6					

These conditions are an indication, optimal values can differ slightly between different LC-MS/MS systems.

4.4 Sample Preparation

4.4.1 Reconstitution of the lyophilised Calibrators / Controls.

Refer to paragraph 2.1.1.1 and the product value data sheets.

4.4.2 Sample preparation (Serum, calibrator or control)

1. 100 µl sample (Calibrator, Control, Patient sample) into a vial.
2. While mixing on a vortex mixer add 50 µl Internal Standard.
3. Keep mixing and add 350 µl Deproteinization Solution.
4. Centrifuge (5min, 10000x g or more)
5. Pipette the centrifuged supernatant into a vial or 96 well plate, which is suitable for the autosampler in use and inject 2-20 µl in the LC-MS/MS.

4.4.3 Sample Preparation with pipette robot

Into a 2 ml 96 well plate:

1. Add 100 µ sample (Calibrator, Control, Patient sample) into a 96 well plate.

2. Whilst mixing the plate, add 50 µl Internal Standard.
3. Whilst continuing to mix the plate, add 350 µl Deproteinization Solution.
4. Once mixing is complete, centrifuge (5 min , 10000x g or more).
5. Transfer the samples into a 1 ml 96 well collection plate for injection on the UHPLC/MS/MS system (Needle placement 2 mm) or inject directly off the pellet (Needle placement 10 mm) 2-20 µl in the LC-MS/MS.

4.5 Interpretation of results

4.5.1 Results from LC-MS and Reference Values

The assay will result in a certain value for the measurand, which will need to be compared to applicable reference values to be interpreted for the specific patient.

For illustrative purposes only, an example of reference values for this device can be used as follows:

As the measured substances do not naturally occur in the human body, scientific literature states the reference values as not applicable. For pharmaceutical substances different levels apply such as regular dosage, upper limit and toxicity. As this information is (patient) specific and of a technical nature, Diagnotix refers to the medical expert under whose authority testing is conducted. As an example we refer to data from the Dutch Association of Hospital Pharmacists, where available[1]. Diagnotix recommends using equivalent national authorities for country specific reference values.

The inclusion of this information is required by Annex I, section 20.4.1 (v) of the IVDR. Diagnotix employs no medically trained professionals and can only indicate possible ways of interpreting results based on published scientific literature. Always consult a trained medical professional with expertise in the area of interest for this kit for interpretation of results.

Interpretation of the results of this test also depends significantly on the individual characteristics of the patient involved. Diagnotix recommends taking these inputs into consideration as well.

5. Summary of Analytical Performance Characteristics

Analytical performance characteristics have been defined by validation of the assay according to IVDR parameters, and using EP Evaluator to extract statistical data from the acquired raw data.

5.1 Repeatability (Simple Precision)

The Repeatability, or Simple Precision, was analyzed by measuring a patient sample, Psychotropic Medication 3 control I and Psychotropic Medication 3 Control III twenty times from one sample within two hours from each other. From these results the Coefficient of

Variation (CV) is calculated and compared to the precision verification goal which is for low samples 10 % and for normal and high samples 7.5 %

	CV		
Sample	**4101 P PS3 Control I Lot: 14B22/16**	**4103 P PS3 Control III Lot: 14B22/18**	**Patient**
Citalopram	3.0 %	2.7 %	2.1 %
Fluoxetine	2.7 %	2.7 %	2.2 %
Fluvoxamine	2.8 %	2.0 %	2.3 %
Mirtazapine	6.6 %	4.9 %	4.8 %
Norcitalopram	4.8 %	2.5 %	3.7 %
Norfluoxetine	3.4 %	1.9 %	2.2 %
Normirtazapine	6.4 %	6.5 %	6.1 %
Norsertraline	3.7 %	3.9 %	2.9 %
Norvenlafaxine	3.2 %	2.9 %	3.2 %
Paroxetine	9.1 %	4.7 %	5.0 %
Sertraline	4.0 %	2.9 %	2.7 %
Venlafaxine	2.9 %	1.9 %	1.8 %

5.2 Reproducibility (Complex Precision)

The Reproducibility, or Complex Precision, was analyzed by measuring a patient sample, Psychotropic Medication 3 control I and Psychotropic Medication 3 control III in duplicate twenty times. Each time the sample preparation had a variance (different analyst, pipet, day, reagent temperature and/or calibration). This to simulate twenty different days in a laboratory. From these results the Coefficient of Variation (CV) is calculated and compared to the precision verification goal which is for low samples 15% and for normal and high samples 12.5%.

	CV		
Sample	**4101 P PS3 Control I Lot: 14B22/16**	**4103 P PS3 Control III Lot: 14B22/18**	**Patient**
Citalopram	5.9 %	5.2 %	6.1 %
Fluoxetine	4.4 %	4.7 %	5.6 %
Fluvoxamine	5.0 %	3.7 %	5.9 %
Mirtazapine	7.7 %	7.4 %	8.4 %
Norcitalopram	5.7 %	4.7 %	5.5 %
Norfluoxetine	4.5 %	3.5 %	4.7 %
Normirtazapine	8.7 %	8.0 %	9.0 %
Norsertraline	6.3 %	5.4 %	6.8 %
Norvenlafaxine	4.8 %	5.0 %	5.3 %
Paroxetine	11.6 %	8.7 %	9.8 %
Sertraline	6.3 %	6.3 %	6.4 %
Venlafaxine	4.7 %	3.9 %	4.3 %

5.3 Linearity

The linearity was analyzed by preparing a series of incrementally increasing psychotropic medication concentrations twice. These samples were measured in duplicate from which the linearity was verified and upper limed of detection was calculated.

Analyte	**Linearity (µg/l)**
Norcitolapram	2000
Fluoxetine	3200
Fluvoxamine	2600
Mirtazapine	550
Norcitalopram	1000
Norfluoxetine	2500
Normirtazapine	490
Norsertraline	2300
Norvenlafaxine	2800
Paroxetine	1800
Sertraline	1100
Venlafaxine	1600

5.4 Limit of Blank

The Limit of Blank (LOB) was analyzed by measuring Psychotropic Medication 3 Calibrator 1 (zero) 20 times and Calibrator 2 (non-zero) 5 times. From the responses the LOB was calculated.

Analyte	**LOB (µg/l)**
Norcitolapram	0.42

Fluoxetine	0.184
Fluvoxamine	0.0683
Mirtazapine	0.0655
Norcitalopram	0.42
Norfluoxetine	0.173
Normirtazapine	0.476
Norsertraline	0.712
Norvenlafaxine	0.897
Paroxetine	0.0944
Sertraline	0.0381
Venlafaxine	0.154

5.5 Accuracy

The accuracy of the method was determined by measuring the subscription schemes from the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML). This organization gathers results from all contributing laboratories and establishes a consensus or average. This in turn is compared to the results from Diagnotix.

All measured samples met the requirement of +/- 20.

5.6 Carry-over

To verify that there is no carryover two samples were prepared. One low (Calibrator 2) and one high (Calibrator 6). The samples were divided into eleven low samples and ten high samples. The samples were measured in a particular order after which the datasets were analyzed.

None of the components showed signs of carryover.

6. Summary of Clinical Performance Characteristics

Not available at the time of this version of the instructions for use.

7. Change log

Version Change	Changed section	What has been changed	Date
V1.1	Several sections	Definitive of final version Several different changes were made	20-12-2024

8. References

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