Supplementary Materials

Automated Screening and Filtering Scripts for GC×GC-TOFMS Metabolomics Data

Seo Lin Nam [1], A. Paulina de la Mata [1] and James J. Harynuk [1, 2]

[1]Department of Chemistry, University of Alberta: delamata@ualberta.ca

[2]Author to whom correspondence should be addressed; E-Mail: james.harynuk@ualberta.ca;

Tel.: +1-780-492-8303 (ext. 123); Fax: +1-780-492-8231. Department of Chemistry University

of Alberta, Edmonton, Alberta, T6G 2G2, Canada

Note: Copies of scripts developed for this research, and other related scripts for metabolomics work have been posted on GitHub at:

https://github.com/seolinnam/Scripts_Metabolomics
https://github.com/orgs/Harynuk-posse/people

Preparation of Standard Mixtures for Evaluation of the Scripts

Table S1. Compounds included in the standard mixtures

Amino acid	Carbohydrate
L-Alanine	D-(−)-Arabinose
L-Arginine	D-(−)-Fructose
L-Aspartic acid	D-(+)-Galactose
L-Cystine	D-(+)-Glucose
L-Glutamic acid	α-Lactose monohydrate
Glycine	D-(+)-Maltose monohydrate
L-Histidine	D-(+)-Mannose
L-Isoleucine	D-(−)-Ribose
L-Leucine	Sucrose
L-Lysine	D-(+)-Xylose
L-Methionine	
L-Phenylalanine	
L-Proline	
L-Serine	
L-Threonine	
L-Tyrosine	
L-Valine	

Fatty acid		Fatty acid methyl ester	
C4:0	Butyric acid	C4:0	Methyl butyrate
C6:0	Hexanoic acid	C6:0	Methyl hexanoate
C8:0	Octanoic acid	C8:0	Methyl octanoate
C10:0	Decanoic acid	C10:0	Methyl decanoate
C12:0	Lauric acid	C11:0	Methyl undecanoate
C13:0	Tridecanoic acid	C12:0	Methyl laurate
C14:0	Myristic acid	C13:0	Methyl tridecanoate
C14:1	Myristoleic acid	C14:0	Methyl myristate
C14:1T	Myristelaidic acid	C14:1	Methyl myristoleate
C15:0	Pentadecanoic acid	C15:0	Methyl pentadecanoate
C15:1	10-Pentadecenoic acid	C15:1	Methyl cis-10-pentadecenoate
C16:0	Palmitic acid	C16:0	Methyl palmitate
C16:1	Palmitoleic acid	C16:1	Methyl palmitoleate
C16:1T	Palmitelaidic acid	C17:0	Methyl heptadecanoate
C17:0	Heptadecanoic acid	C17:1	cis-10-Heptadecanoic acid methyl ester
C17:1	10-Heptadecenoic acid	C18:0	Methyl stearate
C18:0	Stearic acid	C18:1T	trans-9-Elaidic acid methyl ester
C18:1T	Elaidic acid	C18:1	cis-9-Oleic acid methyl ester
C18:1	Oleic acid	C18:2T	Methyl linolelaidate
C18:1T	Petroelaidic acid	C18:2	Methyl linoleate
C18:1T	Transvaccenic acid	C20:0	Methyl arachidate
C18:1	Vaccenic acid	C18:3	Methyl γ-linolenate

Fatty acid		Fatty acid methyl ester	
C18:2TT	Linoelaidic acid	C20:1	Methyl cis-11-eicosenoate
C18:2	Linoleic acid	C18:3	Methyl linolenate
C18:3	Gamma linolenic acid	C21:0	Methyl heneicosanoate
C18:3	Linolenic acid	C20:2	cis-11,14-Eicosadienoic acid methyl ester
C20:0	Arachidic acid	C22:0	Methyl behenate
C20:1	11-Eicosenoic acid	C20:3	cis-8,11,14-Eicosatrienoic acid methyl ester
C21:0	Heneicosanoic acid	C22:1	Methyl erucate
C20:2	11-14 Eicosadienoic acid	C20:3	cis-11,14,17-Eicosatrienoic acid methyl ester
C20:3	Homogamma Linolenic acid	C20:4	cis-5,8,11,14-Eicosatetraenoic acid methyl ester
C20:4	Arachidonic acid	C23:0	Methyl tricosanoate
C20:3	11,14,17 Eicosatrienoic acid	C22:2	cis-13,16-Docosadienoic acid methyl ester
C22:0	Behenic acid	C24:0	Methyl lignocerate
C22:1	Erucic acid	C20:5	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester
C20:5	Eicosapentaenoic acid	C24:1	Methyl nervonate
C22:2	Docosadienoic acid	C22:6	cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester
C23:0	Tricosanoic acid		
C22:4	Docosatetraenoic acid		
C22:5n-6	Docosapentaenoic acid		
C22:5n-3	Docosapentaenoic acid		
C24:0	Lignoceric acid		
C24:1	Nervonic acid		
C22:6	Docosahexaenoic acid		

For the standard mixture of each class of compounds, high and low concentration solutions were prepared with a serial dilution from the stock solution.

A) Amino acid standard

Stock solution: 2.5 µmol/mL for each component (AAS18-10 mL analytical standard, Millipore-Sigma Canada)

- 100-fold dilution (25 nmol/mL) was made in methanol to make a high-concentration standard solution.

- 1000-fold dilution (2.5 nmoles/mL) was made in methanol to make a low-concentration standard solution.

B) Fatty acid standard

Stock solution: 35.1 mg of total fatty acids (GLC-744, Nu-Check, MN, USA) were dissolved in 2.2 mL of methanol and isopropanol solution (2 mL MeOH and 0.2 mL IPA). This corresponds to 319 µg/mL for each component.

- 100-fold dilution (3 µg/g) was made in methanol to make a high-concentration standard solution.
- 1000-fold dilution (0.3 µg/g) was made in methanol to make a low-concentration standard solution.

C) Fatty acid methyl ester standard

Stock solution: 400 µg/g for each component (SUPELCO 37 Component FAME Mix, Millipore-Sigma Canada)
- 100-fold dilution (4 µg/g) was made in dichloromethane to make a high-concentration standard solution.
- 1000-fold dilution (0.4 µg/g) was made in dichloromethane to make a low-concentration standard solution.

D) Carbohydrates standard

Stock solution: 100 µg/mL for each component (Carbohydrates kit, Millipore-Sigma Canada) was made in methanol.
- 100-fold dilution (1 µg/mL) was made in methanol to make a high-concentration standard solution.
- 1000-fold dilution (0.1 µg/mL) was made in methanol to make a low-concentration standard solution.

The indicated volumes (µL) in Table S1 were withdrawn from high or low concentrations of the corresponding standard solution into a GC vial for derivatization.

Table S2. Details of standard mixture preparation µL standard placed in GC vial.

Vial #	Conc	AA	FA	FAME	Carbohydrate
1	Low Con	10	10	10	10
2	Low Con	20	20	20	20
3	Low Con	40	40	40	40
4	Low Con	60	60	60	60
5	Low Con	80	80	80	80
6	Low Con	100	100	100	100
7	High Conc	20	20	20	20
8	High Conc	40	40	40	40
9	High Conc	60	60	60	60
10	High Conc	80	80	80	80
11	High Conc	100	100	100	100
12	Mix	L20	L20	H20	H20
13	Mix	H20	H20	L20	L20
14	Mix	L40	H40	L40	H40
15	Mix	H40	L40	H40	L40
16	Mix	L40	H40	H40	L40
17	Mix	L20	L20	L20	H40
18	Mix	L20	L20	L20	H100

Table S3. Number of compounds in the standard mixtures that were classified for each group.

Classes	Subgroups	mix 1	mix 2	mix 3	mix 4	mix 5	mix 6
Carbohydrates	4TMS	0	0	0	3	1	3
	5TMS	3	4	6	4	7	5
	8TMS	0	0	1	1	2	2
Fatty acids	saturated	8	11	15	17	17	17
	monoenoic	2	6	9	11	11	11
	dienoic	1	0	2	2	1	3
	multienoic	0	1	4	6	9	8
Fatty acid methyl esters	saturated	0	1	5	8	8	8
	monoenoic	0	1	3	4	4	4
	dienoic	0	0	0	2	2	3
	trienoic	0	0	0	1	2	2
	multienoic	0	0	0	0	1	2
Amino acids		0	1	4	3	6	6
Total Number Correct		14	25	49	62	71	74
Total Number Incorrect		1	0	0	2	1	0
Total Number Classified		15	25	49	64	72	74
Classes	Subgroups	mix 7	mix 8	mix 9	mix 10	mix 11	mix 12
Carbohydrates	4TMS	4	4	6	3	4	4
	5TMS	7	10	8	7	5	8
	8TMS	5	5	5	4	3	5
Fatty acids	saturated	17	17	18	20	20	11
	monoenoic	11	11	12	10	10	5
	dienoic	3	3	3	3	4	0
	multienoic	9	9	9	9	11	0
Fatty acid methyl esters	saturated	9	10	10	11	12	8
	monoenoic	7	5	7	6	5	7
	dienoic	2	3	3	3	3	2
	trienoic	3	2	2	2	2	1
	multienoic	2	2	3	2	1	2
Amino acids		7	10	11	12	13	1
Total Number Correct		86	91	97	92	93	54
Total Number Incorrect		0	0	0	1	2	0
Total Number Classified		86	91	97	93	95	54
Classes	Subgroups	mix 13	mix 14	mix 15	mix 16	mix 17	mix 18
Carbohydrates	4TMS	2	5	2	3	4	3
	5TMS	3	8	2	4	9	9
	8TMS	0	5	0	0	5	5
Fatty acids	saturated	16	17	15	16	10	12
	monoenoic	9	10	9	10	4	3
	dienoic	3	3	0	3	0	0
	multienoic	9	9	5	10	0	1
Fatty acid methyl esters	saturated	3	4	10	10	0	0
	monoenoic	2	3	7	6	2	0
	dienoic	0	2	3	3	0	0
	trienoic	0	0	3	2	0	0
	multienoic	0	0	3	2	0	0
Amino acids		8	3	9	3	3	8
Total Number Correct		55	69	68	72	37	41
Total Number Incorrect		0	0	0	0	0	1
Total Number Classified		55	69	68	72	37	42

Details of Sample Preparation

A. SPME (Solid-phase microextraction)

1) Sweat

Sweat was collected on a cotton t-shirt, and the fabric was cut into a 2 cm × 2 cm square from the armpit region. The cut sample was immediately placed in a 10-mL SPME vial and sealed. A pre-cleaned 3-phase SPME fibre divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (SUPELCO, Bellefonte, PA, USA) was used to extract the volatiles for 21 h at 30 °C while the sample vial was immersed in a temperature-controlled oil bath. The fibre was thermally desorbed for 3.5 min at the inlet temperature set to 250 °C.

2) Feces

Immediately after the passage (within 10 min), feces were mixed with a stirring rod for 1 min to homogenize the sample manually. 500 mg aliquot of a fecal sample was transferred into a 10 mL SPME vial and capped immediately. Prior to GC injection, the sample was incubated at 60 °C for 15 min, then extracted with a 3-phase SPME fibre DVB/CAR/PDMS (SUPELCO, Bellefonte, PA, USA) for 2 hours at 60 °C. The fibre was thermally desorbed for 3 min at the inlet temperature set to 250 °C.

B. Derivatization

1) Plasma

P9523-5ML plasma that was prepared from pooled human blood with 4% trisodium citrate as an anticoagulant, was purchased from Sigma-Aldrich (St.Louis, MO, USA). When the product was received, the lyophilized plasma was reconstituted with 5 mL of deionized water and was aliquoted into 2-mL centrifuge tubes. The aliquoted plasma was stored in a –80 °C freezer until the day of analysis. On the day of analysis, a 2-mL centrifuge tube containing plasma was thawed on ice, vortexed for 1 min, and a 40 µL aliquot of plasma was pipetted into a new 2-mL centrifuge tube. 300 µL of methanol was added, and the resulting mixture was vortexed for 3 min, then centrifuged for 15 min at 15,800 g and 4 °C. 100 µL of supernatant was withdrawn, transferred into a 2-mL GC vial and dried under a gentle stream of nitrogen. To the dried residue, 100 µL of toluene dried over anhydrous sodium sulfate was added to enhance the drying of trace amounts of water and dried again at 80 °C under N_2 for 30 min. Next, 50 µL of methoxyamine hydrochloride was added and incubated at 80 °C for 30 min. Subsequently, 50 µL of MSTFA was added and incubated at 80 °C for 30 min. The resulting derivatized sample was cooled for 5

min at room temperature and was transferred into a GC vial with a fused 300 μL insert for GC×GC-TOFMS analysis.

2) Urine

On the night before the sample preparation, a frozen urine sample was thawed on ice overnight. On the day of analysis, the thawed urine sample was vortexed for 1 min, then 40 μL aliquot of urine was transferred into a 2-mL centrifuge tube, and 10 μL of urease suspension was added. The sample was vortexed for 3 min then incubated at 37 °C for 1 h. After incubation, 960 μL of methanol was added to the sample, vortexed for 5 min, then centrifuged for 10 min at 10,000 g and 4 °C. 500 μL of the supernatant was transferred into a 2-mL GC vial and dried under nitrogen at 50 °C. To the dried residue, 100 μL of toluene dried over anhydrous sodium sulfate was added to enhance the drying. To the dried extract, 20 mg/mL methoxyamine hydrochloride in pyridine was added and incubated at 60 °C for 2 h. Following the methoximation, 100 μL of MSTFA was added and incubated at 60 °C for 1 h. After cooling the resulting derivatized urine at room temperature for 5 min, the sample was transferred to a GC-vial with 300- μL inserts for analysis by GC×GC-TOFMS.