APPLICATION INFO

Determination of Docetaxel in Human Plasma by XLC-MS using the Symbiosis™ Pharma System

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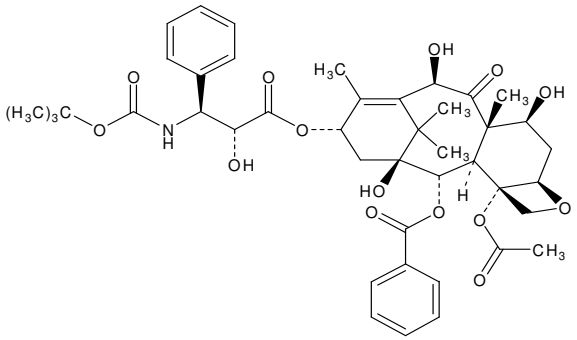
Charles River Laboratories
Clinical Services Montreal

Introduction

The **Symbiosis™ Pharma** is Spark Holland's unique solution for integrated on-line SPE-LC-MS automation (XLC-MS). The system offers large flexibility in processing different types of samples selecting one of the three fully automated operational modes: LC-MS, XLC-MS, or AMD (Advanced Method Development).

A sensitive, specific, accurate and precise assay was developed for the on-line extraction and determination of docetaxel in human plasma by XLC-MS, using the **Symbiosis™ Pharma**. This method was developed as part of an evaluation, to introduce this new technology into our bioanalytical laboratory. This application note demonstrates the ease of transfer of an off-line sample pretreatment to an on-line protocol, with a concomitant increase in productivity.

Docetaxel is a semi-synthetic analogue of paclitaxel, prepared from a non-cytotoxic precursor extract of the European yew, *Taxus baccata L*. This compound has been shown to have broad antitumor activity against various solid tumors.



Docetaxel, $C_{43}H_{53}NO_{14}$, MW 807.89

Docetaxel acts at the cellular level promoting microtubule assembly and inhibiting the tubulin disassembly process, which ultimately inhibits cell division.

Previous Assay

Our previous assay involved off-line protein precipitation, with acetonitrile:methanol (50:50, v/v), which was diluted with water prior to analysis. A reverse phase LC-MS cycle-time of 7.5 min was employed to allow for a column wash, in addition to keeping system carry-over to within FDA bioanalytical guidelines (≤ 20% of the LLOQ response).

Typical preparation time for 80 samples was two thirds of an average working day (7.5 hours), followed by an overnight run (~10 hours). Therefore, a batch of samples would take approximately 18 hours to analyse from start to finish.

XLC-MS Protocol

During the evaluation, our previously developed assay was transferred onto the **Symbiosis™ Pharma.** An SPE elution profile was developed overnight using XLC mode with a spiked plasma sample at the desired ULOQ. This profile was later optimized for breakthrough, recovery and carry-over using AMD mode.

Autosampler Conditions

Control human plasma (K_2 EDTA, 50 µL) was spiked with a docetaxel standard solution (5 µL), and then diluted with internal standard working solution (500 µL; acetonitrile:10 mM ammonium acetate:formic acid 5:95:0.2, v/v/v). This sample cocktail was gently mixed, centrifuged and then injected using the **Reliance™** autosampler with µL-Pickup mode, to ensure the preservation of sample volume.

Autosampler Wash Solvents

Wash Solvent 1	Acetonitrile:10 mM Ammonium Acetate:Formic Acid (5:95:0.2, v/v/v)
Wash Solvent 2	Acetonitrile:Formic Acid (100:0.2, v/v)

Autosampler Wash Routine

Wash Solvent	Wash Volume	Valve Wash
1	700 µL	No
2	1500 µL	Yes
1	700 µL	Yes

SPE Conditions

Cartridge	10 x 2 mm HySphere-C18 HD, 7 µm (Spark PN:0722.609)	
Solvation	Acetonitrile:Formic Acid - 1 mL (100:0.2, v/v)	5 mL/min
Equilibration	Acetonitrile:Water:Formic Acid - 1 mL (5:95:0.2, v/v/v)	5 mL/min
Sample Loading	Acetonitrile:Water:Formic Acid - 1mL (20:80:0.2, v/v/v)	2 mL/min
Washing	Acetonitrile:Water:Formic Acid - 1mL (30:70:0.2, v/v/v)	5 mL/min
LC Elution	2:12 min, with LC Pump	1.2 mL/min
Clamp Wash	Acetonitrile:Water:Formic Acid - 0.5 mL (10:90:0.2, v/v/v)	5 mL/min
Valve Wash	Acetonitrile:Formic Acid (100:0.2, v/v)	-
Sample Matrix	Control Human Plasma (K_2 EDTA) - 50 µL, diluted with Acetonitrile:10 mM Ammonium Acetate:Formic Acid (5:95:0.2, v/v/v) - 500 µL	

LC Conditions

Column	Waters XBridge™ Shield RP_{18}, 3.5 µm, 4.6 x 50 mm
Column Temp	Ambient
Mobile Phase A	Water:Formic Acid (100:0.2, v/v)
Mobile Phase B	Acetonitrile:Formic Acid (100:0.2, v/v)

LC Gradient

Time (mm:ss)	Flow (mL/min)	A (%)	B (%)
00:01	1.20	55	45
00:15	1.20	55	45
02:00	1.20	5	95
02:30	1.20	5	95
02:31	1.20	55	45
03:30	1.20	55	45

MS Conditions

MDS Sciex API 4000™ LC-MS/MS System
Flow Dependent Parameters

Parameter	Setting
Ionisation Mode	(+) ESI
Curtain Gas (N_2)	25 psi
Ion Spray Voltage	5500 V
Source Temperature	450 °C
Nebulizer Gas (Zero Air)	60 psi
Auxiliary Gas (Zero Air)	60 psi
Interface Heater	On
CAD Gas (N_2)	6 dac

MDS Sciex API 4000™ LC-MS/MS System
Mass Dependent Parameters

Parameter	Docetaxel	Internal Standard (Paclitaxel)
Scan Mode	Multiple Reaction Monitoring	
Q1/Q3 Resolution	Unit/Unit	
Q1 mass (amu)	808.4	854.4
Q3 mass (amu)	527.3	286.1
Dwell Time (ms)	100	100
Declustering Potential (V)	30	55
Entrance Potential (V)	-10	-10
Collision Energy (eV)	15	25
Collision Exit Potential (V)	17	7

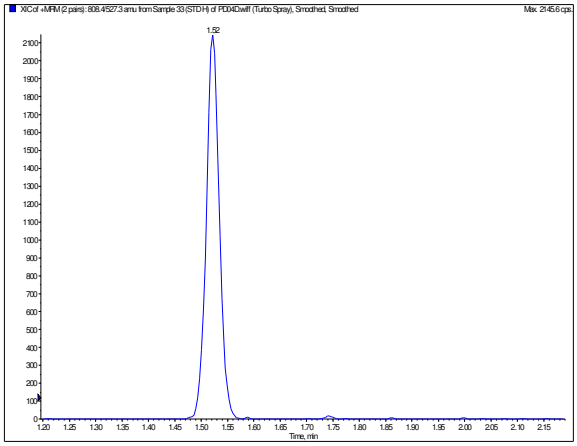
Results

The following samples are prepared in control human plasma:

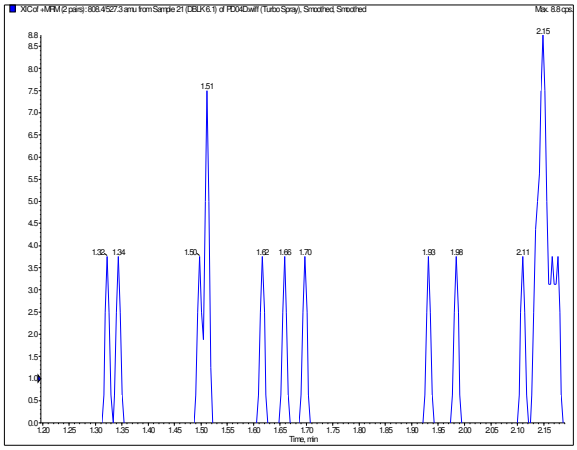
- Calibration standards: 5, 7.5, 10, 25, 50, 75, 100, 250, 500, 750, 1000 and 2000 ng/mL
- QC samples: 5, 10, 200 and 1400 ng/mL, as 6 replicates for each concentration, from 6 individual plasma lots
- Double blanks, from 6 individual plasma lots
- Single blanks, from 6 individual plasma lots

Typical Chromatography

50 ng/mL Docetaxel in Human Plasma (RT 1.52 min)



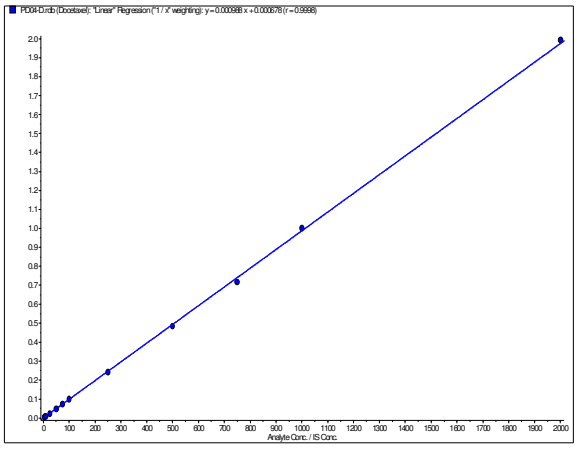
Human Plasma Double Blank



Linearity, Accuracy, Precision And Carry-Over

Linearity was evaluated by injecting a full set of calibration standards. Regression analysis of the calibration data was determined, with a correlation coefficient (r) of 0.9998 and a 1/concentration weighting.

Docetaxel Calibration Curve - 5 to 2000 ng/mL



Calibration Standard Back-Calculated Accuracy

Calibration Standard (ng/mL)	Accuracy (%)
5 (LLOQ)	95.8
7.5	110

10	98.0
25	97.0
50	99.7
75	102
100	102
250	98.4
500	98.1
750	96.9
1000	102
2000 (ULOQ)	101

(Acceptance Criteria: ≤ ±15% of the Theoretical Value; ≤ ±20% for the LLOQ)

QC Sample Mean Accuracy And Precision Over 6 Individual Plasma Lots

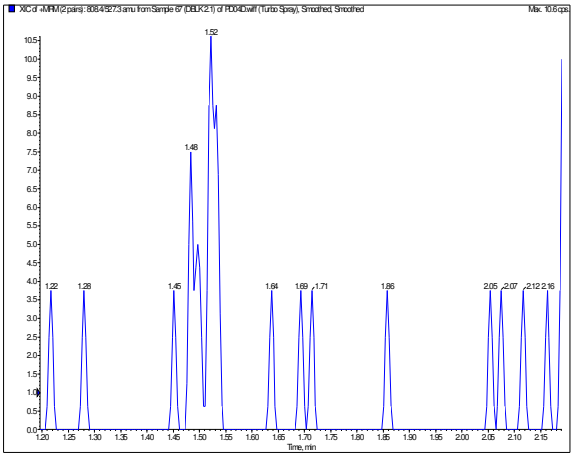
QC Sample (ng/mL)	Acceptable Replicates	Accuracy (%)	CV (%)
5	6/6	97.8	12.6
10	5/6	93.0	8.0
200	6/6	99.3	4.3
1400	6/6	91.5	3.8

(Acceptance Criteria: ≤ ±15% of the Theoretical Value; ≤ ±20% for the LLOQ)

Immediate And Accumulative Carry-Over

A double blank sample injected immediately after the ULOQ showed an insignificant peak at 4.8% of the LLOQ peak area response. While a double blank injected after 6 replicate injections of the high QC sample (1400 ng/mL), showed the absence of any obvious peak.

Human Plasma Double Blank After 6 High QC Sample (1400 ng/mL) Injections



Conclusions

The development of this assay on the **Symbiosis™ Pharma,** demonstrated the speed of transfer from an off-line, to an on-line XLC-MS assay, (~1.5 days, with optimization).

Off-line sample preparation time was reduced from two thirds to one third of a typical working day. In addition, the automated overlap between the extraction of the "next" sample, during the chromatographic run of the "current" sample, the production of a "cleaner" final extract (versus protein precipitation), and the reduced carry-over from the **Reliance™** autosampler, allowed for an XLC-MS cycle-time of 4.3 min. Therefore, in comparison with our previous assay, a typical batch size of 80 samples would take approximately 8.2 hours to analyse from start to finish, versus 17.5 hours. Faster analysis times could be achieved with further optimization of the **Symbiosis™ Pharma.**

About Spark

Since 1982 Spark has provided the HPLC and LC/MS markets with state-of-the-art autosamplers, column ovens and sample preparation solutions. Solid Phase Extraction with on-line elution into HPLC and LC/MS systems was pioneered by Spark and introduced in the early 90's. Spark, ISO 9001 certified, does basic research, product development, production, sales and marketing in-house, guaranteeing quality from start to finish. With 25% of the employees working in research and development Spark continues to invest in the future, making sure we can deliver the solutions you need to improve your business results. Innovation and quality are keywords when talking about our development efforts.

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About Charles River Laboratories Montreal

Charles River Laboratories Montreal, one of the world's largest toxicology CROs, provides a comprehensive range of services dedicated to *in vitro* and *in vivo* drug metabolism / toxicokinetics / pharmacokinetics, and extensive analytical chemistry and bioanalytical capabilities to support both preclinical and clinical trials. Specialties include LC-MS/MS, immunology, immunoassay, CYP450, and QWBA assessments.

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