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Rapid and Simultaneous Determination of Fatty Acid Methyl Esters and Polycyclic Aromatic Hydrocarbons in Diesel Fuel by High-Performance Liquid Chromatography with a Selective Backflush Strategy

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ABSTRACT

The contents and quantitative methods of fatty acid methyl esters (FAMEs) and polycyclic aromatic hydrocarbons (PAHs) in diesel fuel are strictly regulated for process control and product development. However, extensive manual operations or lengthy processes are required for the existing detection methods. In this study, a high-performance liquid chromatography method with a "selective back-flush" strategy was developed for the rapid and simultaneous determination of FAME and PAHs in diesel fuel in one measurement. Compared with the prevailing methods, the proposed method reduced the total measurement time to less than 10 min with satisfactory detection performance, which greatly improved the detection efficiency and saved costs of labor, materials, and time. The feasibility and superiority of this method were demonstrated and the method exhibited great potential for reliable and high-throughput quality control of diesel fuel samples. Furthermore, the selected backflush strategy should expand the realm for developing new chromatographic methods as well as the breadth of applications.

Introduction

Biodiesel, derived from animal fats, vegetable oils, or microbial oils, is one of the most attractive renewable forms of energy and generally obtained by triglyceride transesterification reactions with methanol to produce fatty acid methyl esters (FAMEs). FAMEs have been widely accepted as an alternative diesel fuel for its advantages of renewability, biodegradability, high flash point, reduced hydrocarbon emission, and miscibility with petrodiesel (Vyas, Verma, and Subrahmanyam 2010; Pardo et al. 2012). The maximum permitted amounts of FAMEs for diesel fuels in the USA and the European countries are prescribed according to diesel standards (EN 590:2013/AC 2017; ASTM D975 2019). Therefore, the quantitative determination of FAMEs is of significance for quality control and market surveillance of diesel fuel products.

For FAME detection in diesel fuels, mid-infrared Fourier transform spectroscopy (FTIR) is involved in one of the standard methods (EN 14078 2014). In this method, the FAME content is obtained based on the relative intensity of the carbonyl absorption band, so that the result might be interfered with by matrix effects. Another standard method, based on solid-phase extraction (SPE) with gas chromatography (GC), is also used to determine the FAME content in diesel fuels (ISO 12966 2015). However, extensive manual operations are needed for both the methods.

As a research area of intense interest, FAME determination in petroleum products has been carried out utilizing gas chromatography-mass spectrometry (GC-MS) (Pardo et al. 2012), electrospray ionization mass spectrometry (ESI-MS) (Eide, Neverdal, and Westad 2010), supercritical fluid chromatography-flame ionization detection (SFC-FID) (Diehl and Disanzo 2007), GC-FID (Sobrado et al. 2016), two-dimensional gas chromatography (2 D-GC) (Lissitsyna et al. 2012), high performance liquid chromatography (HPLC) (Kamiński et al. 2006; Jerman et al. 2009), and dye-doped optical sensing (Fong and Xue 2013). Among these detection methods, HPLC stands out for its ease of operation.

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals that consist of two or more aromatic rings (Ahmed et al. 2015). PAHs generally exist in petroleum products and are hazardous to both the environment and human beings. Some PAHs may interact with DNA and cause genetic damage and subsequent malignancies (Wang et al. 2013). As a result, the PAH content in diesel fuels is strictly limited by many countries and regions.

The allowable PAH content in diesel fuels is < 7% for European countries and differs from state to state in the USA (EN 590:2013/AC 2017; ASTM D975 2019). At present, PAH determination in diesel fuels is generally based on chromatographic techniques including HPLC (EN 12916 2016; ASTM D6591 2019) and GC-MS (ASTM D2425 2019). Compared with method ASTM D2425, HPLC methods are more commonly used due to the lower costs in human resources and time. To date, the standard methods for PAH detection have not been applied to other components. As common constituents of diesel fuels, PAH and FAME contents are both regulated by different standards. Therefore, establishing an HPLC method for the simultaneous determination of FAME and PAHs in diesel fuels in one measurement would substantially simplify the detection procedure and improve efficiency.

Kamiński et al. (2006) have developed a method for the simultaneous determination of aromatic hydrocarbons and FAME by HPLC with column backflush. The method design is straightforward, but 30–40 min is needed for FAME detection. Jerman et al. (2009) have qualitatively evaluated the determination of aromatic hydrocarbon and FAME contents in one run using HPLC without backflush. However, in that case, the FAME component, consisting of various FAME species, may elute as more than one peak and a detection time of at least 30–40 min is also needed for this method. The prolonged time for elution results in low detection efficiency and poor component peak patterns, such that an improved HPLC method is urgently needed.

Therefore, based on the commonly used backflush technique, a "selective backflush" strategy was developed in this study for the rapid and simultaneous HPLC determination of complex components. With the proposed strategy, a specific component was

selectively retained in an HPLC column and backflushed at a proper time, modulating the detection procedure as needed. Here the simultaneous detection of FAME and aromatic hydrocarbons in diesel fuels was completed as a model and method. The time required for one measurement was greatly reduced to less than 10 min with good detection performance and low costs, which demonstrated the feasibility and superiority of this strategy. With the elaborated design, selected backflush strategy can be applied to other analytes and more practical HPLC applications will be realized.

Experimental

Apparatus

Chromatographic analyses were performed on a high-performance liquid chromatography (1260 series, Agilent Technologies, USA) equipped with a refractive index detector (RID) and a six-port two-channel backflush valve to switch the flow direction of the mobile phase. The separation was carried out with two Spherisorb NH_2 columns (Column 1: 30 mm × 4 mm i.d.; Column 2: 250 mm × 4 mm i.d.; Particle size: 5 μm; Waters, USA) at 35 °C. The flow rate of the mobile phase (*n*-heptane) was 1.0 mL/min and the injection volume at 10 μL.

Materials

n-Heptane of HPLC grade was used as the mobile phase. *o*-Xylene (≥ 99.0%), fluorene (≥ 98.0%), and phenanthrene (≥ 99.0%) were used as the calibration standards for mono-aromatic hydrocarbons (MAHs), di-aromatic hydrocarbons (DAHs), and tri+-aromatic hydrocarbons (T + AHs). BD100 (Sinopec Research Institute of Petroleum Processing, RIPP, China) was pure soybean-based biodiesel that met the requirements of standard GB/T 20828 and used here as the FAME calibration standard.

A system calibration standard (SCS), composed of a solution containing cyclohexane (≥ 99.0%), 1-phenyldodecane (≥ 99.0%), *o*-xylene (≥ 99.0%), hexamethylbenzene (≥ 99.0%), naphthalene (≥ 99.0%), dibenzothiophene (≥ 98.0%), and 9-methylanthracene (≥ 98.0%), was used as the SCS to aid chromatographic peak identification.

The solvents, calibration standards of aromatic hydrocarbons, SCSs, and chrysene (neat) were all purchased from Sigma-Aldrich (USA). Original diesel fuels were obtained from Sinopec Group (China). Biodiesel from different sources (cooking oil, rapeseed, cottonseed, and acidic oil) was from the Sinopec RIPP.

Samples

Calibration solutions containing o-xylene (0.50–40 mg/mL), fluorene (0.20–20 mg/mL), and phenanthrene (0.10–40 mg/mL) were prepared in *n*-heptane. BD100 was added to each calibration solution at concentrations ranging from 0.10 to 30 mg/mL.

An SCS solution containing cyclohexane (1.0 g ± 0.1 g), 1-phenyldodecane (0.1 g ± 0.01 g), o-xylene (0.5 g ± 0.05 g), hexamethylbenzene (0.1 g ± 0.01 g), naphthalene (0.1 g ± 0.01 g), dibenzothiophene (0.05 g ± 0.005 g), and 9-methylanthracene (0.05 g ± 0.005 g) was diluted with *n*-heptane to 100 mL.

A sample solution containing FAME (5.8 mg/mL) and chrysene (0.3 mg/mL) was pre-pared using *n*-heptane as the solvent.

Solutions of diesel fuel samples A–D were prepared by diluting the original diesel fuels to a concentration of 100 mg/mL with *n*-heptane.

Results and discussion

### *Procedure for the selective backflush*

A schematic illustration of the HPLC system with selected backflush strategy is shown in Figure 1. Two HPLC columns, named Column 1 and 2, were connected in series and a six-port two-channel valve used to control the mobile phase flow direction. At State I, the sample was injected into the system and flowed with the mobile phase through Column 1 and then Column 2. During the procedure, the sample components were sep-arated based on their differential polarities.

The aliphatic hydrocarbons moved to the front, followed by MAHs, PAHs, and then FAME. After the last PAHs group in a sample was introduced to Column 2, the back-flush valve was immediately switched to State II. At this time, FAME was retained in Column 1, with the flow direction of the mobile phase of Column 1 reversed while that of Column 2 stayed the same, such that the connection sequence of the columns and RID was changed. Thus, the components in Column 1 were backflushed to the detector, and the components in Column 2 subsequently eluted in the original order, which was called a "selective backflush strategy."

With this approach, FAME in Column 1 was selectively backflushed before other sample components in Column 2. Afterward, aliphatic hydrocarbons, MAHs, and PAHs sequentially eluted from Column 2 (Figure 2). The identification of aromatic
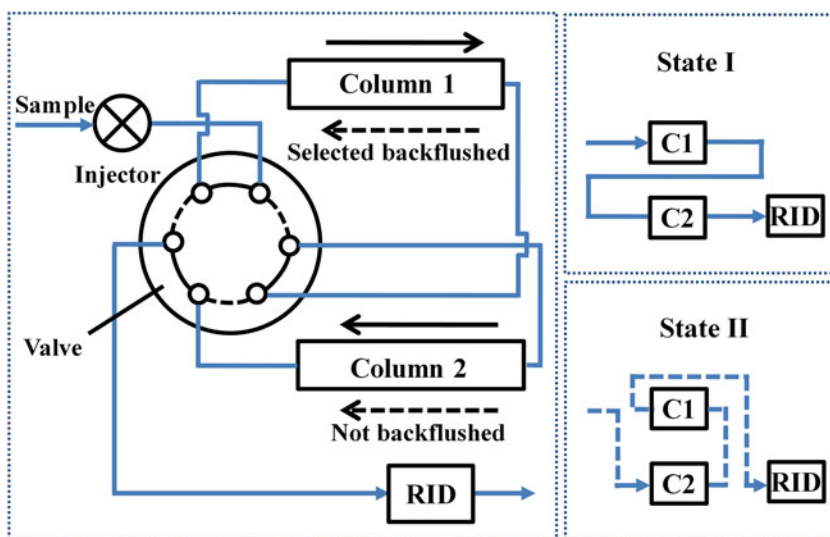
Figure 1. Schematic illustration of the HPLC system with selective backflush for the determination of diesel fuel class composition. Diagrams in the right boxes show the connection sequence of columns 1 and 2 (C1 and C2, respectively) and the refractive index detector (RID). State I: flow path of mobile phase at the initial stage. State II: flow path of mobile phase after the selective backflush.
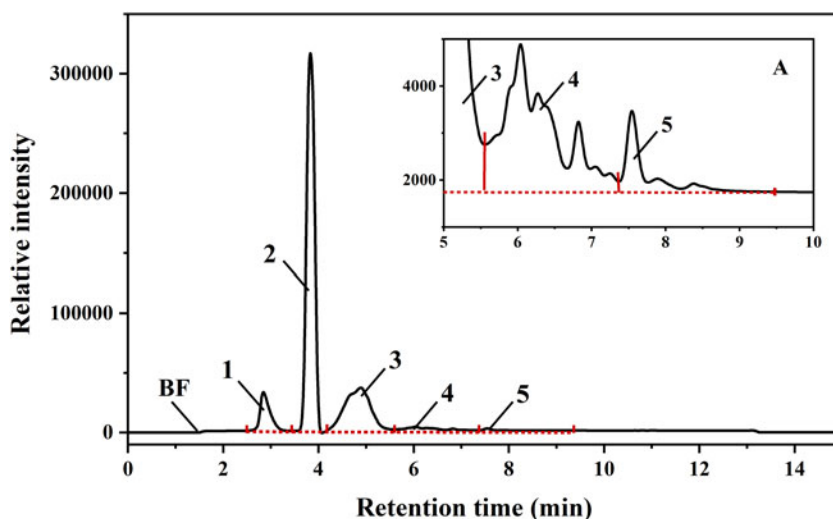
Figure 2. Typical chromatograms obtained with a refractive index detector to determine the class composition of diesel fuel samples containing fatty acid methyl esters. BF was the time for selected backflush and peaks included (1) fatty acid methyl esters, (2) aliphatic hydrocarbons + olefins, (3) mono-aromatic hydrocarbons, (4) di-aromatic hydrocarbons, and (5) tri+-aromatic hydrocarbons. The dotted line in the figure was the baseline for peak integration. Insert A shows details of peaks 4 and 5 with an enlarged scale.

hydrocarbons and the assignment of the peaks were accomplished with the help of the SCS solution (EN 12916 2016).

As shown above, the configuration of the HPLC system, including the types and connection orders of the columns, backflush valve settings, and flow rate of the mobile phase, was well designed to modulate the component separation process. It should be pointed out that the presence of acylglycerols and fatty acids may cause asymmetry or tailing of FAME peaks, thus influencing FAME and PAH determinations. In that case, the HPLC system needs to be modified accordingly. Compared with the frequently used backflush strategy or strategies without column backflush (Kamiński et al. 2006; Jerman et al. 2009), the proposed backflush strategy selectively brought forward the FAME elution time and made good use of the dead time of the HPLC system. This method not only improved detection efficiency but also enhanced the accuracy and precision of FAME quantitation by avoiding peak broadening.

Optimization of the selective backflush time

The backflush time, which meant the switching time of the backflush valve in the experimental process, greatly influenced the elution order and the component quantitative accuracy. The appropriate backflush time varied with different column parameters or flow rates, and, here, the principle of the determination process for particular experimental conditions was exhibited. A mixed solution of FAME and chrysene, which was considered to be the last PAH component in diesel fuels, was used for backflush time optimization. The peak areas of the two components were measured in each experiment with the backflush time ranging from 1.2 to 2.0 min (Figure 3).
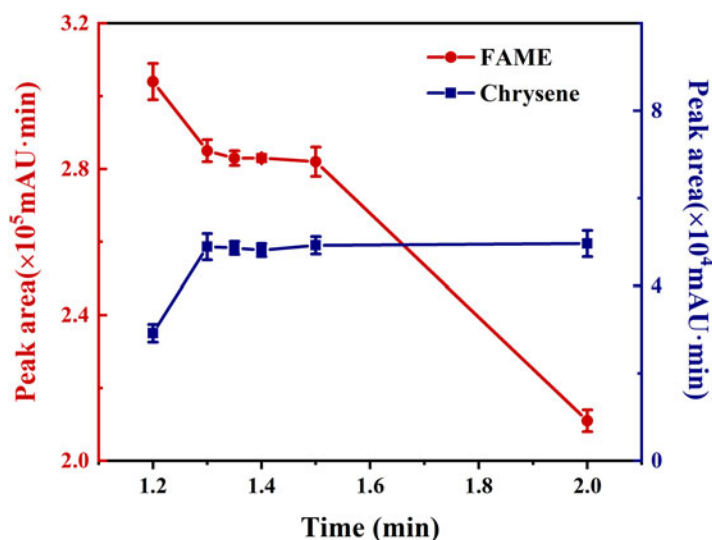
Figure 3. Peak areas of fatty acid methyl esters (FAME) and chrysene with various backflush times. A sample solution containing FAME (5.8 mg/mL) and chrysene (0.3 mg/mL) was used to optimize the backflush time with each result the average of triplicate measurements.

Backflush times shorter than 1.3 min were found to cause the retention of chrysene in Column 1 and the coelution of PAH residues and FAME in the backflush procedure, resulting in a higher FAME and lower chrysene measurements than the actual values. On the other hand, when the backflush time was extended to 2.0 min, the FAME content sharply decreased while that of chrysene remained constant. The reason for this result may be because a portion of the FAME component eluted from Column 1 and flowed into Column 2 with the longer backflush time but did not coelute with chrysene due to its stronger retention in Column 2. Above all, backflush times outside of the range from 1.3 to 1.5 min may cause quantitative errors of FAME or PAHs. Thus, a reasonable backflush time of 1.4 min was used in subsequent experiments.

Quantitative methods for aromatic hydrocarbons and FAME

The accurate detection of sample components is the essential requirement of a good method. Therefore, quantitative methods for FAME, MAH, and PAH detection were first accomplished based on calibration curves. After the injection of the calibration solutions, the components including FAME, *o*-xylene, fluorene, and phenanthrene eluted sequentially. The peak areas of FAME and each group of aromatic hydrocarbons were recorded in triplicate, and the relationships between component concentrations and peak areas were evaluated by linear regression.

The slopes, intercept, and linear correlation coefficients of the calibration curves are presented in Table 1. In addition, the limit of detection (LOD) and limit of quantification (LOQ) were obtained based on the signal-to-noise ratio, according to the International Union of Pure and Applied Chemistry (IUPAC) method. The calibration data showed superior linearity and sensitivity for both FAME and aromatic hydrocarbons, which ensured the feasibility of the proposed method for sample detection.

Table 1. Calibration for the simultaneous determination of aromatic hydrocarbons and fatty acid methyl esters.

Group of compounds	Linear range (mg/mL)	Slope ($\times10^5$)	Intercept	Correlation coefficient	Limit of quantification (mg/mL)	Limit of detection (mg/mL)
Mono-aromatic hydrocarbons	0.50–40	0.81	1572	0.9999	0.014	0.004
Di-aromatic hydrocarbons	0.20–20	1.54	−7014	0.9999	0.016	0.005
Tri+-aromatic hydrocarbons	0.10–40	1.80	−1473	0.9999	0.073	0.022
Fatty acid methyl esters	0.10–30	0.49	376.7	0.9999	0.027	0.008

Validation for PAH determination

For PAH detection, the frequently-used standard methods (EN 12916 2016; ASTM D2425 2019) are built based on HPLC and GC-MS, separately. Therefore, to assess the results of PAH detection, a comparison between the proposed method and standard methods was performed with four diesel fuel samples.

In this study, the aromatic hydrocarbon contents in the diesel fuel sample solutions (Solutions A–D) were determined using two standard methods and the proposed method (Figure 4). The original diesel fuel samples used in this experiment were all considered free of FAME as their FAME contents were measured to be less than 0.1% (v/v).

The proposed method in this study was an innovative development on the basis of method EN 12916. Thus, the results of the two HPLC methods showed high consistency, as anticipated. Because of the disparate detection principle of GC-MS, results with small distinctions and similar trends were found compared to method ASTM D2425. In general, the results of the three methods were in accordance, demonstrating the reliability of the proposed method for PAH detection.

Analytical performance for FAME and PAH determination

This study was focused on the rapid determination of FAME and PAHs contents in diesel fuel samples in one measurement and with superior analytical performance. As stated previously, satisfactory results were obtained for PAH detection in samples free of FAME. To further evaluate the influence of FAME on PAH detection and the detection performance for FAME in diesel fuel samples, varied contents of FAME (0.10–31.2 mg/mL) were added into solutions of diesel fuel samples (Solution D). Then the solutions were analyzed using the proposed method (Table 2).

The present results showed that FAME addition to samples had no noticeable effect on aromatic hydrocarbon quantification. The obtained consistent content determinations demonstrated the applicability of PAH detection in the presence of FAME. For FAME detection, recoveries were in the range from 98.6 to 101%, ensuring the method's accuracy. The repeatability was evaluated by measuring the sample solution containing FAME (0.1 mg/mL) in seven replicates using the proposed method, and the results showed high repeatability with a standard deviation of 2.4%.
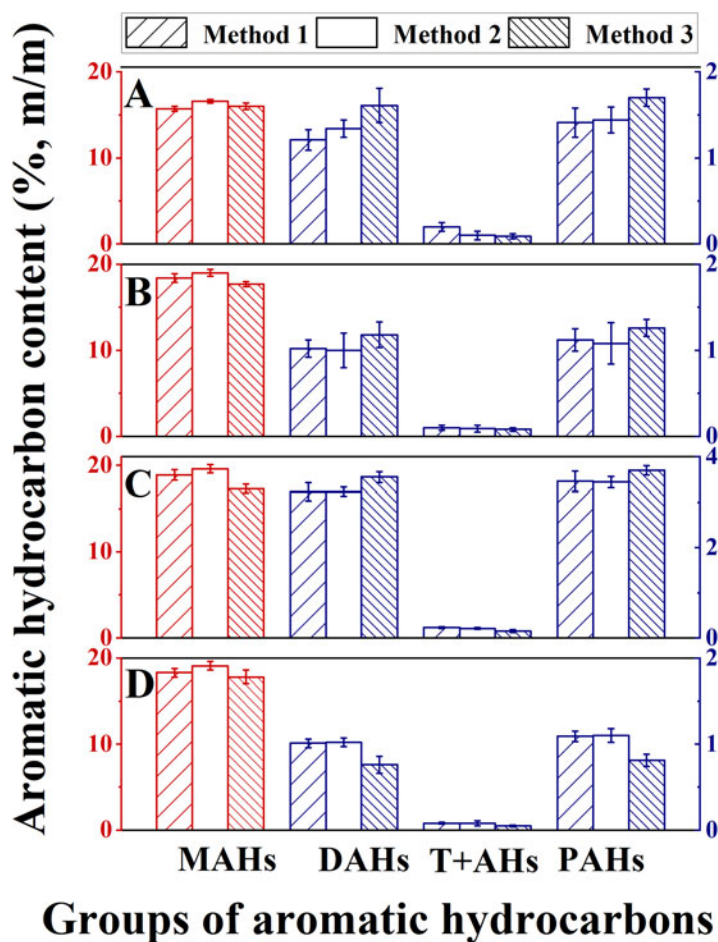
Figure 4. Comparison of aromatic hydrocarbon contents between Method 1 (proposed method with selected backflush strategy), Method 2 (EN 12916:2016), and Method 3 (ASTM D2425:2019). MAHs, mono-aromatic hydrocarbons; DAHs, di-aromatic hydrocarbons; T + AHs, tri+-aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons. Each result was the average of triplicate measurements.

Table 2. Determined contents of aromatic hydrocarbons in diesel fuel samples with various additions of fatty acid methyl esters and their recoveries.

Fatty acid methyl esters		Aromatic hydrocarbon content			
Added (mg/mL)	Recovery (%)	Mono-cyclic (%, m/m)	Di-cyclic (%, m/m)	Tri+-cyclic (%, m/m)	Polycyclic (%, m/m)
0	–	18.3 ± 0.2	1.01 ± 0.03	0.081 ± 0.001	1.09 ± 0.03
0.100	102 ± 2.0	18.2 ± 0.3	0.93 ± 0.01	0.094 ± 0.002	1.02 ± 0.01
0.383	101 ± 2.4	18.3 ± 0.4	0.99 ± 0.02	0.080 ± 0.002	1.07 ± 0.02
2.19	101 ± 1.8	18.4 ± 0.3	0.97 ± 0.01	0.102 ± 0.004	1.07 ± 0.01
9.55	99.0 ± 0.3	18.0 ± 0.1	0.94 ± 0.01	0.144 ± 0.003	1.08 ± 0.01
31.2	99.4 ± 0.9	18.1 ± 0.2	0.95 ± 0.03	0.103 ± 0.001	1.05 ± 0.03

To demonstrate the universality of the method, the recoveries of biodiesel from other common sources, including cooking oil, rapeseed, cottonseed, and acidic oil, were also determined in the same manner (Table 3). Using the calibration curve for BD100 (soybean biodiesel), high recoveries from 96.1 to 104% were obtained for the tested

Table 3. Recoveries of biodiesel from different sources.

Source of biodiesel	Added (mg/mL)	Determined (mg/mL)	Recovery (%)
Soybeans	9.55	9.45 ± 0.03	99.0 ±.0.3
Cooking oil	10.08	9.91 ± 90.08	98.3 ±.3.8
Rapeseed	12.83	13.40 ± 0.03	104 ± 4.2
Cottonseed	11.18	11.42 ± 0.03	102 ± 2.3
Acidic oil	9.94	9.59 ± 0.07	96.1 ±.1.7

biodiesel. Nevertheless, it is recommended that the biodiesel from unusual sources be quantified using corresponding calibration data to avoid possible inaccuracy.

Taken together, the analytical performance of the proposed method fully met the requirements for the simultaneous determination of PAHs and FAME in diesel fuels. In addition, the method offered the advantage of improved detection efficiency and the potential for development into an alternative standard method for product quality control.

Conclusions

This work presented a method for the simultaneous determination of FAME and PAHs in diesel fuels using a selected backflush strategy. Compared with the prevailing backflush method, the proposed method decreased the detection time from 30 to 40 min to less than 10 min without sacrificing detection performance. As HPLC is already a standard laboratory apparatus for aromatic hydrocarbon detections, the satisfactory results from this method indicated that this method could dramatically save costs in time, labor, materials, and instrumentation. Furthermore, the proposed backflush strategy and the detection procedure could be modulated as needed, which would facilitate further chromatographic applications and future method developments.

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