

METFORMIN IN SERUM by UV/VIS - FAST – Code Z88110

INTRODUCTION

The metformin active ingredient with euglycemic action, is the drug of choice for the treatment of type 2 diabetes as monotherapy or in combination with sulfonylureas or insulin; Moreover, in adults, particularly in overweight, it is used as the first line after the failure of diet therapy, in monotherapy or in combination with other oral agents or insulin euglycemic; in children from 10 years of age and adolescents, alone or in combination with insulin (Nathan et al., 2009; AIFA, 2011)

It is a biguanide whose main effect is expressed in the liver through the inhibition of gluconeogenesis. The reduction of hepatic glucose production by metformin is mediated by the complex of the mitochondrial respiratory chain 1, resulting in a decrease of cellular ATP. Since the anabolic process of gluconeogenesis is energetically expensive, accordingly the production of glucose is reduced following treatment with metformin and a concomitant reduction of hepatic energy supply (Viollet and Foretz, 2013).

Metformin does not undergo hepatic metabolism and renal tubular secretion is the major mechanism responsible for the removal of metformin. The estimated average renal clearance is 507 ± 129 ml / min in patients with preserved renal function, approximately 3.5 times higher than the creatinine clearance. As expected, the clearance is reduced in proportion to the decrease in renal function (Graham et al., 2011).

In case of overdose or accumulation during chronic therapy, extracorporeal treatments, such as HD (standard hemodialysis), HF (hemofiltration), hemodiafiltration (HDF), and CVVH (Continuous veno-venous hemofiltration), are considered effective for removing metformin and therefore routinely used in hospital settings. This approach is based on chemical-physical characteristics of the molecule such as the small size and the absence of links with the plasma proteins (Seidowsky et al., 2009).

The most common side effects in about 20% of patients at the beginning of treatment are gastrointestinal disturbances, ie, nausea, loss of appetite, metallic taste, and diarrhea (Chan et al., 1999). Lactic acidosis is a rare but potentially worst side effect, with an incidence of 2-9 cases per 100,000 patients in life-threatening / year (Biradar et al, 2010; Lalau, 2010) and with an overall mortality of 50% (Chan et al, 1999; ... Seidowsky et al, 2009; Lalau et al, 1995; Lalau et al, 1999). In particular, the toxicity associated to metformin accumulation is typically characterized by a triad of acute renal failure, high plasma concentrations of metformin and severe lactic acidosis.

A recent survey (Vecchio et al., 2014) has clearly shown that the accumulation of metformin-associated lactic acidosis may occur below the maximum recommended daily dose of 3000 mg, with a late onset after the start of therapy (60.62 ± 45.74 months) and patients (53%), with no pre-existing contraindications to metformin.

Thus, although the role of the accumulation of metformin in combination with metformin - Lactic acidosis and renal failure is still under debate, the control of the drug should become a good practice during the treatment of patients, in order to prevent the side effects of referred to above, even if this procedure is not required by securities regulators.

**Bibliography*

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 EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



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° 002	Metformin in serum by UV/VIS - FAST	09/2024
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TECHNICAL FEATURES

Principle of Method:

The serum samples are purified by ultrafiltration, and are then ready to be injected into HPLC.

Recovery : >98%

Sensitivity (LLOD): 0.1 mg/l

Minimum concentration analyzable (LLOQ): 0.2 mg/l

Linearity : 100 mg/l

Accuracy intra serie (relative error %) :

Ci	Cs
3 mg/l	10 mg/l
5,0%	3,5%

Reproducibility intra serie (coefficient of variation %):

C LLOQ	Cm	Cs
0,2 mg/l	2 mg/l	20 mg/l
4,8%	3,7%	3,2%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	Cs
0,2 mg/l	2 mg/l	20 mg/l
7,9%	7,2%	3,6%

Coefficient of Correlation + DS: 0,9968 ± 0,0002

Normal Range in serum : < 2 mg/l

See references listed in Page 2

Components of the kit :

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

Reagent A – Test Solution, 1 x 1 ml

See Warnings

Calibrator in serum, 1 x 2 ml

Code Z88116 (packed separately – see data sheets)

Reagent B – Column Conditioning and Wash Mobile Phase, 1 x 500 ml

Reagent M – Mobile Phase, 4 x 500 ml

FILTER 10.000MWCO 1 x 100 pcs

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 µl
Detector UV/VIS $\lambda = 232 \text{ nm}$

Optional Equipment:

Autosampler

Blood Collection Procedure:

Take 3 ml of blood in a tube without anticoagulant.

Centrifuge at 3000 rpm for 10 minutes. Plasma samples should be stored at - 20 ° C. Stable one year.

PREANALYTICAL PROCEDURE

Dispense in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent A - Test Solution**

Vortex for 10 sec.

NOTE : Before starting the analytic session it is advisable to inject 2-10 µl of this solution by HPLC to identify that the retention time of Metformin has similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1 : Sample Preparation

Add 300 µl of Sample, Calibrator and Controls in the upper part of the Vivaspin (the one with the white membrane) and close the cap.

STEP 2 : Centrifuge at 3.500 rpm for 30 minutes.

STEP 3 : Collect the filtrate serum that is located in the collecting cone and insert into vials with reduced volume.

N.B.: at this step, the sample is stable 3 days at 2-8 °C

INJECTION :

- Inject 2-10 µl of this solution in the chromatographic system.
(*Tip: in the case in which the peak appears not symmetrical or is not perfectly reproducible in retention time dilute the filtrate serum 1: 5 or 1:10 with the mobile phase*)

METFORMIN - Warnings

REAGENT A: TEST SOLUTION

METFORMIN	About 100 mg/l
Store at 2-8 °C	

DETECTOR UV/VIS PARAMETERS

λ	232 nm
Sensitivity	Medium

HPLC COLUMN PROTECTION

To save the analytical column Bio SCX the use of Prefilters Bio SCX, NP10 (1 x 4 pcs), cod. 5190-2434 is obligatory.
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HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical Bio SCX, NP10 (250 x 4,6 mm, 10 μ), thermostated at 35°C . NEW HPLC COLUMN CONDITIONING: Disconnect the detector and flux the Reagent B - Column Conditioning and Wash Mobile Phase at 0,2 ml/min growing up till 1,2 ml/min in 30 minutes. Flux further 30 minutes set flow at 1,2 ml/min. Don't recycle the washing solutions. Condition the column with the mobile phase at a flow of 1,2 ml/min for 30 minutes. Finally inject the Chemical Standard and verify the quality of the separation. It is 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.

COLUMN CLEANING

Disconnect the detector. Flux with the column installed in the opposite direction set flow at 1,2 ml/min the Reagent B - Column Conditioning and Wash Mobile Phase for 30 minutes and discharge. It is recommended to perform the washing of Analytical Column at the end of each analytical run. Store the column in a wash solution.
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INJECTION NEEDLE WASH

Wash with Water HPLC grade.

HPLC PARAMETERS

LOOP	10 μ l
Flow	1,2 ml/minute
Pressure	About 50 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z88116	Calibrator in serum for Metformin	4 x 2 ml
Z88119	Control in serum for Metformin – Levels 1 and 2	2 x 5 x 2 ml
Z51902433	Bio SCX, NP10 Analytical Column (250 x 4,6mm –10 μ m)	1 Pc
Z51902434	Prefilters Bio SCX, NP10 (4 x 10 mm)	1 Pc
S51843550	Clear Glass Vials with reduced volume (goblet form) from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for Clear Glass Vials with reduced volume (goblet form) from 1,5 ml to 15 μ l	1 x 100 Pcs

METFORMIN IN SERUM - FAST (Reference Chromatograms)

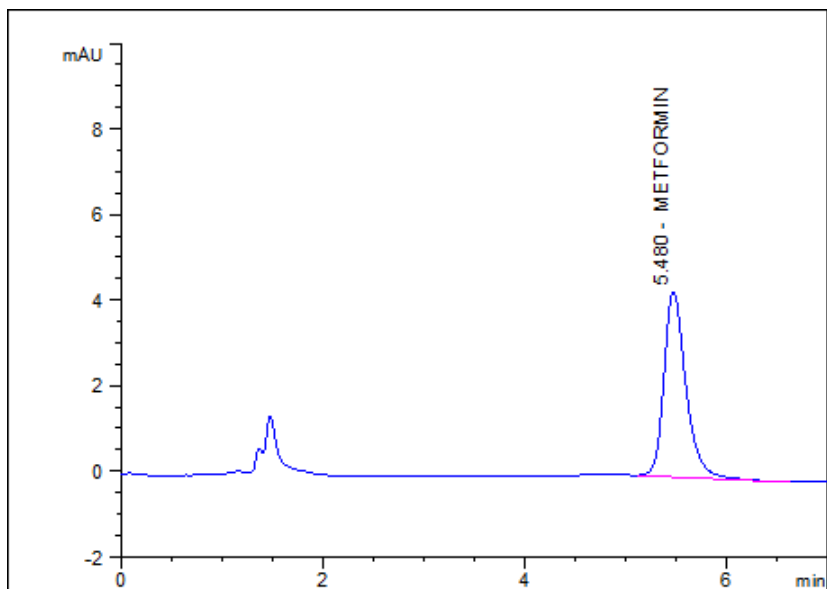


Fig. 1	Test Solution
	R.T. 5.48 Metformin

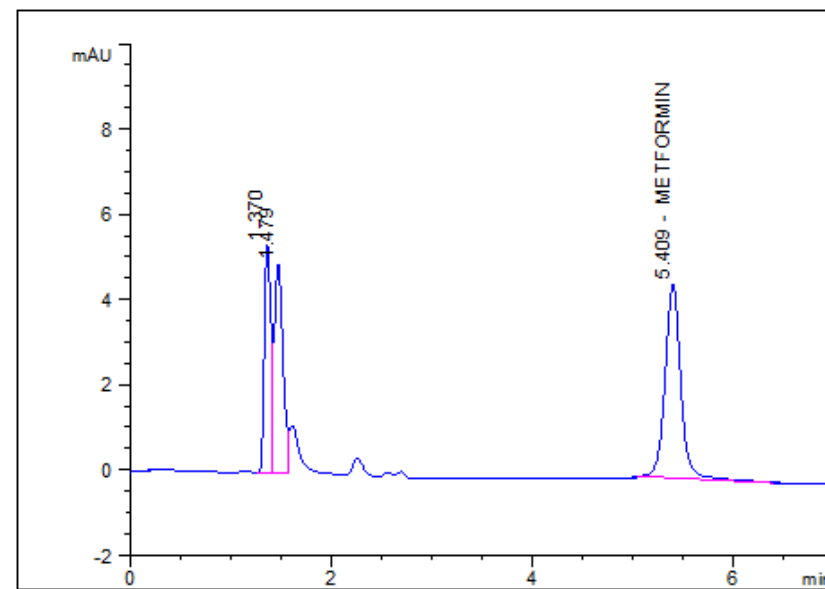


Fig. 2	Calibrator in serum
	R.T. 5.40 Metformin 6,80 mg/l

METFORMIN IN SERUM – FAST (Reference Chromatograms)

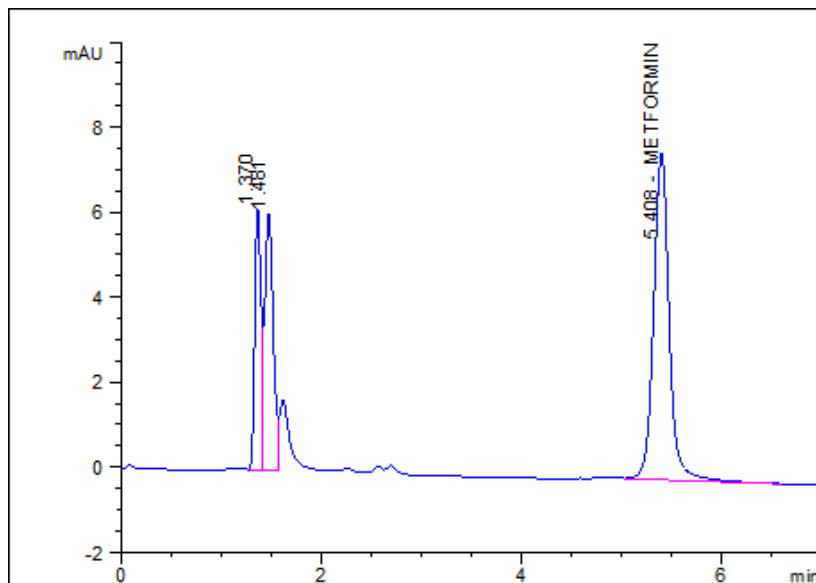


Fig. 3	Control Level 2
	R.T. 5.40 Metformin 5,40 mg/l

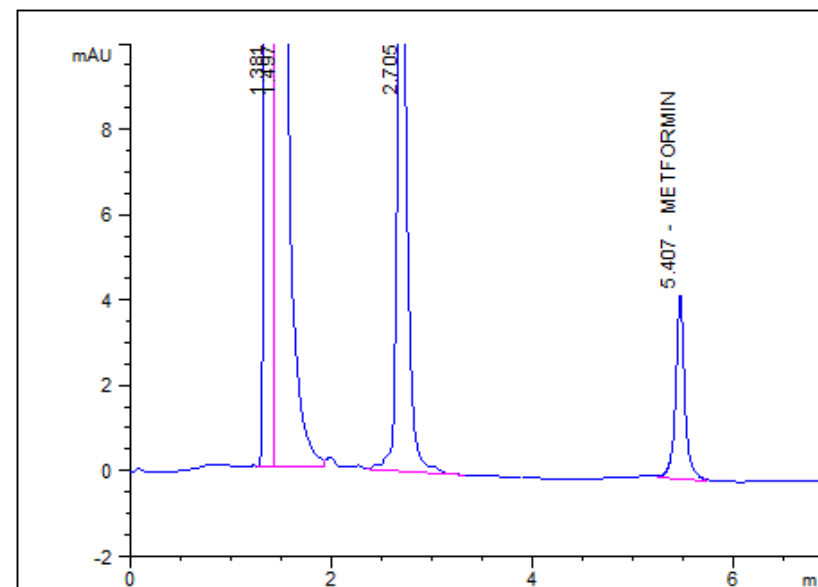


Fig. 4	Serum Sample
	R.T. 5.40 Metformin 6,10 mg/l