

CDT TEST IN SERUM BY UV / VIS- FAST - MONOREAGENT – Code Z68215

INTRODUCTION

The determination of desialated Transferrin or Carbohydrate Deficient Transferrin, CDT is based on dosing the Transferrin which is iron transporting. Various isoforms of transferrin exist with differing levels of sialylation.

Transferrin is a glycoprotein beta 1-globulin and its molecular weight changes from 75.37 to 79.61 kDa. It is formed by only one chain of 679 amino acids (aa) and it is characterized by 2 sub-structural domains (N-terminal aa 1-336 e C-terminal aa 337-679). These domains can each bind one a Fe^{3+} ion independently from one another.

The C- terminal domain has two glucidic chains linked to N of asparagines 413 and 611 (1).

Human serum contains different isoforms so called glycoforms, the most common of which are Trisialo, Tetrasialo and Pentasialo transferrin, while glycoforms such as Asialo, Monosialo and Disialo transferrin are less than 3% (fig.1). Transferrin measured in serum usually contains the total all glycoforms.

The complex Fe-Transferrin has an absorbance maxim at 460-470 nm and this is very important in quantitative analysis

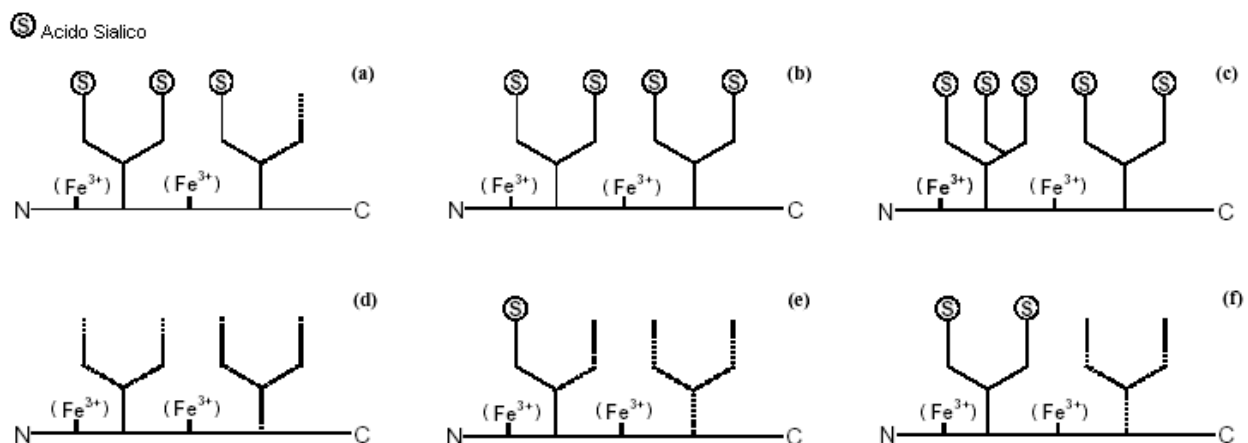


Fig. 1: Glycoforms of transferrin: trisialo, tetrasialo, pentasialo transferrin (a-c); asialo, monosialo, disialo transferrin (d-f)

At the end of the 70's a correlation between alcohol abuse and elevated levels of CDT was demonstrated. **Therefore CDT Test is an important marker for chronic alcohol abuse.** The chemical process has not been yet completely explained and seems to be based on inhibition of some glycan enzymes of ethanol and its derivatives (aldehydes).

STANDARDIZATION OF CDT

The development of numerous methods for easily measuring CDT has led to widespread usage. More than 600 articles about CDT have appeared in pseudoscientific reviews since 2000.

From these publications different and contradictory results appear concerning diagnostic sensitivity and specificity and the results are not comparable. Italian research (2) performed in public labs has demonstrated this homogeneity.

A process of standardization of this analyte is necessary, for this purpose the International Federation of Clinical Chemistry (IFCC), set up a commission for the standardization of CDT

In the first document (7) concerning CDT the following parameters were defined:

- Target Molecule and molecule for standardization
- Definition of measuring a confirmation of nomenclature
- Method of reference and expression of result

TARGET MOLECULE AND NOMENCLATURE

Both Asialo and Disialotransferrin can be correlated to chronic alcohol consumption, even if they have different sensitivity and specificity (3-6). Nevertheless, the IFCC has identified Disialotransferrin as the target analyte for CDT. Even if Asialoform is more specific for alcohol abuse, with the present available methods elevated levels of Disialotransferrin can easily be identified, therefore this form has the highest diagnostic sensitivity

HOW TO EXPRESS THE RESULT

There are different ways to express CDT: the commission of IFCC (7) suggest to calculate the percentage in relation to the total transferrin (%CDT), to cover false-positive or false-negative results linked to high or low values of total transferrin

$$\%CDT = \frac{\text{Area (asialo + monosialo+ disialotransferrin)}}{\text{Area Total Transferrin}} \times 100$$

Where total transferrin is calculated as **Integration baseline**: a-, mono-, di-, tri-, tetra-, pentasialotransferrin.

Associated with other tests such as transaminases, GGT and MCV, CDT can be useful tools in identifying problem drinking i.e. chronic alcohol abuse or alcoholism.

EUREKA srl – LAB DIVISION
VAT N° 01547310423
E-mail: info@eurekaone.com
www.eurekaone.com



Head Quarter:
Via Enrico Fermi 25
60033 Chiaravalle (AN) ITALY
Tel. +39 071 7450790
Fax + 39 071 7496579



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.

Release N° 002

CDT Test in serum by UV/VIS – FAST - Monoreagent

September 2019

TECHNICAL FEATURES

Principle of the Method:

The serum is complexed with appropriate reagent and injected in HPLC system after centrifugation, using a binary gradient pump (pag.6). The kit has been validated with the IFCC Standards, demonstrating good accuracy by integrating with baseline.

Recovery of Method :

Not Applicable

Sensitivity of Method :

Not Applicable

Linearity of Method :

Not Applicable

Accuracy intra serie (relative error %) :

Ci	Cs
1,82 %	3,55 %
3,66%	3,10%

Accuracy inter serie (relative error %) :

Ci	Cs
1,82 %	3,55 %
5,92%	3,72%

Reproducibility intra serie (coefficient of variation %):

C LLOQ	Cm	Cs
1,20 %	2,52 %	4,34%
6,75%	8,95%	7,10%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	Cs
1,20 %	2,52 %	4,34%
7,57%	8,51%	8,28%

Components of the kit (500 tests) :

Reagent A – Complexing Solution, 1 x 25 ml

Serum Control lyophil., 2 x 4 x 1 ml

Reagent M1 – Mobile Phase M1, 20 x 500 ml

Reagent M2 – Mobile Phase M2, 10 x 500 ml

Reagent M3 – Mobile Phase M3, 2 x 500 ml

All the reagents are ready-to-use and stable 3 years at 2-8 °C, except **Serum Control** must stored at – 20 °C.

Code Z68019 (packed separately – see data sheets)

Keep at room temperature for the shortest possible time

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Minimum Instrumental equipment required:

Binary HPLC System with loop of 50 µl in peek Spectrophotometric Detector UV / VIS $\lambda=460$ nm Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Blood Collection Procedure:

It's recommended to take 3 ml of venous blood into a tube for serum without gel. Centrifuge at 4,000 rpm for 5 minutes. Separate the serum and store at - 20 ° C. Stable 4 weeks.

Do not thaw the serum for more than 1 time.

Stable 7 days at 2-8 ° C.

Keep the samples at room temperature for the shortest possible time

Consensus Document of the Scientific Societies SIBioC and GTFI-SIMLA

ANALYTICAL PROCEDURE

STEP 1 : Preparation of Controls and samples

Pipette in a tube :

	Control	Sample
Control	100 µl	
Sample		100 µl
Reagent A – Complexing Solution	50 µl	50 µl

Vortex for 10 seconds

STEP 2 : Centrifuge at 10000 rpm for 5 minutes

STEP 3 : Take 100 µl of supernatant and add 400 µl of H₂O HPLC grade

Vortex for 10 seconds

INJECTION :

- Inject 50 µl into the chromatographic system

GRADIENT			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (ml/min)
0	100	0	1.6
0.5	100	0	1.6
5.0	75	25	1.6
5.1	0	100	1.6
7.5	0	100	1.6
7.6	100	0	1.6
10.0	100	0	1.6

N.B.: the first 5 minutes are devoted to the analysis itself and the second 5 minutes to washing and conditioning of the analytical column before moving to the next sample.

POSTANALYTICAL PROCEDURE

- Disconnect the detector, install the column turned in the opposite direction and flush at 0.5 ml / min 30 ml of **Reagent M3 - Mobile Phase M3**. Invert the column to the original and to flush for 15 minutes, the **Reagent M1 - Mobile Phase M1** at a flow rate of 1.5 ml / min.

CDT TEST FAST - Warnings

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	460 nm
GAIN	0,001 AUFS
INTEGRATION TIME	4 seconds

HPLC PARAMETERS

LOOP	50 μ l in peek
RECOMMENDED FLOW	1,6 ml/min
PRESSURE	30 bar

INITIAL PREPARATION OF SYSTEM and COLUMN CONDITIONING

<ol style="list-style-type: none">1. When column is detached, insert the tubes of the two pumps in a single container containing water2. Insert water as wash solution of autosampler's needle3. Flush water, with opened discharge's valve, at 10 ml/min for 2 minutes in pumps A and B (50:50) and discharge4. Make 2 washes of autosampler's needle (3+3 ml) with water5. Set up a flow of 0,5 ml/min, close the discharge's valve and in the meantime make 2 injections of 100 μl of water (loop's and switching system's washing)6. Insert draught tubes A and B in correspondance of mobile phases M1 and M27. With opened discharge's valve flush mobile phases M1 and M2 at 10 ml/min for 2 minutes (A 50:B 50)8. With closed discharge's valve flush mobile phases M1 and M2 at 0,5 ml/min for 5 minutes (A 50:B 50)9. Set at zero pump's flow10. Connect column for CDT11. Flush mobile phase M1 (100% A) at 1,5 ml/min for 15 minutes12. Inject 100 μl of water so that gradient can start, like mentioned in methodology; naturally the resultant chromatogram has not to be considered (to execute whenever a new serie is made)13. TO ACTIVATE A NEW COLUMN: After preparing the system as shown to flush 15 minutes the mobile phase M1 at a flow rate of 1.5 ml / min, make an injection of HPLC grade water and then injected controls serum until complete activation of the column .
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WASHING OF SYSTEM and COLUMN TO EXECUTE AT THE END OF EVERY WORKING DAY

<ol style="list-style-type: none">1. The column should be left in mobile phase M12. At the end of analysis install the column turned in the opposite direction and flush at 0.5 ml / min 30 ml of Reagent M3 - Mobile Phase M3. Invert the column to the original and to flush for 15 minutes, the Reagent M1 - Mobile Phase M1 at a flow rate of 1.5 ml / min.3. When column is detached wash tubers:<ul style="list-style-type: none">- with opened discharge's valve flush water at 10 ml/min for 2 minutes (A 50 : B 50)- with closed discharge's valve to set up a flow at 1 ml/min and to make 2 injections of 500 μl of water (A 50 : B 50)- with opened discharge's valve flush methanol at 50% at 10 ml/min for 2 minutes (A 50 : B 50)- with closed discharge's valve set up a flow at 1 ml/min and make 2 injections of 500 μl of methanol at 50% (A 50 : B 50)- with opened discharge's valve flush water at 10 ml/min for 2 minutes (A 50 : B 50)- with closed discharge's valve set up a flow at 1 ml/min and make 2 injections of 500 μl of water (A 50 : B 50)4. If the system is going to be left unused, even detector must be washed connecting it with union and flushing (with opened discharge's valve) water at 0,5 ml/min for 10 minutes.
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ANALYTICAL ADVICES

1. After having injected water to make the system conditioned with gradient, inject a control. If you obtain a bad separation of the peaks, it will be necessary to modify the gradient.
2. The computation of the area will always be in percentage.
3. Be careful: you must integrate only the peaks of our interest.
4. The peaks of the mono and asialo are rarely visible, especially with short sensitive detectors.
5. Clean the system with column detached, as indicated.
6. If the peak Asialo > Disialo (for EDTA) do not consider the percentage area of Asialo.
7. If you are in presence of Icterus the peak of Disialo will not be correct.
- 8. It is advisable to integrate the base line of the Disialo up to the Hexasialo to obtain a more accurate result as chromatograms below.**

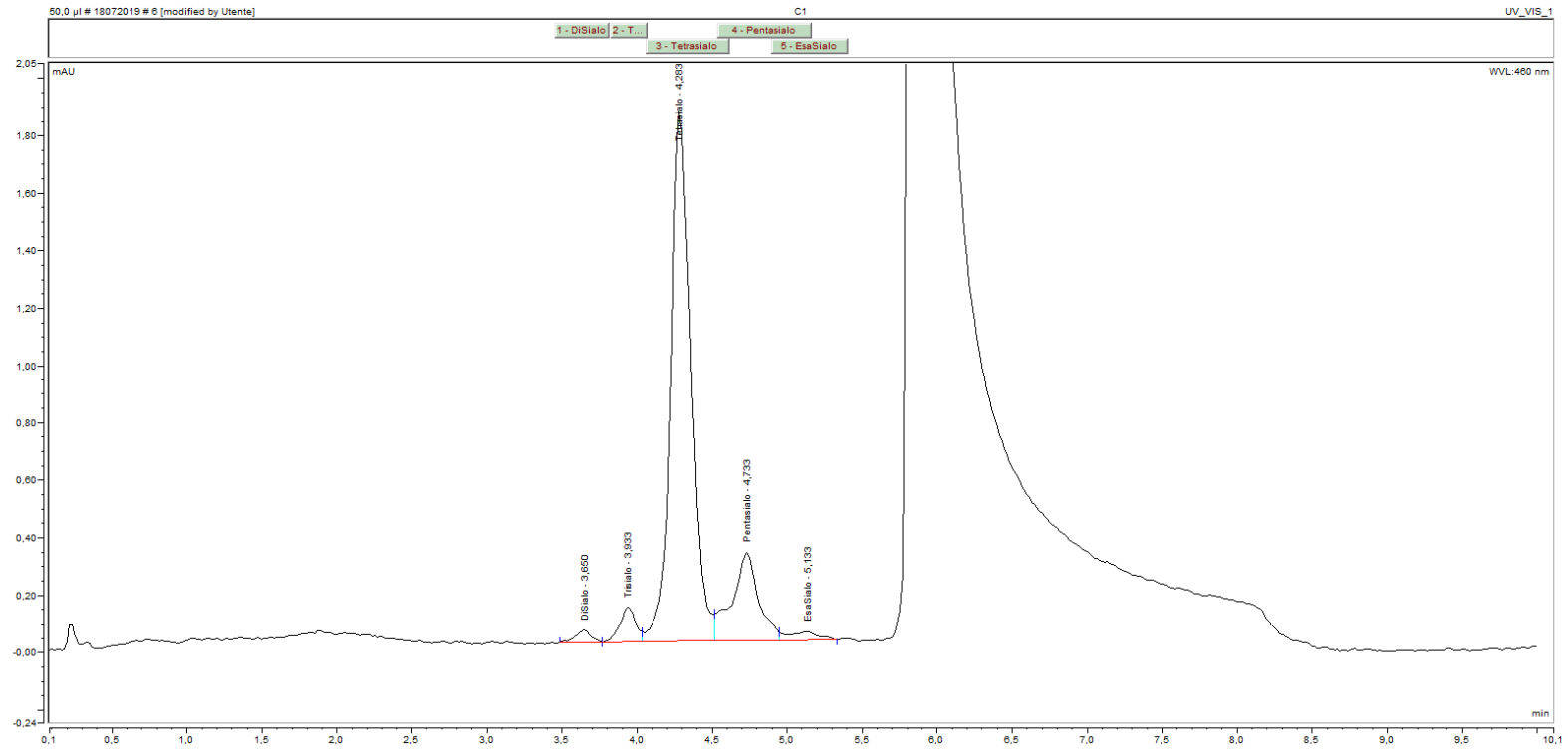
GENERAL CONSIDERATIONS

1. The column should be always left in mobile phase M1.
2. When the peaks of tetra and pentasialo start to overlap, turn the column and be flushed with the mobile phase M3 as described above and work in the opposite direction.
3. Don't leave the mobile phases in HPLC's tubes for a long time, because the mobile phases are strongly saline.
4. Do not use organic solvents and do not leave any trace of them in the system (therefore clean each trace of possible organic phase from the system before every session, included the autosampler).
5. Do not use metallic filters or tubes.
6. Use loop in peek.
7. While the system is working, the pressure must be about 30-80 bar.
8. Use blue peek between the pump and the injector.

ACCESSORIES AND CONSUMABLES

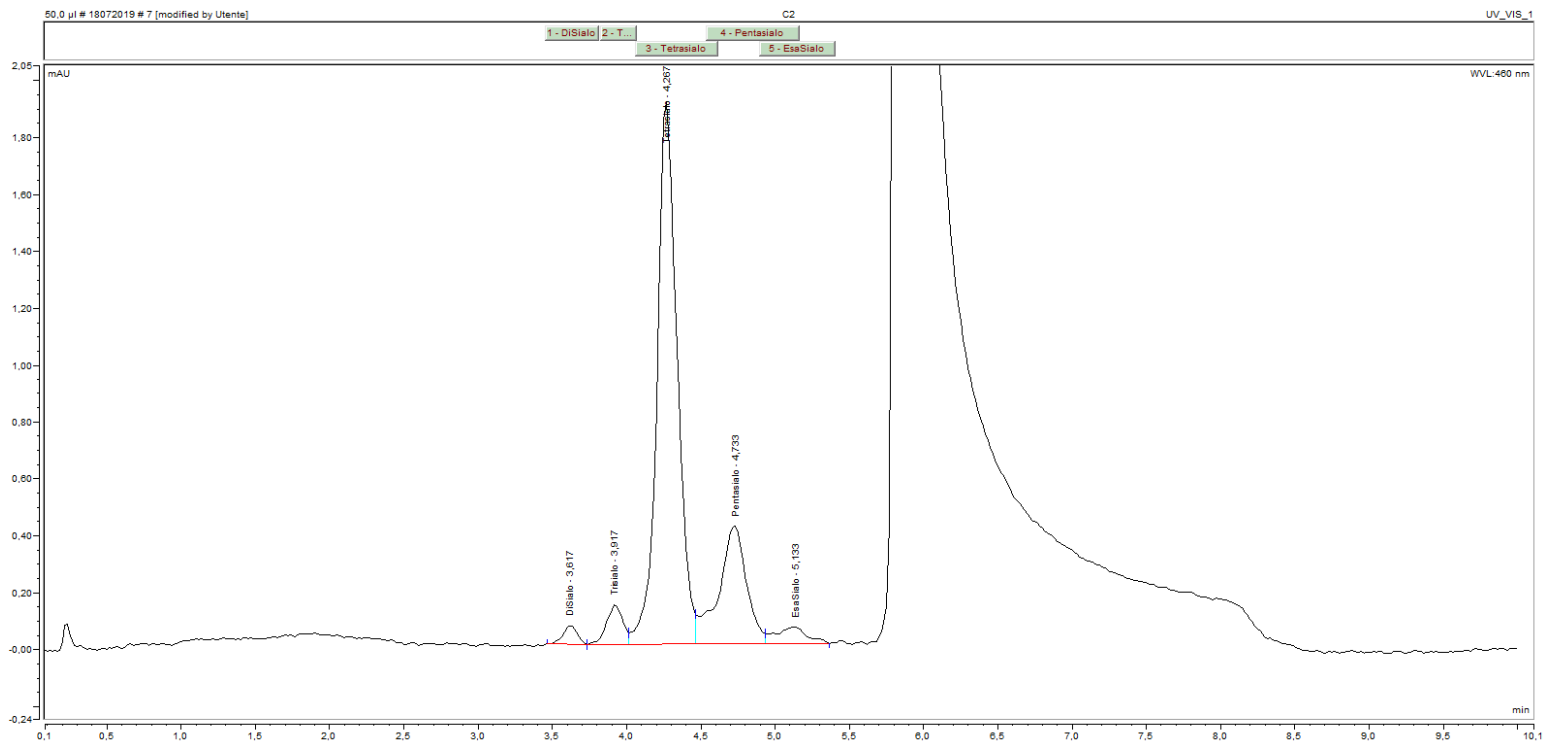
CODE	DESCRIPTION	PACKAGING
Z68017	Serum Control lyophil. for CDT Test, Level 1	5 x 1 ml
Z68018	Serum Control lyophil. for CDT Test, Level 2	5 x 1 ml
Z68019	Serum Control lyophil. for CDT Test, Levels 1 and 2	2 x 5 x 1 ml
Z054998	Analytical Column SAX 10 G (50 x 4 mm -5 um)	1 Pc
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs

CDT TEST IN SERUM - FAST
(Reference Chromatograms)



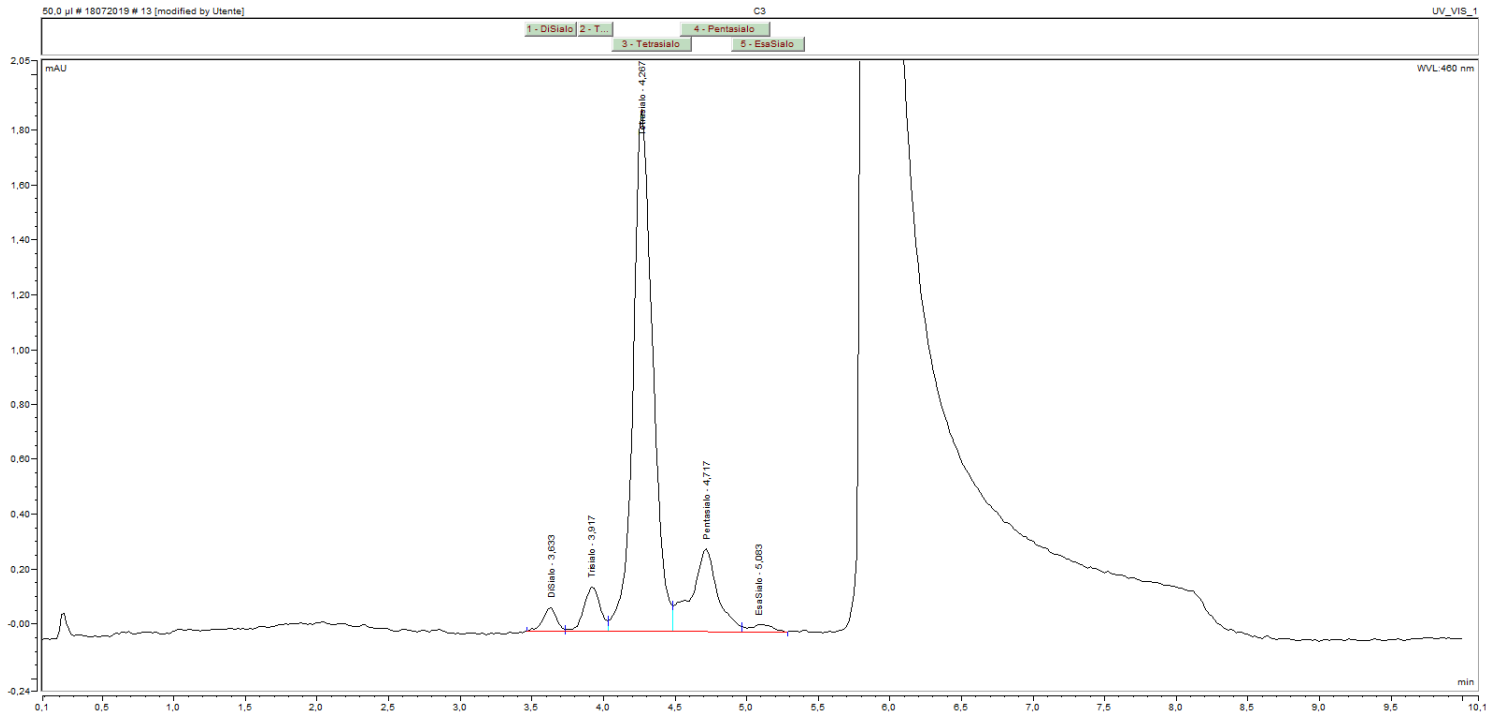
IFCC Calibrator Level 1: % Disialotransferrin = 1,20%

CDT TEST IN SERUM - FAST (Reference Chromatograms)



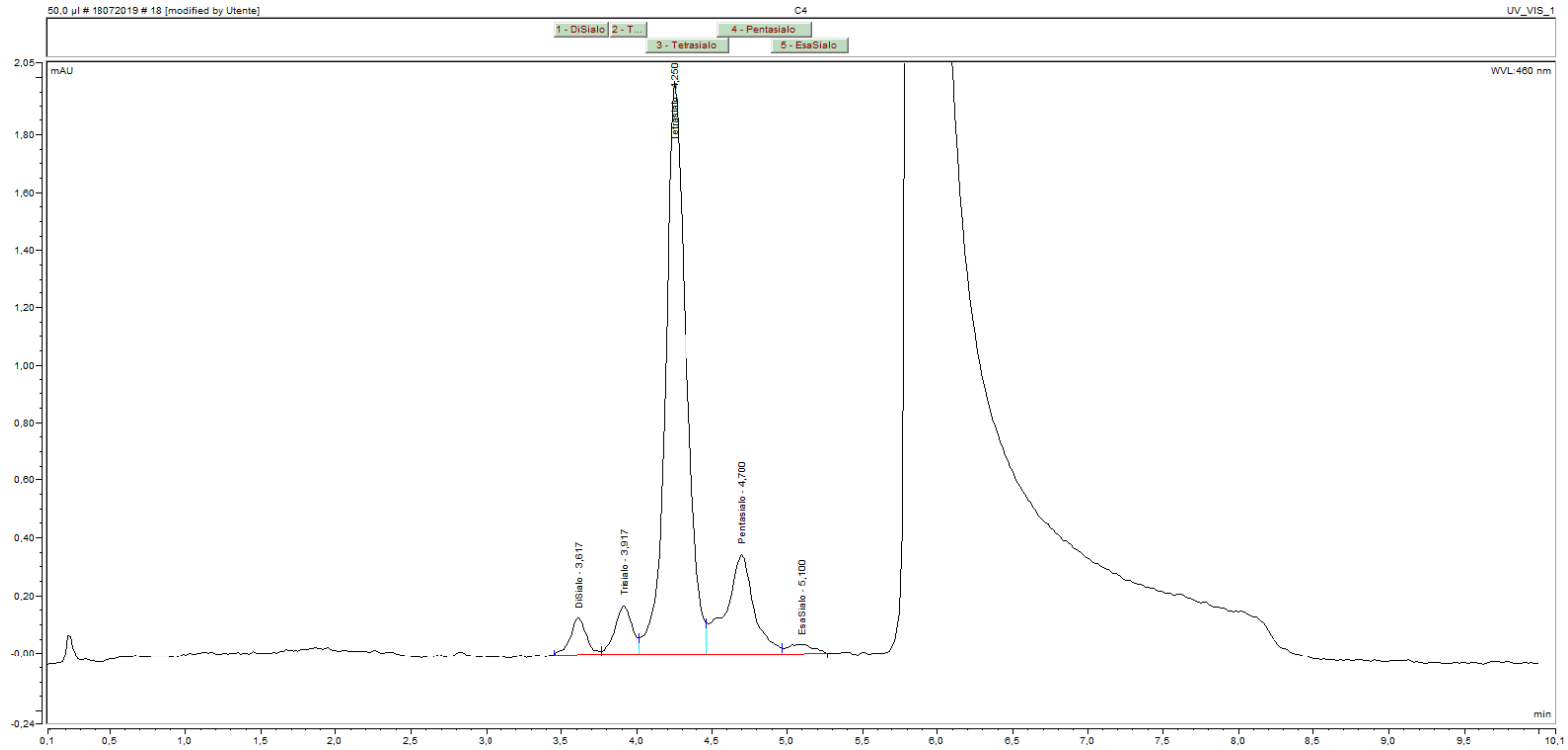
IFCC Calibrator Level 2: % Disialotransferrin = 1,82%

CDT TEST IN SERUM - FAST
(Reference Chromatograms)



IFCC Calibrator Level 3: % Disialotransferrin = 2,52%

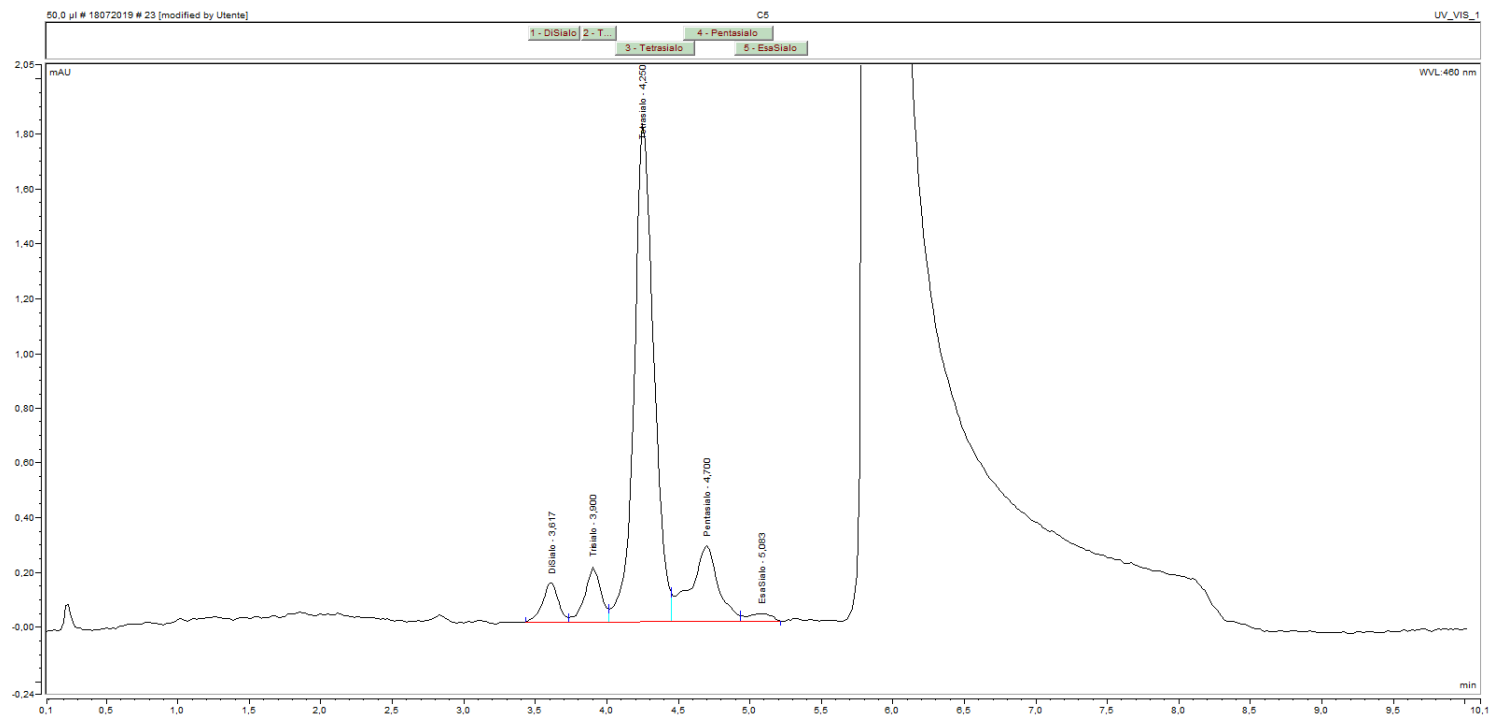
CDT TEST IN SERUM - FAST
(Reference Chromatograms)



IFCC Calibrator Level 4: % Disialotransferrin = 3,55%

CDT TEST IN SERUM - FAST

(Reference Chromatograms)



IFCC Calibrator Level 5: % Disialotransferrin = 4,34%

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