ABSTRACT
Purpose: Development of a compound class specific deep scan for confident annotation of relevant analytes using a high quality curated High Resolution Accurate Mass MS/MS spectral library in conjunction with Real Time Library Search (RTLS).
Methods: A Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Thermo Scientific™ Orbitrap Ascent™ MS/MS mass spectrometer is used for collecting all MS and MS/MS data. For data acquisition, MS/MS is always collected with precursor ions detected in the survey MS scan within 1.2 second cycle time. High order MS n+1 is collected using the built-in spectral library creation tools using LC-MS. The generated MS n+1 tree data are processed using Thermo Scientific™ Mass Frontier 8.0 and Thermo Scientific™ Compound Discoverer™ 3.3 software. Flavonoid standards were used for the library creation and putative flavonoids were annotated in tea extract. Thermo Scientific™ m/zVault™ 2.3 software was used for creating the spectral library to be used for RTLS.
Results: Use of standard spectral library and RTLS allows more unknown flavonoids being identified from the natural products. Nearly 100 more putative flavonoids were annotated using this workflow from tea extracts.

INTRODUCTION
Confident compound annotation is essential for translating untargeted metabolomics data into meaningful biological information. Missing or scarce information from untargeted metabolomic data remains a challenge. Local mass spectral libraries consisting of MS n+1 data from authentic standards provide the opportunity to confidently annotate known and related unknown compounds in biological samples. Here we describe the process of creating and using a high-quality spectral library of high-resolution accurate mass data using authentic flavonoid standards and the built-in library builder method template on the Thermo Scientific™ Orbitrap Ascent™ Trifide™ Mass Spectrometer (MS) and its utility for the confident annotation of flavonoids in biological samples using Real Time Library Search (RTLS) to automatically guide MS n+1 data acquisition on the fly.

MATERIALS AND METHODS
Sample Preparation
The Flavonoid Library consisting of 40 standards was obtained from Metabion (Toronto, ON, CA). The library consists of 4 vials each containing 10 authentic standards which could be directly injected for LC-MS analysis. Three varieties of green tea, black tea and herbal tea were obtained from the local market. Three replicates of each were extracted using 240 mL of hot water in commercially available disposable coffee cup. For blank, hot water without tea in similar coffee cup was used. Pooled samples were prepared by adding 100 µl of each extract. This pooled sample was used for QC.

HPLC Conditions
A Thermo Scientific™ Vanquish™ UHPLC system performed separations. The column was a Thermo Scientific™ Accucore™ Vanquish™ C18 UHPLC column (2.1 x 150mm, 1.5µm).

RESULTS
Spectral Library
Thermo Scientific™ Compound Discoverer™ 3.3 software was used to process the LC-MS data from the standards. The spectral data with the various fragmentation strategies from the standards was transferred to a m/zVault™ library. The m/zVault™ library containing the spectral fragmentation information of the flavonoid standards was populated with the structural and chemical information of individual compounds. This library was used for the RTLS on the instrument.

Data Processing
Thermo Scientific™ Compound Discoverer™ 3.3 software was used to process the LC-MS data from the samples. Differential analysis and compound annotation revealed relative differences between the various tea samples. Several annotation tools are available in the Compound Discoverer 3.3 software including database searching and spectral library matching against the m/zCloud™ spectral library at the MS n+1 level. In parallel it has the ability to search custom mass lists with corresponding chemical structures. Here the data was processed using the Arita Flavonoid Database which has the mass and structural information of around 6500 Flavonoids.

CONCLUSION
The use of RTLS allows more MS n+1 scans for annotation of additional non-flavonoid metabolites.

TRADEMARKS/LICENSING
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