

DIFFERENTIATED PORPHYRINS IN URINE BY FLUORIMETRY – FAST – Code Z73010

INTRODUCTION

Porphyrin from the greek porphyra (purple) is a heterocyclic macrocycle made up of four pyrrole subunits linked by four bridges = CH-lying alpha compared to nitrogen of the heterocycle.

Because it has a total of 22 delocalized π electrons, 18 of which are in a conjugated system, the porphyrin is a heterocyclic aromatic compound. Porphyrin derivatives, known generically as porphyrins are biochemical compounds that perform an important biological role. Porphyrins are classified on the basis of specific enzyme deficiencies.

All porphyrias are caused by the lack of a specific enzyme of the heme biosynthetic pathway (see Fig.1 and Table A). Acute porphyrias are metabolic disorders that cause changes in the biochemical processes especially in the liver. They still do not cause liver damage or hematologic diseases. When porphyrins accumulate in the skin, it becomes extremely sensitive to light and this causes the symptoms typical of cutaneous porphyria. The accumulation in the liver of simpler chemical is a precursors of acute attacks. If a subject is suspected of being a carrier of a porphyria, it is important to understand what form it is, since inheritance and symptoms are different for each.

Other types of classification are based on the main clinical features. They are useful from a clinical point of view, but may overlap. Acute porphyrias are a cause of neurological symptoms that usually have an intermittent pattern. Cutaneous porphyrias causes photosensitivity of the skin.

Acute intermittent porphyria, porphyria deficiency of ALA dehydratase, the coproporphyria hereditary and the porphyria variegata are acute porphyrias. Porphyria cutanea tarda, hereditary coproporphyria, porphyria variegata, the protoporphyria erythropoietic porphyria congenital erythropoietic porphyria and hepato-erythropoietic porphyria are of the skin. In the liver and in those erythropoietic porphyria, excess precursors come from the liver and bone marrow respectively.

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CE

This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 008	Porphyrins in urine by Fluorimetry	06/2024
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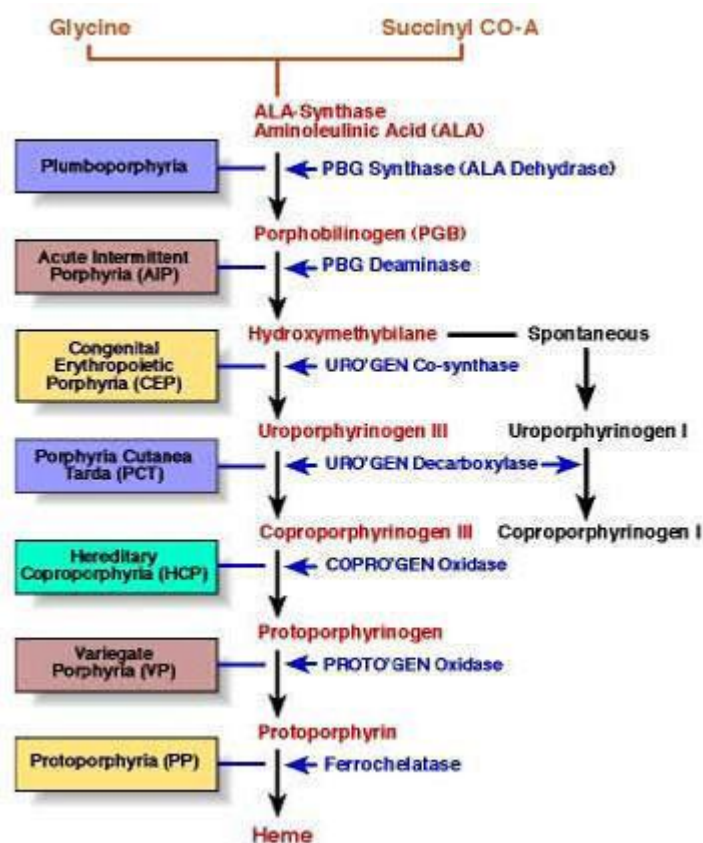


Fig.1 Heme biosynthesis and production of porphyrins

TABLE A: Characteristics of hereditary porphyrias

PATOLOGY	OVER OF ...	ENZYME DEFECT
Acute Intermittent Porphyria (AIP)	ALA, PBG, Uroporphyrin, Coproporphyrin III	Hydroxymethylbilan synthase
Congenital Erythropoietic Porphyria (CEP)	Uroporphyrin I, Coproporphyrin I	Uroporphyrinogen III cosynthase
Porphyria Cutanea Tarda (PCT)	Uroporphyrin, Eptaporphyrin, Tetracarboxiporphyrin	Uroporphyrinogen decarboxilase
Hereditary Coproporphyria (HCP)	ALA, PBG, Coproporphyrin III	Coproporphyrinogen III oxidase
Variegata Porphyria (VP)	ALA, PBG, Coproporphyrin III, Protoporphyrin IX (Coproporphyrin III)	Protoporphyrinogen IX oxidase
Erythropoietic Protoporphyria (EPP)	Protoporphyrin (Coproporphyrin I and III)	Ironchelatasi

Diagnosis is made with a careful examination of porphyrins in blood, urine and faeces. More specific examinations are used in the analysis of the type of porphyria, such as assessment of the activity of individual enzymes in the blood, though not all tests for all enzyme are not available. Genetic analysis is necessary to confirm the diagnosis with certainty. The diagnosis of acute conditions is effected by the dose of ALA and PBG in urine. Porphyrins are able to easily capture different metals. Their measurement in urine is useful to see if there is an intoxication by heavy metals such as mercury, lead, aluminium and arsenic. This investigation gives a precise indication of intoxication.

TECHNICAL FEATURES

Principle of the Method :

This method allows to analyze the absolute concentrations of porphyrins, the samples are diluted with a suitable diluent and directly injected into HPLC using a binary gradient pump (page 7).

Recovery : 100 %

Sensitivity : < 1 µg/l

Dynamic Range of the Method : Up to 5.000 µg/l

CV% : 5,07%

<u>Normal Range :</u>	UROPORPHYRIN I	10 - 40 µg/l
	EPTACARBOXIPORPHYRIN	2,2 – 14 µg/l
	ESACARBOXIPORPHYRIN	< 0,83 µg/l
	PENTACARBOXIPORPHYRIN	0,83 – 6,66 µg/l
	TOTAL COPROPORPHYRIN	24 – 110 µg/l

Components of the kit :

Reagent A – Diluting Solution, 1 x 1 ml

Reagent B – Internal Standard Solution, 1 x 1 ml

Reagent C – Test Solution, 1 x 0,18 ml

Urine Calibrator lyophil., 6 x 1 ml

Reagent M1 – Mobile Phase A, 5 x 500 ml

Reagent M2 – Mobile Phase B, 2 x 500 ml

All the reagents are ready-to-use and stable 3 years stored at 2 - 8 °C.

See Warnings

Code Z73016 (packed separately – see data sheets)

Minimum Instrumental equipment required:

HPLC Instrument with a binary gradient pump and loop of 20 or 50 µl.
Fluorimetric Detector: $\lambda_{EX}=394$ nm, $\lambda_{EM}=624$ nm
Chromatograms Recorder

Optional Equipment:

Autosampler
Automatic preparation of sample / autoinjector
Operational Computer

24 hours Urine Collection Procedure:

Sample of urine collection 24 hours. The sample stored at 4 ° C at pH 6-7 and protected from light is stable 2 days (**Porphyrin photo- and thermo-sensitive**). If stored at - 20 ° C is stable 1 month.

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 990 µl of H₂O HPLC grade
- 10 µl of **Reagent C – Test Solution**

Vortex for 10 sec.

INJECTION :

- Inject 20 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 2. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate

ANALYTICAL PROCEDURE

Calibrator, Samples and Controls preparation.

Dispense in a vial :

	Calibrator	Sample	Control
Reagent A – Diluting Sol.	10 µl	10 µl	10 µl
Reagent B – Internal Standard Sol.	10 µl	10 µl	10 µl
Calibrator	500 µl		
Sample		500 µl	
Control			500 µl

Vortex for 10 sec.

N.B.: at this step, the sample is stable 2 days at 2-8 °C

INJECTION :

- Inject 20 µl of the eluate into the chromatographic system.

GRADIENT			
Time (min)	% PUMP A	% PUMP B	Flow (ml/min)
0	100	0	1,2
11.00	60	40	1,2
11.10	0	100	1,2
14.00	0	100	1,2
14.10	100	0	1,5
18.00	100	0	1,5
18.10	100	0	1,2
20.00	100	0	1,2

DIFFERENTIAL PORPHYRINS IN URINE – FAST - Warnings

REAGENT C : SOLUZIONE TEST

UROPORPHYRIN I	about 33,33 ug/l
EPTACARBOXIPORPHYRIN	about 83,33 ug/l
ESACARBOXIPORPHYRIN	about 83,33 ug/l
PENTACARBOXIPORPHYRIN	about 83,33 ug/l
COPROPORPHYRIN I	about 166,66 ug/l
COPROPORPHYRIN III	about 133,33 ug/l

FLUORIMETRIC DETECTOR PARAMETERS

λ_{EX}	394 nm
λ_{EM}	624 nm
GAIN	100
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ), the use of Javelin Prefilters (1 x 10 pcs), cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Disconnect the detector and flux 30 ml of H ₂ O : Methanol (20 : 80 v/v) at pH > 3 solution and subsequently 30 ml of H ₂ O : Methanol (80 : 20 v / v) , set flow at 1,5 ml/min. Don't recycle the washing solutions. Condition the column with the mobile phase at a flow of 1,5 ml/min. and discharge the first 15 ml. Condition further on the column for 15 min. also at recycling phase. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run. It's NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.
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COLUMN CLEANING

Disconnect the detector. Flux 30 ml of H ₂ O : Methanol or Acetonitrile (80 : 20 v / v) and discharge. Flux a solution of H ₂ O HPLC grade : Methanol or Acetonitrile (30 : 70 v/v) for 30 min. Discharge. A new use of the column needs a flow of 15 ml of H ₂ O : Methanol (20 : 80 v / v) before a new conditioning with the mobile phase. It is recommended to run the washing of the analytical column at the end of each run.

HPLC PARAMETERS

LOOP	20 or 50 μ l
Recommended Flow	See Gradient
Pressure	About 70 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z73016	Urine Calibrator for Differentiated Porphyrins	4 x 1 ml
Z73019	Urine Control for Differentiated Porphyrins - Levels 1 and 2	2 x 5 x 1 ml
Z699975902	Poroshell 120, EC-C18 (50 x 4,6 mm - 2,7 μ m) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

DIFFERENTIATED PORPHYRINS IN URINE (Reference Chromatograms)

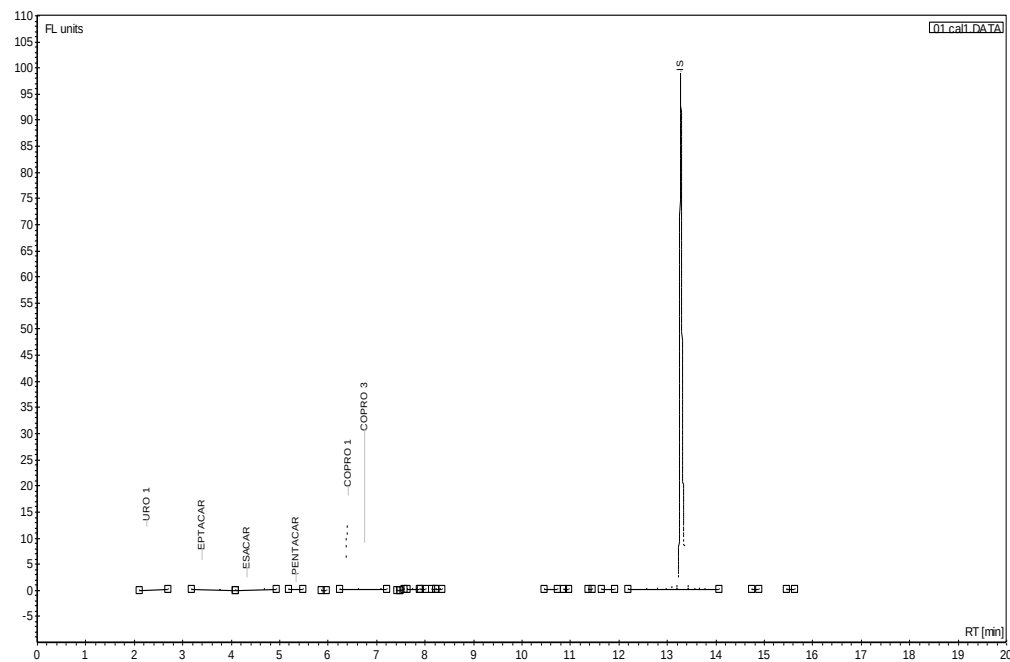


Fig. 2 : Urine Calibrator				
	R.T.	2.26	Uroporphyrin I	38,9 µg/l
	R.T.	3.39	Eptacarboxiporphyrin	21,0 µg/l
	R.T.	4.33	Esacarboxiporphyrin	8,7 µg/l
	R.T.	5.33	Pentacarboxiporphyrin	4,7 µg/l
	R.T.	6.42	Coproporphyrin I	46,9 µg/l
	R.T.	6.75	Coproporphyrin III	26,6 µg/l
	R.T.	13.40	Internal Standard	

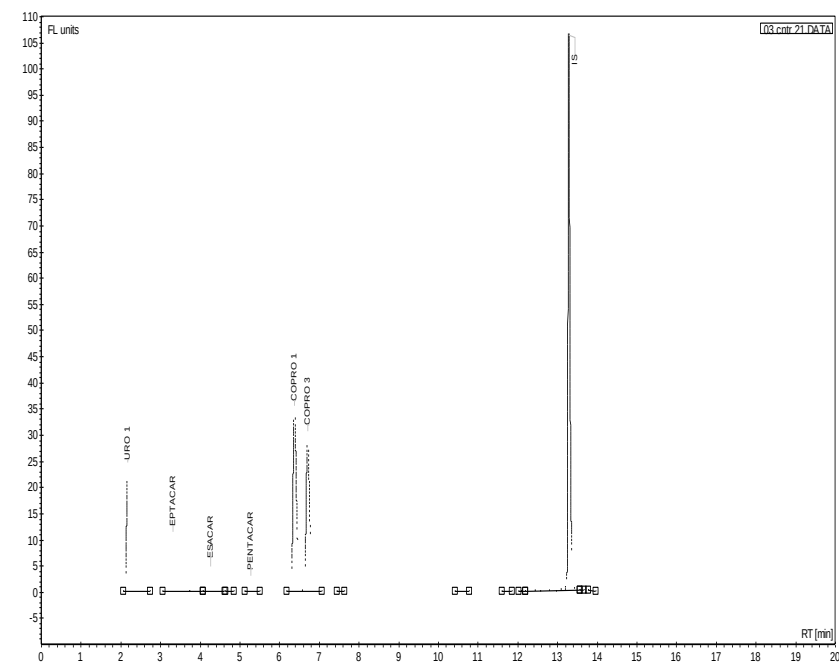


Fig. 3 : Urine Control Level 2				
	R.T.	2.20	Uroporphyrin I	76,6 µg/l
	R.T.	3.34	Eptacarboxiporphyrin	41,3 µg/l
	R.T.	4.32	Esacarboxiporphyrin	18,4 µg/l
	R.T.	5.34	Pentacarboxiporphyrin	9,6 µg/l
	R.T.	6.36	Coproporphyrin I	95,7 µg/l
	R.T.	6.69	Coproporphyrin III	79,6 µg/l
	R.T.	13.30	Internal Standard	