

Measurement of Amino Acids and Acylcarnitines in Dried Bloodspots by Flow-Injection Analysis/Tandem Mass Spectrometry (FIA-MS/MS) for Clinical Research Use

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When time and resources are precious, rely on the Waters ACQUITY UPLC/Xevo TQD IVD System with NeoLynx 4.2 for seamless analysis and data interpretation.

GOAL

To demonstrate the rapid quantification of amino acids and acylcarnitines extracted from dried bloodspots, while making efficient use of operator and instrument time and minimizing solvent consumption.

BACKGROUND

Equipping a laboratory with liquid chromatography and tandem mass spectrometry enables measurement of natural and synthetic compounds in the most challenging matrices. One such matrix encountered in a clinical research setting is the dried filter spot. Plasma, urine, or whole blood may be collected onto filter paper that is transported to the laboratory for quantification of endogenous or exogenous metabolites, toxins, or for functional evaluation of enzyme activity. High volumes of samples generated, while monitoring biomarker response in clinical research trials, or in the *in vivo* phase of drug discovery, place the laboratory under pressure to deliver a cost-effective solution. Results are needed within a short turnaround, to make economical use of laboratory consumables and personnel time.

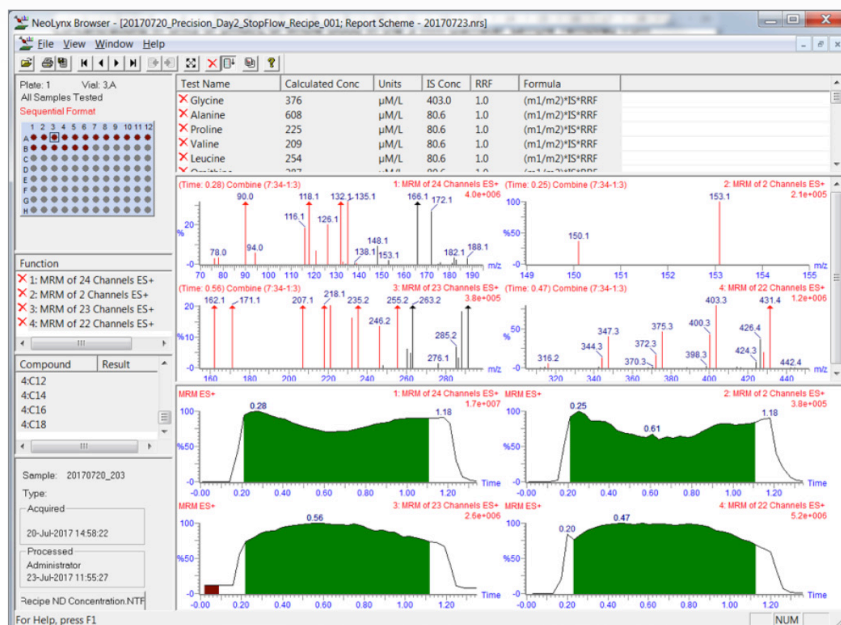


Figure 1. Representative NeoLynx Browser interface.

The Waters® ACQUITY® UPLC®/Xevo® TQD IVD System under the control of MassLynx® Software v4.2 with NeoLynx™ 4.2 Application Manager is designed with features to shorten the injection cycle time and to facilitate the real-time technical review of results for clinical research use. Here, the features of these components are illustrated using the flow-injection analysis of amino acids and acylcarnitines extracted from commercial dried bloodspot control samples.

THE SOLUTION

Control dried bloodspots from the ClinSpot® non-derivatized complete reagent Research Use Only kit (RECIPE Chemicals + Instruments GmbH, Germany) were prepared following the manufacturer's direction for use. Extraction solvent was separated from the bloodspots by centrifugal filtration into a collection plate. Collection plates were queued for analysis in the ACQUITY UPLC Sample Organizer.

FIA-MS/MS was carried out using a variable flow rate pump method. Delivery at low flow rate increases the residence time, allowing the user to optimize the duty cycle for the best possible analytical sensitivity and precision of detection.

Data were acquired for 70 multiple reaction monitoring (MRM) ion transitions for 13 amino acids, 31 acylcarnitines, and 26 internal standards. No data were collected for amino acids or acylcarnitine species not supplemented into the commercial control samples.

Taking advantage of the load ahead and active needle washing features, an injection-to-injection cycle of approximately 1.8 minutes was achieved, allowing a batch of 192 samples to be analyzed and processed in under 6 hours, consuming less than 200 mL of solvent.

Concentrations ($\mu\text{mol/L}$ of whole blood) were calculated using the default concentration formula provided with NeoLynx. The average mass spectral peak height intensity was calculated from the central region of the signal, as shown in the total ion chromatogram pane of the NeoLynx Browser (Figure 1).

The RECIPE quality control bloodspots were measured with precision summarized in Table 1. Within-batch imprecision ($n=10$) was $<15\%$ CV for all compounds listed in Table 1, above the LOD.

| Amino acid | Low QC (%CV) | High QC (%CV) | Acylcarnitine | Low QC (%CV) | High QC (%CV) |
|---------------|--------------|---------------|----------------------------------|--------------|---------------|
| Glycine | 7.3 | 4.4 | Free (C ₀) | LOD | 4.7 |
| Alanine | 7.5 | 4.3 | Acetyl (C ₂) | LOD | 4.6 |
| Proline | 7.1 | 4.0 | Propionyl (C ₃) | 9.0 | 5.9 |
| Valine | 7.4 | 4.2 | Butyryl (C ₄) | 7.5 | 4.1 |
| Leucine | 7.3 | 4.5 | Isovaleryl (C ₅) | 8.5 | 5.4 |
| Ornithine | 12.5 | 9.9 | Hexanoyl (C ₆) | 12.8 | 5.7 |
| Aspartic acid | 11.4 | 7.0 | Glutaryl (C ₅ DC) | LOD | 4.3 |
| Glutamic acid | 7.3 | 4.0 | Octanoyl (C ₈) | 7.8 | 4.8 |
| Phenylalanine | 7.4 | 4.2 | Decanoyl (C ₁₀) | 7.3 | 4.4 |
| Citrulline | 6.4 | 2.3 | Dodecanoyl (C ₁₂) | 8.8 | 4.8 |
| Tyrosine | 7.3 | 4.0 | Tetradecanoyl (C ₁₄) | 8.3 | 4.9 |
| Methionine | 7.6 | 4.2 | Palmitoyl (C ₁₆) | 8.6 | 5.5 |
| | | | Octadecanoyl (C ₁₈) | 9.3 | 6.3 |

Table 1. Between-batch imprecision ($n=5$, in 5 batches). LOD (limit of detection): This compound was measured with a signal to noise ratio of approximately 3:1. Target concentrations available from www.recipe.de/en.

SUMMARY

Flow-injection analysis using the ACQUITY UPLC/Xevo TQD IVD System allowed fast data acquisition with precision for many of the compounds. Low flow rates and short injection cycles minimized solvent consumption. Data reduction with NeoLynx 4.2 Application Manager provided the flexibility to tailor the processing workflow.

Data processed by NeoLynx Application Manager upon completion of the injection, enabled review and approval of results before batch completion. Technical review of the batch was further expedited by presentation of multiple total ion chromatograms and mass spectra in the NeoLynx Browser, using the hands-free Auto Review feature.

For convenience, NeoLynx automatically generated a .txt file electronic report and saved it to a network directory for remote data interrogation. For security, NeoLynx Result Files were encrypted, preventing unauthorized viewing of results with third-party software. For traceability, the NeoLynx Result File was permanently linked to a read-only copy of the processing file, and the NeoLynx Modification History audit trail recorded any changes made to the processing method.

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