Application Note: ANCCSSOLACBINE

Capecitabine in Human Plasma Using SOLA and Accucore Core Enhanced Technology HPLC Column

Joanna Denbigh, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

- SOLA Cartridges and Plates
- Accucore
- Capecitabine
- Bioanalytical

Abstract

This application note demonstrates a simple and rapid method for the analysis of capecitabine in low volumes of human plasma. Extraction of this analyte was achieved on Thermo Scientific SOLA cartridges and plates. The capecitabine in the extract was quantified by a matrix-standard calibration, with extracts from human plasma spiked with increasing amounts of the analyte. Determination of the capecitabine was performed by HPLC-MS/MS, using a Thermo Scienific Accucore PFP column under gradient mobile phase conditions.

Introduction

SOLA™ cartridges and plates 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:

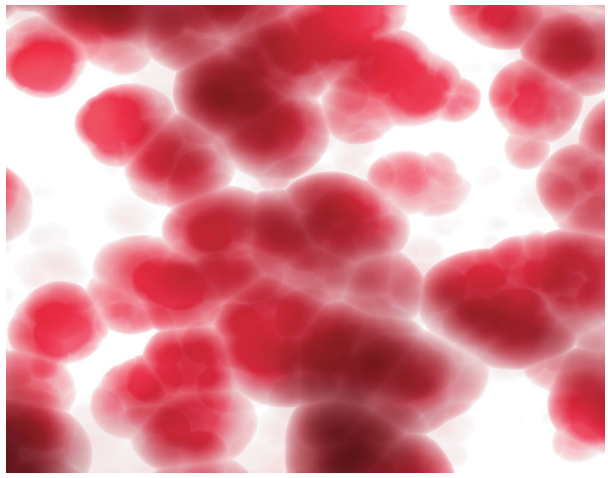
- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

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 form SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development. Method development was carried out using 10 mg cartridges and the method transferred to 96 well plate format for the validation batch.

Accucore™ HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Capecitabine is an oral fluoropyrimidine carbamate prodrug of 5-fluorouracil (5-FU) which is one of the most widely used chemotherapeutic agents for the treatment of



patients with breast and colon cancer. Fluorouracil is not suitable for oral administration because of its highly variable gastro-intestinal tract absorption, however capecitabine is rationally designed as an orally administered precursor of 5-deoxy-5-fluorouridine which is sequentially converted to the cytotoxic 5-FU in a series of metabolic steps.

Figure 1 shows the structure of capecitabine.

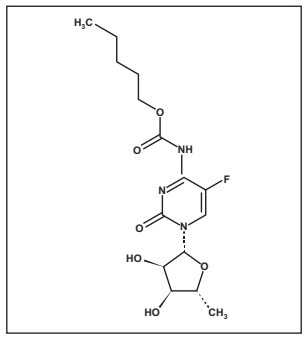


Figure 1. Structure of capecitabine

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific LC-MS grade methanol	M/4062/17
Fisher Scientific LC-MS grade water	W/0112/17
Fisher Scientific LC-MS grade acetonitrile	51101
Capecitabine USP Reference standard (USP Reference standard, Rockville, US)	
Capecitabine-D8 Internal Standard (ArtMolecules, Poitiers, France)	

Sample Handling Equipment	Part Number
Liquid handling hardware:	
Thermo Scientific FinnPipette (100-1000 µL)	642090
Thermo Scientific FinnPipette (10-100 µL)	4642070
Thermo Scientific FinnPipette (1-10 µL)	4642040

Solid Phase Extraction Hardware	Part Number
96 well Thermo Scientific Positive Pressure Manifold	60103-351

Vials and Closures	Part Number
300 µL fixed insert vial, amber, crimp, chromacol	03-FIV(A)
11 mm aluminium crimp cap, type 7, rubber/PTFE	11-AC7

Sample and calibration preparation

Compounds: Capecitabine (USP Reference standard, Rockville, US)

Capecitabine-D8 (ArtMolecules, Poitiers, France)

Matrix: Human Plasma (Seralab, West Sussex, UK)

Samples: A 1 mg/mL stock solution of capecitabine was prepared in 10:90 methanol:water and subsequently diluted with water to produce a set of spiking solutions. These were used to produce a calibration line in Human plasma covering the range 10 - 1000 ng/mL. Capecitabine-D8 was added as internal standard at a concentration of 500 ng/mL.

The concentration range selected was determined by consideration of the published Cmax which is reported to be 5405.9 ng/mL [1]

Further samples were prepared to allow for calculation of reproducibility, recovery and matrix effect. A mid-range concentration was prepared three times and subjected to the same extraction procedure to determine reproducibility. An extracted blank plasma sample was overspiked at a mid-range concentration and compared with the same concentration of extracted spiked plasma to calculate recovery. Matrix effects were determined by comparing the overspiked blank extract with that of the same concentration of extracted plasma.

Sample Preparation - SOLA		Part Number
SOLA 10 mg/1 mL cartridges		60109-001
SOLA 10 mg/2 mL 96 well plate		60309-001
Conditioning stage:	0.5 mL methanol	
Equilibration stage:	0.5 mL water	
Load:	100 µL plasma	
Wash:	200 µL water/methanol (80:20)	
Elute:	250 µL methanol	
Dry under nitrogen		
Reconstitute:	100 µL with water	

Separation Conditions	Part Number
Instrumentation: Thermo Scientific Accela 600 pump, Thermo Scientific CTC autosampler	
Guard Column: Accucore Defender Guard Column PFP, and UNIGUARD direct-connection guard cartridge holder	17426-012105 852-00
Column: Accucore PFP 2.6µm, 30 x 2.1 mm	17426-032130

Mobile Phase

A:	water	
B:	acetonitrile	
Time (min)	% A	%B
0.00	100	0
5.00	0	100
5.1	100	0
6.5	100	0
Flow rate:	1.0 mL/min	
Column temperature:	40 °C	
Injection details:	10 µL partial loop injection	
Injection wash solvent:	water/methanol (80:20)	

Mass Spectrometry Conditions

Instrumentation: Thermo Scientific TSQ Vantage

Ionization conditions	HESI
Polarity	negative
Spray voltage (V)	2500
Vaporizer temp (°C)	350
Sheath gas pressure (Arb)	75
Ion sweep pressure (Arb)	0.5
Aux gas pressure (Arb)	45
Capillary temp (°C)	300
Declustering voltage	0
Collision pressure (mTorr)	1.5
Cycle time (s)	5
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Table 1. TSQ Vantage conditions

Compound	Capecitabine	Capecitabine-D8
Parent (m/z)	358.30	366.00
Products (m/z)	154.21	153.75
Collision energy	21	21
S-lens	94	103

Table 2. Compound transition details

Data Processing

Software: Thermo Scientific Xcalibur LCquan version 2.6

Results

Under the conditions adopted for this analysis, retention of capecitabine and capecitabine-D8 can be achieved in 1.8 minutes. The chromatography is presented in Figure 2.

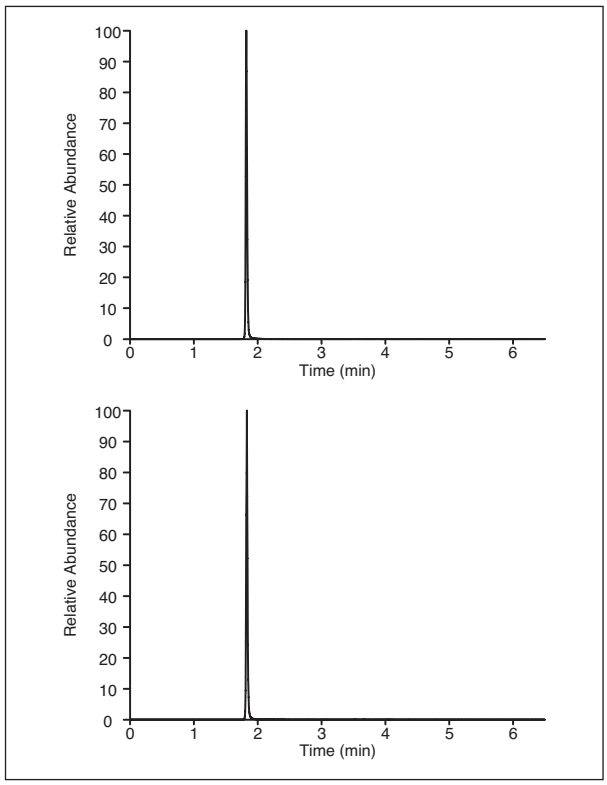


Figure 2: upper trace Capecitabine-D8, lower trace Capecitabine at 500 ng/mL.

The extraction procedure followed used an optimization procedure to obtain the optimum wash and elution conditions for the SPE.

This was achieved by aliquoting 0.5 mL of 100 ng/mL standard mixture (prepared in water) onto SOLA cartridges (following conditioning and equilibration with 0.5 mL of methanol and water respectively). Washes with increasing elutropic strengths of solvent were applied, starting with 0% methanol and increasing sequentially in 10% steps to 100% methanol.

Four elution steps (250 µL of 100 % methanol each) then followed this procedure. Each wash and elution step was collected and analyzed by HPLC. Figure 3 shows the full elution profile for capecitabine. These results show an optimum wash profile of 80:20 water:methanol, before capecitabine started to elute. This was the wash condition adopted throughout this study.

This optimised SPE methodology was transferred onto a SOLA plate for the validation of the methodology.

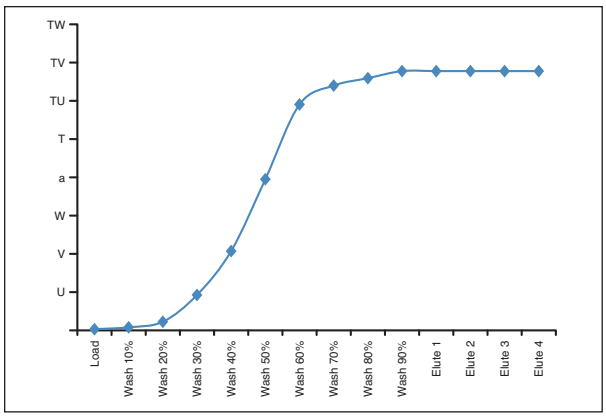


Figure 3. SOLA cartridge elution profile for capecitabine

Linearity

Excellent linearity was observed over a capecitabine concentration range in plasma of 10 ng/mL to 1000 ng/mL with a correlation coefficient value of 0.99. Figure 4 shows the calibration curve plotted for the extracted human plasma.

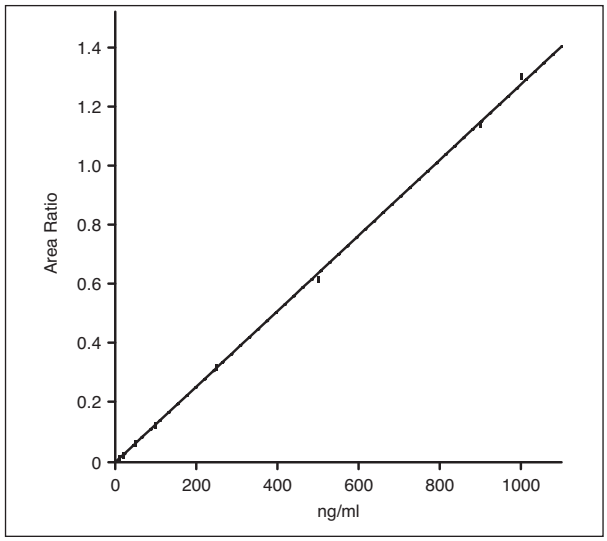


Figure 4. Calibration curve for capecitabine in human plasma

Reproducibility

For the mid range concentration extracted plasma samples, reproducibility was excellent. The calculated %RSD being 2.3% (n=3).

Recovery

Recovery was calculated by comparing the response of the overspiked blank extract with that from an extracted spiked plasma sample. Recovery was calculated to be 73.2%

Conclusion

In this application note SPE-LC-MS/MS methodology for the analysis and quantitation of capecitabine in low volumes of human plasma was developed. Extraction of this analyte was achieved on SOLA well plates using small sample volumes of 100 µL with good recovery and reproducibility.

References

[1]. Farkouh, A, "A Rapid and Simple HPLC Assay for Quantification of Capecitabine for Drug Monitoring Purposes"; *Anticancer Research* (2010), 30, 5207-5212ces

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America
USA and Canada
+1 800 332 3331

Europe
France
+33 (0)1 60 92 48 34

Germany
+49 (0) 2423 9431 -20 or -21

United Kingdom
+44 1928 534110

Asia
Japan
+81 3 5826 1615

China
+86-21-68654588
or +86-10-84193588
800-810-5118

India
+91-22-6742 9494

Thermo Fisher Scientific Australia Pty Ltd
1300 735 292 (free call domestic)

Thermo Fisher Scientific New Zealand Ltd
0800 933 966 (free call domestic)

All Other Enquiries
+44 (0) 1928 534 050

Technical Support

North America
800 332 3331

Outside North America
+44 (0) 1928 534 440

www.thermoscientific.com/chromatography

ANCCSSOLACBINE 1211