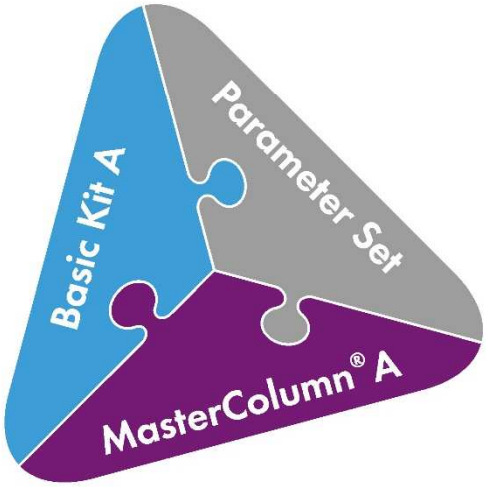
CHROMSYSTEMS

MassTox® TDM Series A



Instruction Manual for LC-MS/MS Analysis

MassTox® TDM Series A PARAMETER Set

Antimycotic Drugs/*EXTENDED* in serum/plasma

92922/XT

CE 0123 IVD

Parameters:

- Anidulafungin
- Caspofungin
- Fluconazole
- 5-Flucytosine
- Isavuconazole
- Itraconazole
- Hydroxyitraconazole
- Ketoconazole
- Micafungin
- Posaconazole
- Voriconazole

Incident reporting:
Any serious incident that has occurred in relation to the 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.

Chromsystems Instruments & Chemicals GmbH is certified according to ISO 13485 (including MDSAP). Products are produced and put into circulation according to regulation (EU) 2017/746 on in vitro diagnostic medical devices.

You can download the declaration of conformity according to IVDR regulation 2017/746 from the secured area of our website.

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1 Ordering information

1.1 Kits

Basic kits:

92111/200		***MassTox*® TDM Series A** **BASIC Kit** **Kit content for 200 analyses:**	
		(Number of packaging units/order number/name/content per packaging unit)	
1 x	92001	Mobile Phase 1	1000 mL
1 x	92002	Mobile Phase 2	1000 mL
1 x	92003	Precipitation Reagent	50 mL
1 x	92005	Extraction Buffer	5 mL
1 x	92007	Dilution Buffer 1	50 mL
1 x	92008	Dilution Buffer 2	50 mL
1 x	92009	Rinsing Solution	1000 mL
2 x	33006	Reaction Vials, transparent	100 pcs.

92111/1000		***MassTox*® TDM Series A** **BASIC Kit** **Kit content for 1000 analyses:**	
		(Number of packaging units/order number/name/content per packaging unit)	
3 x	92001	Mobile Phase 1	1000 mL
3 x	92002	Mobile Phase 2	1000 mL
5 x	92003	Precipitation Reagent	50 mL
5 x	92005	Extraction Buffer	5 mL
5 x	92007	Dilution Buffer 1	50 mL
5 x	92008	Dilution Buffer 2	50 mL
2 x	92009	Rinsing Solution	1000 mL
10 x	33006	Reaction Vials, transparent	100 pcs.

Parameter Set:

92922/XT		***MassTox*® TDM Series A** **PARAMETER Set** **Antimycotic Drugs/*EXTENDED* in serum/plasma** Parameter set for the analysis of: anidulafungin, caspofungin, fluconazole, 5-flucytosine, isavuconazole, itraconazole, hydroxyitraconazole, ketoconazole, micafungin, posaconazole and voriconazole	
		(Number of packaging units/order number/name/content per packaging unit)	
1 x	92051/XT	3PLUS1® Multilevel Plasma Calibrator Set ***MassTox*®** Antimycotic Drugs/*EXTENDED*	4 x 1.0 mL (lyoph.)
1 x	0253/XT	***MassCheck*®** Antimycotic Drugs/*EXTENDED* Plasma Control Level I	5 x 1.0 mL (lyoph.)
1 x	0254/XT	***MassCheck*®** Antimycotic Drugs/*EXTENDED* Plasma Control Level II	5 x 1.0 mL (lyoph.)
1 x	92644/XT	Internal Standard Mix ***MassTox*®** Antimycotic Drugs/*EXTENDED* (content for 200 analyses)	1 x 3.8 mL

1.2 Individual components

1.2.1 Required components

To perform the assay, the Chromsystems components listed in this chapter are required.

For sample preparation:

CE/IVD products		
92003	Precipitation Reagent	50 mL
92005	Extraction Buffer	5 mL
92007	Dilution Buffer 1	50 mL
92008	Dilution Buffer 2	50 mL
92644/XT	Internal Standard Mix	
	MassTox® Antimycotic Drugs/*EXTENDED*	1 x 3.8 mL
General lab equipment		
33006	Reaction Vials, transparent	100 pcs.

For calibration and quality control:

CE/IVD products		
92051/XT	**3PLUS1**® Multilevel Plasma Calibrator Set	
	MassTox® Antimycotic Drugs/*EXTENDED*	4 x 1.0 mL (lyoph.)
0252/XT	***MassCheck***® Antimycotic Drugs/*EXTENDED* Plasma Control	
	Bi-Level (I + II)	2 x 5 x 1.0 mL (lyoph.)
0253/XT	***MassCheck***® Antimycotic Drugs/*EXTENDED* Plasma Control Level I	5 x 1.0 mL (lyoph.)
0254/XT	***MassCheck***® Antimycotic Drugs/*EXTENDED* Plasma Control Level II	5 x 1.0 mL (lyoph.)

For chromatography:

CE/IVD products		
92001	Mobile Phase 1	1000 mL
92002	Mobile Phase 2	1000 mL
92009	Rinsing Solution	1000 mL
92110	***MassTox***® **TDM MasterColumn® Series A**	
	Analytical column (equilibrated, with test chromatogram)	1 pc.

For first installation and optimisation of the method:

CE/IVD products		
92039/XT	Tuning Mix ***MassTox***® Antimycotic Drugs/*EXTENDED*	1 mL

1.2.2 Optional components

The components listed in this chapter are suitable for the use with the assay. However, their use is optional.

Accessories		
15011	PEEK-encased Prefilter, 2 µm	5 pcs.
15071	Stainless Steel Prefilter, 0.5 µm	5 pcs.
General lab equipment		
15010	PEEK Prefilter Housing	1 pc.
15070	Stainless Steel Prefilter Housing	1 pc.

2 Introduction

2.1 Background information

TDM (Therapeutic Drug Monitoring) is generally understood as the determination of the concentration of drugs in a biological matrix, generally blood, with the aim of optimising a patient's treatment regime. Numerous studies and publications confirm the need for individual drug dosing and the associated monitoring of target concentrations in the blood.

Due to the increasing number of immunocompromised patients, invasive mycoses have continuously increased in recent years. Immunodeficiency is usually the result of an infectious disease such as AIDS or is caused by the increased use of highly effective drugs such as immunosuppressants in transplant medicine and chemotherapeutic agents or radiation therapy in cancer treatment.

Most mycoses are caused by *Candida* and *Aspergillus* species, which enter the body either through the air (spores of moulds) or through injuries to the skin. There, they can infect various internal organs or entire organ systems (e.g. gastrointestinal tract), which is why they are also called systemic mycoses. Thanks to improved diagnostic methods, rare mycoses such as fusarioses, scedosporium or zygomycoses are increasingly being detected and made accessible for therapy.

Modern triazole antifungals such as isavuconazole, itraconazole, posaconazole and voriconazole are very suitable for both prophylactic and therapeutic treatment of systemic fungal infections due to their relatively good tolerability and broad spectrum of activity. Posaconazole offers the possibility of oral antifungal therapy also against fusarioses and zygomycoses. Voriconazole is effective against *Fusarium* and *Scedosporium species*, while itraconazole (with its active metabolite hydroxyitraconazole) is limited in its use to the treatment of mycoses caused by yeasts (*Candida* spp.) and filamentous fungi *(Aspergillus* spp.). In 2015, isavuconazole was approved for the treatment of invasive aspergillosis and mucormycosis. Older representatives such as fluconazole and ketoconazole, however, are also still used in the treatment of fungal infections.

The antifungal mechanism of action of all triazole antifungals is based on the inhibition of cytochrome P450-dependent lanosterol-14-α-demethylase, thereby inhibiting the synthesis of ergosterol, which is essential for the maintenance and activity of the fungal membrane. Various secondary effects such as the accumulation of 14-methylated sterols and 3-ketosteroids as well as the uncoordinated synthesis of chitin also impair the stability of fungal membranes, the formation of primary yeast septa and the activity of membrane-bound enzymes. This makes the fungus susceptible to osmotic damage, leading to phagocytosis by host cells and eventual death.

5-flucytosine is an active substance from the group of pyrimidine antifungals. Its spectrum includes generalised candidamycoses, cryptococcoses, chromoblastomycoses and aspergilloses. 5-flucytosine is taken up as a pro-drug by a cytosine permease into the fungal cell, where it is converted to 5-fluorouracil by cytosine deaminase. This deamination does not occur in mammalian cells, or does so only to a small extent. The 5-fluorouracil formed inhibits DNA synthesis and can lead to the formation of defective RNA and thus has a fungistatic effect.

Anidulafungin, caspofungin and micafungin belong to the group of echinocandins. These inhibit glucan synthase, which is necessary for the formation of polymeric carbohydrates, important structural molecules of the fungal cell wall. The cell wall is destabilised and the fungi die. Echinocandins are mainly used to treat invasive candidiasis.

In the systemic treatment of mycoses, monitoring of drug levels is of particular benefit. Depending on the dosage form, type of disease and co-medication, the bioavailability and metabolisation varies greatly. Only adherence to the desired therapeutic concentrations ensures the success of the therapy while minimising the sometimes serious side effects. At the same time, the formation of dangerous resistances is counteracted.

2.2 Principle of the assay

The ***MassTox***® **TDM Series A** assays allow the rapid quantitative determination of a multitude of drugs in serum or plasma by LC-MS/MS. To perform the analysis, you will need the ***MassTox***® **TDM Series A BASIC Kit,** the substance-specific ***MassTox***® **TDM Series A PARAMETER Set** and the ***MassTox***® **TDM MasterColumn® Series A.** The principle of the sample preparation is the same for all ***MassTox***® **TDM Series A PARAMETER Sets**, based on a simple, effective protein precipitation process.

This Chromsystems parameter set allows for the fast quantitative determination of anidulafungin, caspofungin, fluconazole, 5-flucytosine, isavuconazole, itraconazole, hydroxyitraconazole, ketoconazole, micafungin, posaconazole and voriconazole in serum/plasma by LC-MS/MS. The use of stable isotopically labelled internal standards ensures the reliable and reproducible quantitation of the analytes in less than four minutes.

Detailed performance evaluation data for this assay can be found in Appendix II.

2.3 Intended purpose

The Chromsystems "***MassTox***® TDM Series A PARAMETER Set Antimycotic Drugs/*EXTENDED* in serum/plasma" is an *in vitro* diagnostic medical device for professional use in clinical laboratories for the quantitative determination of anidulafungin, caspofungin, fluconazole, 5-flucytosine, isavuconazole, itraconazole, hydroxyitraconazole, ketoconazole, micafungin, posaconazole and voriconazole in human serum or plasma samples via liquid chromatography mass spectrometry (LC-MS/MS).

Manual sample preparation and chromatographic separation is carried out with the Chromsystems "***MassTox***® TDM Series A BASIC Kit" (order no. 92111), which provides the required reagents and buffers, and with the "***MassTox***® TDM MasterColumn® Series A" (order no. 92110).

The Chromsystems "***MassTox***® TDM Series A PARAMETER Set Antimycotic Drugs/*EXTENDED* in serum/plasma" is intended as a therapeutic drug monitoring test, medically indicated for patients treated with one or more of the antimycotic drugs listed above.

2.4 Clinical limitations

There are no universally applicable therapeutic reference ranges for the analytes covered in the ***MassTox***® TDM Series A PARAMETER Set Antimycotic Drugs/*EXTENDED* in serum/plasma. Results obtained using different test methods cannot be compared. Laboratories should indicate the method used for analysis to enable accurate interpretation of the results.

Users must specify their own therapeutic reference ranges based on clinical assessment. Conversion factors between different methods of analysis should not be used to predict results for a specific patient.

Concentration determined on a single specimen may not reflect future concentrations because of changes in adherence, drug dosage, route of administration, absorption, or receipt of other medication affecting absorption or metabolism of the drug(s).

The reagent kit has been developed as a therapeutic drug monitoring test and covers the normal range of concentrations expected with appropriate use of the drug. Concentrations of the antimycotic drugs are quantifiable only up to the ULOQ. Concentrations above the analytical measurement range are not covered by a verified pre-dilution of the sample.

Please consider information on collection and storage of patient specimen (see chapter 5.1) and on possible interferences (see chapter 11) when using the assay.

3 LC-MS/MS system

Caution:
When using the reagents comply with the hazard information in Appendix I.

3.1 General purpose equipment

This chapter lists all general purpose equipment not listed in chapter 1 that is required or recommended for the LC-MS/MS analysis of antimycotic drugs in serum/plasma using the ***MassTox***® **TDM Series A BASIC Kit** in combination with the ***MassTox***® **TDM Series A PARAMETER Set Antimycotic Drugs/***EXTENDED*.

Essential equipment

- Tandem mass spectrometer with evaluation software (sufficient sensitivity provided)
- Gradient HPLC system
- Autosampler (preferably with cooling function)
- Pipettes and pipette tips
- Bench-top centrifuge
- Vortex™

Optional/recommended equipment

- 2-position 6-way selector valve for MS waste switching

3.2 HPLC instrument parameters

Substances are separated chromatographically using the ***MassTox***® **TDM MasterColumn® Series A** (order no. 92110). Keep the mobile phases closed or covered even when in use.

Note:
We recommend the use of a prefilter (PEEK-encased Prefilter, 2 µm, order no. 15011 or Stainless Steel Prefilter, 0.5 µm, order no. 15071) in order to enhance the column lifetime.

The column should only be rinsed with the solutions specified in this instruction manual. Other solvents could irreversibly damage the column.

Instrument settings:

Autosampler:	Cooling recommended
Injection volume:	0.5 to 50 µL (dependent on the mass spectrometer operated)
Run time:	3.2 min
Flow rate:	0.6 mL/min
Column temperature:	+20 to +25 °C
Needle rinsing solution for the injector:	Rinsing Solution (order no. 92009)

To establish the optimum injection volume, inject increasing volumes of a prepared calibrator 1 (92051/1/XT) diluted according to the dilution scheme in chapter 5.6 up to a maximum of 50 µL, until the required peak size and an appropriate signal-to-noise ratio has been established. Then check whether, after calibration of the evaluation system according to chapter 7.1, acceptable deviations from the target value result for all calibrator levels and all analytes.

Gradient:
The separation of the antimycotic drugs is done with gradient elution. The gradient profile shown and the example of a chromatogram in chapter 7.2 is intended as a basis for optimisation. Because of the different void volumes of individual HPLC systems the following gradient profile may have to be modified.

Table 1: Gradient profile (flow rate 0.6 mL/min)

Time	Mobile Phase 1	Mobile Phase 2
0.00 min	70 %	30 %
0.50 min	70 %	30 %
0.51 min	0 %	100 %
2.80 min	0 %	100 %
2.81 min	70 %	30 %
3.20 min	70 %	30 %

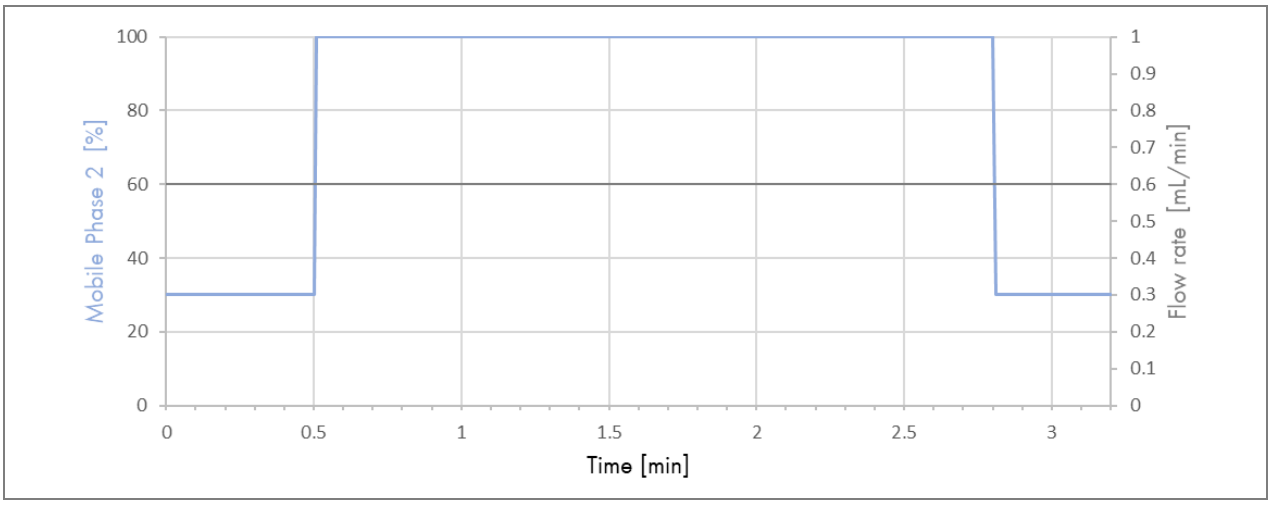

Figure 1: Diagram of the gradient profile

3.3 MS/MS operation

Principle of operation:
Mass spectrometers measure molecules according to their mass to charge ratio (m/z). The analytes first have to be ionised and transferred to the gas phase. Electrospray ionisation (ESI) has proven to be a highly versatile and user-friendly ionisation method for this purpose.

The triple quadrupole tandem MS method uses a variety of measurement modes. The only one used for the assay presented here is multiple reaction monitoring.

Multiple reaction monitoring (MRM):
In MRM mode, both the first and second mass filter are set statically to a particular mass-charge ratio (m/z). In MS 1, the molecular ion of the analyte is usually selected. Ions with a different mass-charge ratio are not shown. The molecular ion undergoes fragmentation in the collision cell and MS 2 detects the characteristic fragment ion. MRM mode enables exceptionally sensitive and selective quantitation. MRM transitions provided in chapter 4 are indicated as MRM 1 (quantifier) and MRM 2/3 (qualifier). For quantitation, only one mass transition is used (MRM 1). For identification of each analyte, a second mass transition (MRM 2 or 3) is regarded as sufficient, provided that the relative fragment ion intensities of both MRMs are consistent in calibrators and samples. MRM mode enables exceptionally sensitive and selective quantitation.

3.4 Optimising the MRMs (tuning)

Before tuning it is highly recommended to check the accuracy and the mass resolution of the MS/MS system according to the manufacturer's instructions. If accuracy and mass resolution are outside the specifications of the manufacturer of the instrument, a re-calibration of the mass spectrometer is recommended. Following this the analyte MRM transitions should be tuned as follows:

1. Dilute the Tuning Mix (order no. 92039/XT) with Mobile Phase 2 (order no. 92002) as appropriate for the specific device (e.g. 1:5 for SCIEX 4500MD™)
2. Inject diluted Tuning Mix or infuse by syringe pump through a T-piece into the solvent stream (100 % Mobile Phase 2 at a flow rate of 0.6 mL/min)
3. Use Q1 Scan (MS Scan) to determine the exact positions of the signal maxima of MS1 masses (precursor/parent ions) (to at least one decimal place)
4. Determine the exact positions of the signal maxima of MS2 masses (product/daughter ion) by product ion scan (to at least one decimal place)
5. Optimise the individual parameters for each MRM transition (e. g. collision energy)
6. Use the optimised MRM transitions to optimise the ion source, especially the capillary voltage, the temperature and the gas flows.

It is recommended to read the operating manual of the LC-MS/MS system. For any questions contact the instrument manufacturer. Training by the manufacturer may be required as necessary.

Note:
- Caspofungin is double charged
- For micafungin and ISTD 11 negative electrospray ionisation mode (ESI–) is applied
- ISTD 9 (internal standard for anidulafungin) and ISTD 11 (internal standard for micafungin) are not included in Tuning Mix 92039/XT. See also product information leaflet of the Tuning Mix. In order to optimise the MRMs of both ISTDs, first optimise the two corresponding unlabelled substances and then calculate the exact MS1and MS2 masses based on this.

3.5 System start-up

Before starting a sequence prepare the LC-MS/MS system as follows:

1. Equilibrate the system for 10 min using the initial conditions of the method
2. Repeatedly inject a prepared sample (e. g. ***MassCheck***® Antimycotic Drugs/*EXTENDED* Plasma Control Level II, order no. 0254/XT) until the retention times, peak shapes and signal intensities of the analytes are consistent
3. Start the sequence

For proper use of your LC-MS/MS system, read the instruction manual of your LC-MS/MS system. If you have any questions, ask the device manufacturer. Training from the device manufacturer may be required.

Check the mass precision and resolution of the mass spectrometer periodically and recalibrate the mass spectrometer if you notice deviations. Read the information in the instruction manual of your mass spectrometer before doing so and contact the device manufacturer if you require further information.

3.6 System shutdown

To pause operation, switch off the HPLC pump and leave the mobile phase in the HPLC system. Salt crystals are unlikely to build up on the piston seals of the HPLC pumps. To protect the ion source and multiplier, switch the MS/MS system to standby mode. Leave the vacuum pumps of the LC-MS/MS system on.

For a longer break, rinse the analytical column with 25 % Mobile Phase 1 and 75 % Mobile Phase 2 and store it tightly closed at ambient temperature. Switch off the HPLC pump and switch the MS/MS system to standby mode.

4 MRM transitions

The following table includes all MRM transitions for the analytes and their isotopically labelled internal standards. Measure the analytes and the internal standards in positive and negative ionisation mode using electrospray ionisation (ESI).

High-sensitivity new-generation mass spectrometers with fast electronics are able to measure all the analytes in one run. Polarity switching between positive and negative ionisation mode is then necessary. When using weaker systems, the analytes should be divided between two methods to achieve enough data points with an adequate measurement time.

Table 2: Recommended MRM transitions (positive ionisation mode)

Substance	Corresponding internal standard (MRM 1)	MRM 1 Quantifier	MRM 2 Qualifier	MRM 3 Qualifier
Anidulafungin	ISTD 9 (1151.5 → 354.1)	1140.5 → 343.1	1140.5 → 1104.5	1140.5 → 1086.5
Caspofungin	ISTD 10 (549.8 → 131.1)	547.3 → 137.1	547.3 → 131.1	n/a
Fluconazole	ISTD 2 (311.1 → 223.1)	308.1 → 220.1	308.1 → 238.1	308.1 → 169.1
5-Flucytosine	ISTD 1 (133.0 → 115.0)	130.0 → 113.0	130.0 → 58.0	130.0 → 85.0
Isavuconazole	ISTD 8 (442.1 → 224.1)	440.1 → 225.1	439.1 → 369.1	440.1 → 215.1
Itraconazole	ISTD 6 (714.3 → 401.1)	705.3 → 392.1	705.3 → 432.1	705.3 → 335.1
Hydroxy-itraconazole	ISTD 7 (729.3 → 400.1)	721.3 → 392.1	721.3 → 430.1	721.3 → 408.1
Ketoconazole	ISTD 4 (539.2 → 497.1)	532.2 → 489.1	532.2 → 83.0	532.2 → 244.1
Posaconazole	ISTD 12 (706.3 → 619.3)	701.3 → 614.3	701.3 → 683.3	701.3 → 343.1
Voriconazole	ISTD 3 (353.1 → 284.1)	351.1 → 281.1	351.1 → 224.1	350.1 → 155.1

Table 3: Recommended MRM transitions (negative ionisation mode)

Substance	Corresponding internal standard (MRM 1)	MRM 1 Quantifier	MRM 2 Qualifier	MRM 3 Qualifier
Micafungin	ISTD 11 (1279.4 → 469.1)	1268.4 → 469.1	1268.4 → 320.1	1268.4 → 300.1

The specified nominal masses are starting points for optimisation. The precise position of the exact masses may vary slightly from MS system to MS system and needs to be determined precisely during method tuning. To set up the method, we recommend specifying mass position to at least one decimal place. Use the Tuning Mix for this purpose (order no. 92039/XT, see section 3.4 on optimising MRM transitions).

If you require more information about setting up the method on your LC-MS/MS system, please contact our Chromsystems support staff by calling our hotline +49 89 18930-111 or by e-mail at support@chromsystems.com.

5 Sample preparation

Caution:
When using the reagents comply with the hazard information in Appendix I.

Ensure that within a sequence the used batch of reagents (including internal standard) for sample preparation as well as the batch of the calibrator and the control are not changed.

5.1 Collection and storage of patient specimens

EDTA/heparin plasma or serum is used for analysis.

For the analysis of anidulafungin, we recommend the use of EDTA/heparin plasma samples and not serum as specimen type. When using different serum sampling systems (see chapter 11.1 "Interferences detected"), absorption of anidulafungin may occur, resulting in falsely low test results.

Table 4: Storage life of patient specimens

Storage temperature	Storage life
+20 to +25 °C	2 days for all analytes except anidulafungin and micafungin: 24 hours
+2 to +8 °C	2 weeks
below –18 °C	2 months
Freeze-thaw cycles	1 cycle

Note:
It is not recommended to use blood sampling systems containing gel separators. Some types of gels may partly absorb analytes and thus produce false-low test results. For further information see chapter 11.1 "Interferences detected".

Storage life data were established from spiked plasma or serum. The stability of the analytes in patient samples may deviate from these results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

5.2 Use and shelf-life of the Internal Standard Mix

Prior to sample preparation, dissolve the Internal Standard Mix in the Precipitation Reagent as follows:

- Pipette 800 µL Internal Standard Mix (order no. 92644/XT) into 12.0 mL Precipitation Reagent (order no. 92003)

Storage life of the resulting solution (Internal Standard Solution):
In the Precipitation Reagent, the internal standards are stable as follows, but not beyond the date indicated on the label:

Table 5: Stability of the Internal Standard Solution

Storage temperature	Storage life	Other storage conditions
+2 to +8 °C	2 weeks	Light protection, tightly closed, glass vials
below –18 °C	2 months	Light protection, tightly closed, glass vials
Freeze-thaw cycles	3 cycles	—

5.3 Reconstitution of the calibrators

The Chromsystems **3PLUS1**® Multilevel Plasma Calibrator Set (order no. 92051/XT) is intended for the calibration of your analysis system. The set contains calibrators in three different concentration levels as well as a blank calibrator. The lyophilised calibrators are based on human defibrinated plasma. After reconstitution, they are handled in the same manner as a patient sample and are analysed under routine conditions analogous to the respective test procedure.

Prior to sample preparation, reconstitute the calibrators as follows:

1. Pipette 1.0 mL high-purity water into the original vial
2. Reconstitute for 10 to 15 min at +20 to +25 °C, swirling repeatedly

Check that the vial contents are homogeneous. If undissolved substances are still visible, extend the reconstitution time.

Immediately after reconstitution, pipette the calibrators into plastic reaction vials/tubes. Do not store in glass receptacles.

The calibrator levels are traceable to initial weights of pure substances (see Appendix IV). The analyte concentrations in the calibrator are batch-dependent. Individual levels are given in the calibrator leaflet. You can also download the values as an excel table in the download centre of our website.

Caution:
This product is manufactured from pooled human plasma which has been tested by the manufacturer and found negative for infections by the human immunodeficiency virus (HIV), the hepatitis B virus (HBV), the hepatitis C virus (HCV) and the bacterium *Treponema pallidum*. Nevertheless, a potential risk of infection cannot be entirely excluded. Consider all products containing human source material as potentially infectious and exercise the same care in the handling of this product as in the handling of potentially infectious patient samples.

Storage life of the calibrators after reconstitution:
The calibrators dissolved in water are stable as follows, but not beyond the date indicated on the label:

Table 6: Stability of the calibrators after reconstitution

Storage temperature	Storage life	Other storage conditions
+20 to +25 °C	7 days for all analytes except: anidulafungin: 8 hours micafungin: 24 hours 5-flucytosin: 2 days caspofungin: 2 days	Light protection, tightly closed, plastic receptacles
+2 to +8 °C	2 weeks for all analytes except anidulafungin and micafungin: 7 days	Light protection, tightly closed, plastic receptacles
below –18 °C	3 months	Light protection, tightly closed, plastic receptacles
Freeze-thaw cycles	3 cycles	—

To avoid unnecessary freeze-thaw cycles, aliquot calibrators before freezing.

5.4 Reconstitution of the controls

The Chromsystems ***MassCheck***® Antimycotic Drugs/*EXTENDED* Plasma Controls (order no. 0253/XT, 0254/XT) are intended to monitor the trueness and precision of each analytical sequence. They are available in two different concentration levels. The lyophilised controls are based on human defibrinated plasma. After reconstitution, they are handled in the same manner as a patient sample and are analysed under routine conditions analogous to the respective test procedure.

Prior to sample preparation, reconstitute the controls as follows:

1. Pipette 1.0 mL high-purity water into the original vial
2. Reconstitute for 10 to 15 min at +20 to +25 °C, swirling repeatedly

Check that the vial contents are homogeneous. If undissolved substances are still visible, extend the reconstitution time.

Immediately after reconstitution, pipette the controls into plastic reaction vials/tubes. Do not store in glass receptacles.

The analyte concentrations in the controls are batch-dependent. Individual levels are given in the leaflet accompanying each control. You can also download the values as an excel table in the download centre of our website.

Caution:
This product is manufactured from pooled human plasma which has been tested by the manufacturer and found negative for infections by the human immunodeficiency virus (HIV), the hepatitis B virus (HBV), the hepatitis C virus (HCV) and the bacterium *Treponema pallidum*. Nevertheless, a potential risk of infection cannot be entirely excluded. Consider all products containing human source material as potentially infectious and exercise the same care in the handling of this product as in the handling of potentially infectious patient samples.

Storage life of the controls after reconstitution:
Controls dissolved in water are stable as follows, but not beyond the date indicated on the label:

Table 7: Stability of the controls after reconstitution

Storage temperature	Storage life	Other storage conditions
+20 to +25 °C	7 days for all analytes except: anidulafungin: 8 hours micafungin: 24 hours 5-flucytosin: 2 days caspofungin: 2 days	Light protection, tightly closed, plastic receptacles
+2 to +8 °C	2 weeks for all analytes except anidulafungin and micafungin: 7 days	Light protection, tightly closed, plastic receptacles
below –18 °C	3 months	Light protection, tightly closed, plastic receptacles
Freeze-thaw cycles	3 cycles	—

To avoid unnecessary freeze-thaw cycles, aliquot controls before freezing.

5.5 Sample preparation procedure

First prepare the multilevel calibrators, controls and internal standard according to chapters 5.2 to 5.4.

Then work through the following steps in the order given:

1. Pipette 50 µL sample/calibrator/control into a 1.5 mL Reaction Vial (order no. 33006)
2. Add 25 µL Extraction Buffer (order no. 92005), vortex briefly and incubate for 2 min at ambient temperature (do not centrifuge)
3. Add 250 µL of the prepared Internal Standard Solution (see chapter 5.2)
4. Vortex for at least 30 s
5. Centrifuge for 5 min at 15000 x g
6. Dilute the supernatant according to the dilution scheme (see chapter 5.6)
7. Inject ≤ 50 µL eluate into the LC-MS/MS system

Information on determining the optimum injection volume is given in chapter 3.2.

5.6 Dilution scheme of prepared samples

Dilution of supernatant is variable and depends on instrument sensitivity. Following table shows verified dilution ranges. Start optimizing with dilution in bold and varying injection volume as described in chapter 3.2. If the signals of the LC-MS/MS system are too low to give accurate and precise results, use a lower dilution in combination with high injection volume. If the signals of the LC-MS/MS systems are too high and detector saturation is reached, use a higher dilution in combination with low injection volume.

Table 8: Dilution of the prepared samples

Dilution	Dilution medium	Injection volume
undiluted	–	0.5–50 µL
1+1	**Dilution Buffer 2**	
1+2	Dilution Buffer 2	

The dilution in **bold** is the recommended starting point for optimising the dilution.

5.7 Storage life of prepared samples

Samples prepared for analysis as indicated in chapter 5.5 have the following storage life:

Table 9: Storage life of the prepared samples

Storage temperature	Storage life	Other storage conditions
+20 to +25 °C	5 days	Light protection, tightly closed, glass vials
+2 to +8 °C	12 days	Light protection, tightly closed, glass vials
below –18 °C	12 days	Light protection, tightly closed, glass vials
Freeze-thaw cycles	1 cycle	—

Thawed eluates must be thoroughly mixed before injection.

5.8 Handling samples above the analytical measuring range

The "***MassTox***® TDM Series A PARAMETER Set Antimycotic Drugs/*EXTENDED* in serum/plasma" is indicated as a therapeutic drug monitoring test. Concentrations above the therapeutic range are quantifiable only up to the ULOQ. Concentrations above the analytical measurement range are not covered by a verified pre-dilution of the sample.

6 Quality control

Monitor trueness and precision of the analyses by including additional controls (0252/XT, 0253/XT, 0254/XT) in each analytical run, at least once during and once at the end of a sample series. If the analysis of these controls yields values outside the range given on the accompanying information leaflet, check the system and take appropriate actions. If the discrepancy continues to exist, re-calibrate the system.

Monitor the quality of chromatographic separation by comparison of the retention times and chromatographic peak shapes of the analytes and internal standards with the chromatogram of the column certificate or with an example chromatogram in chapter 7.2. For a column in use, compare the chromatogram with preceding analytical runs of the same assay (e.g. in the course of system start-up, chapter 3.5). Significant deviations might be due to decreasing performance of the analytical column. Typical indicators would be tailing of the peaks or the formation of double peaks.

For more information see chapter 12 Troubleshooting.

7 Data acquisition and evaluation

7.1 Calibration of the analysis system

Run a full calibration of the analysis system for each series of measurements. Use the **3PLUS1**® Multilevel Plasma Calibrator Set (order no. 92051/XT) for this purpose. The concentrations of the various analytes in the calibrators are batch-dependent. Exact levels are given in the package leaflet. Calibration curves are constructed by calculating the analyte to internal standard (ISTD) peak area ratio on the y axis against calibrator concentrations on the x axis. Then plot a calibration curve for all analytes, regression and weighting can be taken from the following table:

Table 10: Regression and weighting

Analyte	Regression	Weighting
Anidulafungin	linear	$1/x^2$
Caspofungin	linear	$1/x^2$
Fluconazole	linear	$1/x^2$
5-Flucytosine	linear	$1/x^2$
Isavuconazole	linear	$1/x^2$

Analyte	Regression	Weighting
Itraconazole	linear	$1/x^2$
Hydroxyitraconazole	linear	$1/x^2$
Ketoconazole	linear	$1/x^2$
Micafungin	linear	$1/x^2$
Posaconazole	linear	$1/x^2$
Voriconazole	linear	$1/x^2$

7.2 Example of a chromatogram

The following graph provides an example of a chromatogram created using this method.

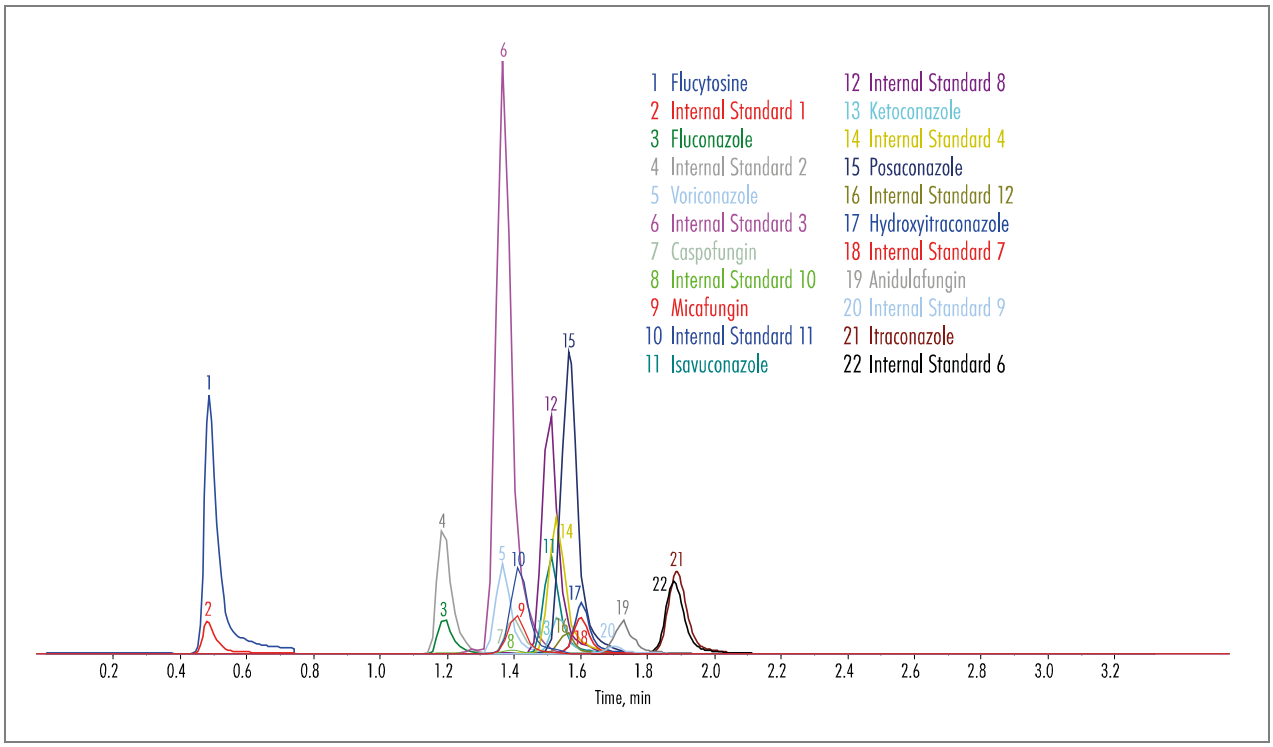

Figure 2: Chromatogram of a calibrator (order no. 92051/XT)

7.3 Conversion factors

The following table lists conversion factors between mass and molar concentrations and conversely.

Table 11: Conversion factors

Substance	µmol/L to mg/L	mg/L to µmol/L
Anidulafungin	1.140	0.8770
Caspofungin	1.093	0.9147
Fluconazole	0.3063	3.265
5-Flucytosine	0.1291	7.747

Substance	µmol/L to mg/L	mg/L to µmol/L
Isavuconazole	0.4375	2.286
Itraconazole	0.7056	1.417
Hydroxyitraconazole	0.7216	1.386
Ketoconazole	0.5314	1.882
Micafungin	1.270	0.7872
Posaconazole	0.7008	1.427
Voriconazole	0.3493	2.863

7.4 Manual calculation

Calibrate the analysis system according to chapter 7.1.

For manual calculation the following data are required.

- Peak area of the analyte A in the MRM chromatogram =A_{sample}
- Peak area of the internal standard in the MRM chromatogram =IS_{sample}
- Slope of the calibration curve =a
- Y-intercept of the calibration curve =b

Calculate the concentration of the analyte A in the sample C_{sample} as follows:

$$C_{sample} = \frac{(A_{sample} / IS_{sample}) - b}{a}$$

8 Storage and lifetime of the assay components

Unopened, and provided that transport and storage conditions are met, the assay components are stable until the expiry date stated on the label. Transport and store the components under the following conditions:

Table 12: Transport conditions for the basic kit and parameter set

Product	Transport temperature
Basic Kit (order no. 92111)	ambient
Parameter Set (order no. 92922/XT)	ambient
All other components listed in chapter 1	ambient

Immediately unpack components after transport and store individually as stated below:

Table 13: Storage conditions for the reagents, calibrators and controls

Product	Storage temperature
Mobile Phase 1 (order no. 92001)	+18 to +30 °C
Mobile Phase 2 (order no. 92002)	+18 to +30 °C
Precipitation Reagent (order no. 92003)	+18 to +30 °C
Extraction Buffer (order no. 92005)	+18 to +30 °C
Dilution Buffer 1 (order no. 92007)	+18 to +30 °C
Dilution Buffer 2 (order no. 92008)	+18 to +30 °C
Rinsing Solution (order no. 92009)	+18 to +30 °C
Internal Standard Mix (order no. 92644/XT)	below –18 °C
Tuning Mix (order no. 92039/XT)	below –18 °C
3PLUS1® Multilevel Plasma Calibrator Set (order no. 92051/XT)	below –18 °C
MassCheck® Plasma Controls (order no. 0252/XT, 0253/XT, 0254/XT)	below –18 °C

Close the reagents immediately after use and store them at the specified temperature. The in-use lifetime of liquid products is one year but does not extend beyond the stated expiry date. Details of the stability of the internal standards after dissolving the Internal Standard Mix in the Precipitation Reagent as well as the reconstituted calibrators and controls are given in sections 5.2 to 5.4.

The analytical column and laboratory materials not listed here can be stored at +18 to +30 °C.

The in-use stability of analytical column and prefilter depends on the individual conditions these components are used under (i.a. frequency of use, number of samples, type of samples, injection volume). Consider quality control measures (chapter 6) to identify decreasing chromatographic performance.

9 Waste disposal

Hazardous waste
Mobile Phase 1 (order no. 92001), Mobile Phase 2 (order no. 92002), Precipitation Reagent (order no. 92003), Dilution Buffer 2 (order no. 92008), Rinsing Solution (order no. 92009), Internal Standard Mix (order no. 92644/XT) and Tuning Mix (order no. 92039/XT) contain organic solvents. Dispose of product residues into a collection container for organic halogen-free solvents.

Residues of patient samples, prepared samples, controls and calibrators as well as laboratory consumables contaminated with human material must be collected and disposed of as potentially infectious waste.

Hazardous waste must not be disposed together with domestic waste. Do not circulate into the main water supply. Dispose of in compliance with Directive 2008/98/EC on Waste and national and local requirements. The waste containers must be stored appropriately and only access permitted to authorised parties.

Non-hazardous waste
Extraction Buffer (order no.92005) and Dilution Buffer 1 (order no. 92007) as well as non-contaminated laboratory consumables are not classified as hazardous. Dispose of in compliance with Directive 2008/98/EC on Waste and national and local requirements.

10 Therapeutic ranges

The stated therapeutic ranges are guides based on the literature. They may differ from other published data. As the levels vary depending on patient population and measurement method, determine specific therapeutic reference ranges for your laboratory. When determining ranges, make sure that you comply with local national requirements.

Itraconazole is rapidly metabolized in the liver to hydroxy-itraconazole, which also has antifungal activity. Therefore, the amount of total itraconazole (= Σ itraconazole + OH metabolite) should always be analysed. The typical ratio of itraconazole to hydroxy-itraconazole in plasma at steady state is 1:1 to 1:2.

Table 14: Therapeutic ranges

Substance	Therapeutic ranges [mg/L]						Peak plasma concentration [mg/L]
	[1]	[2]	[3]	[4]	[5]	[6]	[7]
Anidulafungin	—	—	—	—	—	—	3.32–7.57
Caspofungin	approx. 1–11	—	—	—	—	—	8.7–20.9
Fluconazole	1–5	—	5–15(–40)	approx. 2–6(–15)	—	—	1.5–14.1
5-Flucytosine	25–70	20–50	25–50#	(20–)25–50(–70)	—	—	30–80
Isavuconazole	—	—	—	approx. 2.5	2–5#	1–4.8	0.45–20
Itraconazole + Hydroxy-itraconazole	approx. 0.4–2†	> 0.5 or > 1 to 2†,‡	> 0.25†,#, 1–4	approx. 0.4–2†	—	—	Itraconazole: 1.96–2.86 Hydroxyitraconazole: 1.91–3.49
Ketoconazole	1–6(–10)	—	0.3–0.5#	1–3 (–6)	—	—	—
Micafungin	—	—	—	—	—	—	4.9–60.8
Posaconazole	—	> 0.5 or > 0.5–1.5‡	—	> 0.5–0.7*	> 1#	1–3.5	0.24–2.3
Voriconazole	approx. 2–6	> 0.5 or > 1–2‡, 6 max	0.5–5	1–6	1–2# (<6#)	2–6	0.69–11.4

*for invasive aspergillosis; †only itraconazole; ‡trough levels, for prophylaxis or therapy, respectively; #trough levels

11 Interference testing

Structure-related compounds and drugs were spiked into plasma samples or prepared plasma samples at the highest expected concentrations (see table below) and tested for interferences using SCIEX Triple Quad™ 6500+ and SCIEX Triple Quad™ Citrine™ mass spectrometers.

In addition, different sample conditions were simulated and checked for their influence on the test. Several sampling systems were also analysed for interference.

Table 15: Tested substances and their concentrations

Substance	Test concentrations in plasma in mg/L
Structure-related compounds	
3-methylxanthine	3.32
4-aminophenol	3.27
4-hydroxyphenytoin	0.805
8-hydroxyguanine	0.0000501
Cytosine	2.00
Dihydrouracil	0.228
Enterodiol	0.605
Ganglioside GM3 (d18:1/16:0)	18.8
Glycogen	80.0
Maltotetraose	667
Thymine	189
Xanthine	45.6
Drugs	
Acetaminophen (paracetamol)	200
Acetazolamide	59.9
Acetylcysteine	1663
Acetylsalicylic acid	652
Acyclovir	3.00
Allopurinol	40.0
Alloxanthine	20.0
Amikacin	80.2
Amlodipine	0.100
Amoxicillin	75.2
Ampicillin	53.0
Amprenavir	30.0
Atazanavir	18.0
Azathioprine	2.99
Azithromycin	12.0
Bisoprolol	0.300
Captopril	4.99
Carbamazepine	30.0
Carbamazepine-10,11-epoxide	15.0
Cefepime	6.00
Ceftazidime	24.0
Cephradin	2.00
Chloramphenicol	50.1
Chlordiazepoxide	9.99
Cimetidine	20.0

Substance	Test concentrations in plasma in mg/L
Ciprofloxacin	10.0
Clarithromycin	20.0
Cyclosporine A	6.00
Darunavir	21.6
Delaviridine	10.6
Dexamethasone	0.601
Diazepam	5.13
Diclofenac	50.0
Digitoxin	0.060
Digoxin	0.00600
Dihydrocodeine	0.993
Disopyramide	10.0
Efavirenz	12.0
Elvitegravir	2.13
Emtricitabine	7.50
Enalaprilat	0.300
Erythromycin	59.9
Etravirine	9.00
Everolimus	0.0240
Furosemide	59.9
Ganciclovir	2.00
Gentamicin	9.99
Hydrochlorothiazide	6.02
Ibuprofen	500
Indinavir	9.00
Isosorbiddinitrate	0.150
Levofloxacin	18.0
Levothyroxine	1.00
Lidocaine	12.0
Linezolid	12.0
Lopinavir	30.0
Lorazepam	1.00
M8-nelfinavir	7.50
Maraviroc	1.20
Meropenem	24.0
Metformin	40.0
Methicillin	240
Methylprednisolone	0.150
Metoclopramide	0.450
Metoprolol	4.99

Substance	Test concentrations in plasma in mg/L
Mycophenolic acid	10.0
Mycophenolic acid glucuronide	10.0
N-acetyl-procainamide	39.9
Nadolol	1.20
Natriumfluoride	2.00
N-desmethyldiazepam	5.01
Nelfinavir	7.50
Neomycin	0.200
Nevirapine	19.2
Nifedipine	0.400
Norverapamil	2.00
Omeprazole	6.00
Oxazepam	5.02
Penicillin G	20.0
Penicillin V	20.0
Phenytoin	49.9
Piperacillin	48.0
Prazosin	0.150
Prednisolone	3.00
Prednisone	0.301
Procainamide	24.0
(±)-Propranolol	2.00
Raltegravir	0.300
Ranitidine	6.00
Rifampicin	64.3
Rilpivirine	0.474
Risperidone	0.362
Ritonavir	33.0
Salbutamol	0.399
Salicylic acid	599
Saquinavir	2.40
Streptomycin	10.0
Sulbactam	24.0
Sulfamethoxazole	400
Sirolimus	0.0600
Tacrolimus	0.0540
Tazobactam	48.0
Tipranavir	135
Tramadol	3.00
Triamterene	8.86

Substance	Test concentrations in plasma in mg/L
Trimethoprim	40.0
Valproic acid	499
Vancomycin	100
Verapamil	2.00
Vigabatrin	108
Zaleplon	4.50
Zolpidem	4.50

11.1 Interferences detected

In the presence of the following substances interferences could be observed:
The presence of the stated interferences may affect the accuracy of test results by > 15%. This can be caused either by ion suppression effects or chromatographic interference that does not affect the analyte and internal standard in equal measure.

Table 16: Substances causing interferences

Analyte	Interference	Mass transition affected	Mass transition NOT affected	Comment
Fluconazole	Zaleplon	MRM 2 (308.1 → 238.1)	MRM 1 (308.1 → 220.1) MRM 3 (308.1 → 169.1)	MRM 2 is not applicable to confirm the quantitative results
Fluconazole	Zolpidem	MRM 1 (308.1 → 220.1)	MRM 2 (308.1 → 238.1) MRM 3 (308.1 → 169.1)	—
5-Flucytosine	Metformin	MRM 1 (130.0 → 113.0) MRM 3 (130.0 → 85.0)	MRM 2 (130.0 → 58.0)	—
5-Flucytosine	Emtricitabine	All	—	—
Itraconazole	Atazanavir	MRM 3 (705.3 → 335.1)	MRM 1 (705.3 → 392.1) MRM 2 (705.3 → 432.1)	Interference elutes approx. 0.2 min before analyte peak MRM 3 is not applicable to confirm the quantitative results
Hydroxy-itraconazole	Ritonavir	MRM 1 (721.3 → 392.1) MRM 3 (721.3 → 408.1)	MRM 2 (721.3 → 430.1)	—

Serum sampling systems
The following serum sampling systems may affect the accuracy of test results by > 15%.

Table 17: Serum sampling systems causing interferences

Type	Manu-facturer	Order no.	Volume	Description	Lots tested	Affected analyte(s)
Serum	Sarstedt	01.1601.001	7.5 mL	Clotting activator	4033201	Anidulafungin
Serum	Sarstedt	04.1905.001	2.6 mL	Gel/clotting activator	503111	Anidulafungin Isavuconazole Itraconazole
Serum	BD	369032	4.0 mL	Clotting activator	0230269	Anidulafungin
Serum	BD	367957	3.5 mL	Gel/clotting activator	0288531	Anidulafungin Isavuconazole Itraconazole Hydroxyitraconazole Ketoconazole Micafungin Posaconazole Voriconazole
Serum	greiner BIO-ONE	455071	8.0 mL	Gel/clotting activator	A180035P	Anidulafungin Micafungin

Isolated cases of interferences involving MRM 3 of Isavuconazole (440.1 → 215.1) in association with various blood collection systems were observed. In such cases MRM 3 is not applicable to confirm the quantitative results. We recommend to use MRM 2 (439.1 → 369.1) as qualifier if descrepancies in MRM 3/MRM 1 ratio are detected.

11.2 No interference detected

The following substances were tested and had a negligible influence on the quantitative results (deviation ≤ 15 %).

Commonly used drugs and structure-related compounds:
3-Methylxanthine, 4-aminophenol, 4-hydroxyphenytoin, 8-hydroxyguanine, acetaminophen (paracetamol), acetazolamide, acetylcysteine, acetylsalicylic acid, acyclovir, allopurinol, alloxanthine, amikacin, amlodipine, amoxicillin, ampicillin, amprenavir, azathioprine, azithromycin, bisoprolol, captopril, carbamazepine, carbamazepine-10,11-epoxide, cefepime, ceftazidime, cephradin, chloramphenicol, chlordiazepoxide, cimetidine, ciprofloxacin, clarithromycin, cyclosporine A, cytosine, darunavir, delaviridine, dexamethasone, diazepam, diclofenac, digitoxin, digoxin, dihydrocodeine, dihydrouracil, disopyramide, efavirenz, elvitegravir, enalaprilat, enterodiol, erythromycin, etravirine, everolimus, furosemide, ganciclovir, ganglioside GM3 (d18:1/16:0), gentamicin, glycogen, hydrochlorothiazide, ibuprofen, indinavir, isosorbiddinitrate, levofloxacin, levothyroxine, lidocaine, linezolid, lopinavir, lorazepam, M8-Nelfinavir, maltotetraose, maraviroc, meropenem, methicillin, methylprednisolone, metoclopramide, metoprolol, mycophenolic acid, mycophenolic acid glucuronide, N-Acetyl-Procainamide, nadolol, Natriumfluoride, N-Desmethyldiazepam, nelfinavir, neomycin, nevirapine, nifedipine, norverapamil, omeprazole, oxazepam, penicillin G, penicillin V, phenytoin, piperacillin, prazosin, prednisolone, prednisone, procainamide, (±)-Propranolol, raltegravir, ranitidine, rifampicin, rilpivirine, risperidone, salbutamol, salicylic acid, saquinavir, streptomycin, sulbactam, sulfamethoxazole, sirolimus, tacrolimus, tazobactam, thymine, tipranavir, tramadol, triamterene, trimethoprim, valproic acid, vancomycin, verapamil, vigabatrin, xanthine.

Interference-free analysis is possible with the following sample states:

Haemolysis
Plasma and serum samples were spiked with haemoglobin (to a concentration of 500 mg/dL) and the analyte concentrations measured were compared against those of the original sample:

No significant interferences occurred (deviation ≤ 15 %).

Lipaemia
Plasma and serum samples were spiked with a lipid emulsion (to a concentration of 0.67 to 10 g/L) and the analyte concentrations measured were compared against those of the original sample:

No significant interferences occurred (deviation ≤ 15 %).

Icterus
Plasma and serum samples were spiked with unconjugated and conjugated bilirubin (each 0.2 g/L) and the analyte concentrations measured were compared against those of the original sample:

No significant interferences occurred (deviation ≤ 15 %).

The following sampling systems were tested without significant interference; the quantitative results were not influenced (deviation ≤ 15%):

Table 18: Plasma sampling systems causing no interferences

Type	Manu-facturer	Order no.	Volume	Description	Lots tested
Plasma LH	Sarstedt	03.1628	5.5 mL	Granulate containing 16 I.U./mL Li-Heparine	7034611 9031311 9032011
Plasma NH	Sarstedt	01.1613.100	7.5 mL	Granulate containing 16 I.U./mL Na-Heparine	7032211 9268311
Plasma K2E	Sarstedt	04.1915.100	2.7 mL	1.6 mg K2EDTA/mL liquid	8595111
Plasma K3E	Sarstedt	04.1901	2.7/2.6 mL	1.6 mg K3EDTA/mL liquid	9034911 6030511
Plasma K3E	Sarstedt	06.1644.001	1.2 mL	1.6 mg K3EDTA/mL liquid	7030611
Plasma LH	BD	368886	6.0 mL	17 I.U./ml Li-Heparine spray-dried	7066712
Plasma NH	BD	368480	10.0 mL	17 I.U./ml Na-Heparine spray- dried	8064945
Plasma K2E	BD	368841	2.0 mL	1.8 mg K2EDTA spray-dried	0027522
Plasma K2E	BD	368856	3.0 mL	5.4 mg K2EDTA spray-dried	9347839
Plasma NH	greiner BIO-ONE	454051	4.0 mL	Sodium-Heparin, spray-dried	A19033AM A171039Q
Plasma NH	greiner BIO-ONE	456051	6.0 mL	Sodium-Heparin, spray-dried	A17103QL
Plasma K2E	greiner BIO-ONE	454246	3.0 mL	interior coated spray-dried K2EDTA	A19114J7

Type	Manu-facturer	Order no.	Volume	Description	Lots tested
Plasma K3E	greiner BIO-ONE	454036	4.0 mL	interior coated spray-dried K3EDTA	A19113GY C181033M A1711356

If you have any questions concerning interferences, contact your local Chromsystems representative or our Chromsystems support staff directly by calling our hotline +49 89 18930-111 or by e-mail at support@chromsystems.com.

12 Troubleshooting

Table 19: Troubleshooting

Problem	Possible cause	Remedy
Gradient profile cannot be generated	HPLC pump	Check pump (air, leaks)
	Air in the system	Degas the HPLC system (purge)
	Flow rate not constant	Check pump
Decreasing chromatographic performance (e.g. retention time shift, peak tailing, formation of double peaks)	Prefilter contaminated	Change prefilter
	Analytical column contaminated	Flush column with 100 % Mobile Phase 2 at 0.6 mL/min (as necessary, e.g. 15 minutes)
	Analytical column defective	Change analytical column
Interfering signals	Injection system contaminated	Clean with methanol or inject Rinsing Solution 10 x
	Autosampler vials contaminated	Use new vials
	Vial seals	Use other seals
	Mobile phase contaminated	Replace mobile phase and purge system
	Column contaminated	Replace column
	Mass resolution too low	Optimise system
No signal	Defective injector	Check injector
	Defective pump	Check pump
	MS/MS-System not ready	Check MS/MS system
Poor sensitivity	Ion source contaminated	Clean ion source
	Mass spectrometer contaminated	Clean mass spectrometer
	Injection valve leaking	Check injector
	Mass calibration changes	Re-calibrate MS/MS system
	MRMs not ideal	Re-optimise MRMs
	Conditions for ionisation not ideal	Optimise conditions of ion source
	Mass resolution too high	Optimise system

Problem	Possible cause	Remedy
Unstable signal	Spray instable	Check spray capillary and clean if necessary
	Flow instable	Check HPLC pumps
	Gas flow unstable	Check gas lines
No vacuum	Defective vacuum pump	Check all vacuum pumps
	Vacuum system leaks	Check vacuum tubes and connections
No gas supply	Defective nitrogen generator	Check nitrogen generator
	Defective compressor	Check compressor
	Empty gas cylinder	Replace gas cylinder
	Gas pressure out of rated value	Adjust gas pressure
Quality control outside acceptable range	Interference	Check chromatogram for interference, also MRM2 and MRM3
	Incorrect sample preparation	Check reproducibility of incorrect results
	Calibration unsuitable	Check regression and weighting parameter as well as quality of calibration curve
	HPLC instrument parameter unsuitable	Check HPLC instrument parameters
	Mass spectrometer contaminated	Clean mass spectrometer
	Mass calibration changes	Re-calibrate MS/MS system

If you have any questions concerning troubleshooting, contact your local Chromsystems representative or our Chromsystems support staff directly by calling our hotline +49 89 18930-111 or by e-mail at support@chromsystems.com.

13 Literature

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Appendix I Substance information

Hazardous substances
When using the reagents, note the following hazard information and take the relevant safety measures. More information can be gathered from our safety data sheets. These can be downloaded from our website www.chromsystems.com or are available upon request.

Table 20: Hazard and precautionary statements

Pictograms	Hazard and precautionary statements
Mobile Phase 1 (order no. 92001)	Components: 10–25 % methanol
	**Danger** H226 Flammable liquid and vapour. H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled. H370 Causes damage to the central nervous system and the visual organs. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
Mobile Phase 2 (order no. 92002)	Components: 50–100 % methanol
	**Danger** H225 Highly flammable liquid and vapour. H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled. H370 Causes damage to the central nervous system and the visual organs. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P310 IF SWALLOWED: Immediately call a POISON CENTER/ doctor. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
Precipitation Reagent (order no. 92003)	Components: 25–50 % propan-2-ol, 25–50 % acetonitrile
	**Danger** H225 Highly flammable liquid and vapour. H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 Keep container tightly closed. P241 Use explosion-proof electrical/ventilating/lighting equipment. P243 Take action to prevent static discharges. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Pictograms	Hazard and precautionary statements
Dilution Buffer 2 (order no. 92008)	Components: 10–25 % methanol
	**Danger** H226 Flammable liquid and vapour. H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled. H370 Causes damage to the central nervous system and the visual organs. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
Rinsing Solution (order no. 92009)	Components: 50–100 % methanol
	**Danger** H225 Highly flammable liquid and vapour. H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled. H370 Causes damage to the central nervous system and the visual organs. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P310 IF SWALLOWED: Immediately call a POISON CENTER/ doctor. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
Internal Standard Mix (order no. 92644/XT)	Components: 50–100 % acetonitrile, 3–10 % methanol
	**Danger** H225 Highly flammable liquid and vapour. H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled. H319 Causes serious eye irritation. H371 May cause damage to the central nervous system and the visual organs. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P241 Use explosion-proof electrical/ventilating/lighting equipment. P243 Take action to prevent static discharges. P260 Do not breathe mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection.

Pictograms	Hazard and precautionary statements
Tuning Mix (order no. 92039/XT)	Components: 50–100 % methanol, < 3 % acetonitrile, < 2 % DMSO
	**Danger** H225 Highly flammable liquid and vapour. H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled. H370 Causes damage to the central nervous system and the visual organs. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P310 IF SWALLOWED: Immediately call a POISON CENTER/ doctor. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
These components are not classified as dangerous according to European Union legislation:	
Extraction Buffer (order no. 92005)	Components: aqueous solution
Dilution Buffer 1 (order no. 92007)	Components: aqueous solution
3PLUS1® Multilevel Plasma Calibrator Set (order no. 92051/XT)	Components: human defibrinated plasma
Plasma Controls (order no. 0252/XT, 0253/XT, 0254/XT)	Components: human defibrinated plasma

Active ingredients:

Table 21: Active ingredients ***MassTox***® TDM Series A BASIC Kit

Order no.	Description	Active Component	Specification
92001	Mobile Phase 1	Methanol Water	10–25% 75–90%
92002	Mobile Phase 2	Methanol Water	50–100% 0–50%
92003	Precipitation Reagent	Acetonitrile Propan-2-ol	25–50% 25–50%
92005	Extraction Buffer	Water N-derivate of carbonic acid	90–99% 1–10%
92007	Dilution Buffer 1	Water	> 99%
92008	Dilution Buffer 2	Water Methanol	75–90% 10–25%
92009	Rinsing Solution	Methanol Water	50–100% 0–50%
92110	Analytical column	Polymer based on silica	> 90%

Table 22: Active ingredients ***MassTox***® TDM Series A PARAMETER Set Antimycotic Drugs/*EXTENDED* in serum/plasma

Order no.	Description	Active Component	Specification
92039/XT	Tuning Mix ***MassTox***® Antimycotic Drugs/*EXTENDED*	Analytes (5-flucytosine, fluconazole, voriconazole, ketoconazole, posaconazole, hydroxyitraconazole, itraconazole, caspofungin, isavuconazole, anidulafungin, micafungin) and isotopically labelled analytes	< 0.1 %
92644/XT	Internal Standard Mix ***MassTox***® Antimycotic Drugs/*EXTENDED*	Isotopically labelled analytes (as provided on leaflet)	< 0.1 %
92051/XT	3PLUS1® Multilevel Plasma Calibrator Set ***MassTox***® Antimycotic Drugs/*EXTENDED*	Analytes (5-flucytosine, fluconazole, voriconazole, ketoconazole, posaconazole, hydroxyitraconazole, itraconazole, caspofungin, isavuconazole, anidulafungin, micafungin)	conc. see leaflet
0253/XT	Plasma Control Level I		
0254/XT	Plasma Control Level II		

Appendix II Analytical performance data

The performance features were determined and verified on the following equipment:
– SCIEX Triple Quad™ Citrine™ mass spectrometer with Agilent 1290 Infinity II UHPLC system

Users wishing to use the "***MassTox***® TDM Series A PARAMETER Set Antimycotic Drugs/*EXTENDED* in serum/plasma" (order no. 92922/XT) with a mass spectrometer other than the one specified here should verify the method on that device.

Metrological Traceability and Trueness
For our 3PLUS1® Multilevel Plasma Calibrator Set ***MassTox***® Antimycotic Drugs/*EXTENDED* (order no. 92051/XT) metrological traceability to a reference through a documented unbroken chain of calibrations was demonstrated and is available as traceability chain (see Appendix IV.

Trueness of measurement was demonstrated within the analytical performance process based on following strategies:
- Comparison with commercially available CE/IVD method
- Comparison with spike values
- Determination of the relative recovery (using certified reference material, if available)

Recovery:
Relative recovery was determined with plasma and serum as matrices. The same matrix was spiked with different concentrations of the analytes for this purpose. Two concentration levels within the analytical measuring ranges of the analytes were investigated. Recovery is calculated using the following formula:

$$\text{Recovery [\%]} = \frac{\text{measured concentration in spiked sample} - \text{measured concentration in plain sample}}{\text{reference concentration}} \times 100$$

Table 23: Recovery rates in serum, determination with SCIEX Triple Quad™ Citrine™ mass spectrometer

Substance	Recovery rate in serum (concentration of analyte)	
Anidulafungin	98% (2.56 mg/L)	101% (8.20 mg/L)
Caspofungin	98% (3.48 mg/L)	100% (11.0 mg/L)
Fluconazole	101% (5.34 mg/L)	100% (17.8 mg/L)
5-Flucytosine	100% (33.2 mg/L)	101% (104 mg/L)
Isavuconazole	101% (5.11 mg/L)	101% (18.0 mg/L)
Itraconazole	100% (0.690 mg/L)	97% (2.00 mg/L)
Hydroxyitraconazole	100% (0.710 mg/L)	100% (2.00 mg/L)
Ketoconazole	101% (2.95 mg/L)	97% (10.6 mg/L)
Micafungin	101% (15.2 mg/L)	99% (51.6 mg/L)
Posaconazole	104% (1.26 mg/L)	99% (4.30 mg/L)
Voriconazole	103% (2.12 mg/L)	102% (7.30 mg/L)

Table 24: Recovery rates in plasma, determination with SCIEX Triple Quad™ Citrine™ mass spectrometer

Substance	Recovery rate in plasma (concentration of analyte)	
Anidulafungin	99% (2.56 mg/L)	93% (8.20 mg/L)
Caspofungin	101% (3.48 mg/L)	98% (11.0 mg/L)
Fluconazole	100% (5.34 mg/L)	96% (17.8 mg/L)
5-Flucytosine	103% (33.2 mg/L)	99% (104 mg/L)
Isavuconazole	102% (5.11 mg/L)	94% (18.0 mg/L)
Itraconazole	104% (0.690 mg/L)	96% (2.00 mg/L)
Hydroxyitraconazole	103% (0.710 mg/L)	93% (2.00 mg/L)
Ketoconazole	100% (2.95 mg/L)	95% (10.6 mg/L)
Micafungin	107% (15.2 mg/L)	93% (51.6 mg/L)
Posaconazole	104% (1.26 mg/L)	97% (4.30 mg/L)
Voriconazole	103% (2.12 mg/L)	97% (7.3 mg/L)

Analytical measurement range

Lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ):

Upper limit of quantitation (ULOQ) was determined by spiking plasma and serum samples with defined quantities of standard substances. The lower limit of quantitation (LLOQ) was determined using defined dilutions of plasma and serum samples with analyte-free matrix.

Table 25: Limits of quantitation, determination with SCIEX Triple Quad™ Citrine™ mass spectrometer

Substance	LLOQ	ULOQ
Anidulafungin	0.269 mg/L	20.0 mg/L
Caspofungin	0.153 mg/L	27.6 mg/L
Fluconazole	0.035 mg/L	37.5 mg/L
5-Flucytosine	1.16 mg/L	250 mg/L
Isavuconazole	0.219 mg/L	44.0 mg/L
Hydroxyitraconazole	0.043 mg/L	5.26 mg/L
Itraconazole	0.009 mg/L	4.00 mg/L
Ketoconazole	0.039 mg/L	25.0 mg/L
Micafungin	1.02 mg/L	130 mg/L
Posaconazole	0.068 mg/L	10.0 mg/L
Voriconazole	0.065 mg/L	16.0 mg/L

Precision (repeatability & within-laboratory precision)
The performance data were determined on the basis of 4 different samples by double processing on 10 different days with 2 runs per day. The procedure is based on CLSI EP05-A3 and corresponds to a 10 × 2 × 2 test design.

Table 26: Precision (repeatability & within-laboratory precision), determination with SCIEX Triple Quad™ Citrine™ mass spectrometer

Substance	Sample	Mean	Repeatability		Within-Laboratory Precision	
			Coefficient of variation	95 % confidence interval	Coefficient of variation	95 % confidence interval
Anidulafungin	Serum 1	0.217 mg/L	5.9 %	4.5–8.5 %	9.3 %	7.4–12.8 %
	Serum 2	3.16 mg/L	5.3 %	4.0–7.6 %	6.6 %	5.3–8.7 %
	Serum 3	7.10 mg/L	3.3 %	2.6–4.8 %	5.9 %	4.6–8.2 %
	Serum 4	10.6 mg/L	3.4 %	2.6 – 5.0 %	5.3 %	4.2–7.3 %
	Plasma 1	0.217 mg/L	7.5 %	5.7–10.8 %	10.7 %	8.4–14.5 %
	Plasma 2	3.30 mg/L	4.6 %	3.5–6.6 %	5.8 %	4.7–7.7 %
	Plasma 3	7.05 mg/L	5.2 %	3.9–7.4 %	7.8 %	6.2–10.4 %
	Plasma 4	10.9 mg/L	4.2 %	3.2–6.1 %	5.2 %	4.2–6.8 %
Caspofungin	Serum 1	0.610 mg/L	7.2 %	5.5–10.4 %	12.8 %	10.1–17.6 %
	Serum 2	3.94 mg/L	9.1 %	7.0–13.2 %	9.9 %	8.0–12.8 %
	Serum 3	8.77 mg/L	8.3 %	6.4 – 12.0 %	9.7 %	7.9–12.5 %
	Serum 4	13.6 mg/L	10.2 %	7.8–14.7 %	10.7 %	8.7–13.9 %
	Plasma 1	0.574 mg/L	8.0 %	6.1–11.6 %	9.7 %	7.9–12.7 %
	Plasma 2	4.01 mg/L	9.7 %	7.4–13.9 %	12.5 %	10.0–16.8 %
	Plasma 3	8.68 mg/L	10.1 %	7.7–14.6 %	10.7 %	8.8–13.8 %
	Plasma 4	12.9 mg/L	7.3 %	5.6–10.6 %	9.9 %	7.8–13.5 %
Fluconazole	Serum 1	0.666 mg/L	7.0 %	5.3–10.1 %	10.3 %	8.2–13.9 %
	Serum 2	8.21 mg/L	2.8 %	2.1–4 %	4.3 %	3.4 – 6.0 %
	Serum 3	18.6 mg/L	3.5 %	2.7–5 %	4.8 %	3.9–6.3 %
	Serum 4	28.5 mg/L	2.8 %	2.2–4.1 %	3.8 %	3.0–5.2 %
	Plasma 1	0.667 mg/L	5.4 %	4.1–7.8 %	9.1 %	7.2–12.4 %
	Plasma 2	8.46 mg/L	3.5 %	2.7–5 %	4.3 %	3.5–5.6 %
	Plasma 3	18.2 mg/L	2.6 %	2.0–3.8 %	2.9 %	2.4–3.8 %
	Plasma 4	28.3 mg/L	2.1 %	1.6 – 3.0 %	2.9 %	2.3–3.9 %
5-Flucytosine	Serum 1	8.55 mg/L	12.1 %	9.2–17.4 %	13.6 %	11.1–17.7 %
	Serum 2	40.2 mg/L	3.7 %	2.9–5.4 %	5.0 %	4.0–6.7 %
	Serum 3	90.3 mg/L	2.8 %	2.2–4.1 %	3.4 %	2.8–4.4 %
	Serum 4	137 mg/L	2.6 %	2.0–3.7 %	3.9 %	3.1–5.3 %
	Plasma 1	8.47 mg/L	8.3 %	6.4–12 %	10.2 %	8.3–13.4 %
	Plasma 2	41.8 mg/L	3.8 %	2.9–5.5 %	4.3 %	3.5–5.5 %
	Plasma 3	89.8 mg/L	2.4 %	1.8–3.5 %	2.8 %	2.3–3.6 %
	Plasma 4	137 mg/L	1.4 %	1.1–2.1 %	2.8 %	2.2–3.9 %

Substance	Sample	Mean	Repeatability		Within-Laboratory Precision	
			Coefficient of variation	95 % confidence interval	Coefficient of variation	95 % confidence interval
Isavuconazole	Serum 1	0.299 mg/L	5.8 %	4.5–8.4 %	8.2 %	6.5–10.8 %
	Serum 2	7.57 mg/L	2.2 %	1.6–3.1 %	4.0 %	3.2–5.6 %
	Serum 3	17.3 mg/L	2.5 %	1.9–3.6 %	3.3 %	2.7–4.3 %
	Serum 4	26.2 mg/L	2.1 %	1.6 – 3.0 %	3.1 %	2.5–4.1 %
	Plasma 1	0.299 mg/L	6.0 %	4.6–8.6 %	10.5 %	8.3–14.4 %
	Plasma 2	7.82 mg/L	2.6 %	2.0–3.7 %	3.6 %	2.9–4.8 %
	Plasma 3	17.0 mg/L	2.4 %	1.8–3.5 %	2.7 %	2.2–3.5 %
	Plasma 4	26.3 mg/L	2.6 %	2.0–3.7 %	3.4 %	2.8–4.5 %
Hydroxy-itraconazole	Serum 1	0.234 mg/L	4.5 %	3.5–6.6 %	5.6 %	4.5–7.3 %
	Serum 2	1.26 mg/L	3.1 %	2.4–4.5 %	3.7 %	3.0–4.8 %
	Serum 3	2.75 mg/L	2.5 %	1.9–3.6 %	3.1 %	2.4–4.1 %
	Serum 4	4.14 mg/L	2.8 %	2.1–4.0 %	3.5 %	2.8–4.6 %
	Plasma 1	0.230 mg/L	4.6 %	3.5–6.6 %	6.3 %	5.1–8.4 %
	Plasma 2	1.25 mg/L	2.9 %	2.2–4.2 %	3.6 %	2.9–4.7 %
	Plasma 3	2.80 mg/L	2.3 %	1.7–3.3 %	3.1 %	2.5–4.1 %
	Plasma 4	4.17 mg/L	2.6 %	2.0–3.7 %	3.2 %	2.6–4.3 %
Itraconazole	Serum 1	0.135 mg/L	1.3 %	1.0–1.9 %	3.2 %	2.5–4.5 %
	Serum 2	0.821 mg/L	1.5 %	1.1–2.1 %	2.2 %	1.8–2.9 %
	Serum 3	1.78 mg/L	1.3 %	1.0–1.9 %	1.7 %	1.3–2.2 %
	Serum 4	2.71 mg/L	0.9 %	0.7–1.3 %	1.7 %	1.3–2.3 %
	Plasma 1	0.131 mg/L	1.6 %	1.2–2.3 %	5.4 %	4.1–7.9 %
	Plasma 2	0.801 mg/L	1.2 %	0.9–1.7 %	2.9 %	2.2–4.1 %
	Plasma 3	1.8 mg/L	1.4 %	1.1–2.1 %	3.4 %	2.7–4.9 %
	Plasma 4	2.67 mg/L	1.6 %	1.2–2.3 %	2.7 %	2.2–3.7 %
Ketoconazole	Serum 1	0.269 mg/L	7.5 %	5.7–10.8 %	7.8 %	6.4–10.0 %
	Serum 2	5.14 mg/L	4.0 %	3.1–5.8 %	4.1 %	3.4–5.3 %
	Serum 3	11.5 mg/L	3.0 %	2.3–4.3 %	5.6 %	4.4–7.8 %
	Serum 4	16.9 mg/L	3.0 %	2.3–4.3 %	5.2 %	4.1–7.1 %
	Plasma 1	0.265 mg/L	7.0 %	5.4–10.1 %	8.7 %	6.9–11.5 %
	Plasma 2	5.34 mg/L	2.3 %	1.8–3.4 %	4.6 %	3.6–6.4 %
	Plasma 3	11.2 mg/L	2.7 %	2.1–3.9 %	5.3 %	4.2–7.5 %
	Plasma 4	16.5 mg/L	2.7 %	2.1–4.0 %	5.0 %	3.8–7.0 %
Micafungin	Serum 1	1.57 mg/L	11.4 %	8.7–16.4 %	17.3 %	13.7–23.5 %
	Serum 2	19.1 mg/L	5.3 %	4.0–7.6 %	5.5 %	4.5–7.1 %
	Serum 3	45.2 mg/L	4.2 %	3.2–6.1 %	6.4 %	5.1–8.5 %
	Serum 4	69.9 mg/L	7.9 %	6.0–11.3 %	10.7 %	8.6–14.2 %
	Plasma 1	1.63 mg/L	8.0 %	6.1–11.5 %	13.3 %	10.5 – 18.0 %

Substance	Sample	Mean	Repeatability		Within-Laboratory Precision	
			Coefficient of variation	95 % confidence interval	Coefficient of variation	95 % confidence interval
	Plasma 2	19.7 mg/L	5.0 %	3.8–7.2 %	7.2 %	5.8–9.7 %
	Plasma 3	43.9 mg/L	5.6 %	4.3–8.2 %	7.9 %	6.3–10.6 %
	Plasma 4	69.7 mg/L	5.5 %	4.2–7.9 %	8.0 %	6.3–10.8 %
Posaconazole	Serum 1	0.220 mg/L	12.2 %	9.3–17.6 %	18.5 %	14.7–25.1 %
	Serum 2	1.94 mg/L	5.4 %	4.2–7.8 %	5.7 %	4.7–7.4 %
	Serum 3	4.24 mg/L	5.7 %	4.3–8.2 %	6.6 %	5.4–8.6 %
	Serum 4	6.32 mg/L	4.5 %	3.4–6.5 %	5.4 %	4.3–7.3 %
	Plasma 1	0.216 mg/L	13.9 %	10.6–20.0 %	18.9 %	15.2–25.0 %
	Plasma 2	1.92 mg/L	6.6 %	5.0–9.5 %	7.7 %	6.2–10.0 %
	Plasma 3	4.33 mg/L	5.7 %	4.4–8.2 %	6.6 %	5.4–8.7 %
	Plasma 4	6.4 mg/L	4.1 %	3.1–5.9 %	5.7 %	4.6–7.5 %
Voriconazole	Serum 1	0.326 mg/L	6.2 %	4.7–8.9 %	7.0 %	5.7–9.3 %
	Serum 2	3.06 mg/L	5.0 %	3.8–7.2 %	5.9 %	4.8–7.6 %
	Serum 3	6.82 mg/L	3.9 %	3.0–5.6 %	5.4 %	4.3–7.1 %
	Serum 4	10.5 mg/L	5.6 %	4.3–8.1 %	6.5 %	5.3–8.4 %
	Plasma 1	0.320 mg/L	6.8 %	5.2–9.8 %	9.0 %	7.2–11.8 %
	Plasma 2	2.94 mg/L	5.0 %	3.9–7.3 %	7.1 %	5.6–9.6 %
	Plasma 3	6.72 mg/L	7.0 %	5.4–10.1 %	7.4 %	6.0–9.5 %
	Plasma 4	10.0 mg/L	5.1 %	3.9–7.3 %	5.9 %	4.8–7.7 %

Drift

To identify any drift of analyte concentration over time the concentrations of all analytes in the four samples for each matrix were compared over a 10-day period. No drift was observed for any analytes.

Precision (reproducibility)

The performance data were determined at 3 sites on the basis of 3 different samples by 5-fold processing on 5 different days. The procedure is based on CLSI EP05-A3 and corresponds to a 3 × 5 × 5 test design.

Table 27: Precision (reproducibility), determination with SCIEX Triple Quad™ Citrine™, Waters® Xevo™ TQ-S and SCIEX 4500MD™ mass spectrometer

Substance	Sample	Mean	Reproducibility	
			Coefficient of variation	95 % confidence interval
Anidulafungin	Plasma low	3.53 mg/L	7.5 %	4.9–16.2 %
	Plasma middle	5.93 mg/L	6.1 %	4.6–8.8 %
	Plasma high	8.52 mg/L	6.8 %	5.4–9.1 %
Caspofungin	Plasma low	0.756 mg/L	12.1 %	7.4–31.0 %
	Plasma middle	5.02 mg/L	6.5 %	5.5–7.8 %
	Plasma high	9.44 mg/L	6.3 %	5.4–7.6 %

Substance	Sample	Mean	Reproducibility	
			Coefficient of variation	95 % confidence interval
Fluconazole	Plasma low	0.859 mg/L	6.5 %	4.8–10.1 %
	Plasma middle	7.17 mg/L	6.4 %	3.8–19 %
	Plasma high	16.2 mg/L	5.8 %	3.8–11.9 %
5-Flucytosine	Plasma low	18.5 mg/L	3.3 %	2.3–5.7 %
	Plasma middle	44.4 mg/L	3.2 %	2.1–7.2 %
	Plasma high	68.6 mg/L	3.7 %	2.9–5.0 %
Isavuconazole	Plasma low	0.406 mg/L	6.8 %	5.3–9.3 %
	Plasma middle	9.84 mg/L	5.7 %	4.0–10.1 %
	Plasma high	21.9 mg/L	5.0 %	4.3–6.1 %
Hydroxyitraconazole	Plasma low	0.362 mg/L	6.5 %	4.2–14.1 %
	Plasma middle	2.09 mg/L	5.4 %	3.5–11.3 %
	Plasma high	4.01 mg/L	5.5 %	4.3–7.4 %
Itraconazole	Plasma low	0.386 mg/L	5.1 %	3.4–9.8 %
	Plasma middle	1.63 mg/L	5.7 %	3.4–16.8 %
	Plasma high	2.71 mg/L	5.7 %	4.1–9.1 %
Ketoconazole	Plasma low	0.258 mg/L	11.1 %	8.5–16.1 %
	Plasma middle	3.04 mg/L	6.1 %	3.7–16.3 %
	Plasma high	5.77 mg/L	6.3 %	4.5–10.8 %
Micafungin	Plasma low	4.96 mg/L	6.3 %	4.7–9.3 %
	Plasma middle	34.9 mg/L	5.4 %	3.9–8.9 %
	Plasma high	64.2 mg/L	5.7 %	4.8–6.8 %
Posaconazole	Plasma low	0.531 mg/L	7.1 %	4.4–17.2 %
	Plasma middle	3.06 mg/L	7.9 %	4.7–23.7 %
	Plasma high	5.63 mg/L	7.1 %	4.8–13.5 %
Voriconazole	Plasma low	0.501 mg/L	6.8 %	4.8–12.1 %
	Plasma middle	3.35 mg/L	7.8 %	5.2–15.5 %
	Plasma high	6.56 mg/L	7.7 %	5.9–11.1 %

Carry-over

A prepared plasma sample with an analyte concentration in the range of the upper limit of quantitation was analysed in between several blank samples. The analyte concentrations of blank samples before and after the high-level sample were compared. In case of significant carry-over, the amount was calculated on a percentage basis in relation to the preceding sample.

Review of the data obtained for all of the analytes showed no carry-over effects. In all cases the measured concentration of the blank sample was below the limit of quantitation.

Robustness
The effect of defined modifications in sample preparation and HPLC system setup were evaluated during verification. The method is robust within the following tolerances provided the particular setup remains constant throughout a measurement series:

Table 28: Tolerance ranges HPLC system

HPLC system	Tolerance range
Oven temperature	20–30°C

Table 29: Tolerance ranges sample preparation

Sample preparation (according to chapter 5.5)	**Tolerance range**
Step 4: Vortex for at least 30 s	Vortex 20–40 s
Step 5: Centrifuge for 5 min at 15000 x g	Centrifuge for 5 min at 12500–16000 x g

These data have been established in our laboratory solely in order to verify the performance of the reagent kit and to fulfil regulatory requirements. We particularly emphasize that these data are not suitable to compare the measurement systems used, nor to make any statement concerning their general performance.

Appendix III Clinical performance data

Non-applicability of clinical performance
According to MedTech Europe [8], a Therapeutic Drug Monitoring (TDM) device is a device without medical decision points. Clinical performance data cannot be generated for TDM devices and the clinical benefit lies in the accurate information about the drug concentration for which different subtherapeutic and toxic drug levels may exist, depending on indications and population.

For products for Therapeutic Drug Monitoring (TDM), the assays measure the level of the administered drug and/or its metabolites in plasma or serum. These levels can show tremendous intra- and inter-patient variability, depending on a variety of factors, including time after treatment, concomitant medication, organ function, drug toxicity and others. Since the drug is usually administered to treat an underlying clinical condition and measurement of the concentration of the drug is used to determine whether the levels are within the therapeutic window for that specific patient, there is no direct connection of the device to a clinical condition or physiological process or state. Therefore, none of the clinical performance parameters referenced in IVDR Annex I, 9.1(b), e.g. diagnostic sensitivity, diagnostic specificity, positive or negative predictive value, likelihood ratio, expected values, is applicable.

Appendix IV Traceability of the calibrators

Version 5.0

3PLUS1® Multilevel Plasma Calibrator Set (order no. 92051/XT)

MassTox® TDM Series A PARAMETER Set
Antimycotic Drugs/*EXTENDED* in serum/plasma

Gravimetric preparation of CRMs; Purity and identity are checked by methods provided in the table

Certified Reference Material (CRM)

Analyte	Certified value	Uncertainty U	k	Purity	Identity
Fluconazole	99.3%	± 0.3%	2.0	qNMR	IR
5-Flucytosine	99.9%	± 0.1%	2	HPLC-UV, sec. methods [1]	IR
Hydroxy-itraconazole	100 µg/mL	± 1 µg/mL	2	HPLC-UV, residual water by Karl-Fischer titration	LC-MS, 1H-NMR
Itraconazole	99.6%	± 0.3%	2.0	qNMR	IR, LC-MS, 1H-NMR, qNMR, optical rotation
Ketoconazole	99.9%	± 0.3%	2.0	HPLC-UV, sec. methods [1]	IR, LC-MS, 1H-NMR, melting range, optical rotation
Posaconazole	2.00 mg/mL	± 0.03 mg/mL	2	HPLC-UV, residual water by Karl-Fischer titration	LC-MS, 1H-NMR
Voriconazole	99.96%	± 0.25%	2.0	HPLC-UV, sec. methods [2]	IR

Gravimetric perparation of stock solutions from pure substances of commercial suppliers

Analyte	Purity	Purity	Identity
Anidulafungin	≥96.5%	HPLC	-
Caspofungin	≥95.0%	HPLC	LC-MS
Isavuconazole	≥97.5%	HPLC	-
Micafungin	≥96.5%	HPLC	-

Chromsystems procedure for preparation of master calibrator for all analytes in defibrinated plasma by ID-LC-MS/MS

Chromsystems master calibrator for *3PLUS1*® *Multilevel Plasma Calibrator Set* *MassTox*® *Antimycotic Drugs/EXTENDED*

Chromsystems procedure for assay *MassTox*® *TDM Series A PARAMETER Set Antimycotic Drugs/EXTENDED in serum/plasma* using ID-LC-MS/MS analysis

Chromsystems *3PLUS1*® *Multilevel Plasma Calibrator Set* *MassTox*® *Antimycotic Drugs/EXTENDED*; values are given on insert leaflet in calibrator box

Legend: (1H-/q)NMR: (proton/quantitative) nuclear magnetic resonance; HPLC-UV: high performance liquid chromatography with ultraviolet-visible spectrophotometer; ID-LC-MS/MS: isotopic dilution tandem MS spectroscopy; IR: infrared spectroscopy; LC-MS: liquid chromatography mass spectrometry

[1] secondary methods: residual solvent determination, loss on drying/volatiles and residue analysis
[2] secondary methods: residual solvent determination, residual moisture by Karl-Fischer titration and residue analysis

The master calibrator was prepared gravimetrically by addition of anidulafungin, caspofungin, fluconazole, 5-flucytosine, isavuconazole, itraconazole, hydroxyitraconazole, ketoconazole, micafungin, posaconazole and voriconazole obtained from a commercial supplier. The concentrations were determined in the manufacturer's laboratory using the ***MassTox®*** *TDM Series A PARAMETER Set Antimycotic Drugs/EXTENDED in serum/plasma* assay with Certified Reference Materials (CRMs) obtained from ISO 17025 (and ISO 17034) certified suppliers (concentrations given in the graph above) and pure substances obtained from ISO 9001 certified suppliers. The purity and identity of CRMs were determined by certified suppliers using methods presented in the graph above.

The methodology for the master calibrator is using Chromsystems assay ***MassTox®*** *TDM Series A PARAMETER Set Antimycotic Drugs/EXTENDED in serum/plasma* by ID-LC-MS/MS analysis.

The Chromsystems product *3PLUS1® Multilevel Plasma Calibrator Set* ***MassTox®*** *Antimycotic Drugs/EXTENDED* (working calibrator) has a concentration as shown on the insert leaflet of each batch, determined by the manufacturer's laboratory using Chromsystems ***MassTox®*** *TDM Series A PARAMETER Set Antimycotic Drugs/EXTENDED in serum/plasma* as reference. The assay was calibrated using Chromsystems master calibrator with a known concentration (including uncertainty). The methodology for working calibrator is using Chromsystems ***MassTox®*** *TDM Series A PARAMETER Set Antimycotic Drugs/EXTENDED in serum/plasma* by ID-LC-MS/MS analysis.

Homogeneity is checked for each batch by multiple analyses of several aliquots based on Chromsystems statistic rationale for sample size (based on ISO 13528 with a minimum set of 10 repeats and two runs).

The assigned values and corresponding uncertainties are provided on the insert leaflet for each calibrator.

Appendix V Symbols

We use EN ISO 15223-1 symbols on our labels, specifications and packaging. The meanings of each symbol are given in the table below:

Table 30: Symbols

Symbol	Meaning
	Manufacturer
	Date of manufacture
	Use by
	Order number
	Batch/lot code
	See instructions for use
	Upper temperature limit: Store below a certain temperature
	Temperature limit: Store within a certain temperature range
	*In vitro* diagnostic medical device
	Sufficient for <n> appliances
	Caution
	Serial number
	CE marking of conformity with the relevant EU legislation
	CE marking of conformity with the relevant EU legislation (with affix 0123 – for notified body: TÜV Süd Product Service GmbH)

Appendix VI Version history

Table 31: Version history

Version	Date of release (YYYY-MM-DD)	Description
1.0$_{IVDR}$	2022-09-28	Initial creation IVDR
1.1$_{IVDR}$	2024-12-17	Chapters 1 and 3.1.1: Amended classification of prefilters (CE/IVD) Chapter 3.6: Update on system rinsing during longer breaks Chapters 5.3 and 5.4: Update of data on the storage life of matrix products after reconstitution Chapter 10: Update of therapeutic ranges Appendix IV: New version of the traceability chain
1.2$_{IVDR}$	2025-01-24	Chapters 5.2, 5.3, 5.4 Storage of reconstituted products not beyond the date indicated on the label Chapters 5.3 and 5.4: Storage of reconstituted matrix products in plastic receptacles
2.0$_{IVDR}$	2025-03-14	Chapters 1 and 3.1: Rearrangement Chapters 2.1, 2.2, 2.3, 2.4, 5.8, 7.1, 8, 12, Appendix II: Textual revisions Chapter 3.2: Recommendation to use a prefilter Chapters 5.3, 5.4, Appendix I: Change of matrix: now defribrinated plasma Chapter 10: Additional therapeutic ranges Chapter 13: Addition of two references Appendix IV: Updated traceability chain AppendixV: Addition of a symbol