Library searching capability added to normalization and ion-suppression correction capabilities becomes novel semi-targeted analysis with benefits

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...making metabolic profiling easy®

OVERVIEW

Targeted metabolic analyses are very useful and generally more quantitative than non-targeted analyses, but often miss the appropriate compounds. Non-targeted analyses theoretically avoid this problem but their ability to correctly identify peaks and quantify them is a major challenge. The IROA TruQuant protocol was developed as a targeted analysis for hundreds of compounds in a biochemically-complex Internal Standard (IS) that was quantitatively enhanced by providing a mechanism for the correction of ion-suppression, and an advanced sample-to-sample normalization. This report concerns a technique for addition of hundreds of additional authenticated peaks that can also be normalized even though they will not be associated with an internal standard.

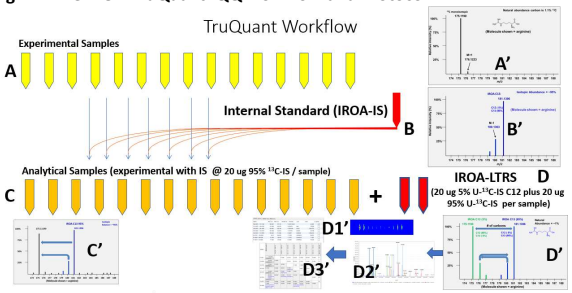
INTRODUCTION

Fig. 1 – Why semi-targeted when we do "just fine now"?

	Targeted analyses	Non-Targeted analyses	Semi-Targeted analyses
Quantitative	Excellent	Poor	Very Good w/ IS Good w/o IS
Identification	Excellent	Poor	Very Good w/ IS Good w/o IS
Comprehensive	Not at all	Reasonably, but with lots of error	All user sought compounds
Suppression Correction	No, possibly not relevant (UV, etc.)	No	Yes w/ IS No w/o IS
Normalize sample	Not always	No	Yes

We really don't do just fine now. Both Targeted (T) and Non-Targeted (NT) analyses have significant problems. A Semi-Targeted (ST) approach could overcome many of these problems by supporting the best aspects of each. The IROA TruQuant Workflow provides one way to approach this as a solution.

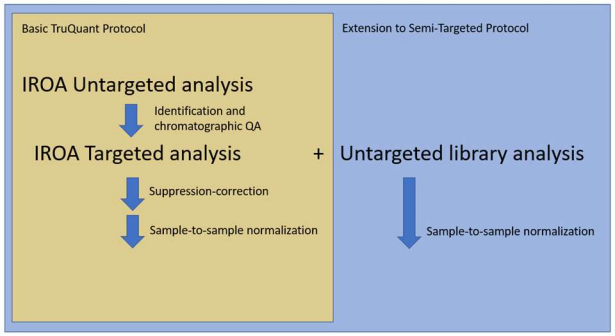
Fig. 2 – The IROA TruQuant IQQ workflow and Protocol.



Acknowledgements
IROA-labeled materials were produced for IROA Technologies (under agreement) by CIL.

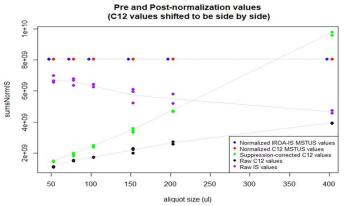
METHODS

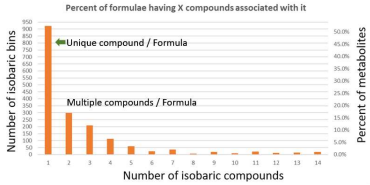
Fig. 3 - The extended IROA workflow embodied into ClusterFinder 4.1.19.



IROA Internal Standards (IROA-IS) and Long-Term Reference Standards (IROA-LTRS) are chemically identical but have specific isotopic probabilities for each carbon, 95% U-^{13}C and 5%/95% U-^{13}C, respectively, and those percentages create mass spectral isotopomeric patterns that are unique for each molecular formula. The IROA Semi-Targeted workflow first runs a Non-Targeted analysis on the highly characterized IROA LTRS for all of its 1,000+ known peaks to determine QA metrics for the instrument, general chromatographic parameters and identity on all peaks. Then these parameters are applied to a Targeted analysis of all the same LTRS compounds that are identified in the analytical samples (identified by spiking the IS into natural abundance experimental sample). During the Targeted analysis phase any additional peaks or compounds that do not have an IS match but would be desirable will also be collected, analyzed and processed to remove errors inherent in them. For those peaks that are associated with an IS this means they will be corrected for suppression and adjusted in a sample-to-sample normalization. Those peaks which are found in the Targeted analysis but do not have an IS will only be sample normalized.

Supplemental materials





REFERENCES

1. de Jong F, Beecher C, "Addressing the current bottlenecks of metabolomics: Isotopic Ratio Outlier Analysis (IROA®), an isotopic-labeling technique for accurate biochemical profiling", *Bioanalysis* 2012, 4(18), 2303-14.
2. Stupp GS, Clendinen CS, Ajredini R, Szewc MA, Garrett T, Menger RF, Yost RA, Beecher C, Edison AS. "Isotopic Ratio Outlier Analysis Global Metabolomics of Caenorhabditis elegans." *Analytical Chemistry* 2013 *85*(24), 11858-11865. doi: 10.1021/ac4025413.
3. Warrack et al. "Normalization Strategies for metabonomic analysis of Urines", J. Chrom B 2009 877(5) 547-552. doi: 10.1016/j.jchromb.2009.01.007

Fig. 4 – Suppression-correction and normalization for IS related peaks

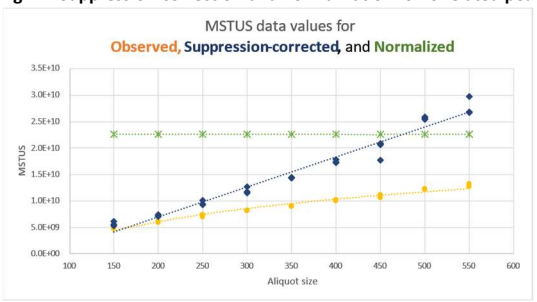
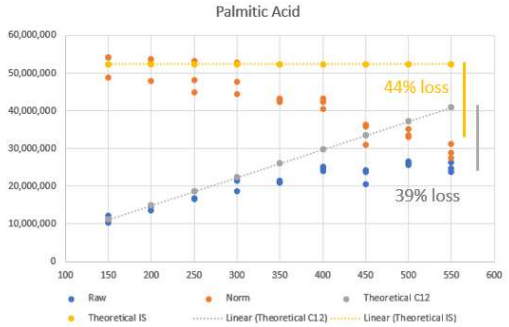


Fig. 5 – Sample-to-sample normalization (compounds without IS).



RESULTS

With the release of ClusterFinder 4.1.19 the analysis of both IS associated peaks and libraries of known peaks not contained in the IS can be targeted for quantitation. The peaks associated with an Internal Standard will be corrected for ion suppression and other in-source losses, and then normalized. Those peaks identified only in libraries will be normalized using the same normalization factors determined for the IS associated peaks.

The ability to collect data from both IS and non-IS associated peaks and to supply data correction parameters to both make the Semi-Targeted approach interesting, and useful. We look forward to hearing about other similar approaches to make Metabolomics a better science.

CONCLUSIONS

- **The IROA TruQuant protocol provides a mechanism for simultaneously tracking instrument performance, verifiable compound identification and the quantitation of compounds in which source inefficiencies, such as ion suppression, or variability of samples may all be corrected.**
- **As of the release of ClusterFinder 4.1.19, libraries of additional non-IS associated peaks may be built and included in the targeted analysis of any sample. The non-IS associated peaks will benefit from normalization but not from suppression correction.**