

# Meeting the requirements of ASTM D 6591-06 (IP548/06) Using Agilent 1200 Series HPLC Systems

## Application

Hydrocarbons

### 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 (aliphatic hexadecane) present in a mixture of Cetane and (aromatic) 1-Methyl-naphthalene. Generally, in order to provide the best performance and maximize the 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 of 150 °C to 400 °C, there exists an ASTM Method (D 6591-06), and identical method IP548/06 that uses HPLC with refractive index detection. The two compound classes (aromatics and non-aromatics) are separated using normal phase HPLC and a column that has little affinity for non-aromatic but has pronounced selectivity for aromatic hydrocarbon classes [1]. The refractive index detector is used because this detector responds to both non-aromatic and aromatic hydrocarbons.

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## About Standard Method ASTM D 6591-06

“This test method covers a high performance liquid chromatographic test method for the determination of monoaromatic, di-aromatic, tri+-aromatic, and polycyclic aromatic hydrocarbon contents in diesel fuels and petroleum distillates boiling in the range of 150 to 400 °C. The total aromatic content in % m/m is calculated from the sum of the corresponding individual aromatic hydrocarbon types.

NOTE 1—Aviation fuels and petroleum distillates with boiling points that range from 50 to 300 °C are not determined by this test method and should be analyzed by Test Method, D 6379 or another suitable equivalent test method.

- 1.2 The precision of this test method has been established for diesel fuels and their blending components, containing from 4 to 40 % (m/m) mono-aromatic hydrocarbons, 0 to 20 % (m/m) di-aromatic hydrocarbons, 0 to 6 % (m/m) tri+-aromatic hydrocarbons, 0 to 26 % (m/m) polycyclic aromatic hydrocarbons, and 4 to 65 % (m/m) total aromatic hydrocarbons.
- 1.3 Compounds containing sulfur, nitrogen, and oxygen are possible interferents. Mono-alkenes do not interfere, but conjugated di- and poly-alkenes, if present, are possible interferents.
- 1.4 By convention, this standard defines the aromatic hydrocarbon types on the basis of their elution characteristics from the specified liquid chromatography column relative to model aromatic compounds. Quantification is by external calibration using a single aromatic compound, which may or may not be representative of the aromatics in the sample, for each aromatic hydrocarbon type. Alternative techniques and methods may classify and quantify individual aromatic hydrocarbon types differently.
- 1.5 Fatty Acid Methyl Esters (FAME), if present, interfere with tri+-aromatic hydrocarbons. If this method is used for diesel containing FAME, the amount of tri+-aromatics will be overestimated.”[2]

This method, also known as IP548/06, is an official method of the American Society of Testing Methods (United States, [www.astm.org](http://www.astm.org)). The method requires a column backflush-capable instrument configuration and analysis scheme, and is similar to other hydrocarbon group analysis methods. Because of this similarity, with respect to mobile phase and detection strategy, the instrument configuration is readily adaptable to those other methods.

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

### Equipment and Conditions

LC:	Agilent 1200 Series LC
Binary pump:	G1312B used isocratically with pump head seals for normal phase, Agilent p/n 0905-1420
Autosampler:	G1367C with needle wash
Therm. Column Compartment:	G1316C with 6 port 2 position switching valve
Refractive Index Detector:	G1362A
Software:	Agilent ChemStation with version B.04.02 software
Columns:	Agilent ZORBAX NH <sub>2</sub> , 4.6 × 250 mm, 5 μm (p/n 880952-708)
Mobile Phase:	n-heptane, HPLC grade
Flow Rate:	1 ml/min
Injection Volume:	10 μl
Oven Temperature:	20 °C
Detection:	Refractive index

### Sample preparation

Samples and standards were prepared according to guidance published in the method, using heptane as the diluent. System qualification and final quantitative results were reported using Agilent ASTM D 6591-06 standard mixtures (p/n 5190-0483 system performance solution SPS, and p/n 5190-0482 quantitative calibrant solutions A-D, respectively).

Table 1. 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

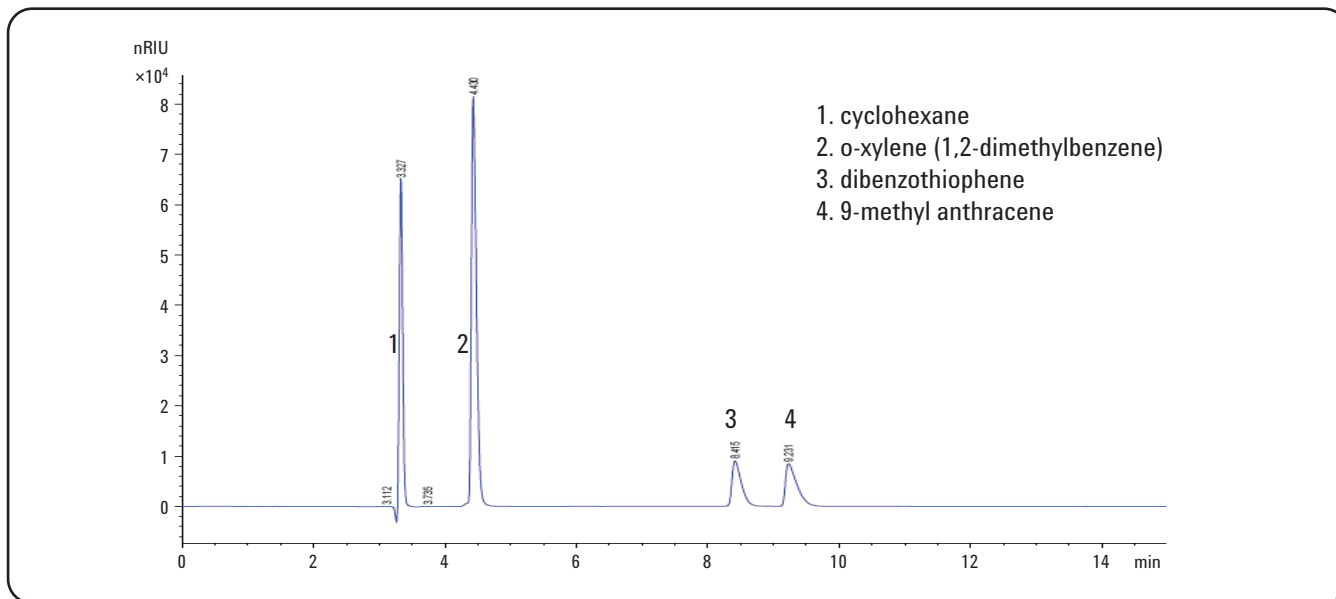


Figure 1. Standard chromatogram of system performance solution (SPS).

## Results and Discussion

The first steps in method implementation are to analyze a system performance solution (SPS) that establishes overall separation selectivity and resolution, and to establish the event time table for column backflushing during the analysis. (Sections 9.4 and 9.6 of the method). Figure 1 illustrates the

results of running the performance solution on the Agilent system without a backflush event.

The SPS is used to determine selectivity and retention data for the saturate and aromatic markers that are used for method acceptance criteria. It is also used to determine the backflush time for eluting tri+aromatics as a single peak.

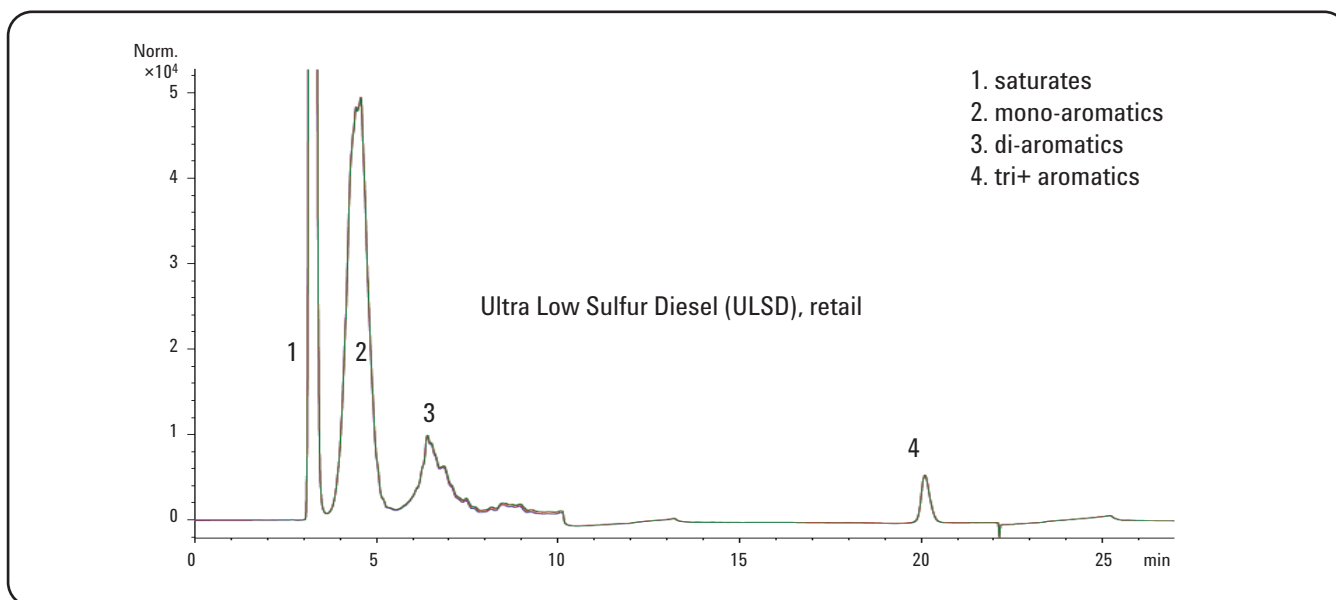


Figure 2. Petroleum diesel sample, n=3 overlay, showing cutpoints for the various compound groups typically present in these samples.

Resolution between cyclohexane and o-xylene (1,2-dimethylbenzene) is part of the method specification and must attain a minimum value of 5.

With a genuine fuel sample, in this case retail quality petrodiesel, greater complexity and overlapping of the various compound class regions are evident. Within the method definitions there are specific “cutpoints” defining the grouping to be performed in the quantitative reports. Manual peak integration is specified in the method for setting the baseline, and inserting valley drop points.

## 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—Any stainless steel HPLC column(s) packed with an approved amino-bonded (or polar amino/cyano-bonded) silica stationary phase is suitable, provided it meets the resolution requirements laid down in 9.4.3. [2]
- 9.4.1 Ensure that baseline separation is obtained between all components of the SPS.
- 8.9 Ensure that the resolution between cyclohexane and 1,2 dimethylbenzene is at least 5 as described in 9.4.3.

- 9.4.3.1 Column Resolution  
Calculate the resolution, R, between cyclohexane and 1,2 dimethylbenzene using the following equation.

$$R = \frac{2(t_2-t_1)}{1.699(y_1+y_2)}$$

difference in retention time  
averaging of peak widths

- 10.1.5: R = >0.999, Intercept <0.01 g / 100 ml

Table 2.

Name	R. Time [min]	width (hh)	Resolution
1. cyclohexane	3.307	0.059	
2. 1,2-dimethylbenzene)	4.477	0.097	8.79
3. dibenzothiophene	8.907	0.186	
4. 9-methyl anthracene (r.t. with backflush)	18.905	0.282	

In Figure 1 there is distinct separation between the markers specified in sections 9.4 and 9.6 of the method. Table 2 confirms the minimum resolution requirement of section 9.4 and shows retention time data obtained with the programmed backflush calculated as defined in section 9.6. With this information, it is possible evaluate calibration standards.

An overlay of calibrant solutions A-D is shown in Figure 3. The backflush time was determined from injections of SPS at the beginning of the analysis sequence.

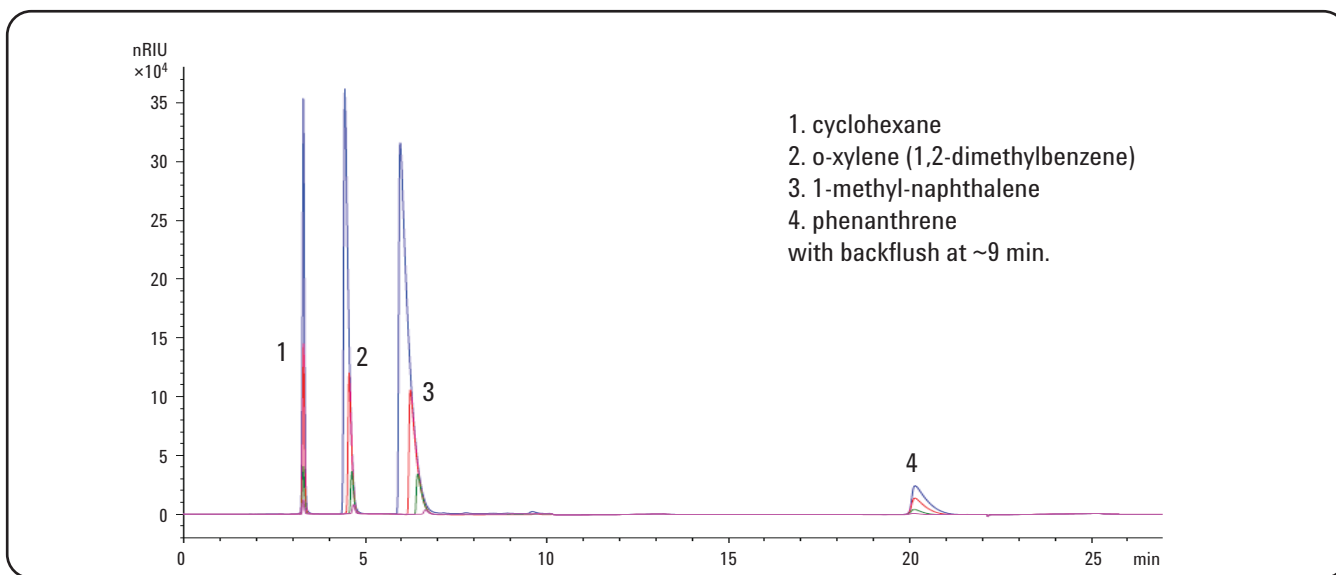


Figure 3. Overlay of calibrant solutions A-D.

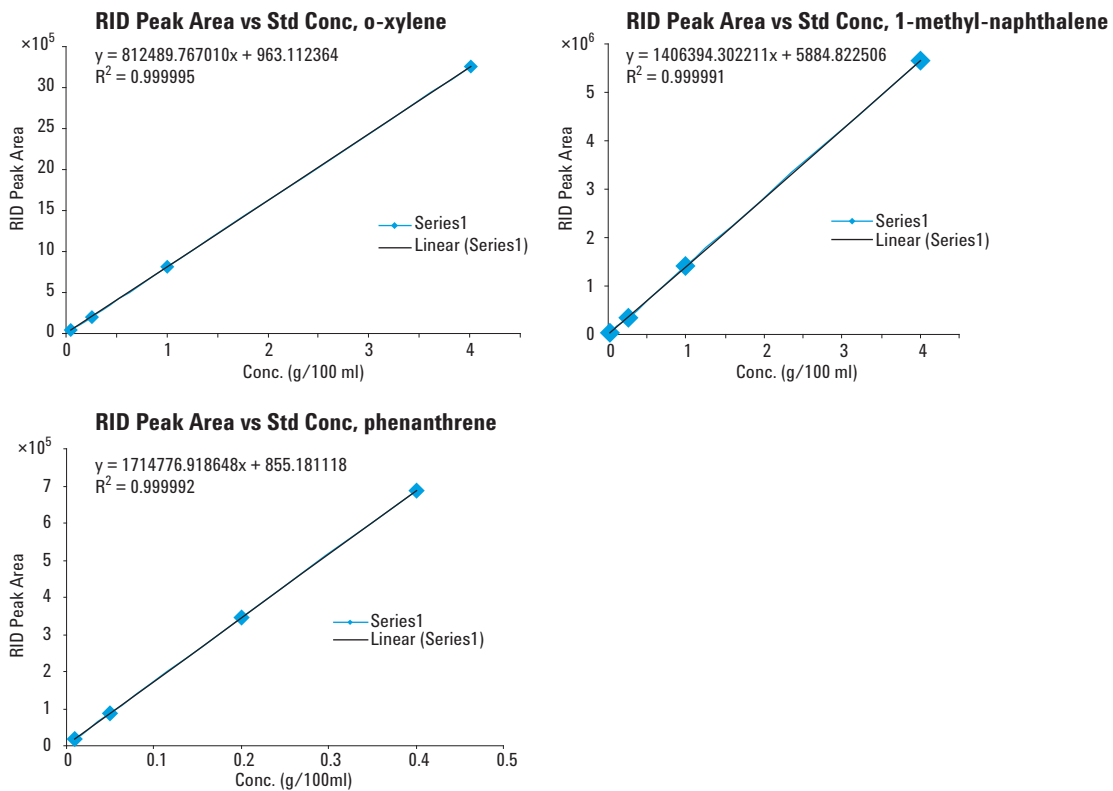


Figure 4. Calibration plots for o-xylene, 1-methyl-naphthalene, and phenanthrene which, are the three components of the four calibration levels specified in the method.

In the calculated results, all calibration plots exceed linearity of 0.9999 and have calculated intercepts well below 0.01 g/100 mL, which are the method specifications of section 10.1.5.

Retention time and peak area precision can be found in Table 3, illustrating that the overall performance of the calibration method is excellent.

Table 3. Calibration Precision

**Calibrant A**

Analyte	RT, Avg, n=3	RT, Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.44	0.0005	0.01%	3.29E+06	755.9	0.02%
1-Methyl-naphthalene	5.96	0.001	0.02%	5.76E+06	2299.2	0.04%
phenanthrene	20.14	0.0020	0.01%	7.12E+05	8351.8	1.17%

**Calibrant B**

Analyte	RT, Avg, n=3	RT Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.55	0.0020	0.05%	8.33E+05	5263.9	0.63%
1-Methyl-naphthalene	6.24	0.0041	0.07%	1.46E+06	14197.7	0.97%
phenanthrene	20.13	0.0023	0.01%	3.55E+05	849.5	0.24%

**Calibrant C**

Analyte	RT, Avg, n=3	RT Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.63	0.0017	0.04%	2.06E+05	536.3	0.26%
1-Methyl-naphthalene	6.44	0.0036	0.06%	3.66E+05	1830.7	0.50%
phenanthrene	20.12	0.0040	0.02%	8.87E+04	139.0	0.16%

**Calibrant D**

Analyte	RT Avg, n=3	RT Stdev	RT, RSD%	Area Avg, n=3	Area Stdev	Area RSD%
xylene	4.67	0.0005	0.01%	4.03E+04	214.7	0.53%
1-Methyl-naphthalene	6.65	0.0020	0.03%	2.96E+04	334.1	1.13%
phenanthrene	20.10	0.0025	0.01%	1.76E+04	176.5	1.00%

**Average RSD% All Runs**

**0.028%**

**0.555%**

**Results for specific petrodiesel and petro/biodiesel blends**

Various samples were collected from local commercial and retail fuel delivery points. An overlay of three samples is shown in Figure 5.

Despite some apparent compositional differences among the samples, the general resolution and valley points are consistent. This should ensure relatively straightforward data reduction.

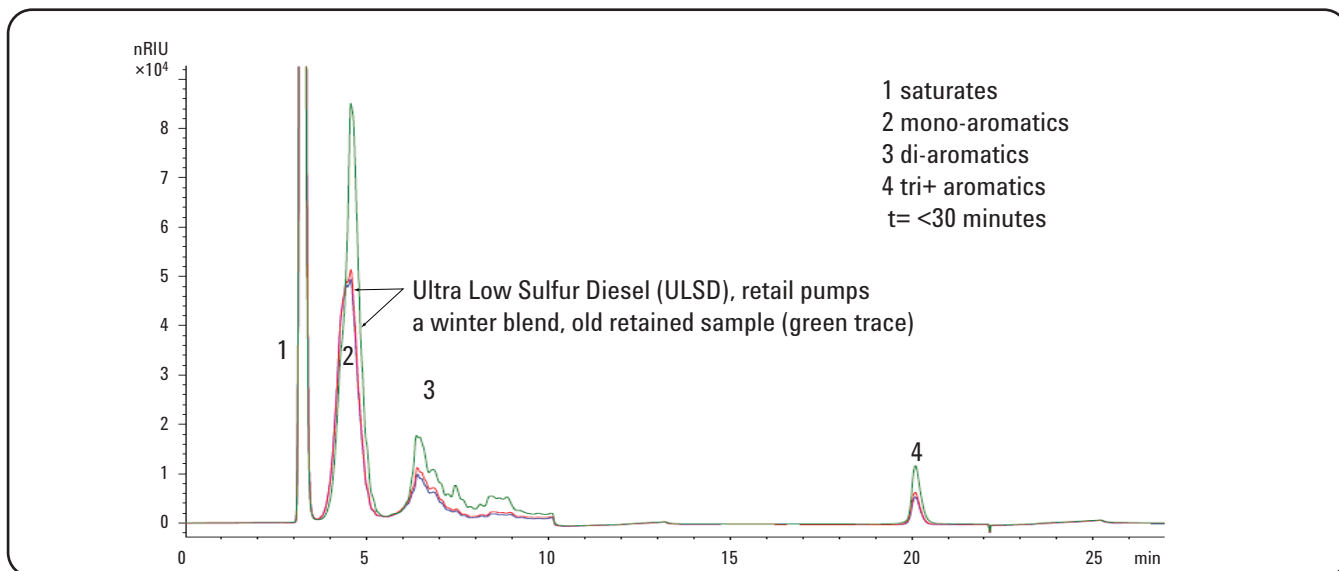


Figure 5. Overlay of three samples.

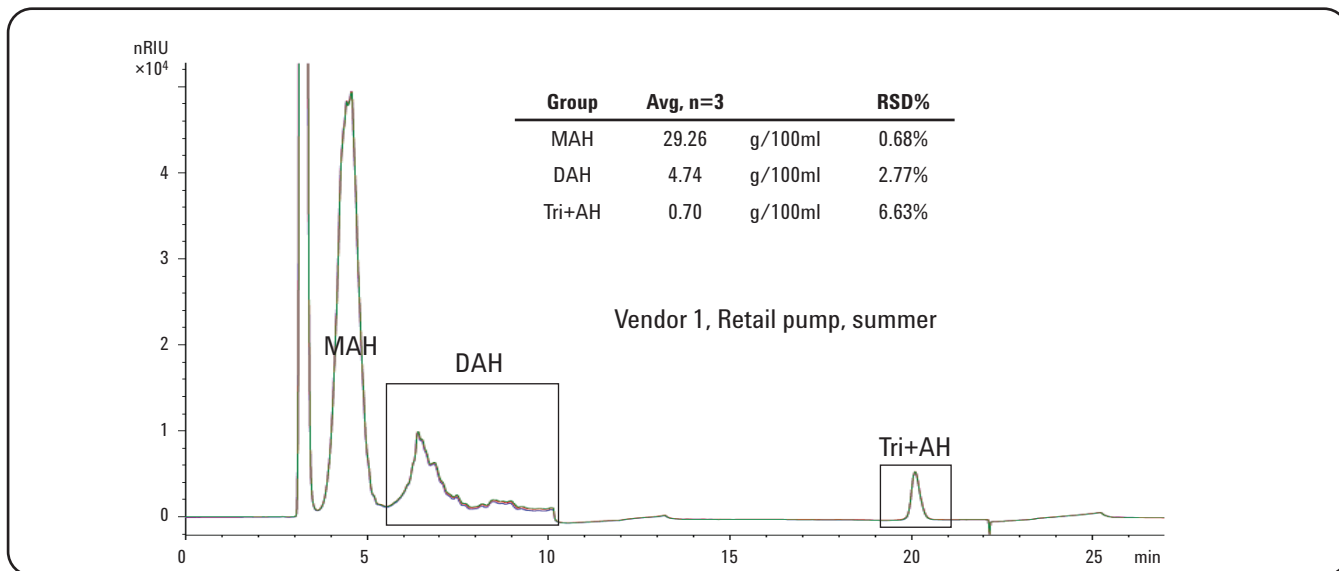


Figure 6. Results and precision for sample designated "Vendor 1".

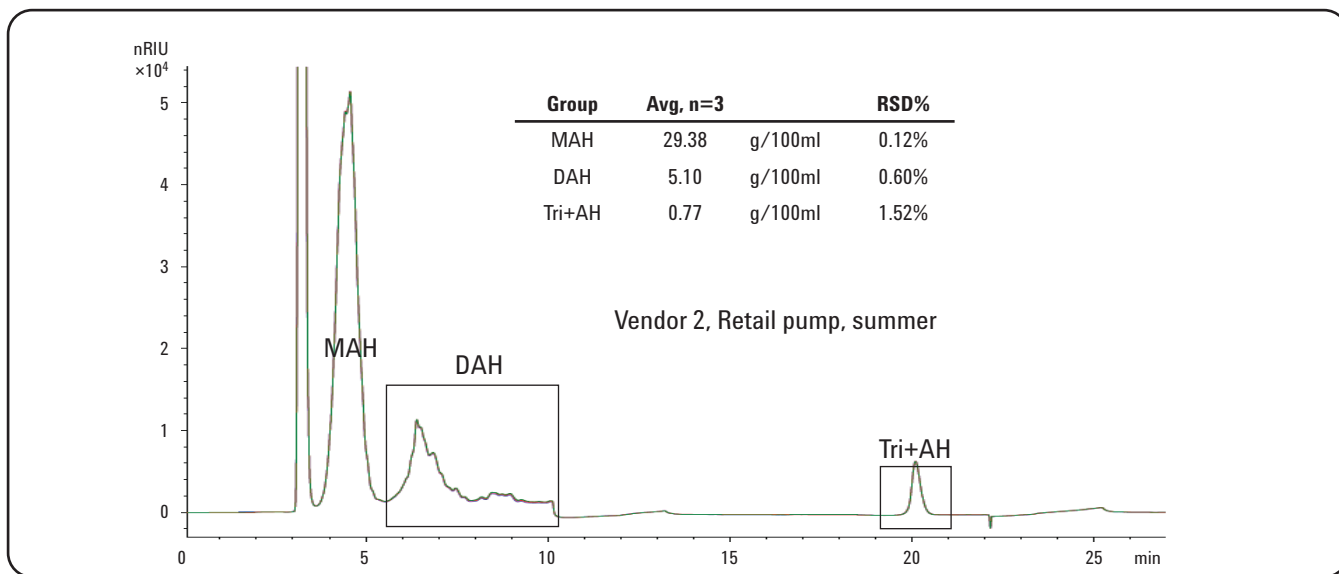


Figure 7. Results and precision for sample designated "Vendor 2".

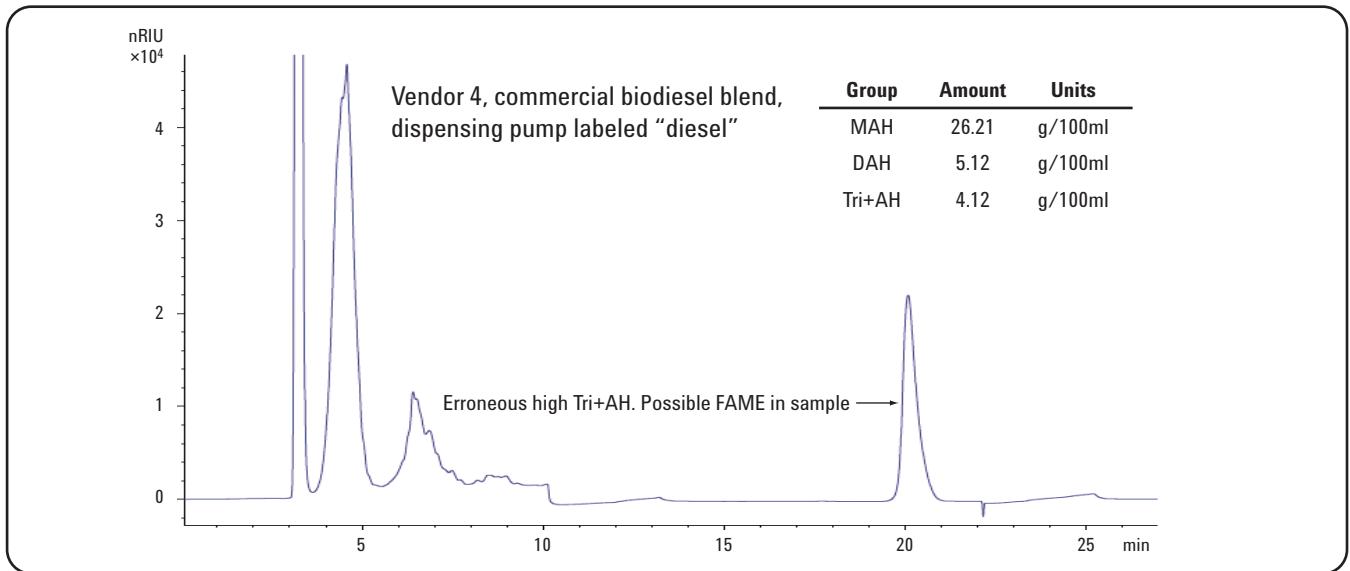


Figure 8. This sample was represented as diesel and was analyzed by the method. Suspiciously high tri+aromatic values compelled an analysis by the alternate, biodiesel approved, method IP391/07.

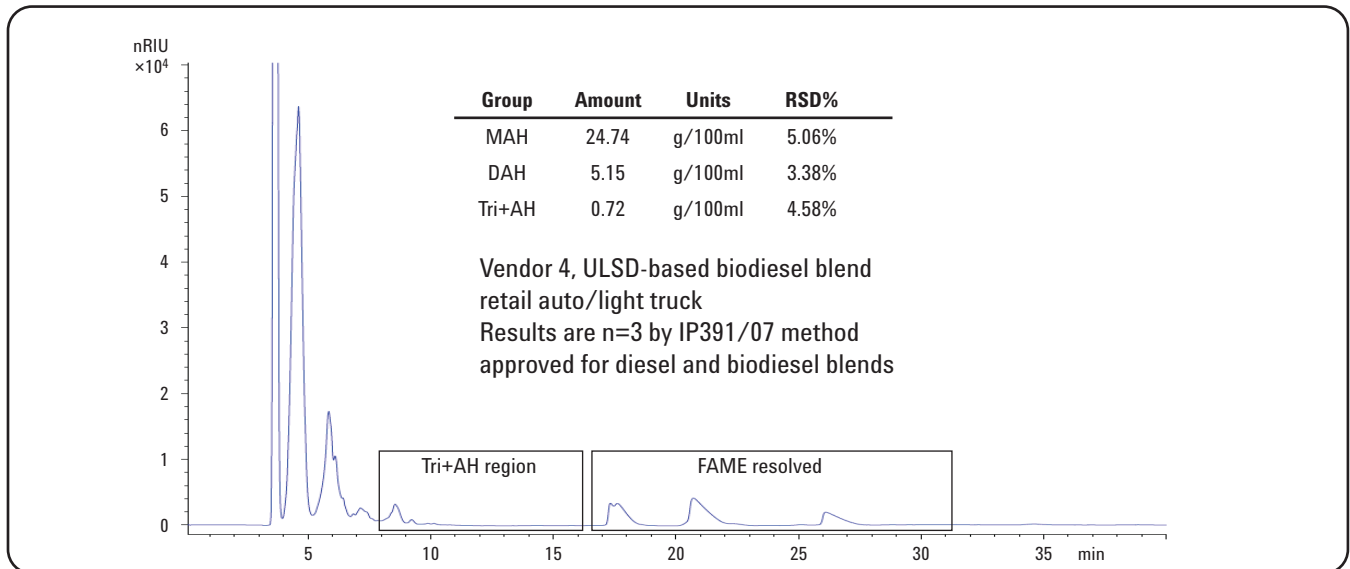


Figure 9. Analysis of suspect biodiesel sample by IP391/07 conditions confirms the contamination or dispensing pump mislabeling and yields a more expected result for typical diesel motor fuel. For further details on the performance and utility of this method, please refer to Agilent application note 5990-4789EN. [3]



## **Ruggedness and Stability of the ASTM D 6591-06 method**

As with most normal phase methods the column is susceptible to adsorption of highly polar components that can affect overall separation performance. Water present in samples or mobile phase also adsorb to the column and somewhat predictably cause reduced elution times for all sample components. Using a high quality anhydrous 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 solvent container, it is also possible and preferable to prepare a high pressure compatible column with prewashed drying agent and placing it inline between the pump and injector.

## **Conclusion**

The performance of the Agilent 1200 Series High Performance LC system with normal phase separation and refractive index detection meets or exceeds the requirements of ASTM D 6591-06 within the range of samples defined in the method. The user should take care to identify samples of petrodiesel containing biodiesel components to ensure adequate analysis modifications are made to prevent erroneous high tri+aromatic values. IP391/07 (EN12916:2006) is required for samples found to contain biodiesel FAME components, and any results showing suspiciously high Tri+aromatics values with ASTM D 6591-06 should be re-analyzed by IP391/07.

## References

1. Angelika Gratzfeld-Huesgen, "Analysis of Aromatic Hydrocarbons in Middle Distillates with HPLC using IP Standard Method 391/95", Agilent Application Note, 5965-9044E, 1997.
2. ASTM D 6591-06 "Standard Test Method for Determination of Aromatic Hydrocarbon Types in Middle Distillates—High Performance Liquid Chromatography Method with Refractive Index Detection".
3. Michael Woodman and Malgorzata Sierocinska, "Meeting the Requirements of EN12916:2006 (IP391/07) Using Agilent 1200 Series HPLC Systems", Agilent Application Note 5990-4789EN, 2009.

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