

IRINOTECAN/7-ETHYL-10-HYDROXYCAMPTOTHECIN (SN38) IN PLASMA by LC/MS –
Code LC94010

INTRODUCTION

Irinotecan is an antineoplastic chemotherapy drug of the class of camptothecins (drugs extracted from the bark of *Camptotheca acuminata*). Irinotecan is a pro drug. Its activation occurs at a hepatic level thanks to the transformation effected by the enzyme carboxesterase in SN 38. Irinotecan and SN 38 are then eliminated mainly through the bile; therefore the dosage should be decreased in patients with hepatic insufficiency. The active metabolite SN 38 is able to inhibit the topoisomerase I, the enzyme responsible for the breaking up of the single strand of DNA in order to cause an increase in the binding number. The inhibition of this enzyme leads to genomic aberrations and consequent triggering of cell apoptosis.

Irinotecan in combination with fluorouracil and leucovorin is the first line treatment for colorectal cancer. Other indications are non-small cell lung cancer.



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 EC declaration of conformity is available on request.

N° 002	Irinotecan/SN38 in plasma by LC/MS	10/2024
--------	------------------------------------	---------

TECHNICAL FEATURES

Principle of the Method:

Irinotecan and its primary active metabolite are analyzed by treating the plasma with a suitable deproteinization reagent. The solution is directly injected into the LC / MS.

Recovery:

Irinotecan 97%
SN38 98%

Sensitivity (LLOD) :

Irinotecan 1 ng/ml
SN38 1 ng/ml

Linearity:

Irinotecan 3 – 10.000 ng/ml
SN38 3 – 1000 ng/ml

Minimum concentration analyzable (LLOQ):

Irinotecan 3 ng/ml
SN38 3 ng/ml

Therapeutic range in plasma :

Irinotecan normal values 50 - 400 ng/ml
SN38 normal values <17 ng/ml

1) Clin Pharmacol Ther. 2002 Sep; 72 (3):265-75
2) J Cancer Res Clin Oncol (2013) 139:1579-1589

Accuracy intra serie (relative error %) Irinotecan :

Ci	Cs
100 ng/ml	500 ng/ml
6,18%	0,73%

Accuracy inter serie (relative error %) Irinotecan :

Ci	Cs
100 ng/ml	500 ng/ml
7,65%	2,44%

Reproducibility intra serie (relative error %) Irinotecan :

Ci	Cm	Cs	
3 ng/ml	250 ng/ml	750 ng/ml	
3,23%	0,29%	0,94%	

Reproducibility inter serie (coefficient of variation %) Irinotecano :

Ci	Cm	Cs	
3 ng/ml	20 ng/ml	40 ng/ml	
6,38%	2,58%	1,64%	

Coefficient of Correlation R2 + Dev Std

0,9978 ± 0,0010 Irinotecan

Accuracy intra serie (relative error %) SN38 :

C1	C2
10 ng/ml	50 ng/ml
3,94%	1,07%

Accuracy inter serie (relative error %) SN38 :

C1	C2
10 ng/ml	50 ng/ml
3,85%	2,40%

Reproducibility intra serie (relative error %)
SN38 :

Ci	Cm	Cs
3 ng/ml	25 ng/ml	75 ng/ml
1,16%	2,01%	1,09%

Reproducibility inter serie (coefficient of variation %)
SN38 :

Ci	Cm	Cs	
3 ng/ml	20 ng/ml	40 ng/ml	
2,07%	3,65%	3,44%	

Coefficient of Correlation R2 + Dev Std

0,9984 ± 0,0008 SN38

Components of the kit :

All the Reagents are ready to use and stable 3 years at 2–8 °C except Reagent A that must be stored at -20 °C.

Reagent A – Deproteinization Sol + Internal Standard, **1 x 40 ml**

Store at -20 °C

Calibrator in plasma – Level 1, 1 x 1 ml

Code LC94016 (packed separately – see data sheets)

Calibrator in plasma – Level 2, 1 x 1 ml

Code LC94016 (packed separately – see data sheets)

Calibrator in plasma – Level 3, 1 x 1 ml

Code LC94016 (packed separately – see data sheets)

Calibrator in plasma – Level 4, 1 x 1 ml

Code LC94016 (packed separately – see data sheets)

Calibrator in plasma – Level 5, 1 x 1 ml

Code LC94016 (packed separately – see data sheets)

Reagent M1 – Mobile Phase M1, **1 x 500 ml**

Reagent M2 – Mobile Phase M2, **1 x 500 ml**

Minimum Instrumental equipment required:

LC-MS Instrument with triple quadrupole MRM work mode, ESI positive

Optional Equipment:

Autosampler

Plasma Collection Procedure:

Collect 3 ml of blood with sodium citrate. Centrifuge **immediately** at 4.000 rpm for 5 min. Plasma samples should be stored at **–20° C** for no longer than **1 month**.

Bibliography

Marangon E. et al., *PLoS One* (2015); **10**: e0118194

Satoh T. et al., *Anticancer Research* (2013); **33**:3845 - 3854

Xun C., et al., *Cancer Res Clin Oncol* (2013); **139**:1579–1589

Sparreboom A. et al., *British Journal of Cancer* (2004); **90**:1508 – 1515

Xie R. et al., *Clinical Pharmacology & Therapeutics* (2002); **72**:265-75

ANALYTICAL PROCEDURE

STEP 1 :

Dispense in eppendorf:

	Matrix Blank	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Matrix Blank	100 µl							
Calibrator – Level 1		100 µl						
Calibrator – Level 2			100 µl					
Calibrator – Level 3				100 µl				
Calibrator – Level 4					100 µl			
Calibrator – Level 5						100 µl		
Sample							100 µl	
Controls								100 µl
Reagent A – Deproteinization Solution +Internal Standard	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl

Vortex for 20 seconds

STEP 2: Centrifuge at 14.000 rpm for 5 minutes

N.B.: at this step, the sample is stable 1 day at 2-8 °C

INJECTION :

- Inject 20 µl of surnatant in LC system

IRINOTECAN/SN38 IN PLASMA - Warnings

COLUMN CONDITIONING

Install a new analytical Hypersil Gold (50 x 2,1 mm, 3,0 µm) and Hypersil Gold (50 x 2,1 mm, 1,9 µm) for UHPLC, thermostated at 25 °C. Disconnect the detector and flush a solution of Mobile Phase M1 : Mobile Phase M2 (95 : 5 v/v) set flow at 0,3 ml / min for 30 minutes. **Don't recycle the washing solutions.** Condition further on the column with a solution of Mobile Phase M2 : Mobile Phase M2 (95 : 5 v/v) set flow at 0,3 ml / min for 15 minutes.

It is NOT possible to make analysis at recycling phase.

If Room Temp. is > 20° C is better to preserve the Mobile Phase at 2 – 8° C among runs.

COLUMN CLEANING

Wash the column with a solution of Mobile Phase M2 : Mobile Phase M1 (95 : 5 v/v) at flow of 300 µl / minutes for 30 minutes. **It recommends flushing the Analytical Column at the end of each run.** Store the column in a solution of Mobile Phase M2 : Mobile Phase M1 (95 : 5 v/v).

INJECTION WASH NEEDLE

Wash with a solution of Methanol or Acetonitrile

ESI Waters TQD PARAMETERS

Capillary: 2.90 KV
Temperature Desolvation: 400 °C
Desolvation (L/Hr): 1000

FLOW SET

GRADIENT			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flux (µl/min)
0.00	95	5	300
0.50	95	5	300
1.50	0	100	300
3.00	0	100	300
3.01	95	5	300
5.00	95	5	300

FRAGMENTATIONS (optimize on TRIPLE QUADRUPOLE Sciex 4500)

Analyte	Precursor Ion	Product Ion	Collision Energy	Fragmentor	Polarity
IRINOTECAN	587.7	167.1 – 124.2	45 – 41	214	Positive
SN-38	393.4	349.1 – 293.1	21 - 33	141	Positive
IRINOTECAN-D10	597.4	177.2 – 133.2	45 - 41	201	Positive

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC94016	Calibrator in plasma for Irinotecan/SN38	5 x 2 x 1 ml
LC94019	Control in plasma for Irinotecan/SN38 – Levels 1 and 2	2 x 5 x 1 ml
For HPLC systems		
ZTF2500305213	Hypersil Gold (50 x 2.1 mm – 3.0 um) Analytical Column	1 Pc
For UHPLC systems		
Z25002052130	Hypersil Gold (50 x 2.1 mm - 1.9 um) Analytical Column	1 Pc
S51843550	Clear glass vials with reduced volume	1 x 100 Pcs
S51820717	Caps for clear glass vials with reduced volume	1 x 100 Pcs
SK94010	Starter kit for Irinotecan/SN38	1 Pc

IRINOTECAN/SN38 IN PLASMA (Reference Chromatograms)

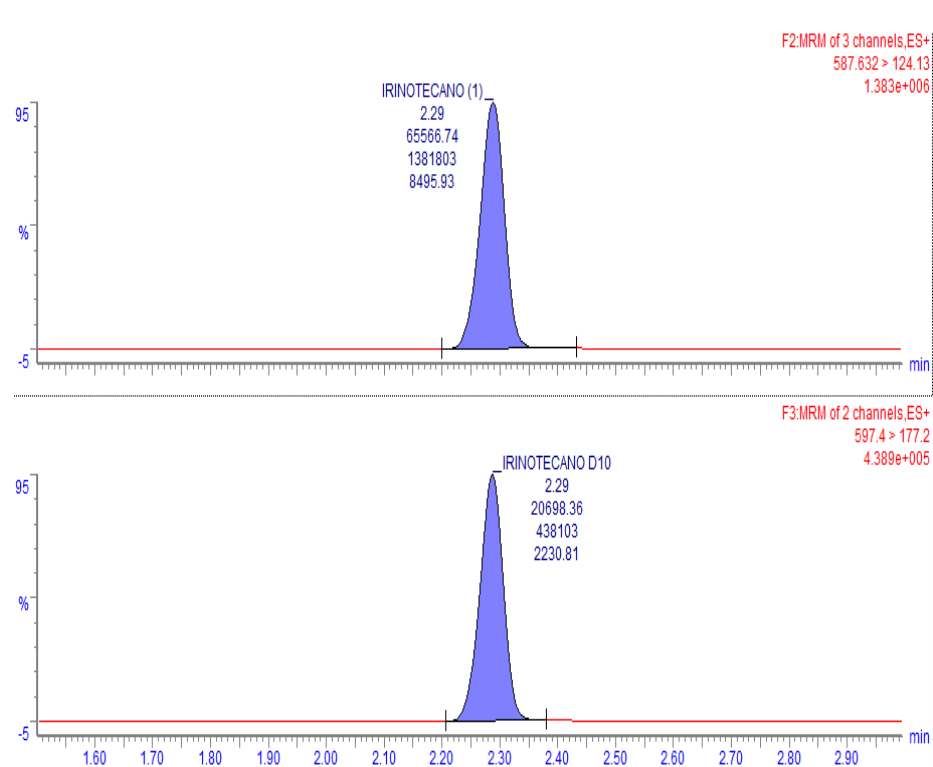


Fig. 1 : Calibrator in plasma
R.T. 2.29 Irinotecan 500 ng/ml
R.T. 2.29 Irinotecan-D10

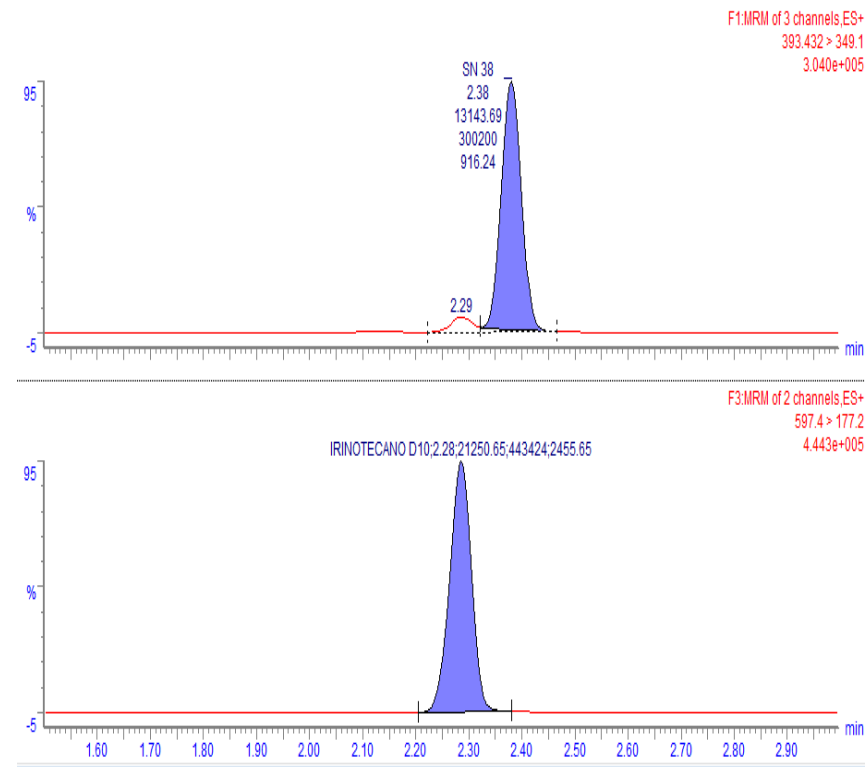


Fig. 2 : Calibrator in plasma
R.T. 2.38 SN38 50 ng/ml
R.T. 2.28 Irinotecan-D10