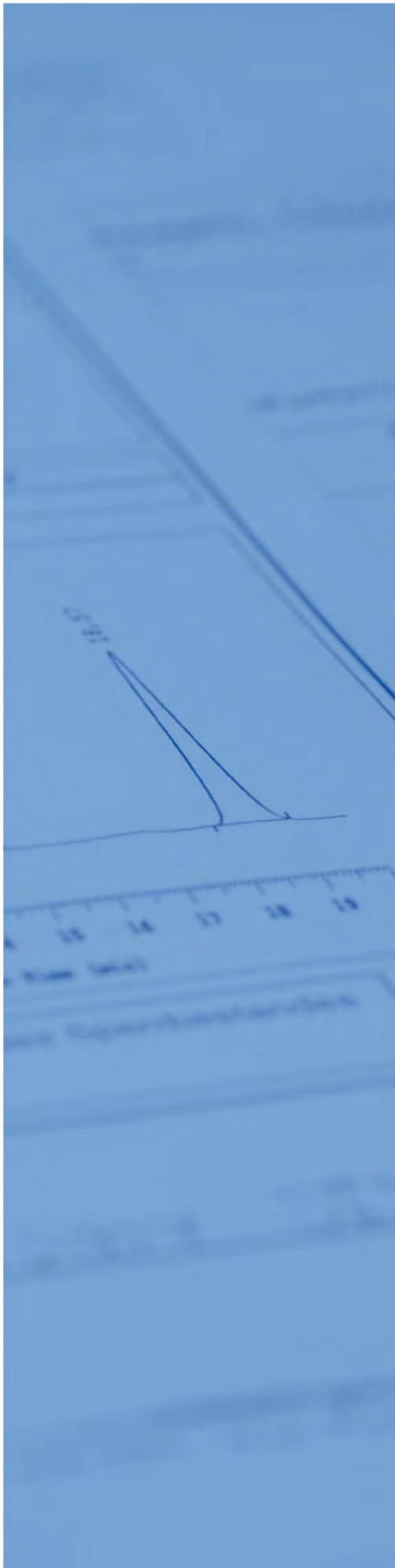
RECIPE®

Instruction Manual

ClinMass® TDM Kit System

Antidepressants in Serum / Plasma



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IVD For in vitro diagnostic use

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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 enlarged analyte panel in section 1.3. Due to the enlarged panel the analytical method has been modified. Please particularly take note of the changes in section 4.3.1 (LC parameters), section 4.3.2 (MS/MS parameters), section 5.1 (Collection and storage of samples), section 5.3.5 (example chromatogram), section 7.1 (validation data) and section 7.4 (interferences).

Please also take notice of the updated reference ranges in section 7.2.

The changes are marked on the outer margins.

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 Antidepressants (order no. MS9400) is intended for the determination of Antidepressants and metabolites from human serum or plasma.

The components of the ClinMass® TDM Platform and the ClinMass® Add-on Set for 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

Many adults are affected by major depressive disorders (MDD) at some point during their lifetime. Treatment for MDD is often started in a primary care setting, and patients generally receive drugs as the standard treatment [1, 2].

In addition to the classical tricyclic antidepressants (TCAs) a variety of newer antidepressants (ADP) is available for the treatment of MDD. These include selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs, NRIs) as well as tetracyclic antidepressants (TeCAs) [1–3].

Therapeutic drug monitoring (TDM), i.e. the quantification of serum concentrations of medications for dose optimization, is required to ensure a patient-matched psychopharmacotherapy and to avoid side effects. Uncertain drug adherence, suboptimal tolerability, non-response at therapeutic doses, or pharmacokinetic drug-drug interactions are typical situations when measurement of medication concentrations is helpful [3].

RECIPE's analytical method provides the reliable quantification of 37 ADPs (see method description in section 1.4). The analytes are listed in Table 1.

Table 1. Analyte list

Analytes	
Agomelatine	Milnacipran
Atomoxetine	Mirtazapine
Bupropion	Moclobemide
Citalopram	Nefazodone
Clomethiazole	O-Desmethyltramadol
Desmethylcitalopram	O-Desmethylvenlafaxine
Desmethylfluoxetine	Opipramol
Desmethylmianserine	Paroxetine
Desmethylmirtazapine	Reboxetine
Desmethylsertraline	Ritalinic acid
Dosulepin	Sertraline
Duloxetine	threo-Dihydro-Bupropion
erythro-Dihydro-Bupropion	Tianeptine
Fluoxetine	Tramadol
Fluvoxamine	Tranylcypromine
Guanfacine	Trazodone
Hydroxybupropion	Venlafaxine
Methylphenidate	Vortioxetine
Mianserine	

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. MS9400 contains the analyte-specific components for the determination of 37 different antidepressants. The analysis is performed by HPLC coupled 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).

The prepared samples are injected into the LC-MS/MS system for chromatographic separation of the compounds. The analytes are then ionised using electrospray ionisation (ESI).

Electrospray ionisation is a soft ionisation technique where a strong electric field is applied to the liquid passing through the ESI-capillary of the MS-source. The ions are mostly preformed in solution before desorption and then transferred into the ion path of the tandem mass spectrometer which consists of three quadrupoles (two mass selectors connected by a collision cell).

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 mass/charge (*m/z*) ratio are isolated in the first quadrupole and subsequently transferred into the collision cell, where they 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 can pass the third quadrupole for final detection. In this way the MRM mode ensures a selective identification and quantification of the target analytes.

The analytical method enables a robust and reliable quantitation in complex biological matrices by use of 28 different isotope-labelled internal standards (see section 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
MS9400	**ClinMass® Add-on Set** **for Antidepressants in Serum / Plasma** for 200 assays	1 pce.
	Content:	
	Internal Standard IS, lyophil.	1 x MS9412
	Serum Calibrator Set, lyophil. (Level 0 - 3)	1 x MS9413
	Manual	
	Separately available components:	
MS9412	Internal Standard IS, lyophil.	5 x 5 ml
MS9413	Serum Calibrator Set, lyophil. (Level 0 - 3)	4 x 1 x 1 ml
	Start Accessory:	
MS9414	Optimisation Mix, lyophil.	2 x 1 x 2 ml
	ClinChek® Controls:	
MS9482	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

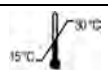
Please unpack all components from the transport packaging **immediately upon receipt** and follow the storage instructions indicated on the product labels and in Table 2.

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 ambient temperature	

Order no.		Product description	Storage conditions	
REF	MS9412	Internal Standard IS, lyophil.		Store below -18 °C*
REF	MS9413	Serum Calibrator Set, lyophil.		Store below -18 °C*
REF	MS9414	Optimisation Mix, lyophil.		Store below -18 °C*
REF	MS9482	Serum Control, lyophil., Level I+II		Store below -18 °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 kit system 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 0.7 ml/min and set the column heater at the temperature of 40 °C. Equilibrate the column with 10 ml of a 95 : 5 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 0.7 ml/min the backpressure of the analytical column should not exceed 230 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 0.7 ml/min).
Autosampler:	Injection volume: 1–10 µl* Injection interval: 3.7 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 gradient programme shown in Table 4 is used for the binary HPLC pump. Please note that according to the dead volume of the HPLC system in use an adaptation of the gradient might be necessary.

With the gradient programme shown in Table 4 all analytes except for the stereoisomers erythro- and threo-dihydro-bupropion are chromatographically separated (see example chromatogram in section 5.3.5). Those are determined as a sum of both compounds (dihydro-bupropion). If a separation of erythro- and threo-dihydro-bupropion is required please refer to section 7.3.

Table 4. Gradient programme

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]	Flow rate [ml/min]
0.01	95	5	0.7
0.10	95	5	0.7
0.20	75	25	0.7
1.50	50	50	0.7
2.50	45	55	0.7
2.60	20	80	0.7
3.00	20	80	0.7
3.10	95	5	0.7
3.70	95	5	0.7

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 shown in Table 5.

The assignments of the isotope-labelled substances to the analytes are shown 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).

The MRM experiments are carried out in ESI positive mode, source settings have to be adjusted to each system in order to reach best sensitivity for all analytes.

Table 5. Mass transitions of the analytes and the isotope-labelled substances in the IS (ESI positive)

Analyte / IS	Quantifier MRM		Qualifier MRM	
	Precursor [*m/z*]	Product [*m/z*]	Precursor [*m/z*]	Product [*m/z*]
Agomelatine	244.1	185.0	244.1	141.0
Atomoxetine	256.2	117.0	256.2	65.1
Bupropion	240.1	184.1	240.1	131.1
Citalopram	325.2	109.1	325.2	262.1
Clomethiazole	162.0	126.0	162.0	71.1
Desmethylcitalopram	311.2	109.0	311.2	234.1
Desmethylfluoxetine	296.1	134.1	296.1	105.0
Desmethylmianserine	251.2	208.1	251.2	118.0
Desmethylmirtazapine	252.1	195.1	252.1	209.1
Desmethylsertraline	292.1	159.0	292.1	275.0
Dosulepin	296.2	202.1	296.2	178.1
Duloxetine	298.3	154.1	298.3	97.1
Dihydro-Bupropion*	242.1	168.0	242.1	116.1
Fluoxetine	310.1	148.1	310.1	65.1

Analyte / IS	**Quantifier MRM**		**Qualifier MRM**	
	Precursor [*m/z*]	**Product [*m/z*]**	**Precursor [*m/z*]**	**Product [*m/z*]**
Fluvoxamine	319.2	71.1	319.2	200.1
Guanfacine	246.0	60.1	246.0	122.9
Hydroxybupropion	256.1	139.0	256.1	238.1
Methylphenidate	234.2	84.0	234.2	56.0
Mianserine	265.2	58.2	265.2	77.1
Milnacipran	247.2	100.1	247.2	230.2
Mirtazapine	266.2	195.1	266.2	72.1
Moclobemide	269.1	182.0	269.1	139.0
Nefazodone	470.2	274.1	470.2	246.1
O-Desmethyltramadol	250.2	58.0	250.2	232.2
O-Desmethylvenlafaxine	264.2	107.1	264.2	58.2
Opipramol	364.3	171.2	364.3	143.1
Paroxetine	330.2	70.1	330.2	192.1
Reboxetine	314.2	176.1	314.2	91.1
Ritalinic acid	220.1	84.1	220.1	56.2
Sertraline	306.1	159.0	306.1	275.0
Tianeptine	437.1	292.0	437.1	228.0
Tramadol	264.1	58.0	264.1	42.1
Tranylcypromine	134.1	117.1	134.1	65.1
Trazodone	372.2	120.0	372.2	78.1
Venlafaxine	278.2	58.2	278.2	260.2
Vortioxetine	299.1	150.0	299.1	109.0
d_3-Agomelatine	247.1	185.0		
d_3-Atomoxetine	259.2	117.0		
d_9-Bupropion	249.1	185.1		
d_6-Citalopram	331.2	109.1		
d_3-Dosulepin	299.2	203.1		
d_7-Duloxetine	305.3	154.1		
d_3-Desmethylcitalopram	314.2	109.0		
d_5-Desmethylfluoxetine	301.1	139.1		
d_4-Desmethylsertraline	296.1	160.0		
d_5-Fluoxetine	315.1	153.1		
d_6-O-Desmethyltramadol	256.2	64.0		
d_3-Fluvoxamine	322.2	74.1		
d_6-Hydroxybupropion	262.1	139.0		
d_9-Methylphenidate	243.2	93.0		
d_3-Mianserine	268.2	61.2		
d_{10}-Milnacipran	257.2	110.1		
d_3-Mirtazapine	269.2	195.1		
d_8-Moclobemide	277.1	182.0		
d_6-Nefazodone	476.2	280.1		
d_4-Opipramol	368.3	175.2		
d_4-Paroxetine	334.2	74.1		
d_5-Reboxetine	319.2	176.1		
d_3-Sertraline	309.1	159.0		
d_6-Tramadol	270.1	64.0		

Analyte / IS	Quantifier MRM		Qualifier MRM	
	Precursor [*m/z*]	Product [*m/z*]	Precursor [*m/z*]	Product [*m/z*]
d_9-threo-Dihydro-Bupropion	251.1	169.0		
d_5-Tranylcypromine	139.1	122.1		
d_6-Venlafaxine	284.2	58.2		
d_8-Vortioxetine	307.1	153.0		

*Dihydro-bupropion is determined as a sum of erythro- and threo-dihydro-bupropion. For the separation of these analytes please refer to section 7.3.

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

Analyte	RT [min]	Internal Standard IS	RT [min]
Agomelatine	1.56	d_3-Agomelatine	1.56
Atomoxetine	2.16	d_3-Atomoxetine	2.16
Bupropion	1.99	d_9-Bupropion	1.94
Citalopram	1.91	d_6-Citalopram	1.90
Clomethiazole	1.31	d_9-threo-Dihydro-Bupropion	1.56
Desmethylcitalopram	1.73	d_3-Desmethylcitalopram	1.73
Desmethylfluoxetine	2.33	d_5-Desmethylfluoxetine	2.32
Desmethylmianserine	1.98	d_5-Reboxetine	1.82
Desmethylmirtazapine	1.41	d_3-Mirtazapine	1.95
Desmethylsertraline	2.71	d_{4-}-Desmethylsertraline	2.70
Dosulepin	2.55	d_3-Dosulepin	2.52
Duloxetine	2.33	d_7-Duloxetine	2.32
Dihydro-Bupropion*	1.59	d_9-threo-Dihydro-Bupropion	1.56
Fluoxetine	2.62	d_5-Fluoxetine	2.61
Fluvoxamine	2.25	d_3-Fluvoxamine	2.24
Guanfacine	1.19	d_6-Tramadol	1.19
Hydroxybupropion	1.30	d_6-Hydroxybupropion	1.29
Methylphenidate	1.34	d_9-Methylphenidate	1.31
Mianserine	2.71	d_3-Mianserine	2.68
Milnacipran	1.36	d_{10}-Milnacipran	1.33
Mirtazapine	1.96	d_3-Mirtazapine	1.95
Moclobemide	1.09	d_8-Moclobemide	1.07
Nefazodone	2.81	d_6-Nefazodone	2.77
O-Desmethyltramadol	0.85	d_6-O-Desmethyltramadol	0.85
O-Desmethylvenlafaxine	0.98	d_6-Venlafaxine	1.55
Opipramol	1.95	d_4-Opipramol	1.95
Paroxetine	2.13	d_4-Paroxetine	2.13
Reboxetine	1.83	d_5-Reboxetine	1.82
Ritalinic acid	0.73	d_6-O-Desmethyltramadol	0.85
Sertraline	3.05	d_3-Sertraline	3.03
Tianeptine	1.34	d_{10}-Milnacipran	1.33
Tramadol	1.22	d_6-Tramadol	1.19
Tranylcypromine	0.95	d_5-Tranylcypromine**	0.94

Analyte	RT [min]	Internal Standard IS	RT [min]
Trazodone	1.74	d_4-Opipramol	1.95
Venlafaxine	1.56	d_6-Venlafaxine	1.55
Vortioxetine	3.41	d_8-Vortioxetine	3.40

*Dihydro-Bupropion is determined as a sum of erythro- and threo-Dihydro-Bupropion. For the separation of these analytes please refer to section 7.3.

**Alternatively d_6-tramadol can be used as an internal standard for tranylcypromine.

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 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 [5].

At room temperature (15–30 °C) the samples can be stored at least one day (5 hours for clomethiazole, tranylcypromine and two hours for bupropion, methylphenidate). At temperatures between 2–8 °C the samples can be stored at least 7 days (1 day for clomethiazole, tranylcypromine and 7 hours for bupropion, methylphenidate). At temperatures below -18 °C the samples can be stored for at least three months (multiple freeze-thaw cycles should be avoided).

Annotation: The samples should be cooled for transportation.

5.2 Sample preparation

5.2.1 Reconstitution of the lyophilised serum calibrators / controls

The ClinCal® Serum Calibrators and ClinChek® Serum Controls (order nos. MS9413 and MS9482, see section 2.1) are lyophilised and therefore need to be reconstituted before use.

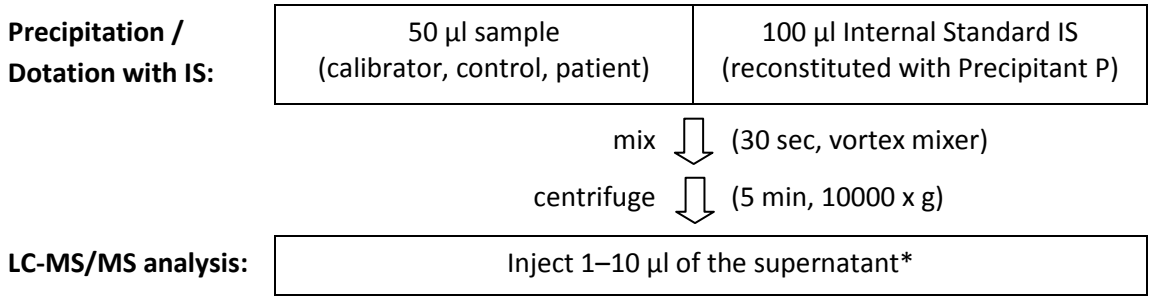
Information on reconstitution, analyte concentration, storage and stability is indicated in the respective product data sheets.

5.2.2 Reconstitution of the lyophilised Internal Standard IS

The ClinMass® Internal Standard IS (order no. MS9412) 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.

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). If necessary dilute the supernatant with Diluting Solution D (order no. MS9022).

5.2.3.3 Stability of the prepared samples

At room temperature (15–30 °C) the samples can be stored for at least two days. At temperatures between 2–8 °C the samples can be stored for at least 7 days.

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. MS9414 is available, which contains all analytes. **The packing unit of the Optimisation Mix** contains two bottles, **Optimisation Mixes 1 and 2**.

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

The Optimisation Mixes 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. MS9414.

If necessary the optimisation mixes 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. MS9414) 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

*mixture of equal volumes

5.3.3 Calibration run

A ClinCal® 4-Level Serum Calibrator Set (Level 0–3, order no. MS9413) is available for calibration.

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. MS9482).

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

Figure 1 shows a chromatogram of the ClinCal® Serum Calibrator (order no. MS9413), level 2, acquired with the LC system Shimadzu Nexera2 and the MS/MS system Sciex TQ5500

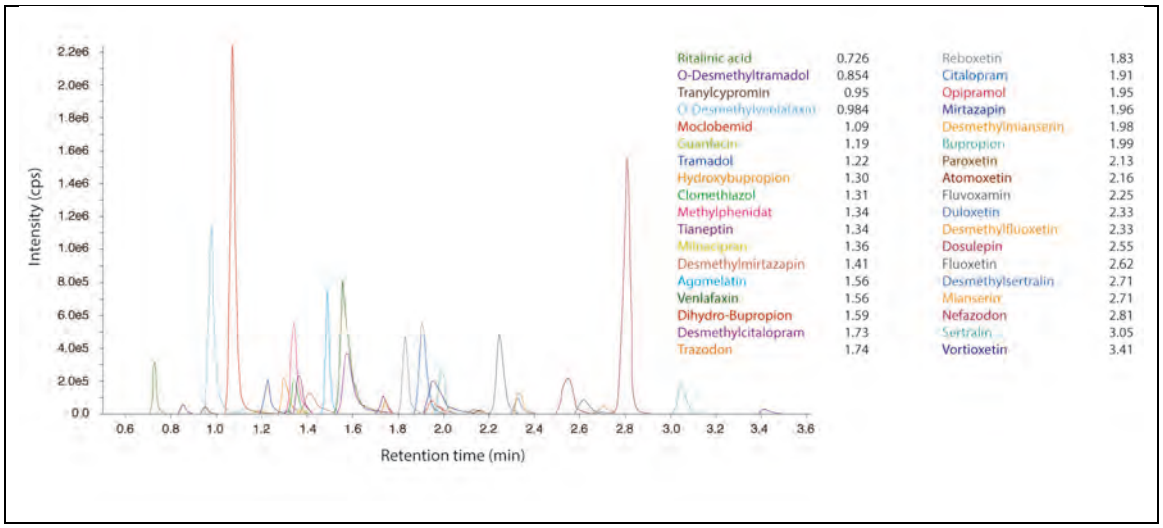


Figure 1. Chromatogram of the ClinCal® Serum Calibrator (order no. MS9413), level 2

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**
Agomelatine	243.3	0.243	4.110
Atomoxetine	255.4	0.255	3.915
Bupropion	239.7	0.240	4.172
Citalopram	324.4	0.324	3.083
Clomethiazole	161.7	0.162	6.184
Desmethylcitalopram	310.4	0.310	3.222
Desmethylfluoxetine	295.2	0.295	3.388
Desmethylmianserine	250.3	0.250	3.995
Desmethylmirtazapine	251.3	0.251	3.979
Desmethylsertraline	292.3	0.292	3.421
Dosulepin	295.5	0.296	3.384
Duloxetine	297.4	0.297	3.362
erythro-Dihydro-Bupropion	241.8	0.242	4.136
Fluoxetine	309.3	0.309	3.233
Fluvoxamine	318.3	0.318	3.142
Guanfacine	246.1	0.246	4.063
Hydroxybupropion	255.7	0.256	3.912
Methylphenidate	233.3	0.233	4.286
Mianserin	264.4	0.264	3.782
Milnacipran	246.4	0.246	4.058
Mirtazapine	265.4	0.265	3.768
Moclobemide	268.7	0.269	3.722
Nefazodone	470.0	0.470	2.128
O-Desmethyltramadol	249.3	0.249	4.011
O-Desmethylvenlafaxine	263.4	0.263	3.797
Opipramol	363.5	0.364	2.751
Paroxetine	329.4	0.329	3.036
Reboxetine	313.4	0.313	3.191
Ritalinic acid	219.3	0.219	4.560

Analyte	**Molecular weight [g/mol]**	**Conversion: nmol/l → µg/l**	**Conversion: µg/l → nmol/l**
Sertraline	305.3	0.305	3.275
threo-Dihydro-Bupropion	241.8	0.242	4.136
Tianeptine	437.0	0.437	2.288
Tramadol	263.4	0.263	3.797
Tranylcypromine	133.2	0.133	7.508
Trazodone	371.9	0.372	2.689
Venlafaxine	277.4	0.277	3.605
Vortioxetine	298.5	0.299	3.350

7 Test data

7.1 Validation data

The validation data was established with the LC system Agilent 1290 and the MS/MS system Agilent 6460.

7.1.1 Linearity, detection limit and quantitation limit

Linearity, detection limit 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]
Agomelatine	0.750–2250	0.250	0.750
Atomoxetine	21.9–6579	7.30	21.9
Bupropion	2.50–750	0.833	2.50
Citalopram	1.00–750	0.333	1.00
Clomethiazole	100–30000	33.3	100
Desmethylcitalopram	2.50–1000	0.833	2.50
Desmethylfluoxetine	5.00–1500	1.67	5.00
Desmethylmianserine	2.50–750	0.833	2.50
Desmethylmirtazapine	1.25–500	0.417	1.25
Desmethylsertraline	2.50–750	0.833	2.50
Dihydro-Bupropion*	11.2–6717	3.73	11.2
Dosulepin	1.00–1500	0.333	1.00
Duloxetine	5.00–1500	1.67	5.00
Fluoxetine	4.46–1786	1.49	4.46
Fluvoxamine	2.00–2000	0.667	2.00
Guanfacine	0.217–65.2	0.0723	0.217
Hydroxybupropion	11.0–6579	3.67	11.0
Methylphenidate	0.500–300	0.167	0.500
Mianserine	1.25–750	0.417	1.25
Milnacipran	3.75–2250	1.25	3.75
Mirtazapine	1.00–750	0.333	1.00
Moclobemide	1.50–6000	0.500	1.50
Nefazodone	2.00–3000	0.667	2.00
O-Desmethyltramadol	7.50–4500	2.50	7.50
O-Desmethylvenlafaxine	7.50–2250	2.50	7.50
Opipramol	5.00–2000	1.67	5.00
Paroxetine	2.50–1500	0.833	2.50
Reboxetine	5.00–2000	1.67	5.00
Ritalinic acid	2.50–1000	0.833	2.50
Sertraline	1.00–750	0.333	1.00
Tianeptine	1.19–714	0.397	1.19
Tramadol	2.63–1974	0.877	2.63
Tranylcypromine	2.50–750	0.833	2.50
Trazodone	9.10–6818	3.03	9.10
Venlafaxine	2.50–1000	0.833	2.50
Vortioxetine	0.980–591	0.327	0.980

*Dihydro-bupropion as a sum of erythro and threo-dihydro-bupropion

7.1.2 Recovery

The recovery rate for all analytes lies between 91–99 %.

7.1.3 Precision

The intra- and interassay precisions of the method 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 [%]**		**Interassay Precision [%]**	
Analyte	**Level**		**Level**		**Level**	
	I	**II**	**I**	**II**	**I**	**II**
Agomelatine	26.9	290	3.7	4.2	8.3	5.0
Atomoxetine	407	952	4.8	5.3	8.9	6.5
Bupropion	35.3	78.7	4.7	4.5	7.9	8.1
Citalopram	49.0	116	3.1	3.7	3.5	6.3
Clomethiazole	518	2828	11.4	7.4	8.9	3.8
Desmethylcitalopram	24.7	58.9	6.1	4.6	6.3	6.7
Desmethylfluoxetine	118	275	5.8	4.6	3.3	4.0
Desmethylmianserine	30.0	67.6	5.3	3.7	5.6	5.2
Desmethylmirtazapine	34.9	80.6	3.5	2.6	4.4	4.0
Desmethylsertraline	38.7	91.7	7.1	6.2	6.5	6.3
Dihydro-Bupropion*	279	662	4.5	4.3	4.9	5.9
Dosulepin	44.6	102	3.2	2.9	4.8	2.8
Duloxetine	50.3	115	5.2	4.5	7.8	4.5
Fluoxetine	105	249	6.1	5.7	4.8	4.7
Fluvoxamine	101	244	2.6	2.7	2.7	3.9
Guanfacine	2.52	5.97	5.4	8.9	9.9	6.3
Hydroxybupropion	398	903	2.9	4.4	6.6	3.6
Methylphenidate	11.1	25.3	1.4	3.0	5.8	3.2
Mianserine	29.3	69.3	2.4	2.9	3.9	4.1
Milnacipran	77.4	180	2.2	2.3	5.5	2.9
Mirtazapine	33.9	79.0	2.6	3.3	3.4	4.7
Moclobemide	474	1089	2.4	3.0	7.3	4.3
Nefazodone	103	232	2.3	3.8	4.6	4.4
O-Desmethyltramadol	227	518	0.8	2.7	7.8	5.9
O-Desmethylvenlafaxine	104	239	4.6	3.9	5.9	5.9
Opipramol	108	257	2.6	3.8	7.8	7.1
Paroxetine	50.6	121	3.3	4.3	6.5	4.7
Reboxetine	134	318	1.5	2.8	2.9	3.5
Ritalinic acid	63.2	151	6.3	5.2	5.4	6.2
Sertraline	28.9	145	3.8	2.4	6.3	4.4
Tianeptine	31.7	73.4	2.3	4.5	5.8	4.9
Tramadol	231	501	1.6	2.9	8.5	7.0
Tranylcypromine	19.6	46.9	5.6	5.0	5.1	4.9

	Concentration [µg/l]		Intraassay Precision [%]		Interassay Precision [%]	
Analyte	**Level**		**Level**		**Level**	
	I	**II**	**I**	**II**	**I**	**II**
Trazodone	548	1224	2.6	3.1	4.4	6.5
Venlafaxine	60.9	145	1.9	1.9	1.9	3.7
Vortioxetine	22.4	51.1	2.2	3.2	4.4	5.9

*Dihydro-bupropion as a sum of erythro- and threo-dihydro-bupropion

7.2 Reference ranges

The following reference ranges are taken from the „ Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017" [3].

Table 11. Reference ranges

Analyte	**Therapeutic Range [µg/l]**	**Laboratory Alert Level† [µg/l]**
Agomelatine	7–300 (1–2 h after 50 mg)	600
Atomoxetine	200–1000 60–90 min after intake of 1.2 mg/kg/day	2000
Bupropion plus Hydroxybupropion	Antidepressant drug: 850–1500 Drug for treatment of substance related disorders: 550–1500	Antidepressant drug: 2000 Drug for treatment of substance related disorders: 2000
Citalopram	50–110	220
Clomethiazole	100–5000 (at 4 to 8 h)	n. a.
Desmethylcitalopram	n. a.	n. a.
Desmethylmianserine	n. a.	n. a.
Desmethylmirtazapine	n. a.	n. a.
Dosulepin*	45–100	200
Duloxetine	30–120	240
erythro-Dihydro-Bupropion	n. a.	n. a.
Fluoxetine plus Desmethylfluoxetine	120–500	1000
Fluvoxamine	60–230	500
Guanfacine	n. a.	n. a.
Methylphenidate	For children and adolescents: 6–26 2 h after 20 mg IR (immediate release) or 4–6 h after 40 mg XR (retarded formulations) formulations For adults: 12–79 2 h after 20 mg IR or 4–6 h after 40 mg XR formulations	50
Mianserin	15–70	140
Milnacipran	100–150	300
Mirtazapine	30–80	160
Moclobemide	300–1000	2000
Nefazodone	n. a.	n. a.
O-Desmethyltramadol	n. a.	n. a.
O-Desmethylsertraline	n. a.	n. a.
Opipramol	50–500	1000
Paroxetine	20–65	120
Reboxetine	60–350	700
Ritalinic acid**	80–300	
Sertraline	10–150	300
threo-Dihydro-Bupropion	n. a.	n. a.
Tianeptine	30–80	160

Analyte	**Therapeutic Range [µg/l]**	**Laboratory Alert Level† [µg/l]**
Tramadol	n. a.	n. a.
Tranylcypromine	≤ 50	100
Trazodone	700–1000	1200
Venlafaxine plus O-Desmethylvenlafaxine	100–400	800
Vortioxetine	10–40	80

n.a.: not available;

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

*Dosulepin is in [3] described as dothiepin

**withdrawn from reference [4]

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 Separation and quantification of erythro- and threo-Dihydro-Bupropion

For the separation and quantification of the stereoisomers erythro- and threo-dihydro-bupropion the gradient programme shown in Table 12 has to be used. **Please note that this programme is only suitable for those stereoisomers.** The programme is not suitable for the separation and quantification of the remaining analytes. The retention times are shown in Table 13.

Table 12. Gradient programme for the separation of erythro- and threo-dihydro-bupropion*

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]	Flow rate [ml/min]
0.01	89	11	0.7
7.50	89	11	0.7
7.60	20	80	0.7
7.80	20	80	0.7
7.90	89	11	0.7
8.50	89	11	0.7

*Note:

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

Table 13. Assignment of erythro- and threo-dihydro-bupropion to the isotope labelled substances in the IS

Analyte	RT [min]	Internal Standard IS	RT [min]
erythro-Dihydro-Bupropion	5.48	d_9-threo-Dihydro-Bupropion	5.99
threo-Dihydro-Bupropion	6.24		

7.4 Interferences

7.4.1 O-Desmethylvenlafaxine and Tramadol

O-Desmethylvenlafaxine and tramadol are isobaric substances (molecular weight 263.4 g/mol). Only O-desmethylvenlafaxine shows a selective mass transition as the quantifier. Its qualifier and both mass transitions of tramadol show both substances. However, these analytes 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 the Optimisation Mixes (see section 5.3.2).

7.4.2 Guanfacine

O-Desmethylvenlafaxine and tramadol interfere with both mass transitions of guanfacine. However, the analytes are baseline separated, thus a selective quantification is ensured.

7.4.3 Desmethylmianserine

Lacosamide interferes with the quantifier mass transition of desmethylmianserine, the qualifier mass transition is not affected by this interference. However, these analytes are chromatographically separated, so in case of co-medication with lacosamide a selective quantification of desmethylmianserine is still ensured.

7.4.4 Mirtazapine

7.4.4.1 Nordoxepin

Nordoxepin, the active metabolite of doxepin, interferes with both mass transitions of mirtazapine. However, the analytes are chromatographically separated, so in case of co-medication with doxepin a selective quantification of mirtazapine is still ensured.

7.4.4.2 Mycophenolic acid

Mycophenolic acid interferes with both mass transitions of mirtazapine. However, the peaks are baseline separated, thus a selective quantification of mirtazapine is still ensured.

7.4.5 d_4-Desmethylsertraline

In case of low sensitivity for d_4-desmethylsertraline (system dependent), the mass transition of the internal standard can show an interference peak from dosulepin. However, the two analytes are baseline separated, thus d_4-desmethylsertraline can still be evaluated.

7.4.6 d_9-Methylphenidate

d_9-Methylphenidate is a racemic mixture of its erythro and threo isomers. Therefore, the mass transition of d_9-methylphenidate shows two peaks which are baseline separated. As normally the threo diastereomers of methylphenidate are given, also the threo form of the internal standard (second eluting peak) should be used for quantification.

8 References

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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
Dessauerstraße 3
80992 München / Germany

declares that the CE labelled products

ClinMass® TDM Platform (order no.: MS9000) and
ClinMass® Add-on Set for Antidepressants in Serum / Plasma (order no.: MS9400)

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

München, 25.05.2018

Alfred Bauer
General Manager

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