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

pro training
ASSISTANCE

KIT ADVANTAGES

- ✓ The serum sample volume required for the anaytical phase is **100 µl**.
- ✓ **No** reagent to reconstitute
- ✓ A single reagent is dispensed
- ✓ A binary pump **is sufficient**

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INTRODUCTION

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

Transferrin is a glycoproteic beta 1-globuline 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 substructural domains (N-terminal aa 1-336 e C-terminal aa 337-679). These domains could bind each one a Fe3+ ion independently one from another.
The C- terminal domain has two glucidic chains linked to N of asparagines 413 e 611 (1).

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

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

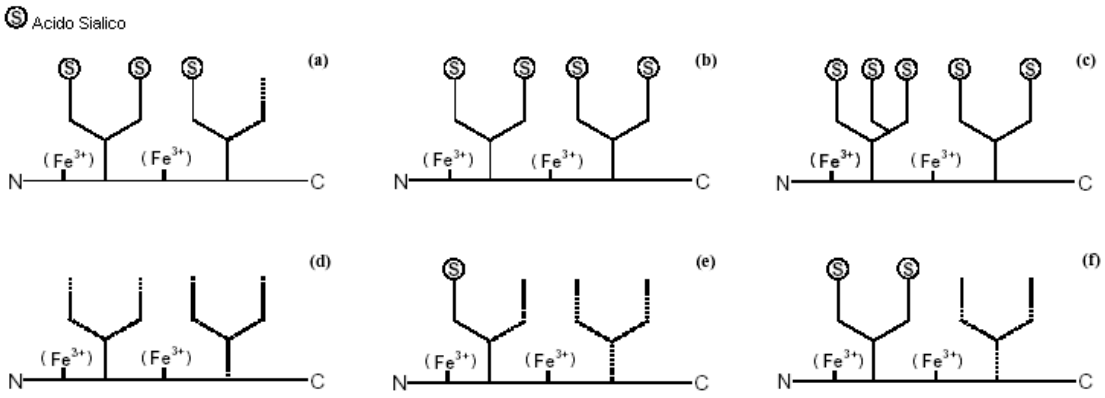


Fig. 1: Glicoforms of transferrin: trisialo, tetrasialo, pentasialo transferrin (a-c); asialo, monosialo, disialo transferrin (d-f)

At the end of 70's it has been demonstrated a correlation between alcohol abuse and elevated levels of CDT. **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 inibition of some glycan enzymes of ethanol and its derivates (aldheydes).

STANDARDIZATION OF CDT

The development of numerous methods for measuring CDT which have made an easier determination is supporting the spreading in different circles. There are more than 600 the publications about CDT appeared in pseudo scientific reviews since 2000 up to now.

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

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

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

- Target Molecule and molecule for standardization
- Definition of measuring an 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 commissiono of IFCC had identified the Disialotransferrin as target analyte for CDT. Actually even if Asialoform is more specific than Disialotransferrin for alcohol abuse, with the present available methods it can be identified when Disialo is already elevated, therefore this form has the highest diagnostic sensitivity.

HOW TO EXPRESS THE RESULT

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

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

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

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

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).

RECOVERY OF THE METHOD: Not Applicable

SENSITIVITY OF THE METHOD: Not Applicable

LINEARITY OF THE METHOD: Not Applicable

ACCURACY INTRA SERIE (RELATIVE ERROR %):

Ci	Cs
1,6%	4,2%
8,54%	5,40%

ACCURACY INTER SERIE (RELATIVE ERROR %):

Ci	Cs
1,6%	4,2%
5,69%	4,52%

REPRODUCIBILITY' INTRA SERIE (COEFFICIENT OF VARIATION %):

C LLOQ	Cm	Cs
1,6%	3%	4,2%
7,53%	3,55%	3,21%

REPRODUCIBILITY' INTER SERIE (COEFFICIENT OF VARIATION %):

CLLOQ	Cm	Cs
1,6%	3%	4,2%
7,83%	5,06%	427%

COMPONENTS OF THE KIT

Reagent A - Complexing Solution, ***1 x 5 ml***	
Serum Control lyophil, *1 x 2 x 1 ml* ,	**Code Z68019** (packed separately – see data sheets)
Reagent M1 - Mobile Phase M1, ***5 x 500 ml***	**Keep at room temperature for the shortest possible time**
Reagent M2 – Mobile Phase M2, ***2 x 500 ml***	**Keep at room temperature for the shortest possible time**
Reagent M3 – Mobile Phase M3, ***1 x 500 ml***	**Keep at room temperature for the shortest possible time**

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

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED:

Binary HPLC System with loop of 100 Bl in peek
Spectrophotometric Detector UV / VIS λ**=460 nm**
Chromatograms Recorder

OPTIONAL EQUIPMENT:

Autosampler
Operational Computer

BLOOD COLLECTION PROCEDURE:

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

STEP 1

PREPARATION OF CONTROLS AND SAMPLES

Pipette in a tube:

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

Vortex for 10 seconds

STEP 2

Centrifuge at 10000 rpm for 10 minutes

STEP 3

Take 100 ul of surnatant and add 400 ul of H2O HPLC grade

Vortex for 10 seconds

INJECTION

Inject 50 or 100 ul into the chromatographic system

GRADIENT FAST CDT Test			
Time (min)	**% M1 (PUMP A)**	**% M2 (PUMP B)**	**Flow (ml/min)**
0	100	0	1,2
0.50	100	0	1,2
0.60	93	7	1,2
5.0	75	25	1,2
5.1	0	100	1,2
7.5	0	100	1,2
7.6	100	0	1,2
10.0	100	0	1,2

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.

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 1.2 ml / min.

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

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

HPLC PARAMETERS

LOOP	100 µl in peek
Recommended Flow	1,2 ml/min
Pressure	30 bar

INITIAL PREPARATION OF SYSTEM and COLUMN CONDITIONING

1. When column is detached, insert the tubes of the two pumps in a single container containing water
2. Insert water as wash solution of autosampler's needle
3. Flush water, with opened discharge's valve, at 10 ml/min for 2 minutes in pumps A and B (50:50) and discarge
4. Make 2 washes of autosampler's needle (3+3 ml) with water
5. Set up a flow of 0,5 ml/min, close the discharge's valve and in the meantime make 2 injections of 100 Bl of water (loop's and switching system's washing)
6. Insert draught tubes A and B in correspondence of mobile phases M1 and M2
7. 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 flow
10. Connect column for CDT
11. Flush mobile phase M1 (100% A) at 1,2 ml/min for 15 minutes
12. Inject 100 Bl of water so that gradient can start, like mentioned in methodology; naturally the resulting 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 1.2 ml / min, make an injection of HPLC grade water and then injected controls serum until complete activation of the column .

WASHING OF SYSTEM and COLUMN TO EXECUTE AT THE END OF EVERY WORKING DAY

1. The column should be left in mobile phase M1
2. 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 1.2 ml / min.
3. When column is detached wash tubers:
- 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 Bl 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 Bl 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 Bl 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.

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.

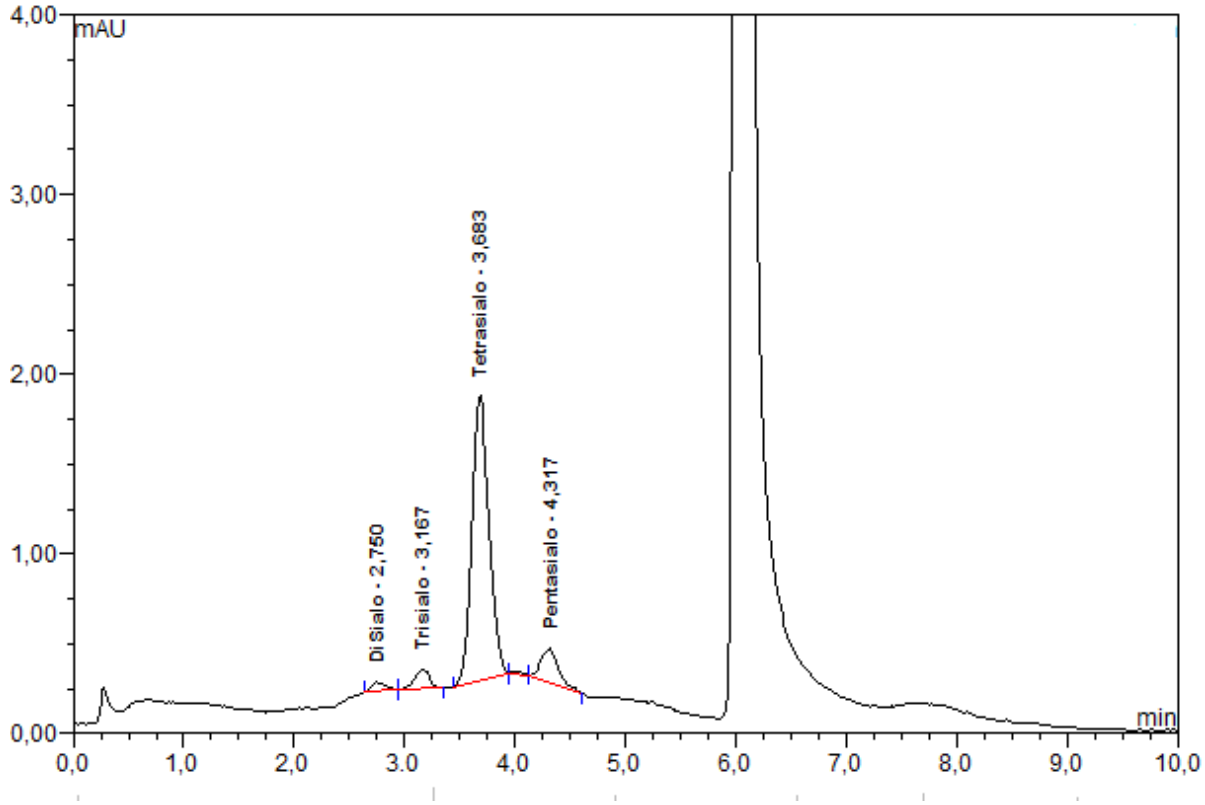
GENERAL CONDITIONS

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.

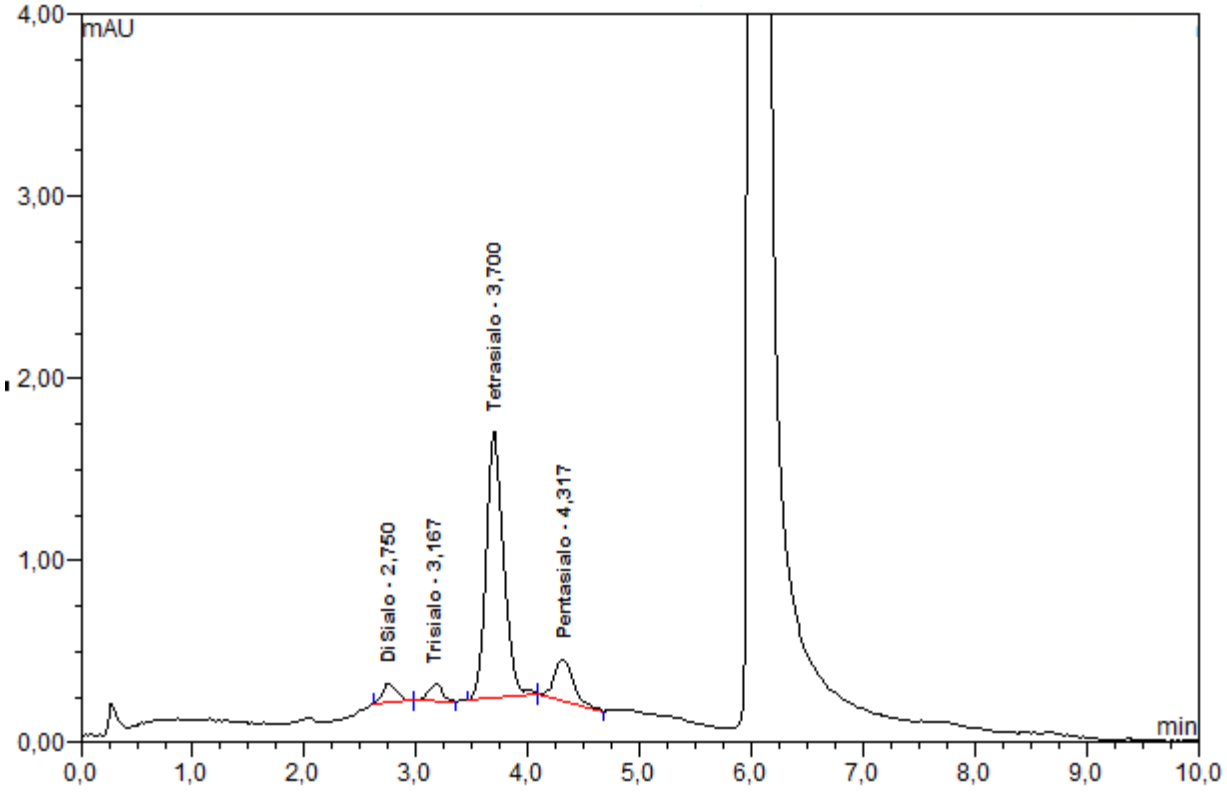
ACCESSORIES AND CONSUMABLES

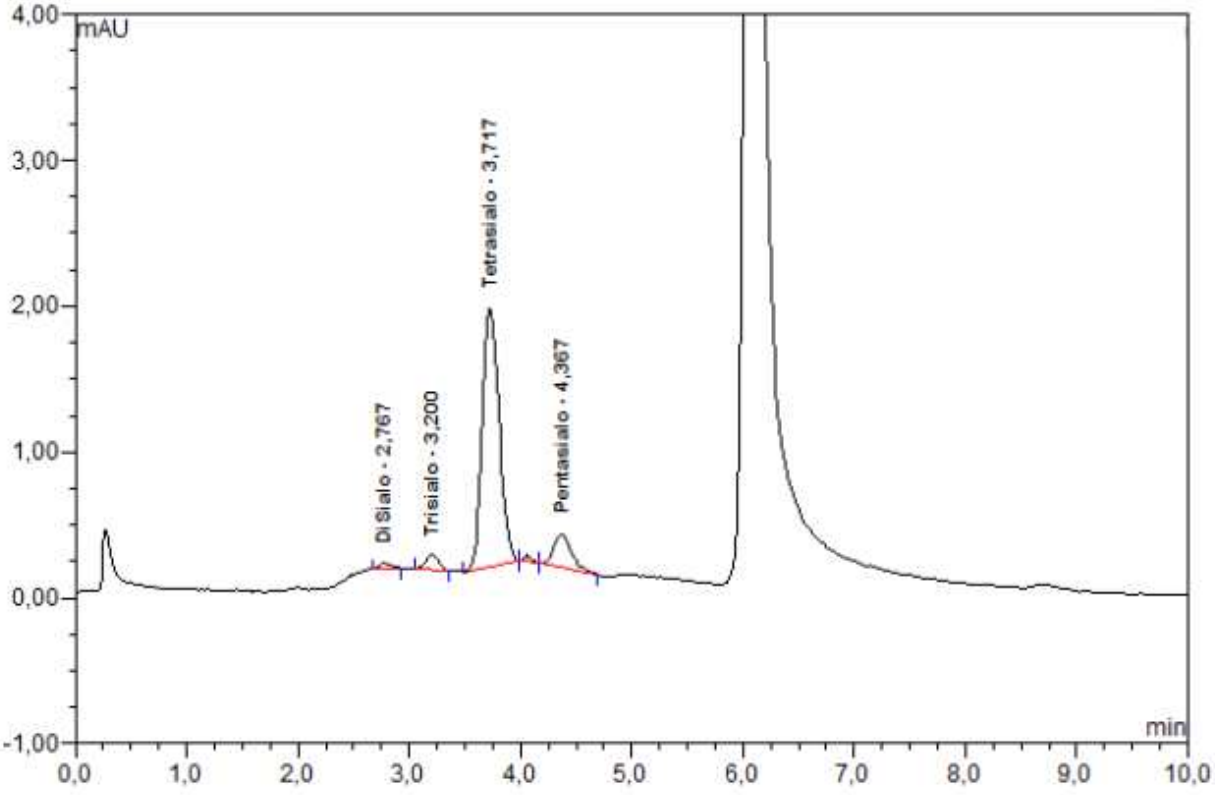
CODE	DESCRIPTION	PACKAGING
Z68017	Serum Control lyophil. for CDT Test, Level 1	5 x 1 ml
Z68018	Serum Control lyophil. for CDT Test, Level 2	5 x 1 ml
Z68019	Serum Control lyophil. for CDT Test, Levels 1 and 2	2 x 5 x 1 ml
Z054998	Analytical Column SAX 10 G (50 x 4 mm -5 um)	1 Pc
S29307U	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S29046U	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs

REFERENCE CHROMATOGRAM - Serum Control Level 1

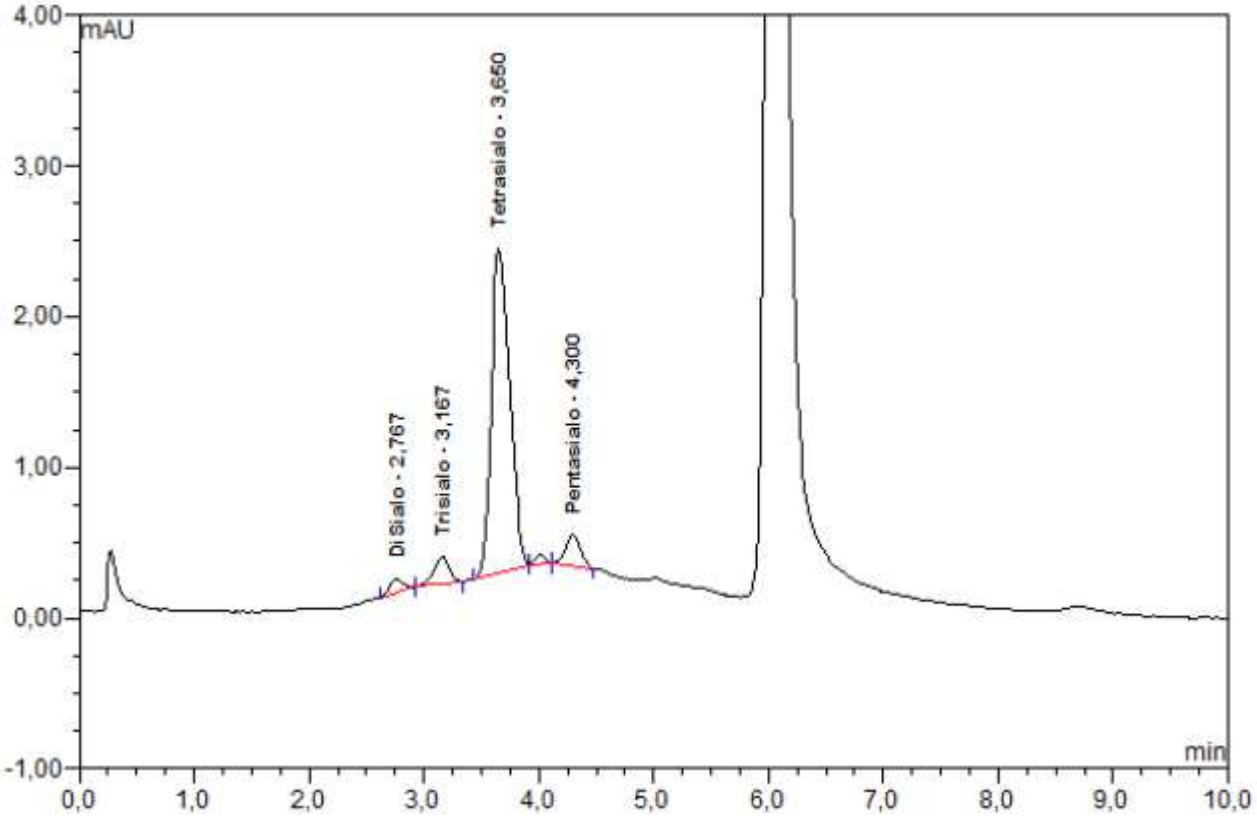


REFERENCE CHROMATOGRAM - Serum Control Level 2




mAU
4,00
3,00
2,00
1,00
0,00
-1,00
Di Sialo - 2,767
Trisialo - 3,200
Tetrasialo - 3,717
Pentasialo - 4,367
min
0,0
1,0
2,0
3,0
4,0
5,0
6,0
7,0
8,0
9,0
10,0

REFERENCE CHROMATOGRAM – Positive Sample



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