Psychotropic Medication 1 in serum

Tricyclic Antidepressants

4050 P PS1

Instructions for use, LC-MS/MS assay

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Contents

1. General Information

1.1 Information for the Device

4050 P PS1 - Psychotropic Medication 1 Reagent Set
UDI-DI: 08720514311776

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

1.2 Intended Purpose

This Psychotropic Medication 1 kit is intended for the determination of ten (10) tricyclic antidepressants (TCA's) in serum, conducted by laboratory professionals on LC-MS/MS.

1.2.1 Measurands

Clozapine

Norclozapine
N-Desmethylclozapine

Nortriptyline

E-10-OH-Nortriptyline

Z-10-OH-Nortriptyline

Amitriptyline

Clomipramine

Norclomipramine
N-Desmethylclomipramine

Imipramine

Desipramine

1.2.2 Function

The function of this device is to aid in the monitoring of several psychotropic medication, 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

Deviant measurand values can be an indication of the following physiological or pathological states and/or conditions:

Incorrect dose/use of specified psychotropic medication.

1.2.5 Required Specimen

Human serum.

1.2.5.1 Conditions for collection, handling and preparation of specimen

Serum tubes are suitable for collecting specimen. Sample stability is 4 weeks at -20 °C.

1.2.6 Testing Population

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

1.3 Intended User

Laboratory Professional Use.

1.4 Test Principle

In this analytical method, 6 TCA's and their 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 C-18 column, the compounds are ionized by electrospray ionization (ESI) and detected by LC-MS/MS.

1.5 Clinical background

Tricyclic antidepressants (amitriptyline, nortriptyline, clomipramine, imipramine and desipramine) and antipsychotics (clozapine).

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" [1]. The Diagnotix kit for measuring tricyclic antidepressants (TCAs) and antipsychotics (PS1) includes the TCAs amitriptyline, nortriptyline, clomipramine, imipramine and desipramine (including the drugs' metabolites) and the antipsychotic clozapine (including the metabolite norclozapine).

The TCAs amitriptyline, nortriptyline, clomipramine, imipramine and desipramine are used for the treatment of several types of depression, obsessive compulsive disorder, neuropathic pain, nocturnal enuresis, and for the prophylactic treatment of chronic tension type headache and migraine [2-5]. TCAs have a pronounced inter-individual pharmacokinetic variability and a narrow therapeutic window. Studies on the relation between blood concentration and clinical improvement have supported the relation for the TCAs [1]. Systematic reviews and meta-analyses led to convincing evidence of a significant relationship between clinical outcomes and plasma concentrations for the TCAs which are associated with a high probability of response [1,6-8].

TDM of amitriptyline, nortriptyline, clomipramine, imipramine and desipramine is strongly recommended in the consensus guidelines for TDM in Psychiatry [1]. The guidelines state that reported drug concentrations are established and evaluated therapeutic reference ranges. Controlled clinical trials have shown beneficial effects of TDM, and reports on decreased tolerability or intoxications are present. TDM is therefore strongly recommended for dose titration at the start of the treatment, and for special indications, such as in patients with therapeutic failure, adverse events, drug-drug interactions, relevant comorbidities, altered CYP2D6 or CYP2C19 metabolic activity, and if nonadherence is suspected. For the TCAs, reference concentrations are based on literature and an overview of target concentrations can be found in several articles and the consensus guidelines for TDM in Psychiatry [1,6-8]. Furthermore, the therapeutic range for amitriptyline and nortriptyline is stated in the Summary of Product Characteristics (SPC) of these drugs [2,3].

Clozapine is indicated in treatment-resistant schizophrenic patients and in schizophrenia patients who have severe adverse reactions to other antipsychotic agents, including atypical antipsychotics. Clozapine is also indicated in psychotic disorders occurring in Parkinson's disease, in cases where standard treatment has failed. TDM of clozapine is advised in the SPC in certain clinical situations, such as when a patient ceases smoking or switches to e-cigarettes (altered metabolism of clozapine can lead to altered clozapine exposure), when concomitant medicines may interact and increase of decrease clozapine blood concentration, where poor clozapine metabolism is suspected, when a patient has pneumonia or other serious infection (altered metabolism of clozapine can lead to altered clozapine exposure), and in the event of onset of symptoms suggestive of toxicity (adverse events) [9]. Furthermore, a high inter-patient pharmacokinetic variability of clozapine is seen [10]. This pharmacokinetic variability, in combination with a good correlation between clozapine blood concentrations and efficacy/toxicity makes TDM also useful at the start of clozapine treatment for dose titration, in case of an insufficient response to the treatment, in case of suspected non-adherence, and with the use of high clozapine doses [11-14]. TDM of clozapine is therefore strongly recommended in the consensus guidelines for TDM in Psychiatry [1].

Besides for TDM, measuring blood concentrations of TCAs and antipsychotic drugs is helpful in the management of an intoxication with one of these drugs [15-17]. It is known that these drugs have a small therapeutic window and signs of toxicity are not always easily recognized purely on clinical grounds. Therefore, measuring blood concentrations will help to identify intoxications and guide in clinical patient management. In such situations, the Diagnotix kit could provide in quick measurement of blood concentrations to guide the treatment of intoxications with amitriptyline, nortriptyline, clomipramine, imipramine, desipramine and clozapine.

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.

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.

2.1.1 Calibrators and Controls

4051 CAL P PS1 | Psychotropic Medication 1 Calibrator Set
UDI: 8720514311318

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.

4052 CON P PS1 | Psychotropic Medication 1 Control Set
UDI: 8720514311325

4061 P PS1 | Psychotropic Medication 1 Control I
UDI: 8720514311349
4062 P PS1 | Psychotropic Medication 1 Control II
UDI: 8720514311356
4063 P PS1 | Psychotropic Medication 1 Control III
UDI: 8720514311363

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 1 Calibrator Set and Controls with exactly 500 µl distilled or deionised water using a volumetric pipette.
   Note: Diagnotix strongly recommends the use of type 1 ultrapure (LC-MS grade) water.
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
After reconstitution:	2 - 8 °C	48 hours
After reconstitution:	- 20 °C	2 weeks

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

2.1.1.3 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 1 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 Value Data Sheet for this specific set for more information.

2.1.2 Deproteinization Solution + Internal Standard

4059 P PS1 | Psychotropic Medication 1 Deproteinization Solution + Internal Standard
UDI: 8720514311332

Deuterated versions of the measurands, dissolved in a deproteinizing fluid. The internal standard is used to identify and correct potential deviating values, due to errors or varying circumstances in sample preparation or within the LC-MS/MS.

The Deproteinization Solution in which the internal standard is dissolved is used to remove any interfering substances from the matrix.

Active ingredient(s):

Amitriptyline-D3, Clozapine D4, Clomipramine D3, Desipramine D4, Imipramine D4, Nortriptyline D3 (also used for E-10-OH-Nortriptyline & Z-10-OH-Nortriptyline).

Acetonitrile	75 - <100%
Methanol	10 - <25%

2.1.2.1 Handling

The Reagent is liquid and ready for use.

2.1.2.2 Stability and Storage

The stability of the reagent is:

Store at 2 - 8 °C. After first opening the Reagent can be used for 4 weeks if closed and stored at 2 - 8 °C.

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

2.1.3 Mobile Phases

4064 P PS1 | Psychotropic Medication 1 Mobile Phase I
UDI: 8720514311370

4065 P PS1 | Psychotropic Medication 1 Mobile Phase II
UDI: 8720514311387

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

Active ingredient(s):

Mobile Phase I:	Ultrapure water	
	Methanol	2.5 - <10%
	Ammonium Formate	<1 %
	Formic Acid	<1 %
Mobile Phase II:	Methanol	75% - <100%
	Formic Acid	<1 %

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.

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

2.1.5 Autosampler Washing Solution

4066 P PS1 | Psychotropic Medication 1 Autosampler Washing Solution
UDI: 8720514311394

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

Active ingredient(s):

Methanol	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

4050 KIT P PS1 - Complete Kit for Psychotropic Medication 1 in serum

Contents (for 300 assays):

Psychotropic Medication 1 Calibrator Set (Calibrator 1 – 6)	4051 CAL P PS1	6 x 2 x 500 µl
Psychotropic Medication 1 Deproteinization Solution + Internal Standard	4059 P PS1	3 x 100 ml
Psychotropic Medication 1 Mobile Phase I	4064 P PS1	1 x 500 ml
Psychotropic Medication 1 Mobile Phase II	4065 P PS1	1 x 500 ml
Psychotropic Medication 1 Autosampler Washing Solution	4066 P PS1	1 x 1000 ml
Psychotropic Medication 1 Manual		

2.3 Separately available materials and components

Psychotropic Medication 1 Calibrator Set (Calibrator 1 – 6)	4051 CAL P PS1	6 x 2 x 500 µl
Psychotropic Medication 1 Internal Standard in Deproteinization Solution	4059 P PS1	100 ml
Psychotropic Medication 1 Mobile Phase I	4064 P PS1	500 ml
Psychotropic Medication 1 Mobile Phase II	4065 P PS1	500 ml
Psychotropic Medication 1 Autosampler washing solution	4066 P PS1	1000 ml

Analytical column Acquity uplc HSS T3 1,8 µm 2.1x100 mm	186003539	1 pc
Psychotropic Medication 1 Control I	4061 P PS1	10 x 500 µl
Psychotropic Medication 1 Control II	4062 P PS1	10 x 500 µl
Psychotropic Medication 1 Control III	4063 P PS1	10 x 500 µl
Psychotropic Medication 1 Control Set	4052 CON P PS1	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.

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: HBsAg and antibody to HIV-1, HIV-2 and HIV p24 Ag by combo assay, anti-HTLV 1&2 and to HCV and HIV genome as well as syphilis. Nevertheless, the serum controls should be considered as potentially infectious and treated with appropriate care.

3.2 CMR substances

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

3.3 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 4-8 ml/min and flush the system for 4 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

Please note that the provided LC-MS/MS Parameters and Conditions are derived from the system used by Diagnotix to perform the validation of the analytical performance of this assay kit. Conditions may vary between LC-MS/MS, even between systems of the same type from the same manufacturer. End-user systems used to perform this assay may require optimization.

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 Retention Times (RT) through evaporation of the mobile phases.
Column	The column is installed in the column heater at 40°C. For the complete UHPLC system the backpressure should not exceed 800 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:	40 °C ± 2 °C alarm
Needle wash:	Wash twice for 6 seconds, post injection 12 seconds
Seal Wash:	10:90 MeOH:H2O
Wash Solvent:	Autosampler Washing Solution; 40:60 H2O:MeOH

4.3.3 Gradient

Time (min)	**Flow Rate (mL/min)**	**%A**	**%B**	**Curve**
Initial	0.4	90	10	Initial
1.00	0.4	70	30	6
2.60	0.4	40	60	6
3.10	0.4	30	70	6
3.31	0.4	30	70	6
4.00	0.4	10	90	6
4.50	0.4	0	100	6
5.00	0.4	0	100	6
5.01	0.4	90	10	6
7.00	0.4	90	10	6

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)

MS System:	(Waters Xevo TQS)
Ion mode:	Electrospray
Capillary voltage:	3.0 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	600 °C
Desolvation gas flow:	1200 L/hr
Detection mode:	MRM
Collision gas:	Argon
Cone:	150 L/hr

Substance	Precursor	Product	Cone (V)	Collision (V)
Nortriptyline	264.2	91.15	25	20
Nortriptyline	264.2	233.25	25	15

Substance	Precursor	Product	Cone (V)	Collision (V)
Desipramine	267.1	43.8	25	40
Desipramine	267.1	72.1	25	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Amitryptiline	278.2	91.15	35	22
Amitryptiline	278.2	105.2	35	20
Amitryptiline	278.2	233.3	35	15

Substance	Precursor	Product	Cone (V)	Collision (V)
Imipramine	281.2	58.2	25	25
Imipramine	281.2	86.2	25	20

Substance	Precursor	Product	Cone (V)	Collision (V)
E/Z-OH-Nortriptyline	280.2	215.2	35	40
E/Z-OH-Nortriptyline	280.2	231.2	35	18
E/Z-OH-Nortriptyline	280.2	262.2	35	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Norclomipramine	301.1	44.1	25	40
Norclomipramine	301.1	72.1	25	13
Norclomipramine	301.1	270.1	25	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Norclozapine	313.1	192.1	25	35
Norclozapine	313.1	270.1	25	25

Substance	Precursor	Product	Cone (V)	Collision (V)
Clomipramine	315.2	58.1	25	40
Clomipramine	315.2	86.1	25	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Clozapine	327.2	192.2	25	40
Clozapine	327.2	270.2	25	25

Substance	Precursor	Product	Cone (V)	Collision (V)
Nortriptyline-D3	267.2	91	25	33
Nortriptyline-D3	267.2	191.2	25	20
Nortriptyline-D3	267.2	233.2	25	14

Substance	Precursor	Product	Cone (V)	Collision (V)
Desipramine-D4	271.2	44.1	25	40
Desipramine-D4	271.2	72.1	25	13
Desipramine-D4	271.2	194.8	25	13
Desipramine-D4	271.2	212	25	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Amitriptyline-D3	281.2	91.1	25	22
Amitriptyline-D3	281.2	105.1	25	15
Amitriptyline-D3	281.2	233.2	25	12

Substance	Precursor	Product	Cone (V)	Collision (V)
Imipramine-D4	285.2	58.2	25	25
Imipramine-D4	285.2	86.2	25	20

Substance	Precursor	Product	Cone (V)	Collision (V)
Norclomipramine-D3	304.1	47.1	25	13
Norclomipramine-D3	304.1	72.1	25	13
Norclomipramine-D3	304.1	270.1	25	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Clomipramine-D3	318.2	61.1	25	30
Clomipramine-D3	318.2	89.1	25	13

Substance	Precursor	Product	Cone (V)	Collision (V)
Clozapine-D4	331.2	192.2	25	40
Clozapine-D4	331.2	272.2	25	25

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. Add 25 µl sample (Calibrator, Control, Patient sample) to a container.
2. Add 1000 µl Psychotropic Medication 1 Internal Standard in Deproteinization Solution in the containers using a vortex mixer for 30 seconds.
3. Make sure that all the containers have been shaken for at least 30 seconds.
4. Centrifuge (5 min, 10000 x g or more).
5. Transfer centrifuged supernatant to a vial or 96 well plate, which is suitable for the auto sampler in use and inject 2-20 µl in the LC-MS/MS.

4.4.3 Sample Preparation with pipette robot

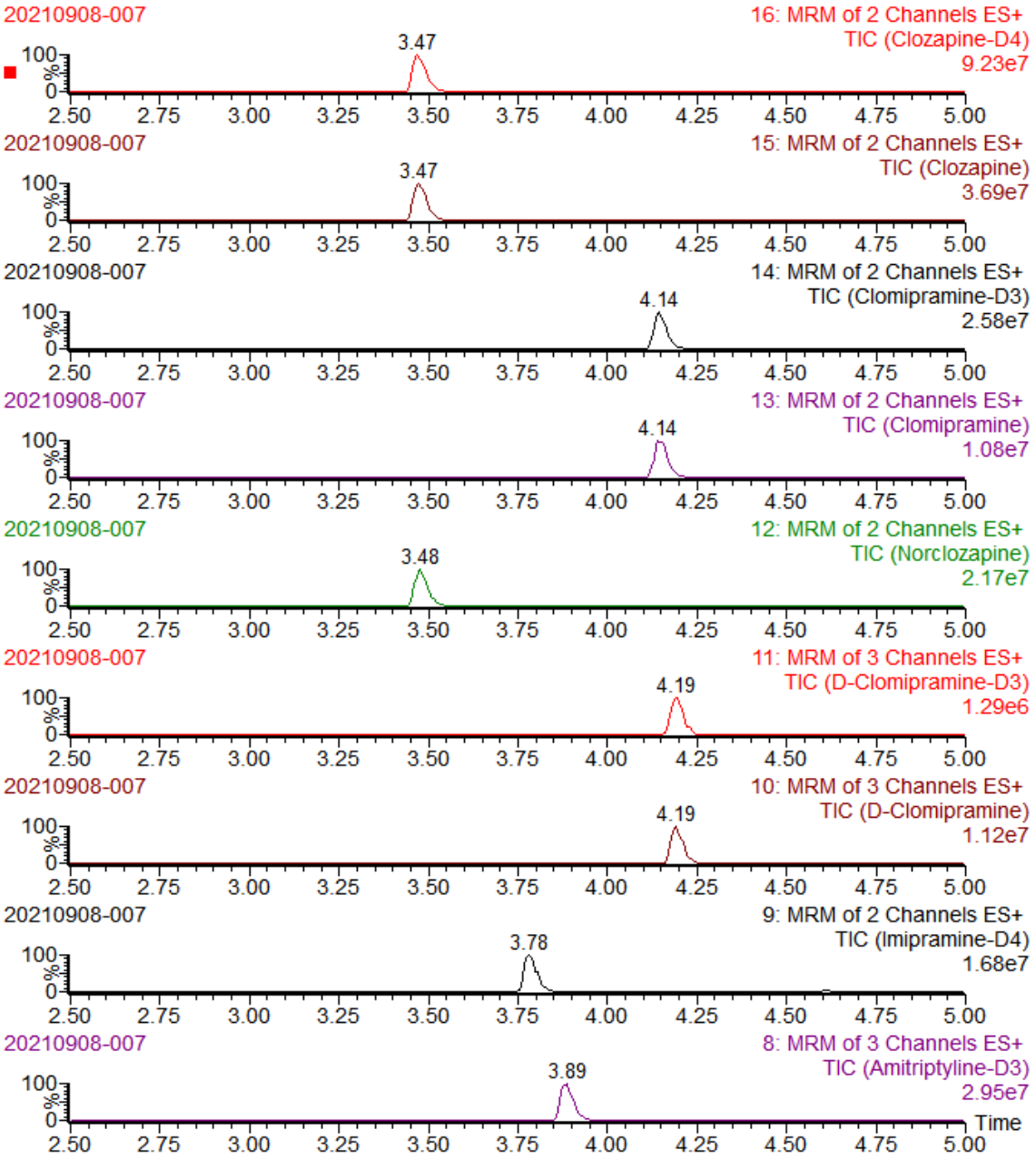
Into a 2 ml 96 well plate:

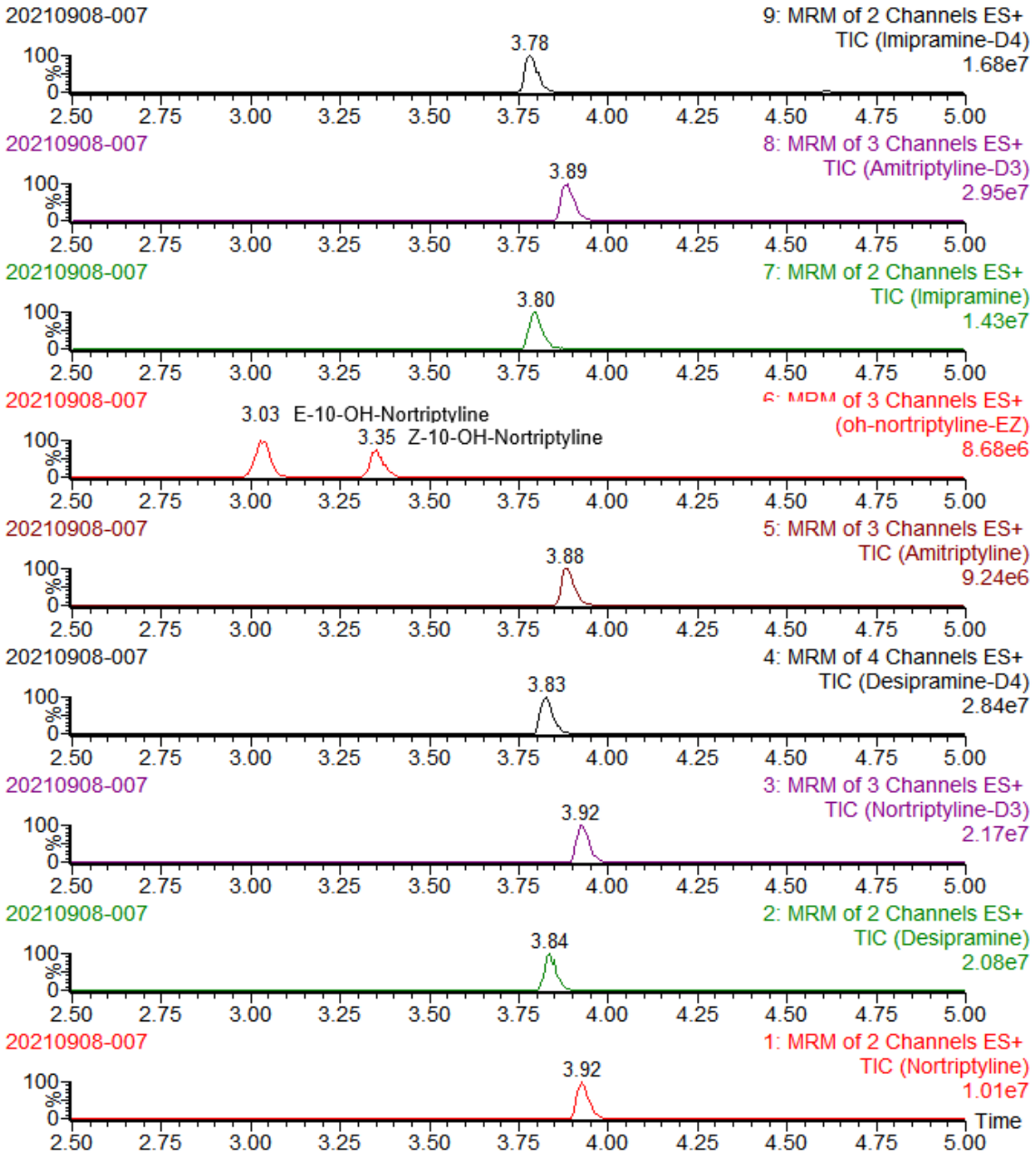
1. Whilst mixing the plate, add 25 µl sample (Calibrator, Control, Patient sample), and leave mixing for 15 minutes.
2. Whilst continuing to mix the plate, add 1000 µl Psychotropic Medication 1 Internal Standard in Deproteinization Solution.
3. Once mixing is complete, centrifuge (5 min , 10000x g or more).
4. Transfer the samples into a 1 ml 96 well collection plate for injection on the UHPLC/MS/MS system or inject 2-20 µl of the pellet directly into the LC-MS/MS.

4.5 Interpretation of results

4.5.1 Examples of chromatograms

Example chromatogram of hypothetical patient sample, recorded with a Waters LC-MS/MS TQS:




20210908-007
9: MRM of 2 Channels ES+
TIC (Imipramine-D4)
1.68e7
3.78
20210908-007
8: MRM of 3 Channels ES+
TIC (Amitriptyline-D3)
2.95e7
3.89
20210908-007
7: MRM of 2 Channels ES+
TIC (Imipramine)
1.43e7
3.80
20210908-007
(oh-nortriptyline-EZ)
8.68e6
3.03 E-10-OH-Nortriptyline
3.35 Z-10-OH-Nortriptyline
20210908-007
5: MRM of 3 Channels ES+
TIC (Amitriptyline)
9.24e6
3.88
20210908-007
4: MRM of 4 Channels ES+
TIC (Desipramine-D4)
2.84e7
3.83
20210908-007
3: MRM of 3 Channels ES+
TIC (Nortriptyline-D3)
2.17e7
3.92
20210908-007
2: MRM of 2 Channels ES+
TIC (Desipramine)
2.08e7
3.84
20210908-007
1: MRM of 2 Channels ES+
TIC (Nortriptyline)
1.01e7
3.92
100
%
0
2.50 2.75 3.00 3.25 3.50 3.75 4.00 4.25 4.50 4.75 5.00
Time

4.5.2 Results from LC-MS/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 so (patient) specific and of a technical nature Diagnotix refers to the medical expert under whose authority testing is conducted. [18]

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 in a significant way 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 1 control I and Psychotropic Medication 1 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 in turn is calculated using the biological variation.

	CV		
Sample	**4061 P PS1 Control I Lot: 15B21/07**	**4063 P PS1 Control III Lot: 15B21/09**	**Patient**
Amitriptyline	3.7 %	1.8 %	2.3 %
Clomipramine	2.0 %	1.2 %	1.7 %
Clozapine	2.4 %	1.3 %	1.6 %
Desipramine	2.8 %	1.5 %	2.2 %
Imipramine	4.1 %	2.2 %	3.3 %
Norclomipramine	2.3 %	1.2 %	1.8 %
Norclozapine	3.3 %	1.9 %	2.2 %
Nortriptyline	6.6 %	4.6 %	4.5 %
E-10-OH-Nortriptyline	4.0 %	3.5 %	3.5 %
Z-10-OH-Nortriptyline	4.1 %	3.5 %	5.3 %

5.2 Reproducibility (Complex Precision)

The Reproducibility, or Complex Precision, was analyzed by measuring a patient sample, Psychotropic Medication 1 control I and Psychotropic Medication 1 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 10% and for normal and high samples 7.5%.

	CV		
Component	**4061 P PS1 Control I Lot: 15B21/07**	**4063 P PS1 Control III Lot: 15B21/09**	**Patient**
Amitriptyline	8.0 %	5.0 %	4.7 %
Clomipramine	6.4 %	4.6 %	5.9 %
Clozapine	4.9 %	4.8 %	4.7 %
Desipramine	4.9 %	4.4 %	5.5 %
Imipramine	7.1 %	6.6 %	6.0 %
Norclomipramine	6.8 %	4.9 %	5.5 %
Norclozapine	5.0 %	4.4 %	4.7 %
Nortriptyline	10.6 %	4.6 %	5.4 %
E-10-OH-Nortriptyline	8.1 %	5.0 %	6.0 %
Z-10-OH-Nortriptyline	8.1 %	5.1 %	6.1 %

5.3 Linearity

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

Analyte	**Linearity (µg/l)**
Amitriptyline	1800
Clomipramine	1400
Clozapine	2200
Desipramine	1200
Imipramine	2000
Norclomipramine	1175
Norclozapine	2600
Nortriptyline	1250
E-10-OH-Nortriptyline	1500
Z-10-OH-Nortriptyline	288

5.4 Limit of Blank

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

Analyte	**LOB (µg/l)**
Amitriptyline	0.236
Clomipramine	0.229
Clozapine	0.0717
Desipramine	0.0575
Imipramine	0.145
Norclomipramine	0.192
Norclozapine	0.139
Nortriptyline	1.28
E-10-OH-Nortriptyline	0.304
Z-10-OH-Nortriptyline	0

5.5 Limit of Quantification

The Limit of Quantification (LOQ) was analyzed by preparing a series of 8 incrementally decreasing concentrations. These samples were measured twelve times and the limit of quantification was calculated.

Analyte	**LOQ (µg/l)**
Amitriptyline	< 3.6588
Clomipramine	< 6.1184
Clozapine	< 11.0454
Desipramine	< 4.2668
Imipramine	< 4.9726
Norclomipramine	< 4.3137
Norclozapine	< 6.4608
Nortriptyline	5.6061
E-10-OH-Nortriptyline	15.7476
Z-10-OH-Nortriptyline	4.3244

5.6 Carryover

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.

5.7 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 +/- 15%.*

* The scheme did not include Norclozapine due to lack of reference material.

6. Summary of Clinical Performance Characteristics

Not available at the time of this version of the Instructions for Use.

7. References

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