Comprehensive analysis of human sebum lipids by using GCxGC-HRTOFMS

Introduction

Skin is an active metabolic tissue that synthesizes a variety of complex lipid compounds. Sebum, an oily material secreted by the skin, is known to provide a moisturizing effect, sunlight protection, and antibacterial protection for the skin surface. Sebum consists of a complex mixture of free fatty acids, squalene, cholesterol, wax esters, diacylglycerols and triacylglycerols. These species and their concentrations vary depending on skin conditions.

Lipid compounds are generally measured by using GC–FID, GC–MS or LC–MS. However, these methods are often unable to separate all of the individual compounds under the same measurement conditions. In addition, it can be difficult to definitively identify each lipid compound due to co-elution.

On the other hand, two-dimensional GC - high resolution time-of-flight mass spectrometry (GCxGC–HRTOFMS) is a powerful tool for identifying analytes in complex mixtures such as crude oils. The purpose of this work is the comprehensive detection and identification of lipid compounds in sebum by using GCxGC-HRTOFMS.

Experiment

Samples were collected onto square aluminum sheets (3cm x 3cm) gently wiped across a human forehead for 2 minutes. This was repeated for a total of 3 sheets. The lipid compounds were then extracted with sonication from the aluminum sheets using a 3mL of a 50/50 volume solution of methanol/dichloromethane. Next, the extraction solution was centrifuged and the supernatant was concentrated to 200 µL. The resulting solution was analyzed using GCxGC–HRTOFMS and the conditions shown in table 1.

Table 1. Measurement Conditions

- Instruments -	JMS-T200GC "*AccuTOF GCx-plus*" (JEOL Ltd.) ZX-2 (GCxGC module：ZOEX Corporation)
- GCxGC conditions -	
Inlet	Cool on column
Inlet mode	Track Oven
1st column	DB-5HT (15 m x 0.25 mm, firm thickness 0.1 µm)
Modulator loop	Deactivated capillary tube (1.0 m x 0.15 mm)
2nd column	Rxi-17Sil MS (1 m x 0.15 mm, firm thickness 0.15 µm)
Transfer line	Deactivated capillary tube (1.0 m x 0.15 mm)
Oven temp. program	100˚C (1min) => 5˚C/min => 360˚C (10min)
Carrier gas flow	2.0 mL/min (He, Constant flow)
Modulation period	14 sec
Injection Volume	0.1 µL
- MS conditions -	
Ion source	Dedicated EI ion source
Ionization method	EI(+) : 70 eV, 300 µA
GC Interface temp.	350˚C
Ion source temp.	280 ˚C
Spectrum recording interval	25 Hz (0.04 sec/spectrum)
m/z range	35 ～ 1,000

Results

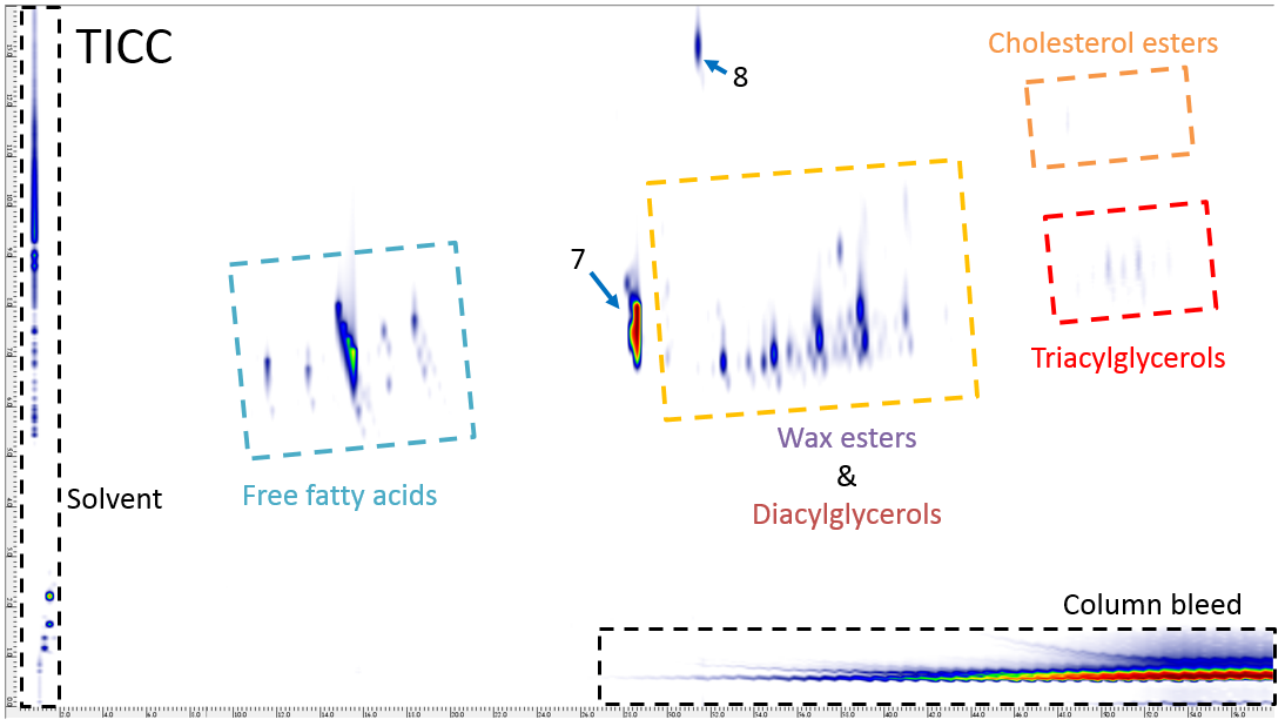

Figure 1. 2D map (TICC)

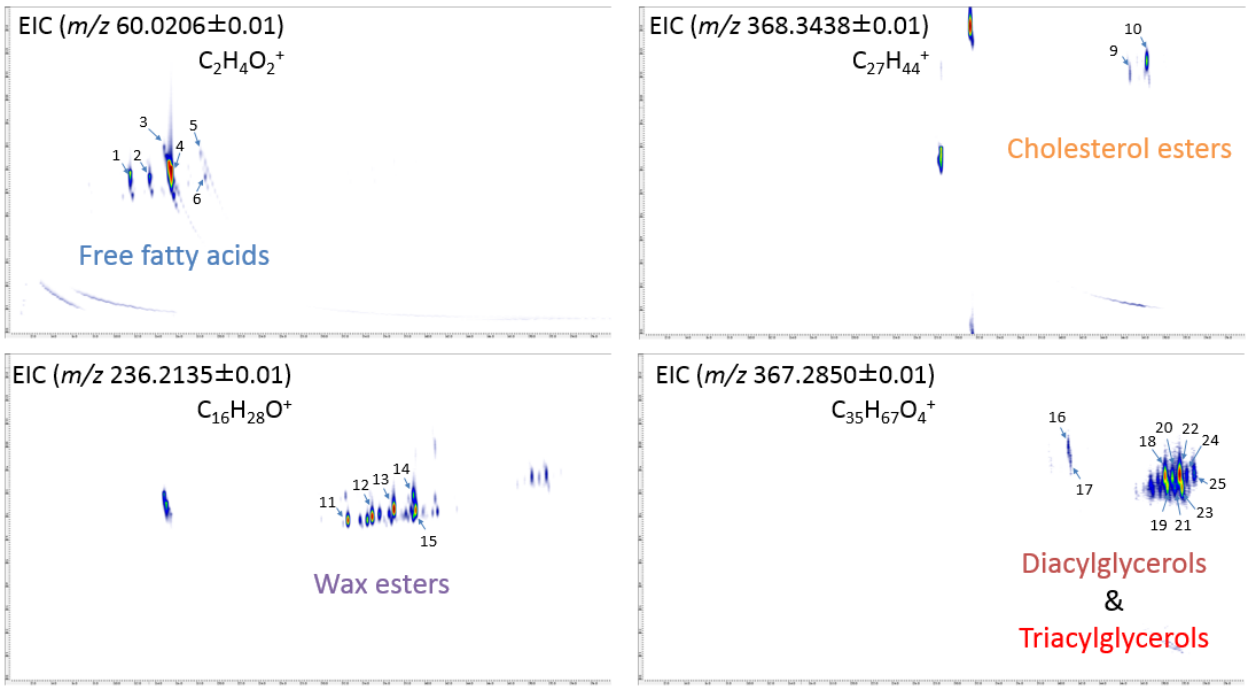

Figure 2. 2D map of EIC (Free fatty acids (Top left), Cholesterol esters (Top right), Wax esters (Bottom left) and Diacylglycerols/ Triacylglycerols (Bottom right))

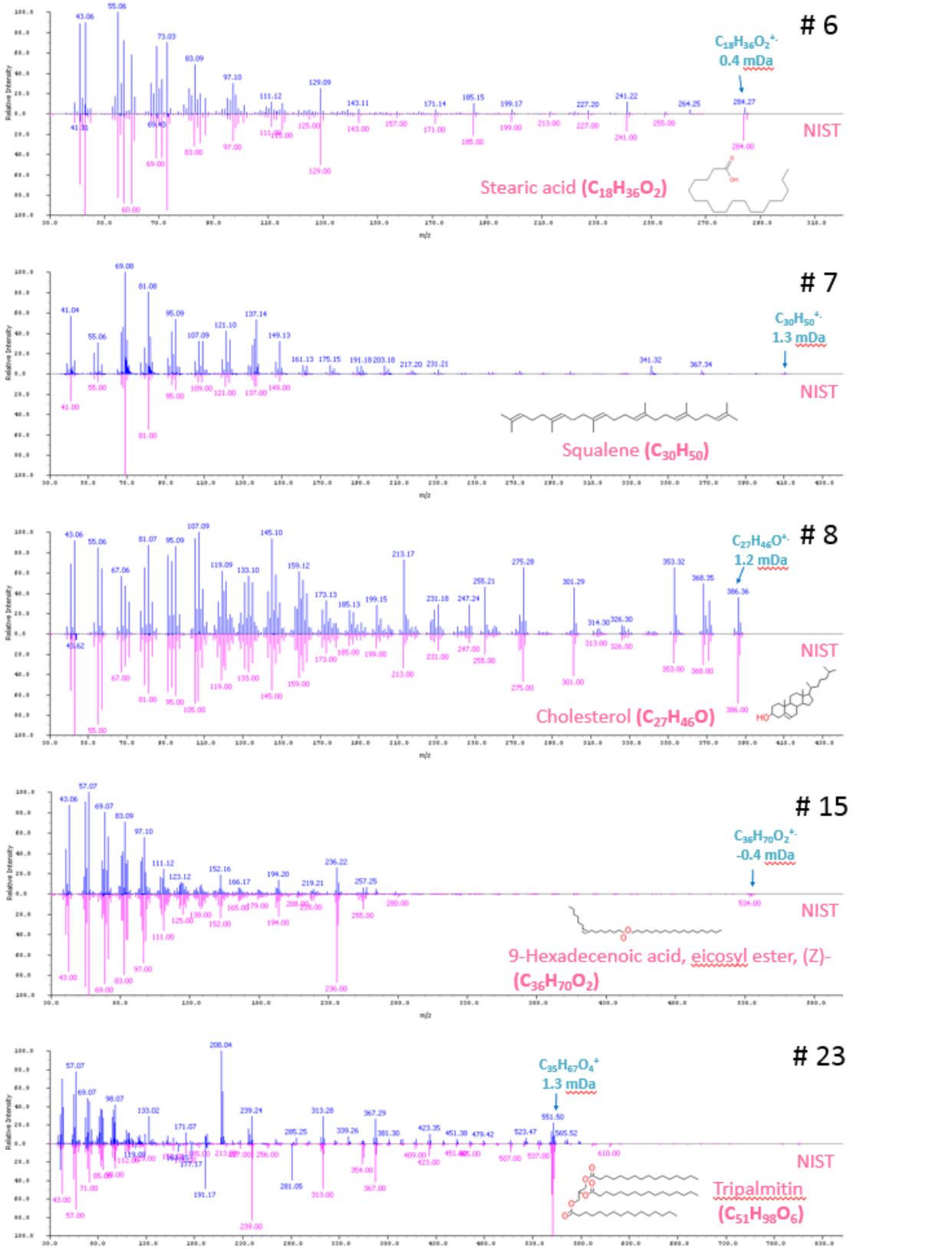

Figure 3. Mass spectra

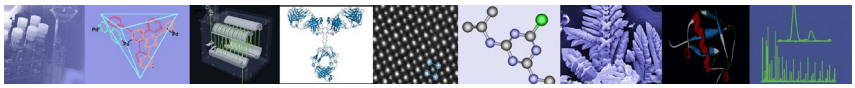

Table 2. Major Lipid Classes

Classification	#	RT 1st	RT 2nd	NIST results Compound name	Formula	Match factor	Exact mass Measured m/z	Measured Ions	Error (mDa)
Free fatty acids	1	11.45	6.84	Tetradecanoic acid	$C_{14}H_{28}O_2$	924	228.2100	$M^{+\cdot}$	1.6
	2	13.32	6.67	Pentadecanoic acid	$C_{15}H_{30}O_2$	921	242.2253	$M^{+\cdot}$	1.2
	3	14.72	8.01	Palmitoleic acid	$C_{16}H_{30}O_2$	922	254.2252	$M^{+\cdot}$	1.2
	4	15.65	6.55	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	870	256.2456	$M^{+\cdot}$	-1.3
	5	18.22	7.76	cis-Vaccenic acid	$C_{18}H_{34}O_2$	916	282.2563	$M^{+\cdot}$	0.9
	6	18.68	6.59	Stearic acid	$C_{18}H_{36}O_2$	811	284.2713	$M^{+\cdot}$	0.4
Squalene	7	28.48	7.63	Squalene	$C_{30}H_{50}$	889	410.3920	$M^{+\cdot}$	1.3
Cholesterol	8	31.28	13.26	Cholesterol	$C_{27}H_{46}O$	909	386.3555	$M^{+\cdot}$	1.2
Cholesterol esters	9	46.68	11.18	CHOLEST-5-EN-3-YL PALMITATE	$C_{43}H_{76}O_2$	702	368.3482	$C_{27}H_{44}^{+}$	-1.5
	10	48.32	11.67	CHOLEST-5-EN-3-YL STEARATE	$C_{45}H_{80}O_2$	817	368.3484	$C_{27}H_{44}^{+}$	-1.2
Wax esters	11	32.45	6.76	9-Hexadecenoic acid, tetradecyl ester, (Z)-	$C_{30}H_{58}O_2$	836	450.4417	$M^{+\cdot}$	1.4
	12	34.78	7.05	9-Hexadecenoic acid, hexadecyl ester, (Z)-	$C_{32}H_{62}O_2$	829	478.4809	$M^{+\cdot}$	0.6
	13	36.88	7.30	9-Hexadecenoic acid, octadecyl ester, (Z)-	$C_{34}H_{66}O_2$	860	506.5053	$M^{+\cdot}$	-0.4
	14	38.75	7.97	9-Octadecenoic acid (Z)-, 9-octadecenyl ester, (Z)-	$C_{36}H_{68}O_2$	610	532.5218	$M^{+\cdot}$	0.4
	15	38.98	7.26	9-Hexadecenoic acid, eicosyl ester, (Z)-	$C_{36}H_{70}O_2$	808	534.5370	$M^{+\cdot}$	-0.4
Diacylglycerols	16	40.85	10.00	1,3-DIPALMITIN	$C_{35}H_{68}O_5$	685	313.2746	$C_{19}H_{37}O_3^{+}$	0.9
	17	41.08	8.84	1,2-Dipalmitin	$C_{35}H_{68}O_5$	709	313.2744	$C_{19}H_{37}O_3^{+}$	0.6
Triacylglycerols	18	50.18	8.63				549.4888	$C_{35}H_{65}O_4^{+}$	1.1
	19	50.42	8.38	3-(Palmitoyloxy)-2-(tetradecanoyloxy)propyl palmitate	$C_{49}H_{94}O_6$	651	551.5039	$C_{35}H_{67}O_4^{+}$	0.5
	20	50.88	8.63				563.5030	$C_{36}H_{67}O_4^{+}$	-0.4
	21	51.12	8.22				565.5190	$C_{36}H_{69}O_4^{+}$	0.0
	22	51.58	8.59				549.4892	$C_{35}H_{65}O_4^{+}$	1.5
	23	51.82	8.26	Tripalmitin	$C_{51}H_{98}O_6$	625	551.5047	$C_{35}H_{67}O_4^{+}$	1.3
	24	52.28	8.63				577.5205	$C_{37}H_{69}O_4^{+}$	1.5
	25	52.98	8.72	1,3-Dipalmitoyl-2-stearin	$C_{53}H_{102}O_6$	495	577.5206	$C_{37}H_{69}O_4^{+}$	1.6

Figure 1 shows that the TICC 2D map resulted in high chromatographic separation of the six lipid groups corresponding to free fatty acids, squalene, cholesterol/ cholesterol esters, wax esters, diacylglycerols and triacylglycerols. Figure 2 shows that EICs made from group-specific fragment ion masses can clearly separate every compound in each group. Additionally, the lipid compounds in each group were comprehensively identified by HRTOFMS using a combination of accurate mass measurements and library searches. As an example, peak number 6 detected at retention time 18.68 minute (1st column), 6.59 seconds (second column) was matched to stearic acid by using an NIST library search. Furthermore, this mass spectrum showed a molecular ion that coincided with the calculated accurate mass of steric acid (error of 0.4 mDa). The results for other compounds present in the sebum sample are presented in Fig. 3 and table 2.

Conclusion

GCxGC-HRTOFMS has the following advantages for sebum analysis:

- Signal detection from low boiling point fatty acids up to high boiling triglycerides in a single measurement
- High chromatographic separation for cholesterol esters and triacylglycerols which are difficult to separate by 1D GC
- Comprehensive identification using a combination of accurate mass measurements and library searches

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