Application Note:
ANCCSSOLADXCIN

Doxorubicin in Human Serum Using SOLA and Hypersil GOLD Column

Jamil Ali, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

- Anti-cancer drug
- Doxorubicin
- SOLA Cartridges and Plates
- Bioanalysis
- DOX
- DAU

Abstract

A liquid chromatography method for doxorubicin from human serum has been developed using Thermo Scientific SOLA cartridges. The sample preparation is fast and efficient giving good reproducibility and accuracy. A Thermo Scientific Hypersil GOLD column was used to give a fast run time of 4 minutes.

Introduction

SOLA™ products are a revolutionary new Solid Phase Extraction (SPE) product range. This first in class SPE product range introduces next-generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, phospholipid and protein precipitation products. This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA products have significant advantages for the analyst when processing compounds in complex matrices particularly in high throughput bioanalytical and clinical laboratories where reduced failure rate, higher analysis speed and lower sample/solvent requirements are critical.

The increased performance from SOLA products provides higher confidence in analytical results and lowers cost, without compromising ease of use or requiring complex method development.

Doxorubicin is a drug used for treating a wide range of cancers, including hematological malignancies, many types of carcinoma and soft tissue sarcomas. It is an anthracycline antibiotic related to daunomycin and like all anthracyclines, it functions by intercalating DNA.

This is typically dosed at 40-75 mg every 3-4 weeks. A C_{max} value of 638 ng/mL has been reported for 60 mg doses.[1, 2] In this application the extraction and quantification of doxorubicin in human serum are demonstrated.

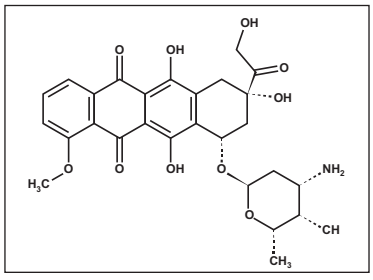
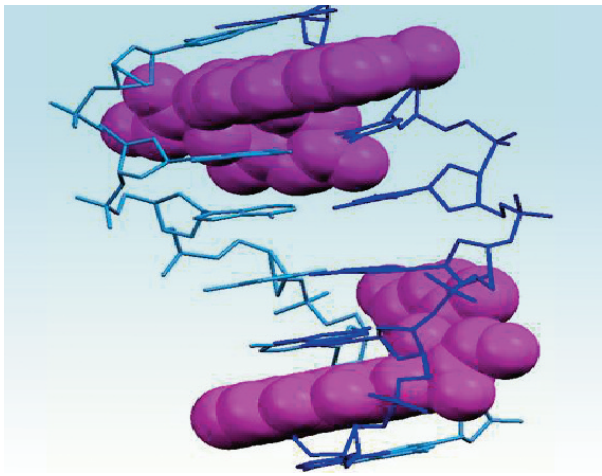


Figure1. Structure of doxorubicin



Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade methanol	M/4056/17
Ammonium acetate (Fisher Scientific)	A/3440/50

Sample Handling Equipment	
Finn pipettes (Thermo Fisher Scientific) 02-707-408, 02-707-423)	9402151
Vacuum manifold	60104-232
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap MSCERT 4000-34W	
0.3 mL Screw Top Fixed Insert Vial Chromacol	03-FISV
9 mm Screw Cap (blue) Pre-fitted Silicon/PTFE Liner	9-SC(B)-ST101

Sample and Calibration Preparation		
Compound(s):	doxorubicin HCl (DOX) and daunorubicin HCl (DAU) (IS)	
Matrix:	human serum	
Cartridge type:	SOLA, 10mg/1mL	60109-001
Stock Solution:	1000 µg/mL stock solutions of DOX in methanol and daunorubicin (IS) in water were prepared,	
	120 µL daunorubicin solution was diluted with 9880 µL water to give the internal standard stock solution.	
Calibration standards:	S1-S8 calibration standards were prepared as shown in table 1. 180 µL of serum was spiked with 10 µL internal standards and 10 µL of doxorubicin (S1-S8) solution to give 5, 10, 25, 100, 200, 500, 850 and 1000 ng/mL solution.	

Standards	Concentration wanted in plasma (ng/mL)	Take spike from	Amount to Spike (µL)	Solvent Added (MeOH)(µL)
S8	1000	Stock	100.0	4900.0
S7	850	S8	1700.0	300.0
S6	500	S8	1000.0	1000.0
S5	200	S8	400.0	1600.0
S4	100	S8	200.0	1800.0
S3	25	S6	100.0	1900.0
S2	10	S5	100.0	1900.0
S1	5	S4	100.0	1900.0

Table 1. Preparation of calibration standards

Single Level Accuracy Check

180 µL of serum was spiked with 10 µL DOX (400ng/mL) and 10 µL IS solution to give 20 ng/mL doxorubicin solution in the serum. SPE was performed and the extract analyzed by HPLC. This was repeated 6 times to check the accuracy and the precision.

Sample Preparation - SOLA		Part Number
Cartridge type:	SOLA 10 mg/1mL cartridges	60109-001
Conditioning stage:	500 µL methanol	
Equilibration stage:	500 µL water	
Load:	200 µL plasma (spiked with IS)	
Wash:	200 µL water/methanol 90:10 (v/v)	
Elute:	200 µL methanol + 0.1% formic acid	

Chromatographic Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 600 pump, Thermo Scientific CTC autosampler	
Column:	Hypersil GOLD, 3µm	25003-104630

Mobile Phase		
A:	Ammonium acetate 25 mM, (native pH)	
B:	Acetonitrile	
T/min	% A	%B
0.00	80.0	20.0
1.80	70.0	30.0
2.30	60.0	40.0
2.50	80.0	20.0
4.00	80.0	20.0
Flow rate:	1.50 mL/min	
Column temperature:	Ambient	
Injection details:	25 µL	
Injection wash solvent:	water/acetonitrile (80:20)	
Pressure recorded(t=0min):	170 Bar	
Fluorescence detection parameters:		
Excitation wavelength:	480nm	
Emission wavelength:	560nm	
Rise time (s):	0.05	
Sampling period (ms):	200	
PMT voltage:	high	

Data Processing	
Software:	ChromQuest 5
Integration parameters:	
Width:	0.5
Threshold:	10
Additional manual integration was applied as necessary.	

Results

The dynamic range was shown to be linear between 1 and 100 ng/mL with a r^2 (goodness of fit) of 0.9991.

Single level standards	Actual amount /ng.mL^{-1}	Calculated amount /ng.mL^{-1}	Accuracy
1	20	19.4	96.7%
2	20	19.4	96.0%
3	20	18.8	93.7%
4	20	20.1	100.2%
5	20	21.8	108.8%
6	20	22.1	110.6%
Mean	-	20.2	101.2%
RSD	-	6.9%	6.9%

Table 2. Determination of doxorubicin in spiked serum samples using an internal standard

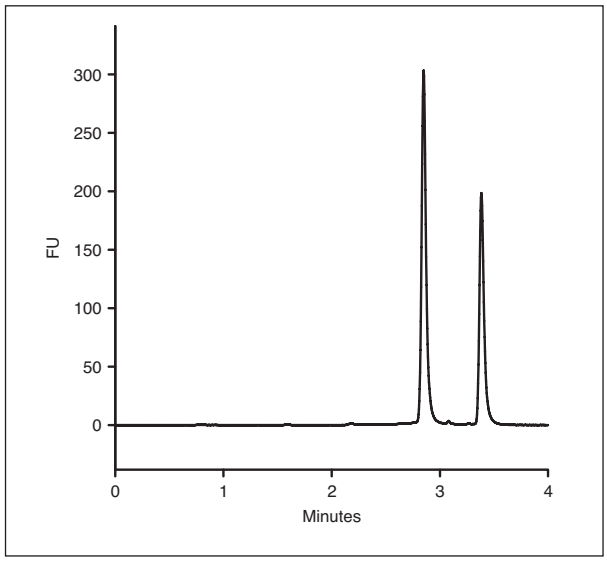


Figure 2. Chromatogram of doxorubicin (T_r 2.85min) in extracted human serum using daunorubicin (T_r 3.38min) as the internal standard

Conclusion

SOLA cartridges and Hypersil GOLD HPLC columns can be used to extract and quantify doxorubicin from human serum using a quick and simple method. In this application we have demonstrated that:

- SOLA cartridges require less elution solvent volume, resulting in reduced solvent cost and shorter drying times.
- SOLA cartridges allow for high accuracy and precision
- SOLA cartridges are very effective in removing endogenous interferences.

References

1. Barpe, D. R, Rosa, D. D, Froehlich P, E. "Pharmacokinetic evaluation of doxorubicin plasma levels in normal and overweight patients with breast cancer and simulation of dose adjustment by different indexes of body mass". Eur J Pharm Sci. 2010, 41(3-4), 458-463

2. 2011 Current Cancer, http://currentcancer.com/doxorubicin.html, Dated 06/09/2011

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India
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