

Determination of dyes and marker in diesel using high performance liquid chromatography

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A fast, precise and accurate high performance liquid chromatography method has been developed for the determination of dyes (Solvent Red 19 and Solvent Blue 35) and a marker (Solvent Yellow 124) in diesel. Separation was carried out on a 250 × 4.60 mm Agilent Zorbax Rx-SIL column (5 µm particle size). Detection was done in a visible wavelength range. The best performance of fuel dye separation and the shortest retention times were achieved when using hexane, toluene and ethyl acetate as a mobile phase. During this research the eluent composition and the elution gradient were optimized consequently that helped to perform the analysis within 15 min. The developed method was applied for the analysis of real samples of dyed diesel fuel. Preparation of the samples for the analysis simply consisted of filtering through a 0.45 µm filter previous to direct injection of the sample into the HPLC system for analysis.

Keywords: HPLC, fuel, dyes, diesel, marker, Solvent Red 19, Solvent Blue 35, Solvent Yellow 124

INTRODUCTION

Fuel dyes provide colour to fuel, and could be identified visually. Fuel dyes can also be used as some fuel markers, they must be of different colour and exact concentration that can be determined by analytical methods. Fuel dyes are used to differentiate fuel with differently applied taxes. It is important that dyed fuel stands out by its colour from the fuel which is not dyed and does not have lower taxes [1].

Dyes are added to fuel which is used for heating in agriculture, aquaculture and commercial fishing in inland waters.

Also, without dyes there are fuel markers which are used to mark the fuel. Fuel markers are the same materials as fuel dyes can change fuel colour, but must be in an exact concentration and could be determined with analytical methods. Fuel markers are used to track the origin of the fuel.

According to the Order on Fuels of the Ministry of Energy of Lithuania, in Lithuania there are two types of fuel dyes: Solvent Red 19 (SR 19) and Solvent Blue 35 (SB 35), and one marker: Solvent Yellow 124 (SY 124) [1]. Furthermore, there are fixed concentrations of the dyes and the marker used in fuels with lower taxes. Fuel dyeing and marking must be carried out using a specific methodology [1, 6]. However, an exact chemical analysis method which allows the determination of

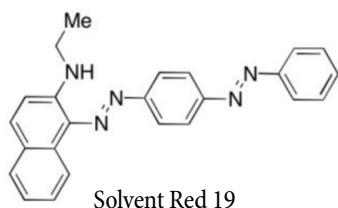
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various fuel dyes, markers and, also, their concentration effectively in one run is not yet available [2–10]. The difference and variety of analytical methods suitable for the determination of fuel dyes and markers have appeared for all EU countries using different combinations of dyes and markers. There are suggestions for electrochemical [4, 5], spectrophotometric [6, 7], gas chromatographic [8, 9] and HPLC [10] analytical methods to determine chosen fuel dyes or markers. But the variety of fuel dyes and markers set up the problem if country has not adopted a method for their own dyes and markers because there is no suitable analytical method to determine all fuel dyes or markers in one run.

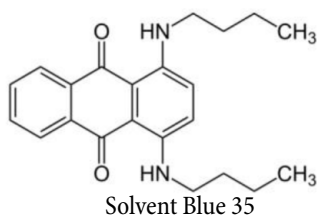
The aim of this study was to develop, optimize and adopt a HPLC method which would allow sensitive detection and accurate determination of the concentrations of the dyes and the marker in fuels (SR 19 and SB 35; and the marker: SY 124) using single injection.

Structural formulas of fuel dyes and marker

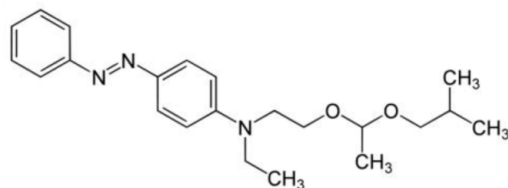
1. Solvent Red 19 (N-ethyl-1-(phenyldiazophenylazo)-2-aminonaphthalen):



2. Solvent Blue 35 (1,4-bis (butylamino)-9,10 anthraquinone):



3. Solvent Yellow 124 (N-ethyl-N-[2-(1-izobutoxyethoxy) ethyl]-4-phenyldiazenyl) aniline



EXPERIMENTAL

Reagents and solutions

Toluene (HPLC grade), hexane (HPLC grade), MTBE (methyl tert-butyl ether) (99.8%), Solvent Red 19 (N-ethyl-1-(phenyldiazophenylazo)-2-aminonaphthalen) (96%), Sol-

vent Blue 35 (1,4-bis(butylamino)-9,10-anthraquinone) (98%), Solvent Yellow 124 (N-ethyl-N-[2-(1-izobutoxyethoxy) ethyl]-4-phenyldiazenyl) aniline) (98%) were obtained from Sigma-Aldrich (Germany). Ethyl acetate (HPLC grade) was purchased from Merck (Germany). Stock solutions of the fuel dyes and the marker (SR 19, SB 35 and SY 124) (10 mg/L each) were prepared in toluene. Working solutions of the fuel dyes and the marker were prepared by dilution of the stock solution with toluene. All solutions were stored in the dark at 4°C.

Dyed fuel samples were purchased from the local petrol stations.

Sample preparation

The real samples of dyed fuel were filtered through 0.45 μm PTFE Chromafil Xtra PTFE-45/25 filters (Macherey-Nagel, Germany) filters. After filtering, the samples were transferred into 2 mL chromatographic vials.

Instrumental

The chromatographic analysis was performed on a Liquid Chromatograph PerkinElmer Series 200 (PerkinElmer Instruments, USA) using UV/Vis spectrophotometric detection. A 200 μL volume injection loop was used for automated injection. The HPLC system was equipped with a Zorbax Rx-SIL column (250 mm × 4.60 mm ID, sorbent particle size 5 μm) (Agilent Technologies, USA). A 150 μL high pressure mixer (PerkinElmer Instruments, USA) was used.

Parameters of HPLC analysis

Real samples were filtered through a 0.45 μm PTFE filter right into a 2 mL vial. The vials with the prepared samples were placed into an autosampler for injection. The best separation of analytes with the shortest retention times was achieved while using a mobile phase consisting of hexane, toluene and ethyl acetate. A constant mobile phase flow of 1 mL/min was set. A gradient composition of the mobile phase was used to get the best results: firstly, 7 min the column was conditioned with a hexane/toluene (30/70 vol.%) mixture. The first 2 minutes after the injection the mobile phase composition was the same as used for preconditioning; after 8 min the mobile phase composition changed to the toluene/ethyl acetate (93/7 vol.%) solution; in the last step the isocratic mobile phase composition was held for 5 min. The total time of the analysis of dyes and markers in fuel was 22 min. The HPLC column must be preconditioned for 7 min before every analysis to remove ethyl acetate from the column.

RESULTS AND DISCUSSION

Selection of detection wavelengths

The sample analysis was performed with a PerkinElmer Series 200 (PerkinElmer Instruments, USA) equipped with a UV/Vis detector. The absorption wavelength of each

analyte should be optimized in order to improve the analysis sensitivity. The spectrophotometric analysis was performed for each dye and marker solution using a solution with a 5 mg/L concentration. The results presented in Fig. 1 show that the highest absorption is at 535 nm for SR 19, 650 nm for SB 35, and 390 nm for the marker SY 124. By setting the UV/Vis detector to those wavelengths for each analyte, the highest detection sensitivity was achieved. Our detector could record only two signals at two different wavelengths, consequently it was decided to use the reference wavelength mode to monitor the third one.

All measurements were made with a two-channel UV/Vis detector. In the first channel, the 535 nm main wavelength was chosen to detect SR 19, and the 390 nm reference wave-

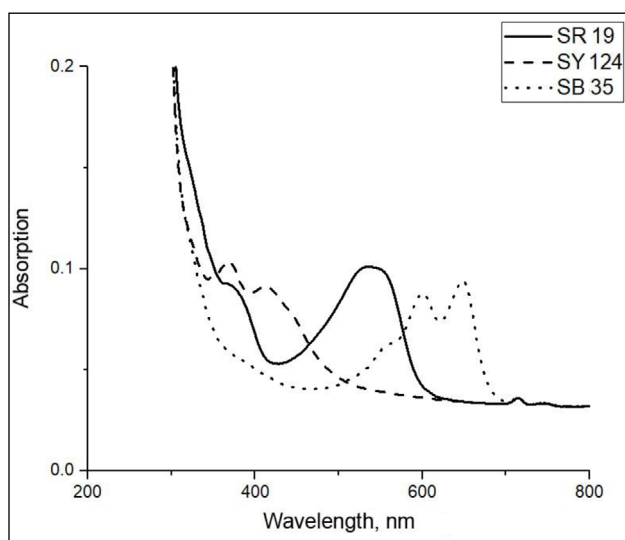


Fig. 1. Results of the spectrophotometric analysis of the fuel dyes and the marker (concentration of each analyte 5 mg/L)

length was chosen to detect SY 124 so this peak in all chromatograms is shown as a negative peak. In the second channel, the 650 nm main wavelength was chosen to detect SB 35.

Optimization of separation conditions

As the first eluent composition, hexane, toluene and MTBE were chosen. However, this composition is appropriate only for SR 19 and SY 124, with no results for SB 35. The analysis started with the hexane and toluene (75/25 vol.%) eluent composition, after 18 min MTBE was added to the mobile phase for the next 5 min, so at the end of the analysis the eluent composition was hexane, toluene and MTBE (71/21/8 vol.%). Using this methodology, the retention time of analytes was very long. Furthermore, increasing the MTBE concentration in the mobile phase decreases the measurement repeatability and prolongs the preconditioning time of the HPLC column.

A different eluent composition could be used for the detection of SB 35 and SY 124. Using the mobile phase of toluene and ethyl acetate (98/2 vol.%) in an isocratic mode was not suitable for SR 19. After few injections it was clear that SR 19 was eluted with the dead volume. Increasing or decreasing the ethyl acetate concentration has no effect on SR 19 retention. Also there was a large retention time difference between SB 35 and SY 124.

After series of separations using different eluents, it seems that ethyl acetate is a good eluent additive for the dyes SR 19 and SB 35 and the marker SY 124. In order to achieve the shortest analyte retention times the eluent consisting of hexane/toluene/ethyl acetate (49.5/49.5/1 vol.%) was tried. This analysis showed that by using less ethyl acetate in the eluent dyes SR 19 and SB 35 and the marker SY 124 retentions can be managed. All retention times with different mobile phases are shown in Fig. 2. For further analysis, the last mobile phase composition was chosen.

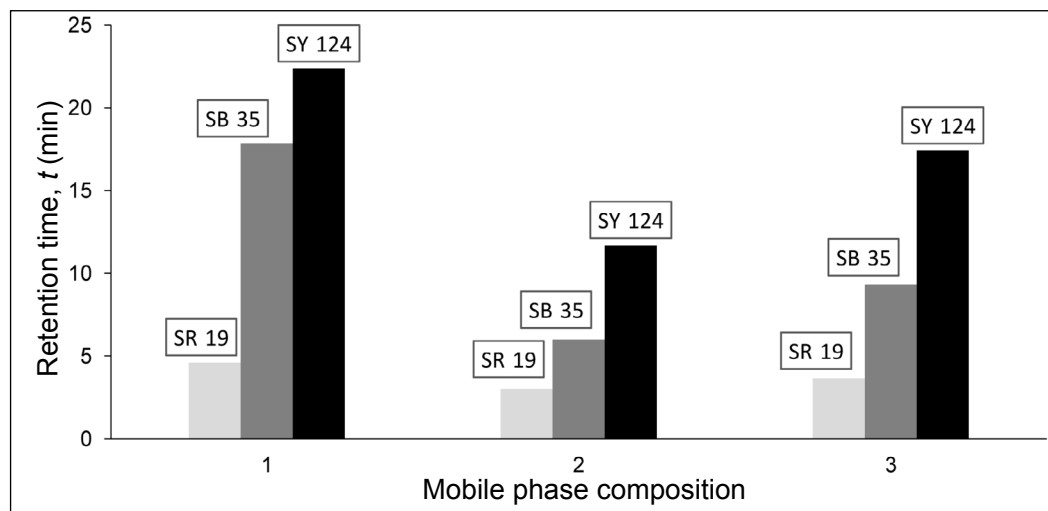


Fig. 2. Comparison of the retention times of analytes according to the composition of different mobile phases: 1, hexane/toluene/MTBE (71%/21%/8%); 2, toluene/ethyl acetate (98%/2%); 3, hexane/toluene/ethyl acetate (49.5%/49.5%/1%)

The optimized and adopted mobile phase composition for the analysis of the dyes and the marker in fuel is shown in Table 1. The experiment showed that the shortest retention and the best separation of analytes was when the three different solvents were used: hexane, toluene and ethyl acetate. Moreover, using a gradient elution with these solvents the best results can be obtained. In addition to that, analyses were performed to find out the shortest time for column conditioning (i.e. to eliminate all ethyl acetate from it). The optimized conditioning time was found to be 7 min. The total time of the analysis is 22 min.

Table 1. Mobile phase composition for the analysis

Step	Time, min	Flow rate, mL/min	Hexane, %	Toluene, %	Ethyl acetate, %
Conditioning	7.0	1.0	30	70	0
1	2.0	1.0	30	70	0
2	8.0	1.0	0	93	7
3	5.0	1.0	0	93	7

Determination of method parameters

The main chromatographic parameters were determined and calculated. Column efficiency could be evaluated according to the theoretical plate number (N) and its height (H):

$$N = 5.54 \times \left(\frac{t_R}{w_{0.5}} \right)^2 \cdot \text{eq.}, \quad (1)$$

$$H = \frac{L}{N} \cdot \text{eq.} \quad (2)$$

All the calculations were made measuring the SR 19 standard solution with the 5 mg/L concentration. The theoretical plate number (N) and its height (H) were 4986 and 0.05014 mm, respectively.

After the mobile phase composition and separation optimization, five standard solutions with a concentration range from 1 to 10 mg/L were prepared. All results of the calibration curves are shown in Fig. 3 and Table 2.

Limit of detection (LOD) and limit of quantification (LOQ)

The linearity of the response of the chromatographic system was tested by recording chromatograms for different concentrations in the range 1–10 mg/L, using the previously optimal conditions and with the standard solution corresponding to each point of the calibration curve. Each calibration curve was constructed using five concentration levels, prepared by the serial dilution of working standard solutions. From Fig. 3 it can be seen that a good linearity was achieved, with a correlation coefficient (r) greater than

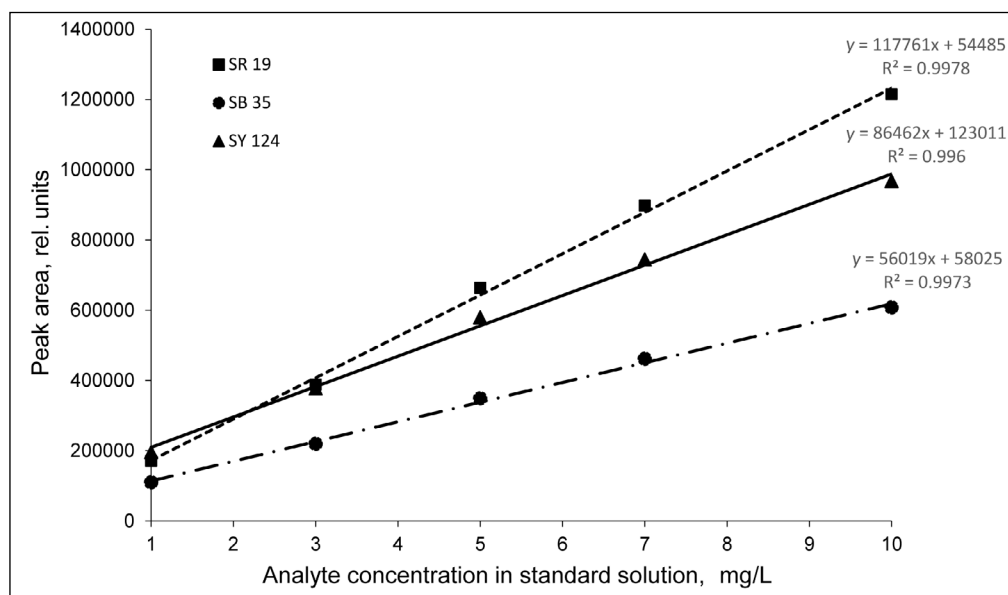


Fig. 3. Calibration curves of the analytes measured using series of the standard solutions prepared in hexane

Table 2. Main analytical characteristics

Analyte	Retention time, min	Line equation	r^2	LOD, mg/L	LOQ, mg/L	Repeatability RSD, % ($n = 7$)
SR 19	3.8	$y = 117761x + 54485$	0.998	0.56	1.88	2.1
SB 35	12.0	$y = 86462x + 123011$	0.997	0.63	2.10	5.0
SY 124	12.4	$y = 56019x + 58025$	0.996	0.77	2.55	1.0

0.99 for all the compounds within the calibration range used. The parameters of the regression equations are given in Table 2. The standard deviation of the y-intercept (S_A) of the regression line calculated for each compound was used to calculate the limit of detection (LOD) and quantification (LOQ), as 3 and $10 \cdot S_A/m$, respectively, where m is the slope of the calibration curve. The limits of detection using this relationship were between 0.56 and 0.77 mg/L, the limits of quantification were between 1.88 and 2.55 mg/L shown in Table 2, showing that the method is capable of determining dye concentrations at the levels that must be found in real samples.

Analysis of diesel samples

The optimized method was successfully applied for the measurements of real samples – diesel fuel. Two different samples of coloured diesel were measured using the developed method. The concentrations of the dyes and the marker for fuel

colouring were determined. The samples were received from the company engaged in fuel dyeing and marking. Two types of fuel with low taxes were analysed. The first was the red colour fuel suitable for heating systems which dyed with Solvent Red 19 and marked using Solvent Yellow 124. The second was the green colour fuel suitable for agriculture or aquaculture aggregates which dyed with Solvent Blue 35 and marked using Solvent Yellow 124. The results of the analysis are shown in Table 3 and Figs. 4–6. The first analysis of red dyed fuel showed that the in-house developed method was fully suitable for the determination of Solvent Red 19 and Solvent Yellow 124, but the dye and the marker used for dyeing fuel are not pure from their isomers or mixed up with another dyes and markers of the same colour. Consequently, a similar situation has been obtained with the data of the analysis of the second sample which should be dyed with Solvent Blue 35 and marked with Solvent Yellow 124.

Table 3. Results of the analysis of real samples

Sample	Dye	Measured concentration, mg/L	Officially compulsory concentration*, mg/L	Marker	Measured concentration, mg/L	Officially compulsory concentration*, mg/L
Green colour diesel fuel	SB 35	0.61	5.0	SY 124	4.42	8.5–12.7
Red colour diesel fuel	SR 19	0.45	6.0	SY 124	4.63	8.5–12.7

* Officially compulsory concentration, the lowest concentration of the necessary dye or marker in fuel, as required according to the regulation of the Ministry of Energy [1].

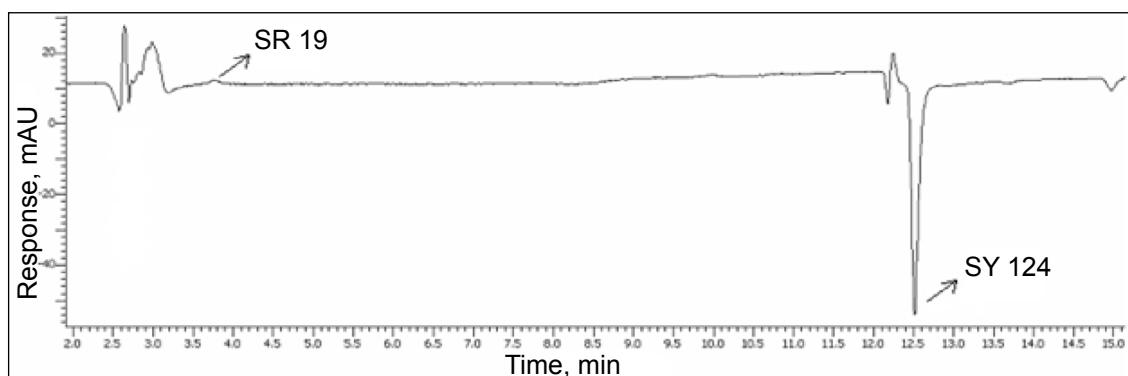


Fig. 4. Chromatogram of the red coloured fuel (dyed with SR 19 and marked with SY 124). Eluent composition: hexane, toluene, ethyl acetate; detection at 535 nm as the main wavelength and at 390 nm as the reference wavelength; injection volume 20.0 μ L

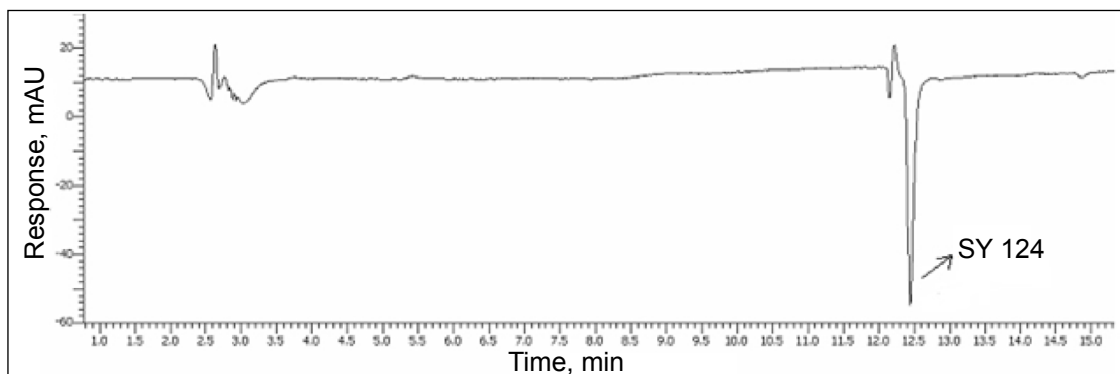


Fig. 5. Chromatogram of the green coloured fuel (dyed with SB 35 and marked with SY 124). Eluent composition: hexane, toluene, ethyl acetate; detection at 535 nm as the main wavelength and at 390 nm as the reference wavelength. Injection volume 20.0 μ L

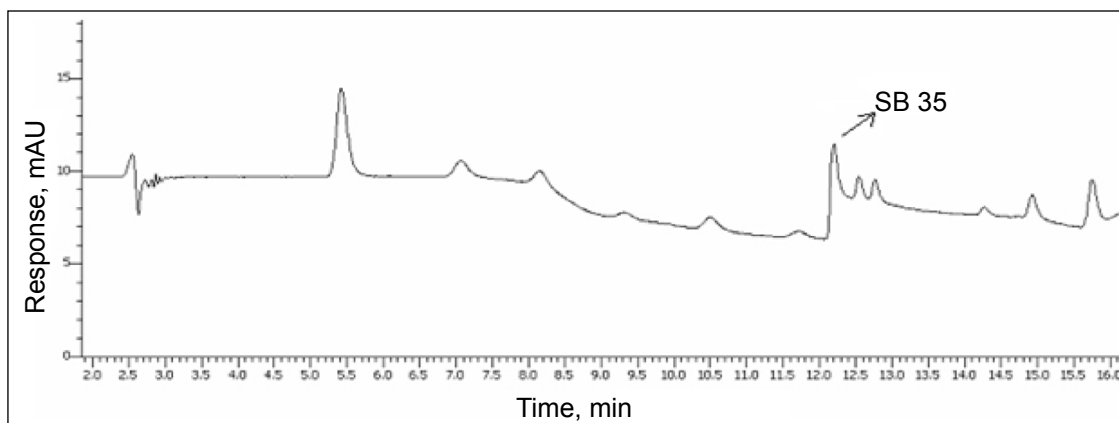


Fig. 6. Chromatogram of the green coloured fuel (dyed with SB 35 and marked with SY 124). Eluent composition: hexane, toluene, ethyl acetate; detection at 640 nm as the main wavelength. Injection volume 20.0 μ L

CONCLUSIONS

The developed method was applied for the analysis of dyed diesel fuel samples. The method is able to separate and to detect the analytes which are used according to the national regulation for colouring and marking of the low taxed diesel fuels in one run. Using this method concentrations of 0.45 and 4.63 mg/L of SR 19 and SY 124, respectively, were determined in the red dyed diesel, and 0.61 and 4.42 mg/L of SB 35 and SY 124, respectively, in the green dyed diesel sample. It is possible to adopt this HPLC method for other types of dyes and markers in real samples.

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DAŽIKLIŲ IR ŽYMIKLIO NUSTATYMAS DYZELINIAME KURE EFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODU

Santrauka

Paruoštas, ištirtas ir optimizuotas dyzeliniame kure naudojamų dažiklių („Solvent Red – 19“, „Solvent Blue – 35“) ir žymiklio („Solvent Yellow – 124“) nustatymo metodas. Optimalios šio metodo sąlygos, kai iš pradžių judrioje fazėje taikant gradientinį režimą heksanas ir toluenas sudaro 70 % / 30 %, nuo antros analizės minutės iki analizės pabaigos judrios fazės sudėtį sudaro toluenas ir etilacetatas 93 % / 7 %, o judrios fazės srauto greitis 1 mL/min. Iš viso analizė trunka 22 min. Analitės detektuojamos, kai: SR-19 – 535 nm; SB-35 – 640 nm; SY-124 – 390 nm. Metodas pritaikytas dviejų tipų (raudonai nudažyto ir žaliai nudažyto) realių kuro mėginių analizei. Sėkmingai nustatytos koncentracijos dviejuose dyzelinio kuro mėginiuose siekė 0,61 mg/L SB-35; 0,45 mg/L SR-19 ir atitinkamai 4,42 bei 4,63 mg/L žymiklio SY-124. Šios nustatytos koncentracijos neatitiko minimalių reikalaujamų koncentracijų pagal LR Energetikos ministerijos ministro įsakymą [1].