Open-Tube Flow Injection Liquid Chromatography-Tandem Mass Spectrometry for In-Born Errors of Metabolism Disorder Research Using a Meta Calculation Software

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Overview

Purpose: The research reported here describes a new approach in analyzing donor samples for the quantitation of amino acids and acylcarnitines using open-tube flow injection liquid chromatography tandem mass spectrometry (LC-tandem MS) with a meta calculation software.

Methods: The open-tube flow injection liquid chromatography provides an automated front-end sample introduction for a tandem mass spectrometer. The tandem MS used selected-reaction monitoring (SRM) for the detection of amino acids and acylcarnitines. This beta version software is developed for an automatic calculation of mass ion ratio and user defined formulas using data files generated from tandem MS.

The performance of this beta software is compared with manual multiple-step calculation using a Excel® worksheet.

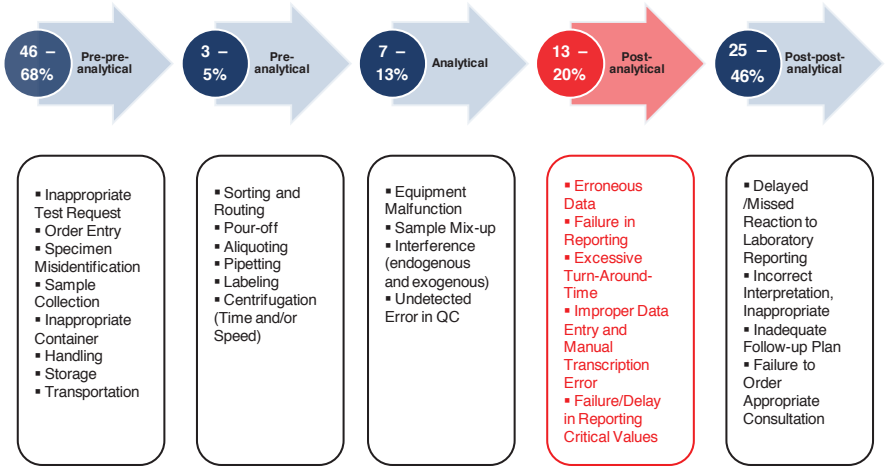
Results: The preliminary research result shows that the agreement between these two approaches is within 10% of bias for the analyzed donor samples. This software reduces manual calculation time and also applies calculations to all analytes that can be detected by tandem MS.

Introduction

The technology used in research of in-born errors of metabolism disorders has changed drastically from EIA, RIA, FIA, ELISA to LC and tandem MS for the past 50 years. The research areas include metabolic, genetic and endocrine disorders. The current focus of research activities varies worldwide due to demographics and ethnic background, including amino acid disorders, fatty acid oxidation disorders, endocrinopathies, lysosomal storage disorders and others.

Even though LC combined with tandem MS allows for higher quality results compared to the old approaches, data processing in the post-analytical phase still remains a common cause of errors in the total testing processing (Fig.1) [1].

FIGURE 1. Common causes of errors in total testing processing



In order to reduce the opportunity of errors during this post-analytical phase, we developed a meta calculation software aimed at the automatic calculation of mass ion ratios and user-defined formulas using the raw data files acquired by a tandem MS. The software was used to process raw data files acquired by open-tube flow injection LC-tandem MS to support in-born error metabolism disorder research. Results were exported in Excel format and compared to the results obtained by manual multi-step calculation using an Excel worksheet and the peak areas obtained through the use of Thermo Scientific™ Xcalibur™ software.

Methods

Sample Preparation

Samples from in-born error metabolism disorder research 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. QC samples were also added to the batch.

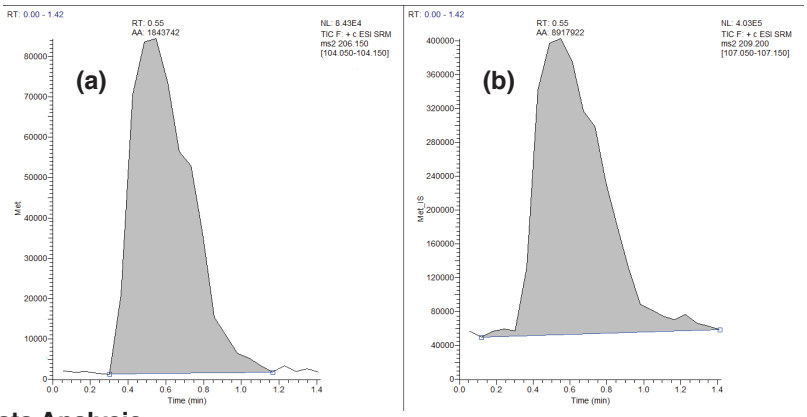
Liquid Chromatography Tandem Mass Spectrometry

Flow injection was used to elute samples through an LC system; no chromatography was involved in this step. The typical cycle time was below three minutes per sample.

Data acquisition was performed using a triple quadrupole mass spectrometer operated in selected-reaction monitoring (SRM) mode acquiring one precursor/fragment transition for each acylcarnitine or amino acid and their corresponding internal standards.

A representative chromatogram for methionine and its internal standard d3-methionine is reported in Fig. 2.

FIGURE 2. Representative chromatogram for methionine (a) and its internal standard d3-methionine (b) from a real DBS sample



Data Analysis

Software calculation

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

Customized 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 customized formulas.

A processing method is created by selecting the peak areas, analyte concentrations and customized formula results that will be displayed by the software.

Results can be exported in Excel or text format.

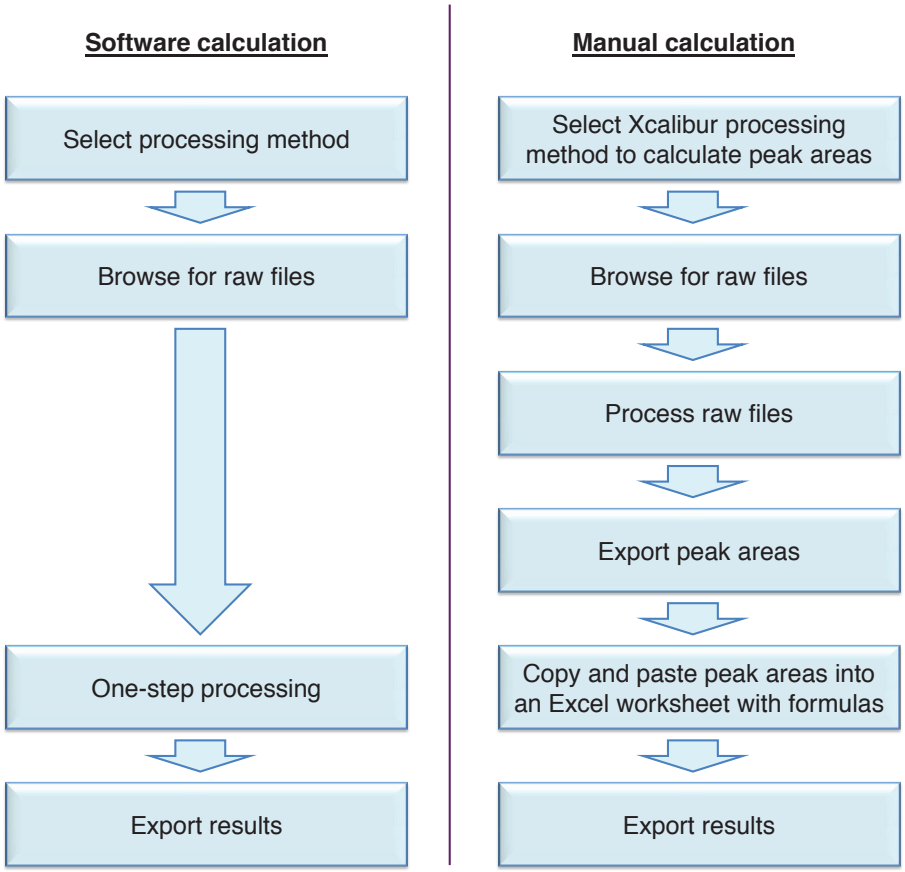
Manual calculation

Manual calculation for comparison was performed by creating a processing method to extract chromatograms and calculate peak areas for each analyte and IS using 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.

A comparison between the two approaches is reported in Fig. 3.

FIGURE 3. Workflow comparison between the software and the manual approach



Results

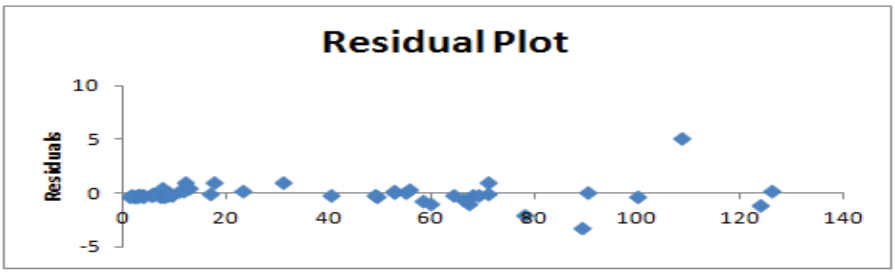
The percentage bias between results obtained using our meta-calculation software and the manual calculation approach was calculated for each sample and each analyte. Comparison of results obtained for a set of acylcarnitines and amino acids in a representative sample is reported in Fig. 4.

FIGURE 4. Comparison of results obtained for a set of acylcarnitines and amino acids in a representative sample using software and manual calculation with peak areas (a), concentrations (b) and customized formulas (c)

	ID	SOFTWARE CALCULATION	MANUAL CALCULATION	Bias (%)
(a) PEAK AREAS (counts)	C0	5795418	5774456	0.4
	C0 IS	7095972	7045751	0.7
	C8	73063	71421	2.3
	C8 IS	2305724	2305496	0.0
	C14	146333	145030	0.9
	C14:1	48140	45931	4.8
	C14 IS	1868221	1846426	1.2
	C16	5144998	5093274	1.0
	C16 IS	4990605	4973729	0.3
	Cit	639420	618699	3.3
	Cit IS	6018614	5948050	1.2
	Met	3505563	3449235	1.6
	Met IS	12939330	11912912	8.6
	Orn	2353515	2306890	2.0
	Orn IS	1218512	1206264	1.0
	Phe	34814320	34408624	1.2
	Phe IS	34840593	34455654	1.1
	Tyr	12865891	12854171	0.1
	Tyr IS	15722225	15604857	0.8
(b) CONCENTRATIONS (ng/mL)	C0	52.3	52.5	-0.3
	C8	2.0	2.0	2.3
	C14	5.0	5.0	-0.3
	C14:1	1.6	1.6	3.6
	C16	66.0	65.5	0.7
	Cit	6.8	6.7	2.1
	Met	17.3	18.5	-6.4
	Orn	123.6	122.4	1.0
	Phe	64.0	63.9	0.1
	Tyr	52.4	52.7	-0.7
(c) FORMULAS	C0+C14:1	53.9	54.0	-0.2
	(Orn-Phe)/Tyr	1.14	1.11	2.7
	(C8+C14:1-C16)/(Orn+Tyr)	-0.35	-0.35	0.0

Results obtained for the first set of samples analyzed in 5 different laboratories on a total of over 100 analytes showed a good agreement between the two approaches with a percentage bias always within ±10%.

FIGURE 5. Residual plot of 50 calculations from 5 donor samples
($Y = 0.9978X + 0.1497$ $R^2 = 0.9992$)



Conclusion

We evaluated a beta software to perform one-step calculations on peak areas, concentrations and user-defined formulas from raw files acquired by flow-injection LC-tandem MS.

The results obtained using this software on a first set of donor samples from in-born error metabolism disorder research showed a good agreement with performing the same calculations using a multi-step manual approach.

This offline automated data process tool is easy to use, has an intuitive workflow and improves the turn around time by eliminating manual calculation process and removing transcription errors.

References

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

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