

A novel algorithm for peak  
alignment and multivariate  
analysis applied to  
simultaneous polarity  
switching LC/MS/MS analysis

ASMS 2011 WP384

Mitsuhiro Kanazawa<sup>1</sup>, Hisae Anyoji<sup>1</sup>, Jun Watanabe<sup>2</sup>,  
Junko Iida<sup>2</sup>

<sup>1</sup> Reifycs Inc, Tokyo, Japan

<sup>2</sup> SHIMADZU CORPORATION, Kyoto, Japan

## A novel algorithm for peak alignment and multivariate analysis applied to simultaneous polarity switching LC/MS/MS analysis

### Introduction

The use of multivariate analysis of mass spectrometry data has been well established for a diverse range of research fields including the metabonomics analysis of biological fluids, food and beverage analysis and environmental samples. Although a number of tools exist to help data reduction in large samples studies, there is a limitation in

using MS data sets which includes polarity switching. In this study, we describe the application of a novel algorithm for MS peak alignment which takes into account both positive and negative ion data to create a single data array using a fast polarity switching LC/MS/MS system.

### Materials and Methods

Several commercial carbonated drinks were analyzed by LC/MS/MS using a UFLC HPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan). Chromatographic separations were carried out using Shim-pack XR-ODSII (2.0mmID x 75 mm; 2.2  $\mu$ m) maintained at 40 °C. Components were separated using a gradient elution with a flow rate of 0.25mL/min; solvent A, 5mmol/L ammonium acetate, and solvent B, acetonitrile. MS scan data in both positive and negative ionization was acquired using a switching speed of 15msecs using an electrospray ion source. Data analysis was achieved using a novel software application (*Reifycs Signpost™ MS*, Japan) using both positive and negative data streams.

#### Sample Preparation:

4 kinds of the carbonated drink on market were prepared. The portions of the drink were decarbonated by ultrasonic and analyzed on LC/MS/MS.

#### LC/MS/MS:

Samples were analyzed using a UFLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan).

#### Key features of LCMS-8030 triple quadrupole mass spectrometer

- # ultra fast polarity switching of 15msec
- # ultra fast scan speed of up to 15,000 u/sec
- # UFsweeper™ technology dramatically minimizes cross talk
- # excellent linearity with wide dynamic range



Fig. 1 LCMS-8030 triple quadrupole mass spectrometer

#### Analytical Conditions

##### HPLC (UFLC system)

- Column: Shim-pack XR-ODSII (2.0mmID x 75 mm; 2.2  $\mu$ m)
- Mobile phase A: 5mM ammonium acetate
- Mobile phase B: acetonitrile
- Gradient program: 2%B (0 min) - 90%B (8-9min) - 2%B (9.1-14min)
- Flow rate: 0.25 mL/min
- Column temperature: 40 °C

##### Mass (LCMS-8030 triple quadrupole mass spectrometry)

- Ionization: ESI
- Polarity: positive & negative
- Scan mode: Q1 scan

#### Software for the fast polarity switching LC/MS/MS system:

For this fast polarity switching LC/MS/MS Data analysis, we apply a new function to the existing *Reifycs Signpost™ MS* Software. The *Signpost™ MS* developed for peak alignment and subsequent multivariate analysis software takes into account the molecular ion location (RT-*m/z*) and volume of each molecular ion. By applying a shape detection technique for molecular elution and isotopic distribution, this process significantly reduces the amount of comparative information employed in the analysis. Since it uses a data reduction technique only on the molecular ions rather than all ion peaks which includes a significant contribution from background noise, the computational time and comparative accuracy are significantly improved. The molecular ion locations are detected and processed as spots, the spots are subsequently aligned for all samples to create a data array (or alignment table). We develop the function of LC/MS data simultaneous loading from both positive and negative polarity measurement data and apply into *Signpost™ MS*.

# A novel algorithm for peak alignment and multivariate analysis applied to simultaneous polarity switching LC/MS/MS analysis

## Results

### Data Acquisition

A single data array was generated using a series of LC/MS/MS sample files following the analysis of a batch of commercially available carbonated drinks (the array included replicate analysis, n=5 for 4 different drink products; all data included positive and negative data streams).

By using a fast polarity switching MS time of 15msecs, there is no compromise in acquiring a high number of data points across a peak for each polarity data stream.

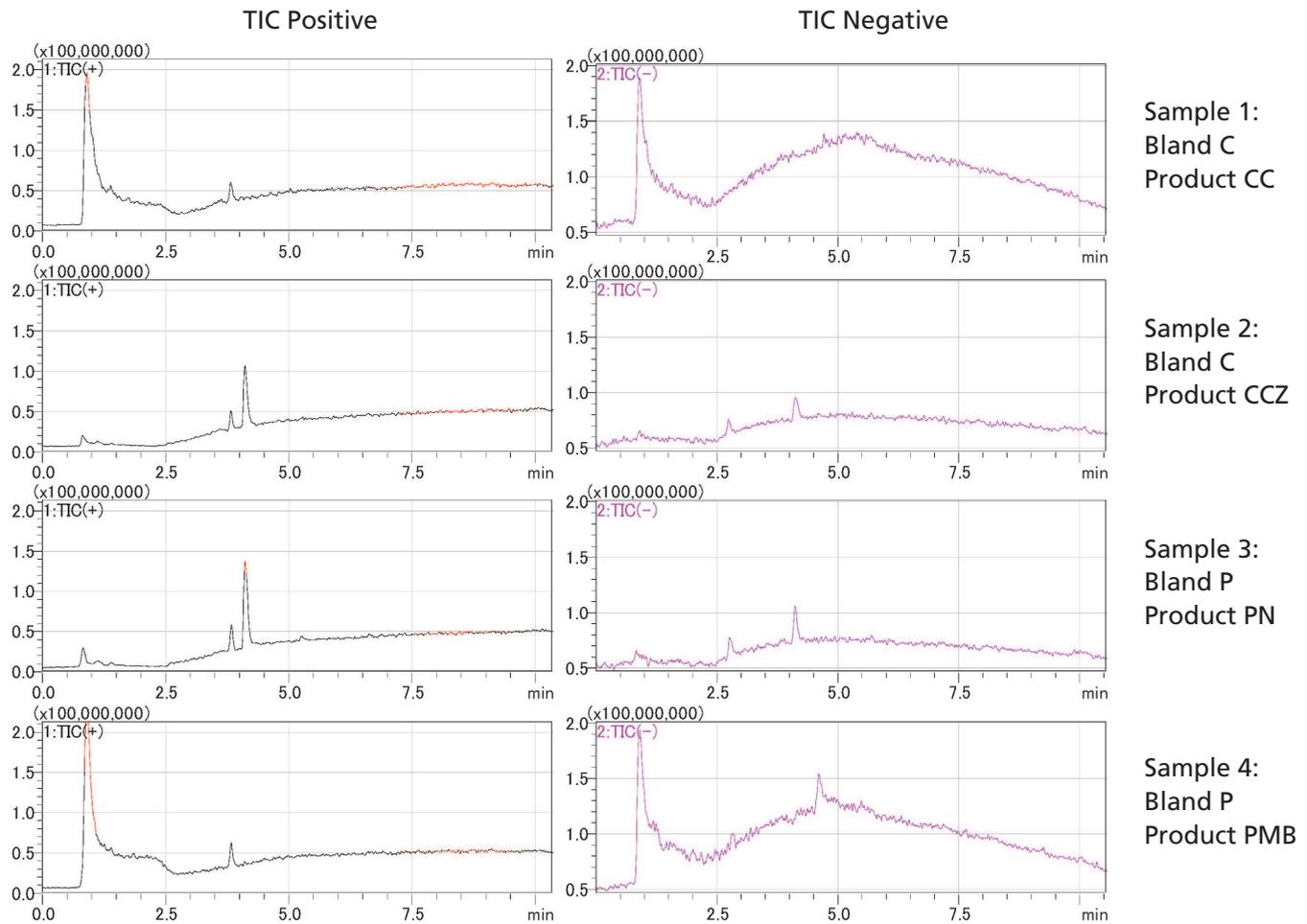


Fig. 2 TIC comparison of 4 drink samples  
Sample 1 & 4, Sample 2 & 3 resulted similar TIC each other.

# A novel algorithm for peak alignment and multivariate analysis applied to simultaneous polarity switching LC/MS/MS analysis

## Data Analysis for Multiple Data

The developed software for this presentation can analyze both Positive and Negative polarity

simultaneously and displays either one or both at the same time. (Figure 3)

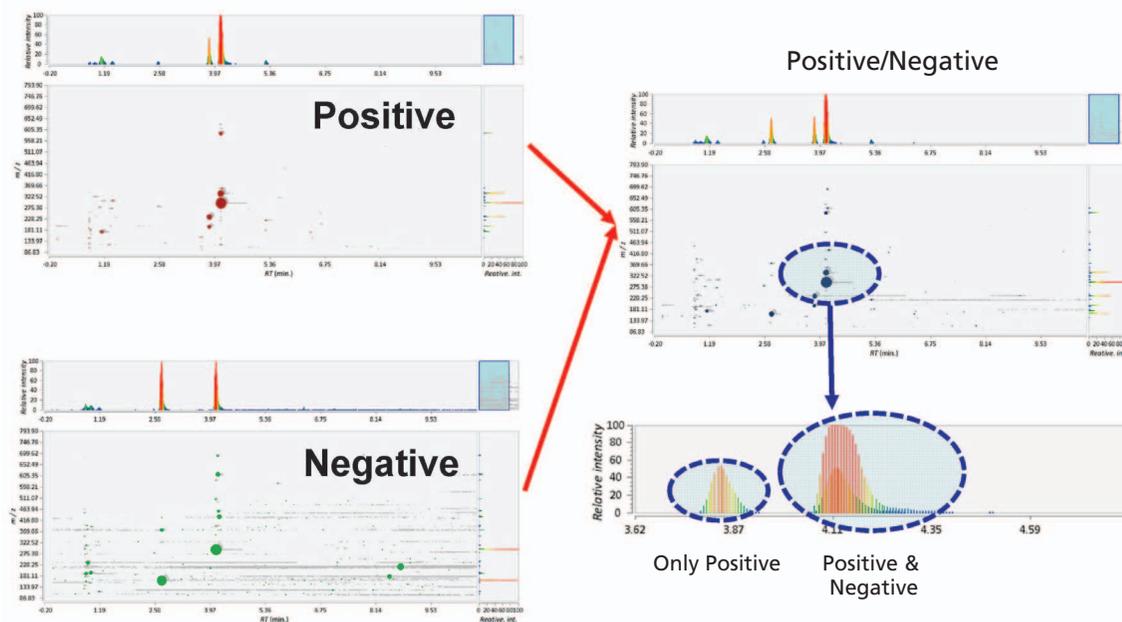


Fig. 3 Simultaneous analysis for both Positive-Negative polarity  
Peaks of both Positive and Negative polarities are displayed with an overlap.

Once Positive and Negative polarity data are loaded into the software, multivariate analysis lists up

differentiate target molecules among comparative samples.

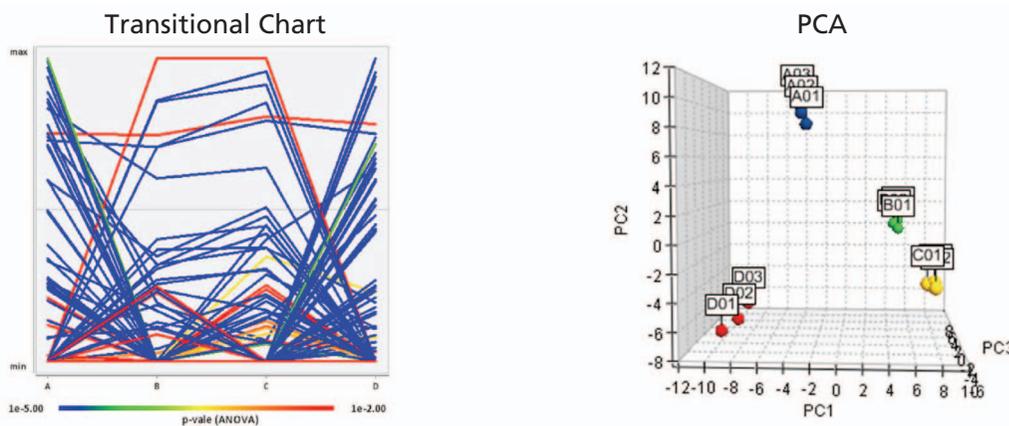


Fig. 4 Multivariate analysis for both Positive-Negative peaks  
List up target molecules among comparative samples by multivariate analysis

## A novel algorithm for peak alignment and multivariate analysis applied to simultaneous polarity switching LC/MS/MS analysis

### Peak Confirmation by LC/MS/MS

Peaks detected in multivariate analysis were then analyzed by LC/MS/MS with multiple product ion scans, collision energy low, medium, high. The peak, RT 5.6min was detected only in sample 3 as the result of multivariate analysis. MS and

MS/MS spectra of the peak were shown in Figure 5. Owing to fast scanning capacity of LCMS-8030 (up to 15000u/sec), these spectra could be acquired at one LC/MS run.

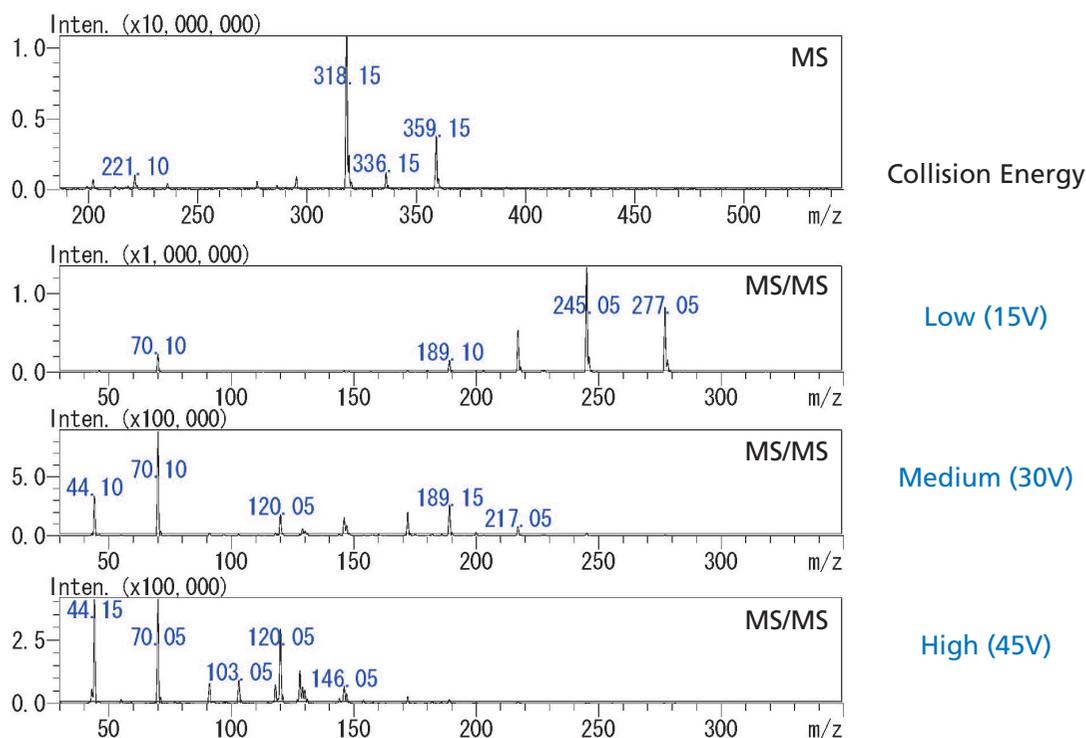


Fig. 5 Mass spectra of Peak, RT 5.6min, detected in sample 3

## Conclusion

This study introduced the simultaneous polarity switching LC/MS/MS based multivariate analysis method to compare

measurement samples and the analytical ability using drink products comparison.

- Spot Detection and alignment techniques facilitated comparative analysis by LC/MS measurement data, which consisted in both positive and negative full scan.
- We utilized drink sample comparison as one of example in this analysis, but this approach enables to apply many kinds of sample comparison.
- Multiple MS/MS analysis were available for peak conformation because of comparison in LC/MS/MS spectra.

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. We maintain a global network of sales, service, technical support and applications centers on six continents, and have established long-term relationships with a host of highly trained distributors located in over 100 countries. For information about Shimadzu, and to contact your local office, please visit our Web site at **[www.shimadzu.com](http://www.shimadzu.com)**



**SHIMADZU CORPORATION. International Marketing Division**

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan

Phone: 81(3)3219-5641 Fax. 81(3)3219-5710

**URL <http://www.shimadzu.com>**