CHROMSYSTEMS

VITAMINSTATUS
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DOSAGE DE VITAMINES
PROFILO DELLE VITAMINE
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Instruction Manual for HPLC Analysis

Vitamin B_1 in whole blood

35000

CE 0123 IVD

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 (IVDR).

You can download the declaration of conformity according to IVDR from the download centre of our website.

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1 Ordering information

1.1 Kits

Reagent kit for the analysis of Vitamin B$_1$ in whole blood

35000	**HPLC Reagent Kit** **Vitamin B$_1$ in whole blood** Kit content for 100 analyses:	
	Mobile Phase	1000 mL
	Extraction Buffer	10 mL
	Precipitation Reagent	30 mL
	Derivatisation Reagent 1	5 x 0.3 mL (lyoph.)
	Derivatisation Reagent 2	20 mL
	Stabilisation Buffer	10 mL
	Whole Blood Calibration Standard	5 x 1.0 mL (lyoph.)
	Neutralisation Buffer	10 mL
	Reaction Vials 1.5 mL, amber colour (light protection)	2 x 100 pcs.

The following CE/IVD products and accessories are not included in the kit 35000 but are required for the application of the method:

35110	HPLC column (equilibrated, with test chromatogram)	1 pc.
0034	Whole Blood Control Level I Vitamins B1 and B2 in whole blood	5 x 2.0 mL (lyoph.)
0035	Whole Blood Control Level II Vitamins B1 and B2 in whole blood	5 x 2.0 mL (lyoph.)

Basic kit for sample preparation

35000-BK	**HPLC Basic Kit** **Vitamin B$_1$ in whole blood**	
	Kit content for 100 sample preparations:	
	Extraction Buffer	10 mL
	Precipitation Reagent	30 mL
	Derivatisation Reagent 1	5 x 0.3 mL (lyoph.)
	Derivatisation Reagent 2	20 mL
	Stabilisation Buffer	10 mL
	Neutralisation Buffer	10 mL
	Reaction Vials 1.5 mL, amber colour (light protection)	2 x 100 pcs.

The following CE/IVD products and accessories are not included in the kit 35000-BK but are required for the application of the method:

35110	HPLC Column (equilibrated, with test chromatogram)	1 pc.
35021	Mobile Phase	1000 mL
37008	Whole Blood Calibration Standard Vitamins B1 and B2 in whole blood	5 x 1.0 mL (lyoph.)
0034	Whole Blood Control Level I Vitamins B1 and B2 in whole blood	5 x 2.0 mL (lyoph.)
0035	Whole Blood Control Level II Vitamins B1 and B2 in whole blood	5 x 2.0 mL (lyoph.)

1.2 Individual components

CE/IVD products and accessories

For sample preparation:

37003	Extraction Buffer	10 mL
37004	Precipitation Reagent	30 mL
35005	Derivatisation Reagent 1	5 x 0.3 mL (lyoph.)
35006	Derivatisation Reagent 2	20 mL
35009	Neutralisation Buffer	10 mL
35007	Stabilisation Buffer	10 mL

For chromatography:

35021	Mobile Phase	1000 mL
35022	Mobile Phase	10 x 1000 mL
35110	HPLC Column (equilibrated, with test chromatogram)	1 pc.
15009	PEEK-encased Prefilter 5 µm	5 pcs.
18010	Precolumn Cartridge 4/10	1 pc.

For calibration and quality control:

37008	Whole Blood Calibration Standard Vitamins B_1 and B_2 in whole blood	5 x 1.0 mL (lyoph.)
0033	Whole Blood Control Bi-Level (I+II) Vitamins B_1 and B_2 in whole blood	2 x 5 x 2.0 mL (lyoph.)
0034	Whole Blood Control Level I Vitamins B_1 and B_2 in whole blood	5 x 2.0 mL (lyoph.)
0035	Whole Blood Control Level II Vitamins B_1 and B_2 in whole blood	5 x 2.0 mL (lyoph.)

Non-CE/IVD products

33005	Reaction Vials 1.5 mL, amber colour (light protection)	100 pcs.
15010	PEEK Prefilter Housing	1 pc.
18001	Precolumn Cartridge Holder 4/10	1 pc.

2 Introduction

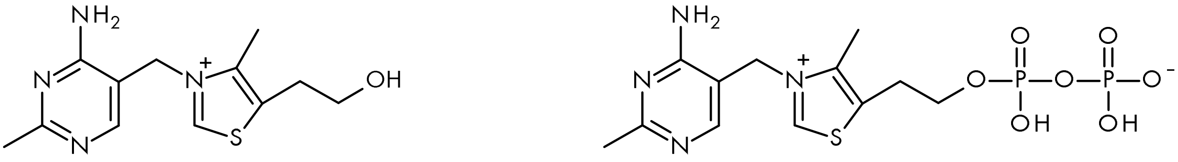
2.1 Background information

B vitamins play an essential role in a number of basic metabolic pathways. They are among the water-soluble vitamins that are poorly stored in the body, which is why a regular supply by food or dietary supplements is essential to avoid deficiency symptoms.

Vitamin B_1

Vitamin B_1 (syn. thiamine) contains two heterocyclic rings (a pyrimidine and a thiazole) connected by a methylene bridge, and a quaternary nitrogen atom (Fig. 1). It is sensitive to alkaline solutions (which split the thiazole ring) and to oxidising and reducing agents.

The majority of thiamine is located in the erythrocytes [1] where it can be found in its active form, thiamine pyrophosphate (TPP).



Thiamine

Thiamine pyrophosphate (TPP, Thiamine diphosphate)

Figure 1: Vitamin B_1

Vitamin B_1 is synthesised naturally by a variety of plants and microorganisms, but for most animals it is an essential nutrient. After phosphate residues are split off, thiamine is absorbed from the small intestine both by passive and active transport. Subsequent phosphorylation yields the physiologically most important coenzyme form TPP. Thiamine is excreted via the kidney after dephosphorylation to the free form or as a conjugated sulphate ester.

TPP is an essential cofactor in multiple enzyme complexes involved in carbohydrate and amino acid metabolism, production of energy as adenosine triphosphate (ATP), synthesis of essential cellular molecules and neurotransmitters and control of oxidative stress, among others. It also plays a crucial role for cognitive and neurological health.

Worldwide, thiamine deficiency occurs predominantly in populations whose diets are comprised mainly of polished rice, milled cereals and white wheat flour. The best known disease associated with vitamin B_1-deficiency is beriberi. Its clinical signs may cause neurological symptoms or involve cardiovascular system. "Dry beriberi" describes neurological symptoms such as confusion, eye disorders and ataxia (Wernicke's encephalopathy) or memory disorder (Korsakoff syndrome). In "wet beriberi", symptoms include lactate acidosis and congestive heart failure. Both forms of beriberi are most commonly seen in individuals with alcoholism, poor diet or malabsorption syndrome [2–4].

Another group at high risk for thiamine deficiency are people who start a normal nutrition after a long period of starvation or malnutrition. They run the risk of developing refeeding syndrome, a serious metabolic imbalance characterised by disturbances in electrolyte and fluid homeostasis after the initiation of nutritional support. The high demand of TPP during carbohydrate administration during the refeeding phase cannot be covered due to (existing) exhausted or insufficient deposits resulting in lactate increase and reduced cellular energy production [5,6].

Milder cases of vitamin B_1 deficiency manifest themselves as general physical and mental fatigue, irritability, confusion, sleep disorders and gastrointestinal symptoms [7].

Vitamin B_1 deficiency

Our modern diet differs from the evolutionary diet, which was mainly plant-based consisting of vegetables, fruits and nuts, along with small amounts of fish and meat. Energy-rich, easily digestible and micronutrient-poor nutrition leads to vitamin deficiency even in developed societies and is linked to obesity, cardiovascular diseases and dementia [7]. Evaluation of the vitamin status should also be considered in the case of unbalanced diets.

In addition, certain diseases are associated with vitamin deficiency. Patients with diabetes mellitus type 2 are often deficient in vitamin B_1 [8].

People with alcohol-use disorder, renal dialysis patients, elderly, pregnant women, as well as patients after surgery are at high risk for vitamin B_1 deficiency. Disturbances in absorption, e.g. in inflammatory bowel diseases, genetic defects causing impairment of thiamine cell uptake (e.g. Roger's Syndrome) or surgical procedure such as bariatric surgery also lead to a reduced supply of important nutrients [3,9].

Some medications e.g. chemotherapeutic agents, diuretics, isoniazid, and metformin interfere with vitamin B metabolism and may contribute to deficiency [3,10].

Vitamin B_1 excess

Excess amounts of water-soluble B vitamins are excreted in the urine. No toxicity of high vitamin B_1 have been reported, apart from anaphylactic reactions to intravenous administration. However, symptoms and signs of vitamin B_1 overdose may include nausea, lethargy, ataxia and diminution of gut tone [11,12].

2.2 Principle of the assay

High-pressure liquid chromatography and an appropriately optimised system offer the possibility of direct determination of thiamine pyrophosphate (TPP), the active form of vitamin B_1, in whole blood. The present rapid, simple and specific method requires an isocratic HPLC system with fluorescence detector. The usual post-column derivatisation using extensive equipment is replaced by a simple pre-column derivatisation. Sample preparation is restricted to a single precipitation step after addition of the extraction buffer and subsequent derivatisation in a reaction vial. A selected HPLC column and a mobile phase optimised for this specific separation, permit certain and reliable quantification.

2.3 Intended purpose

The Chromsystems reagent kit 35000 "Vitamin B_1 in whole blood" is an in vitro diagnostic medical device for professional use in clinical laboratories for the quantitative detection of the physiologically active form of vitamin B_1, thiamine pyrophosphate, in human whole blood samples.

Sample preparation is carried out manually, and analytic separation is done via high performance liquid chromatography (HPLC).

The test kit is intended to be used for screening and/or monitoring of vitamin B_1 levels where indicated

- in patients with suspected Vitamin B_1 deficiency,
- in patients with suspected Vitamin B_1 excess, and/or
- in patients under Vitamin B_1 supplementation therapy.

2.4 Clinical limitations

There are no universally applicable reference ranges for thiamine pyrophosphate. 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 reference ranges based on clinical assessment. Conversion factors between different methods of analysis should not be used to predict results for a specific patient.

3 HPLC system

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

3.1 Additionally required equipment

In addition to the components included in the Chromsystems reagent kit (order no. 35000), the HPLC analysis of Vitamin B$_1$ in whole blood requires the following materials:

3.1.1 Essential equipment

Products directly associated with the kit, available from Chromsystems:

Required for the application of the method:

35110	HPLC column (equilibrated, with test chromatogram)
0033	Whole Blood Control Bi-Level (I + II)
0034	Whole Blood Control Level I
0035	Whole Blood Control Level II

Laboratory devices for HPLC analysis, only partially available from Chromsystems:
- Isocratic HPLC system
- Autosampler
- Fluorescence detector

Laboratory devices for sample preparation, not available from Chromsystems:
- Vortex mixer
- Suitable centrifuge

3.1.2 Optional/recommended equipment

Accessories, available from Chromsystems:

15009	PEEK-encased Prefilter 5 µm
18010	Precolumn Cartridge 4/10

General lab-equipment (non-CE/IVD products), available from Chromsystems:

15010	PEEK Prefilter Housing
18001	Precolumn Cartridge Holder 4/10

The PEEK Prefilter Housing (order no. 15010) is intended for combination with the PEEK-encased Prefilter 5 µm (order no. 15009), the Precolumn Cartridge Holder 4/10 (order no. 18001) for combination with the Precolumn Cartridge 4/10 (order no. 18010).

Laboratory devices:

- Thermostatted column compartment

3.2 Instrument parameters

The substances are separated chromatographically using an analytical column (order no. 35110). Keep the Mobile Phase closed or covered even when in use. The use of a thermostatted column compartment will avoid temperature variations and ensure optimal stability and reproducibility of the chromatographic separation.

Instrument settings:

Autosampler:	Magazine should be light protected, amber coloured glass vials
Injection volume:	50 µL
Run time:	6 min
Flow rate:	1.0 mL/min
Column temperature:	+20 to +25 °C
Fluorescence detector:	EX 367 nm, EM 435 nm
Needle rinsing solution for the injector:	Ultrapure water (HPLC grade) with 5–10 % methanol

3.3 HPLC column

The HPLC column for the analysis of thiamine pyrophosphate (TPP) is supplied equilibrated and tested, and is ready for use. The backpressure of a new column at a flow rate of 1.0 mL/min is about 80 bar and may increase with column age and/or use. As long as the separations are satisfactory, a raised backpressure is of no consequence.

Note:
We recommend the use of a prefilter (PEEK-encased Prefilter 5 µm, order no. 15009) in order to enhance the column life-time. A pre-column (Precolumn Cartridge 4/10, order no. 18010) can be used for additional protection of the column.

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

3.4 System start-up

Before starting a sequence, prepare the HPLC system as follows:

1. Rinse the system with approx. 30 mL Mobile Phase before installing the HPLC column
2. Install the column and equilibrate the system at a flow rate of 1.0 mL/min for about 15 to 20 min with Mobile Phase, until the baseline has stabilised
3. Inject the prepared calibrator several times until the retention times and peak areas/heights are consistent
4. Compare the chromatogram with those in chapter 7.2
5. Start the sequence

Thereafter, the mobile phase can be recirculated for approximately 100 injections.

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

3.5 System shutdown

For interruptions in operation of up to 3 days, pump the Mobile Phase at a low flow rate (0.2 mL/min). The HPLC column remains connected in the system. To protect the lamp, turn off the detector.

For longer periods of disuse, the HPLC column should be disconnected. Rinsing or conservation is not necessary. Store the column in the Mobile Phase at +18 to +30 °C. Insert a union to replace the column and rinse the HPLC system using about 50 mL of ultrapure water (HPLC grade)/methanol (80/20).

4 Chromatographic separation

The following table shows the retention time of thiamine pyrophosphate (TPP):

Table 1: Retention times

Substance	Retention time (ca.)
Thiamine pyrophosphate (TPP)	3.2 min

Retention times may vary slightly, for instance if there is a change in ambient temperature, if you use a new batch of Mobile Phase, or if you replace the HPLC column. Therefore, use a calibration chromatogram to determine current values.

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 for sample preparation as well as the batch of the calibrator and the controls are not changed.

5.1 Collection and storage of patient specimens

For the analysis of thiamine pyrophosphate (TPP) use whole blood.

Both EDTA (K3-EDTA and K2-EDTA) and heparin (lithium, sodium and ammonium heparin) are suitable as anticoagulants. EDTA whole blood is recommended because heparin whole blood is much more susceptible to enzymatic falsification of results (both increased and decreased analyte concentrations are possible) and is more prone to clotting, which may reduce pipetting precision.

Sample stability
Storage/transport temperature is the main factor likely to affect sample stability. Other factors identified in preliminary tests include the type of anticoagulant (EDTA or heparin) and differences between donors or blood samples, probably due to differing enzyme status. Stability differences between EDTA types (K2-EDTA, K3-EDTA) or heparin types (lithium heparin, sodium heparin, ammonium heparin) have not been identified.

Stability of TPP was determined in K3-EDTA blood (representing both types of EDTA blood) and in lithium heparin blood (representing all three types of heparin blood) from five different donors.

Deviations in analyte concentrations of ≤ 15% from the reference were accepted.

Table 2: Analyte stability in patient samples

Storage temperature	EDTA whole blood	Heparin whole blood
+20 to +25 °C	24 hours	24 hours
+2 to +8 °C	7 days	7 days
below –18 °C	3 months	3 months
Freeze-thaw cycles	1 cycle	1 cycle

There are no restrictions on the use of haemolytic, lipaemic and icteric samples (see chapter 11).

Note:
It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine laboratory-specific stability criteria.

5.2 Reconstitution of the calibrator

The Chromsystems Whole Blood Calibration Standard (order no. 37008) is intended for the calibration of your analysis system. The lyophilised calibrator is a single point calibrator. It is based on human whole blood. After reconstitution, it is handled in the same manner as a patient sample and is analysed under routine conditions analogous to the respective test procedure.

Prior to sample preparation, reconstitute the whole blood calibrator 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. Avoid exposure to direct sunlight.

The calibrator level is traceable to certified reference material (see Appendix IV). The analyte concentration in the calibrator is batch-dependent. Individual level is given in the calibrator leaflet.

Caution:
This product is manufactured from pooled human whole blood 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 calibrator after reconstitution:
The calibrator dissolved in water has the following storage live, but not beyond the date indicated on the label:

Table 3: Stability of thiamine pyrophosphate (TPP) in the whole blood calibrator (order no. 37008) after reconstitution

Storage temperature	Storage life	Other storage conditions
+20 to +25 °C	3 days	Light protection, tightly closed
+2 to +8 °C	7 days	Light protection, tightly closed
below –18 °C	3 months	Light protection, tightly closed
Freeze-thaw cycles	3 cycles	-

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

5.3 Reconstitution of the controls

The Chromsystems Whole Blood Controls (order no. 0034, 0035) are intended to monitor the accuracy and precision of each analytical sequence. They are available in two different concentration levels. The lyophilised controls are based on human whole blood. 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 whole blood controls as follows:

1. Pipette 2.0 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. Avoid exposure to direct sunlight.

The analyte concentrations in the controls are batch-dependent. Individual levels are given in the leaflet accompanying each control.

Caution:
This product is manufactured from pooled human whole blood 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 have the following storage lives, but not beyond the date indicated on the label:

Table 4: Stability of thiamine pyrophosphate (TPP) in the whole blood controls (order no. 0034, 0035) after reconstitution

Storage temperature	Storage life	Other storage conditions
+20 to +25 °C	3 days	Light protection, tightly closed
+2 to +8 °C	7 days	Light protection, tightly closed
below –18 °C	3 months	Light protection, tightly closed
Freeze-thaw cycles	3 cycles	-

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

5.4 Preparation of the derivatisation mix

Prior to sample preparation, prepare a derivatisation mix as follows:

1. Reconstitute a bottle of Derivatisation Reagent 1 (order no. 35005) with exactly 0.3 mL high-purity water. At +2 to +8 °C, this solution is stable for 2 weeks.

2. Mix 100 µL of it with 2.0 mL Derivatisation Reagent 2 (order no. 35006), proportion: 1:21

Per sample, 200 µL of this mixture (= derivatisation mix) are used for sample preparation.

Note:
The derivatisation mix should be prepared **immediately prior to sample preparation**. At room temperature, it is stable for 12 hours.

5.5 Sample preparation procedure

Important:
Sufficient light protection during the whole sample preparation is essential. The samples must not be exposed to direct sunlight.

Before sample preparation, allow reagents/calibrator/controls/samples that are stored frozen or refrigerated to reach ambient temperature and mix thoroughly.

To prepare patient samples, controls and calibrator for analysis, work through the following steps in the order given:

1. Pipette 200 µL sample/calibrator/control into an amber-coloured Reaction Vial (order no. 33005)
2. Add 100 µL Extraction Buffer (order no.: 37003)
3. Shake 2 s (vortex)
4. Add 300 µL Precipitation Reagent (order no.: 37004)
5. Shake 30 s (vortex)
6. Centrifuge 5 min at 9000 x g
7. Pipette 200 µL of the derivatisation mix (see chapter 5.4) into a new amber-coloured Reaction Vial
8. Add 100 µL of the supernatant and mix briefly
9. Add 100 µL Neutralisation Buffer (order no.: 35009)
10. Add 100 µL Stabilisation Buffer (order no.: 35007) and mix briefly
11. Let stand for 20 minutes
12. Transfer into an amber coloured autosampler glass vial, inject 50 µL into the HPLC system

5.6 Storage life of prepared samples

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

Table 5: Storage life of the prepared samples

Storage temperature	Storage life	Other storage conditions
+20 to +25 °C	3 days	Light protection, tightly closed, glass vials
+2 to +8 °C	3 days	Light protection, tightly closed, glass vials

6 Quality control

Monitor precision and accuracy of the analyses by including additional controls (order no. 0034, 0035) in each analytical run. If the analysis of these controls yields values outside the range given on the accompanying information leaflet, check the system. If the discrepancy continues to exist, re-calibrate the system.

Monitor quality of chromatographic separation by comparison of retention times and chromatographic peak shapes of the analyte with the chromatogram of the column certificate or with an example chromatogram (chapter 7.2). In case of a column in use, compare to preceding analytical runs of the same assay (e.g. in the course of system start-up, chapter 3.4). Significant deviations might be due to decreasing performance of the prefilter, precolumn and/or analytical column. Typical indicators would be tailing of the peaks.

For more information, see chapter 12 Troubleshooting.

7 Data acquisition and evaluation

7.1 Calibration of the data analysis system

Run a calibration of the analysis system for each series of measurements. Use the whole blood calibrator (order no. 37008) for this. The concentration of the analyte in the calibrator is batch-dependent. Exact levels are given in the package leaflet.

Calibration curve is constructed by calculating the analyte peak area or peak height on the y-axis against calibrator concentration on the x-axis. Then plot a calibration curve through origin (single point calibration).

Select the external standard method for calibration in your analysis system.

7.2 Examples of chromatograms

The following graphs provide examples of chromatograms created using this method.

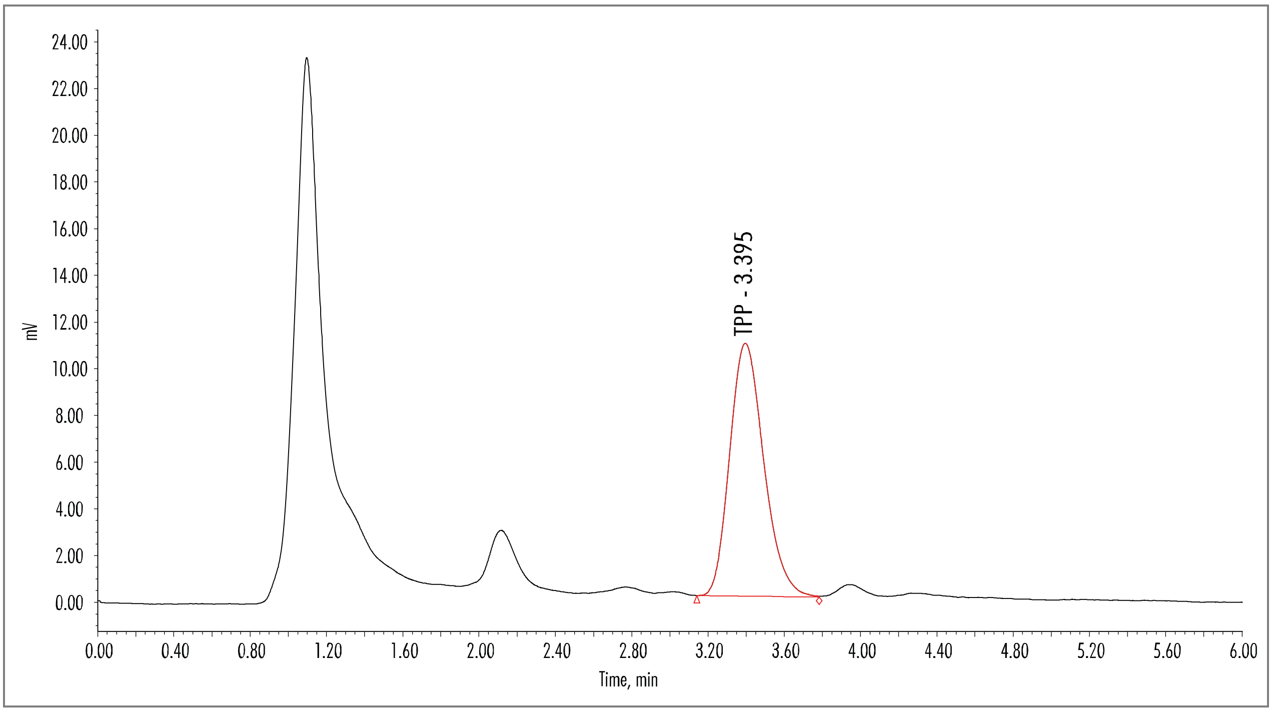


Figure 2: Chromatogram of a whole blood calibrator
Concentration of the analyte: Thiamine pyrophosphate (TPP): 46.1 µg/L

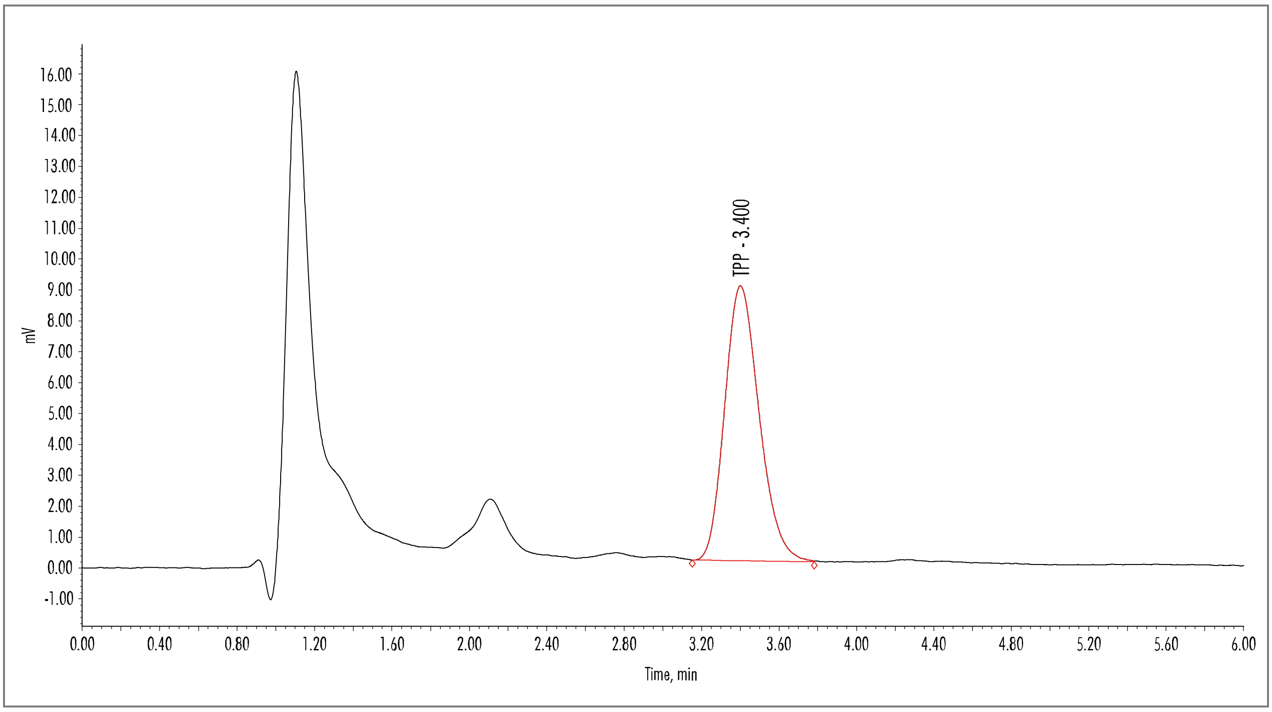


Figure 3: Chromatogram of a patient sample
Concentration of the analyte: Thiamine pyrophosphate (TPP): 39.5 µg/L

7.3 Conversion factors

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

Table 6: Conversion factors

Substance	µg/L to nmol/L	nmol/L to µg/L
Thiamine pyrophosphate (TPP)	x 2.357	x 0.4243

7.4 Manual calculation

For the manual calculation the following data are required:

- Peak area/height of substance A in the chromatogram of the sample $=A_{Sample}$
- Peak area/height of substance A in the chromatogram of the calibrator $=A_{Calibrator}$
- The concentration of substance A in the calibrator $=C_{Calibrator}$

The concentration of the substance A in the sample C_{Sample} is then calculated as follows:

$$C_{Sample} = \frac{A_{Sample}}{A_{Calibrator}} \times C_{Calibrator}$$

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 7: Transport conditions for the reagent kit and components

Product	Transport temperature
Reagent kit (order no. 35000)	ambient
Basic kit (order no. 35000-BK)	ambient
All other components listed in chapter 1	ambient

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

Table 8: Storage conditions for the reagents, calibrator and controls

Product	Storage temperature
Mobile Phase (order no. 35021, 35022)	+18 to +30 °C
Extraction Buffer (order no. 37003)	+18 to +30 °C

Product	Storage temperature
Precipitation Reagent (order no. 37004)	+18 to +30 °C
Derivatisation Reagent 1 (order no. 35005)	+18 to +30 °C
Derivatisation Reagent 2 (order no. 35006)	+18 to +30 °C
Neutralisation Buffer (order no. 35009)	+18 to +30 °C
Stabilisation Buffer (order no. 35007)	+2 to +8 °C
Whole Blood Calibration Standard (order no. 37008)	below –18 °C
Whole Blood Controls (order no. 0033, 0034, 0035)	below –18 °C

Close the reagents immediately after use and store them at the specified temperature. The in-use shelf-life is one year but does not extend beyond the stated expiry date. Details of the stability of the reconstituted calibrators and controls are given in chapters 5.2 and 5.3.

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

The in-use shelf-life of HPLC column, precolumn and prefilter is dependent 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
The Mobile Phase (order no. 35021, 35022) and the Derivatisation Reagent 2 (order no. 35006) contain organic solvents. Dispose product residues into a container for organic halogen-free solvents. The Precipitation Reagent (order no. 37004) and the Neutralisation Buffer (order no. 35009) contain strong acids. Neutralise the solutions and dispose into a container for salt solutions. The Stabilisation Buffer (order no. 35007) contains a dangerous salt. Dispose product residues into a container for salt solutions.

Residues of patient samples, prepared samples, controls (order no. 0034, 0035) and calibrator (order no. 37008) 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. 37003), Derivatisation Reagent 1 (order no. 35005) and 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 Reference ranges

Note:
Reference ranges listed below are representing levels found in a proportion of a healthy population (e.g. of 95% of the investigated healthy blood-donors). Consequently, they might or might not align with clinically relevant decision levels or cut-off-values regarding the intended purpose of this device. For clinical performance data, including values expected in affected and non-affected persons, see Appendix III.

The stated reference ranges are based on the literature [13]. They may differ from other published data. As the levels vary depending on patient population and measurement method, determine specific reference ranges for your laboratory. When determining ranges, make sure that you comply with local national requirements.

Lithium heparin blood samples from at least 15 men and 15 women of various age groups (21–30, 31–40, 41–50, 51–60, 61–70 and > 70 years old) were collected for the G. Steen and M. van der Zwaal study [13]. A questionnaire was administered to check for the following exclusion criteria: use of oral vitamin or iron supplements, anticoagulant use, diabetes, pregnancy, and oral contraceptive use. Testing was done using HPLC fluorescence assay after derivatisation.

Table 9: Reference ranges for thiamine pyrophosphate (TPP) in whole blood

Reference range	**33.1–60.7 µg/l** **78–143 nmol/l**
Number of results obtained	247
Statistical method of the reference range	Mean ± 2x standard deviation
Correlation between results obtained and years of age	None
Correlation between results obtained and gender	None
Source	[13]

11 Interference testing

Metabolites and drugs were spiked into whole blood samples or prepared whole blood samples prior derivatisation at the highest expected concentrations (see table below) and tested for interferences.

In addition, different sample conditions were simulated and their influence on the test was examined. Different sampling systems were also tested for interference.

Table 10:Tested substances and their concentrations – metabolites

Substance	**Test concentration in µg/L**
Thiamine	500
Thiamine monophosphate	500
Thiamine triphosphate	4.39

Table 11: Tested substances and their concentrations - drugs

Substance	Test concentration in mg/L
Acetaminophen (paracetamol)	156
Acetylsalicylic acid	30.0
Allopurinol	60.0
Alprazolam	0.258
Amlodipine	0.0750
Amoxicillin	54.0
Amphetamine/dexamfetamine	0.330
Apixaban	0.315
Ascorbic acid	1044
Atenolol	9.00
Atorvastatin	0.750
Azithromycin	11.1
Bisoprolol	0.258
Bupropion	0.396
Candesartan	0.551
Carvedilol	0.233
Cefuroxime	543
Citalopram	5.43
Clonazepam	0.300
Clopidogrel	0.00169
Cyclobenzaprine	0.102
Diclofenac	24.0
Donezepil	6.00
Duloxetine	0.162
Edoxaban	1.01
Empagliflozin	0.929
Enalaprilat	0.819
Fenoterol	0.00358
Fluoxetine	1.42
Fluticasone	0.00126
Formoterol	0.000273
Furosemide	15.9
Gabapentin	26.7
Galantamine	14.4
Glimepiride	1.64
Glipizide	3.00
Hydrochlorothiazide	1.13
Hydrocodone	0.0720
Ibuprofen	219

Substance	Test concentration in mg/L
Ipratropium bromide	0.00300
Lercanidipine	0.0407
Levothyroxine	0.429
Lisinopril	0.246
Lorazepam	0.720
Losartan	0.836
Meloxicam	6.00
Memantine	12.0
Metamizole/dipyrone (as 4-methylaminoantipyrine)	206
Metformin	12.0
Methocarbamol	123
Methylphenidate	0.108
Metoclopramide	2.25
Metoprolol	1.50
Montelukast	1.65
Nebivolol	0.0300
Nimodipine	54.0
Noscapine	0.810
Omeprazole/esomeprazole	8.40
Pantoprazole	30.0
Phenprocoumon	15.0
Pravastatin	0.207
Prednisolone	1.20
Prednisone	0.0990
Pregabalin	22.5
Propranolol	1.01
Ramipril	0.156
Ranitidine	10.5
Rivaroxaban	2.70
Rivastigmine	7.20
Rosuvastatin	0.111
Salbutamol/albuterol	0.0450
Sertraline	0.927
Simvastatin	0.0831
Sitagliptin	1.15
Spironolactone	0.555
Tamsulosin	0.0338
Tilidine (as nortilidine)	0.368
Torasemide	83.1
Tramadol	3.14

Substance	Test concentration in mg/L
Trazodone	14.7
Valsartan	11.7
Venlafaxine	0.696
Zolpidem	0.816

11.1 Interferences detected

Acetaminophen (paracetamol), a drug to treat fever and mild to moderate pain, leads to false low determination of thiamine pyrophosphate (TPP).

11.2 No interference detected

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

Metabolites
Thiamine, thiamine monophosphate, thiamine triphosphate
These metabolites can be converted into each other and into TPP by enzymatic reactions. They are not analytical but rather pharmacokinetic interferences.

Drug substances
Acetylsalicylic acid, allopurinol, alprazolam, amlodipine, amoxicillin, amphetamine/dexamfetamine, apixaban, ascorbic acid, atenolol, atorvastatin, azithromycin, bisoprolol, bupropion, candesartan, carvedilol, cefuroxime, citalopram, clonazepam, clopidogrel, cyclobenzaprine, diclofenac, donepezil, duloxetine, edoxaban, empagliflozin, enalaprilat, fenoterol, fluoxetine, fluticasone, formoterol, furosemide, gabapentin, galantamine, glimepiride, glipizide, hydrochlorothiazide, hydrocodone, ibuprofen, ipratropium bromide, lercanidipine, levothyroxine, lisinopril, lorazepam, losartan, meloxicam, memantine, metamizole/dipyrone as 4-methylaminoantipyrine, metformin, methocarbamol, methylphenidate, metoclopramide, metoprolol, montelukast, nebivolol, nimodipine, noscapine, omeprazole/esomeprazole, pantoprazole, phenprocoumon, pravastatin, prednisolone, prednisone, pregabalin, propranolol, ramipril, ranitidine, rivaroxaban, rivastigmine, rosuvastatin, salbutamol/albuterol, sertraline, simvastatin, sitagliptin, spironolactone, tamsulosin, tilidine as nortilidine, torasemide, tramadol, trazodone, valsartan, venlafaxine, zolpidem

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

Haemolysis
Freshly collected whole blood samples were haemolysed by freezing and thawing. Analyte concentrations were compared against those of the original sample:

No significant interferences occurred (deviation ≤ 15 %).

Lipaemia
Whole blood samples were spiked with different concentrations of a lipaemic emulsion (0.67 to 10 g/L) and analyte concentrations were compared against those of the original sample:

No significant interferences occurred (deviation ≤ 15 %).

Icterus
Whole blood samples were spiked with unconjugated and conjugated bilirubin (each 0.4 g/L) and the analyte concentrations 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 affected (deviation ≤ 15%):

Table 12: Sampling systems causing no interferences

Type	Manu-facturer	Order no.	Volume	Description	Lots tested
Whole blood EDTA (K3E)	Sarstedt	06.1664.001	1.2 mL	S-Monovette® 1.6 mg K3-EDTA/mL, spray-dried	7030611
Whole blood EDTA (K3E)	Greiner	454036	4.0 mL	Vacuette® K3-EDTA, spray-dried	A161036E
K2-EDTA whole blood (K2E)	Sarstedt	04.1915.100	2.7 mL	S-Monovette® 1.6 mg K2-EDTA/mL, spray-dried	8595111
K2-EDTA whole blood (K2E)	BD	368841	2.0 mL	Vacutainer® 1.8 mg K2-EDTA/mL, spray-dried	0027522
Li-heparin whole blood (LH)	Sarstedt	03.1628	5.5 mL	S-Monovette® 16 I.U./mL Li-heparin, granulate	7034611
Li-heparin whole blood (LH)	BD	368886	6.0 mL	Vacutainer® 17 I.U./mL Li-heparin, spray-dried	7066712
Na-heparin whole blood (NH)	Sarstedt	01.1613.100	7.5 mL	S-Monovette® 16 I.U./mL Na-heparin, granulate	7032211
Na-heparin whole blood (NH)	Greiner	454051	4.0 mL	Vacuette® Na-heparin, spray-dried	A19033AM
NH4-heparin whole blood (AH)	Sarstedt	05.1105	4.5 mL	S-Monovette® 16 I.U./mL NH4-heparin, granulate	7033011
NH4-heparin whole blood (AH)	Greiner	455031	9.0 mL	Vacuette® NH4-heparin, spray-dried	A180936D

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 13: Troubleshooting

Problem	Possible cause	Remedy
Baseline drifts	Detector lamp not yet warm	Wait until baseline is stable
	Detector lamp old	Replace lamp
	System not yet in equilibrium	Inject calibrator repeatedly, until two successive chromatograms are identical
	Temperature drift	Use column oven
	Flow rate not constant	Check pump
Baseline unstable	HPLC pump	Check pump (air, seals)
	Air in the system	Degas mobile phase
	Detector cell contaminated	Clean detector cell
	Mobile Phase contaminated	Replace Mobile Phase
Interference peaks	Air in the system	Degas mobile phase
	Injector contaminated	Clean injector
	Injection syringe contaminated	Clean syringe with methanol
	Prefilter or precolumn contaminated	Replace prefilter or precolumn
	HPLC column contaminated	Replace column
	Late-eluting peaks from previous sample	Inject Mobile Phase several times
	Interference	Check sample for known interferences
Broad peaks, tailing	Prefilter or precolumn contaminated	Replace prefilter or precolumn
	HPLC column too old	Replace column
Double peaks	Dead volume in fittings	Replace fittings
	Dead volume in precolumn	Replace precolumn
	Dead volume in HPLC column	Replace column
No peaks	System leaking	Check injector and fittings
Reduced sensitivity	Detector lamp ageing	Replace lamp
	Detector cell contaminated	Clean cell
	Defective injection valve	Check injector
Retention times changed	Temperature drift	Use column oven
	Unstable flow rate, pump pulsing	Check HPLC pump
	Leak	Check system for leaks
	Mobile Phase has evaporated	Replace Mobile Phase and keep it closed or covered even when in use
	System not in equilibrium	Repeat injections of calibrator until two successive chromatograms are identical

Problem	Possible cause	Remedy
No signal	Connection to data system defective or interrupted	Check signal cable and connection
	Detector lamp	Check lamp, replace lamp if necessary
Quality control outside acceptable range	Interference	Check chromatogram for interference
	Incorrect sample preparation	Check reproducibility of incorrect results
	Insufficient light protection	Use amber coloured vials

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.

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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 14: Hazard and precautionary statements

Pictograms	Hazard and precautionary statements
Mobile Phase (order no. 35021, 35022)	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 water. P403+P233 Store in a well-ventilated place. Keep container tightly closed.
Precipitation Reagent (order no. 37004)	Components: 10–25 % perchloric acid
 	**Danger** H272 May intensify fire; oxidiser. H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P309+P311 IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician.
Derivatisation Reagent 2 (order no. 35006)	Components: 25–50 % methanol; 5–10 % sodium hydroxide
   	**Danger** H226 Flammable liquid and vapour. H290 May be corrosive to metals. H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled. H314 Causes severe skin burns and eye damage. 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. P301+P330+P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P403+P233 Store in a well-ventilated place. Keep container tightly closed.

Pictograms	Hazard and precautionary statements
Neutralisation Buffer (order no. 35009)	Components: 10–25 % phosphoric acid
	**Warning** H290 May be corrosive to metals. H315 Causes skin irritation. H319 Causes serious eye irritation. 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. P309+P311 IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician. P332+P313 If skin irritation occurs: Get medical advice/attention.
Stabilisation Buffer (order no. 35007)	Components: 3–10 % sodium metabisulphite
	**Danger** H318 Causes serious eye damage. P280 Wear 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. P313 Get medical advice/attention.
These components are not classified as dangerous according to European Union legislation:	
Extraction Buffer (order no. 37003)	Components: aqueous solution
Derivatisation Reagent 1 (order no. 35005)	Components: aqueous solution
Whole Blood Calibration Standard (order no. 37008)	Components: human whole blood
Whole Blood Controls (order no. 0033, 0034, 0035)	Components: human whole blood

Active ingredients:

Table 15: Active ingredients

Order no.	Description	Active component	Specification
35021/ 35022	Mobile Phase	Methanol	10–25 %
		Water	75–90 %
37003	Extraction Buffer	Water	97–97.5 %
37004	Precipitation Reagent	Perchloric acid	10–25 %
35005	Derivatisation Reagent 1	Chelating iron complex	Lyophilised powder
35006	Derivatisation Reagent 2	Methanol	25–50 %
		Sodium hydroxide	5 - 10 %
35009	Neutralisation Buffer	Phosphoric Acid	10 - 25 %
35007	Stabilisation Buffer	Sodium metabisulphite	3 - 10 %
35110	Analytical Column	Polymer based on silica	> 90 %
37008	Whole Blood Calibration Standard	Analyte (thiamine pyrophosphate (TPP))	Conc. see leaflet
0033	Whole Blood Control Level I+II	See 0034 and 0035	—

Order no.	Description	Active component	Specification
0034	Whole Blood Control Level I	Analyte (thiamine pyrophosphate (TPP))	Conc. see leaflet
0035	Whole Blood Control Level II	Analyte (thiamine pyrophosphate (TPP))	Conc. see leaflet

Appendix II Analytical performance data

The performance features were determined and verified on the following equipment:

- Shimadzu fluorescence detector RF-20A

Users wishing to use the HPLC assay “Vitamin B1 in whole blood” (order no. 35000) with an HPLC system other than the one specified here should validate the method on that device.

Peak areas were used to calculate results throughout the analytical performance evaluation.

Metrological traceability and trueness:
For the Whole Blood Calibration Standard (order no. 37008) metrological traceability was demonstrated and is available as traceability chain (see Appendix IV).

Trueness of measurement was demonstrated within the analytical performance evaluation process based on following strategies due to the absence of a reference method or reference material:

- Comparison with a CE/IVD method
- Proficiency scheme participation (available in the download centre of our homepage: www.chromsystems.com/downloadcenter.html)

Analytical measuring range
Lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ):
The lower limit of quantitation (LLOQ) has been determined by a defined dilution of samples with low endogenous analyte concentrations with phosphate buffered sodium chloride solution. Upper limit of quantitation (ULOQ) was determined by spiking a whole blood sample with defined quantities of standard substances.

The method is linear from the lower limit of quantitation (LLOQ) to the stated upper limit of quantitation (ULOQ).

Table 16: Lower and upper limit of quantitation

Substance	LLOQ	ULOQ
Thiamine pyrophosphate (TPP)	0.8 µg/L	750 µg/L

Precision (intra-assay):
Determination of the intra-assay precision was done by 10 preparations of the same sample and determination of the analyte concentration at 3 different concentrations in whole blood:

Table 17: Intra-assay precision

Substance	Coefficient of variation (concentration of analyte)		
Thiamine pyrophosphate (TPP)	4.4 % (26.0 µg/L)	4.0 % (38.7 µg/L)	3.8 % (95.4 µg/L)

Precision (inter-assay precision)

Determination of the inter-assay precision was done by 10 sample preparations and determination of the analyte concentrations in three whole blood pools in 10 different test series:

Table 18: Inter-assay precision

Substance	Coefficient of variation (concentration of analyte)		
Thiamine pyrophosphate (TPP)	4.9 % (26.0 µg/L)	4.4 % (38.7 µg/L)	4.7 % (95.4 µg/L)

Drift

To identify any drift of analyte concentration over time the analyte concentration in the three whole blood samples was compared over a 10-day period. No drift was observed.

Precision (reproducibility)

The performance data were determined at 3 sites on the basis of 4 different samples. All samples were prepared 5-fold on 5 different days. The procedure is based on CLSI EP05-A3 and corresponds to a 3 x 5 x 5 test design.

Table 19 : Reproducibility

Substance	Sample	Mean	Reproducibility	
			Coefficient of variation	95 % confidence interval
Thiamine pyrophosphate (TPP)	native	59.7 µg/L	5.1 %	4.1–6.9 %
	low	27.0 µg/L	6.1 %	4.8–8.2 %
	medium	114 µg/L	4.4 %	3.6–5.8 %
	high	375 µg/L	5.5 %	4.3–7.4 %

Carry over

A prepared whole blood 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 samples 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 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. The method is robust within the following tolerances provided the particular setup remains constant throughout a measurement series:

Table 20: Tolerance ranges HPLC system

HPLC system	Tolerance range
Column temperature	+20 to +30 °C
Injection volume	10 to 100 µL

Table 21: Tolerance ranges sample preparation

Sample preparation (according to chapter 5.5)	**Tolerance range**
Step 6: Centrifuge 5 min at 9000 x g	Centrifuge 5 min at 4500 to 9000 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

The Chromsystems reagent kit 35000 "Vitamin B1 in whole blood" is used to screen specific populations for abnormal levels or to monitor vitamin B1 status in patients, e.g., under supplementation therapy.
As vitamin B1 status never serves to diagnose a disease directly, clinical performance parameters such as diagnostic specificity, diagnostic sensitivity, positive predictive value, negative predictive value or likelihood ratio are not applicable for this devices. Instead, relevant clinical parameters are expected values in normal and affected populations. The table below exhibits clinical performance data using Chromsystems vitamin B1 assays.

Clinical performance data for vitamin B1

Monitoring of vitamin B1 levels or screening for deficiency or excess is relevant in population groups who have shown an increased risk for deficiency or excess. These include

for vitamin B1 deficiency:

- Patients with restrictive eating disorders
- Patients who undergo hemodialysis

for vitamin B1 excess:

- Patients under vitamin B1 supplementation therapy

Table 22: Clinical performance for vitamin B_1

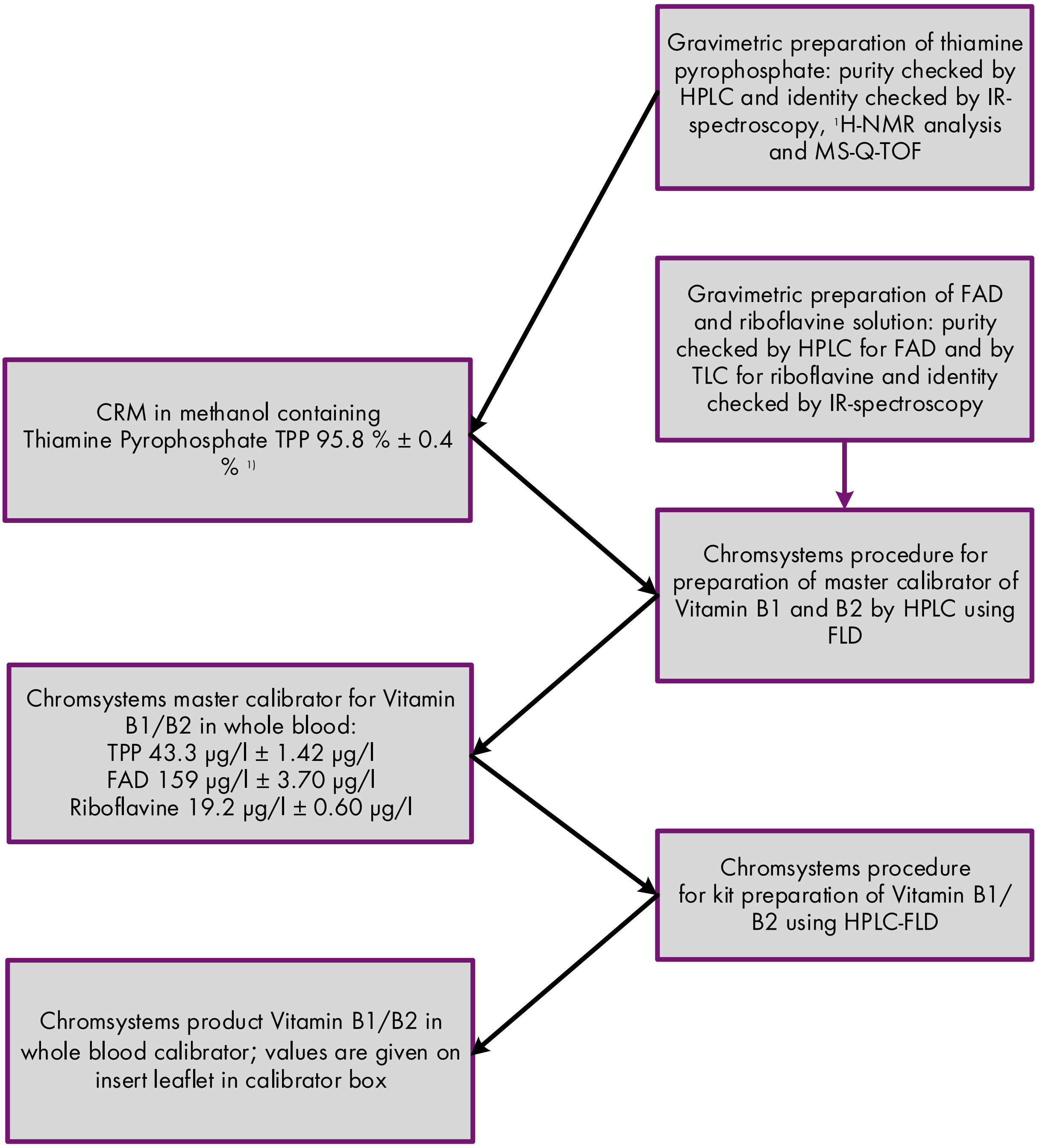
Aim/research question of the study	Matrix used to determine TPP levels	Expected values in affected population	Expected values in non-affected population	Ref
Retrospective audit of hospitalised adolescents with a restrictive eating disorder that were commenced on a high caloric refeeding regimen were investigated for vitamin B_1 deficiency or excess at admission or during admission	Whole blood	Deficiency: < 67 nmol/L Excess: > 200 nmol/L	67–200 nmol/L	[14]
Screening for thiamine deficiency was performed in pediatric eating disorder patients admitted for the first time to an outpatient hospital	Whole blood	Deficiency: < 66 nmol/L	66–200.3 nmol/L	[15]
Study to screen biochemical, nutritional and hormonal parameters for nutritional abnormalities in adolescent anorexia nervosa patients and to establish whether certain abnormalities persist after short-term refeeding	Whole blood	Deficiency: < 82.5 nmol/L	82.5–167.3 nmol/L	[16]
Study to measure the water-soluble vitamins and trace elements in blood and dialysate in patients treated by online post-dilution hemodiafiltration (HDF)	Whole blood	Deficiency: < 66 nmol/L	66-200 nmol/L	[17]
Study to investigate the intradialytic loss of vitamins and of homocysteine in patients before and after haemodialysis treatment and whether the loss is dependent on the dialyser type	Whole blood	Deficiency: < 47 nmol/L	-	[18]

Appendix IV Traceability of the calibrators

Whole Blood Calibration Standard (order no. 37008)

Vitamins B_1 and B_2 in whole blood

Version 1.0



The master whole blood calibrator (37008) was prepared gravimetrically by addition of TPP, FAD and riboflavine obtained from a commercial supplier. The concentrations were determined in the manufacturer's laboratory using the HPLC reagent kit Vitamin B_1 in whole blood and Vitamin B_2 in whole blood.

The methodology for master whole blood calibrator (37008) is using Chromsystems reagent kit no. 35000 (reagent kit for vitamin B_1 in whole blood) and kit no. 37000 (reagent kit for vitamin B_2 in whole blood) by HPLC-FLD analysis.

The Chromsystems calibration standard vitamins B_1/B_2 (working calibrator) (order no 37008) has a concentration as shown on the insert leaflet of each batch, determined by the manufacturer's laboratory using the Chromsystems reagent kit method as reference. The assay was calibrated using Chromsystems master calibrator with a concentration (including uncertainty) as shown in the diagram above. The methodology for working calibrator is using Chromsystems reagent kit no. 35000 (Vitamin B_1 in whole blood) and 37000 (Vitamin B_2 in whole blood) by HPLC-FLD 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.

1) Uncertainty of the concentration is expressed as an „expanded uncertainty" at the approximate 95 % confidence interval using a coverage factor of k=2.

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 23: Symbols

Symbol	Meaning
🏭	Manufacturer
🏭	Date of manufacture
⌛	Use by
REF	Order number
LOT	Batch/lot code
ℹ	See instructions for use
🌡	Upper temperature limit: Store below a certain temperature
🌡	Temperature limit: Store within a certain temperature range
IVD	*In-vitro* diagnostic medical device
Σ	Sufficient for <n> appliances
⚠	Caution
SN	Serial number
CE	CE marking of conformity with the relevant EU legislation
CE 0123	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 24: Version history

Version	**Date of release** (YYYY-MM-DD)	**Description**
1.0IVDR	2023-12-19	Initial creation IVDR