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LC/MS – Putative Identification – Food and Beverage

A Putative Identification of Compounds in Yerba Mate Tea Leaves with LC-MS/MS

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Abstract

Yerba mate tea is a beverage in South America prepared from the leaves of *Ilex paraguariensis*. Its consumption is associated with beneficial health effects and has been gaining popularity globally, both as tea and an ingredient in health supplements. In this study, a targeted qualitative analysis workflow for the putative identification of compounds in yerba mate tea was performed with liquid chromatography and tandem-in-space mass spectrometry (LC-MS/MS) technique, specifically enabled by triple quadrupole mass spectrometer (TQMS). The putative identification of known-unknown compounds was achieved by matching the acquired product ion mass spectra against public and NIST MS/MS mass spectral libraries. A total of 14 compounds were putatively identified in yerba mate tea sample in both positive and negative scan modes. The targeted qualitative analysis workflow herein can be extended to support the preliminary identification of unknown compounds.

Targeted qualitative analysis of Yerba mate tea for putative identification

Keywords:
Yerba Mate, Tea Leaves, Putative Identification, Triple Quadrupole, NIST MS Library

Highlights

- Shimadzu LCMS-8050 enables putative identification of known-unknown compounds in tea samples by matching product ion mass spectra of the targeted precursor against NIST mass spectral libraries

Technologies Featured

LCMS-8050

LabSolutions™ LCMS and NIST MS Search

1. INTRODUCTION

Yerba mate (*Ilex paraguoriensis*) tea is a popular beverage in South America and has recently seen a surge in demand globally due to its natural health-promoting benefits. The tea is believed to provide beneficial health effects such as hypocholesterolemic properties, enhanced antioxidant activities and improved cardiovascular function. These pharmacological properties were reported to derive from the high content of caffeoyl derivatives and other phenolic components found in yerba mate leaves. Hence, efforts are put into identifying and characterising these components.

Liquid chromatography and tandem-in-space mass spectrometry (LC-MS/MS) instrument based on triple quadrupole mass spectrometer (TQMS) is widely used for the analysis of yerba mate tea. Bravo *et* al. and Vieira *et* al. reported the presence of polyphenols, methylxanthines and flavonols as the major groups of compounds found in the tea samples [1,2]. Additionally, Ferreira da Silveira *et* al. and Matei *et* al. extracted and determined the presence of hydroxycinnamic acid derivatives and flavonols during the preparation of yerba mate tea [3,4].

Triple quadrupole mass spectrometry filters ions via a quadrupole mass filter and subsequently fragments ions via collision-induced dissociation (CID) in the collision cell filled with collision gas. This confers higher sensitivity and selectivity, which explained its wide application in targeted quantitative screening. In this work, we demonstrate the possibility of extending the usage of TQMS beyond targeted quantitative analysis. Herein, a targeted qualitative analysis workflow enabled by screening against MS/MS mass spectral libraries is shown for the analysis of yerba mate tea sample. This workflow enables the usage of TQMS for putative identification of compounds with possibilities to extend to non-targeted qualitative analysis.

2. EXPERIMENT

2.1 Sample Preparation

The yerba mate tea leaves were cut into small pieces and weighed into a 15 mL conical centrifuge tube. The tea extract was then prepared by adding boiling ultrapure water and allowing to stand for 30 minutes. This simple sample preparation is to simulate the tea brewing process with just hot water for consumption. The extract was then filtered with a 0.22 µm size nylon syringe filter before transferring into a 1.5 mL glass injection vial for analysis.

2.2 Analytical Setup

The tea extract was then analysed on Shimadzu LCMS-8050 triple quadrupole MS. The analytical conditions for UHPLC and LCMS are described in Table 1 and Table 2, respectively.

Table 1. Analytical LC conditions for detection of compounds in yerba mate tea

Nexera X2 LC-30

Column	Velox C18 column (100 mm x 2.1 mm x 2.7 µm)
Mobile phase	A : 0.1% formic acid in water B : acetonitrile
Gradient program	0.0 – 6.0 min (5 %B), 16.0 – 22.0 min (25 %B), 22.5 – 30.0 min (5 %B)
Flow rate	0.4 mL/min
Oven temperature	40 ˚C
Injection volume	1 µL

Table 2. MS conditions for detection of compounds in yerba mate tea on LCMS-8050

LCMS-8050 (LC/MS/MS)

Interface	Heated ESI
Acquisition Mode	Q1 scan and product ion scan, positive and negative mode
Heat block temperature	400 ˚C
DL temperature	250 ˚C
Interface temperature	300 ˚C
Nebulising gas	N_2, 3 L/min
Drying gas	N_2, 10 L/min
Heating gas	Zero air, 10 L/min

2.3 Software and Processing

All operations and data acquisition were controlled with Shimadzu LabSolutions™ LCMS v5.99 software. Data processing was performed with Shimadzu LabSolutions™ LCMS v5.99 Postrun. A suspect screening list containing compound names, monoisotopic masses and chemical formulae was used for targeted screening. NIST MS Search v2.4 was utilised as the mass spectral database for isotopic mass pattern library matching for putative identification.

3. RESULTS AND DISCUSSION

3.1 Triple Quadrupole MS (LC-MS/MS)

The targeted qualitative analysis workflow with LabSolutions™ LCMS and NIST MS/MS spectral library is described in Scheme 1. The tea sample extract was first analysed with Q1 scan mode to determine the precursor ions *m/z* from a list of suspected compounds. Secondly, product ion scan (PIS) was performed on the precursor ions at collision energy of 35 V to confirm and characterise each of the suspected compounds, Shimadzu UFswitching™ technology enables a high-sensitivity and high-speed positive/negative ionisation switching in 5 msec which minimises ion losses and ensures the collection of sufficient scan data points across a chromatographic peak. As a result, both positive and negative ions could be successively analysed within a single acquisition run.

Figure 1 shows the product ion scan acquisition setting on LabSolutions™ LCMS software. Thirdly, the product ion mass spectra of the peaks were extracted from the precursor ion m/z chromatogram, exported and matched against public libraries or NIST MS/MS spectral libraries using NIST MS Search software. A list of possible hits with respective scores and compound information was provided by the NIST library tool. The library hit results could be used to aid putative identification of targeted precursor ions.

The major components found in yerba mate tea sample were xanthines, alkaloids, flavonoids and polyphenols. Figure 2 shows the product ion scan chromatograms of the targeted precursor ion m/z including five positive ions and nine negative ions in the yerba mate tea leaves sample.

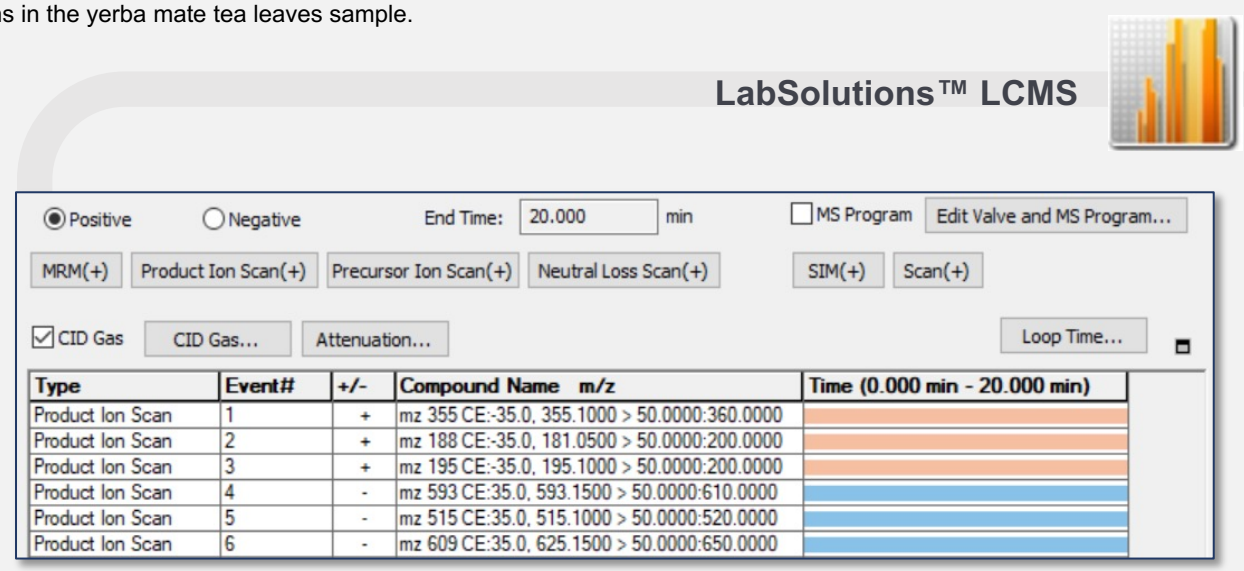


Figure 1. LabSolutions™ LCMS software interface on MS parameters for product ion scan acquisition mode

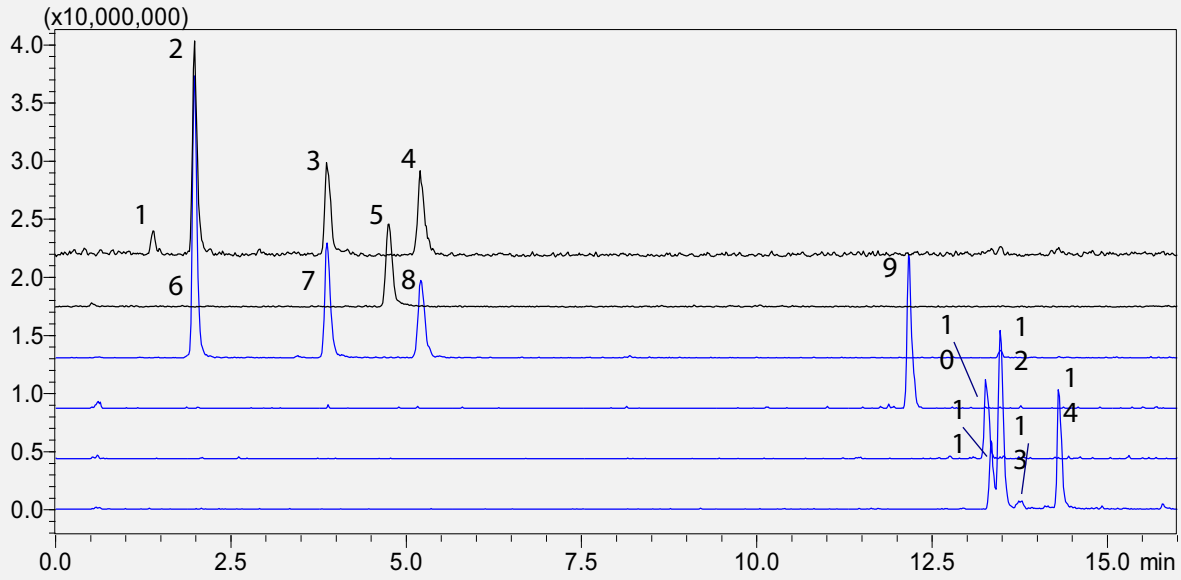
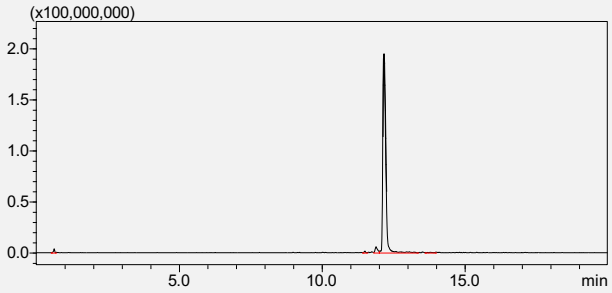


Figure 2. Product ion scan chromatogram in positive mode (black) and negative mode (blue) for yerba mate tea leaves sample at 50x dilution

Data Acquisition

- **Precursor *m/z* Determination**
  - Q1 Scan is carried out to determine all the precursor ions *m/z* from the list of suspected compounds

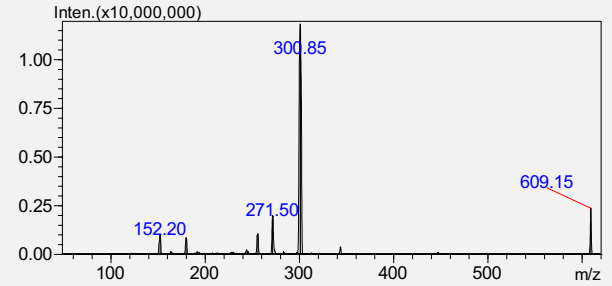
- **Product Ion Scan**
  - This scan enables the collection of mass spectra of the suspected compounds triggered by the selected precursor ion *m/z*



TIC(-) of product ion scan for *m/z* 609.15

MS Data Analysis

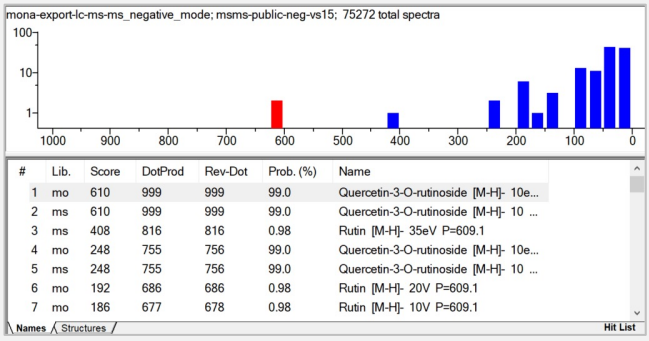
- **Mass Spectrum Pattern**
  - Postrun allows extraction of the precursor ion *m/z* chromatogram and the MS/MS spectrum of the peak
  - The extracted spectrum would be exported to NIST MS Search library as text format

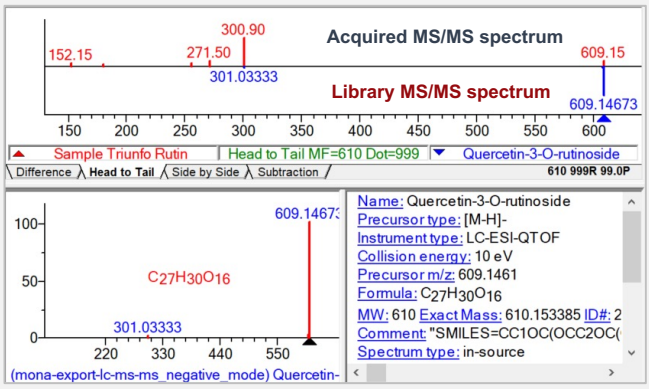


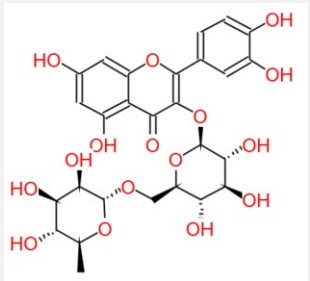
Mass spectrum of product ion scan for *m/z* 609.15

NIST MS Search

- **Registered Library Search**
  - The library search option enables the modification of search criteria and library registration
  - NIST Library tool provides the hit list of compounds with respective score and information
  - Mirror plot displaying acquired and theoretical isotopic patterns provides fast visual comparison



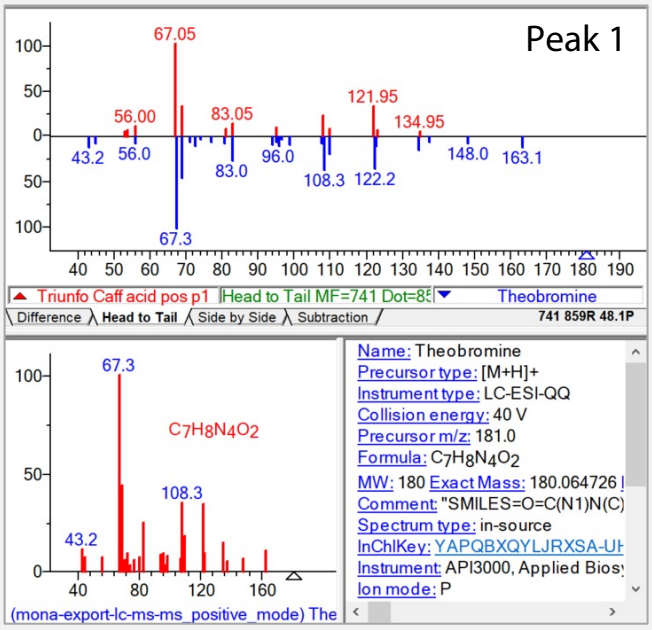


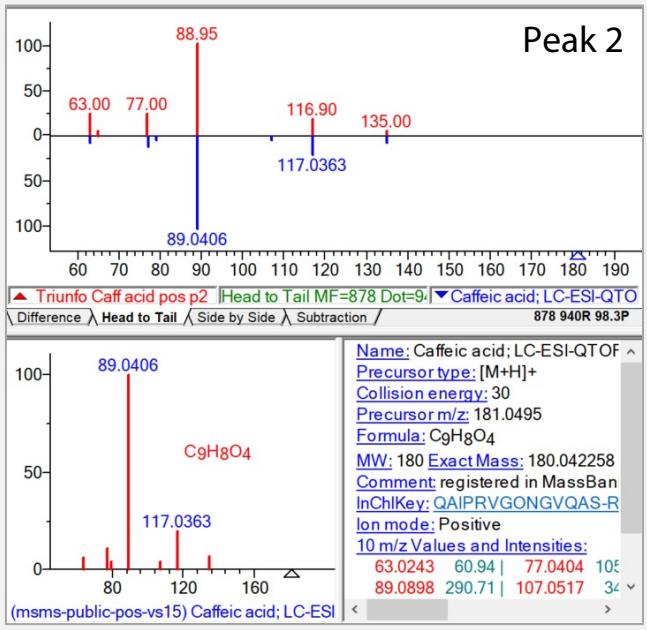


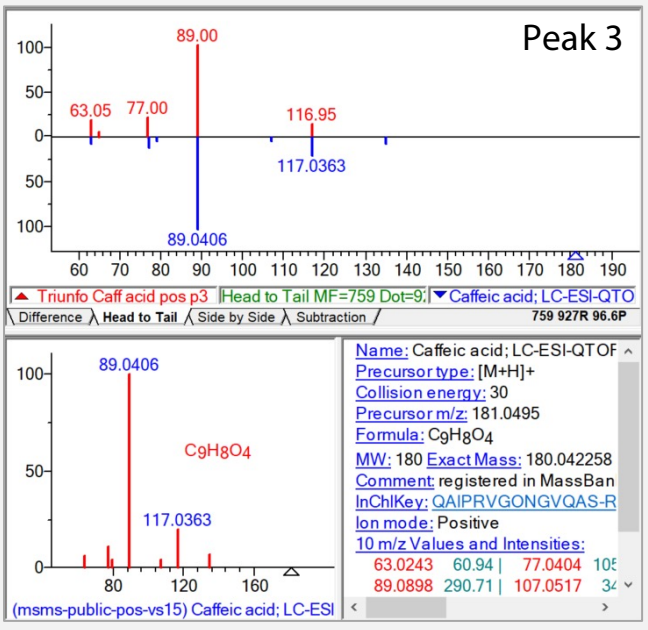
Quercetin-3-O-rutinoside (Rutin)
Score: 610
Library: MassBank of North America

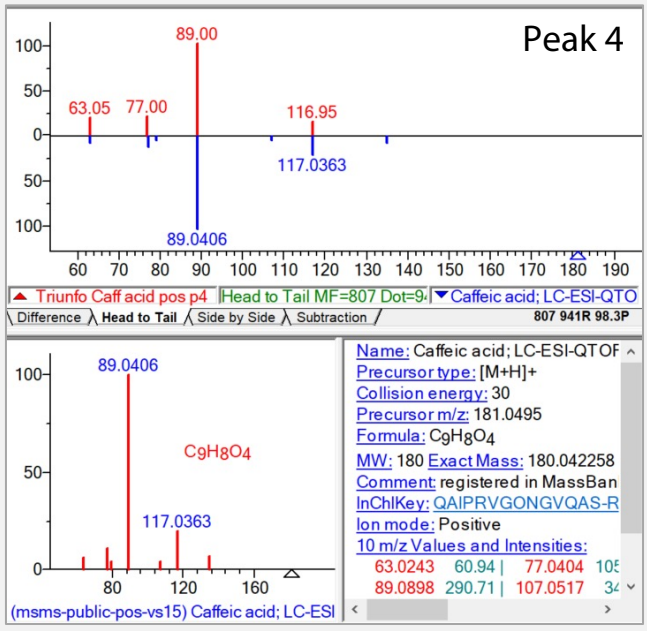
Scheme 1. Targeted qualitative analysis workflow with LabSolutions™ LCMS and NIST MS Search software

Each chromatographic peak was subsequently integrated and their respective mass spectra were exported for library match. A compound hit list would be generated and arranged according to respective matching scores after the ion *m/z* search criteria and library options have been established. Figure 3 shows the best library match for the product ion mass spectra of the 14 targeted precursor ions. The mirror plot function in NIST MS Search software allows fast visual comparison between the acquired product ion mass spectra and library mass spectra. The mirror plots showed similar fragmentation patterns between the experimental and library mass spectra. However, the intensities of the fragment ions might differ since the mass spectra in the libraries were curated from various MS/MS instruments and analysis parameters. Nevertheless, attaining a good match for crucial fragment ions is often adequate for putative identification.









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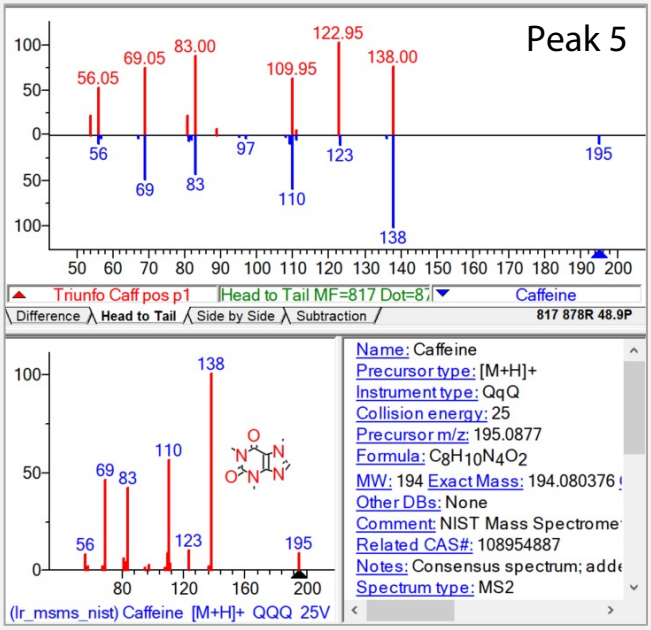

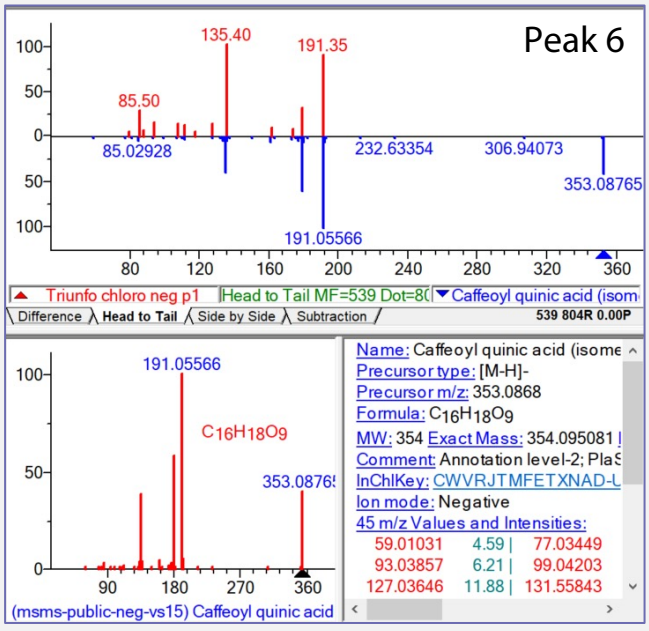

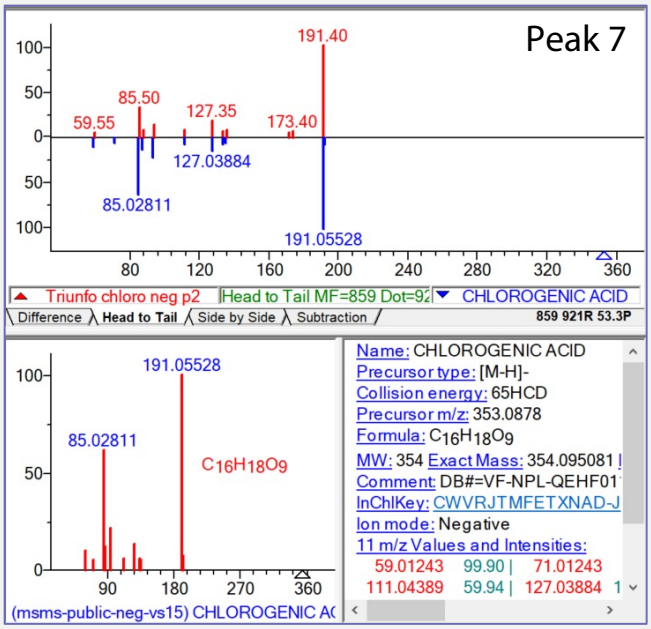

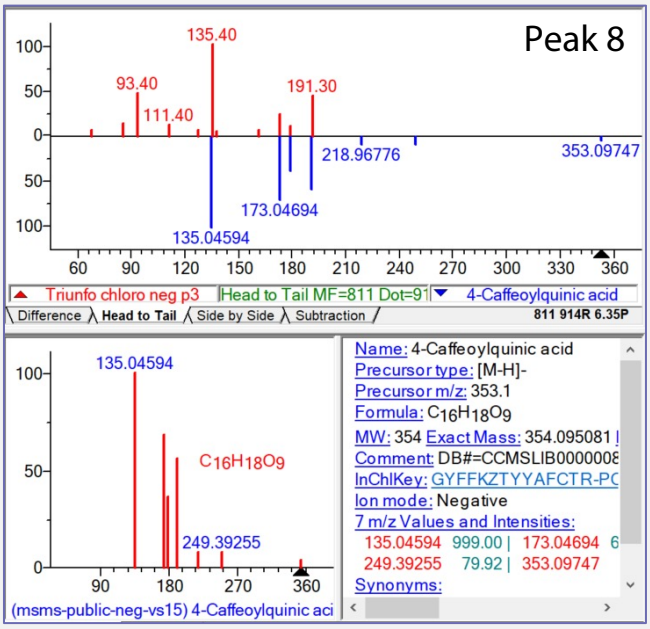

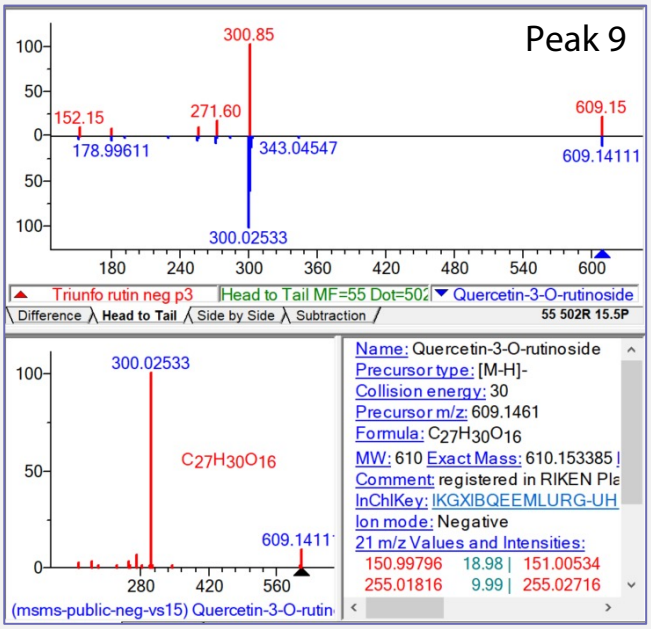

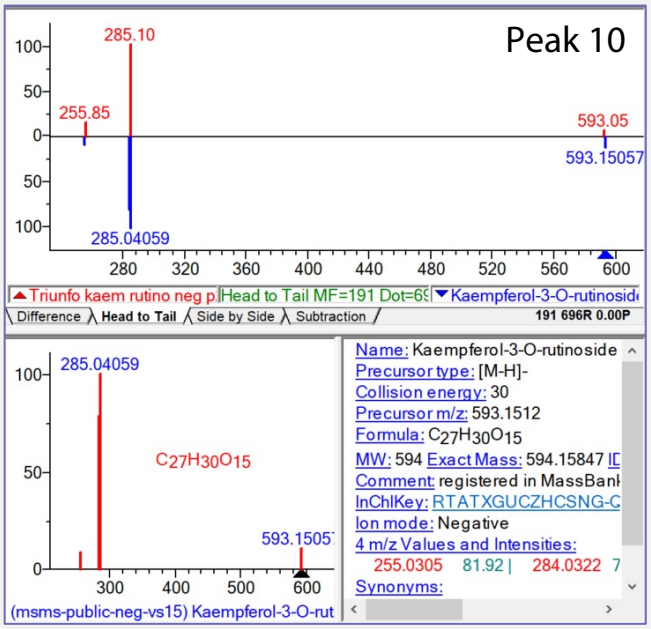

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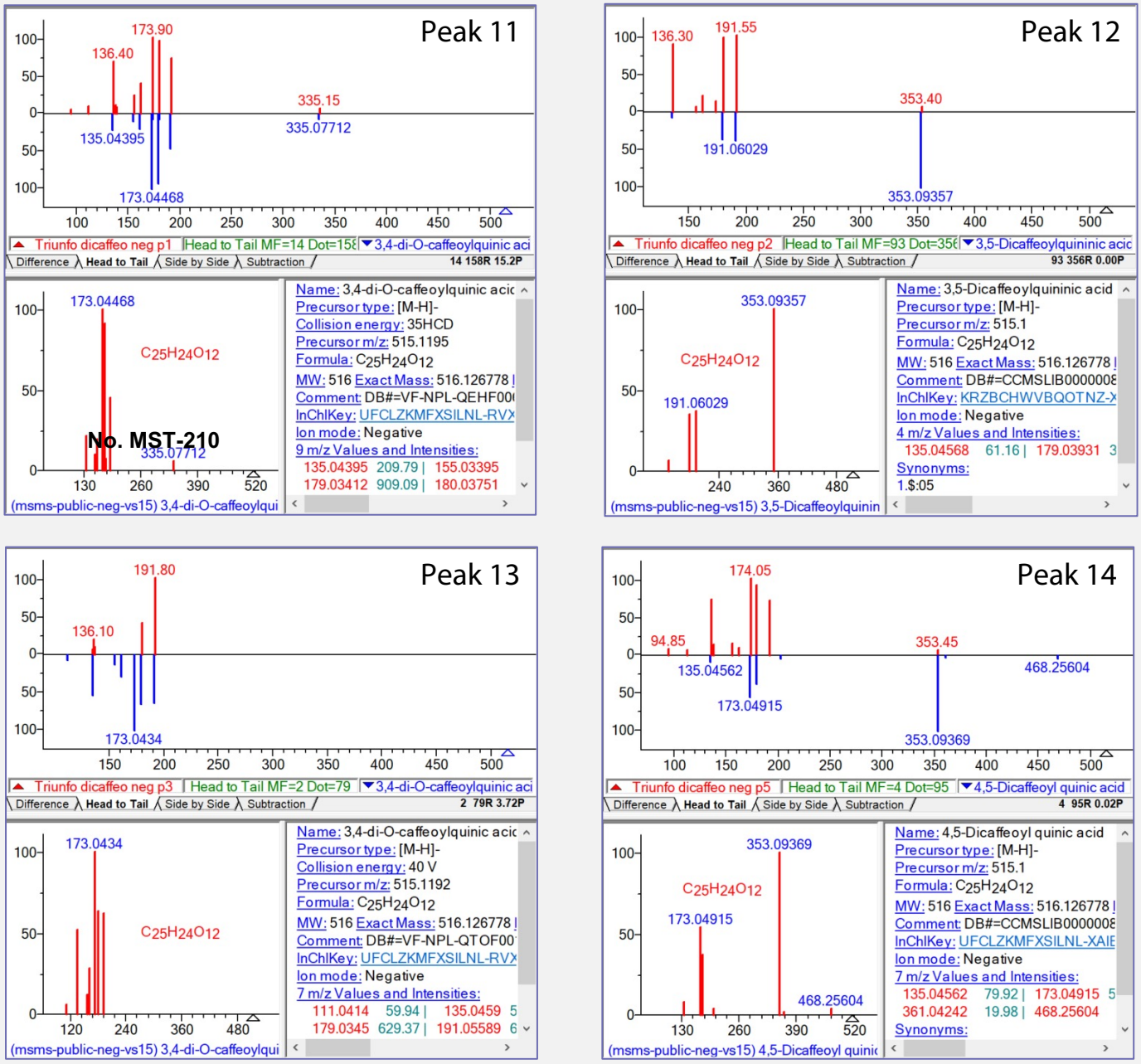

Figure 3. Library match results for 14 chromatographic peaks arising from the analysis of targeted precursor ions for yerba mate tea sample

Table 3 summarises the putatively identified compounds in the yerba mate tea sample. With successful identification using TQMS, distinguishing isomers would require additional retention time information or reference standards. For example, peak 2 – 4, 6 – 8, and 11 – 14 were collectively identified as caffeic acids, chlorogenic acids and di-caffeoyl quinic acids, respectively.

Table 3. Putative identification of compounds in yerba mate tea by LC/MS/MS

Peak	Mode	*m/z*	NIST identified compound
1	+	181.05	Theobromine
2	+	181.05	Caffeic acid
3	+	181.05	Caffeic acid
4	+	181.05	Caffeic acid
5	+	195.15	Caffeine
6	-	353.10	Caffeoyl quinic acid
7	-	353.10	Chlorogenic acid
8	-	353.10	4-caffeoylquinic acid
9	-	609.15	Quercetin-3-O-rutinoside
10	-	593.15	Kaempferol-3-O-rutinoside
11	-	515.10	3,4-di-O-caffeoylquinic acid
12	-	515.10	3,5-dicaffeoylquinic acid
13	-	515.10	3,4-di-O-caffeoylquinic acid
14	-	515.10	4,5-dicaffeoylquinic acid

4. CONCLUSION

A targeted qualitative analysis workflow performed with LCMS-8050 on yerba mate tea sample has enabled the putative identification of 14 compounds by obtaining their respective mass spectra and comparing against NIST MS/MS spectral library. For instance, theobromine, caffeic acid and its isomers, and caffeine were determined under positive mode. On the other hand, caffeoyl quinic acid and its isomers, quercetin-3-O-runtinoside, kaempferol-3-O-rutinoside, and di-caffeoyl quinic acid and its isomers were identified under negative mode.

By systematic analysis using Q1 scan mode and product ion scan mode followed with mass spectral match against MS/MS spectral libraries, putative identification can be achieved with LC-MS/MS. While high confidence identification and differentiation of isomers could be obtained with the availability of reference standards, this workflow extends the usage of TQMS from routine targeted quantitative analysis to preliminary unknown identification.

5. REFERENCES

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LabSolutions LCMS is an integrated workstation software used to control Shimadzu HPLC/UHPLC systems and LCMS instruments from a single user interface. Equipped with a variety of data processing features, the software not only allows targeted qualitative analysis (as shown here), but also enables the creation of quantitation methods for multi-component analysis. This translates to the ability for anyone to perform quantitative analyses with ease.

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