Psychotropic Medication 2 in serum

Neuroleptics

4070 P PS2

Instructions for use, LC-MS/MS assay

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Contents

1. General Information

1.1 Information for the Device

4070 P PS2 - Psychotropic Medication 2 Reagent Set
UDI-DI: 08720514311813

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 2 kit is intended for the determination of seven (7) antipsychotic medications (several of which can be referred to as neuroleptics) in serum, conducted by laboratory professionals on LC-MS/MS.

1.2.1 Measurand

Aripiprazole

Clozapine

Haloperidol

Olanzapine

Quetiapine

Risperidone

9-OH-risperidone
paliperidone

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 2 days at 2-8 °C and at least a week 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, seven components 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.

1.5 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 antipsychotics includes aripiprazole, olanzapine, risperidone (including the metabolite 9-OH-risperidone, also paliperidone), quetiapine, haloperidol, and clozapine.

The antipsychotics are used for the treatment of schizophrenia and schizoaffective disorder, moderate to severe manic episodes, bipolar disorder, treatment of persistent aggression and psychotic symptoms in patients with moderate to severe Alzheimer's dementia, psychosis during the course of Parkinson's disease, acute treatment of delirium, treatment of tic disorders including Tourette's syndrome, and treatment of mild to moderate chorea in Huntington's disease[3,4,5,6,7,8].

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[1] https://tdm-monografie.org/monografieen/tdm-monografieen/
[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 Olanzapine. Available via: https://www.ema.europa.eu
[4] Summary of Product Characteristics Quetiapine. Available via: https://www.ema.europa.eu
[5] Summary of Product Characteristics Aripiprazole. Available via: https://www.ema.europa.eu
[6] Summary of Product Characteristics Risperidon. Available via: https://www.ema.europa.eu
[7] Summary of Product Characteristics Zaponex. Available via: https://www.ema.europa.eu.
[8] Summary of Product Characteristics Haloperidol. Available via: https://www.ema.europa.eu.

The antipsychotic drugs have a pronounced inter-individual pharmacokinetic variability and a narrow therapeutic window. Studies on the relation between blood concentration and clinical improvement have supported this relation for the antipsychotic drugs. TDM of aripiprazole, risperidone, and quetiapine is therefore recommended in the consensus guidelines for TDM in Psychiatry. 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 clozapine, olanzapine, and haloperidol is strongly recommended in the consensus guidelines for TDM in Psychiatry. 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[1].

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 such as patients with altered hepatic and/or renal clearance, presence of an infection, or patients who start or stop smoking, patients with altered CYP2D6, CYP1A2, CYP3A4, or CYP2C19 metabolic activity, and if nonadherence is suspected. 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[1].

TDM of clozapine is also advised in the Summary of Product Characteristics (SPC) in clinical situations, such as when a patient ceases smoking (altered metabolism of clozapine can lead to altered clozapine exposure), when concomitant drugs may interact and increase or 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)[6]. Furthermore, a high inter-patient pharmacokinetic variability of clozapine is seen[9]. 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[10,11,12,13].

[9] Summary of Product Characteristics Haloperidol. Available via: https://www.ema.europa.eu.
[10] Perry et al. Clozapine and norclozapine plasma concentrations and clinical response of treatment-refractory schizophrenic patients. Am J Psychiatry 1991;148(2):231-5
[11] Couchman et al. Plasma clozapine, norclozapine, and the clozapine:norclozapine ratio in relation to prescribed dose and other factors: data from a therapeutic drug monitoring service, 1993-2007. Ther Drug Monit 2010;32(4):438-47
[12] Couchman et al. Plasma clozapine, norclozapine, and the clozapine:norclozapine ratio in relation to prescribed dose and other factors: data from a therapeutic drug monitoring service, 1993-2007. Ther Drug Monit 2010;32(4):438-47
[13] Khan et al. Examining concentration-dependent toxicity of clozapine: role of therapeutic drug monitoring. J Psychiatr Pract 2005;11(5):289-301

TDM of haloperidol is highly recommended because of the high inter-individual variability in blood concentration, and especially when other drugs like anti-epileptics or antiparkinsonian drugs are administered[14,15,16]. TDM of haloperidol is also advised in the SPC due to the high inter-subject variability in haloperidol pharmacokinetics and the concentration-effect relationship[7].

Also for newer antipsychotic drugs like aripiprazole, olanzapine, quetiapine, risperidone, and 9-OH-risperidone, relationships between drug concentration in blood and clinical effectiveness have been reported[11,13,14,17,18,19,20,21,22,23].

Besides for TDM, measuring blood concentrations of antipsychotic drugs is helpful in the management of an intoxication with one of these drugs[1,24,25,26] [1,23-25]. Measuring blood concentrations will help to identify intoxications and guide clinical patient management. In such situations, the Diagnotix kit could provide quick, sensitive and reliable measurement of blood concentrations to guide the treatment of intoxications with aripiprazole, olanzapine, risperidone, paliperidone, quetiapine, haloperidol, or clozapine by LC-MS/MS.

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.

[14] Patteet et al. Therapeutic drug monitoring of common antipsychotics. Ther Drug Monit. 2012;34(6):629-51
[15] Patteet et al. Genotype and co-medication dependent CYP2D6 metabolic activity: effects on serum concentrations of aripiprazole, haloperidol, risperidone, paliperidone and zuclopenthixol. Eur J Clin Pharmacol. 2016;72(2):175-84
[16] Ulrich et al. The relationship between serum concentration and therapeutic effect of haloperidol in patients with acute schizophrenia. Clin Pharmacokinet. 1998;34(3):227-63
[17] Kirschbaum et al. Serum levels of aripiprazole and dehydroaripiprazole, clinical response and side effects. World J Biol Psychiatry. 2008;9(3):212-8
[18] Lin et al. Aripiprazole and dehydroaripiprazole plasma concentrations and clinical responses in patients with schizophrenia. Clinical Trial J Clin Psychopharmacol. 2011;31(6):758-62
[19] Perry et al. Olanzapine plasma concentrations and clinical response in acutely ill schizophrenic patients. J Clin Psychopharmacol. 1997;17(6):472-7
[20] Yasui-Furukori et al. Clinical response to risperidone in relation to plasma drug concentrations in acutely exacerbated schizophrenic patients. J Psychopharmacol. 2010;24(7):987-94
[21] Dragicevic et al. Serum Concentrations of Quetiapine and Clinical Effects. Pharmacopsychiatry 2005;38-23
[22] Bergemann et al. Olanzapine plasma concentration, average daily dose, and interaction with co-medication in schizophrenic patients. Pharmacopsychiatry. 2004;37(2):63-8
[23] Bergemann et al. Olanzapine plasma concentration, average daily dose, and interaction with co-medication in schizophrenic patients. Pharmacopsychiatry. 2004;37(2):63-8
[24] Reith et al. Features and toxicokinetics of clozapine in overdose. Ther Drug Monit 1998;20(1):92-7.
[25] Robertson et al. Olanzapine concentrations in clinical serum and postmortem blood specimens--when does therapeutic become toxic? J Forensic Sci. 2000;45(2):418-21.
[26] Hefner et al. Inflammation and psychotropic drugs: the relationship between C-reactive protein and antipsychotic drug levels. Psychopharmacology. 2016;233(9):1695-705.

2. Components and Accessories

2.1 Description of Components

All components are for LC-MS/MS use only, components may also 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

4071 CAL P PS2 | Psychotropic Medication 2 Calibrator Set
UDI: 8720514311400

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.

4072 CON P PS2 | Psychotropic Medication 2 Control Set
UDI: 8720514311417
4081 P PS2 | Psychotropic Medication 2 Control I
UDI: 8720514311493
4082 P PS2 | Psychotropic Medication 2 Control II
UDI: 8720514311509
4083 P PS2 | Psychotropic Medication 2 Control III
UDI: 8720514311516

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 2 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
After reconstitution:	2 - 8 °C	5 days
After reconstitution:	-20 °C	1 month

The 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 2 the highest available order of reference material has been established to be the reference laboratory network of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML).

Refer to the Value Data Sheet of this specific set for more information.

2.1.2 Internal Standard

4079 P PS2 | Psychotropic Medication 2 Internal Standard
UDI: 8720514311752

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):

Aripripazole-D8, Haloperidol-D4, Olanzapine-D3, Quetiapine-D8, Risperidon-D4, OH-Risperidon-D4, Clozapine-D4

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 2 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
After reconstitution:	2 - 8 °C	5 days
After reconstitution:	-20 °C	1 month

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

2.1.3 Deproteinization Solution

4080 P PS2 | Psychotropic Medication 2 Deproteinization Solution
UDI: 8720514311769

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

4084 P PS2 | Psychotropic Medication 2 Mobile Phase I
UDI: 8720514311523
4085 P PS2 | Psychotropic Medication 2 Mobile Phase II
UDI: 8720514311530

Two mobile phases are added to carry the sample through the LC-MS/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.

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

2.1.5 Autosampler Washing Solution

4086 P PS2 | Psychotropic Medication 2 Autosampler Washing Solution
UDI: 8720514311547

A solution used to clean the LC-MS/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.

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

2.2 List of components provided

4070 KIT P PS2 - Complete Kit for | Psychotropic Medication 2 in serum

Contents (for 300 assays):

Psychotropic Medication 2 Calibrator Set (Calibrator 1 – 6)	4071 CAL P PS2	6 x 2 x 500 µl
Psychotropic Medication 2 Internal Standard	4079 P PS2	3 x 2.5 ml
Psychotropic Medication 2 Deproteinization Solution	4080 P PS2	3 x 40 ml
Psychotropic Medication 2 Mobile Phase I	4084 P PS2	2 x 250 ml
Psychotropic Medication 2 Mobile Phase II	4085 P PS2	2 x 250 ml
Psychotropic Medication 2 Autosampler washing solution	4086 P PS2	1 x 1000 ml
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2.3 Separately available materials and components

Psychotropic Medication 2 Calibrator Set (Calibrator 1 – 6)	4071 CAL P PS2	6 x 2 x 500 µl
Psychotropic Medication 2 Internal Standard	4079 P PS2	1 x 2.5 ml
Psychotropic Medication 2 Deproteinization Solution	4080 P PS2	1 x 46 ml
Psychotropic Medication 2 Mobile Phase I	4084 P PS2	1 x 250 ml
Psychotropic Medication 2 Mobile Phase II	4085 P PS2	1 x 250 ml
Psychotropic Medication 2 Autosampler washing solution	4086 P PS2	1 x 1000 ml

Analytical column Raptor Biphenyl Column 2.7 µm, 2.1 mm x 150 mm		1 pc
Psychotropic Medication 2 Control I	4081 P PS2	500 µl*
Psychotropic Medication 2 Control II	4082 P PS2	500 µl*
Psychotropic Medication 2 Control III	4083 P PS2	500 µl*
Psychotropic Medication 2 Control Set	4072 CON P PS2	3 x 3 x 500 µl

* sold as separate control levels per 10 pieces

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 any significant quantity 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 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 or until pressure is stable.

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 systems, 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 RT's through evaporation of the mobile phases
Column	The column is installed in the column heater 40°C. The backpressure should not exceed 1000 bar for the complete UHPLC system. 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:	pre-injection 0 sec. post-injection 12 sec.
Seal Wash:	10:90 ACN:H2O
Wash Solvent:	Autosampler Washing Solution; 50:50 H2O:ACN

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
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:	Positive
Capillary voltage:	2.3 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	500°C
Desolvation gas flow:	800L/hr
Detection mode:	ESI
Dwell time:	Auto
Collision gas:	Argon

Substance	Precursor	Product
Olanzapine	313	84.15
Olanzapine	313	169.05
Olanzapine	313	198
Olanzapine	313	213
Olanzapine	313	256.1

Substance	Precursor	Product
Olanzapine D3	316.1	198
Olanzapine D3	316.1	256.1

Substance	Precursor	Product
Clozapine	327	192.1
Clozapine	327	227.05
Clozapine	327	270.1

Substance	Precursor	Product
Clozapine D4	331.2	192.2
Clozapine D4	331.2	272.2

Substance	Precursor	Product
Haloperidol	376.2	95.1
Haloperidol	376.2	123.1
Haloperidol	376.2	165.15
Haloperidol	376.2	358.15

Substance	Precursor	Product
Haloperidol D4	380.1	95.1
Haloperidol D4	380.1	123.1

Substance	Precursor	Product
Quetiapine	384.1	210.05
Quetiapine	384.1	221.2
Quetiapine	384.1	247.15
Quetiapine	384.1	253.1
Quetiapine	384.1	279.1

Substance	Precursor	Product
Quetiapine D8	392.3	226
Quetiapine D8	392.3	258.2

Substance	Precursor	Product
Risperidon	411.3	110.1
Risperidon	411.3	191.1

Substance	Precursor	Product
Risperidon D4	415.3	195.1

Substance	Precursor	Product
9-OH-risperidon	427.3	69.15
9-OH-risperidon	427.3	82.15
9-OH-risperidon	427.3	110.05
9-OH-risperidon	427.3	179.15
9-OH-risperidon	427.3	207.1

Substance	Precursor	Product
9-OH-risperidon D4	431.2	114.1

Substance	Precursor	Product
Aripiprazole	448	98.15
Aripiprazole	448	146.2
Aripiprazole	448	176.1
Aripiprazole	448	218.15
Aripiprazole	448	285.15

Substance	Precursor	Product
Aripiprazole D8	456.2	146.2
Aripiprazole D8	456.2	293.1

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. Pipette 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 (5 min, 10000 x g or more).
5. Pipette the centrifuged supernatant into 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. Add 100 µl 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
6. Inject 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 2 control I and Psychotropic Medication 2 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	**4081 P PS2 Control I Lot: 14B22/07**	**4083 P PS2 Control III Lot: 14B22/09**	**Patient**
Aripripazole	2.5 %	2.0 %	3.0 %
Clozapine	2.6 %	1.9 %	2.0 %
Haloperidol	3.4 %	2.2 %	2.6 %
Olanzapine	4.0 %	3.4 %	1.9 %
Quetiapine	2.2 %	3.2 %	2.6 %
Risperidon	2.4 %	2.7 %	2.0 %
9-OH-Risperidon	2.1 %	2.5 %	2.4 %

5.2 Reproducibility (Complex Precision)

The Reproducibility, or Complex Precision, was analyzed by measuring a patient sample, Psychotropic Medication 2 control I and Psychotropic Medication 2 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		
Sample	**4081 P PS2 Control I Lot: 14B22/07**	**4083 P PS2 Control III Lot: 14B22/09**	**Patient**
Aripripazole	4.7 %	6.9 %	8.8 %
Clozapine	4.6 %	5.5 %	5.4 %
Haloperidol	4.6 %	4.5 %	6.5 %
Olanzapine	7.0 %	5.2 %	6.6 %
Quetiapine	3.9 %	4.1 %	4.2 %
Risperidon	4.6 %	7.9 %	9.8 %
9-OH-Risperidon	4.0 %	5.7 %	6.8 %

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 limit of detection was calculated.

Analyte	**Linearity (µg/l)**
Aripripazole	950
Clozapine	2700
Haloperidol	140
Olanzapine	700
Quetiapine	1800
Risperidon	250
9-OH-Risperidon	300

5.4 Limit of Blank

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

Analyte	**LOB (µg/l)**
Aripripazole	0.18
Clozapine	0.0977
Haloperidol	0.032
Olanzapine	0
Quetiapine	0.0736
Risperidon	0.0286
9-OH-Risperidon	0.0103

5.5 Limit of Quantification

The Limit of Quantification (LOQ) was analyzed by preparing a series of 6 incrementally decreasing concentrations. These samples were measured twelve times and the limit of quantification was calculated.

Analyte	**LOQ (µg/l)**
Aripripazole	6.61
Clozapine	14.59
Haloperidol	< 0.52
Olanzapine	< 1.48
Quetiapine	< 3.57
Risperidon	< 0.48
9-OH-Risperidon	< 0.91

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%.

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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