

# Comparison of three automated data processing and reporting approaches for inborn errors of metabolism by LC-MS flow injection analysis for clinical research

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## Goal

Evaluate and compare three automated data processing and reporting approaches for workflow capability, completeness, and ease of use, including data acquisition, peak integration, concentration calculation, meta-calculation, annotation, and data reporting.

## Introduction

With the advances of tandem mass spectrometry (MS), it is now easier to identify inborn errors of metabolism (IEM) for clinical research. However, there are some practical challenges in processing and interpreting MS data, for example:

- An increasing number of targets for research
- The complexity, time, and effort of data interpretation
- The lack of hands-on experience due to the occurrence of rare conditions



The success of an IEM workflow depends largely on how it processes, interprets, and integrates data and information from multiple sources. Currently many labs export data from instrument software and import it into an external spreadsheet for data interpretation calculations. Automated reporting and interpretation tools are solutions that could streamline or expand the capability of data processing software for efficient data review and rapid report delivery.

## Experimental

### Sample preparation

Samples were extracted from dried blood spot cards. Internal standards (IS) were added during the extraction procedure, and extracted samples were injected onto an LC-MS system. Quality control (QC) samples were used for software performance evaluation.

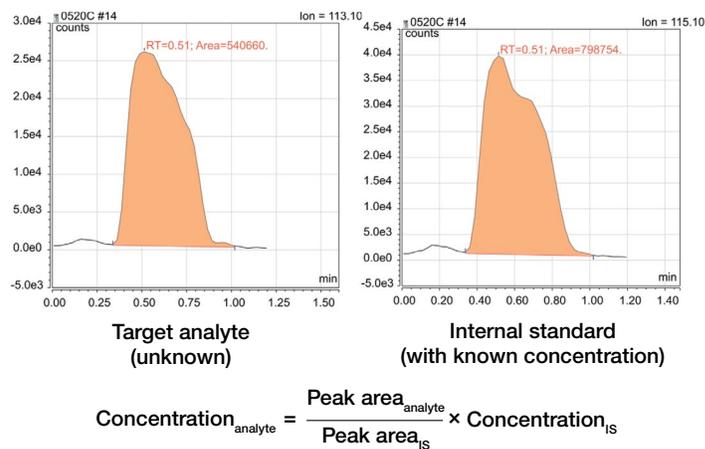
### Liquid chromatography-mass spectrometry

Dried blood spot extracts were injected by flow injection analysis (FIA) onto a triple quadrupole mass spectrometer. The flow injection was conducted using a Thermo Scientific™ Vanquish™ Flex UHPLC system with open tube, providing an automated sample introduction to a Thermo Scientific™ TSQ Endura™ or TSQ Fortis™ triple quadrupole mass spectrometer, without chromatographic separation. Data were collected in selected reaction monitoring (SRM) mode for the detection of amino acids and acylcarnitines.

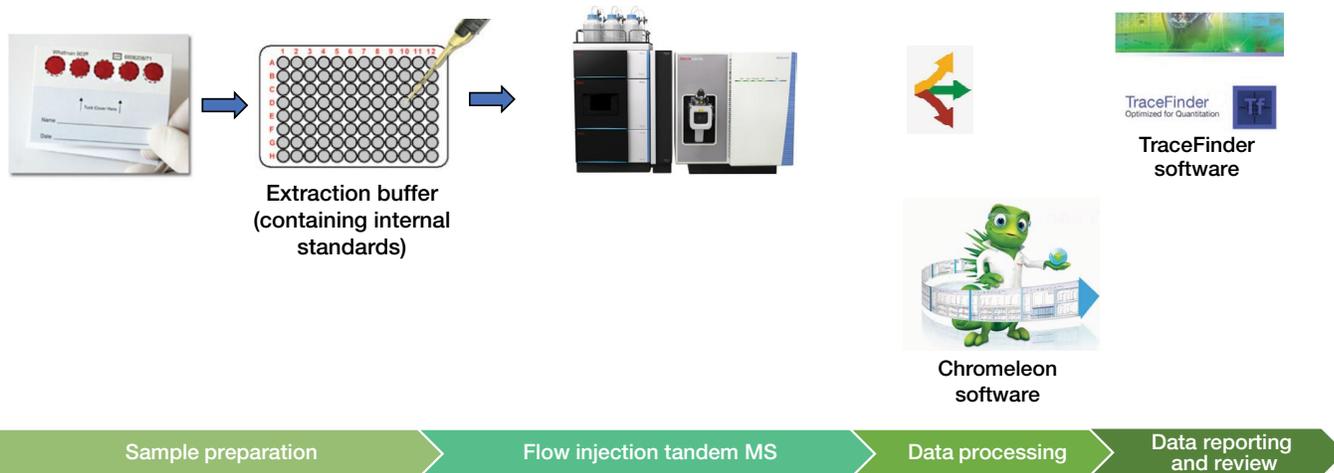
### Data analysis

Thermo Scientific™ TraceFinder™ software version 4.1 (Chinese version) and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.2.10, both with customized meta-calculation reporting templates and iRC PRO™ 1.3.0.40 software, were used for automated data processing of raw data generated by the mass spectrometers. Custom report plug-in templates were created for TraceFinder software and Chromeleon software to perform data meta-calculations and reporting (Figure 1).

Integration algorithms were compared between the software packages by comparing final concentration values. Analyte concentrations were calculated by internal calibration (Figure 2). Functionality differences of the software packages were also compared for reproducibility of results, completeness of workflow, and ease of use.



**Figure 2. Semi-quantitative method for inborn errors of metabolism by LC-MS flow injection analysis.** Internal standards were spiked in samples. Both peak areas of target analyte and internal standard were measured in a single injection. No calibration curve is needed.

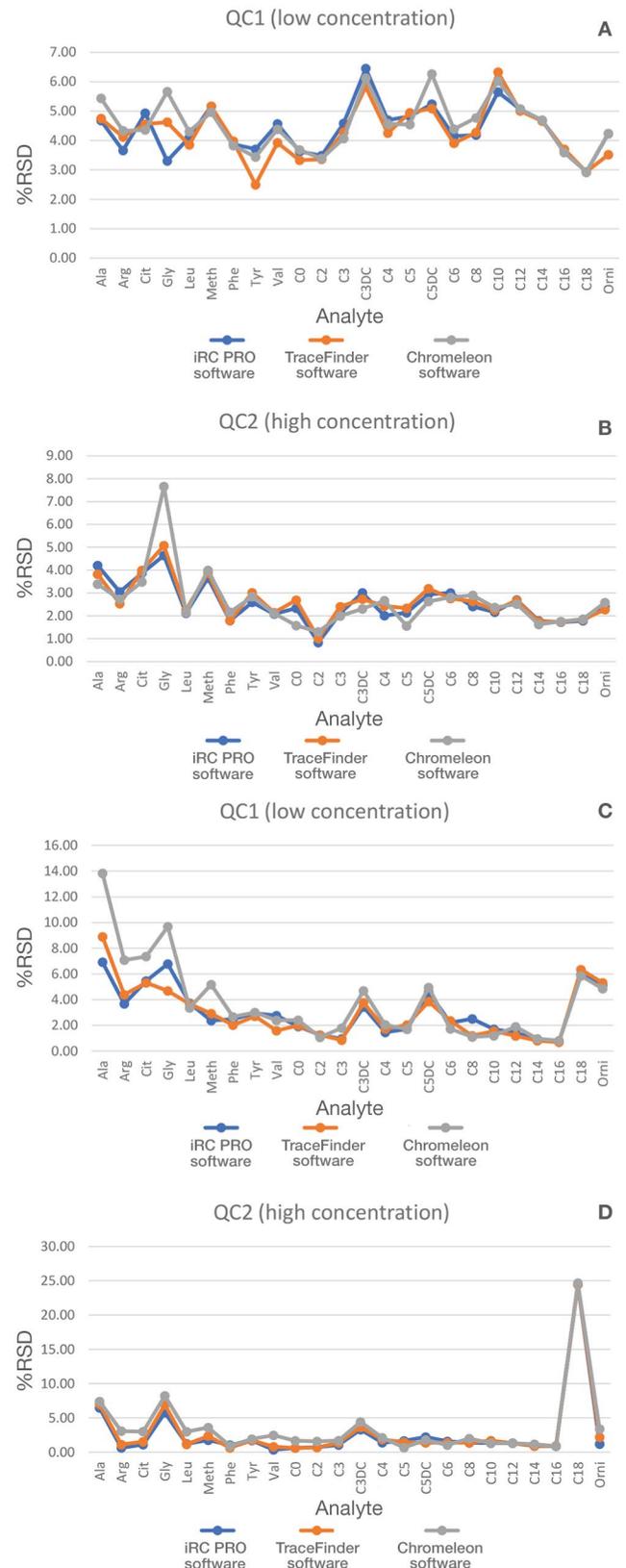


**Figure 1. Software comparison between TraceFinder, iRC PRO, and Chromeleon software was performed using a workflow of inborn errors of metabolism by LC-MS flow injection analysis on a Vanquish Flex system coupled to a TSQ Endura mass spectrometer or TSQ Fortis mass spectrometer.**

## Results and discussion

All three approaches can generate the desired results and perform user-defined meta-calculations. From the view of the completeness of a start-to-finish strategy, both TraceFinder software and Chromeleon CDS software can control instruments, acquire, process, review, and report data, while iRC PRO software can only process and report data.

For peak integration, all can perform user-defined integration, which is desired for FIA data analysis. However, IRC PRO SW does not allow user review of peak integration. TraceFinder software and Chromeleon CDS software allow full user review of integrated peaks, as well as adjusting compound specific integration parameters. iRC PRO and TraceFinder software have a built-in internal calibration semi-quantification mode, which can calculate target analyte concentrations from IS concentration and peak areas. A comparison of the integration algorithms for concentration calculation (%RSD) was performed using low and high concentration QC samples from different batches. The batches were chosen for their different data quality as indicated by %RSD of peak areas. The results from three software platforms using different peak integration algorithm are consistent (Figure 3). The results suggest variations from peak integration of three software are not significant and could truly reflect the raw data quality.

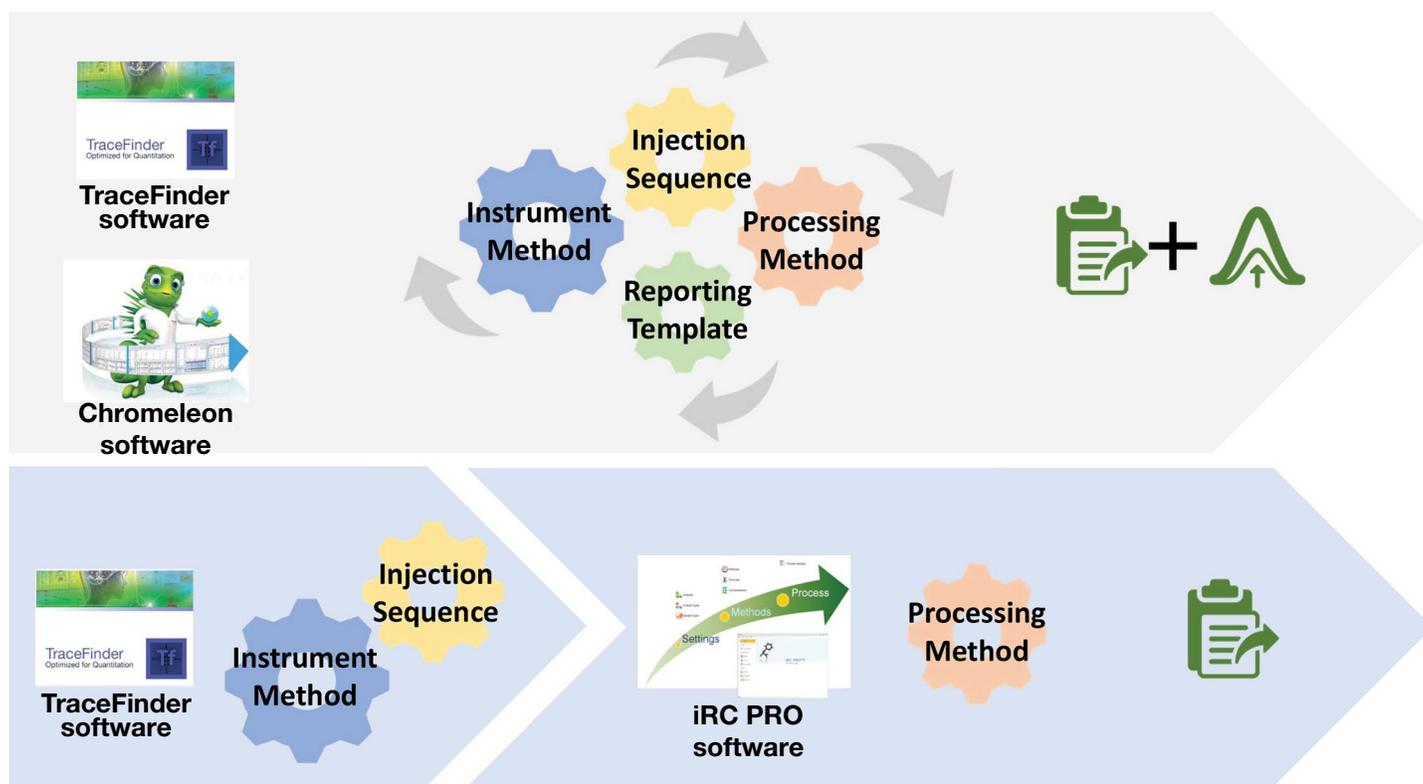


**Figure 3. Comparison of integration algorithms for concentration calculation (%RSD).** A) Low concentration QC (n=7), high quality; B) high concentration QC (n=7), high quality; C) low concentration QC (n=6), low quality; D) high concentration QC (n=6), low quality.

All three software platforms could streamline and automate the workflow from samples to reports (Figure 4). iRC PRO software has built-in data reporting and meta-calculations, while both TraceFinder software and Chromeleon CDS software require a plug-in template to include those functions. Meta-calculations can automate customer defined calculations and functions from different sources, and streamline the automated data acquisition and processing. The plug-in templates can add value of reporting adeptness and flexibility and be tailored to user needs. For ease of use, iRC PRO software is very simple, straightforward, and easy to learn, but it requires the importing of data from data acquisition software. TraceFinder software and Chromeleon CDS software could initiate the reporting function from sample sequence submission, with even less manual intervention for the end user. However, software learning curves for the method development user are longer than for iRC Pro software.

Both TraceFinder software and Chromeleon CDS software can use one platform for data acquisition, processing, and reporting (Figure 4). When the instrument method, processing method, reporting template, and injection sequence are defined and ready, one click will initiate sample sequence submission with automated reporting. The strategy can provide a single, complete audit trail for acquisition, processing, and reporting, which reduces and simplifies audit preparation. On the other hand, iRC PRO software has a simple user interface. It can process raw data to reports with behind-the-scenes, built-in peak integration, calculation method, and reporting template. Set up of the automated data processing can be started without learning the details of TraceFinder software.

A comparison of the capabilities of each software platform is given in Table 1.



**Figure 4. Workflow comparison between TraceFinder software, Chromeleon CDS software, and iRC Pro software.** TraceFinder software and Chromeleon CDS software have a seamless start-to-finish workflow, including data acquisition, data processing and reporting, while iRC PRO software depends on TraceFinder software for instrument control and data acquisition.

**Table 1. Software capability comparison chart**

| Capability   | TraceFinder software | Chromeleon software | iRC Pro software |
|--|----------------------|---------------------|------------------|
| <b>1. Controlling and monitoring instrument</b>      | ●                    | ●                   | ○                |
| <b>2. Creating a sequence</b>                        | ●                    | ●                   | ○                |
| a. Instrument method                                 | ●                    | ●                   | ○                |
| b. Processing method                                 | ●                    | ●                   | ●                |
| c. Report template                                   | ●                    | ●                   | ○                |
| d. Creating a sequence                               | ●                    | ●                   | ○                |
| <b>3. Data acquisition</b>                           | ●                    | ●                   | ○                |
| a. Checking instrument's readiness                   | ●                    | ●                   | ○                |
| b. Starting a sequence                               | ●                    | ●                   | ○                |
| c. Monitoring an ongoing analysis                    | ●                    | ●                   | ○                |
| <b>4. Processing data</b>                            | ●                    | ●                   | ●                |
| a. Detecting and integrating peaks                   | ●                    | ●                   | ●                |
| b. Identifying peaks                                 | ●                    | ●                   | ●                |
| c. Adjusting parameters for individual analytes      | ●                    | ●                   | ○                |
| d. Built-in concentration calculation                | ●                    | ●                   | ●                |
| <b>5. Reporting data</b>                             | ●                    | ●                   | ●                |
| a. Customer-tailored reporting                       | ●                    | ●                   | ◐                |
| b. "Out of Range" flagging                           | ●                    | ●                   | ●                |
| c. Customer formula                                  | ●                    | ●                   | ●                |
| <b>6. Reviewing large dataset (peak integration)</b> | ●                    | ●                   | ○                |
| <b>7. Managing data</b>                              | ◐                    | ●                   | ◐                |
| a. Data storage                                      | ●                    | ●                   | ◐                |
| b. 21 CFR Part 11, GLP, GMP capable                  | ◐                    | ●                   | ○                |
| c. Legacy data and method transfer                   | ●                    | ●                   | ◐                |
| d. Template security                                 | ○                    | ●                   | ●                |
| <b>8. User learning curve</b>                        | ◐                    | ◐                   | ●                |

**Conclusions**

- The three software packages—Thermo Scientific TraceFinder software version 4.1, Thermo Scientific Chromeleon CDS software version 7.2, and iRC PRO software—all offer automated reporting approaches that streamline data processing and meta-calculations of IEM data for clinical research.

- All three packages can significantly improve productivity, timeliness, quality management, and communication.
- Users have multiple options depending on their needs and desired flexibility.

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