A Reduced Workflow Method For The Extraction Of Vitamin B7 (biotin) From Human Serum With No Drydown Prior To Mixed Mode LC-MS/MS

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Introduction

The metabolic precursor, vitamin B7, is detailed here as an analytical target for consideration of reduced workflow sample preparation strategies. Recent reports suggest concentrations levels for healthy adults are on the order of µg/mL. This elevated range creates a forgiving field for analytical requirements in method development. Peadiatric and adult deficient patients have been determined to be <100 ng/mL requiring a different approach in sample prep. The structure of this analyte is detailed in Figure 1. In this study, the sorbent conditioning and equilibration steps familiar to traditional sample preparation methods have been eliminated by leveraging recent advances in SPE 96 well plate technology. A third method step is considered for elimination. The evaporation/reconstitution step is considered by decision making in the formulation of elution chemistry and mobile phase. The analytes of interest were fortified into pooled mixed gender serum. The samples were loaded on to a 10mg 96 well plate. A comparison of extracts prepared in a traditional polymeric ion exchange cartridge format is provided. The extracted serum samples were measured using a gradient mixed-mode LC-MS/MS method.

Analytes of Interest

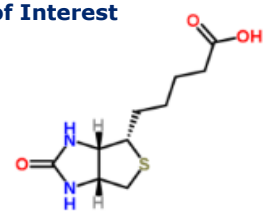

Figure 1: Structure of biotin

Experimental Procedure

Reagents

HPLC grade water, methanol, ammonium hydroxide, biotin and formic acid (FA) were purchased from Sigma-Aldrich Co. (Atlanta, GA.). The biological fluids were obtained from BioChemEd services (Winchester, VA).

Mass Spectrometry

Detection of the target analyte was optimized using an Applied Biosystems /MDS Sciex 4000 Q-Trap hybrid triple quadrapole / linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface operated in positive ion mode. The MRM transition used in this study was detailed in Table 1.

Table 1: *MS/MS transitions for the detection of vitamin B7*

Analyte	Mol. Wt. (g/mole)	MRM transition (m/z)	DP	CE	Dwell time (ms)
biotin	244	245 → 227	40	30	300
qualifier ion	244	245→ 166	40	36	300

Chromatography

Chromatographic separation was accomplished using the Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK). Optimized gradient chromatographic conditions were identified using a IMTAKT Scherzo SM-C18 column (2 mm x 150mm, 3.0 µm). The injection volume was 20 µL. The gradient conditions were given in Table 2. The mobile phase A and B were 0.1% formic acid (aq) and 0.1% formic acid in MeOH respectively.

Table 2: *Gradient parameters for vitamin B7 (biotin)*

Time (min)	flow rate (µL/min)	A(%)	B(%)
0	150	98	2
0.5	150	98	2
5	150	2	98
5.5	150	2	98
6.55	150	98	2
9.5	150	98	2

Optimized SPE methods

Samples were extracted using Biotage EVOLUTE EXPRESS AX extraction materials. The sorbent chemistry is an EVOLUTE® backbone, surface modified with quaternary amine groups (Figure 2). Relative recovery comparisons between plates and cartridges are detailed in Figs 3 and 4.

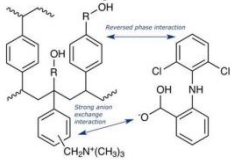

Figure 2: *The Biotage EVOLUTE AX sorbent*

EVOLUTE AX EXPRESS 96 well plates / 10mg

The method for plates was described in Table 4. Samples were processed under positive pressure with a Biotage Positive Pressure Manifold PPM96. The sample pretreament was 25 µL serum + 75 µL 0.1% NH_4OH(aq). Samples were vortexed prior to loading. Automation for this method was not available at the time of this study. *required mobile phase modification: isocratic 80/20%, solvents A/B: A = 2% FA (aq) ; B = MeOH.

Table 3: *Sample preparation method – EVOLUTE EXPRESS AX*

Step	Source	Volume (µL)
Load	pretreated sample	100
Wash 1	0.1% NH_4OH(aq)	1,000
Wash 2	MeOH	1,000
Elute	98/2 MeOH/FA	2 x 75
Post-elution	Dilute sample with H_2O	37.5

EVOLUTE AX-50 cartridges 100mg / 3mL

The optimized method for cartridges was described in Table 3. These samples were processed under positive pressure with a Biotage Positive Pressure Manifold PPM48. The evaporation step was performed using a Biotage TurboVap LV (45°/N_2 20psi). Automation of this method was demonstrated using a Biotage RapidTrace$^+$ workstation. The sample pretreament was 250 µL serum + 750 µL 0.1% NH_4OH(aq). Samples were vortexed prior to loading.

Table 4: *Sample prep method – EVOLUTE AX*

Step	Source	Volume (mL)
Condition	MeOH	3
Equilibration	0.1% NH_4OH(aq)	3
Load	pretreated sample	1
Wash 1	0.1% NH_4OH(aq)	3
Wash 2	MeOH	3
Elute	98/2 % MeOH/FA	2 x 1
Post – extraction Evaporate/ recon	0.1% FA	0.1

Results

A comparison of relative recovery data for the cartridge method processed manually and with automation is detailed in Figure 3. An improvement in recovery was noted possibly due to improved control of flow rates. In addition, analyte suppression experiments demonstrated 190% when comparing extracted matrix (spiked post-elution) versus an unprocessed solution standard. These results suggest an enrichment factor of ~1.9. The reduced workflow 96 well plate method was also evaluated for relative recovery. Results were comparable (Figure 4); however, replacing the enrichment factor with a dilution factor signficantly affected method sensitivity. It is anticipated that patient samples from healthy adults could be processed by the reduced workflow 96 well plates. Patient samples collected from pediatric or deficient patients should follow the cartridge format. Screening versus confirmation perhaps? The analysis of patient samples is planned for future work.

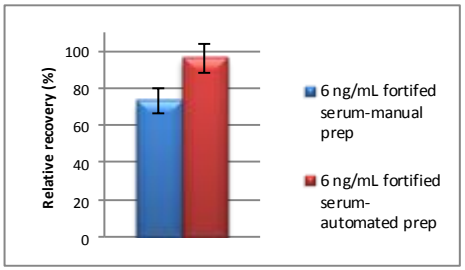

Figure 3: *Relative recovery comparison for the extraction of biotin from human serum using traditional polymeric ion exchange cartridges*

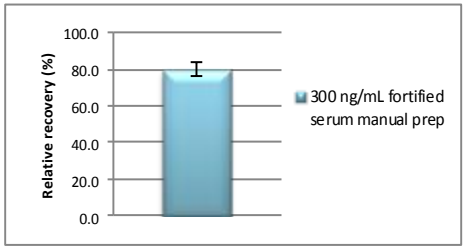

Figure 4: *Relative recovery for the extraction of biotin from human serum using reduced workflow 96 well plates*

Conclusions

The EVOLUTE EXPRESS AX 96 well plate demonstrated the ability to remove 3 steps from a traditional SPE workflow for measuring vitamin B7 in human serum samples.