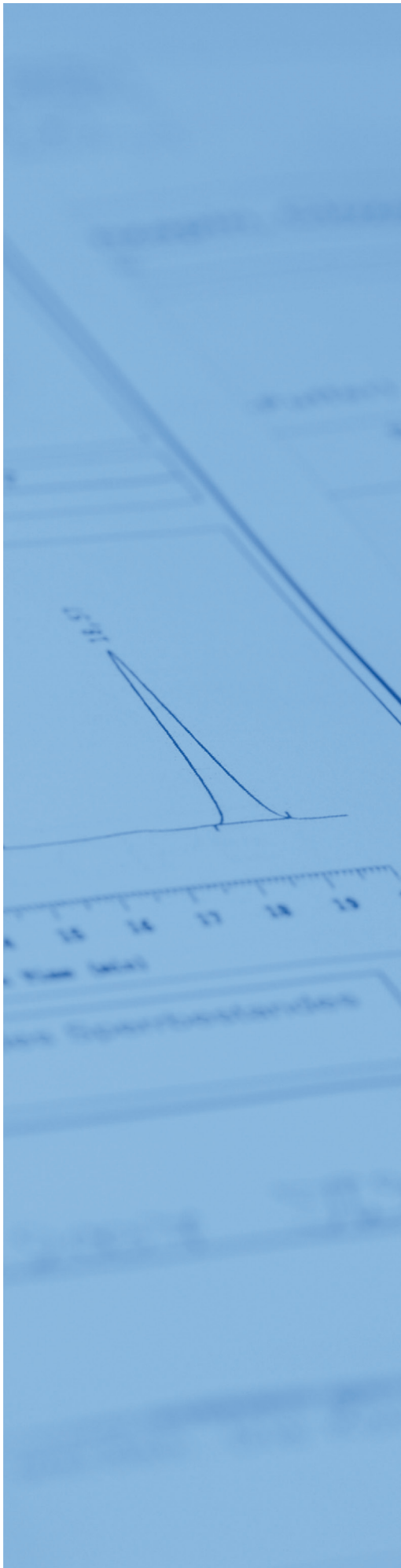
RECIPE

Instruction Manual

ClinMass® TDM Kit System

Tricyclic Antidepressants in Serum / Plasma



REF MS9000, MS9100

IVD For in vitro diagnostic use

CE IVDD, 98/79/EC

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Contents

Contents

1 Introduction

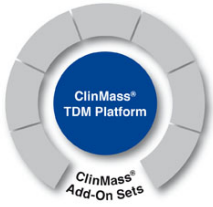
1.1 Information on changes in this instruction manual

This instruction manual (version 3.0) was revised and replaces the previous version 2.0.

Please note the updated reference ranges in section 7.2.

The changes are marked on the page margin.

1.2 Intended use



ClinMass® TDM Kit System

The ClinMass® TDM Kit System is based on a universal TDM Platform (order no. MS9000), which can be used with various Add-On Sets for the Therapeutic Drug Monitoring (TDM) with LC-MS/MS.

The ClinMass® Add-On Set for Tricyclic Antidepressants (order no. MS9100) is intended for the determination of Tricyclic Antidepressants from human serum or plasma.

The components of the ClinMass® TDM Platform and the ClinMass® Add-On Set for Tricyclic Antidepressants have to be used in accordance with the instructions in this user manual. A combination with components from other manufacturers is not intended.

1.2.1 IVD symbols

Symbols according to the EU directive 98/79/EC for in vitro diagnostic medical devices (IVDD), which are used on the product labels and in this user manual:

IVD	For in vitro diagnostic use	REF	Order number
	Manufacturer	LOT	Lot number
			Upper temperature limit: … °C
			Temperature limits: … °C to … °C
			Expiry date: …
			See instructions for use

1.3 Clinical background

Tricyclic antidepressants (TCAs) are a group of psychoactive drugs which are mainly used for the therapy of endogene depressions, anxiety and pain management [1]. Their name is derived from a common chemical structure with a tripartite ring system. The following figure exemplifies the structure with the compounds amitryptiline, imipramine, doxepin and clomipramine.

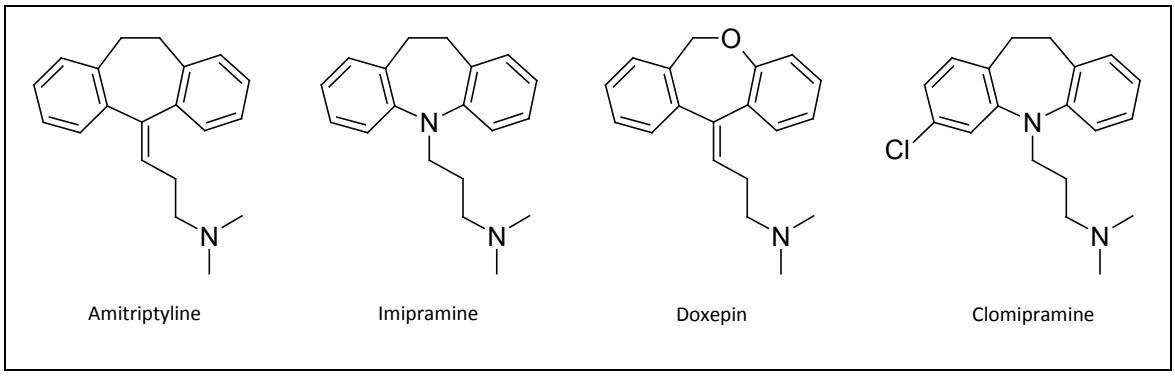


Figure 1. Chemical structure of the TCAs (examples)

TCAs are widely established compounds since the 1960s, however due to their side effects they have a narrow therapeutic range [1], which requires therapeutic drug monitoring (TDM) [2]. The consequences of overdosing may vary from mild agitation or drowsiness to delirium and coma resulting in death [1].

The drug monitoring is mainly performed from serum. Besides dosage optimisation, the monitoring is used for the verification of compliance as well as for the early detection of pharmacokinetic changes of the compound.

RECIPE's analytical method provides the reliable quantification of 13 TCAs and two atypical neuroleptics and their metabolites (see method description in section 1.4). The analytes are listed in Table 1.

Table 1. Analyte list

Analytes		
Amitriptyline	Imipramine	Normaprotiline
Clomipramine	Maprotiline	Nortrimipramine
Clozapine*	Norclomipramine	Nortriptyline
Desipramine	Norclozapine*	Protriptyline
Doxepin	Nordoxepin	Trimipramine

*Atypical neuroleptics

1.4 General description of the analytical method

The analytical procedure is based on a universal ClinMass® TDM Platform (order no. MS9000), which can be used with various ClinMass® Add-On Sets (ClinMass® TDM Kit System).

The ClinMass® Add-On Set with order no. MS9100 contains the analyte-specific components for the determination of 13 different TCAs and two atypical neuroleptics. The analysis is performed by HPLC with tandem mass spectrometry (LC-MS/MS).

Prior to the LC-MS/MS analysis a short sample preparation is carried out in order to remove the sample matrix and to spike the samples with an internal standard (see sample preparation section 5.2).

After sample preparation the samples are injected into the LC-MS/MS system, where the analytes are detected subsequent to electrospray ionisation (ESI).

In the electrospray ionisation the sample components are ionised and subsequently passed into the gas phase. They are then transferred into the MS/MS system, which consists of two quadrupoles connected through a collision cell.

The measurement of the analytes is carried out in MRM mode (MRM: Multiple Reaction Monitoring). In this mode only selected ions (known as “precursor ions”) with a defined ratio mass/charge (*m/z*) are isolated in the first quadrupole and subsequently transferred into the collision cell. There the ions are fragmented by impact with an inert gas (argon or nitrogen) at defined voltage settings. Among the fragments generated (known as “product ions”) only those with a defined m/z ratio are isolated in the final quadrupole for subsequent detection. Thus the measurement in MRM mode ensures identification and quantification with high selectivity and sensitivity with the analyte identification based on characteristic mass transitions for the compound of interest.

The analytical method enables a robust and reliable quantitation in complex biological matrices by use of 12 different isotope-labelled internal standards (see sectiont 4.3.2). If required, two transitions are used per analyte (quantifier, qualifier).

A ClinMass® Optimisation Mix is available for the optimisation of the MS/MS parameters (see section 5.3.1) and for the test run of the analytical system (see section 5.3.2).

The calibration of the analytical system is performed by use of ClinCal® Serum Calibrators. For this purpose a 4-Level Serum Calibrator Set is available (see section 5.3.3).

Quality control is performed by the use of ClinChek® Serum Controls. These controls are available in two different concentrations (see section 5.3.4).

2 Components of TDM platform and Add-On Set, accessories

2.1 Ordering information

Order No.	Description	Quantity
MS9000	**ClinMass® TDM Platform** for 400 assays	1 pce.
	Content:	
	Autosampler Washing Solution	1 x MS9005
	Mobile Phase A	2 x MS9007
	Mobile Phase B	1 x MS9008
	Sample Preparation Vials	4 x MS9020
	Precipitant P	2 x MS9021
	Separately available components:	
MS9005	Autosampler Washing Solution	1000 ml
MS9007	Mobile Phase A	1000 ml
MS9008	Mobile Phase B	1000 ml
MS9020	Sample Preparation Vials	100 pcs.
MS9021	Precipitant P	25 ml
	Start Accessories:	
MS9030	Analytical Column with test chromatogram	1 pce.
MS9032	Prefilter	1 pce.
	Accessory:	
MS9022	Diluting Solution D	50 ml
MS9100	**ClinMass® Add-on Set** **for Tricyclic Antidepressants in Serum / Plasma** for 200 assays	1 pce.
	Content:	
	Internal Standard IS, lyophil.	1 x MS9112
	Serum Calibrator Set, lyophil. (Level 0 - 3)	1 x MS9113
	Manual	
	Separately available components:	
MS9112	Internal Standard IS, lyophil.	5 x 5 ml
MS9113	Serum Calibrator Set, lyophil. (Level 0 - 3)	4 x 1 x 1 ml
	Start Accessory:	
MS9114	Optimisation Mix, lyophil.	3 x 1 x 2 ml
	ClinChek® Controls:	
MS9182	Serum Control, lyophil., Level I, II	2 x 5 x 1 ml

2.1.1 Safety information

Components such as mobile phases and reagents are chemical preparations and may contain hazardous substances. For safety information please consult the respective safety data sheet (SDS) for each component.

The calibrator and control materials are manufactured from human serum. Although the products are tested for the absence of common infection markers, they still should be considered as potentially infectious. For this reason we recommend the product to be handled with the same precautions as patient samples. Detailed safety information is indicated in the respective SDS.

2.1.2 Storage conditions and lifetime

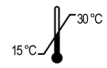
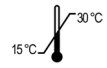
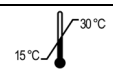
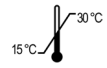
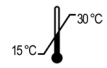
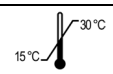
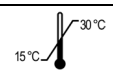
Please unpack all components from the transport packaging **immediately upon receipt** and follow the storage instructions indicated on the product labels and in Table 2.

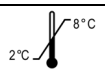
Unused components, stored under appropriate conditions, can be used until the expiry date indicated on the product label.

After use of ClinMass® Reagents and ClinMass® Mobile Phases the bottles must be closed tightly and stored immediately under the required conditions. Provided that instructions for proper use and storage procedures are followed, the lifetime of the reagents is the same as for the unused products.

Storage conditions and lifetime of the ClinMass® Internal Standard, the ClinMass® Optimisation Mix as well as the ClinCal® Calibrators and ClinChek® Controls (lyophilised and after reconstitution) are indicated in the respective product data sheets.

Table 2. Storage conditions

Order no.		Product description	Storage conditions	
REF	MS9005	Autosampler Washing Solution		Store at 15–30 °C
REF	MS9007	Mobile Phase A		Store at 15–30 °C
REF	MS9008	Mobile Phase B		Store at 15–30 °C
REF	MS9020	Sample Preparation Vials	Store at ambient temperature	
REF	MS9021	Precipitant P		Store at 15–30 °C
REF	MS9022	Diluting Solution D		Store at 15–30 °C
REF	MS9030	Analytical Column		Store at 15–30 °C
REF	MS9032	Prefilter		Store at 15–30 °C

Order no.		Product description	Storage conditions	
REF	MS9112	Internal Standard IS, lyophil.		Store below -18 °C*
REF	MS9113	Serum Calibrator Set, lyophil.		Store at 2–8 °C*
REF	MS9114	Optimisation Mix, lyophil.		Store below -18 °C*
REF	MS9182	Serum Control, lyophil., Level I+II		Store at 2–8 °C*

*refers to the lyophilised product. Please consult the product data sheet for information regarding storage conditions after reconstitution.

2.1.3 Disposal of laboratory waste

For disposal laboratory waste should be collected separately according to the different chemical properties. Recommendations for the disposal of the product and the respective packaging are indicated in section 13 of the respective safety data sheet (SDS).

3 Required instruments

The use of this test kit requires an LC system with tandem mass spectrometer (LC-MS/MS) with sufficient sensitivity and evaluation software. Data regarding the suitability of the various LC-MS/MS systems is available upon request (info@recipe.de).

Required LC modules:

- Autosampler
- Binary HPLC pump (Mobile Phases A and B)
- Column heater (40 °C)
- Degasser (optional)

For sample preparation the following laboratory instruments are required:

- Pipettes, pipette tips
- Tabletop centrifuge
- Vortex mixer

4 Operation of the analytical system

4.1 Flushing of the LC system

Connect the LC modules **with exception** of the column. Put the outlet capillary into a safe waste container.

Set the HPLC pump at a flow rate of 1 ml/min and flush the LC system with 10 ml of a 50 : 50 mixture of the Mobile Phases A and B.

Connect the analytical column in the column heater and make sure that the flow direction follows the arrow marking on the column!

Please also make sure to use the proper fittings for the connection to the column. A new fitting should be used and adapted to the column. For information regarding the proper connection please contact RECIPE.

4.2 Equilibration of the LC system

After the flushing (see section 4.1) equilibrate as follows:

- Set the HPLC pump at a flow rate of 0.8 ml/min and set the column heater at the temperature of 40 °C. Equilibrate the column with 10 ml of a 85:15 mixture of the Mobile Phases A and B. This corresponds to the start conditions of the gradient programme (see table 4).
- Then **stop the pump** and connect the outlet capillary of the analytical column with the tandem mass spectrometer.

4.3 Starting the analytical system

The following sections provide the parameters for the LC system (see section 4.3.1) and the tandem mass spectrometer (see section 4.3.2). Please consult section 5.3 regarding optimisation, equilibration, test run and calibration of the LC-MS/MS system.

Please consult the user manual of the tandem mass spectrometer to ensure proper handling. User trainings provided by the instrument manufacturer, may also be advisable.

4.3.1 LC parameters

Table 3. LC parameters

HPLC pump (Mobile Phases A, B):	Gradient programme of the binary pump: See Table 4 Make sure the bottles are closed well to avoid alteration of the retention times, which could occur due to evaporation of the mobile phase components.
Analytical column:	The analytical column and the prefilter are installed in the column heater (40° C). At a flow rate of 0.8 ml/min the backpressure of the analytical column should not exceed 250 bar. The prefilter should be renewed after 300 injections at the latest. It should also be replaced if the backpressure of prefilter plus column is increased by 10 % (at a flow rate of 0.8 ml/min).
Autosampler:	Injection volume: 1 - 10 µl* Injection interval: 3.8 min Needle washing: The injection needle needs to be flushed after sample injection (minimisation of sample carryover). Please refer to the recommended needle wash settings in the instruction manual of the autosampler manufacturer. For flushing please use the autosampler washing solution with order no. MS9005. *depending on the sensitivity of the mass spectrometer in use

The following gradient programme is used for the binary HPLC pump*:

Table 4. Gradient programme

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]	Flow rate [ml/min]
0.01	85	15	0.8
0.05	85	15	0.8
0.06	70	30	0.8
2.10	70	30	0.8
2.11	62	38	0.8
2.70	62	38	0.8
3.00	25	75	0.8
3.35	25	75	0.8
3.40	85	15	0.8
3.80	85	15	0.8

*Note:
Please note that according to the dead volume of the HPLC system in use an adaptation of the gradient might be necessary.

4.3.2 MS/MS-Parameter

The mass transitions of the analytes and of the respective isotope-labelled substances in the ClinMass® Internal Standard IS are contained in Table 5.

The assignments of the isotope-labelled substances to the analytes are contained in Table 6.

The indicated mass transitions should be considered as starting points for the optimisation. As the optima may vary slightly between the different MS/MS systems, these have to be determined for the respective system in use (see section 5.3.1).

Table 5. Mass transitions of the analytes and the isotope-labelled substances in the IS

Analyte / IS	**Quantifier MRM**		**Qualifier MRM**	
	Precursor [amu]	**Product [amu]**	**Precursor [amu]**	**Product [amu]**
Amitriptyline	278.2	105.1	278.2	233
Clomipramine	315.2	86.1	315.2	58.1
Clozapine	327.1	192	327.1	270
Desipramine	267.2	72.1	267.2	44.1
Doxepin	280.2	107	280.2	58.1
Imipramine*	281.2	58.1	281.2	86.1
Maprotiline	278.2	250.1	278.2	178.1
Norclomipramine	301.2	72.1	301.2	44
Norclozapine	313.1	192	313.1	270
Nordoxepin**	266.2	107	266.2	44.1
Normaprotiline	264.2	169.1	264.2	219.1
Nortrimipramine	281.2	44.2	281.2	55.2
Nortriptyline	264.2	233	264.2	91.1
Protriptyline***	264.2	233	264.2	155.1
Trimipramine	295.2	100.1	295.2	58.1
d_3-Amitriptyline	281.2	105.1	---	---
d_3-Clomipramine	318.2	89.1	---	---
d_4-Clozapine	331.2	192	---	---
d_3-Desipramine	270.2	75.1	---	---
d_3-Doxepin	283.2	107	---	---
d_3-Imipramine	284.2	61.1	---	---
d_5-Maprotiline	283.2	255.2	---	---
d_3-Norclomipramine	304.2	75.1	---	---
d_8-Norclozapine	321.2	192.1	---	---
d_3-Nordoxepin	269.2	107	---	---
d_3-Nortriptyline	267.2	233	---	---
d_3-Trimipramine	298.2	103.1	---	---

Please note:

*for co-medication with Trimipramine see section 7.3.1.

**for co-medication with Mycophenolic Acid see section 7.3.3

***for co-medication with Normaprotiline see section 7.3.2

Table 6. Assignment of the analytes to the isotope-labelled substances in the IS

Analyte	**RT [min]**	**Internal Standard IS**	**RT [min]**
Amitriptyline	3.22	d_3-Amitriptyline	3.18
Clomipramine	3.40	d_3-Clomipramine	3.39
Clozapine	2.94	d_4-Clozapine	2.90
Desipramine	2.27	d_3-Desipramine	2.25
Doxepin	2.14	d_3-Doxepin	2.07
Imipramine	2.84	d_3-Imipramine	2.80
Maprotiline	2.66	d_5-Maprotiline	2.65
Norclomipramine	3.20	d_3-Norclomipramine	3.19
Norclozapine	1.40	d_8-Norclozapine	1.37
Nordoxepin	1.52	d_3-Nordoxepin	1.52
Normaprotiline	2.27	d_3-Desipramine	2.25
Nortrimipramine	2.78	d_3-Imipramine	2.80
Nortriptyline	2.65	d_3-Nortriptyline	2.63
Protriptyline	2.29	d_3-Nortriptyline	2.63
Trimipramine	3.32	d_3-Trimipramine	3.29

4.3.2.1 System-specific settings of various MS/MS systems

Device-specific data for the various MS/MS systems by different suppliers is available upon request (info@recipe.de).

4.4 Standby mode

When the analytical system is not in use, the pumps have to be switched off. The mobile phases may remain in the LC system.

The vacuum pumps of the tandem mass spectrometer (MS/MS system) should be in permanent operation. In order to protect the ion source and the multiplier the MS/MS system should be switched into standby mode.

For a longer operation pause the analytical column should be disconnected and stored tightly closed. The LC system should then be flushed with a water/acetonitrile mixture (1:1).

5 Implementation of the analytical procedure

5.1 Collection and storage of samples

The therapeutic monitoring of tricyclic antidepressants is primarily performed from serum. Plasma may be used alternatively.

Serum extraction should not be performed by use of collection tubes with gel separators. Some gels could partially absorb the analytes and thus lead to false low analytical values [3].

At room temperature (15 - 30 °C) the samples can be stored at least three days. At temperatures between 2 – 8 °C the samples can be stored at least 7 days and below -18 °C at least three months (multiple freeze-thaw cycles should be avoided).

5.2 Sample preparation

5.2.1 Reconstitution of the lyophilised serum calibrators / controls

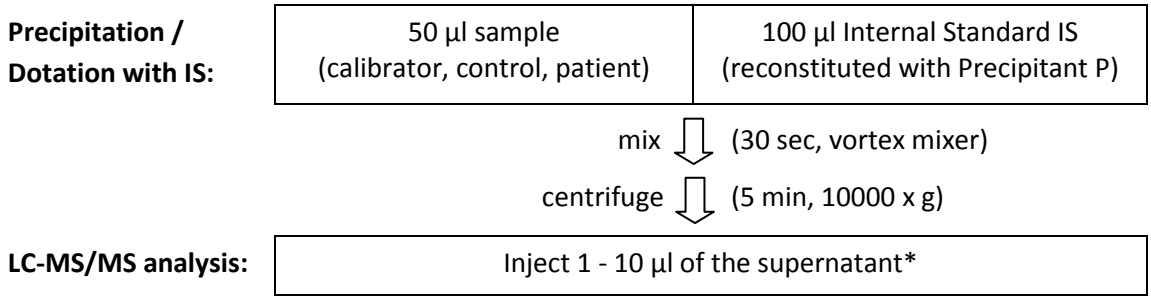
The ClinCal® Serum Calibrators and ClinChek® Serum Controls (order nos. MS9113 and MS9182, see section 2.1) are lyophilised and therefore need to be reconstituted before use.
Information on reconstitution, analyte concentration, storage and stability is indicated in the respective product data sheets.

5.2.2 Reconstitution of the lyophilised Internal Standard IS

The ClinMass® Internal Standard IS (order no. MS9112) is lyophilised and is **reconstituted with Precipitant P (order no. MS9021)**.

Information on reconstitution, storage and stability is indicated in the product data sheet of the Internal Standard IS.

5.2.3 Work flow



*Note 1:
The injection volume needs to be selected with respect to the sensitivity of the MS/MS system in use. If necessary the supernatant needs to be diluted with the Diluting Solution D (order no. MS9022).

Note 2:
Within one analytical series no different lots of a reagent should be used.

5.2.3.1 Precipitation / Dotation with IS

Pipette 50 µl sample (calibrator, control, patient) in the sample preparation vial (order no. MS9020). Add 100 µl of the Internal Standard IS (reconstituted with Precipitant P, see section 5.2.2) and mix for 30 sec on a vortex mixer. Subsequently centrifuge for 5 min at 10000 x g.

Transfer approx. 100 µl of the centrifuged supernatant into a suitable autosampler glass vial.

5.2.3.2 LC-MS/MS analysis

Inject 1 - 10 µl of the sample into the LC-MS/MS system. If necessary dilute the supernatant with Diluting Solution D (order no. MS9022).

The injection volume needs to be selected with respect to the sensitivity of the MS/MS system in use. Device-specific data for the various MS/MS systems by different suppliers is available upon request (info@recipe.de).

5.2.3.3 Stability of the prepared samples

At room temperature (15 - 30 °C) the samples can be stored for at least three days. At temperatures between 2 - 8 °C the samples can be stored for at least 7 days and at temperatures below - 18 °C for at least three months (multiple freeze-thaw cycles should be avoided).

5.3 LC-MS/MS analysis

Regardless of the analytical method the mass accuracy of the tandem mass spectrometer should be checked at regular intervals. A mass calibration may be required.

For information regarding the check-up of the MS/MS system, please refer to the instructions for use provided by the instrument manufacturer.

5.3.1 Optimisation of the tandem mass spectrometer

The optimisation of the MS/MS system comprises the optimisation of the ion source parameters as well as the compound-specific mass transitions. For this purpose the Optimisation Mix with order no. MS9114 is available, which contains all analytes. **The packing unit of the Optimisation Mix** contains three bottles, **Optimisation Mixes 1, 2 and 3**.

The analyte composition of the Optimisation Mixes 1, 2 and 3 has been selected in a way so the mass transitions will sufficiently differ from each other and thus provide an analyte-specific optimisation.

Optimisation Mixes 1, 2 and 3 are lyophilised and therefore need to be reconstituted prior to use. Information regarding reconstitution, storage and stability are indicated in the product data sheet of order no. MS9114.

If necessary the optimisation mixes 1, 2 and 3 need to be diluted with Mobile Phase A with respect to the sensitivity of the MS/MS system in use. Device-specific data for the various LC-MS/MS systems by different suppliers is available upon request (info@recipe.de).

5.3.2 Equilibration of the analytical system and test run

Equilibrate the entire analytical system for at least 30 min before injecting samples.

At least three „blank-injections“ need to be carried out at the beginning of each analytical series (injection volume: 0 µl or injection of Mobile Phase A). This procedure facilitates reproducible analytical results from the first sample injection.

To perform a test run, repeatedly inject the optimisation mixes, until two consecutive chromatograms, comparable in retention times and peak areas, are obtained.

Depending on the required analytes (see product data sheet for order no. MS9114) the injection of the optimisation mixes can be performed subsequently or as a mixture of equal volumes.

Further dilution of the reconstituted optimisation mixes with Mobile Phase A may be necessary with respect to the sensitivity of the MS/MS system in use, see examples in Table 7.

Table 7. Dilution with Mobile Phase A (examples)

Target dilution	Opti Mixes in use	Dilution with Mobile Phase A
1:20	1	1:20
1:20	2*	1:10
1:20	3*	1:(6.67)

*Mixture of equal volumes

5.3.3 Calibration run

A ClinCal® 4-Level Serum Calibrator Set (Level 0 - 3, order no. MS9113) is available for calibration.

Please always consider the information provided in the product data sheet of the calibrator lot in use.

After reconstitution (see section 5.2.1) the calibrators need to be prepared as described for the patient samples (see section 5.2).

For each analytical series freshly prepared calibrators have to be used.

5.3.4 Accuracy control

For the quality control of the analytical measurements ClinChek® Serum Controls in two different concentrations are available (Level I+II, order no. MS9182).

After reconstitution the controls need to be prepared like patient samples (see section 5.2).

For each analytical series freshly prepared controls have to be used. In case of large analytical series we recommend injecting these controls additionally at the end of the series.

5.3.5 Example chromatogram

Chromatogram of the ClinCal® Serum Calibrator (order no. MS9113), level 2, acquired with the LC system Thermo Scientific Dionex UltiMate™ 3000 and the MS/MS system Thermo Scientific TSQ Endura™:

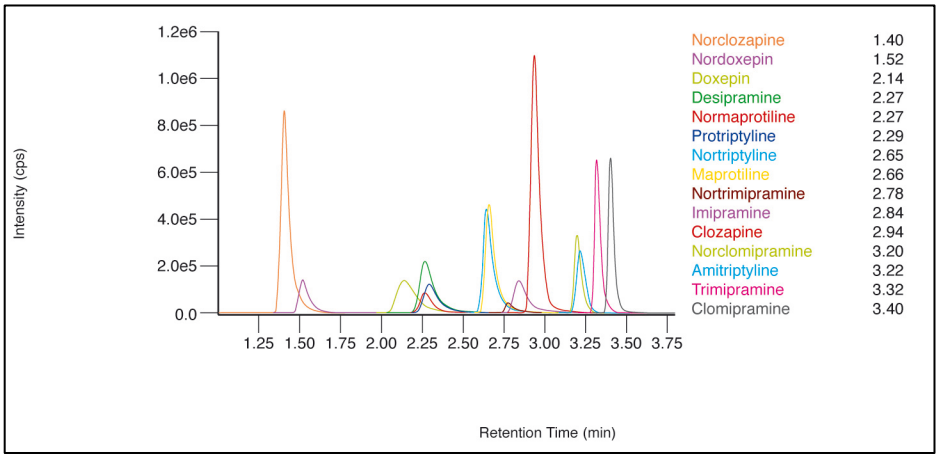


Figure 2. Chromatogram of the ClinCal® Serum Calibrator (order no. MS9113), level 2

6 Evaluation

The analyte detection is performed via compound-specific mass transitions, see section 4.3.2.

The analyte concentration is calculated with the internal standard method via the peak areas.

The respective calibration curve is obtained from the calibrators by plotting the ratio of *peak area „analyte / internal standard“* against c*oncentration „analyte*“.

The analyte concentrations in the samples and the controls are calculated from the calibration curves.

Please consult the software user manual of the MS/MS manufacturer in order to ensure correct evalution of the results.

For the conversion of the mass concentrations [µg/l] into molar concentrations [nmol/l] and vice versa, the analytical results should be multiplied with the factors listed in Table 8.

Table 8. Conversion factors

Analyte	**Molecular weight [g/mol]**	**Conversion: nmol/l --> µg/l**	**Conversion: µg/l --> nmol/l**
Amitriptyline	277.40	0.2774	3.605
Clomipramine	314.85	0.3149	3.176
Clozapine	326.82	0.3268	3.060
Desipramine	266.38	0.2664	3.754
Doxepin	279.38	0.2794	3.579
Imipramine	280.40	0.2804	3.566
Maprotiline	277.41	0.2774	3.605
Norclomipramine	300.82	0.3008	3.324
Norclozapine	312.80	0.3128	3.197
Nordoxepin	265.35	0.2654	3.769
Normaprotiline	263.40	0.2634	3.797
Nortrimipramine	280.40	0.2804	3.566
Nortriptyline	263.38	0.2634	3.797
Protriptyline	263.37	0.2634	3.797
Trimipramine	294.43	0.2944	3.396

7 Test data

7.1 Validation data

The validation data was established with LC system Thermo Scientific Dionex UltiMate™ 3000 and the MS/MS system Thermo Scientific TSQ Endura™.

7.1.1 Linearity, detection- and quantitation limit

Linearity, detection- and lower quantitation limit are listed in Table 9.

Table 9. Linearity, detection- and lower quantitation limit (LOD, LLOQ)

Analyte	**Linearity [µg/l]**	**LOD [µg/l]**	**LLOQ [µg/l]**
Amitriptyline	1.79-650	0.597	1.79
Clomipramine	2.24-864	0.747	2.24
Clozapine	1.10-2332	0.367	1.10
Desipramine	0.301-696	0.100	0.301
Doxepin	1.48-570	0.493	1.48
Imipramine	1.86-718	0.620	1.86
Maprotiline	0.900-900	0.300	0.900
Norclomipramine	2.07-826	0.690	2.07
Norclozapine	0.852-1884	0.284	0.852
Nordoxepin	0.238-550	0.079	0.238
Normaprotiline	1.14-1286	0.380	1.14
Nortrimipramine	2.83-630	0.943	2.83
Nortriptyline	0.655-688	0.218	0.655
Protriptyline	0.564-658	0.188	0.564
Trimipramine	1.84-726	0.613	1.84

7.1.2 Recovery

The recovery rate for all 15 analytes lies between 84–96 %.

7.1.3 Precision

The method intra- and interassay precisions were determined with samples in two different concentrations. The analyte concentrations were selected according to the respective therapeutic reference range and are contained in Table 10 together with the precision results.

Table 10. Precision results

	Concentration [µg/l]		**Intraassay Precision [%] (mean value)**		**Interassay Precision [%]**	
Analyte	**Level**		**Level**		**Level**	
	I	**II**	**I**	**II**	**I**	**II**
Amitriptyline	59.2	135	1.9	1.7	1.9	2.2
Clomipramine	73.9	171	2.1	1.4	2.1	2.0
Clozapine	217	510	1.8	1.8	2.6	1.9
Desipramine	64.3	152	2.3	1.0	2.8	2.2
Doxepin	50.8	117	2.6	2.3	2.6	3.6

	Concentration [µg/l]		Intraassay Precision [%] (mean value)		Interassay Precision [%]	
Analyte	**Level**		**Level**		**Level**	
	I	**II**	**I**	**II**	**I**	**II**
Imipramine	63.9	148	1.6	1.1	2.1	2.0
Maprotiline	82.7	193	1.8	2.2	1.4	1.9
Norclomipramine	79.9	187	2.3	2.2	2.9	2.4
Norclozapine	179	418	1.6	1.3	1.5	2.5
Nordoxepin	49.4	116	3.0	0.9	3.6	1.5
Normaprotiline	121	280	3.2	3.0	5.0	3.5
Nortrimipramine	55.1	133	5.3	2.5	4.7	1.8
Nortriptyline	64.5	145	2.2	1.1	2.3	1.9
Protriptyline	58.1	143	3.0	2.1	3.1	5.4
Trimipramine	64.2	155	4.5	2.3	4.2	4.9

7.2 Reference ranges

The following reference ranges are taken from the „ Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017” [2].

Table 11. Reference ranges

Analyte	**Therapeutic Range [µg/l]**	**Laboratory Alert Level† [µg/l]**
Amitriptyline plus Nortriptyline	80-200	300
Clomipramine plus Norclomipramine[1]	230-450	450
Clozapine	Antipsychotic: 350-600	Antipsychotic: 1000
Desipramine	100-300	300
Doxepin plus Nordoxepin[2]	50-150	300
Imipramine plus Desipramine	175-300	300
Maprotiline	75-130	220
Norclomipramine[1]	see: Clomipramine plus Norclomipramine	
Norclozapine	n.a.	n.a.
Nordoxepin	see: Doxepin plus Nordoxepin	
Normaprotiline	n.a.	n.a.
Nortrimipramine	n.a.	n.a.
Nortriptyline	70–170	300
Protriptyline	n.a.	n.a.
Trimipramine	150-300	600

n.a.: not available;

†at values above the „Alert level“ the physician in charge should be informed immediately

[1]Description in [2]: N-desmethyl-clomipramine

[2]Description in [2]: N-desmethyldoxepin

The indicated reference ranges are taken from thoroughly selected and current scientific literature. Their actuality corresponds to the printing date of this document. Please note that these ranges do not reflect any recommendations by the manufacturer of this product, but may be used as a guideline for the assessment of the reference range by the clinical laboratory.

7.3 Interferences

7.3.1 Imipramine

Nortrimipramine, the active metabolite of trimipramine, interferes with the qualifier transition of imipramine (m/z 281.2 → 86.1). The quantifier transition (m/z 281.2 → 58.1) is not affected by this interference.

In case of co-medication with trimipramine the qualifier transition for imipramine can therefore not be evaluated.

7.3.2 Nortriptyline and Protriptyline

Nortriptyline and protriptyline are isobaric substances (molecular weight 263.4 g/mol), which do not have selective mass transitions. These two analytes however are chromatographically separated, thus a selective quantification is ensured.

We recommend to regularly check the chromatographic separation performance by a test run with a 1:1 mixture of optimisation mixes 1 and 2 (see section 5.3.2).

Furthermore normaprotiline, the active metabolite of maprotiline, interferes with the qualifier transition of protriptyline (m/z 264.2 → 155.1). The quantifier transition (264.2 → 233) is not affected by this interference.

In case of co-medication with maprotiline the qualifier transition for protriptyline can therefore not be evaluated.

7.3.3 Nordoxepin

Mycophenolic acid interferes with the qualifier transition of nordoxepin (m/z 266.2 → 44.1). The quantifier transition (m/z 266.2 → 107) is not affected by this interference.

In case of co-medication with mycophenolic acid the qualifier transition for nordoxepin can therefore not be evaluated.

8 References

[1] Tietz (Ed.): Fundamentals of Clinical Chemistry, 6th Edition, Saunders Elsevier 2008, p. 554-557 and p. 572-573.

[2] Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K et al. Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. Pharmacopsychiatry 2018;51(01/02):9–62; DOI: https://doi.org/10.1055/s-0043-116492.

[3] J. Karppi, K. Åkerman, M. Parviainen: Suitability of Collection Tubes with Separator Gels for Collecting and Storing Blood Samples for Therapeutic Drug Monitoring (TDM), Clin Chem Lab Med 2000; 38(4), 313–320; DOI: https://doi.org/10.1515/CCLM.2000.045.

9 Troubleshooting

Problem	Possible Cause	Corrective Measures
Retention times shifted	Defective HPLC pump	Check pumps
	Air within the system	Degas the mobile phases and flush HPLC
	Fluctuations of the flow rate	Check pumps
Interference signals	Injection system contaminated	• Rinse with methanol or inject 10 x mobile phase • Flushport: check solvent level • Clean/exchange injection needle and needle seat assembly
	Sample vials contaminated	Use new vials
	Vial septum contaminated	Use another septum
	Mobile phase contaminated	Change the mobile phases and flush the system
	Column(s) (guard / analytical column) contaminated	Change guard / analytical column
	Mass resolution too low	Optimise mass resolution
	System not properly installed	Check all connections
No signals	Defective injector	Check injector
	Defective HPLC pump	Check pump
	MS/MS system not ready for operation	Check MS/MS system
Decrease of sensitivity	Ion source contaminated	Clean ion source
	Mass spectrometer contaminated	Clean mass spectrometer
	Shift of mass calibration	Recalibrate MS/MS
	Mass resolution too high/low	Optimise mass resolution
	Injection valve leaking	Check injector

Problem	**Possible Cause**	**Corrective Measure**
Fluctuation of signal intensity	Spray unstable	Check spray needle capillary and clean if necessary
	Gas flow unstable	Check gas lines
No vacuum	Defective vacuum pumps	Check the pre- and high-vacuum pumps
	Vacuum system leaking	Check vacuum tubes and fittings
No gas supply	Defective nitrogen generator	Check nitrogen generator
	Defective compressor	Check compressor
	Gas bottle empty	Replace gas bottle
	Inlet gas pressures not within the specified range	Regulate the inlet gas pressures

10 Appendix: EC Declaration of Conformity

EC Declaration of Conformity

for in-vitro diagnostic medical devices, acc. to article 9 (1) of the directive 98/79/EC

The company

RECIPE Chemicals + Instruments GmbH
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80992 München / Germany

declares that the CE labelled products

ClinMass® TDM Platform (order no.: MS9000) and
ClinMass® Add-On Set for Tricyclic Antidepressants in Serum / Plasma (order no.: MS9100)

meet all applicable provisions of the directive on in-vitro diagnostic medical devices 98/79/EC. The conformity assessment was perfomed according to annex III. The technical documenation is held according to annex III no. 3.

München, 27.08.2018

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