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Equivalent GC systems performance for regulatory method compliance and validation

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Migration of methods between laboratories or from one analytical system to another when replacing technologies such as gas (GC) or liquid chromatographic (LC) system, either as a result of updating analytical equipment or changing from one supplier to another, could be a time consuming and difficult task. The instruments used in analytical laboratories are diverse and can belong to various brands. Often the same analytical method is used on instruments that are manufactured by different vendors with the expectation that the performance is equivalent. As part of the method transfer and validation, federal and governmental agencies, such as the United States Food and Drug Administration (US-FDA), and the European Medicines Agency (EMA) released specific guidelines.[1,2] Moreover, the USP Chapter 621 of the current United States Pharmacopeia has suitability procedures to test analytical methods and demonstrate equivalency when transferring them from one system to another.[3] This is also applicable for GC methods where strict chromatographic separation criteria are defined.

In this white paper, several examples of how the Thermo Scientific™ TRACE™ 1300 and 1600 Series Gas Chromatograph systems perform with typical, well known GC methods are detailed. The compatibility with common consumables, like liners and capillary columns, simplify the method portability assuring equivalency of the analytical performance. The examples considered in this white paper cover food safety, environmental and pharmaceutical methods where expected chromatographic conditions and compound separation criteria must be fulfilled.

Determination of gasoline range organics (GRO) in water by static headspace gas chromatography

Introduction

Crude oil is a complex mixture of hydrocarbons, metals and trace elements. When spills occur, it is important to determine the magnitude of the contamination using quantitative analytical methods. The most volatile hydrocarbon fraction (C_6-C_{10}) of crude oil is commonly known as Gasoline Range Organics (GRO). These compounds can be determined in soil and water by applying the EPA Method 8015C[4] or the Wisconsin modified GRO method.[5] Due to their high volatility GROs can be easily extracted from the matrix using headspace sampling coupled to gas chromatography and flame ionization detection.

Experimental

A Thermo Scientific™ TriPlus™ 500 Headspace (HS) Autosampler was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph* equipped with a Thermo Scientific™ Instant Connect split/splitless SSL Injector and a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID) and used to assess the GRO content in water samples. Additional details regarding the method parameters can be found in Table 1 and in the related application note AN 10702.[6]

Results and discussion

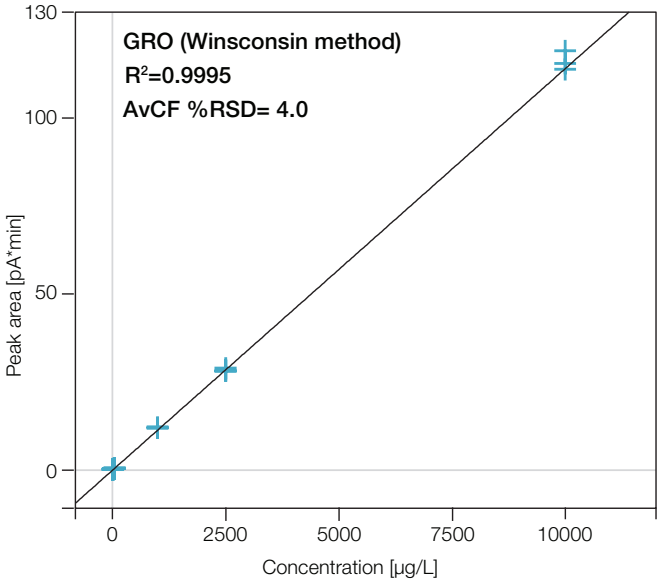
Chromatographic resolution (R_s) was achieved for all the target compounds, including methanol and MTBE (R_s=31.4) and the critical pair ethylbenzene and *m,p*-xylene (R_s=1.9) in < 12.5 minutes as per Wisconsin method requirements.[5] Gaussian peak shapes were obtained for the target compounds with average asymmetry (A_s) factor of 1.0 (Figure 2) indicating a high inertness of the system and an efficient chromatographic process. Linearity was assessed by serially diluting a GRO standard mix (1000 µg/mL, Restek®, P/N 30095) in tap water to obtain seven stock solutions ranging from 6.25 to 10,000 µg/L. Tap water samples (n=10) were spiked with raw gasoline solution (5%) and quantified using the generated calibration curve (Figure 1). A "baseline to baseline" integration was obtained integrating all the chromatographic peaks within the windows specified in the Wisconsin method (from MTBE to naphthalene) and EPA method 8015C (from 2-methylpentane to 1,2,4-trimethylbenzene). Wisconsin and EPA method 8015C performance criteria were met with coefficient of determination (R^2) >0.99, percentage recovery between

Table 1. TRACE 1310 Gas Chromatograph and FID parameters

TRACE 1310 GC Parameters	
Inlet module and mode:	SSL, split
Split ratio:	20:1
Septum purge mode, Flow (mL/min):	Constant, 5
Carrier gas, carrier mode, Pressure (kPa):	He, constant pressure, 150
Oven Temperature Program	
Temperature 1 (°C):	50
Hold time (min):	1
Temperature 2 (°C):	220
Rate (°C/min):	15
Hold time (min):	5
FID	
Temperature (°C):	300
Air flow (mL/min):	350
H_2 flow (mL/min):	35
N_2 flow (mL/min):	40
Acquisition rate (Hz):	25
Chromatographic Column	
Thermo Scientific™ TraceGOLD™ TG-1MS (P/N 26099-4840)	30 m × 0.32 mm × 3.0 µm

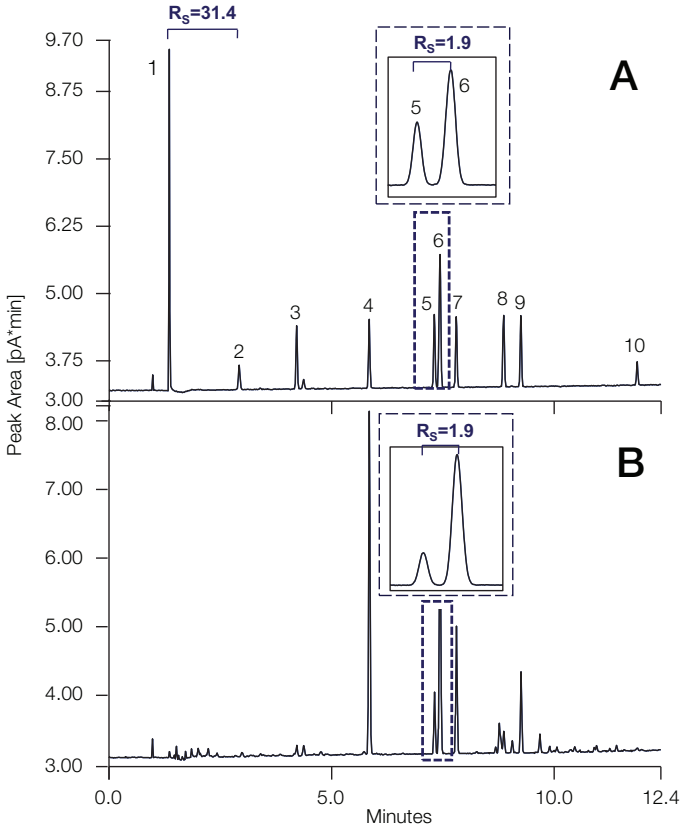
TriPlus 500 HS Autosampler Parameters	
Incubation temperature (°C):	85
Incubation time (min):	30
Vial shaking:	Fast
Vial pressurization mode:	Pressure
Vial pressure (kPa) (Auxiliary Gas Nitrogen):	200
Vial pressure equilibration time (min):	1
Loop size (mL):	1
Loop/sample path temperature (°C):	105
Loop filling pressure (kPa):	150
Loop equilibration time (min):	1
Needle purge flow level:	5
Injection mode:	standard
Injection time (min):	1

*Equivalent or better performance with the Thermo Scientific™ TRACE™ 1600 Series Gas Chromatograph systems



GRO		
	Wisconsin method	EPA 8015C method
Coefficient of determination (R^2)	0.9995	0.9995
AvCF %RSD	4.0	4.1
Calculated MDL (µg/L)	1.9	2.2
Calculated LOQ (µg/L)	6.1	7.0
Average recovery (%, n=5)	97	91
% RSD (n=10)	1.6	1.6
Calculated sample concentration (µg/L)	53.3	56.0

Figure 1. GRO linearity, recovery and repeatability of measurement



1=Methanol (A_s=1.2), 2=Methyl-*tert*-butylether (MTBE, A_s=1.0), 3=Benzene (A_s=1.0), 4=Toluene (A_s=1.0), 5=Ethylbenzene (A_s=1.0), 6=*m,p*-xylene (A_s=1.0), 7=*o*-xylene (A_s=1.0), 8=2,3,5-trimethylbenzene (A_s=1.0), 9=1,2,4-trimethylbenene (A_s=1.0), 10=Naphthalene (A_s=1.0)

Figure 2. Example chromatogram of a tap water sample spiked with standard solutions at 50 µg/L (A) and 5% raw gasoline solution (B)

80–120% (calculated using n=5 tap water samples spiked with raw gasoline solution (5%) and 100 µg/L standard solution) and peak area %RSD <20% (calculated using n=10 tap water samples spiked with raw gasoline solution (5%)) as reported in Figure 1. According to the Wisconsin method, calculated LOQ should be ≤ 100 µg/L for water samples. As an example, chromatograms of tap water samples spiked with standard solutions at 50 µg/L (A) and 5% raw gasoline solution (B) are reported in Figure 2.

Summary

The results presented in this work demonstrate that TRACE 1310 GC-FID combined with the TriPlus 500 static headspace ensures compliance to both Wisconsin and EPA 8015 C methods providing the requested chromatographic resolution for the critical pairs (methanol/MTBE and ethylbenzene/*m,p*-xylene) and fulfilling the linearity criteria for reliable quantitation of analytes.

The analytical conditions used to run the method are in a standard range of the instrument performance and assure system equivalency with different GC brands for streamlined method validation procedures.

Residual solvent analysis in pharmaceutical products according to USP <467> method

Introduction

Solvents are widely used in the synthesis of pharmaceutical products, substances and excipients. To ensure patients' safety, the International Conference on Harmonization (ICH)[7] and the United States Pharmacopeia (USP)[8] have published some guidelines to set the acceptable limits and to support the assessment of the residual solvents used during the production and purification processes. Residual solvents (RS) have low boiling points and thermal stability therefore they can be determined using headspace-gas chromatography (HS-GC) coupled to flame ionization detection.

The workflow for residual solvent assessment is reported using a simplified schematic in Figure 3. When the residual solvents that are likely to be present are known, they can be determined using a limit test, such as Procedure A or Procedure B, or by a quantitative test, such as Procedure C. When the residual solvents are not known, then a screening test using Procedure A must be used. If the article does not meet the acceptance criteria of Procedure A, then Procedure B must be used to demonstrate compliance. If the article does not meet the criteria using Procedure A and Procedure B, then Procedure C must be used to quantify the residual solvents present in the article.

Table 2. TRACE 1310 Gas Chromatograph and FID parameters

TRACE 1310 GC Parameters According to USP <467> Method	
	Procedure A/C
Inlet module and mode:	SSL, split
Split ratio:	10:1
Septum purge mode, flow (mL/min):	Constant, 5
Carrier gas, carrier mode, flow:	He, constant flow, 2.2 mL/min
Oven temperature program:	Procedure A/C
Temperature 1 (°C):	40
Hold time (min):	20
Temperature 2 (°C):	240
Rate (°C/min):	10
Hold time (min):	20
FID	
Temperature (°C):	250
Air flow (mL/min):	350
H_2 flow (mL/min):	35
N_2 flow (mL/min):	40
Acquisition rate (Hz):	25
Chromatographic Column	
Thermo Scientific™ TraceGOLD™ TG-624 (P/N 26085-3390)	30 m × 0.32 mm × 1.8 µm

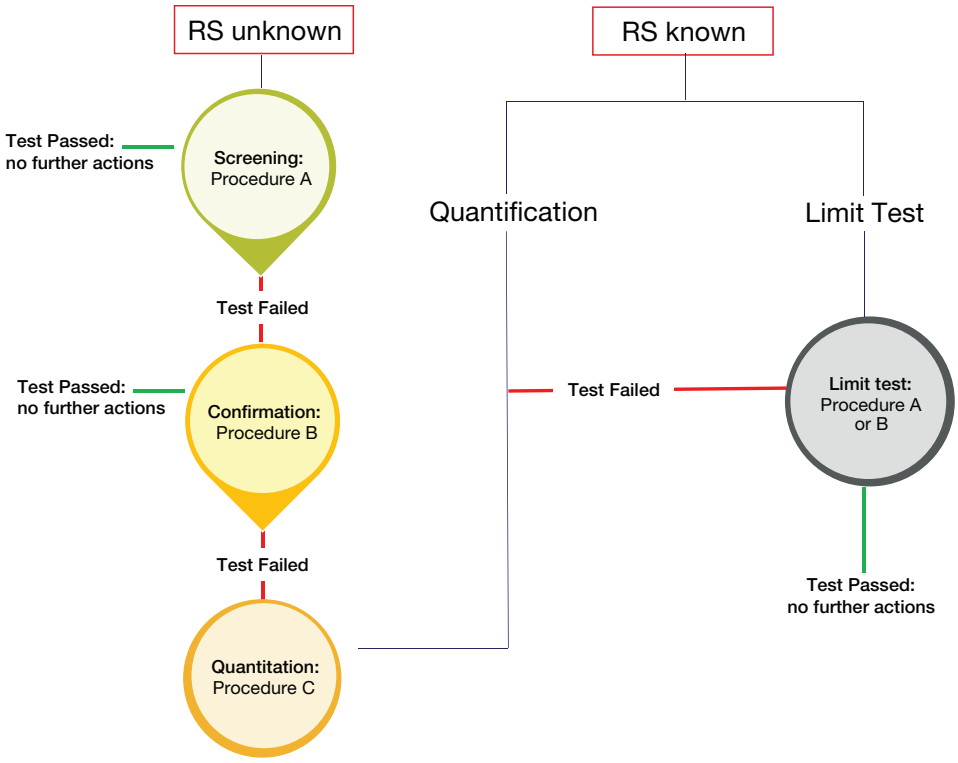


Figure 3. Simplified diagram of the analytical workflow used for residual solvent assessment

*Equivalent or better performance with the Thermo Scientific TRACE 1600 Series Gas Chromatograph systems

Results and discussion

Procedure A – Screening of unknown residual solvents

Stock, standard and test solutions were prepared according to the USP <467> method. An over-the-counter acetylsalicylic acid (dispersive aspirin, 75 mg) was purchased locally and analyzed according to the USP <467> workflow in Figure 3.

System suitability criteria for sensitivity (peak-to-peak (PtP) signal-to-noise ratio (S/N)) and chromatographic resolution (R_s) were met with:

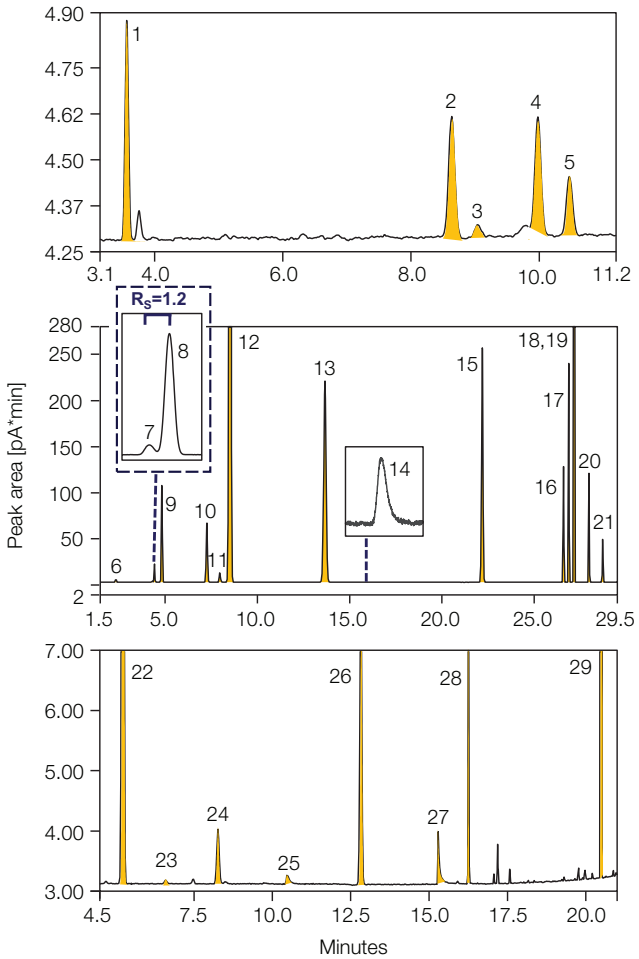
- S/N > 5:1 for 1,1,1-trichloroethane in Class 1 standard solution
- S/N >3:1 for all peaks in Class 1 system suitability solution (Figure 4)
- R_s between acetonitrile/dichloromethane >1 in Class 2A standard solution (Figure 4).

The innovative system design with direct connection between the gas chromatograph and the autosampler combined with the high inertness and the precise temperature and flow controls of the TRACE 1310 Gas Chromatograph allowed for an efficient chromatographic process ensuring Gaussian peak shapes with average asymmetry factor (A_s) of 1.2. Peak responses obtained for the un-spiked sample were lower than the corresponding peaks in Class 1 and Class 2 standard injections. According to the regulation, the test solution met the requirements for residual solvent content with no other actions required.

A streamlined method transfer from a different HS-GC system using the Valve-and-Loop headspace technology is ensured by the consistency of the method parameters. The name to report the method parameters may differ within different brands, especially for the headspace autosampler. The equivalency of the parameters is clearly explained in a previous published white paper.[10]

Summary

The results presented in this work demonstrate that the TRACE 1310 GC-FID fulfills the USP <467> requirements, meeting the suitability criteria for chromatographic separation as required for regulated c-GMP pharma laboratories. The equivalency of the method parameters assures a safe portability of the method from different HS-GC brands using the Valve-and-Loop headspace technology.



Class 1 Residual Solvents:

1=1,1-Dichloroethene (RT=3.60 min, S/N 126, A_S=1.0)
2=1,1,1-Trichloroethane (RT=8.65 min, S/N 82, A_S=1.0)
3=Carbon Tetrachloride (RT=9.06 min, S/N 8, A_S=1.1)
4=Benzene (RT=9.97 min, S/N 26, A_S=1.0)
5=1,2-Dichloroethane (RT=10.39 min, S/N 41, A_S=1.0)

Class 2A Residual Solvents:

6=Methanol (RT=2.33 min, A_S=1.3)
7=Acetonitrile (RT=4.31 min, A_S=0.9)
8=Dichloromethane (RT=4.42 min, A_S=1.0)
9=*trans*-1,2-Dichloroethene (RT=4.82 min, A_S=1.0)
10=*cis*-1,2-Dichloroethene (RT=7.25 min, A_S=1.0)
11=Tetrahydrofuran (RT=7.96 min, A_S=1.3)
12=Cyclohexane (RT=8.43 min, A_S=0.9)
13=Methylcyclohexane (RT=13.63 min, A_S=1.0)
14=1,4-Dioxane (RT=15.80 min, A_S=1.3)
15=Toluene (RT=22.14 min, A_S=0.9)
16=Chlorobenzene (RT=26.55 min, A_S=1.0)
17=Ethylbenzene (RT=26.84 min, A_S=1.0)
18=*p*-Xylene (RT=27.13 min, A_S=0.9)
19=*m*-Xylene (RT=27.13 min, A_S=0.9)
20=*o*-Xylene (RT=27.93 min, A_S=1.3)
21=Cumene (RT=28.67 min, A_S=1.0)

Class 2B Residual Solvents:

22=Hexane (RT=5.22 min, A_S=1.0)
23=Nitromethane (RT=6.58 min, A_S=1.2)
24=Chloroform (RT=8.25 min, A_S=1.1)
25=1,2-dimethoxyethane (RT=10.46 min, A_S=1.7)
26=Trichloroethylene (RT=12.80 min, A_S=1.0)
27=Pyridine (RT=15,28 min, A_S=4.0)
28=2-hexanone (RT=16.25 min, A_S=1.1)
29=Tetralin (RT=20.48 min, A_S=1.0)

Figure 4. Chromatographic separation of class 1, class 2A and class 2B residual solvents with annotated compound number as well as chromatographic resolution (Rs) for critical pair acetonitrile/dichloromethane.

Separation of US EPA 16 priority polycyclic aromatic hydrocarbons by GC-FID

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic chemicals containing two or more benzene rings and are found ubiquitously in the environment. There are many different possible chemical structures for PAHs with varying, physical, chemical and toxicological properties. Many PAHs have toxic and/or carcinogenic properties[11] and so the monitoring of these compounds is vital.

The United States Environmental Protection Agency (US EPA) has designated 16 of these possible PAHs as high priority pollutants due to their toxicity and abundance in the environment.[12] These are: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene.

Experimental

A Thermo Scientific™ AI/AS 1310 Liquid Autosampler was coupled to a TRACE 1310 Gas Chromatograph* configured with an Instant Connect split/splitless SSL Injector and an Instant Connect Flame Ionization Detector (FID). The system was used to detect the 16 PAHs designated high priority pollutants by the US EPA. Chromatographic separation was achieved using TraceGOLD TG-5MS 30m × 0.25 mm × 0.25 µm column (P/N 26098-1420)

A standard solution was prepared by diluting a Restek SV Calibration Mix #5/610 PAH Mix 2000 µg/mL in dichloromethane (P/N 31011) to 1 µg/mL in dichloromethane. Instrument conditions are shown in Table 3.

Results and discussion

Sample injection

The injection method using a surged pressure is optimized to accelerate the transfer 2µL of sample in splitless mode and maintain the column efficiency. Those inlet conditions assure the complete transfer of the target compounds up to the less volatile components without peak broadening, achieving the required separation of the critical pairs.

Chromatography

To reliably identify and quantify compounds when using an FID, chromatographic separation of the analytes is key. An example of the chromatography produced from a 2 µL injection of the 1 µg/mL mixed standard (2 ng per analyte on column) is shown in Figure 5. Chromatographic resolution (R_s) ≥ 1.0 was achieved for all analytes.

Table 3. TRACE 1310 Gas Chromatograph instrument parameters

Thermo Scientific TRACE 1310 Gas Chromatograph Conditions	
Inlet module and mode:	SSL, Splitless with surge
Surge pressure and duration:	30 psi, 0.2 min
Splitless time and flow:	0.2 min, 60 mL/min
Liner:	4 mm id single taper splitless liner (P/N 453A1345-UI)
Inlet temperature:	250 °C
Injection volume:	2 µL
Column:	TG-5MS 30 m × 0.25 mm × 0.25 µm (P/N 26098-1420)
Carrier gas, flow:	Helium, 1.3 mL/min, constant flow
Oven ramp:	70 °C (0.2 min hold), 25 °C/min to 265 °C, 5 °C/min to 315 °C (2 min hold)
Run time:	20 min
FID temperature:	350 °C
Hydrogen flow:	35 mL/min
Air flow:	350 mL/min
Makeup gas and flow:	40 mL/min nitrogen
Data collection rate:	10 Hz

*Equivalent or better performance with the Thermo Scientific™ TRACE™ 1600 Series Gas Chromatograph and the Thermo Scientific™ AI/AS 1610 Liquid Autosampler

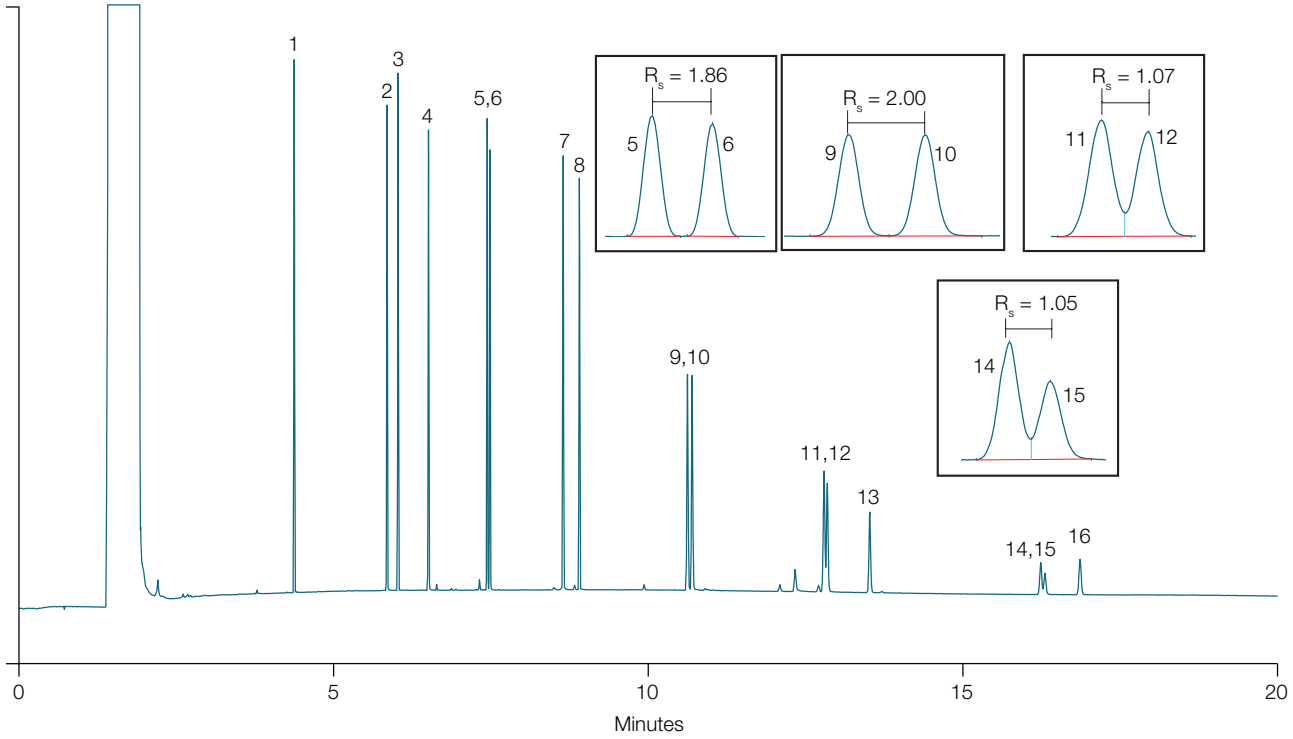


Figure 5. Chromatogram of the 16 PAHs in DCM with insets showing the zoomed in regions of the 4 closely eluting pairs annotated with their chromatographic resolution and peak number. Analytes elute as follows. 1 – naphthalene, 2 – acenaphthylene, 3 – acenaphthene, 4 – fluorene, 5 – phenanthrene, 6 – anthracene, 7 – fluoranthene, 8 – pyrene, 9 – benzo(a)anthracene, 10 – chrysene, 11 – benzo(b)fluoranthene, 12 – benzo(k)fluoranthene, 13 – benzo(a)pyrene, 14 – indeno(1,2,3-cd)pyrene, 15 – dibenzo(a,h)anthracene, 16 – benzo(g,h,i)perylene

Summary

The chromatography shown here demonstrates that the TRACE 1310 Gas Chromatograph in combination with a TG-5MS column can separate the 16 high priority PAHs for quantitative analysis in line with the US EPA suitability requirements. The injection parameters allowed a quick transfer of the sample across the entire volatility range preserving the separation efficiency of the column.

Separation of 37 fatty acid methyl esters according to AOAC method 996.06 by GC-FID

Introduction

Food fat mainly consists of triglycerides and assessing the fat (*trans* and saturated) composition of food products as part of the nutritional information is a fundamental test for the food industry. The AOAC method 996.06 describes the determination of total, saturated and unsaturated fat in foods using capillary GC-FID by a multiple steps procedure: hydrolytic extraction followed by the derivatization (methylation) of fatty acids to produce fatty acids methyl esters (FAMEs) which are the derivatives suitable for GC analysis.[13]

Experimental

A TRACE 1610 Gas Chromatograph configured with an Instant Connect split/splitless SSL Injector and an Instant Connect Flame Ionization Detector (FID) was coupled with an AI/AS 1610 Autosampler and used to assess the chromatographic separation performance according to AOAC method 996.06.

A standard solution was prepared by diluting Restek Food Industry FAMEs mix (30 mg/mL in dichloromethane) (P/N 35077) to 1000 µg/mL in dichloromethane/hexane.

Instrument conditions are shown in Table 4.

Results and discussion

Chromatographic resolution (R_s) is fundamental for FAMEs separation, identification and quantitation and specific resolution requirements for critical peaks pair are included in AOAC method 996.06: (R_s) ≥ 1.0 for FAMEs pair of adjacent peaks ($C_{18:3}$ - $C_{20:1}$ and $C_{22:1}$ – $C_{23:3}$ – $C_{20:4}$).

The chromatographic profile of 37 FAMEs separation obtained with TRACE 1610 Gas Chromatograph (equipped with Restek Rt-2560 column) is shown in Figure 6; critical pair peaks are highlighted, and the achieved resolution meets and exceeds the requirements. Peak identification and retention times are reported in Table 5.

Table 4. TRACE 1610 GC instrument parameters

TRACE 1610 GC Conditions	
Inlet module and mode:	SSL, Split
Split ratio and flow:	1:20, 20 mL/min
Liner:	LinerGOLD Precision Split/Splitless liner (P/N 453A1255-UI)
Inlet temperature:	225 °C
Injection volume:	1 µL
Column:	Restek Rt-2560 100 m × 0.25 mm × 0.20 µm (P/N 13198)
Carrier gas, flow:	Helium, 1 mL/min, constant flow
Oven ramp:	100 °C (4 min hold), 3 °C/min to 240 °C (15 min hold)
FID temperature:	285 °C
Hydrogen flow:	35 mL/min
Air flow:	350 mL/min
Makeup gas and flow:	Nitrogen 40 mL/min
Data collection rate:	25 Hz

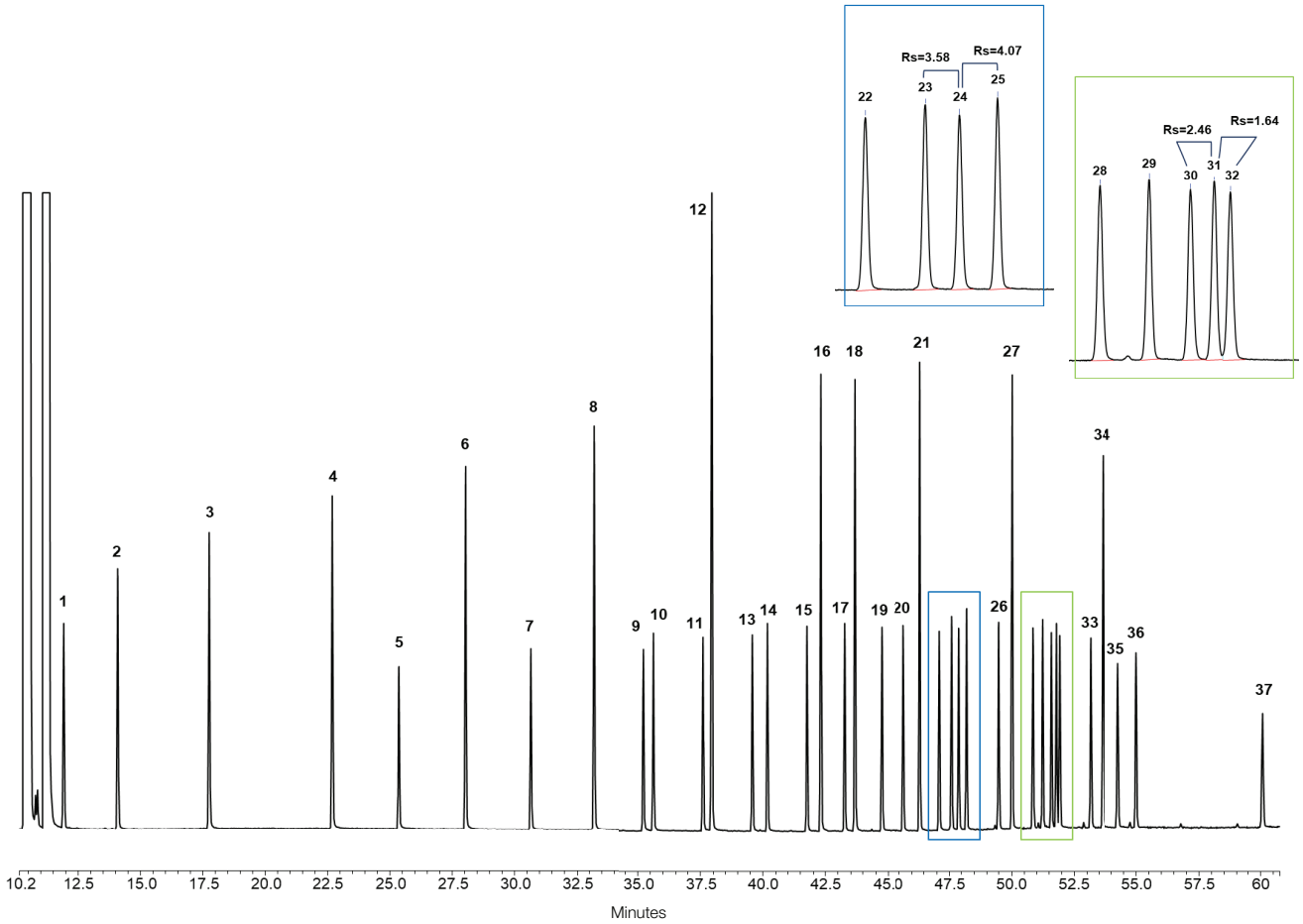


Figure 6. Chromatographic separation of a solvent standard of the Food Industry FAMEs mix (37 components) at 50 ppm (µg/mL) on column using the TRACE 1610 Gas Chromatograph and a Restek Rt-2560 column.

Table 5. Food Industry FAMEs mix (37 components) - identification and retention times.

Peak	Compound		Retention Time (min)	Peak	Compound		Retention Time (min)
1	C4:0	Methyl butyrate	11.937	20	C18:2 (c9,c12)	Methyl linoleate	45.679
2	C6:0	Methyl caproate	14.101	21	C20:0	Methyl arachidate	46.341
3	C8:0	Methyl octanoate	17.770	22	C18:3 (c6,c9,c12)	Methyl linolenate	47.125
4	C10:0	Methyl decanoate	22.704	23	C20:1 (c11)	Methyl eicosenoate	47.622
5	C11:0	Methyl undecanoate	25.375	24	C18:3 (c6,c9,c15)	Methyl linolenate	47.904
6	C12:0	Methyl dodecanoate	28.050	25	C21:0	Methyl heneicosanoate	48.220
7	C13:0	Methyl tridecanoate	30.667	26	C20:2 (c11,c14)	Methyl eicosadienoate	49.504
8	C14:0	Methyl myristate	33.201	27	C22:0	Methyl behenate	50.044
9	C14:1 (c9)	Methyl myristoleate	35.235	28	C20:3 (c8,c11,c14)	Methyl eicosatrienoate	50.877
10	C15:0	Methyl pentadecanoate	35.634	29	C22:1 (c13)	Methyl erucate	51.283
11	C15:1 (c10)	Methyl pentadecenoate	37.619	30	C20:3 (c11,c14,c17)	Methyl eicosatrienoate	51.625
12	C16:0	Methyl palmitate	37.972	31	C23:0	Methyl tricosanoate	51.823
13	C16:1 (c9)	Methyl palmitoleate	39.593	32	C20:4 (c5,c11,c14,c17)	Methyl arachidonate	51.955
14	C17:0	Methyl heptadecanoate	40.194	33	C22:2 (c13,c16)	Methyl docosadienoate	53.182
15	C17:1 (c10)	Methyl heptadecenoate	41.779	34	C24:0	Methyl lignocerate	53.669
16	C18:0	Methyl stearate	42.333	35	C20:5 (c5,c8,c11,c14,c17)	Methyl eicosapentaenoate	54.297
17	C18:1 (t9)	Methyl octadecenoate	43.284	36	C24:1 (c15)	Methyl nervonate	55.026
18	C18:1 (c9)	Methyl oleate	43.695	37	C22:6 (c4,c7,c10,c13,c16,c19)	Methyl docosahexaenoate	60.107
19	C18:2 (t9,t12)	Methyl linolelaidate	44.776				

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Summary

The TRACE 1610 Gas Chromatograph equipped with Restek RT-2560 100 m, 0.25 mm, 0.2 µm capillary column is suitable for FAMEs separation in food samples according to AOAC method 996.06, meeting or exceeding resolution requirements and providing reliable peaks integration and quantification.

Conclusion

The examples considered in this white paper demonstrate that the TRACE Series Gas Chromatograph systems allows for equivalent chromatographic performance ensuring that suitability requirements of specific regulatory methods are met. The application of method parameters within a standard working range for the gas chromatographic system, along with the use of standard consumables, allow for a smooth transfer maintaining the required analytical performance. Method equivalency was demonstrated for:

- Environmental laboratories assessing GRO in water samples using GC-FID combined with static headspace.
- Regulated c-GMP pharma laboratories analyzing residual solvents according to the USP <467> requirements.
- Environmental laboratories analyzing priority PAHs in line with the US EPA suitability requirements
- Laboratories focusing on determination of total, saturated and unsaturated fat in foods with GC-FID according to the AOAC method 996.06

Taken together, these experiments show method equivalency for the TRACE Series Gas Chromatograph systems and that chromatographic performance criteria are met with ease, ultimately ensuring the data quality requirements of the intended application.

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