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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ABSTRACT

Purpose: This study was to evaluate and compare three automated data processing and reporting approaches. We evaluated each workflow's capabilities, completeness, and ease of use including data acquisition, peak integration, concentration calculation, meta-calculation, annotation and data reporting.

Methods: Dried blood spot samples were extracted and injected by flow injection analysis (FIA) onto a triple quadrupole mass spectrometer. The generated data files were processed using three different software packages(SW): 1) Thermo Scientific™ TraceFinder™ SW Version 4.1; 2) Thermo Scientific™ Chromeleon™ 7.2 SW; and 3) iRC PRO SW. Custom report plug-in templates were created for TraceFinder and Chromeleon SW to perform data meta-calculations and reporting (Figure 1).

Results: 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 and Chromeleon SW can control instruments, acquire, process, review, and report data, while iRC PRO SW 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 and Chromeleon SW allow full user review of integrated peaks as well as adjusting compound specific integration parameters. iRC PRO and TraceFinder SW have a built-in internal calibration mode for concentration calculation. Chromeleon SW needs to take advantage of a plug-in template to perform the calculation. iRC PRO SW has built-in data report and interpretation, while both TraceFinder and Chromeleon SW need a plug-in template to include those functions and bridge the gaps. However, the plug-in templates can expand the functions according to user needs and have more reporting flexibility. For easy of use, iRC PRO SW is very simple and straight-forward, but needs to import data from data acquisition software. TraceFinder and Chromeleon SW can integrate plug-in templates into the data acquisition step, requiring even less manual intervention.

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, 1) an increasing number of targets for research, 2) the complexity, time, and effort of data interpretation, and 3) the lack of hands-on experience due to the occurrence of rare diseases. 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 which could streamline or expand the capability of data processing software for efficient data review, and rapid report delivery.

MATERIALS AND METHODS

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

The flow injection was conducted using a Thermo Scientific™ Vanquish™ Flex UHPLC with open-tube, providing an automated sample introduction to a Thermo Scientific™ TSQ Endura™ or Fortis™ triple quadrupole mass spectrometer, without chromatographic separation. Data was collected in selected reaction monitoring (SRM) mode for the detection of amino acids and acylcarnitines.
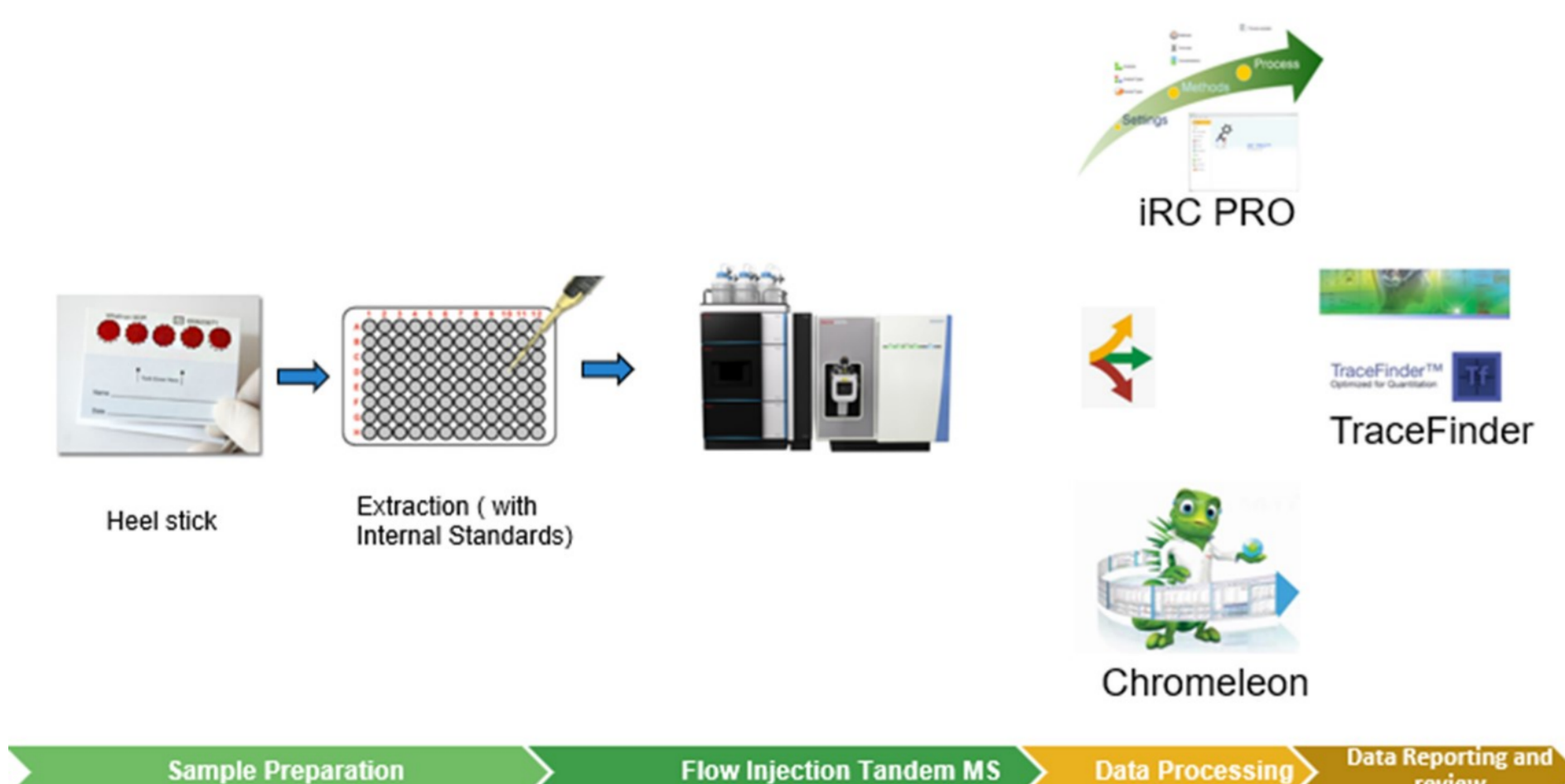
Figure 1. Software comparison between TraceFinder, iRC PRO and Chromeleon SW was performed using a workflow of inborn errors of metabolism by LC-MS flow injection analysis on a Vanquish Flex system coupled to a TSQ Endura or Fortis mass spectrometer.

Data Analysis

TraceFinder and Chromeleon SW both with customized meta-calculation reporting templates, and iRC PRO SW were used for automated data processing of raw data generated by the mass spectrometers. 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.
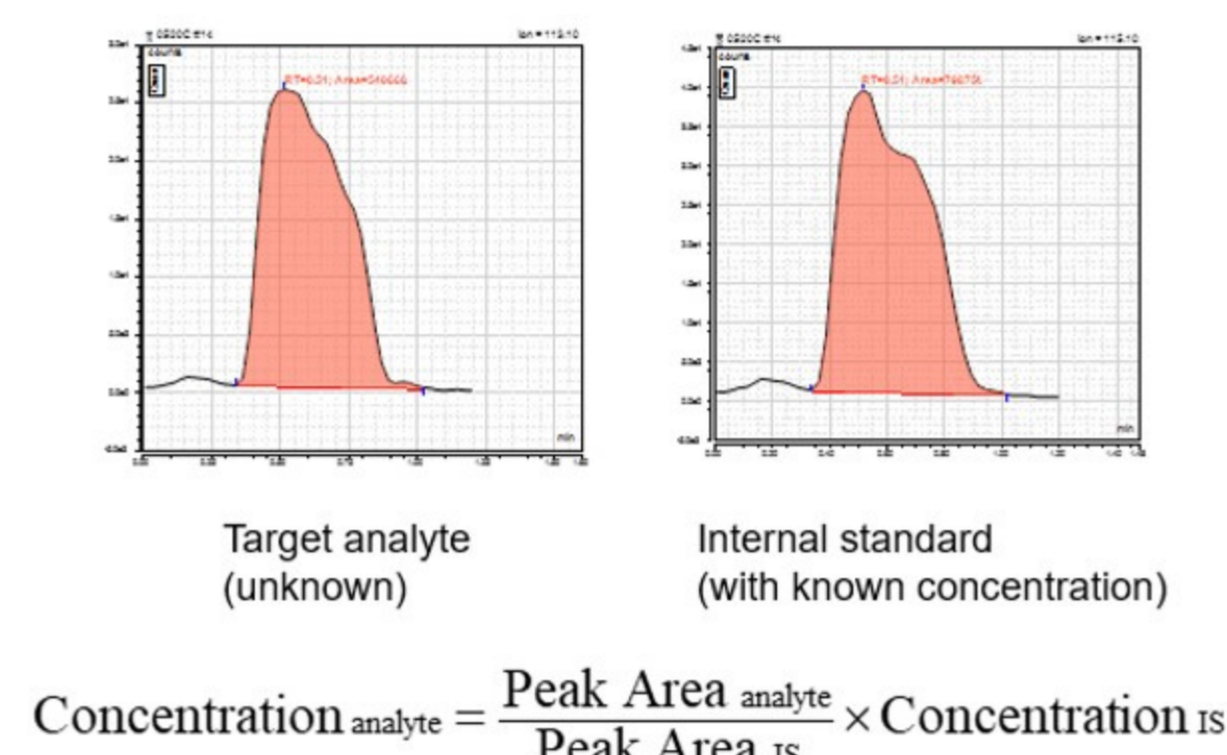
$$\text{Concentration}_{analyte} = \frac{\text{Peak Area}_{analyte}}{\text{Peak Area}_{IS}} \times \text{Concentration}_{IS}$$

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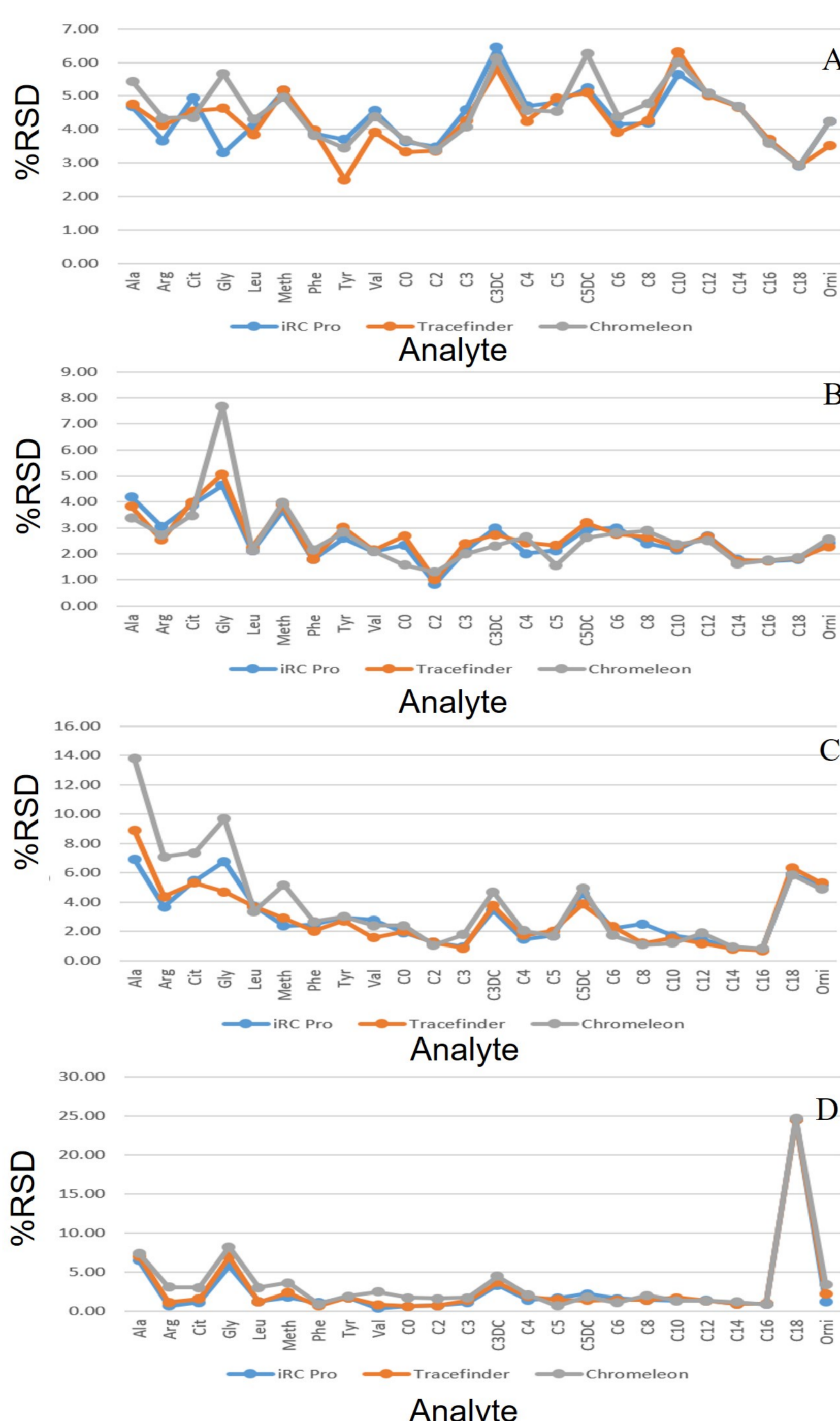
RESULTS

Figure 3. Comparison of integration algorithms for concentration calculation (%RSD) was performed using low and high concentration QC samples from different batches with low and high quality. The results from three software using different peak integration algorithm are consistent. The results suggest %RSD variations from peak integration of three software are not significant and could truly reflect the raw data quality 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.
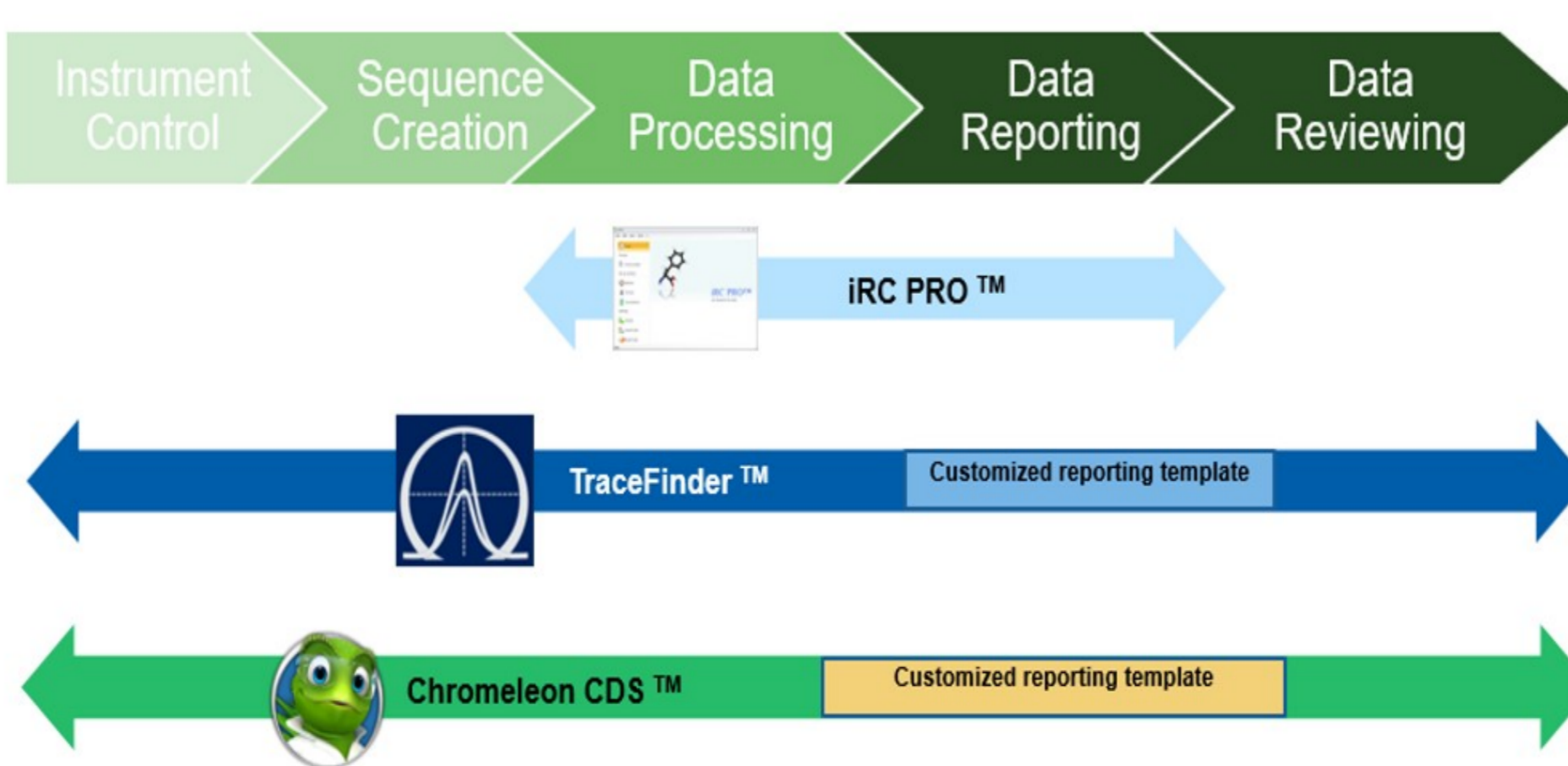
Figure 4. The completeness of a start-to-finish strategy. All three software could streamline and automate the workflow from samples to reports. iRC PRO SW has built-in data report and meta-calculations, while both TraceFinder and Chromeleon SW need a plug-in template to include those functions. The plug-in templates can add value of reporting adeptness and flexibility and be tailored to user needs. For easy of use, iRC PRO SW is very simple and straight-forward, and easy to learn, but needs to import data from data acquisition software. TraceFinder and Chromeleon SW could initiate reporting function from sample sequence submission, with even less manual intervention for end user. However software learning curves for method development user are longer than for iRC Pro SW.

Figure 5. Workflow comparison between TraceFinder, Chromeleon and iRC Pro SW. A) Both TraceFinder and Chromeleon SW can use one software platform for data acquisition, processing, and reporting. When 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 would reduce and simplify audit preparation. B) iRC PRO SW has simple user interface. It can process raw data to reports, with behind-the-scene built-in peak integration, calculation method, and reporting template. Setting up of the automated data processing can be started without learning the details of TraceFinder SW.

Table 1. Software capability comparison chart

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

CONCLUSIONS

- The three software packages, Thermo Scientific TraceFinder SW version 4.1, Thermo Scientific Chromeleon 7.2 SW, and iRC PRO SW all offer automated reporting approaches which 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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TRADEMARKS/LICENSING

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