Metabolite Profiling in Fruit Juice using X500B QTOF System for Determining its Antioxidant Activity

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ABSTRACT

Recent advancements in LC-MS/MS technology, including hybrid systems like quadrupole-quadrupole Time-of-Flight (QTOF), now provide the ability to perform targeted and non-targeted screening in food samples on a routine basis. Plants contain high concentrations of numerous redox-active antioxidants, such as polyphenols, anthocyanins, and flavonoids with antioxidant activity, which fight against hazardous oxidative damage of plant cell components. In animal cells, antioxidant production is much more limited and oxidative damage is involved in the pathogenesis of most chronic degenerative diseases (including cancer and heart diseases) and aging. In the present study, the new SCIEX X500B QTOF system was used for profiling of metabolite levels in different fruit juice samples to indicate its antioxidant properties for health benefits. This is mainly contributed by phenolic compounds, anthocyanins and flavonoids. Together, these contribute to major health benefits of the fruit juice.

INTRODUCTION

The SCIEX X500B QTOF system is a robust, high performance high resolution MS/MS system designed for routine use providing high sensitivity, mass accuracy and resolving power along with confident identification based on MS/MS spectra. It has the industry leading robustness of Turbo V™ source and Curtain Gas™ interface.

The SCIEX OS software is a single platform for MS control, data processing, and reporting and provides:
Simple software workflows that deliver reliable results
Simultaneous identification and quantitation
Quick data review and reporting utilizing customizable flagging and filtering of results

Figure 1: The new benchtop X500B high resolution mass spectrometry system

MATERIALS AND METHODS

Materials:
The solvents which were used for the experiment, methanol, acetonitrile and water were purchased as LCMS grade from Sigma. For this analysis, pomegranate juice was used which was purchased from a general store of Gurugram in India. Pomegranate juice was purchased from 4 different brands. One of the brands manufactured 2 varieties of juice, each with variations in the process of preparation of the juice.

Sample Preparation:
The packaged juice was diluted with equal amount of water and methanol mixture (50:50). The diluted juice was then centrifuged at 4000 rpm for 2-3 minutes. The clear supernatant was then passed through 0.2micron filter and then injected into the mass spectrometer.

LC Method:
The LC-MS/MS analysis was carried out in 3 replicates for each of the juice sample. The ExionLC™ system (SCIEX) coupled to the X500B system (SCIEX) was used for analysis of the extracts. The extracts were run on Thermo Hypersil Gold 100mm X 2.1mm, 1.9µ column maintained at 40° C. The mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The samples were run for 20 minutes at a flow rate of 500µl/min with the following gradient program of solvent B: 0-1min, 2%; 1-8min, 2-40%; 8-10min, 40%; 10-14min, 40-95%; 14-16min, 95%; 16-16.2min, 95-2% and 16.2-20min, 2% of B. The injection volume was 10 µl.

Mass Spectrometry Method:
The new benchtop SCIEX X500B system with SCIEX OS Software was used for the analysis. This platform comes with the revolutionary N geometry TOF flight path. SCIEX OS Software is a single software platform for LC and MS control, data processing and reporting. It has three workspaces for Acquisition, Processing and Management (Figure 2). TOF MS scan mode acquisition with simultaneous Information Dependent Acquisition MS/MS was performed. Each sample was injected in minimum 3 replicate injections in both positive and negative polarity. Data was acquired over a mass range of 100 – 1200m/z with IDA MS/MS performed over the mass range of 50-1200m/z with collision energy of 40 eV and a spread of ±15eV. The ion spray capillary voltage was kept at 5500V, GS1 and GS2 was 40 psi each. Curtain gas was maintained at 30 psi. The source temperature was maintained at 500 ° C.

Data Analysis using SciexOS and MarkerView™ Software :
Data processing was done using the Analytics workspace in SCIEX OS. The result table was generated for qualitative and quantitative analysis in a single pane. Targeted screening for the metabolites was performed for identification and confirmation by structural elucidation. A traffic light system indicated the confidence in identification based on accurate mass, isotopic pattern and retention time. The different juice samples were analysed for cross-sample comparison using MarkerView™ Software 1.3.1 for statistical analysis, including principal component analysis, principal component variable grouping and t-tests.

RESULTS

Targeted screening of metabolites
TOF MS and MS/MS data was used for simultaneous identification of precursor masses followed by confirmation of metabolites by MS/MS fragmentation pattern matching. Using the accurate mass elemental composition analysis by FormulaFinder, the suggested formulas can then be linked to ChemSpider to automatically generate the list of possible compounds with the predicted formula. Identification and confirmation was further done by structural elucidation by theoretical and experimental fragmentation pattern matching. This identified and confirmed the presence of the various anthocyanins and phenolic metabolites in the juice samples.

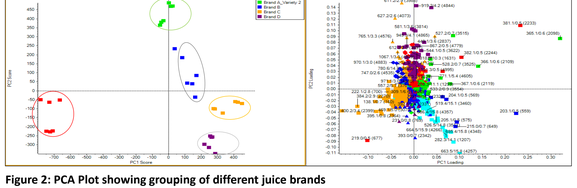

Figure 2: PCA Plot showing grouping of different juice brands

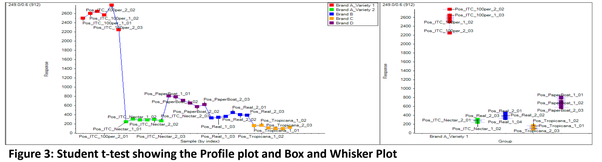

Figure 3: Student t-test showing the Profile plot and Box and Whisker Plot

Sample Type	Component Name	Formula	Precursor Mass	Expected RT	Area	Retention Time	Mass Error (ppm)	Mass Error...	RT Confi...	Isotope Confi...	Found At Mass	Isotope Ratio Dif...
Unknown	delphinidin-3,5-digl...	C27H31O17	627.1556	2.61	20860149	2.61	-1.0	✓	✓	✓	627.1550	3.4
Unknown	cyanidin-3,5-diglyc...	C27H31O16	611.1607	3.02	33916019	2.99	-0.7	✓	✓	✓	611.1602	1.3
Unknown	pelargonidin-3,5-d...	C27H31O15	595.1657	3.28	6749482	3.28	-0.9	✓	✓	✓	595.1652	0.9
Unknown	delphinidin-3-glyc...	C21H21O12	465.1028	3.37	5269507	3.34	-0.8	✓	✓	▲	465.1024	5.1
Unknown	cyanidin-pentoside...	C26H27O15	579.1348	3.21	33010	3.31	24.6	▼	✓	▼	579.1487	Infinity
Unknown	(epi) afzelechin-delp...	C6H13O17	737.1712	3.21	90915	3.15	-0.9	✓	✓	▲	737.1706	17.9
Unknown	(epi) gallocatechin-...	C6H13O17	737.1712	3.21	90915	3.15	-0.9	✓	✓	▲	737.1706	17.9
Unknown	cyanidin-3-glycosi...	C21H21O11	449.1078	3.65	16326849	3.69	-1.0	✓	✓	▲	449.1074	6.3
Unknown	(epi) gallocatechin...	C6H13O14	753.1661	2.59	75396	2.60	-0.8	✓	✓	✓	753.1656	20.1
Unknown	cyanidin-3-rutinosi...	C27H31O15	595.1657	3.28	6749482	3.28	-0.9	✓	✓	✓	595.1652	0.9
Unknown	pelargonidin-3-glyc...	C21H21O10	433.1129	4.07	6449848	4.08	-0.9	✓	✓	✓	433.1126	2.2
Unknown	(epi) catechin-cyan...	C6H13O17	737.1712	3.21	90915	3.15	-0.9	✓	✓	▲	737.1706	17.9

Figure 4: Screening and identification results from IDA experiment

Mass accuracy of the instrument was maintained at less than 1 ppm for most of the metabolites identified for both the positive and negative polarity (Figure 4).

Index	Sample Name	Sample Type	Component Name	Formula	Precursor Mass	Expected RT	Area	Retention Time	Mass Error (ppm)	Mass Error...	RT Confi...	Isotope Confi...	Found At Mass	Isotope Ratio D...
1	Pos_Conc_01	Unknown	delphinidin-3,5-digl...	C27H31O17	627.1556	2.61	20860149	2.61	-1.0	✓	✓	✓	627.1550	3.4
▶ 2	Pos_Conc_01	Unknown	cyanidin-3,5-diglyc...	C27H31O16	611.1607	3.02	33916019	2.99	-0.7	✓	✓	✓	611.1602	1.3
3	Pos_Conc_01	Unknown	pelargonidin-3,5-d...	C27H31O15	595.1657	3.28	6749482	3.28	-0.9	✓	✓	✓	595.1652	0.9

Figure 5: Identification of Cyanidin 3,5 glucoside showing the mass accuracy as 0.7ppm, extracted ion chromatogram of M+ ion, Isotopic distribution pattern and the MS/MS spectrum. FormulaFinder also predicts the formula for the precursor mass.

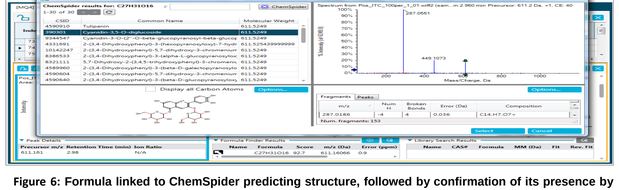

Figure 6: Formula linked to ChemSpider predicting structure, followed by confirmation of its presence by theoretical and experimental fragmentation matching.

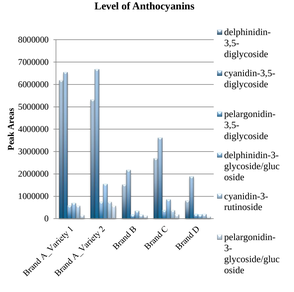

Figure 7: Levels of Anthocyanins: Analysis done in the positive ionization mode

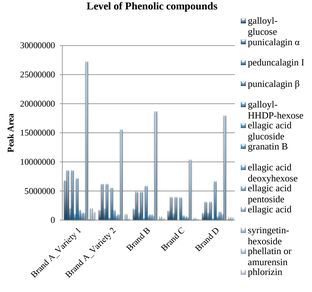

Figure 8: Levels of Phenolic compounds: Analysis done in the negative ionization mode

CONCLUSIONS

- High resolution X500B system can be used for metabolite profiling for simultaneous analysis of
  - Chemical compound identification
  - Confirmation using TOF MS and MS /MS
  - Comparison of different products based on chemical compounds identified and using statistical tools
- Easy user interface sand workflow assisted in the identification of the metabolites and its confirmation by structural elucidation. Data was acquired in both polarities with high mass accuracy.
- MarkerView™ Software facilitated in the statistical analysis of the juice samples showing the difference in the various juice brands. The levels of anthocyanins and phenolic compounds varied in different brands, indicating some brands with superior antioxidant property.

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TRADEMARKS/LICENSING

Document number: RUO-MKT10-7856-A