Flow Injection-Tandem Mass Spectrometry for Inborn Error Metabolism Research Using a Meta Calculation Software

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Overview

Purpose: Streamline tedious and multiple steps of manual calculations; remove transcription errors in post-analytical phase of testing processing.

Methods: Use a new software to automatically process raw data files generated from flow injection tandem MS analysis of amino acids and acylcarnitines in dried blood spot cards.

Results: A total of 1312 calculations from 41 donor samples were compared between single step software processing with multiple-steps manual calculations, including 779 analyte peak areas, 410 analyte concentrations and 123 user defined formulas. An agreement of results was demonstrated, and processing time reduced from hours to minutes.

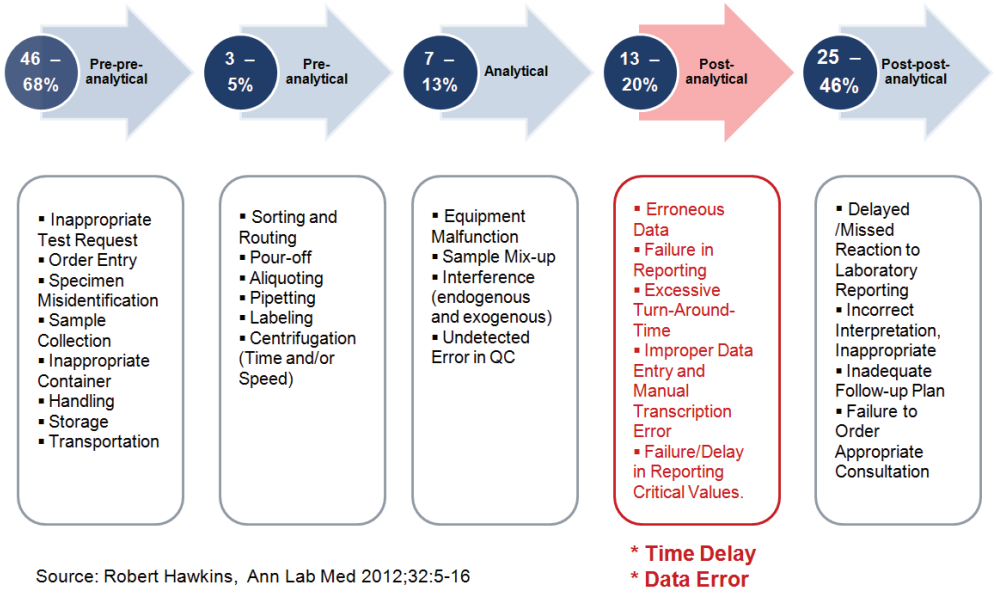
Introduction

Since bacterial inhibition method was first developed for research of inborn error metabolism in 1960, the technology has changed drastically from EIA, RIA, FIA, ELISA to LC and Tandem MS [1] over the past 50 years.

Tandem MS allows for higher quality results compared to the old approaches. However, manual data processing in the post-analytical phase still remains a common cause of errors in the total testing processing (Figure 1) [2].

This research describes a method of flow injection-tandem MS in analyzing donor samples for the quantitation of amino acids and acylcarnitines with a meta calculation software.

FIGURE 1. Common Errors in Total Testing Processing



Source: Robert Hawkins, Ann Lab Med 2012;32:5-16

Methods

Sample Preparation

Samples were extracted from dried blood spot cards; the internal standards were added during the extraction procedure and extracted samples were derivatized prior to injection onto an LC-Tandem MS system. Quality Control (QC) samples were added to the batch.

Liquid Chromatography Tandem Mass Spectrometry

The flow injection was conducted using a LC with open-tube providing an automated sample introduction to a Tandem MS (Thermo Fisher Scientific, San Jose, CA) without chromatographic separation. The Tandem MS used selected reaction monitoring (SRM) scanning for the detection of amino acids and acylcarnitines. Transitions used in this study are listed in Table 1.

TABLE 1. SRMs Monitored for Amino Acids and Acylcaritines

Analyte	Precursor (m/z)	Product (m/z)	Analyte	Precursor (m/z)	Product (m/z)
Cit	232.10	113.10	C0	218.25	85.00
Cit IS	234.10	115.10	C0 IS	227.25	85.00
Met	206.15	104.10	C8	344.25	85.00
Met IS	209.20	107.10	C8 IS	347.25	85.00
Orn	189.20	70.20	C14	428.35	85.00
Orn IS	191.20	72.20	C14:1	426.35	85.00
Phe	222.10	120.10	C14 IS	437.35	85.00
Phe IS	228.20	125.90	C16	456.35	85.00
Tyr	238.10	136.10	C16 IS	459.35	85.00
Tyr IS	244.10	142.10			

A new meta calculation software, iRC PRO™ was used for offline automated calculation of raw data files generated from Tandem MS in SRM scanning mode. This beta version software is developed for an automatic calculation of mass ion ratio and user defined formulas. Software workflow is shown in Figures 2 and 3.

FIGURE 2. Intuitive Workflow – Icon Based User Interface

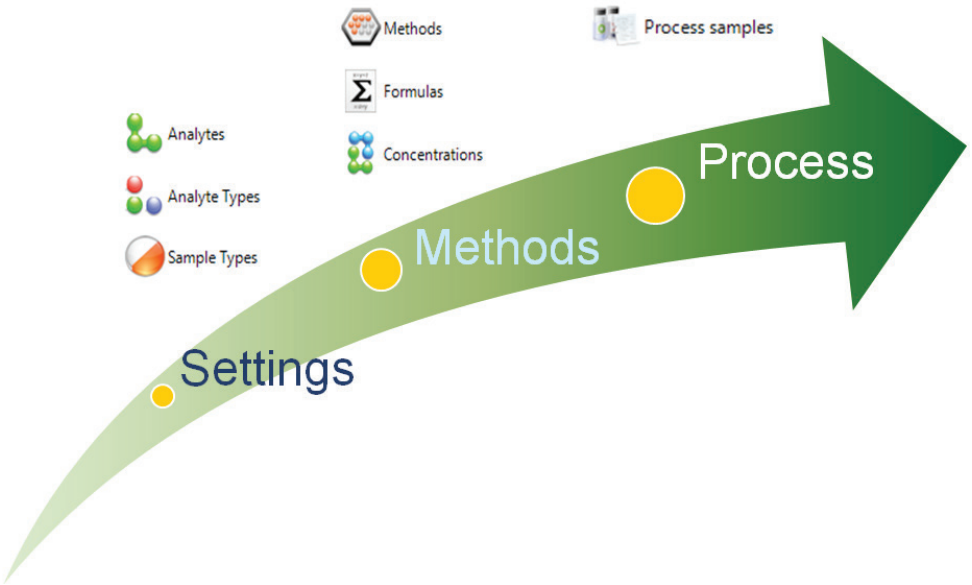
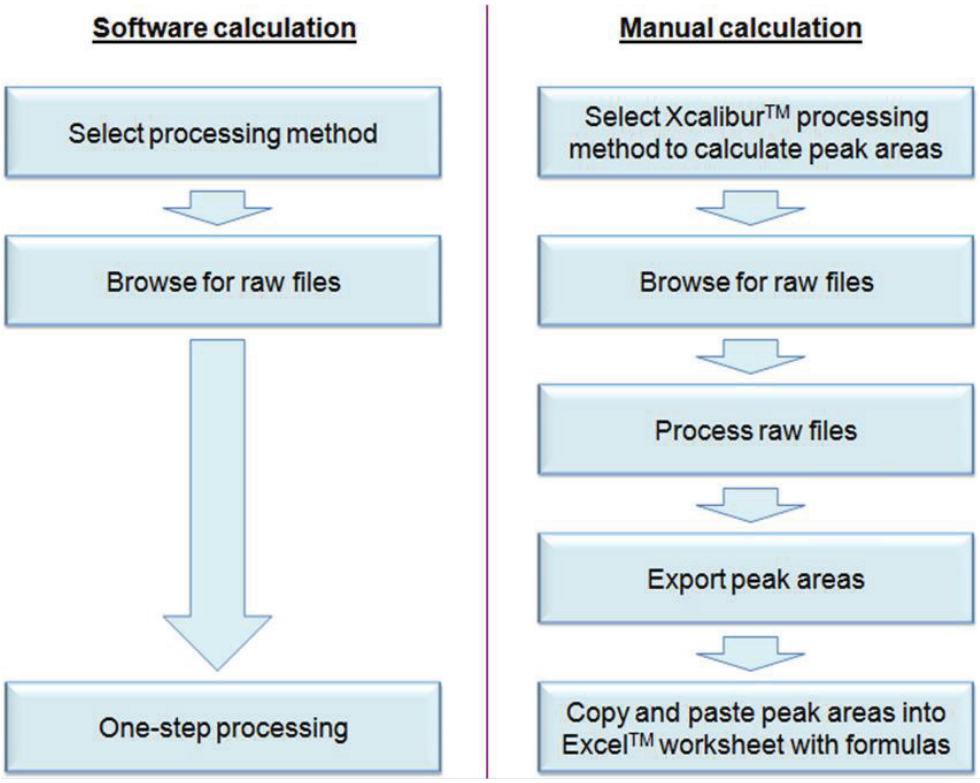


FIGURE 3. Workflow Comparison between Software and Manual Approach



Data Analysis

Manual Calculation: Manual calculation was performed by creating a processing method to extract chromatograms and calculate peak areas for each analyte and IS using Thermo Scientific™Xcalibur™ software.

Peak areas were exported in Excel™ format and copied and pasted into an Excel worksheet setup to calculate analyte concentrations and values based on the same formulas used by the meta-calculation software.

Software Calculation: The SRM transitions for each analyte and internal standard are entered in the software for data analysis; IS concentration and analyte/IS relative response factor are also entered to calculate analyte concentration.

User defined formulas can be created to perform calculations using peak areas or analyte concentrations.

Upper and lower concentration limits can be set for each analyte; different values can be used for unknown and quality control samples; the software will flag samples outside these acceptance ranges. The same applies to user defined formulas.

A processing method is created by selecting the peak areas, analyte concentrations and user defined formula results that will be displayed by the software. Results can be exported in Excel or text format.

Results

A total of 41 samples and 779 analytes were processed, and a total of 1312 calculations were performed, including 799 analyte peak areas, 410 analyte concentrations and 123 user defined formulas.

The comparison of result of this sample set shows that over 99% of calculations of analyte peak area and concentration (Analytes and Formulas) are within 10% of bias. Over 87% of Formulas Ratios are within 10% of bias. Table 2 below shows comparison between software calculations (One-Step) and manual calculations (Multiple-Steps). Figures 4 and 5 show additional statistics for the comparison.

TABLE 2. Comparison between Software and Manual Calculations

Type of Calculations	Analyte/ Formula	Number (N)	Bias %	R2	Linearity Equation	Value Range
Analyte Peak Area	19 Analytes	779	<20%	0.9997	Y = 16296 + 0.9998 X	17,058 – 53,942,008
		774	<10%			
		721	<5%			
Analyte Concentrations	C0, C8, C14, C14:1, C16, Cit, Met, Orn, Phe, Tyr	410	< 20%	0.99864	Y = 0.05927 + 0.99932 X	0.81 – 199.15
		407	< 10%			
		378	< 5%			
Formula Concentrations (User Defined)	F1=C0+C14:1	41	< 5%	0.99916	Y = -0.77030 + 0.99958 X	43.98 – 167.04
Formulas Ratios (User Defined)	F2=(Orn-Phe) /Tyr F3=(C8+C14:1-C16)/ (Orn+Tyr)	82	< 40%	0.99661	Y = 0.00394 + 0.99830 X	-0.98 - 2.25
		79	< 20%			
		72	< 10%			
		61	< 5%			

FIGURE 4. Residual Plot of 779 Calculations of Analyte Peak Area from 41 Donor Samples (Y = 16296 + 0.9998 X R2 = 0.9997)

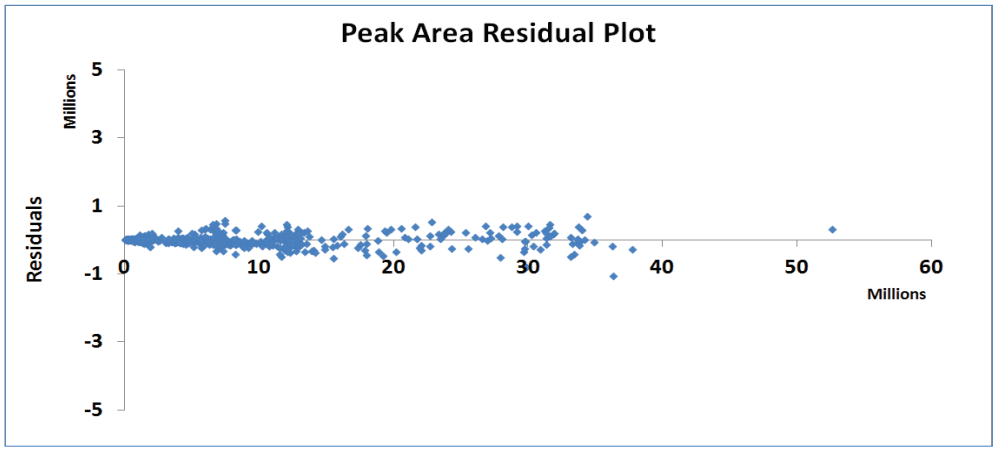
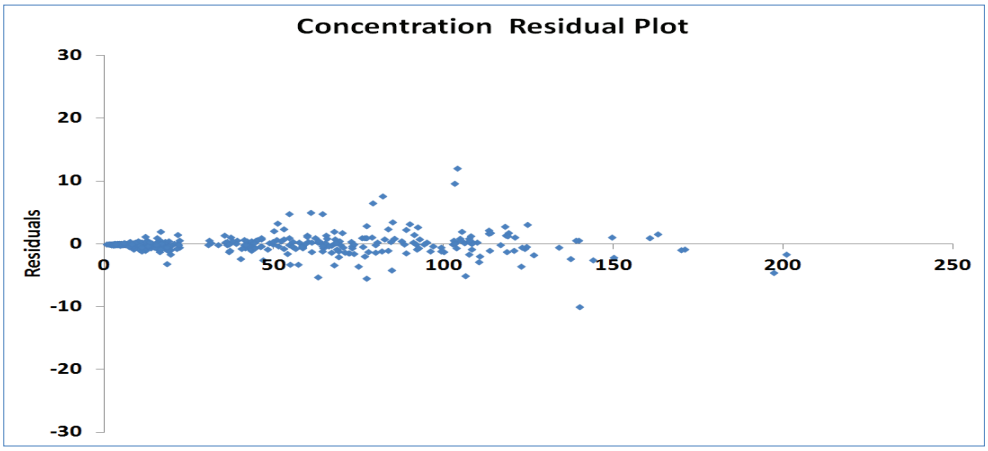


FIGURE 5. Residual Plot of 410 Calculations of Analyte Concentrations from 41 Donor Samples (Y = 0.0593 + 0.9993 X R2 = 0.9986)



Conclusion

This off-line automated data processing tool shows a good agreement with manual calculation process, and it can process both concentration and user defined formulas.

This meta calculation software improves time effectiveness by eliminating the manual calculation process and removing transcription errors in the post-analytical phase. The processing time is reduced from hours to minutes.

References

1. Millington DS, Kodo N, Norwood DL, Roe CR. J Inherit Metab Dis 1990;13:321-4.
2. Robert Hawkins, Ann Lab Med 2012; 32: 5-16

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