Meeting the Requirements of EN12916:2006 (IP391/07) Using Agilent 1200 Series HPLC Systems

Application Note

Hydrocarbons

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Important Note: For reliable column performance, it is essential that Agilent publication 5990-9202EN be accessed and followed.

Abstract

The performance of diesel fuel is predominantly determined by its ignition quality. This parameter is known as the Cetane number. The Cetane number describes the volume % Cetane (hexadecane) present in a mixture of Cetane and 1-Methylnaphthalene. Generally, in order to provide the best performance and lifetime of an engine, the amount of aromatics in diesel should be as low as possible. For the analysis of non-aromatics and aromatics in diesel fuel and petroleum distillates boiling in the range 150 °C to 400 °C, there exists an IP Method (391/07), which uses HPLC with refractive index detection. The two compound classes (aromatics and non-aromatics) are separated using normal phase HPLC and a column which has little affinity for non-aromatic but pronounced selectivity for aromatic hydrocarbons [1]. Recent growth in biodiesel production created a demand for analysis of petrodiesel and petrodiesel/biodiesel blends. In this method revision, fatty acid methyl esters (FAME) originating from biodiesel sources must elute after a tetra-aromatic marker peak, chrysene, which facilitates improved accuracy of large PAH molecules without interference from FAME. The refractive index detector is used because this detector responds to both non-aromatic and aromatic hydrocarbons.

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About Standard Method IP391/07

"This European Standard specifies a test method for the determination of the concentration of mono-aromatic, di-aromatic and tri+-aromatic hydrocarbons in diesel fuels that may contain fatty acid methyl esters (FAME) up to 5 % (v/v) and petroleum distillates in the boiling range from 150 °C to 400 °C. The polycyclic aromatic hydrocarbon content is calculated from the sum of di-aromatic and tri+-aromatic hydrocarbons and the total content of aromatic compounds is calculated from the sum of the individual aromatic hydrocarbon types. Compounds containing sulfur, nitrogen and oxygen may interfere in the determination; mono-alkenes do not interfere, but conjugated di-alkenes and polyalkenes, if present, may do so.

The precision statement of the test method has been established for diesel fuels with and without FAME blending components, with a mono-aromatic content in the range from 6 % (m/m) to 30 % (m/m), a di-aromatic content from 1 % (m/m) to 10 % (m/m), a tri+-aromatic content from 0 % (m/m) to 2 % (m/m), a polycyclic aromatic content from 1 % (m/m) to 12 % (m/m), and a total aromatic content from 7 % (m/m) to 42 % (m/m)." [2]

This method, also known as EN12916:2006, is an official method of the Energy Institute (United Kingdom, www.energyinst.org.uk) which maintains IP (Institute of Petroleum) standards since their acquisition of the IP. Earlier IP391 revisions are similar to ASTM D-6591-06 and include a column backflush-capable instrument configuration and analysis scheme. This requirement was discontinued in the current IP391/07 revision due to erroneous reporting of tri+aromatic hydrocarbons when FAME were present. The main IP391 changes from earlier revisions include the elimination of the backflushing valve, allowing compatibility with biodiesel/petrodiesel fuel blends (up to 5% v/v FAME) and modifications to calibrants to improve data accuracy.

The various methods associated with middle distillate fuel analysis are shown in Table 1.

Equipment and Conditions

LC:	Agilent 1200 Series LC including
G1312B:	Binary pump, used isocratically with pump head seals for normal phase, Agilent p/n 0905-1420
G1367C:	Autosampler with needle wash
G1316C:	Thermostatted column compartment
G1362A:	Refractive index detector
Software:	Agilent ChemStation with version B.04.01 software
Columns:	ZORBAX NH$_2$ 4.6 mm × 150 mm, 5 µm (p/n 883952-708) and ZORBAX SB-CN 4.6 mm × 150 mm, 5 µm (p/n 883975-905) connected in series using 0.12 × 70 mm ss connector tubing, Agilent p/n G1316-87303
Mobile phase:	n-heptane, HPLC grade
Flow rate:	1 mL/min
Injection volume:	10 µL
Oven temperature:	25 ºC
Detection:	Refractive index

Sample preparation

Samples and standards were prepared according to Standard Method IP391/07, using heptane as the diluent. Final quantitative results were reported using Agilent IP391/07 standard mixtures (p/n 5190-0485 system calibration standards SCS1 and SCS2, and p/n 5190-0484 quantitative calibration standards A-D).

Results and Discussion

The first step in implementing of IP391/07 is the analysis of calibrants that establish overall separation selectivity and resolution, and confirmation of the elution order of the calibrant components. (Sections 8.6, 8.7, 8.9 IP391/07). Figure 1 shows the results of running these calibrants on the Agilent system.

Table 1.   Middle Distillate Fuel Analysis Methods

IP method and revision	Method overview	Special parameters	ASTM method	Comments
IP391/07	150-400 °C diesel fuel petro/bio blends up to B-5	No backflush, amino and/or cyano column	No current equivalent available	Same as method EN12916:2006 *MAH, DAH, Tri+AH are reported
IP436/01	50-300 °C aviation fuel, kerosene	No backflush, amino and/or cyano column	D-6379-04	MAH and DAH reported not for samples with Tri+AH
IP548/06	150-400 °C diesel fuel	Backflush required, amino and/or cyano column	D-6591-06	MAH, DAH, Tri+AH reported FAME interferes with result

*MAH – monoaromatic hydrocarbon, DAH –- diaromatic hydrocarbon, Tri+AH – tri and higher ring aromatic hydrocarbons

RID1 A, Refractive Index Signal (083109_HGA\083109_N15CN15_ALLGO2 2009-09-01 12-44-12\N15CN15_0901044.D)

SCS1

1. Cyclohexane
2. 1-phenyldodecane
3. o-xylene (dimethylbenzene)
4. Hexamethylbenzene
5. Naphthalene
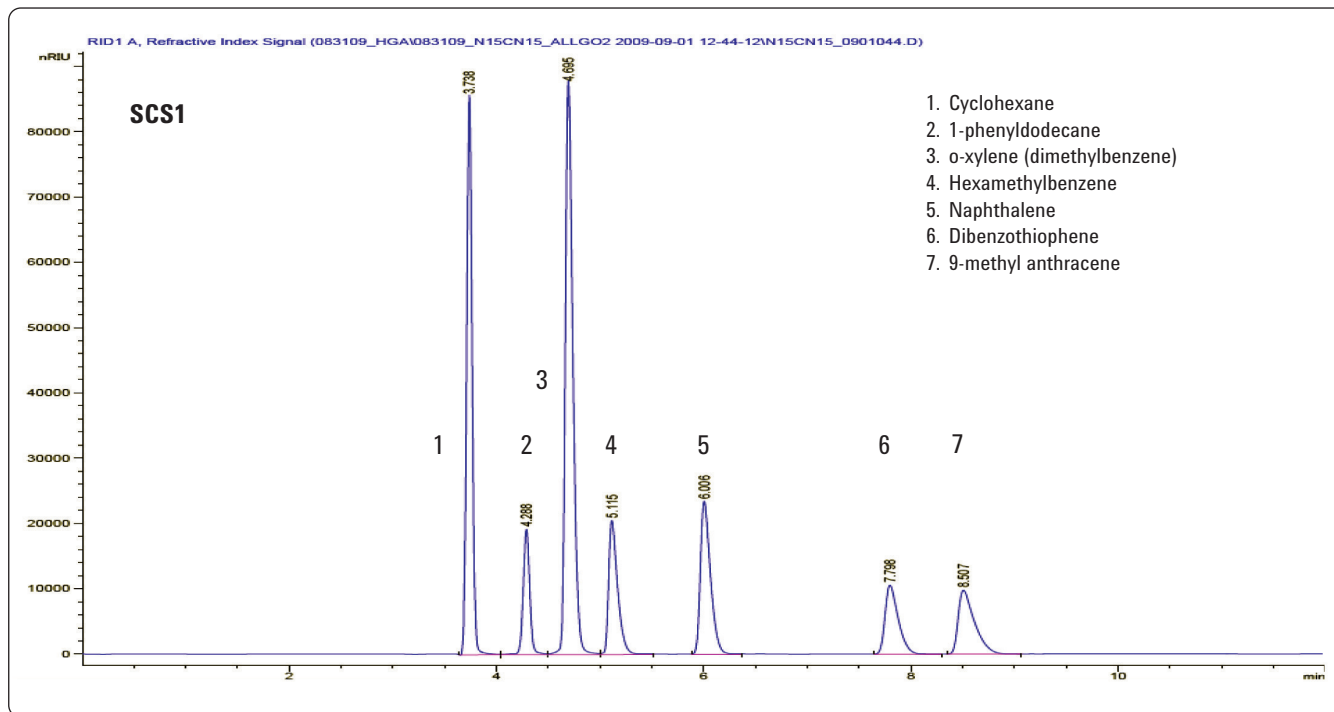6. Dibenzothiophene
7. 9-methyl anthracene

Figure 1.   Standard chromatogram of SCS1.

The system calibration standard 1 (SCS1) determines selectivity and retention data for the saturate and aromatic markers used for method acceptance criteria. The SCS1 also determines retention time grouping parameters for sample reporting. Resolution between cyclohexane and o-xylene (1,2-dimethylbenzene) is part of the method specification and must conform to a minimum and maximum value.

System calibration standard 2 (SCS2) establishes the selectivity for components present in petro/bio diesel blends, demonstrating that there is no interference with tri+aromatic components by FAME. Petro/bio fuel blends require longer analysis time to elute all FAME peaks before the next analysis is begun. This applies whether there is an interest in quantifying the FAME or not. When FAME is present in the sample, but does not need to be quantified, it is possible to reduce analysis time by programming the flow rate through the column to increase with time. This will rapidly wash FAME components from the column.

The method requirements state that chrysene, a tetra-aromatic marker peak, must elute with or before the first FAME peak. As shown in Figure 2, the selected operating conditions provide ample separation of the chrysene from the first FAME peak (C16:0 and C18:0 partially resolved).
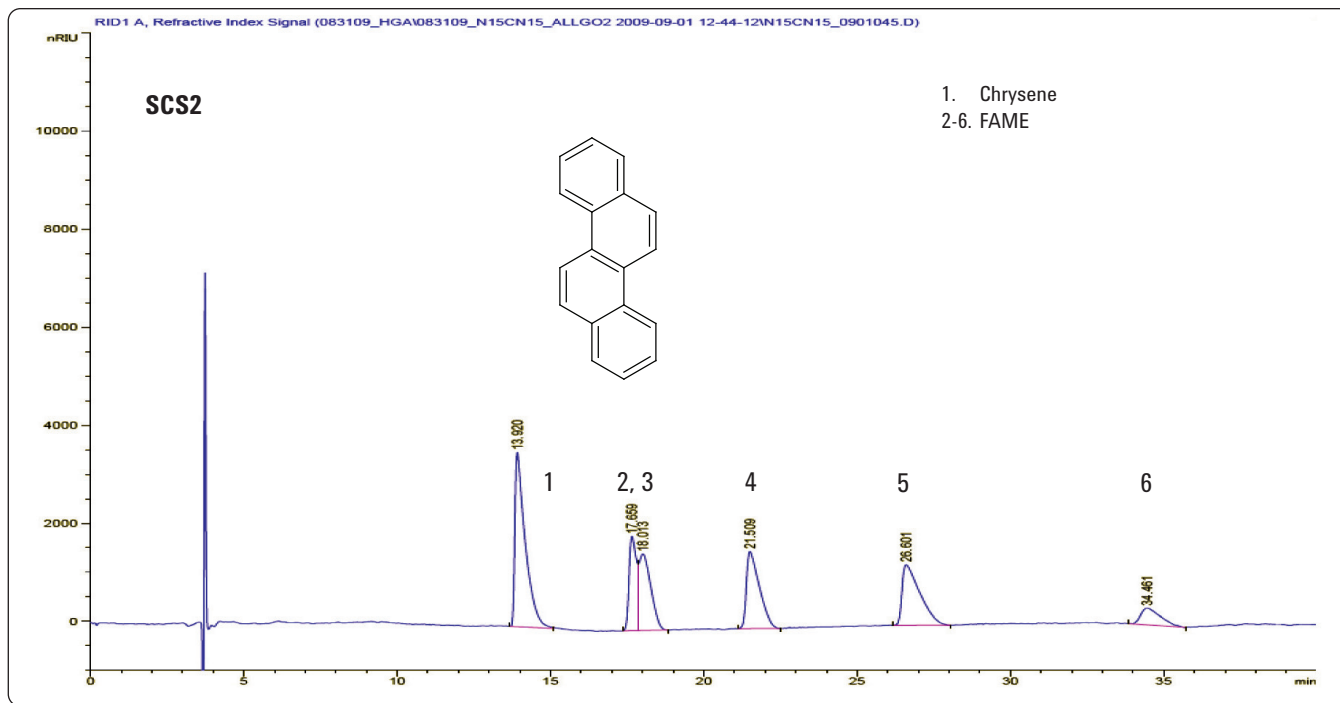
3

Figure 2.   Standard chromatogram of SCS2.

With a genuine fuel sample, in this case retail quality petrodiesel, we can see greater complexity and overlapping of the various compound class regions (Figure 3). Within the method definitions there are specific "cut" points defining the grouping to be performed in the quantitative reports. These are calculated from retention and peak width data in SCS1.
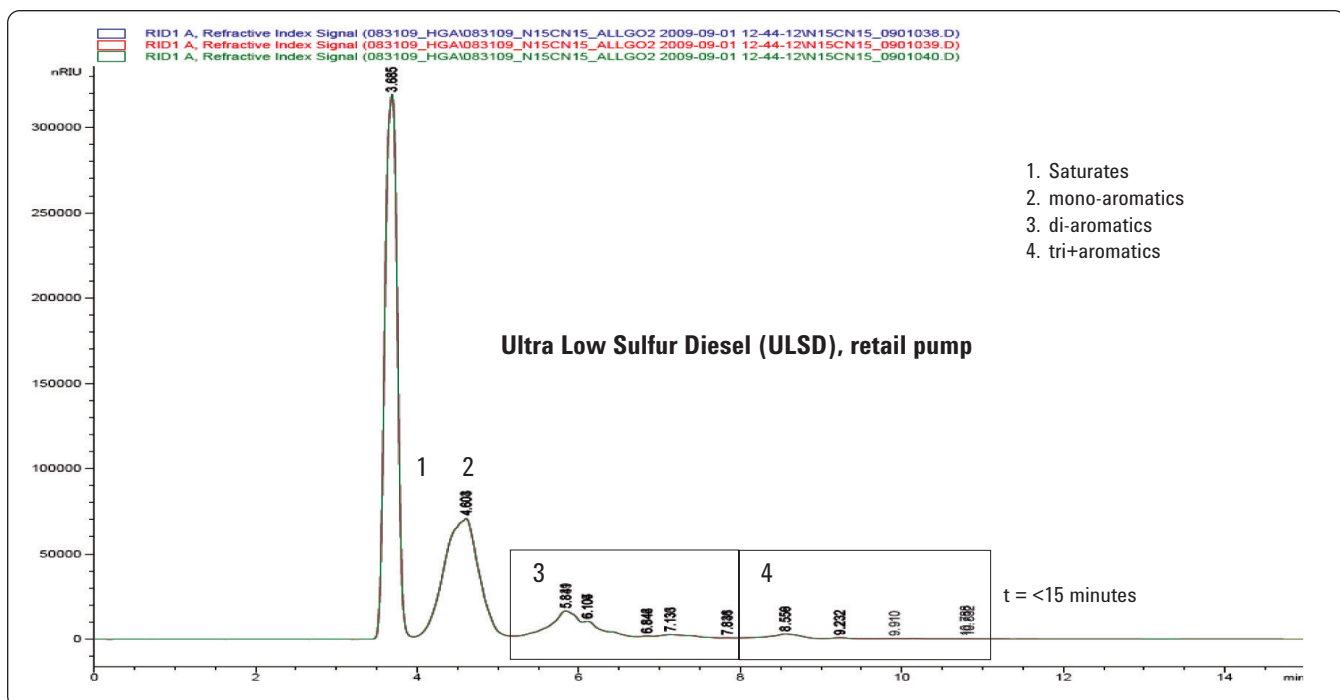

Figure 3.   Petroleum diesel sample showing cut points for the various compound groups typically present in these samples.

Results and Discussion

Method Performance

As with most official methods, there are specific performance criteria that allow qualification of the separation system and its subsequent use for reporting quantitative results of diesel fuel analysis.

- 6.4 Column system, consisting of a stainless steel HPLC column(s) packed with a commercial 3 µm, 5 µm or 10 µm amino-bonded (or amino/cyano-bonded) silica stationary phase meeting the resolution requirements given in 8.6, 8.7 and 8.9.

- 8.6 Ensure the components of the SCS1 are eluted in the order: cyclohexane, phenyldodecane, 1,2, dimethylbenzene, hexamethylbenzene, naphthalene, dibenzothiophene and 9-methylanthracene.

- 8.7 Ensure that baseline separation is obtained between all components of the SCS1.

- 8.9 Ensure that the resolution between cyclohexane and 1,2 dimethylbenzene is between 5.7 and 10. [calculated as described in 11.2]

- 11.2 Column Resolution

Calculate the resolution, R, between cyclohexane and 1,2 dimethylbenzene using the following equation.

$$R = \frac{2(t3-t1)}{1.699(y1+y3)}$$

(difference in retention time)
(averaging of peak widths)

Name	Ret Time [min]	Resolution	W hh [min]	Cut ref
1. Cyclohexane	3.738	–	0.0558	t1
2. 1-phenyldodecane	4.288	–	0.0648	t2
3. 1,2-dimethylbenzene	4.695	8.57	0.0756	t3

- 8.11 Ensure the retention time peak of chrysene is higher than the ... 9-methylanthracene peak....and check that the chrysene peak elutes just before or with the first peak of FAME.

In Figure 4, we see that there is distinct separation between the markers specified in section 8.11 of the method. With this information in hand, it is possible to proceed to the evaluation of calibration standards.
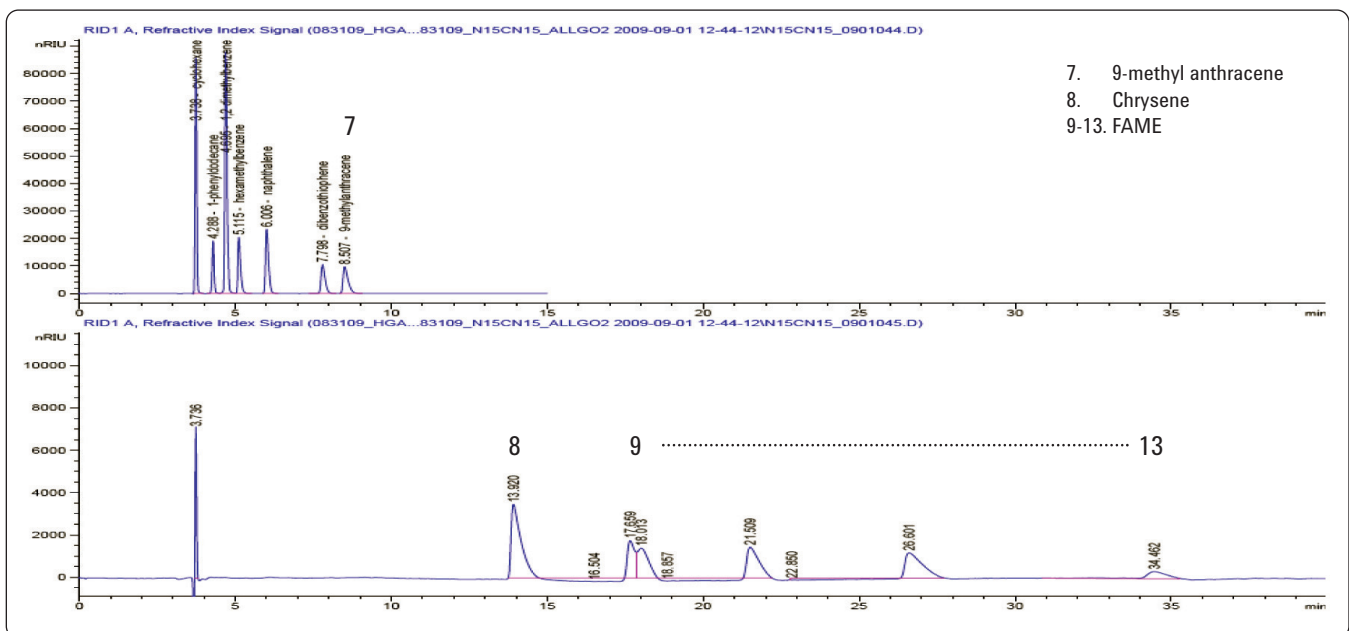
- 9.4 R = >0.999, Intercept <0.01 g/100 mL)

7. 9-methyl anthracene
8. Chrysene
9-13. FAME

Figure 4.   Stack plot of SCS1 and SCS2 showing proof for section 8.11.
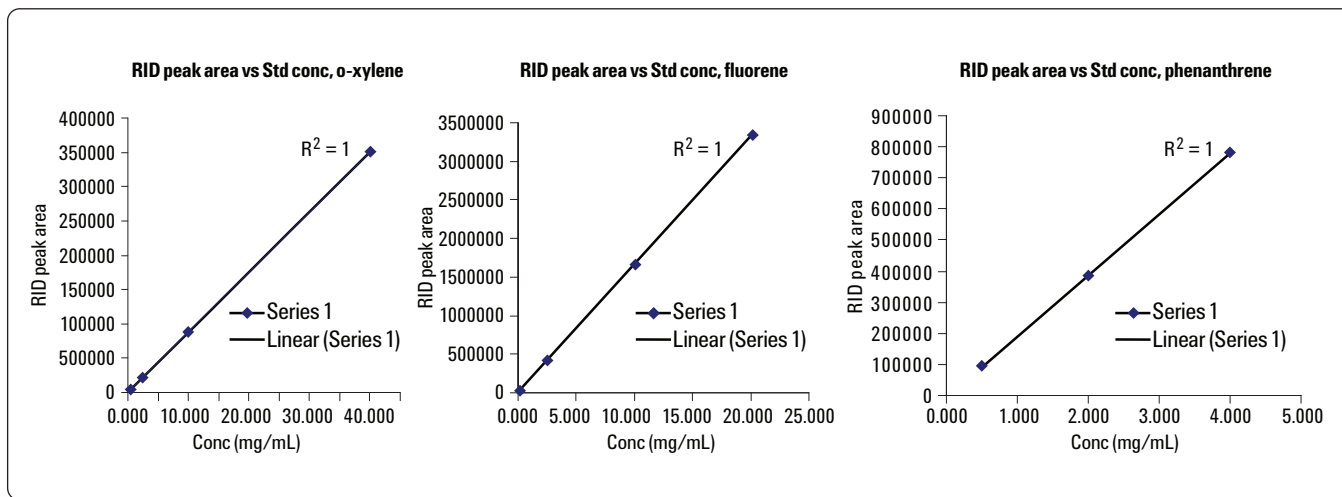
Figure 5. Calibration plots for o-xylene, fluorene and phenanthrene, the three components of the four calibration levels specified in the method.

In the calculated results, all calibration plots exceed linearity of 0.9999 and have calculated intercepts well below 0.01 g/ 100 mL, the method specification of section 9.4.

Retention time and peak area precision can be found in Table 2, in which we see that the overall performance of the method is excellent.

Table 2. Retention Time and Peak Area Precision

Calibrant A

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.57	0.003	0.06	3.54E+06	5829.9	0.16
Fluorene	6.82	0.004	0.05	3.38E+06	2500.5	0.07
Phenanthrene	8.32	0.004	0.04	8.05E+05	594.03	0.07

Calibrant B

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.65	0.001	0.02	9.23E+05	636.28	0.07
Fluorene	6.95	0.001	0.02	1.70E+06	1731.17	0.10
Phenanthrene	8.44	0	0.00	4.00E+05	473.79	0.12

Calibrant C

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.70	0.002	0.03	2.24E+05	474.36	0.21
Fluorene	7.15	0.002	0.03	4.29E+05	507.38	0.12
Phenanthrene	8.62	0.002	0.02	1.00E+05	291.04	0.29

Calibrant D

Analyte	R.T. Avg, n=3	R.T. Std dev	R.T. RSD%	Area Avg, n=3	Area Std dev	Area RSD%
Xylene	4.72	0.001	0.02	4.45E+04	321.90	0.72
Fluorene	7.36	0.002	0.02	1.70E+04	145.91	0.86
Phenanthrene	8.70	0.002	0.02	3.80E+04	551.43	1.45

| **Average RSD% All Runs** | | | **0.029** | | | **0.355** |

Results for Specific Petrodiesel and Petro/Biodiesel Blends

Various samples were collected from local commercial and retail fuel delivery points. An overlay of four samples is shown in Figure 6.
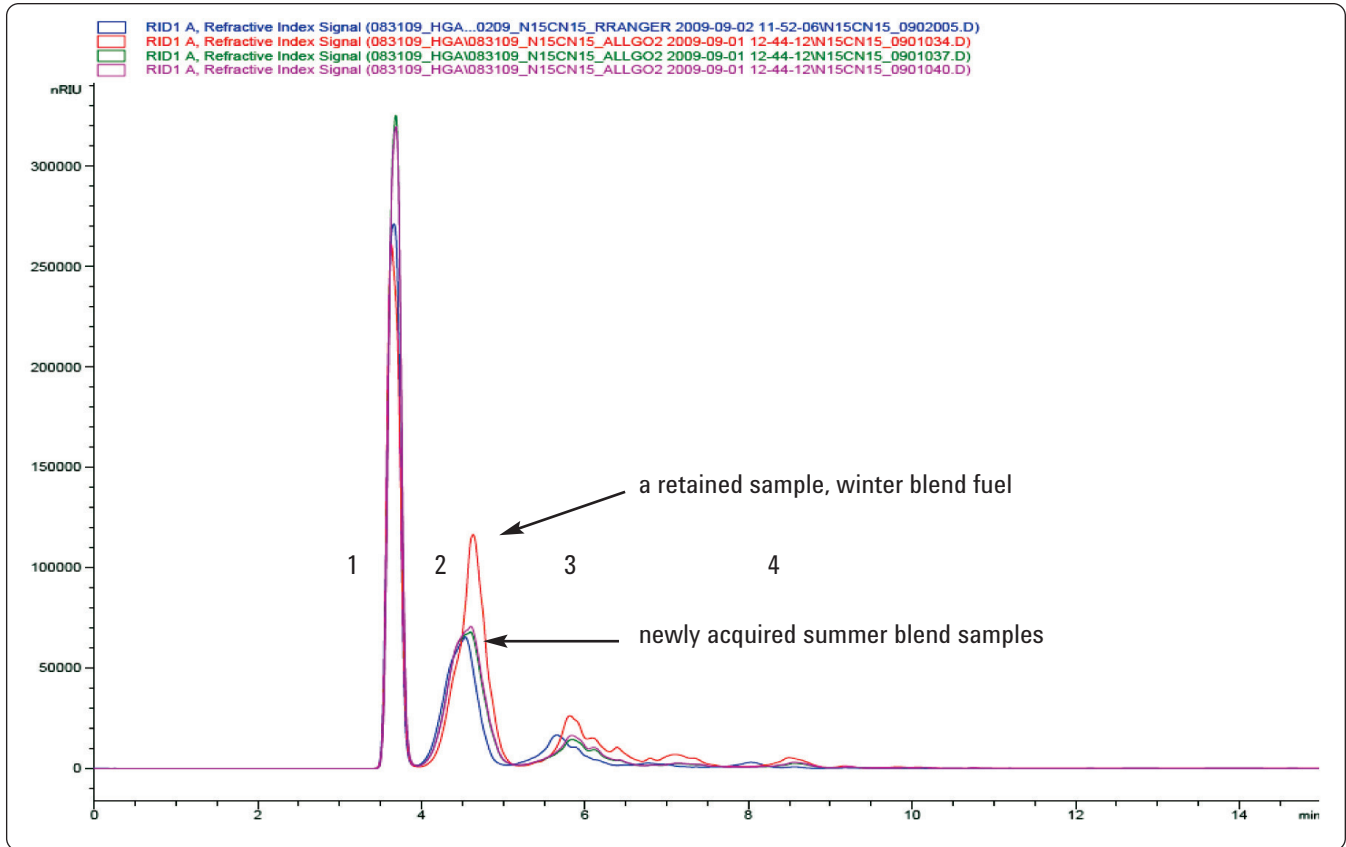

Figure 6.   Overlay of four samples.

Results (n=3 each sample) and precision data are reported in Table 3, where vendor 4 results are n=3 based on one sampling of each of the three B-11 biodiesel delivery points (commercial heavy truck, commercial auto/light truck and retail auto/light truck).

Table 3.   Results (n=3 each sample) and Precision Data for Four Vendors.

Vendor	Group	Avg, n=3		RSD%	Std dev
1	MAH	36.0	g/100 mL	0.06	0.022
	DAH	8.4	g/100 mL	0.09	0.008
	Tri+AH	1.2	g/100 mL	2.78	0.033
2	MAH	28.5	g/100 mL	0.05	0.016
	DAH	4.8	g/100 mL	0.48	0.023
	Tri+AH	0.6	g/100 mL	3.24	0.020
3	MAH	29.0	g/100 mL	0.07	0.021
	DAH	5.3	g/100 mL	0.37	0.020
	Tri+AH	0.7	g/100 mL	6.81	0.047
4	MAH	24.7	g/100 mL	5.06	1.252
	DAH	5.1	g/100 mL	3.38	0.174
	Tri+AH	0.7	g/100 mL	4.58	0.033

Because of very low level response and broadenend peaks, it is much more difficult to get high precision for the tri+aromatic hydrocarbons group of components. If no biodiesel components are present, it would be practical to consider using Method IP548/01, which uses a backflush valve to elute the tri+aromatic hydrocarbons group as a single peak via the backflush configuration.

Ruggedness and Stability of the IP391/07 Method

As with most normal phase methods the column is suscepti-ble to the adsorption of highly polar components which can affect overall separation performance. Water present in sam-ples or the mobile phase also adsorbs into the column and somewhat predictably causes reduced elution times for all sample components. Using a high quality HPLC grade mobile phase is essential, and the user may consider using a drying agent such as molecular sieve to dehydrate the mobile phase. While this is often done by adding molecular sieve to the sol-vent container, it is also possible and preferable to prepare a high pressure compatible column with pre-washed drying agent and placing it inline between the pump and injector.

Conclusion

The performance of the Agilent 1200 Series HPLC with normal phase separation and refractive index detection meets or exceeds the requirements of IP391/07 within the range of samples defined in the method. The user should take care identifying samples of petrodiesel that may contain biodiesel components to ensure adequate analysis time before proceeding to the next analytical run.

References

1. ASTM 6591-06 and ASTM 6379-04, www.astm.org

2. IP391/07, Energy Institute (formerly Institute of Petroleum Test Methods)

3. EN12916/06, Energy Institute (formerly Institute of Petroleum Test Methods)

4. Agilent Technologies publication 5965-9044EN, (1997)

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