AUTOMATED SOLID PHASE EXTRACTION OF TRIPTORELIN USING ANDREW+ PIPETTING ROBOT FOR BIOANALYTICAL LC-MS/MS QUANTITATION

Anita Thorimbert[1], Christophe Chardonnens[1], Melvin Blaze[2]

[1]Debiopharm International SA, Lausanne, Switzerland; [2]Waters Corporation, Milford, USA

INTRODUCTION

Solid phase extraction (SPE) is a commonly used sample preparation technique in bioanalytical liquid chromatography mass spectrometry (LC-MS) quantitation of analytes in complex biological samples. Most SPE workflows involve several steps of pipetting and transfer of samples, reagents, and solvents. Automation of these pipetting and transfer workflows using expensive liquid handlers often involve complex programming, needing expert, trained and dedicated personnel to perform the task. Performing workflows manually on the other hand can be extremely tedious and prone to errors, requiring good analytical skills to produce reproducible result. In this abstract an automated SPE workflow for a quick, reliable and reproducible quantitation of triptorelin from rat serum using the Andrew+ pipetting robot, connected and operated using OneLab™ software, an easy-to-use browser-based interface is demonstrated.

Figure 1. Andrew+ Pipetting Robot together with OneLab software cloud-native software

Objectives:

- To adapt and automate the manual SPE extraction of triptorelin from rat serum using the Andrew+ pipetting robot operated using OneLab software.
- Compare robustness, accuracy and reproducibility of the manual to automated SPE workflow using triptorelin spiked rat serum calibrants (C) and quality control (QC) samples.

METHODS

Method automation strategy

❖ **Automation**: Automate the whole rat serum SPE extraction procedure into two OneLab software protocols: one for the working solutions (WS) preparation and the second for the rat serum spiked calibrants (C) and quality control (QC) sample preparation and solid phase extraction.

❖ **Manual Sample Preparation:** Blank rat serum manually spiked and then processed with the Andrew+ as unknow samples.

❖ **LC-MS analysis:** The SPE extracted C/QC/manual samples were then analyzed using Waters™ ACQUITY UPLC™ I-Class coupled to Xevo™ TQ-S in multiple reaction monitoring, positive ionization mode (MRM).

Triptorelin rat serum SPE method summary

Calibration-curve and QC at low-mid-high concentrations were prepared in replicates by spiking blank rat-serum with working-solution of triptorelin (previously prepared using Andrew+ with a dedicated protocol) and IS solution (^{13}C6.^{15}N Leu^{7} Triptorelin).

❖ 5µL of corresponding calibration working solutions (set A) were added to 95 µL of blank rat serum
Calibration range: 50 pg/ml to 20'000 pg/mL
❖ 5µL of corresponding QC working solutions (set B) were added to 95 µL of blank rat serum
QC levels: low QC (150 pg/ml), mid QC (3'000 pg/mL) and High QC (18'000 pg/ml)

The spiked serum C/QC/samples were diluted in water and processed using Oasis™ MAX µelution SPE plate after conditioning and equilibrating with methanol and 0.2% aqueous ammonium hydroxide respectively prior to loading.

Oasis MAX µelution SPE plates were successively washed using 0.2% aqueous ammonium hydroxide and 5% methanol, followed by analyte elution using methanol.

ANDREW+ PROTOCOLS SETUP

Working solution preparation protocol

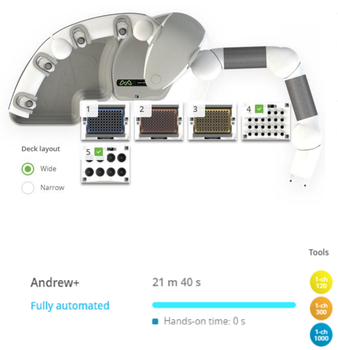

Figure 2 Andrew+ deck configuration for the triptorelin working solutions preparation protocol.

[1-2-3] Tips insertion system with 5-120 µL / 10-300 µL / 50-1000 µL Optifit tips

[4] Microtubes domino with 2 mL safe-Lock tubes

[5] 50mL conical tubes domino with 50 mL conical centrifuge tubes

C/QC spiking + C/QC/samples solid phase extraction (SPE) protocol

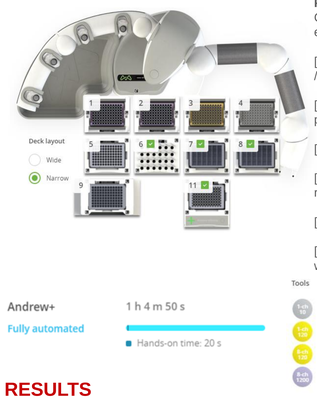

Figure 4 Andrew+ deck configuration for the triptorelin C/QC preparation and then the C/QC/samples SPE extraction protocol.

[1-2-3-4] Tips insertion system with 0.1-10 µL / 5-120 µL / 50-1200 µL Optifit tips

[5] Storage plate domino with 0,5 mL 96-deep well protein LoBind plate -> elution plate

[6] Microtubes domino with 2 mL safe-Lock tubes

[7-8] Deepwell microplate domino with 6-column reagent reservoir

[9] Vacuum+ device with Oasis MAX µelution SPE plate

[11] Microplate Shaker+ device with 0,5 mL 96-deep well protein LoBind plate -> sample plate

LC-MS conditions

Instruments: ACQUITY UPLC I-CLASS coupled to Xevo TQ-S triple quadrupole mass spectrometer
Data Analysis: MassLynx™ v4.2
LC method conditions:
Column: ZORBAX™ Eclipse Plus C8, 2.1 x 50mm 1,8µm (with pre-column)
Column temperature : 40 ºC
Injection Volume: 10 µL
Flow rate: 0.6 mL/min
Mobile Phase A: 0.1 % (v/v) Formic acid in Water
Mobile Phase B: 0.1 % (v/v) Formic acid in Methanol
Gradient Conditions:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
Initial	80	20
1,2	80	20
2,0	40	60
2,6	40	60
4,2	2	98
4,7	2	98
5	80	20
5,5	80	20

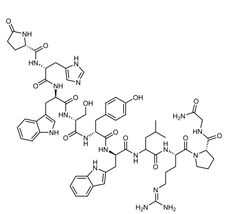

Figure 3. Structure of Triptorelin

MS conditions: ESI Positive; Source Temp(ºC): 150; Desol Temp(ºC): 600; Cone Gas Flow (L/Hr): 200; Desol Gas Flow (L/Hr): 1000; Collision Gas Flow (mL/min): 0.15; Nebuliser Gas Flow (Bar): 7.0
MRM detection parameters:

Compound Name	Precursor (m/z)	Product (m/z)	Cone (V)	Collision (V)	Dwell time (s)
Triptorelin	656.5	249.1	60	30	0.06
^{13}C6.^{15}N Leu^{7} Triptorelin	660.0	249.1	8	30	0.06

RESULTS

Linearity and reproducibility

The duplicate calibration curves (Nine-point calibration ranging from 50 pg/ml to 20'000 pg/mL of spiked rat serum) produced a linear regression co-efficient using a weighing factor of 1/X^2.

Figure 5 Triptorelin spiked rat serum calibration curve established with TargetLynx XS v4,2 software

Calibrant and Quality Control accuracy

Accuracy of quality control and calibration spiked and then extracted with the Andrew+ robot were within the acceptance criteria.

Calibrant level	Accuracy % Calibration curve1	Accuracy % Calibration curve 2
C1	excluded	92.3
C2	111.9	110.1
C3	96.6	92.1
C4	104.0	96.1
C5	98.8	94.5
C6	97.4	94.2
C7	96.4	97.0
C8	100.5	106.3
C9	excluded	111.6

Quality Control level	Accuracy % Replicate 1	Accuracy % Replicate 2	Accuracy % Replicate 3	Accuracy % Replicate 4
QC Low	97.8	94.6	86.0	104.6
QC Med	105.0	107.7	104.7	113.7
QC High	106.3	105.1	108.6	103.9

RESULTS

Manually spiked vs robot spiked quality controls

The accuracy of the manually spiked QC (used to mimic unknow samples) processed with the Andrew+ and the accuracy of the QC spiked by the Andrew+ robot were within the accuracy acceptance thresholds.

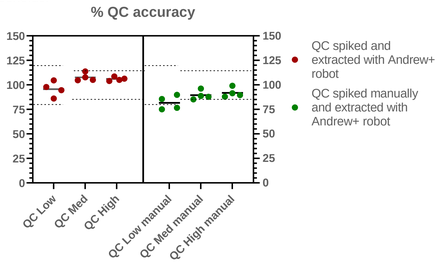

Figure 6 Comparison of QC spiked manually vs QC spiked with the robot; all then processed with the Andrew+

DISCUSSION

❖ The accuracy of calibration curve and QC standards were within the acceptance limits of ±15% for Andrew+ prepared samples.

❖ Manually spiked vs robot spiked quality controls demonstrated its reliability and precision.

❖ The entire automated workflows (working solutions, C-QC preparation and SPE extraction of 50 incurred samples) using the Andrew+ were performed in 1,5 hour demonstrating throughput in addition to accurate and reproducible quantitation, comparable to manual sample processing.

SPE extraction of triptorelin from rat serum was completely automated using the Andrew+ pipetting robot and the accuracy and reproducibility of the automated workflow was within the acceptable limit and comparable to the manual workflow. This allowed for quick, reliable and reproducible sample preparation for quantitation on UHPLC-MSMS systems.

CONTACT

Debiopharm International S.A., Lausanne, Switzerland.
www.debiopharm.com
christophe.chardonnens@debiopharm.com

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