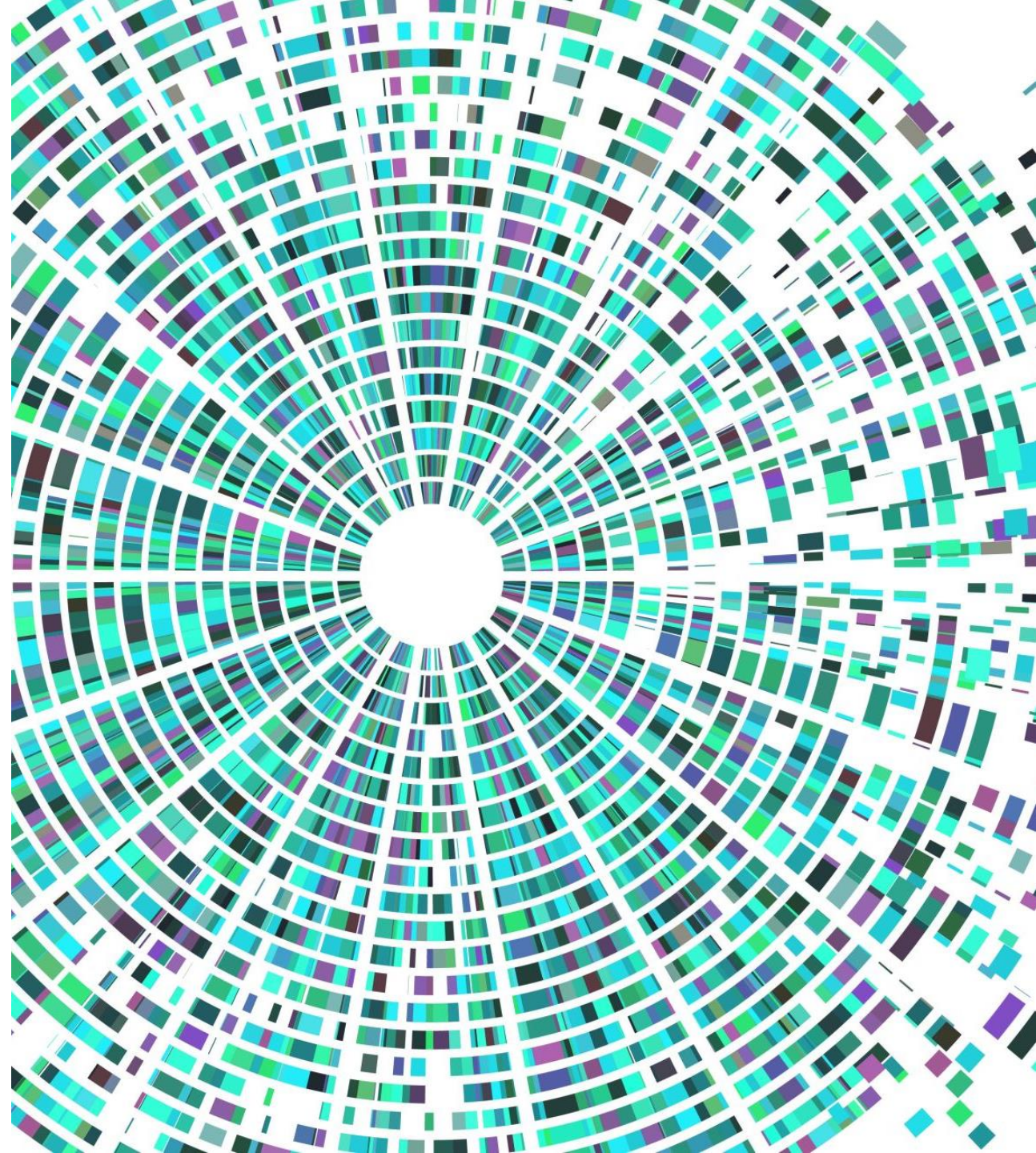


APLIKASI DAN INTERPRETASI DATA GCMS

Chandra W. Purnomo

Teknik Kimia UGM



OUTLINE

1. Kemampuan GCMS
2. Protokol penyiapan sampel
3. Setting operasi
4. Interpretasi hasil
5. Contoh aplikasi

1. KEMAMPUAN GC/MS

GCMS cocok dipakai untuk menganalisis zat volatil dengan aplikasi: environmental pollutants, industrial by products, food contaminants/trace, pestisida, and metabolit dari penyalahgunaan obat.



2. PROTOKOL PENYIAPAN SAMPEL

- Sampel yang akan dianalisa GC-MS sering mengandung komponen yang masih banyak pengotor, zat yang tidak stabil yang perlu diproses lebih lanjut sebelum masuk ke GC.
- Protokol yang umum:
 - A. Headspace
 - B. Pyrolysis
 - C. Solid Phase Extraction
 - D. Solvent Extraction

A. Headspace sampling

- Sampel cairan atau padatan ditambahkan kedalam sebuah glass vial dan ditunggu sampai terjadi kesetimbangan fasa.
- Beberapa analit akan teruapkan dan mengisi ruang kosong bagian atas (headspace) vial.
- static headspace sampling jika fasa gas di headspace langsung disuntikkan ke GC
- Jika gas ditangkap memakai absorbant surface atau cryogenic trap, disebut dynamic headspace sampling.
- Metode ini banyak diaplikasikan untuk sampel darah, kosmetik, plastic, padatan dan sampel dengan kadar air yang tinggi.

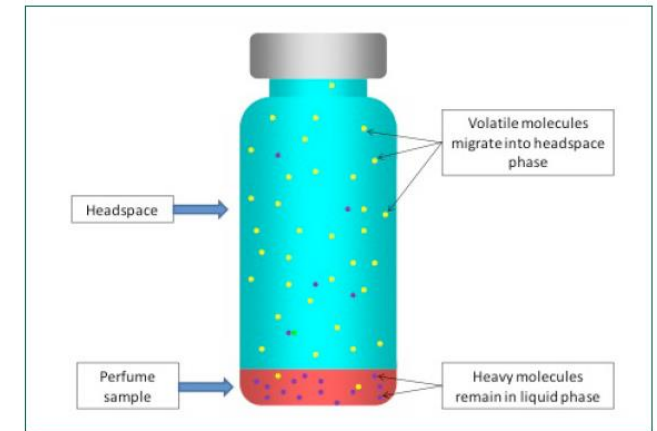
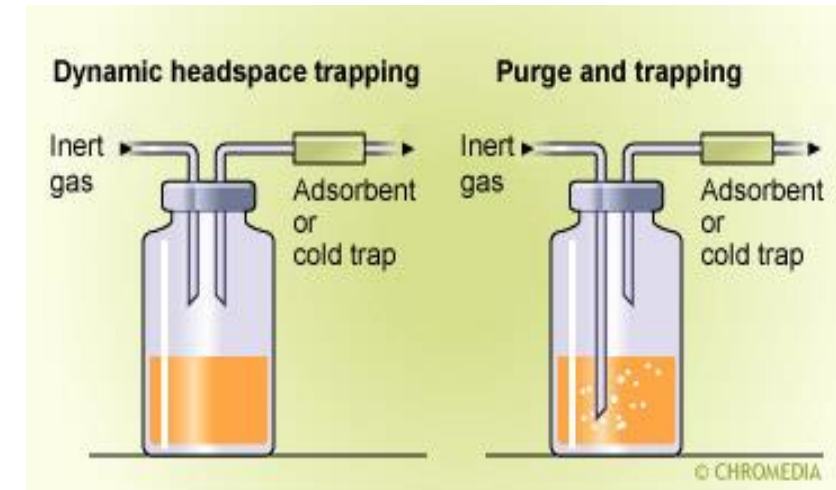


Figure 2. Movement of perfume molecules within a sealed and heated vial.



Contoh kromatogram dengan dan tanpa metode headspace

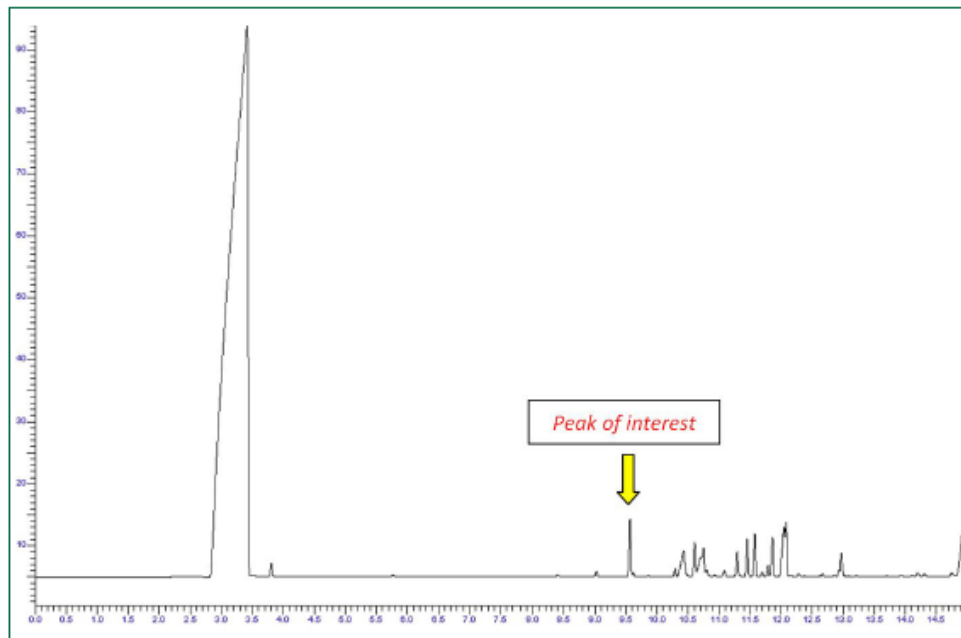


Figure 1. Chromatogram from direct injection of a perfume sample.

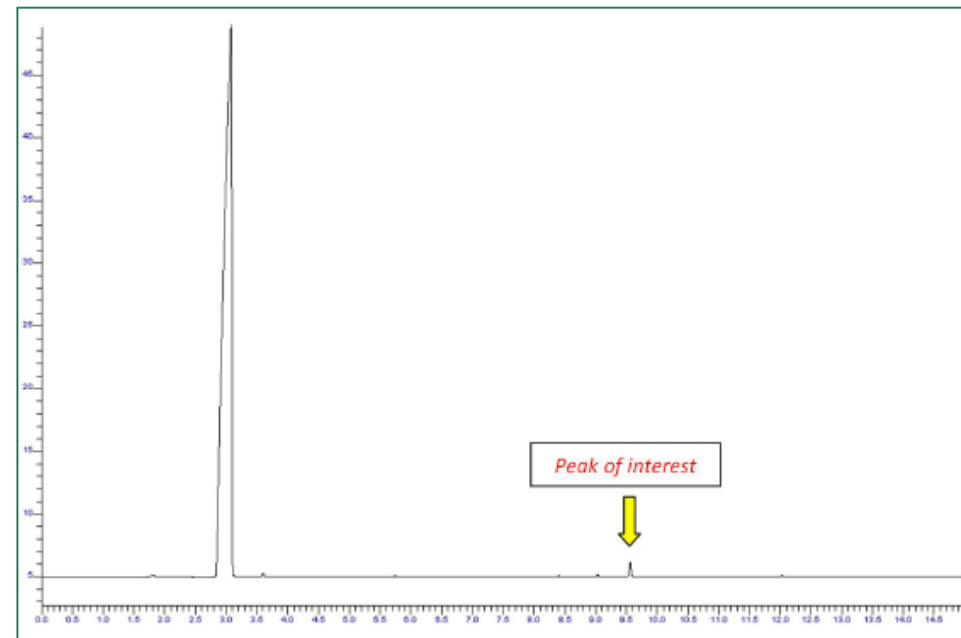


Figure 3. Chromatography of a perfume sample with headspace sampling.

Menghitung balik komposisi

$$K = C_s / C_g$$

C_s = konsentrasi sampel

C_g = konsentrasi gas

K – koefisien partisi (kesetimbangan)

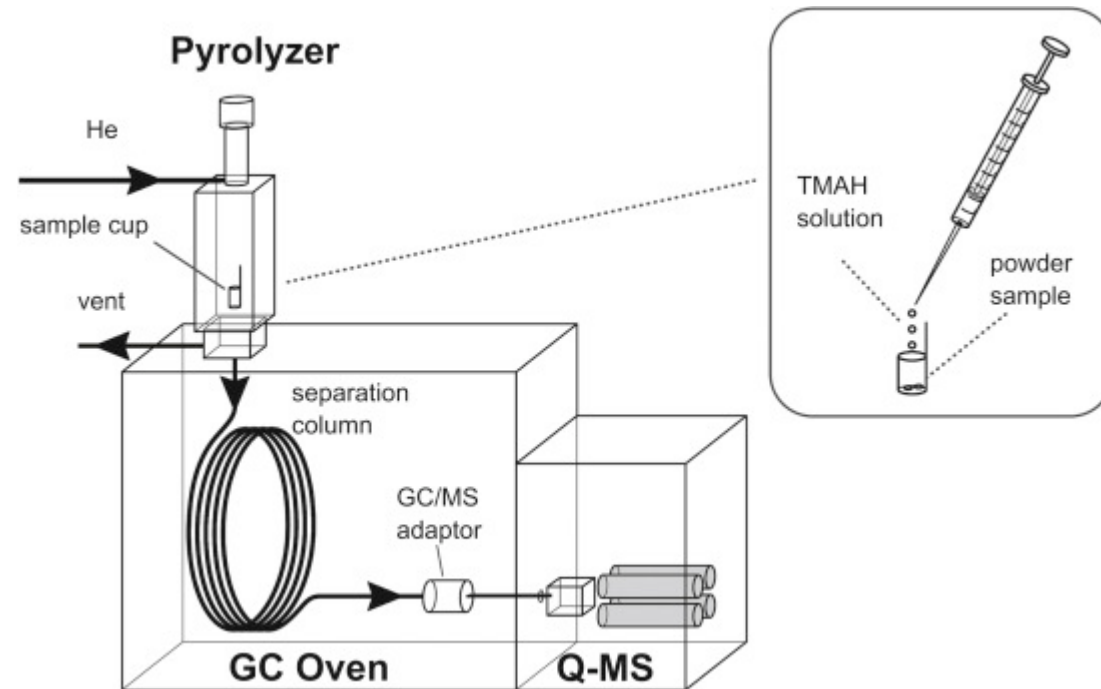
Table 1: Partition coefficients of various compounds between water and air phases at 60 °C.

Compound	K
Dioxane	642
Ethanol	511
Isopropyl alcohol	286
n-Butanol	238
Methyl ethyl ketone	68.8
Ethyl acetate	29.3
n-Butyl acetate	13.6
Benzene	2.27

Compound	K
Toluene	1.77
o-Xylene	1.31
Dichloromethane	3.31
1,1,1-Trichloroethane	1.47
Tetrachloroethylene	1.27
n-Hexane	0.043
Cyclohexane	0.040

B. Pyrolysis

- Sampel dipanaskan sampai 120 C dengan inert atau udara sehingga terdekomposisi menjadi senyawa-senyawa lebih sederhana.
- Banyak diaplikasikan di plastik, paints, dyes, resins, cellulose, wood, oils, dan rubber, juga sampel yang punya banyak crosslinked atau insoluble.
- Karena metode ini tidak memerlukan solvent maka bermanfaat untuk analisa solvents dan additives.



C. Solid Phase Extraction (SPE)

- Metode ini memakai solid packing material umumnya didalam cartridge untuk memisahkan komponen tertentu dalam sampel, menghilangkan interferensi atau memisahkan matrik.
- Umumnya sampel berupa cairan terutama seperti urine, saliva, dan plasma, environmental samples seperti air, dan produk minuman.



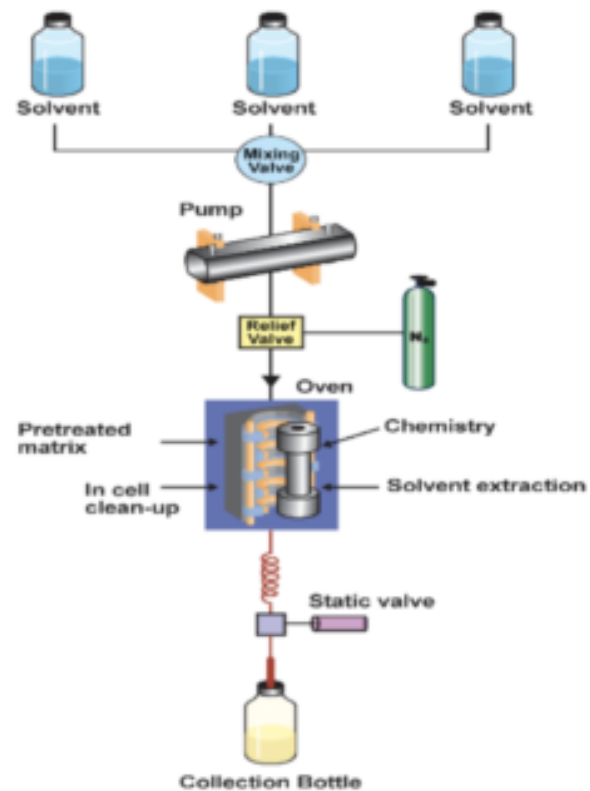
D. Solvent extraction:

- Banyak analit seperti pestisida bersifat sangat polar sehingga bisa diekstrak memakai solven seperti acetonitrile.
- Cocok untuk menganalisa kontaminan dalam tanah, sedimen atau lumpur.
- Beberapa Teknik ekstraksi: soxhlet extraction, Accelerated Solvent Extraction (ASE) dan ultrasonically assisted extraction (UAE)

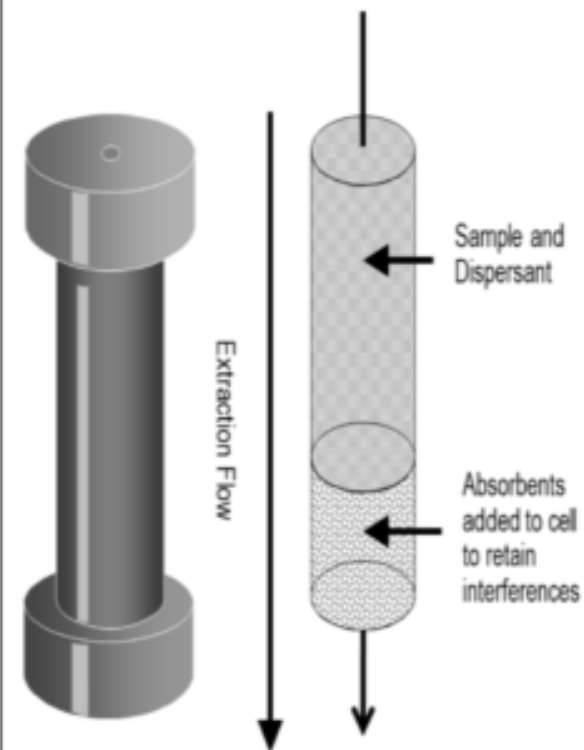
ASE



ASE 350 system



ASE 350 Schematic



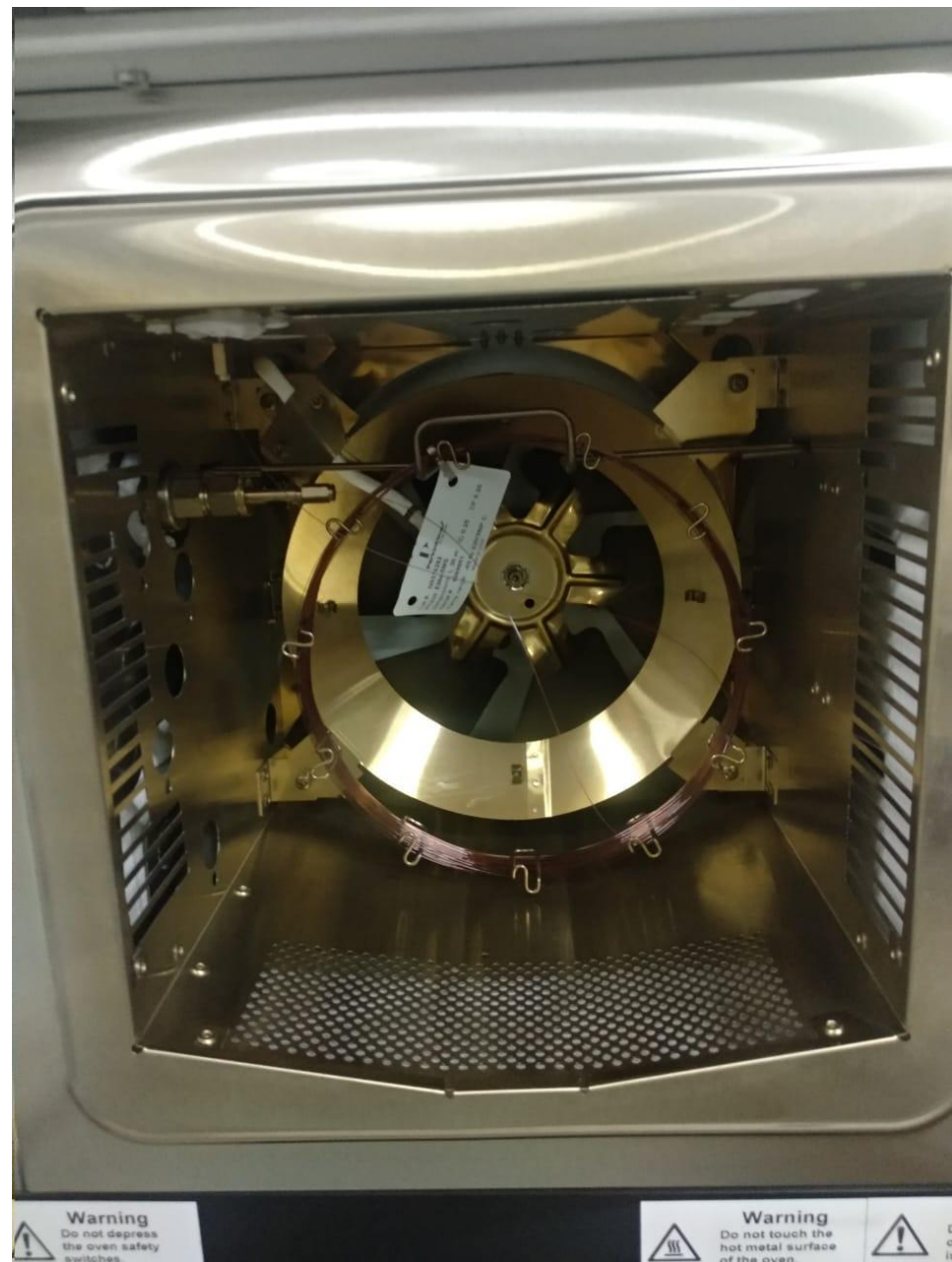
In-Cell Cleanup with ASE

3. SETTING OPERASI

Umumnya GCMS perlu diseting beberapa hal sebagai berikut:

- Split ratio/splitless
- Suhu injeksi, suhu oven dan suhu detektor
- Ramp program suhu oven
- Kecepatan aliran carrier gas







lenovo

TurboMass - DEFAULT - Ilham_18082020.SPL

File Edit Samples Run View Quantify Configure GC Tools Help

GC

0.00

Oven Temp
0°C

General Status
No Method

GC Status

MS
Operate
Pressures
Filament

	File Name	MS Method	GC Method	Vial #	Injector	Sample ID	File Text	Conditions	Quantify Method	Calibration Curve	Qualitative Method
1	SMPL_Liquid Ter	TerpentinMS	TERPENTIN_16		A	SMPL_Liq					
2	SMPL_Injek										Qualitative Method_50_New
3	SMPL_Miny										AA

Method Editor - (untitled) - [Method Summary]

File Instrument Equilibrate View Help

GC Control

Instrument Name : inst1
Experiment Time : 20.00
Delay Time : 0.00
Run Time : 20.00

Oven Temperature Program
Initial Temperature

Instrument Control

Autosampler Oven/Inlets Carrier Detectors Instrument Timed Events

Program time (min)

Oven 20.00
Inj A: PSSI 20.00
Inj B: NONE 0.00
Data end time 20.00

Cryo

Coolant Off
Cut-in temp (°C) 60
Timeout (min) 999

Oven

Max temp (°C) 350
Equil time (min) 2.0

Heated zone setpoints (°C)

Injector A On
Injector B 0

Auxiliary 0.0

OK Cancel Apply

Index Acquiring

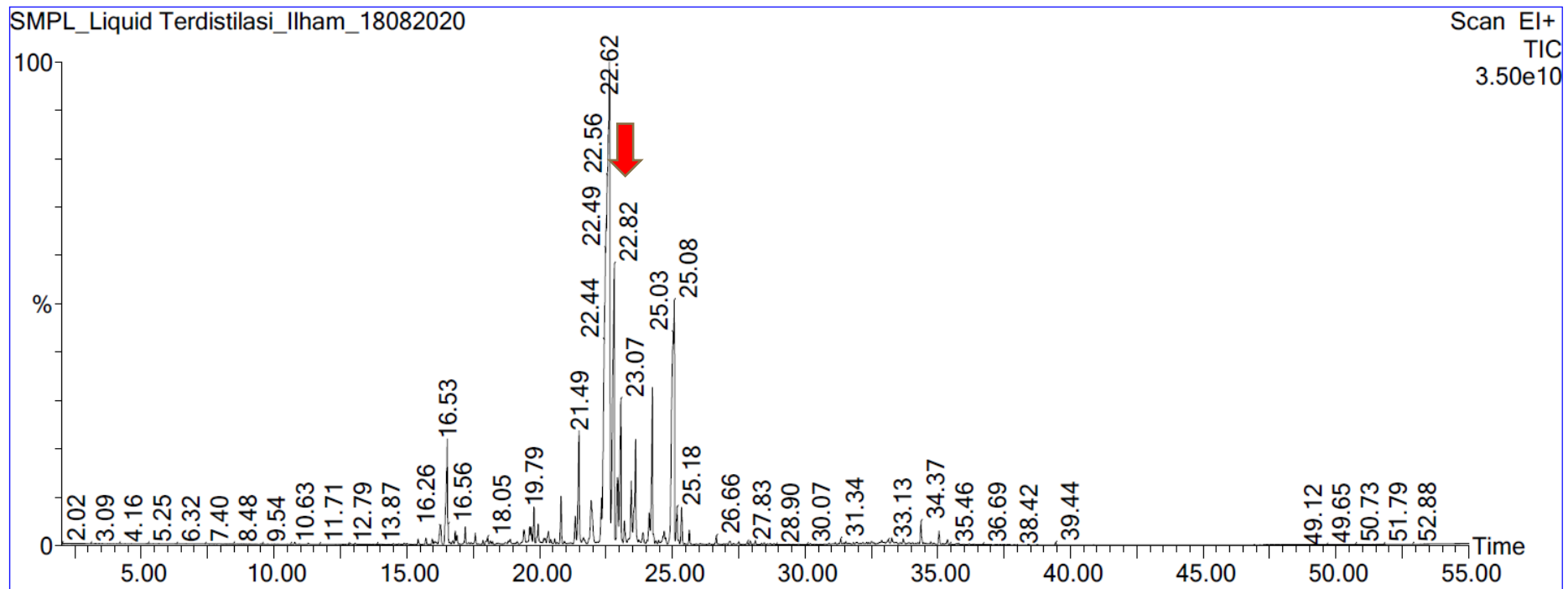
Ready

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9/14/2020

4. INTERPRETASI HASIL

- Uji GC_MS bisa kualitatif dan kuantitatif
- Contoh hasil analisis sampel penelitian terpentin



PEAK SELECTION

#	RT	Scan	Height	Area	Area %	Norm %
1	2.248	50	130,030,616	27,171,406.0	0.173	0.45
2	2.884	177	114,002,744	29,445,026.0	0.187	0.49
3	3.444	289	105,316,832	39,937,772.0	0.254	0.66
4	4.835	567	94,740,856	27,175,522.0	0.173	0.45
5	5.695	739	96,147,096	17,850,094.0	0.114	0.29
6	5.960	792	92,956,208	22,622,946.0	0.144	0.37
7	15.424	2684	421,648,736	18,399,172.0	0.117	0.30
8	15.724	2744	463,821,984	20,596,516.0	0.131	0.34
9	16.284	2856	1,416,343,936	105,359,240.0	0.670	1.74
10	16.529	2905	7,474,477,568	475,494,656.0	3.024	7.84
11	16.829	2965	946,333,504	33,167,802.0	0.211	0.55
12	16.894	2978	647,385,280	28,832,180.0	0.183	0.48
13	17.204	3040	1,233,019,136	52,075,928.0	0.331	0.86
14	17.585	3116	792,861,760	34,561,368.0	0.220	0.57
15	18.050	3209	492,449,376	32,915,950.0	0.209	0.54
16	18.900	3379	347,783,968	19,268,970.0	0.123	0.32
17	19.415	3482	923,694,912	60,457,928.0	0.384	1.00
18	19.625	3524	1,108,014,336	44,992,764.0	0.286	0.74
19	19.685	3536	1,093,316,480	45,484,048.0	0.289	0.75
20	19.790	3557	2,559,654,656	114,887,368.0	0.731	1.89

#	RT	Scan	Height	Area	Area %	Norm %
21	19.955	3590	1,369,969,920	76,648,552.0	0.487	1.26
22	20.341	3667	822,140,352	47,901,984.0	0.305	0.79
23	20.816	3762	3,467,480,576	153,926,608.0	0.979	2.54
24	21.346	3868	1,855,388,416	94,234,328.0	0.599	1.55
25	21.491	3897	8,004,314,112	422,929,696.0	2.689	6.97
26	21.661	3931	378,238,304	32,961,430.0	0.210	0.54
27	21.941	3987	3,116,313,344	313,373,376.0	1.993	5.16
28	22.331	4065	2,750,882,560	99,136,832.0	0.630	1.63
29	22.632	4125	33,521,158,144	6,067,388,928.0	38.583	100.00
30	22.817	4162	19,696,427,008	1,371,532,672.0	8.722	22.60
31	22.947	4188	3,856,449,792	225,408,304.0	1.433	3.72
32	23.067	4212	9,884,366,848	499,686,368.0	3.178	8.24
33	23.197	4238	1,579,616,896	81,749,952.0	0.520	1.35
34	23.457	4290	3,913,858,560	177,669,008.0	1.130	2.93
35	23.622	4323	7,128,519,168	384,485,632.0	2.445	6.34
36	23.897	4378	745,465,664	45,436,544.0	0.289	0.75
37	24.132	4425	2,207,649,024	118,616,608.0	0.754	1.95
38	24.252	4449	11,286,536,192	568,749,120.0	3.617	9.37
39	24.702	4539	881,441,856	67,556,768.0	0.430	1.11
40	24.757	4550	441,739,616	18,385,064.0	0.117	0.30
41	25.078	4614	17,338,728,448	1,817,659,392.0	11.559	29.96
42	25.188	4636	2,531,933,952	119,978,888.0	0.763	1.98
43	25.353	4669	2,646,502,656	137,449,776.0	0.874	2.27
44	25.638	4726	1,033,778,816	44,309,328.0	0.282	0.73
45	26.658	4930	587,458,496	27,042,540.0	0.172	0.45
46	33.131	6224	252,589,488	18,977,348.0	0.121	0.31
47	33.271	6252	408,826,336	23,265,452.0	0.148	0.38
48	33.701	6338	388,556,384	22,600,158.0	0.144	0.37
49	34.366	6471	1,633,265,664	80,832,728.0	0.514	1.33
50	35.047	6607	954,361,792	44,485,140.0	0.283	0.73

LIBRARY SEARCH

Pk #	RT	Hit	Compound Name	Match	R.Match	Prob.	CAS	Library
29	22.632	1	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1à,3aá,4à,8aá)]-	916	917	14.6	475-20-7	replib
		2	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1à,3aá,4à,8aá)]-	908	911	14.6	475-20-7	replib
		3	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1à,3aá,4à,8aá)]-	898	898	14.6	475-20-7	replib
30	22.817	1	Caryophyllene	918	918	22.3	87-44-5	MAINLIB
		2	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]-	898	902	10.1	118-65-0	replib
		3	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]-	894	898	10.1	118-65-0	MAINLIB
31	22.947	1	1R,3Z,9S-2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene	890	893	10.9		MAINLIB
		2	Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-	889	891	10.5		MAINLIB

5. CONTOH APLIKASI

A SIMPLE EXTRACTION METHOD FOR DETERMINATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS BY GC-MS (J. CHIN. CHEM. SOC. 2015, 62, 766-771)

- Weigh 2.50 g of dried sediment sample (spike with 40 g/kg of CS and SS) or SRM, 5 mL of distilled toluene and methanol (9/1, v/v) were added together in a 50 mL test tube.
- Those was capped and heated to 100 C staying for 10 minutes.
- After the temperature of tubes returned to room temperature, sample was repeated this procedure two more times.
- Extracted solution was collected after filtration combined and dried by anhydrous sodium sulfate.
- This solution was concentrated to nearly dry and added with n-hexane to 1 mL.
- The final solution was cleaned up by 5 g silica column with 15 mL DCM and n-hexane (2/3, v/v).
- The clean-up sample volume was reduced to 0.5 mL by purged with a nitrogen stream.
- Then, this extracts were added with 5 μ L of the internal standard (benzo[e]pyrene-d12, 155 mg/L) and dilute to 1.0 mL in a volumetric flask with n-hexane.
- After mixing completely, it could be ready for GC-MS analysis

ASE

- ASE method, accurately weigh 2.50 g of dried sediment sample (spike with 40 µg/kg of CS and SS) or SRM and place the sample into 10 mL stainless steel extracting cell of accelerated solvent extractor.
- Distillated toluene and methanol (9/1, v/v) were also used as extracting solution.
- Extracting temperature 120 C, 10 min static extraction time, three static cycles, 1500 psi (10.3 MPa), flush volume 60%, purge time 100 sec.

Soxhlet

- For Soxhlet method, accurately weigh 2.50 g of dried sediment sample (spike with 40 µg/kg of CS and SS) or SRM into the thimble.
- The thimble was loaded on the Soxhlet extraction
- system and extracted with 150 mL of distilled DCM for
- 16 hrs (about 2 ~ 3 reflux cycles/hr). Extracted solution was collected
- via the same procedures of filtration, dehydration, clean-up
- and concentration described previously

ANALISIS BIODIESEL (FUEL 280 (2020) 118579)

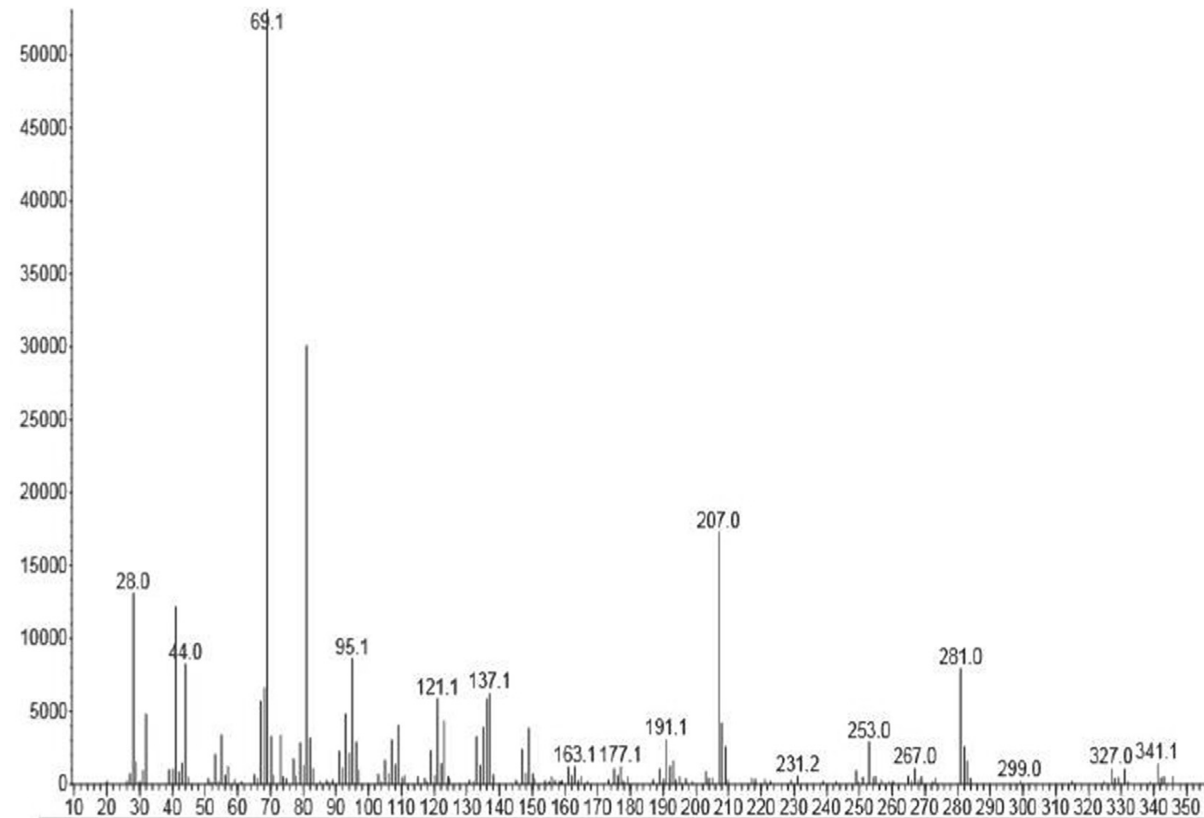
- The composition of methyl esters was determined in a GC-MS method, with the column description of 200 °C operating temperature, 2 °C/min ramp rate, 2 μ l/min flow rate and 80:1 split ratio.
- The FFME of methyl ester of julifora oil composition is determined by GC-MS method



a) Seeds of julifora

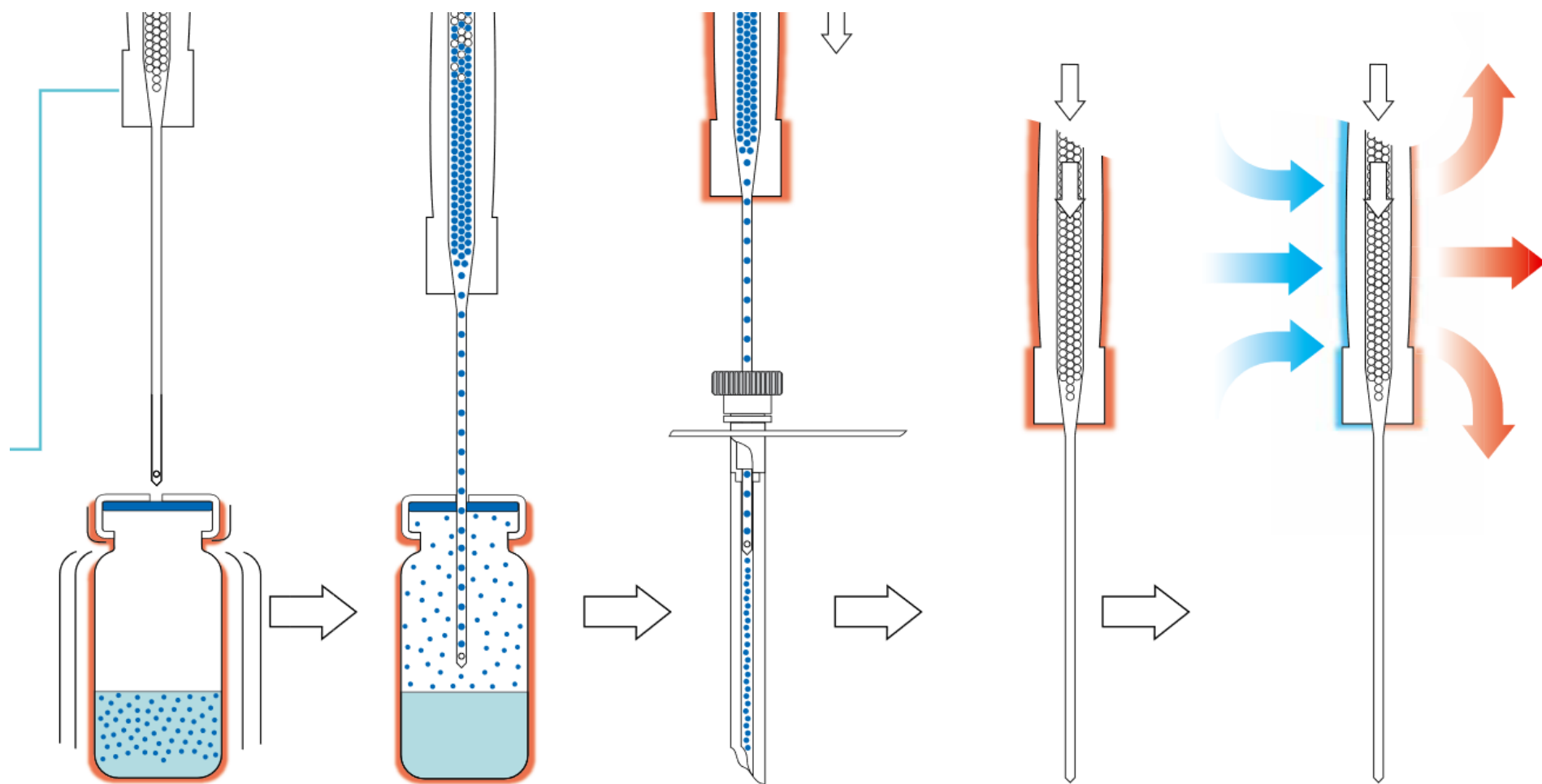


- The first peak in the GC spectrum indicates the methyl hexadecanoate and methyl 14-methyl pentadecanoate in the second peak.
- Methyl cis, cis-9,12-octadecadienoate and methyl cis, cis-10,13-octadecadienoate are indicated in the third peak of the chromatogram while the fourth peak is methyl cis-9-octadecenoate.
- The fifth peak is recognized as methyl octadecenoate and methyl 16-methyl-heptadecanoate.
- All the five components separated by GC are probably methyl palmitoleate, methyl linoleate, methyl palmitate, methyl stearate and methyl oleate



VALIDATION OF SPME-GCMS METHOD FOR THE ANALYSIS OF VIRGIN OLIVE OIL VOLATILES RESPONSIBLE FOR SENSORY DEFECTS (TALANTA134(2015)394-401)

- Volatile compounds are responsible for the aroma of virgin olive oil and also for its quality.
- The oil sample (2 g) was placed in a 20 mL glass vial, tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40°C to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial was pierced with a SPME needle and the fiber was exposed to the headspace for 40 min. The SPME fiber (1 cm length and 50/30 µm film thickness) was purchased from Supelco (Bellefonte, PA), and it was endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS).



Sample conditioning

Adsorption

Desorption

Trap cleaning

Active cooling

SETTING GCMS

- gas chromatograph 7820 coupled to quadrupole mass spectrometer Series MSD 5975 (Agilent Technologies, Santa Clara, CA) for 5 min at 300 C (splitless mode).
- ATR-WAX capillary column (60 m 0.25 mm i.d., 0.25 μ m coating) (Teknokroma, Spain) was used.
- The carrier gas was hydrogen, at a flow rate of 0.9 mL/min.
- The oven temperature was held at 40 C for 10 min and then programmed to rise a final temperature of 200 C at 3C/min.
- The GC-MS interface was heated at 280 C with the actual temperature reaching 180 C in MS source and 150 C in MS-quadrupole

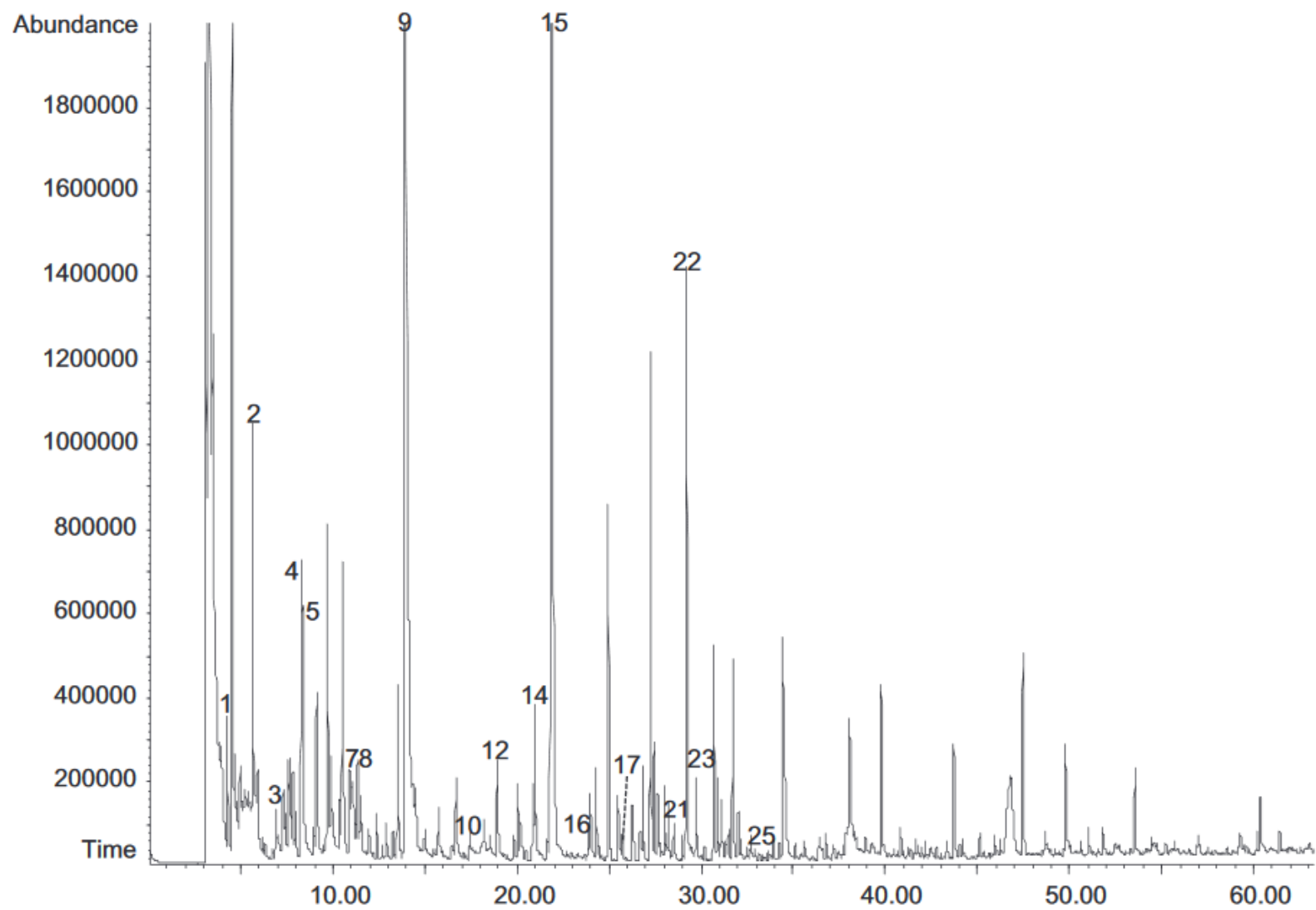


Fig. 1. SPME–GCMS chromatogram of EVOO sample. Codes are described in [Table 2](#).

ANALYTICAL DETERMINATION OF NICOTINE IN TOBACCO LEAVES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (ARABIAN JOURNAL OF CHEMISTRY (2013) 6, 275-278)

- The leaves were cut into small pieces and dried by sunlight or oven below temperature 40°C.
- The dried leaves samples were pulverized into powder form.
- The dried powder (0.1 g) was extracted three times with methanol (5 ml) by sonication at 30 min.
- It was then filtered and the filtrate was evaporated near to dryness by Kuderna-Danish evaporator.
- The extract was passed through the cleanup column (i.d. = 1 cm), which was filled with cotton in the bottom.
- An activated silica gel (10 g) soaked with solvent was loaded into the cleanup column (5 cm), which was then topped with 1.5 cm of anhydrous sodium sulfate.
- Five milliliters of solvent were added to wash the sodium sulfate and the silica gel.

Isolasi

- The pre-concentrated dried crude extracts, 1 ml of each extract sample, were then separately transferred into the column, and the vessel was rinsed twice with 2 ml loaded solvent, which was also added to the column.
- Sixty milliliters of loaded solvent were added to the column and allowed to flow through the column at a rate of 3–5 ml/min, and the eluent was collected.
- The collected eluent from the cleanup procedure was reconcentrated to 2 ml by using K-D concentrator.
- Finally the extract (2 ml) from leaves was filtered through a 0.45 μ m Millex HA filter (Millipore, Molsheim, France) prior to GC–MS analysis.
- The methanol extract (1 ml) was diluted with 5 ml of methanol and the samples were filtered through 0.45 μ m membrane filters (Molsheim, France) prior to GC–MS analysis.

Sekian dan terimakasih