Application News

No. M305A

GCMS™ GCMS-QP™2020 NX, GCMS-TQ™ NX series

Quantitation of Policosanols and Phytosterols in Brans by GC/MS

Y. Nakagawa

User Benefits

◆ No dilution re-runs of high positives by an extended calibration curve range

◆ Automatic positive confirmation by relative ion intensities according to 2002/657/EC

■ Introduction

Functional foods such as brans, wheat and tea have gained popularity in modern diet due to an increasing number of scientific discoveries indicating that certain dietary factors are connected to disease. With the growth of the functional foods market, quantitation of existing functional nutrients as well as qualification of new ones are of paramount interest to both food producers and consumers.

Shimadzu Corporation Japan has conducted a collaborative research with National Agriculture and Food Research Organization (NARO) on the relation between the origin of food products and the composition of their functional nutrients (e.g. policosanols and phytosterols).

In this article, 6 policosanols and 3 phytosterols were quantitated in 48 bran samples (Fig. 2). The otherwise time-consuming analysis of the 48 samples was expedited by LabSolutions Insight ™ with its automatic positive confirmation features regarding relative ion intensities and retention times.

Multivariate analysis was also performed with Orange to explain which compounds, besides the 6 policosanols and 3 phytosterols, played a significant role in distinguishing sample clusters among the 48 samples.
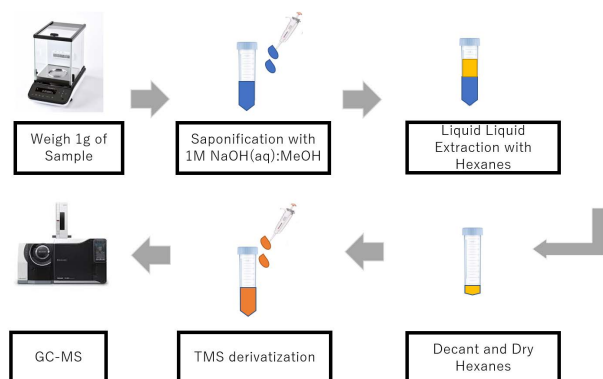
■ Methods – Extraction

Fig. 1 Extraction Flowchart

■ Methods – Analysis

Table 1 Instrument Configurations

GC-MS	: GCMS-QP 2020 NX
Auto Injector	: AOC™-20i Plus
Auto Sampler	: AOC-20s Plus
Analytical Column	: SH-I-5Sil MS (15 m × 0.25 mm I.D., df=0.25 µm) [*1]
Guard Column	: Rxi guard column (5m × 0.25 mm I.D.) P/N: 10029
Glass Insert	: Topaz 3.5 mm ID Single Taper inlet Liner w/ Wool P/N: 23336, Restek Corp.

*1 P/N: 227-36036-01

Table 2 Analytical Conditions

GC	
Inlet temp.	: 270 ˚C
Injection Mode	: Splitless High Pressure Injection (200 kPa, 1.1 min)
Sampling Time	: 1 min
Carrier gas	: Helium
Control Mode	: Constant linear velocity (77.3 cm/s)
Column oven temp.	: 200 ˚C (1 min) → (25 ˚C /min) → 250 ˚C → (15 ˚C /min) → 320 ˚C (1 min) Total 8.67 mins
Purge flow rate	: 3 mL/min
Sample Inj. volume	: 2 µL

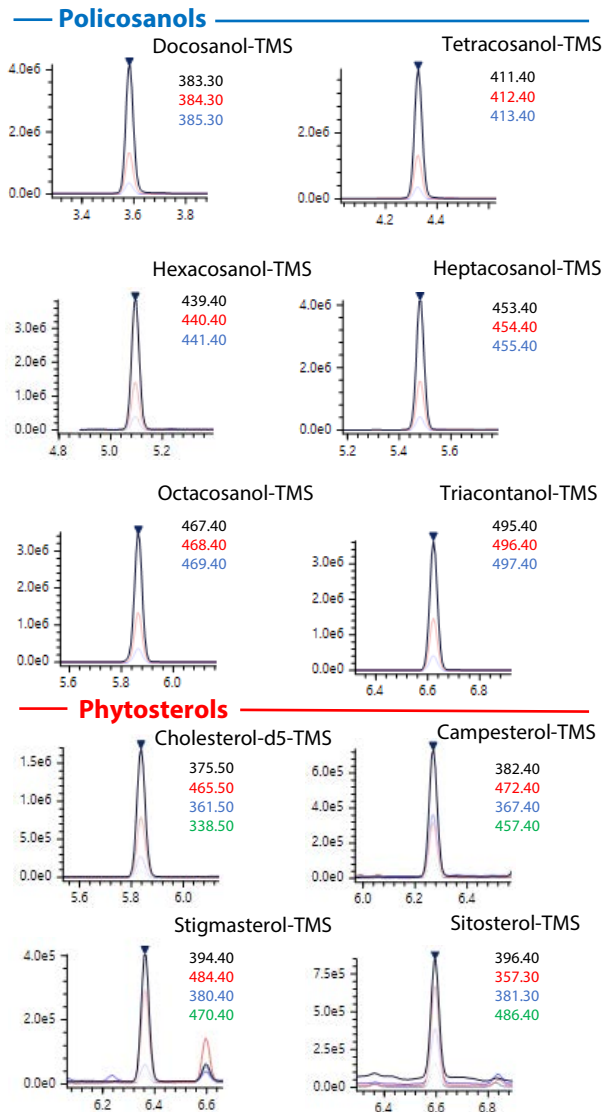| MS	
Ion Source Temp.	: 230 ˚C
Interface temp.	: 270 ˚C
Measurement Mode	: Simultaneous Scan/SIM (FAAST)
Scan mass range (*m/z*)	: 35-600 at scan speed of 10000 µ/s
SIM ions (*m/z*)	: Refer to Fig. 2

■ Results

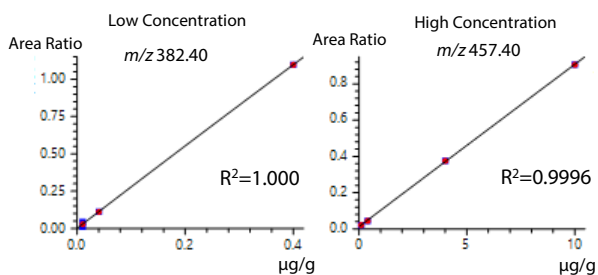Fig. 2 Chromatograms at 0.1 μg/mL

Fig. 3 Calibration Curve of Campesterol-TMS

■ Results-Ion Ratio Comparison

For those compounds commonly found in foods, it is essential to confirm positive hits with peak shape, retention time and ion ratio. While peak shape and retention time are rather easy to spot, ion ratio requires calculation and can be time consuming. With Flexible Ion Ratio Settings in LabSolutions Insight™, relative ion intensities can be set as in Fig. 4. This is an example by EU 2002/657/EC and SANCO.12495, but the same function can be applied to other regulations with relative ion intensity criteria   (e.g. WADA in sports doping, FDA ORA-LB.10 for pesticides in foods).

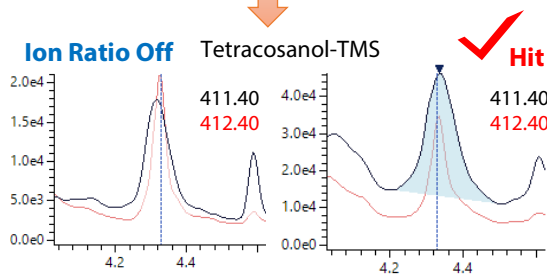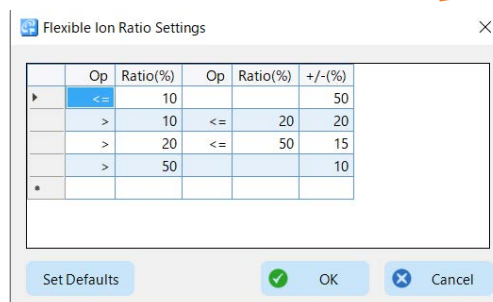| Relative Abundance (% of base peak)	Maximum Tolerance
> 50 %	± 10 %
20 % to 50 %	± 15 %
10 % to 20 %	± 20 %
≤ 10 %	± 50 %

2002/657/EC

Fig. 4 Flexible Ion Ratio Settings

With this setting, the number of false hits were reduced and analysis time was cut by hours.

To further facilitate the analysis, an extended calibration range by choosing an high intensity *m/z* for low concentration range and a low intensity *m/z* for high concentration range (Fig. 3) was employed to circumvent dilution re-runs, or even re-extractions in case of analysis involving derivatizations as in this experiment. The linear range spanned from 0.01 to 10 μg/g in the case of campesterol-TMS.

When the linear range runs more than a few orders of magnitude, it is possible that peaks of small and large intensities have different ion ratios despite the best efforts during method development not to. With LabSolutions Insight, different ion ratios can be set at low and high concentration ranges and Flexible Ion Ratio Settings can be applied to both ranges to avoid missing true positive hits at either range.

■ Results-Spike Recoveries

Table 3 Spike Recoveries at 0.2 μg/g

Compound	SPK1	SPK2	SPK3	SPK4	SPK5	SPK6	Average	RSD (%)
Docosanol-TMS	103	100	108	112	111	104	107	4.9
Tetracosanol-TMS	104	99	111	117	114	101	108	7.2
Hexacosanol-TMS	101	97	107	111	111	98	104	6.5
Heptacosanol-TMS	94	86	90	86	88	57	83	13.5
Octacosanol-TMS	89	89	91	90	94	85	89	2.8
Triacontanol-TMS	89	92	75	74	77	76	81	7.8
Stigmasterol-TMS	66	73	58	61	53	57	61	7.3

The 6 spikes in Table 3 were extracted for the batch of the 48 samples and the first two ran consecutively after the calibration curve run and each of the rest of the 4 spikes ran at an interval of 10-12 sample runs to serve as a continuing calibration verification (CCV).

A bran blank used for spike contained high concentrations of endogenous Campesterol and Sitosterol, making the spikes at 0.2 μg/g too low to be calculated Stigmasterol spike also suffered from its endogenous counterpart with the blank possibly containing a pocket of high concentration of Stigmasterol.

On the whole, however, spike recoveries and repeatability were acceptable for the purpose of this experiment.

Below is the results of sample 1 as an example.

Table 4 Sample 1 results in μg/g

	Docosanol	Tetracosanol	Hexacosanol	Heptacosanol	Octacosanol	Triacontanol
Sample 1	0.069	0.036	0.037	0.042	0.018	0.020

	Campesterol	Stigmasterol	Sitosterol
Sample 1	1.894	0.668	2.224

■ Addition into Smart Metabolites Database™

If it so happens where target analytes are not registered in Smart Metabolites Database, those compounds can be simply added to the database with optimized MRM and/or SIM, mass spectrum, retention time, retention index and calibration curve.

MRM, SIM, MRM/Scan or MRM/SIM method can be created from the database with automatically adjusted MS elution windows by AART (Automatic Adjustment of Retention Times). In other words, retention time shifts caused by column trimming are adjusted by one injection of n-alkane homologs and a new method is instantly.

■ Scan Results

Scan and SIM modes were run simultaneously in this experiment. SIM results did not separate the sample clusters well. Scan results, however, showed the four distinct clusters (Fig. 5) and produced a list of important peaks that contribute to the distinction of the clusters.
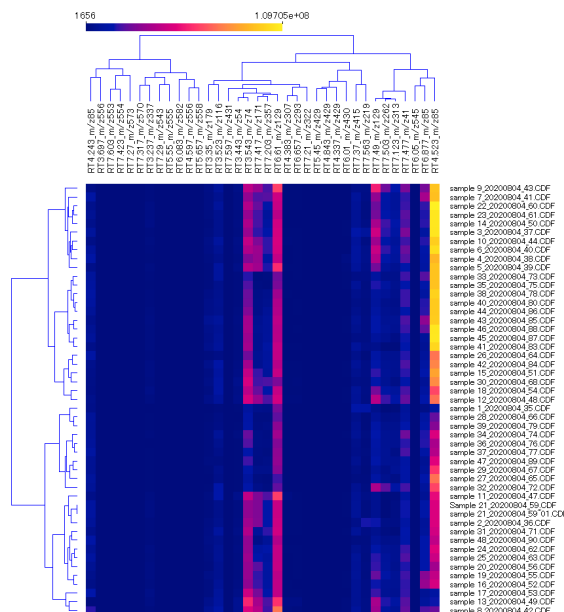

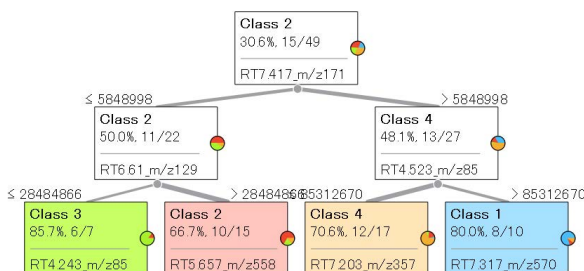Fig. 5 Heatmap by Scan portion of the simultaneous Scan/SIM by Orange Data Mining

Fig. 6 Decision Tree by Scan portion of the simultaneous Scan/SIM by Orange Data Mining

■ Summary

As a part of the ongoing collaborative research with National Agriculture and Food Research Organization (NARO) on functional foods, bran samples from all over Japan were analyzed for a variety of policosanols and phytosterols.

GCMS-QP2020 NX has been the work horse in this project, running hundreds, if not thousands, of samples of heavy matrix with a minimum maintenance. In this experiment, 48 bran samples were run, resulting in acceptable spike recoveries and repeatability.

Data analysis time was greatly reduced with the help of Flexible Ion Ratio Settings of LabSolutions Insight. The number of dilution re-runs was cut by an extended calibration curve.

Shimadzu Corporation would like to thank Hironori Juichi, Yayoi Ichiki and Mari Yamamoto from NARO for their guidance in leading the project.

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