SHIMADZU

Comprehensive analysis of functional ingredients using LC-MS/MS

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1. Introduction

Currently in Japan, the strategic innovation creation program SIP 2nd phase is being promoted as a project of the Cabinet Office. Under the leadership of Dr. Yamamoto of National Agriculture and Food Research Organization (NARO), Shimadzu Corporation is also participating in an initiative that contributes to the extension of healthy life expectancy by establishing a health system through food.

In this poster, we report the comprehensive analysis method which we are promoting in this initiative.

2. Methods

As a rough pretreatment, homogenize, mixture, centrifugation, filtration, dry-up, and reconstitution were done, and final sample solution were used as a sample for LC/MS/MS.

For LC-MS/MS, a triple quadrupole mass spectrometer with high quantitative performance; NexeraTM X3 system and LCMS-8060NX (Shimadzu Corporation) were used for this analysis.

Comprehensive analysis methods were classified into 5 groups according to target functional ingredients, and LC/MS analysis methods were constructed for each.





Table 1. Number of compounds for 5 analytical methods

Target analytes	Compounds
Polyphenols	147
Amino acids/di-peptides	62
Carotenoids	23
Primary metabolites	61
Phospholipids	10
Total	303

Sample preparation methods for each analyte group.

- LC-MS/MS based method.
- Semi-quantitation based on single-point calibration.
 - 1) estimated LLOQ is equal to or more than 100 ppb
 - 2) Individual analysis may be required for accurate quantitation.

For example, the polyphenol analysis method targeted 147 polyphenols, and the carotenoid analysis method targeted 23 carotenoids. In total, the five analytical methods covered approximately 300 functional ingredients.

Compound Name	MRM trasition (Q1>Q3)	Retention time (min)	Event number / Porarity
6"-O-Acetyldaidzin	459.1500>255.0500	8.417	48:MRM(+)
6"-O-Acetylgenistin	475.1500>271.1000	9.069	54:MRM(+)
Acetylglycitin	489.1500>285.0500	8.461	49:MRM(+)
Apigenin	271.0578>153.0500	10.255	62:MRM(+)
Apigenin 7-glucuronide	447.0878>271.2000	8.628	51:MRM(+)
Apigenin 7-glucoside	433.1000>271.0500	8.261	45:MRM(+)
Apigeninidin	255.0700>171.2000	8.042	40:MRM(+)
Apiin	565.1500>271.0500	8.054	42:MRM(+)
Astragalin	447.1000>284.0500	6.599	94:MRM(-)
Biochanin A	285.0778>213.1000	12.518	80:MRM(+)
3-Caffeoylquinic acid	353.0922>135.1000	6.449	92:MRM(-)
4-Caffeoylquinic acid	353.1000>173.1500	6.870	97:MRM(-)
5-Caffeoylquinic acid	353.1000>191.1000	6.955	98:MRM(-)
3,5-Dicaffeoylquinic acid	515.1000>353.3000	8.363	133:MRM(-)
4,5-Dicaffeoylquinic acid	515.1000>353.2000	8.464	134:MRM(-)
Caffeic acid	179.0322>135.1500	7.438	104:MRM(-)
Caffeine	195.0877> 83.1000	7.080	28:MRM(+)
Catalpol	407.1000>199.1000	5.861	89:MRM(-)
Catechin	291.0878>139.0500	6.981	21:MRM(+)
Catechin gallate	441.1000>169.1000	7.983	123:MRM(-)

Table 2. Some of the compounds included in the polyphenol method

All methods were developed by using a single standard compound for all analytes. The investigation includes selection of ISTDs for each methods, calibration point, dynamic range, and dilution factors etc...

3. Results

In the SIP2 initiative, we measured 32 types of food using 5 analytical methods (polyphenols, carotenoids, amino acids, primary metabolites, and phospholipids) and provided semi-quantitative values. The 32 types of food have been selected from widely consumed foods such as rice, soybean, and potato. There have never been reports of such a large amount of data being collected, so the amount of information in this study is extremely large.

Although it was a semi-quantitative method, the analysis was performed using standard products and internal standard substances, and the approximate concentration value was calculated by single-point calibration.

The analysis was not performed for the group of methods that did not contain or expect functional ingredients in the food. Table 2 presents a matrix table showing which method was used for the analysis of 32 foods.

An example of measurement results was shown in Figure 2.

	Analytical Method				
Foods	Polyphenols	Carotenoids	Amino acids	Primary metabolites	Phospholipids
Rice	Ο	Ο	0		Ο
Soybeam	Ο	Ο	0		О
Shiitake	О		0	О	
Potato	Ο		0		
Sesame Seed		Ο	Ο		
Squach	Ο	Ο	Ο		
Carrot	Ο	Ο	Ο		
Green onion	Ο		Ο		
Burdock	Ο		Ο		
Sweet potato	О		Ο		
Apple	О		Ο	Ο	
Mandarin	Ο	Ο	Ο	Ο	
Broccoli	Ο	Ο	Ο		
Onion	Ο		0		
Komatsuna	Ο	Ο	Ο		
Salmon	Ο	Ο	0		
Spinach	Ο	Ο	0	Ο	
Egg plant	Ο	Ο	0		
Wakame seaweed	Ο	Ο	0		
Green tea	Ο	Ο	Ο	Ο	
Barley	Ο	Ο	Ο		
Green pepper	Ο		Ο		
Tomato	Ο	Ο	Ο		
Cabbage	Ο		0		
Shiso leaves	Ο	Ο	Ο		
Chicken egg			Ο		Ο
Wheat		Ο	Ο		
Daikon radish	Ο		0		
Chicken breast			0		
Paprika	Ο	Ο	0		
Edamame (green soybeans)	Ο	Ο	Ο		
Lettue	О	О			

Table 3. Target foods and measurement items



- (A) Glucoraphanin in Broccoli, Komasuna, Onion
- (B) β-Carotene in Broccoli, Komatsuna
- (C) Glutamine in Broccoli, Komatsuna, Carrot, Onion

4. Conclusion

- ingredients contained in major foods were ✓ Functional comprehensively semi-quantified by LC-MS/MS.
 - The measurement results will be published as a database on the web.

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