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Application News

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

Fast Analysis of Organic and Amino Acids in Café Late by Solid Phase-Derivatization method

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User Benefits

- 10 mins sample extraction with SP-Deriv. method followed by 23 mins MRM run (Smart Metabolites Database™)
- 5 min analysis time with TraverseMS
- Selective removal of carbohydrates from sugar rich food samples

■ Introduction

Food products with a high concentration of sugars are known to pose extraction issues both during a freeze drying (e.g. loss of sample by explosive boiling) as well as in the subsequent derivatization steps (e.g. derivatization reagent quenching).

As a solution to these issues, SP-Deriv. method using Presh-SPE (Wakayama, Japan, AiSTI Co., Ltd) was employed to selectively remove carbohydrates from sugar rich samples before derivatization steps.

In this article, results from the conventional overnight method (i.e. Bligh and Dyer extraction, drying and derivatization) and the SP-Deriv. method were compared.

Fig. 1 Presh (left) and GCMS-TQ™8040 NX (right)

■ Methods – Extraction and Analysis

The same sample was extracted separately by two methods; the conventional and the Solid Phase-Derivatization.

For the conventional method, a Café Late sample (n=5) was extracted in accordance with the Shimadzu sample pretreatment handbook for metabolomics (document number: C146-2181).

For comparison, the same Café Late sample (n=5) was extracted as per Presh-SPE ACXs instruction (P/N SA-5589-003, AiSTI Co., Ltd) with the sample weight of 50 µL.

Both method employed 2-isopropylmalic acid as an internal standard and analyzed with the instrument configurations and analytical conditions according to Smart Metabolites Database (Table 1 and 2).

Table 1 Instrument Configurations

GC-MS	: GCMS-TQ 8040 NX
Auto Injector	: AOC™-20i Plus
Auto Sampler	: AOC-20s Plus
Analytical Column	: BPX-5 (30m × 0.25 mm I.D., df=0.25 µm) P/N: 054101
Glass Insert	: Split liner with wool

Table 2 Analytical Conditions

GC	
Inlet temp.	: 250 ˚C
Injection Mode	: Split
Spit ratio	: 30
Carrier gas	: Helium
Control Mode	: Constant linear velocity (39.0 cm/s)
Column oven temp.	: 60 ˚C (1 min) → (15 ˚C /min) → 330 ˚C (1 min) Total 23.0 mins
Purge flow rate	: 5 mL/min
Sample Inj. volume	: 1 µL
MS	
Ion Source Temp.	: 200 ˚C
Interface temp.	: 280 ˚C
Measurement Mode	: MRM
SIM ions (*m/z*)	: Refer to Smart Metabolites Database

■ Results

The number of compounds detected were almost identical between the conventional method and the SP-Deriv. method (Table 3).

Table 3 The Number of Compounds Detected

# of cpds found	Conventional	Presh
Organic Acid	41	40
Amino Acid	7	7

As shown below in Table 4, reproducibility (n=5) were also comparable between the conventional method and the SP-Deriv. method (Table 4). The % RSDs were calculated by averaging % RSD of each of the 41 compounds in organic acid and each of 7 compounds in amino acid respectively.

Table 4 Average Reproducibility (n=5) in % RSD

% RSD	Conventional	Presh
Organic Acid	18.0	25.2
Amino Acid	47.2	27.2

Carbohydrates that would cause extraction issues (e.g. sample loss by sudden boiling, and poor derivatization of difficult compounds by quenching) in conventional methods were effectively and selectively removed as illustrated in the chromatograms below in Fig. 2.

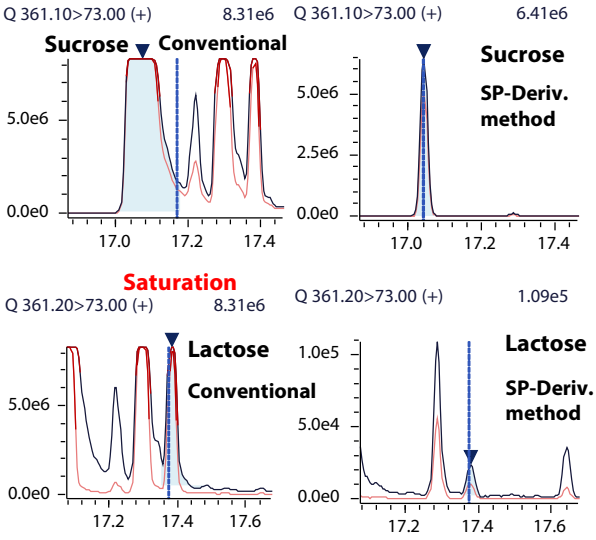

Fig. 2 Carbohydrates Removal

The removal of carbohydrates as illustrated by the chromatograms on the left bottom, bears particular importance in GC-MS as they are notorious in shortening a maintenance cycle of the instrument.

The total numbers of compounds detected (e.g. organic acids, amino acids, fatty acids, carbohydrates) by Smart Metabolites DB are listed below in Table 5.

Table 5 The Total Number of Identified Peaks

# of cpds detected	Conventional	Presh
Total	121	102

It should be noted here that with the use of TraverseMS (Reifycs Inc), the data analysis time can be greatly reduced by automation. The acquired data can be simply loaded onto TraverseMS software and multivariate analysis (e.g. PCA, HCA) can be conducted on the same software platform.

■ Summary

A quick sample extraction using SP-Deriv. method (Wakayama, Japan, AiSTI Co., Ltd) was performed on café late for organic and amino acids. The extraction took approximately 10 mins per sample.

The results were compared against those obtained with a conventional overnight method using the Bligh and Dyer extraction solution followed by overnight drying and derivatization on the second day as per the Shimadzu Metabolomics handbook (document number C146-2181).

The results obtained with SP-Deriv. method and the conventional method were comparable in the number of organic and amino acids found and reproducibility (n=5), though the sample extraction time became >10-100 times faster with SP-Deriv. method.

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