Column Conditioning Procedure for the IP391/07 Diesel/Biodiesel Aromatics Test Method

White Paper

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Introduction

For the analysis of non-aromatics and aromatics in diesel fuel and petroleum distillates boiling in the range 150 °C to 400 °C, the official IP Method 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. Recent growth in biodiesel production created a demand for analysis of petrodiesel and petrodiesel/biodiesel blends. In revised IP391/07 method, 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 method is far more demanding than the original IP391/95 version using a backflush, and this white paper reports a reliable method of pre-conditioning Agilent ZORBAX columns for consistent performance with the updated method.

Please note that previously used columns from various vendors have proven unpredictable in terms of IP391/07 performance and in their response to the pre-conditioning procedure described here. This conditioning procedure is thus recommended only for new, unused Agilent ZORBAX columns. The result of using this procedure on any other brand of columns has not been tested and no certainty exists regarding the success or failure of the procedure with other columns.

Sample Preparation

The SCS1/SCS2 sample mixture was prepared using Agilent IP391/07 System Calibration Standards 1 and 2, p/n 5190-0485. The sample mixture was prepared in the ratio SCS1/SCS2 1:2 (v/v) by mixing 0.5 mL of SCS1 (p/n 5190-0485-1) with 1 mL of SCS2 (p/n 5190-0485-2).

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Preparation of Columns for Automated Drying Sequence

The SB-CN column 4.6 × 150 mm, 5 µm (p/n 883952-708) is shipped in 50% acetonitrile/50% water and needs to be purged with IPA (~ 20 column volumes) before being connected in series to the NH2 column 4.6 × 150 mm, 5 µm (p/n 883975-905) that is packed in hexane. Once the SB-CN has been purged with IPA, it can be connected in series to the NH2 column. In the series, the NH2 column is positioned first, and connected to both the injector and the SB-CN column. The SB-CN column is positioned second and is connected both to the NH2 column and the refractive index detector (RID). Heptane mobile phase is then run through both columns at 1 mL/min and 25 °C until a fairly stable baseline is achieved with the RID. Once a fairly stable RID baseline is achieved, the following automated drying sequence can be run. The automated drying sequence can be set up to run overnight so that the IP391/07 method can be run the next day. The drying sequence is set up to run the series of three samples listed in Table 1 a total of six times. It is possible that the required separation may be achieved in fewer than six cycles. However, running the full sequence results in no adverse effects to the chrysene/FAME separation.

ChemStation Automated Drying Sequence

Fill the vials as follows:

1. Fill one 2-mL vial with heptane for the Blank Heptane sample.
2. Fill one 2-mL vial with the SCS1/SCS2 standard in a ratio of 1:2 (v/v) for the SCS1/2 sample.
3. Fill three 2-mL vials with at least 1.5 mL of the Silica Column Regeneration Solution (CRS) (Sigma-Aldrich p/n 33175, mfr. Supelco)

Table 1. The Drying Sequence is Basically Made Up of the Repetition of the Following Series of Samples

Line	Location	Sample name	Method name	Inj/vial
1	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
2	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
3	Vial 3	CRS	HEPT_10MIN_100UL_25C_COL1	5

Table 2 . ChemStation Drying Sequence

Line	Location	Sample name	Method name	Inj/vial
1	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
2	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
3	Vial 3	CRS	HEPT_10MIN_100UL_25C_COL1	5
4	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
5	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
6	Vial 3	CRS	HEPT_10MIN_100UL_25C_COL1	5
7	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
8	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
9	Vial 4	CRS	HEPT_10MIN_100UL_25C_COL1	5
10	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
11	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
12	Vial 4	CRS	HEPT_10MIN_100UL_25C_COL1	5
13	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
14	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
15	Vial 5	CRS	HEPT_10MIN_100UL_25C_COL1	5
16	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	2
17	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	1
18	Vial 5	CRS	HEPT_10MIN_100UL_25C_COL1	5
19	Vial 1	Blank Heptane	HEPT_40MIN_10UL_25C_COL1	5
20	Vial 2	SCS1/2	HEPT_40MIN_10UL_25C_COL1	5
21	Vial 1	Blank Heptane	MIN_LOWFLOW_25C_COL1	1

Experimental Conditions for Methods used in the Automated Drying Sequence

Method HEPT_40MIN_10UL_25C_COL1

Column flow	1.0 mL/min
Mobile phase	100% HPLC grade heptane
Run/stop time	40 min
Injection volume	10 µL
Column oven temp	25 °C
Refractive index detector temp	30 °C
Optional UV detector λ	250 nm (for chrysene detection)

Method HEPT_10MIN_100UL_25C_COL1

Column flow	1.0 mL/min
Mobile phase	100% HPLC grade heptane
Run/stop time	10 min
Injection volume	100 µL
Column oven temp	25 °C
Refractive index detector temp	30 °C
UV detector λ	250 nm (for chrysene detection)

Method MIN_LOWFLOW_25C_COL1

Column flow	0.100 mL/min
Mobile phase	100% HPLC grade heptane
Run/stop time	Can be set to any time
Injection volume	1 µL
Column oven temp	25 °C
Refractive index detector temp	30 °C
UV detector λ	250 nm (for chrysene detection)

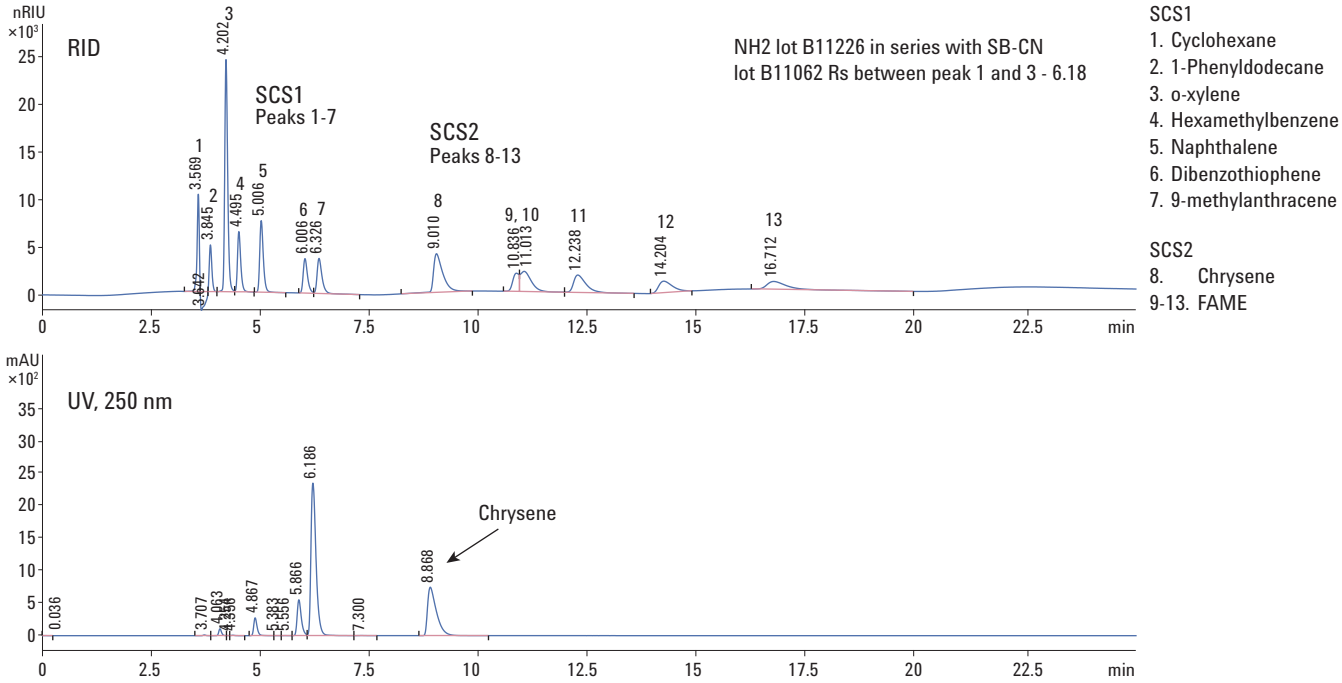

Figure 1. Separation of chrysene from FAME after drying sequence. Required resolution between peaks 1 and 3 is achieved. Use of UV detection to identify chrysene.

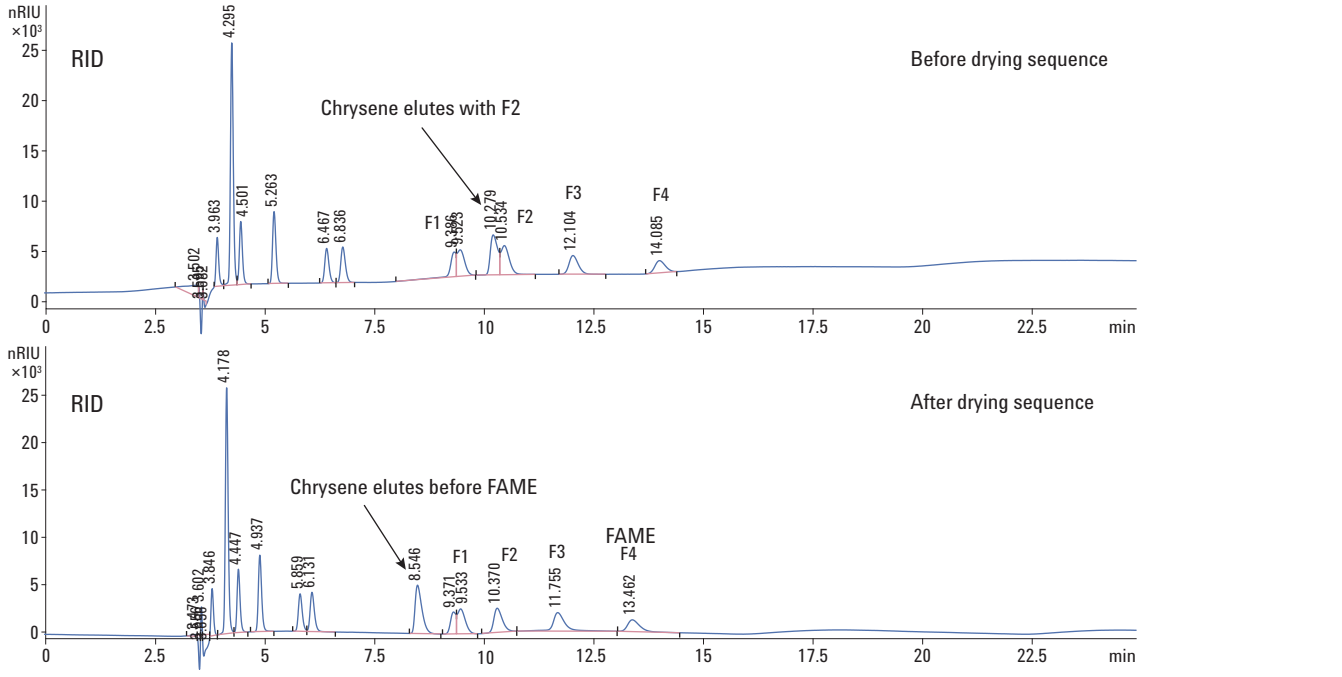

Figure 2. Effect of drying sequence on chrysene/FAME separation using Agilent ZORBAX NH2 lot B10285 USU0005655 in series with SB-CN lot B11062 USSJ015730.

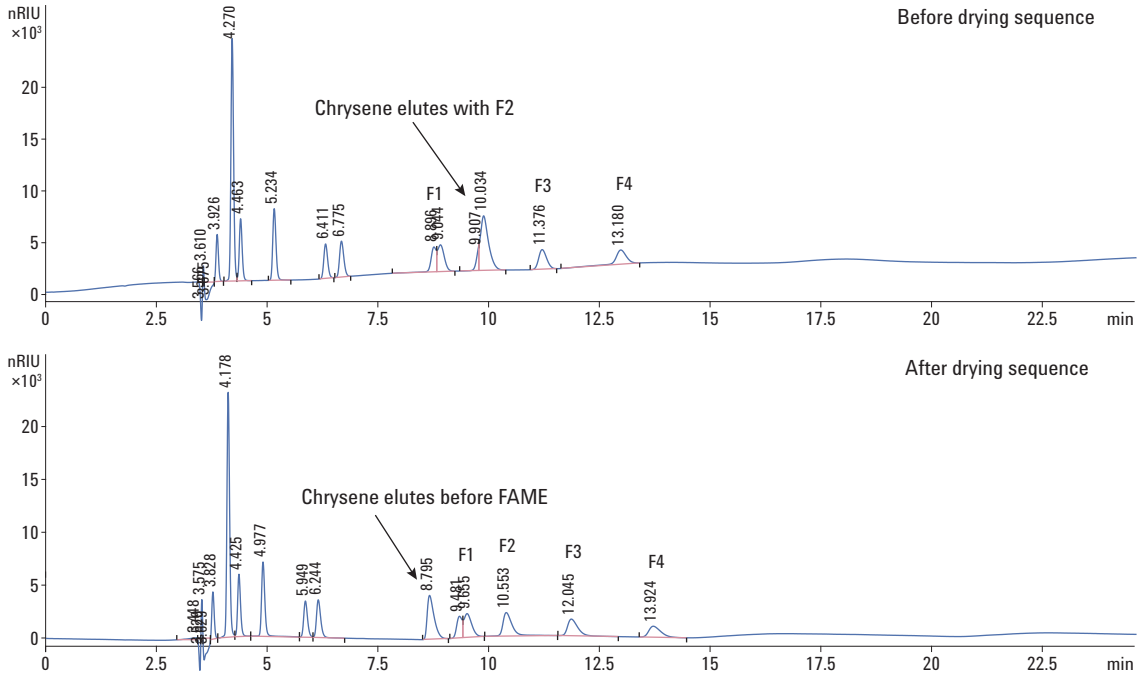

Figure 3. Effect of drying sequence on chrysene/FAME separation using Agilent ZORBAX NH2 lot B11069 USU0005657 in series with SB-CN lot B11062 USSJ015714.

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