

The Use of A New Meta-calculation Software for Automated Data Processing of Tandem MS for Inborn Error Metabolism Research

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Overview

Purpose: Streamline tedious and multiple steps of manual calculations; remove transcription errors in post-analytical phase of testing processing; improve turn-around-time of data analysis.

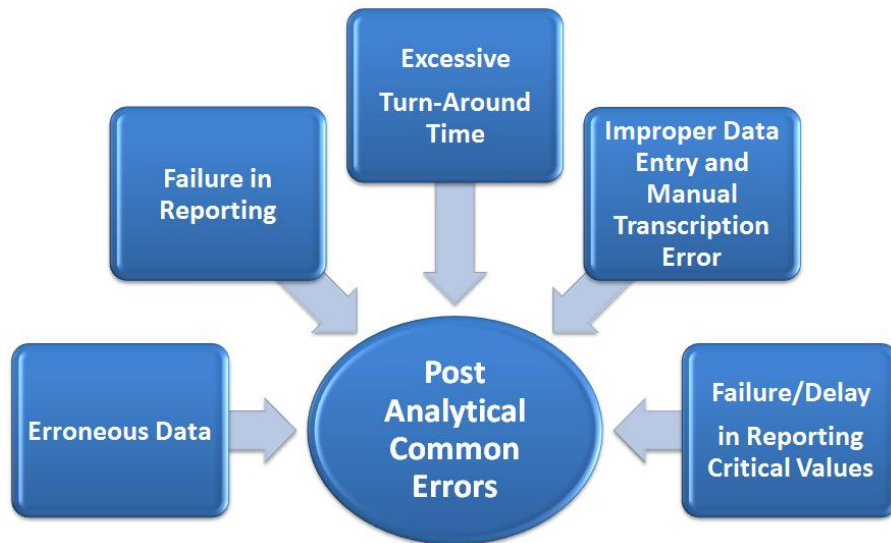
Methods: Use a new software for research to automatically process raw data files generated from flow injection tandem MS analysis of amino acids and acylcarnitines in dried blood spot cards.

Results: A total of 3200 calculations from 100 donor samples were compared between single step software processing with multiple-steps manual calculations, including 1,900 analyte peak areas, 1,000 analyte concentrations and 300 user defined formulas. An agreement of results was demonstrated, and processing time reduced from hours to minutes.

Introduction

The use of Tandem MS for inborn error metabolism research started in early 1990 [1]. With advancement of Tandem MS technology, more compounds can be detected and quantified using a simple sample introduction method such as flow injection with isotopic internal standards. A major challenge is to process a large quantity of generated data efficiently without transcription errors [2] (Figure 1).

FIGURE 1. Common errors in post analytical phase of testing



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 Acylcarnitines

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			

Meta Calculation Software

A new meta calculation software, iRC PRO (2Next srl, Prato, Italy) was used for offline automated calculation of raw data files generated from Tandem MS in SRM scanning mode. This software is designed specifically for Thermo Scientific™ TSQ Tandem MS. This beta version of software is developed for an automatic calculation of mass ion ratio and user defined formulas.

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 (Microsoft Co.) 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.

As depicted below, Figure 2 shows the workflow of software, and Figure 3 shows workflow comparison between software and manual process.

FIGURE 2. Intuitive Workflow – icon based User Interface

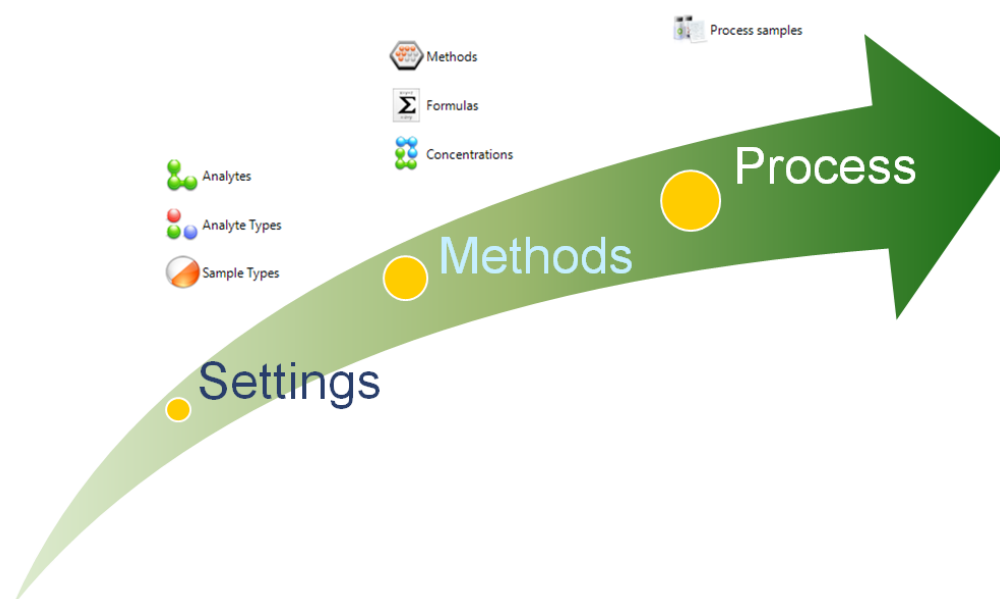
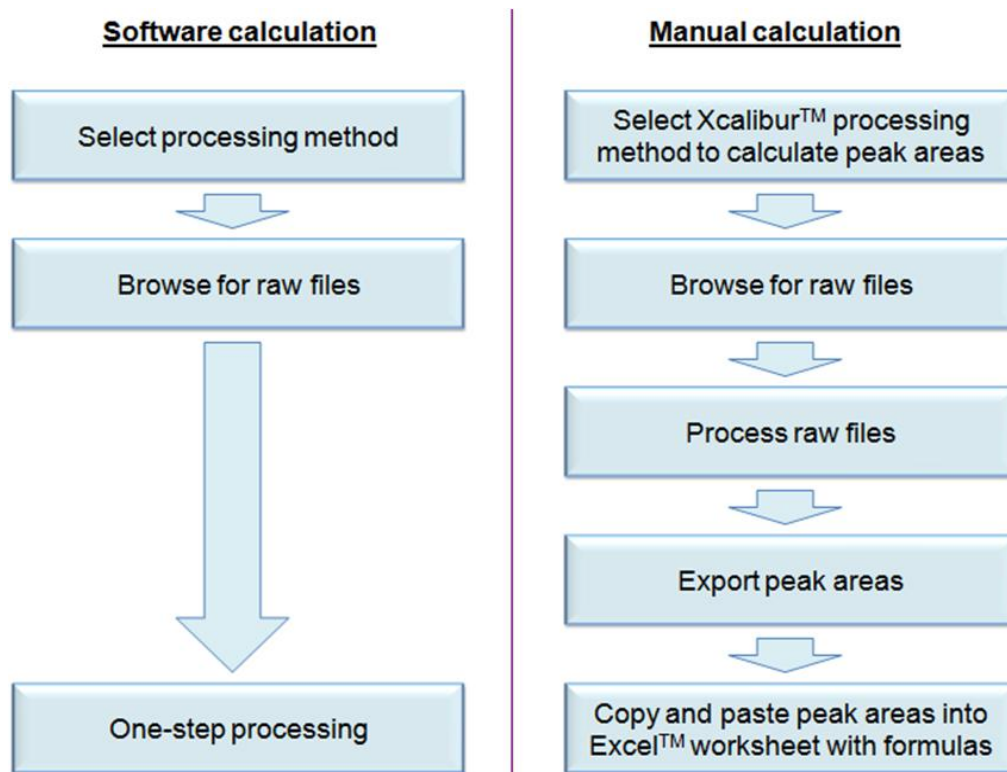


FIGURE 3. Workflow Comparison between Software and Manual Approach



Results

Over 96% of calculations of analyte peak area and concentration (Analytes and Formulas) are within 10% of bias. Over 82% of Formulas Ratios are within 10% of bias. Table 2 below shows comparison between software calculations and manual calculations.

TABLE 2. Comparison between software and manual calculations

Type	Analyte/ Formula	Number (N)	Bias%	Value Range
Analyte Peak Area	19 Analytes	1,900	< 48%	17,400 – 174,827,146
		1898	< 30%	
		1894	< 20%	
		1842	< 10%	
		1672	< 5%	
R2 = 0.999438 Y = - 7879 + 0.999719X				
Analyte Concentration	Cit, Met, Orn, Phe, Tyr, C0, C8, C14, C14:1, C16	1,000	< 45%	0.62 – 431.51
		998	< 30%	
		993	< 20%	
		958	< 10%	
		845	< 5%	
R2 = 0.997733 Y = - 0.19774 + 0.998866X				
Formula Concentration (User Defined)	F1=C0+C14:1	100	< 5%	20.83 – 386.55
	R2 = 0.999544 Y = 0.174589 + 0.999772X			
Formula Peak Area (User Defined)	F2=(Orn-Phe)/Tyr F3=(C8+C14:1- C16)/(Orn+Tyr)	200	< 30%	-1.4403 – 3.534176
		192	< 20%	
		165	< 10%	
		113	< 5%	
		R2 = 0.991617 Y = 0.004626 + 0.995799X		

Figures 4 and 5 show additional statistics for the comparison.

FIGURE 4. Residual plot of 1900 calculations of analyte peak area from 100 donor Samples

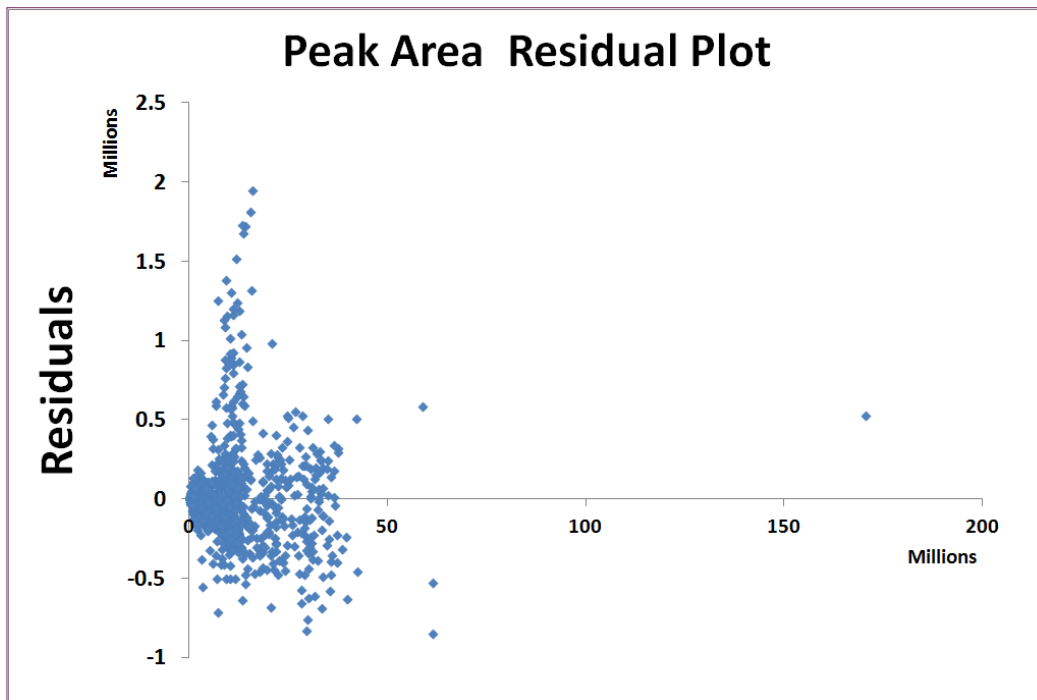
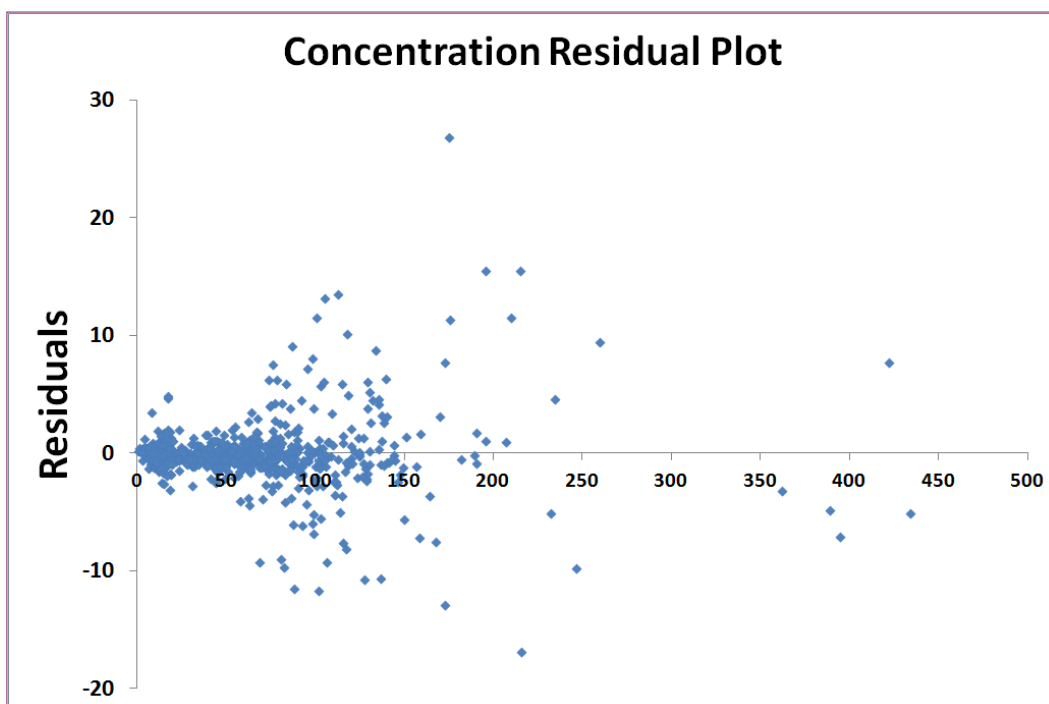


FIGURE 5. Residual plot of 1000 calculations of analyte concentrations from 100 donor Samples



Conclusion

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

This meta calculation software for research 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

Acknowledgements

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