RECIPE®

SHIMADZU
Excellence in Science

ClinMass® LC-MS/MS Complete Kits for

Free Metanephrines in Plasma

SPE-column extraction or
ONE-STEP sample preparation

Benefits:

- Highly sensitive and selective CE-IVD LC-MS/MS methods
- Fast and easy sample preparation
- Isotope labelled internal standards for all analytes

Two methods available

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Clinical Background

Pheochromocytomas and paragangliomas (PPGL) are rare tumors that arise from adrenal and extraadrenal chromaffin tissue. PPGL produce excessive amounts of catecholamines (epinephrine, norepinephrine, dopamine) and their metabolites, the metanephrines (metanephrine, normetanephrine and 3-methoxytyramine). Measurement of metanephrines provides a particularly sensitive test for the diagnosis of pheochromocytoma [1]. Compared to tests using catecholamines or other metabolites it offers the best diagnostic performance [2].

Higher accuracy of plasma over urinary metanephrines

Plasma free metanephrines (PFM) are well-established parameters with adequate reference values [3, 4] and have a higher diagnostic accuracy than metanephrines in urine, which has been confirmed by several independent studies (Table 1) [5, 6]. These results suggest higher sensitivity and specificity of the plasma than the urine test [2].

Table 1: Comparison of diagnostic performance of plasma free vs. urinary fractionated metanephrines [6]

	Sensitivity	Specificity
Plasma	99 %	89 %
Urine	97 %	69 %

LC-MS/MS is the method of choice

To establish a biochemical diagnosis of PPGL, the use of liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) is suggested rather than other laboratory methods [6]. LC-MS/MS represents the method of choice for high-throughput, low-cost, precise and accurate measurements of the catecholamine metabolites [7].

The ClinMass® LC-MS/MS Complete Kits represent highly sensitive and selective CE-IVD methods for the determination of plasma free metanephrines (PFM). **There are two methods available:**

PFM analysis with SPE-column extraction using standard LC systems (MS11100)

- No dilution or evaporation steps required
- measurement of highly concentrated samples guaranteed
- injection interval of 4 min

PFM analysis with one-step sample preparation using two-dimensional LC-systems (on-line, MS11000)

- LC-MS/MS Complete Kit with simple and short one-step sample pretreatment (protein precipitation)
- designed for high sample throughput via on-line SPE
- injection interval of 6 min

Our calibrators are intended for calibration of the LC-MS/MS system. For this, we offer matrix based ClinCal® Multilevel Calibrator Sets (5 levels). For internal quality assurance, matrix based ClinChek® Controls are available in 3 levels. The accuracy is being checked by regular successful participation at RCPA Quality Assurance Programs (RCPAQAP). Matrix effects are compensated using isotope labelled internal standards.

References:

[1] Lenders JWM, Willemsen JJ, Eisenhofer G, Ross HA, Pacak K, Timmers HJ, Sweep CG. Is supine rest necessary before blood sampling for plasma metanephrines? Clin Chem. 2007; 53:352–354.

[2] van Berkel A, Lenders JWM, Timmers HJLM. Diagnosis of endocrine disease: biochemical diagnosis of phaeochromocytoma and paraganglioma. European Journal of Endocrinology. 2014; 170 R109–R119.

[3] Eisenhofer G, Lenders JWM, Siegert G, Bornstein SR, Friberg P, Milosevic D, Mannelli M, Marston Linehan W, Adams K, Timmers HJ, Pacak K. Plasma methoxytyramine: A novel biomarker of metastatic pheochromocytoma and paraganglioma in relation to established risk factors of tumor size, location and SDHB mutation status. Eur J Cancer. 2012; 48(11), 1739–1749.

[4] Eisenhofer G, Lattke P, Herberg M, Siegert G, Qin N, Därr R, Hoyer J, Villringer A, Prejbisz A, Januszewicz A, Remaley A, Martucci V, Pacak K, Alec Ross H, Sweep FCGJ, Lenders JWM. Reference intervals for plasma free metanephrines with an age adjustment for normetanephrine for optimized laboratory testing of phaeochromocytoma. Ann Clin Biochem. 2013; 50:62–69.

[5] Lenders JWM, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G. Biochemical diagnosis of pheochromocytoma: which test is best? Jama. 2002; 287(11): 1427–34.

[6] Lenders JWM, Duh Q-Y, Eisenhofer G, Gimenez-Roqueplo A-P, Grebe SKG, Murad MH, Naruse M, Pacak K, Young WF. Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2014; 99(6), 1915–1942.

[7] Eisenhofer G, Peitzsch M, McWhinney BC. Impact of LC-MS/MS on the laboratory diagnosis of catecholamine-producing tumors. Trends in Analytical Chemistry. 2016; 84, 106–116

Analysis with SPE-column extraction

Order no. MS11100

LC-MS/MS Parameters

LC-Parameters

Binary HPLC pump:	Mobile Phases A / B (0.6 ml/min)
Injection volume:	20 - 50 µl
Injection interval:	4.0 min
Column heater:	25 °C

MS-Parameters

Ion-Source:	ESI positive
MS/MS-Mode:	MRM

Test Data

Linearity (up to):	20970 ng/l (M), 19860 ng/l (NM), 5094 ng/l (MT)
Recovery:	95 - 98 %
LOD:	0.97 ng/l (M), 0.92 ng/l (NM), 0.94 ng/l (MT)
LLOQ:	2.91 ng/l (M), 2.76 ng/l (NM), 2.83 ng/l (MT)
Intraassay precision:	2.0 % (M), 4.4 % (NM), 3.7 % (MT)
Interassay precision:	3.1 % (M), 3.2 % (NM), 3.4 % (MT)

M: Metanephrine
NM: Normetanephrine
MT: 3-Methoxytyramine

Sample Preparation

Extraction:

Apply (conditioned* sample preparation column):	
500 µl Sample (calibrator, control, patient)	500 µl Internal Standard IS

↓ wash with Washing Solution W2, W1 and W3

Elution:

Elute with 2 x 100 µl Eluting Reagent E

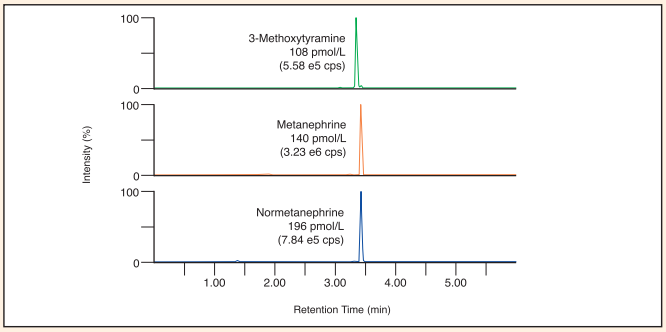
↓ collect the eluate

LC-MS/MS Analysis:

Inject 20 - 50 µl

* Conditioning with Washing Solution W1 and W2

Chromatogram of the ClinCal® Calibrator, Level 1 (order no. MS11013)



Analysis with one-step sample preparation (on-line)

Order no. MS11000

LC-MS/MS Parameters

LC-Parameters

Pump 1:	SPE-buffer (0.1 - 1.0 ml/min)
Pump 2:	Mobile phase A / B (0.6 ml/min)
Injection volume:	50 µl
Injection interval:	6.0 min
Switching valve:	automatic switching valve, 6-port/3-channel
Column heater:	25 °C

MS-Parameters

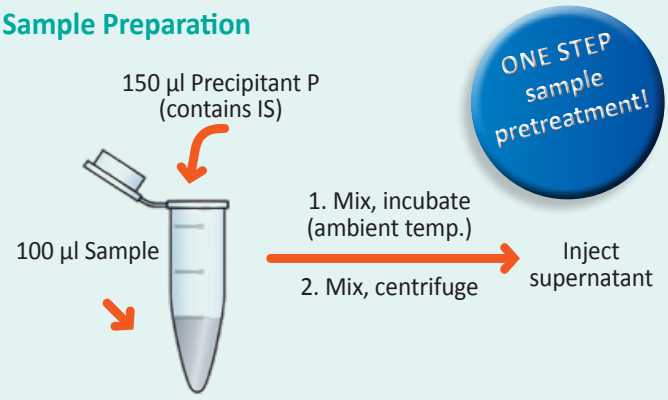
Ion-Source:	ESI positive
MS/MS-Mode:	MRM

Test Data

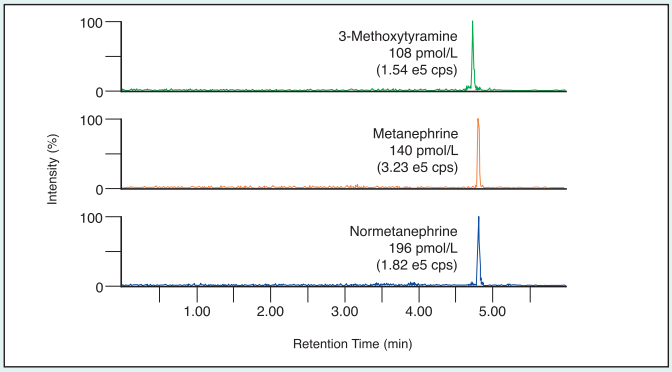
Linearity (up to):	5200 ng/l (M), 10000 ng/l (NM, MT)
Recovery:	83 - 116 %
LOD:	1.94 ng/l (M), 3.67 ng/l (NM), 0.94 ng/l (MT)
LLOQ:	5.82 ng/l (M), 11.0 ng/l (NM), 2.83 ng/l (MT)
Intraassay precision:	3.1 % (M), 4.5 % (NM), 3.4 % (MT)
Interassay precision:	3.2 % (M), 4.7 % (NM), 4.1 % (MT)

M: Metanephrine
NM: Normetanephrine
MT: 3-Methoxytyramine

Sample Preparation



Chromatogram of the ClinCal® Calibrator, Level 1 (order no. MS11013)



Ordering Information

CE

Analysis with SPE-column extraction

Order No.	Description	Quantity
MS11100	**ClinMass® Complete Kit for Free Metanephrines in Plasma, for 200 assays**	**1 pce.**
Contents (order no. MS11100)		
MS11005	Autosampler Washing Solution	1000 ml
MS11007	Mobile Phase A	250 ml
MS11008	Mobile Phase B	2 x MS11008 (2 x 1000 ml)
MS11112	Internal Standard IS	2 x MS11112 (2 x 50 ml)
MS11013	Serum Calibrator Set, lyophil. (Level 0 - 4)	5 x 1 x 3 ml
MS11120	Sample Preparation Columns	4 x MS11120 (4 x 50 pcs.)
MS11121	Washing Solution W1	400 ml
MS11122	Washing Solution W2	400 ml
MS11123	Washing Solution W3	200 ml
MS11124	Eluting Reagent E	40 ml
	Manual	
Additional Components		
MS11014	Optimisation Mix	2 ml
Start Accessories		
MS11030	Analytical Column with test chromatogram	1 pce.
Accessories		
MS11161	Elution Vials	100 pcs.
ClinChek® Controls		
MS11080	Serum Control, lyophil., Level I	10 x 3 ml
MS11081	Serum Control, lyophil., Level II	10 x 3 ml
MS11082	Serum Control, lyophil., Level III	10 x 3 ml
MS11083	Serum Control, lyophil., Level I, II, III	3 x 3 x 3 ml

Analysis with one-step sample preparation (on-line)

Order No.	Description	Quantity
MS11000	**ClinMass® Complete Kit for Free Metanephrines in Plasma – on-line Analysis, for 200 assays**	**1 pce.**
Contents (order no. MS11000)		
MS11005	Autosampler Washing Solution	1000 ml
MS11007	Mobile Phase A	250 ml
MS11008	Mobile Phase B	2 x MS11008 (2 x 1000 ml)
MS11009	SPE-Buffer Load L	2 x MS11009 (2 x 1000 ml)
MS11010	SPE-Buffer Wash W	1000 ml
MS11012	Precipitant P with Internal Standard	30 ml
MS11013	Serum Calibrator Set, lyophil. (Level 0 - 4)	5 x 1 x 3 ml
MS11020	Sample Pretreatment Vials	2 x MS11020 (2 x 100 pcs.)
	Manual	
Additional Components		
MS11014	Optimisation Mix	2 ml
Start Accessories		
MS11030	Analytical Column with test chromatogram	1 pce.
MS11031	SPE-Column	1 pce.
ClinChek® Controls		
MS11080	Serum Control, lyophil., Level I	10 x 3 ml
MS11081	Serum Control, lyophil., Level II	10 x 3 ml
MS11082	Serum Control, lyophil., Level III	10 x 3 ml
MS11083	Serum Control, lyophil., Level I, II, III	3 x 3 x 3 ml

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SHIMADZU RECIPE

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