Bias, limit of detection, and limit of quantitation for the ASTM D2425 method updated in 2019

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ARTICLE INFO

ABSTRACT

An update for ASTM D2425, "Standard Test Method for Hydrocarbon Types in Middle Distillates by Mass Spectrometry," was recently released to expand its scope by adding modern mass spectrometric instrumentation and a new sample type, synthesized hydrocarbons. While the method update is a proper step towards modernization, it still lacks validation parameters. The description of the updated standard method contains precision estimations but no consideration for a possible bias, meaning that the method accuracy was not evaluated. The 2019 update does not either provide the lowest concentration of an analyte that can be detected and quantitatively determined, 'limit of detection' (LOD) and 'limit of quantification' (LOQ). In the present study, the LOD, LOQ, and bias were evaluated as an intra-laboratory study. The LOD and LOQ determination was based on the adjusted standard deviation of the least abundant hydrocarbon type in a sample, and were 0.004 and 0.01 w/w% in renewable jet fuel (RJF) and 0.01 and 0.02 w/w% in gas oil (GO), respectively. The bias was determined by measuring relative hydrocarbon recovery percentages for spiked samples. The renewable jet fuel was spiked with cycloparaffins and alkylbenzenes and the recovery percentages were 140% and 63%, respectively. The gas oil was spiked with paraffins and alkylbenzenes and the recovery percentages were 117% and 90%, respectively. Both sample sets were positively biased for paraffin content (recovery percentage over 100%) and negatively biased for alkylbenzene content (recovery percentage less than 100%). Based on above results, this method may not work well for RJF samples.

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1. Introduction

An update for ASTM D2425, "Standard Test Method for Hydrocarbon Types in Middle Distillates by Mass Spectrometry," was released in January 2019 to expand its scope by adding modern mass spectrometric instrumentation and a new sample type, synthesized hydrocarbons [1]. ASTM defines synthesized hydrocarbons as hydrocarbons derived from alternative sources, such as coal, natural gas, biomass, and hydrogenated fats and oils, by processes such as gasification, Fischer-Tropsch synthesis, and hydroprocessing, while conventional hydrocarbons are hydrocarbons derived from the following conventional sources: crude oil, natural gas liquid condensates, heavy oil, shale oil, and oil sands. This update was necessary because this method is a mandatory test for synthesized renewable jet fuels (RJF) as specified by ASTM D7566 [2].However, RJF had not been included in the scope of previous version

of ASTM D2425 which only covered virgin middle distillates such as Gas Oil (GO) fraction of crude oil distillation unit Furthermore, the previous method was based on the use of an obsolete Consolidated Electrodynamics Corporation Type 103 mass spectrometer last manufactured in 1970.

The updated D2425 standard method workflow is presented in Fig. 1. Briefly, the analysis begins with separation of the sample into saturated and aromatic hydrocarbon fractions by one of three liquid chromatography separation procedures suggested by ASTM methods D2549, D1319 and D6379 [3–5]. The resulting fractions are analyzed either by an electron ionization magnetic sector mass spectrometer ((EI) B MS) or a gas chromatograph coupled with an electron ionization quadrupole mass spectrometer (GC/(EI) Q MS) (Procedure A and Procedure B, respectively). The produced mass spectra, one for each fraction, show the stable analyte molecular ions and their fragment ions. These ions are assigned to eleven predefined hydrocarbon types based on their mass-to-charge ratios (m/z). ASTM D2425 defines the eleven hydrocarbons types as paraffins, mononaphthenes, dinaphthenes, trinaphthenes, alkylbenzenes, indanes and tetralins, indenes (C_nH_{2n-10}), naphthalenes, acetylnaphthenes (C_nH_{2n-14}), acetylnaphthylenes (C_nH_{2n-16}), and

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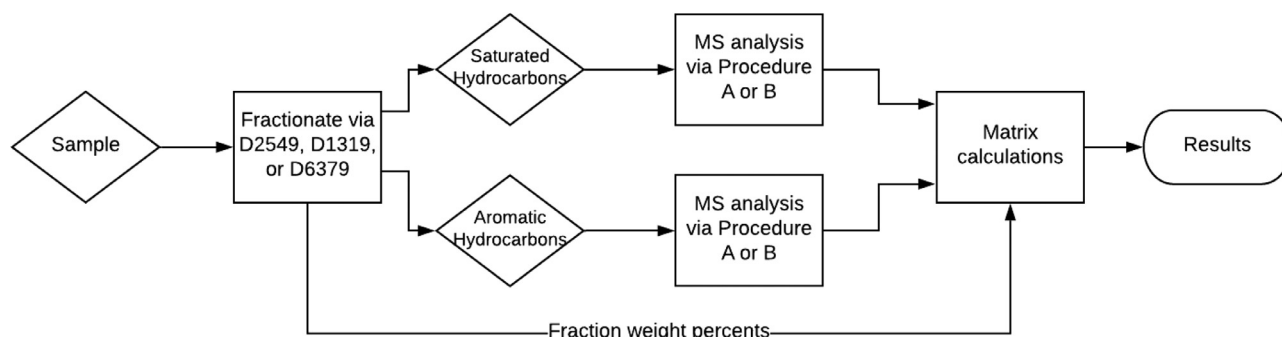[1] Elias and Katherine have contributed equally to this paper.

Fig. 1. The 2019 updated ASTM D2425 analysis workflow.

Table 1

Internal standard (PFTBA) was used as the calibrant for targeted automatic tuning. The MS was tuned automatically five times and each time reached the ion abundances in the ranges specified below.

m/z-value	Target ion abundance ratio (%)	Measured ion abundance ratio (%) range
50	1.0	0.9–1.2
69	100.0	100.0
131	55.0	55.7–69.1
219	45.0	44.5–49.8
414	3.5	3.2–3.8
502	2.5	2.5–3.1

tricyclic aromatic compounds (where n is any whole number). In addition, D2425 uses terms cycloparaffins, saturates and aromatics for the sample compounds. These compound classifications will be used throughout this paper.

For the above classification approach, the weight percentage (wt%) of different hydrocarbon types in the sample are determined using the calibration data given in Table 1 in the 2019 update of ASTM D2425. The table provides the theoretical abundance proportions of the ions in the mass spectrum measured for each hydrocarbon type to facilitate the generation of the weight percentage for each hydrocarbon type by utilizing measured ion abundances (obtained from measured peak heights) determined by MS [1]. This categorization is based on the assumption that the ions in the table are the most characteristic for the respective hydrocarbon type and average number of carbons. The D2425 method provides guidance for estimation of the average number of carbons in each hydrocarbon type. To determine the weight percentages of different hydrocarbon types for the whole sample, the results obtained for each hydrocarbon fraction are normalized to the gravimetric weight percent of the saturated and aromatic hydrocarbon fractions. However, the updated D2425 method does not introduce new calibration data necessary for the analysis of the new sample type, synthesized hydrocarbons, such as RJFs, or the new instrument type utilized in Procedure B. Therefore, it is unknown whether the calibration data given in the 2019 method can be used to appropriately determine the content of different hydrocarbon types in the new sample type or in any sample when using the new instrument.

While the January 2019 update of ASTM D2425 is a proper step towards modernization, it lacks validation parameters. First, the updated description does not present all the essential performance characteristics to ensure the validity of the method. For example, knowing the magnitude of total error is essential for result interpretation and use but is not included in the 2019 method update. The description of the updated standard method contains precision estimations but no consideration for a possible bias, meaning that accuracy was not evaluated. Second, it is essential to know the lowest concentration of an analyte that can be detected and quantitatively determined at a specified level of confidence. Terms 'limit

of detection' (LOD) and 'limit of quantification' (LOQ) are used for these concepts. Unfortunately, the 2019 update of D2425 does not provide either of these limits, nor guidelines for the lowest analyte concentration that may be determined.

The D2425 procedure is required to verify that the amounts of cycloparaffins and aromatic compounds are below the specified maximum allowed limits for renewable jet fuels. Therefore, bias determination is imperative. For example, the maximum allowed level for aromatic compounds is only 0.5 wt% for most synthetized RJF sample types, making the LOQ an essential figure to be determined. To improve the 2019 version of the ASTM method D2425, this study evaluated the LOD and LOQ and estimated the method bias for Procedure B (GC/(EI) Q MS) using both conventional and synthesized hydrocarbons as defined by ASTM. The LOD, LOQ, and bias were evaluated as an intra-laboratory study in accordance with the Eurachem guide [6].

2. Experimental section

2.1. Instrumentation hardware

Experiments were performed following Procedure B, which is based on the use of a gas chromatograph/(electron ionization) quadrupole mass spectrometer (GC/(EI) Q MS). An Agilent 7890B GC was utilized with helium ($>$99.996% pure) as the carrier gas and liquid carbon dioxide ($>$99.7% pure) for cryogenic oven cooling. Samples were injected via an Agilent 7693A automatic liquid sampler with a 10 μl Agilent ALS Syringe (5181-3354) by using dichloromethane as syringe cleaning solvent. The GC contained two parallel GC columns, each with an individual inlet. These experiments only utilized one of the columns with a split/spitless inlet. The other column was reserved for other purpose and was not used in this research. The column utilized in this research had no active phase (10 m x 100 μm x 0 μm; Agilent Part No: 160-2635-10), but the separation of the solvent from the sample hydrocarbons was done with means of GC oven temperature and carrier gas flow ramp as discussed below in the methods Section 2.2. The second column (not used in this research) was a ZB-1 ms col-

umn (60 m x 250 μm x 1 μm) and had a constant 0.4 mL/min carrier gas flow slightly increasing the back pressure of the first column (used in the research). Both columns were connected into a tee-connection that was attached to the EI source of the mass spectrometer. The GC was coupled with an Agilent 5977B single quadrupole MS with an inert plus extractor EI source and triple-axis electron multiplier detector. Specific GC parameters are discussed below as the parameters were adjusted for each sample that was analyzed.

2.2. Methods

Two different sets of GC conditions were utilized in this investigation. One was used for both the saturated compound fraction of the RJF sample as well as the saturated and aromatic compound fractions of the GO sample. A separate set of GC conditions was used for the aromatic compound fraction of the RJF sample. The reason for this was the low absolute weight of the fraction resulting in lower sample solution concentration (10 mg/mL, not 100 mg/mL). This in turn led to the use of lower GC inlet split ratio (50:1, not 140:1), which together with the low sample solution concentration forced a lowered oven temperature and carrier gas flow ramp to be used in order to accommodate the elevated on-column solvent volume in the analysis (nearly 200% increase of solvent volume). The same MS and data analysis method was used for all samples, as discussed below.

2.2.1. GC method conditions

The conditions of the GC methods are discussed below separately for each sample type, the aromatic compounds and the saturated compounds derived from the RJF and the GO samples.

2.2.1.1. Aromatic compound fraction of RJF. An inlet split ratio of 50:1 was utilized. The concentration of sample solutions was 10 mg/mL. A 1 μl volume of each sample was injected into the inlet held at a temperature of 280 °C. The GC oven temperature was set to −30 °C and was held there for 7 min, after which it was ramped at a rate of 50 °C/min until a temperature of 250 °C was reached, which was maintained for 0.5 min. The initial helium carrier gas flow was set to 0.06 mL/min and held there for 7 min, after which it was quickly ramped at a rate of 20 mL/min until a flow rate of 0.7 mL/min was achieved. This flow rate was maintained until the end of the program.

2.2.1.2. Saturated compound fraction of RJF and both saturated and aromatic compound fractions of GO. An inlet split ratio of 140:1 was utilized. The concentration of the sample solutions was 100 mg/mL. A 1 μl volume of each sample was injected into the inlet held at a temperature of 280 °C. The GC oven temperature was set to −30 °C and held there for 2 min, after which it was ramped at a rate of 50 °C/min until a temperature of 250 °C was reached. This temperature was held for 0.5 min. The initial helium carrier gas flow was set to 0.2 mL/min and held there for 2 min, after which it was quickly ramped at a rate of 20 mL/min until a flow rate of 0.7 mL/min was achieved. This flow rate was maintained until the end of the program.

2.2.2. MS conditions

The MS was operated as outlined in the 2019 version of ASTM D2425: positive electron ionization mode using 70 eV electron kinetic energy, 230 °C ion source temperature, 300 °C transfer line temperature, and 150 °C quadrupole temperature. Mass spectra were collected at a scan speed of 9.8 scans/s over the *m/z* range 40–292. The instrument was autotuned with standard spectrum tune program (Stune), which monitors the target ion relative abundances (%) for an internal calibration mixture of perfluorotributylamine (PFTBA).

2.2.3. Data analysis

The average mass spectra of the runs, one measured for each fraction, show analyte molecular ions and their fragment ions. These ions were assigned to eleven predefined hydrocarbon types based on their characteristic *m/z* values as described in the 2019 update of ASTM D2425 [1]. The summed abundances of the ions (determined from the relevant peak heights) assigned to each hydrocarbon type, together with the calibration data given in the 2019 version of ASTM D2425, were mathematically processed to produce the mass percent of each hydrocarbon type as described below.

After summing the abundances of the ions characteristic for each hydrocarbon type, the average number of carbons for the alkylbenzenes and naphthalenes in the aromatic hydrocarbon fraction were determined. The average number of carbons determines the calibration matrices and sensitivities used in further calculations for the aromatic and saturated hydrocarbon fractions. The matrix calculations consisted of solving a set of simultaneous linear equations. These calculations were performed separately for the aromatic and saturated hydrocarbon fractions. Each linear equation illustrated the distribution of the summed abundances of the characteristic ions for each specific hydrocarbon type compared to all hydrocarbon types. The linear equation coefficients are given in D2425 as matrices. The coefficients were the sums of the abundances of the ions characteristic to each hydrocarbon type. The variables were the unknowns to be solved, and they reflected the mass fractions of the hydrocarbon types in the sample. The unknowns (variables) were solved by multiplying the inverse of the square matrix (5×5 for the saturated compound fraction, 10×10 for the aromatic compound fraction) of the linear equation coefficients by constants (the sum intensities of the hydrocarbon types) that were tabulated as a column matrix (5×1 for the saturated compound fraction, 10×1 for the aromatic compound fraction). The results were output as a column matrix (5×1 for the saturated compound fraction, 10×1 for the aromatic compound fraction). The results were then converted to hydrocarbon type mass fractions by dividing each value by the corresponding sensitivity (response factor) given in D2425. To obtain the final result, the mass fractions were normalized to the gravimetric weight percentages of the fractions. The most convenient way to conduct the calculations was via automated calculation software. An in-house macro-based Microsoft Excel calculation sheet was used to generate the hydrocarbon type contents (wt%) of the samples.

2.3. Samples

Neat model compounds were utilized to evaluate the performance of the analytical method. After this, real mixtures were fractionated and utilized to evaluate the updated ASTM D2425 method.

2.3.1. Sample sets for evaluation of instrument performance

Three types of performance were evaluated, the accuracy of MS tuning, instrument signal as a function of the amount of the analyte and instrument ruggedness. Each required a unique sample set. They are discussed below in this order.

Validation of MS tuning: The MS instrument was automatically tuned using the factory Stune function on the instrument software. The 2019 version of ASTM D2425 dictates that MS tuning completed this way must be validated using n-hexadecane. A solution of 0.6 mg/mL n-hexadecane (Acros Organics, 99%) in dichloromethane (DCM) (Merck Millipore Dichloromethane, purity >99.8%) was used in this work.

Investigation of the linearity of the instrument signal with respect to the amount of the analytes: Instrument signal linearity as a function of analyte mass percent was investigated by spiking
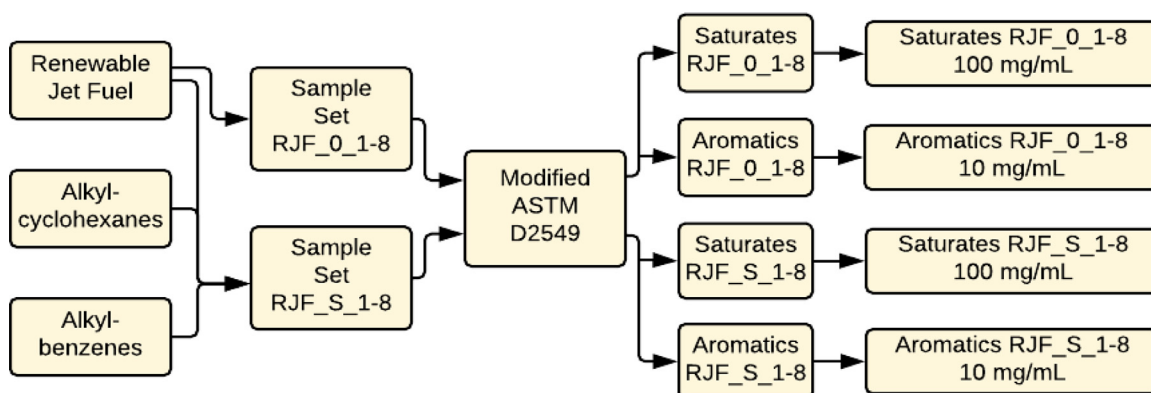
Fig. 2. Scheme for the preparation of renewable jet fuel (RJF) sample for LOD, LOQ, and bias investigation.

a GO sample and an RJF sample with n-hexadecane-d_{34} (CDN Isotopes, Quebec, Canada, 98.6% enrichment) in an increasing weight percent from 0 to 10 w/w%. The spiked fuel samples, RJF and GO, were analyzed in duplicate in the order of increasing n-hexadecane-d_{34} mass percent with a blank of DCM between each sample.

Investigation of instrument ruggedness: Test samples were prepared by fractionating a GO sample into saturated and aromatic hydrocarbon types (according to a modified method of ASTM D2549 as described below) and then diluting the fractions with DCM to have a concentration of 100 mg/mL. Each sample was then divided into five sub-aliquots, one for each tune testing. For each tune test, both saturated and aromatic hydrocarbon fractions were injected a total of eight times with a DCM solvent blank between each set such that the analysis order per MS tune was: solvent, saturated hydrocarbons, aromatic hydrocarbons, solvent, saturated hydrocarbons, aromatic hydrocarbons, solvent, ..., saturated hydrocarbons, aromatic hydrocarbons. This led to a total of 24 acquisitions per tune file and 120 acquisitions in total for all five tune files.

2.3.2. Sample fractionation

The separation of the samples into saturated and aromatic hydrocarbon fractions prior to GC/(EI) Q MS analysis was completed according to a modified version of the D2549 method to make it suitable for the samples studied here that mostly contained compounds with low boiling points. The modifications are specified in the supplementary data (Method S1). The main modification was the way the solvent was removed, which was performed via distillation rather than evaporation. The modified method is referred to as D2549M from now on. n-Pentane (VWR Chemicals, Analar, purity >99%), diethyl ether (VWR chemicals, emsure® for analysis, purity >99%), chloroform (VWR chemicals, Analar, purity >99%), ethanol (ETAX A, Altia, purity >94%) and n-hexane (VWR chemicals, HiPer Solv, purity >97%) were used in the fractionation procedure.

2.3.3. Sample sets for evaluation of the 2019 updated ASTM D2425 method

The complex mixtures investigated in this study were a synthesized hydrocarbon sample of renewable jet fuel (RJF) and a conventional hydrocarbon sample of gas oil (GO). The RJF sample was a synthesized paraffinic kerosene obtained via hydroprocessing of esters and fatty acids at Neste refinery, with a boiling point range of 172.6–287.9 °C. This sample type would be included in D7566-19 standard specification for aviation turbine fuels containing synthesized hydrocarbons. The GO sample was a virgin middle distillate of a crude oil sample with a boiling point range of 197.6–304.7 °C

obtained from a refinery crude oil distillation unit utilizing mostly Russian sour heavy crude oil (Urals oil).

Replicate samples of RJF and GO were used to determine the limit of detection (LOD), limit of quantitation (LOQ), and bias. The sample set for RJF was prepared as shown in Fig. 2. Alkylcyclohexane and alkylbenzene model compounds, n-hexylcyclohexane, n-heptylcyclohexane, n-octylcyclohexane, n-nonylcyclohexane, n-decylcyclohexane, n-undecylcyclohexane, n-dodecylcyclohexane, n-hexylbenzene, n-heptylbenzene, n-octylbenzene, n-nonylbenzene, n-decylbenzene, and n-undecylbenzene, were all purchased from abcr (Karlsruhe, Germany; purity >98%) and used as received. The sample set of RJF (RJF_S_1-8) was spiked with model compounds in such a manner that the mass fraction of each alkylcyclohexane model compound was 0.75 w/w% in solution and the mass fraction of each alkylbenzene model compound was 0.23 w/w% in solution. The mass fractions of the spiked samples were corrected based on the purities of the model compounds. Additionally, a set of eight neat RJF fuel samples (RJF_0_1-8), with no model compound spiking, was also tested. The sample sets were fractionated into saturated and aromatic hydrocarbons according to ASTM D2549M (described above). After fractionation, the aromatic hydrocarbons were diluted with DCM to yield a 10 mg/mL solution (with a minimum of 100 µL DCM added) and the saturated hydrocarbons were diluted in DCM to yield a 100 mg/mL solution. The RJF_0_1-8 saturated hydrocarbon sample set and the RJF_0_1-8 aromatic hydrocarbon sample set were analyzed in one day. The RJF_S_1-8 saturated hydrocarbon sample set and the RJF_S_1-8 aromatic hydrocarbon sample set were analyzed the following day. Analysis order was set to be the same as described above for the investigation of the instrument ruggedness, with DCM solvent blanks between the sample sets.

The sample set for GO was prepared similarly to that for RJF. However, for GO, the alkane and alkylbenzene model compounds used were n-undecane (purity 99.3%), n-tridecane (purity 99.7%), n-pentadecane (purity 99%), n-octadecane (purity 99.3%), 1,2,4,5-tetramethylbenzene (purity 98%), 1,4-diethylbenzene (purity 95%), pentamethylbenzene (purity 98%), 1-phenyloctane (purity 98%), 1-phenyldecane (purity 98%), and 1-phenylpentane (purity 99%) and they were purchased from Sigma Aldrich Chemistry with the following exceptions. n-Dodecane was purchased from Merk Millipore (purity 99%). n-Tetradecane (purity 99.4%), n-hexadecane (purity 99.1%), and n-heptadecane (purity 99.1%) were purchased from Acros Organics and used as received. The sample set GO_S_1-8 was spiked with the model compounds in such a manner that the mass fraction of each alkane model compound was 2 w/w% in solution and the mass fraction of each alkylbenzene model compound was 1 w/w% in solution. The mass percentages of the spiked samples were corrected based on the purities of the model compounds. Ad-

ditionally, a set of eight neat samples (GO_0_1-8), with no model compound spiking, were also tested. After fractionation, the samples were diluted with DCM in such a manner that both aromatic and saturated hydrocarbon fractions had a concentration of 100 mg/mL. The GO_0_1-8 saturated hydrocarbon sample set and the GO_0_1-8 aromatic hydrocarbon sample set were analyzed in one day. The GO_S_1-8 saturated hydrocarbon sample set and the GO_S_1-8 aromatic hydrocarbon sample set were analyzed the following day. Analysis order was performed as described above, with DCM solvent blanks between the sample sets.

3. Results and discussion

First, the MS instrument was evaluated in order to determine whether it satisfied the ASTM standards. After this, it was utilized to evaluate the 2019 version of ASTM D2425 method by determining the LOD, LOQ, and bias for several samples.

3.1. MS instrument evaluation

The accuracy of MS tuning with respect to ion relative abundances was first evaluated as dictated by the 2019 updated ASTM D2425 method. Then the linearity of the magnitude of the MS signal as a function of the amount of the analyte was examined using n-hexadecane-d_{34} dopant at different mass percentages. Finally, the instrument ruggedness for tuning was assessed. They are discussed below in this order.

3.1.1. MS tuning validation

The MS instrument was automatically tuned using the Stune function on the instrument software. A sample of n-hexadecane was used to determine whether the tuning of the relative abundances of ions measured using the MS instrument was within ASTM specifications. The sum of the heights of the peaks corresponding to ions of *m/z* 67, 68, 69, 81, 82, 83, 96, 97 (noted as $\Sigma 67$) and to ions of *m/z* 71 and 85 (noted as $\Sigma 71$) were used to verify the tuning of the MS instrument. As outlined in the 2019 version of ASTM D2425 [1], an acceptable range for the $\Sigma 67/\Sigma 71$ is between 0.2 and 0.3. Performing the test in triplicate, the average was determined to be 0.23, which falls within the required range. Therefore, the developed GC/(EI) Q MS method met the requirements of the 2019 updated ASTM D2425 tuning validation test, confirming that the instrument performed comparably with the instrument used to generate the calibration data given in the ASTM method. Appropriate tuning of the MS is imperative because the magnitude of the signal will be used to derive the weight percentages of the various hydrocarbon types and no external calibration method was offered in the 2019 updated ASTM D2425.

3.1.2. Magnitude of the instrument signal as a function of the amount of the analyte

The 2019 version of ASTM D2425 does not specify the type of the GC/(EI) Q MS instrument to be used. Therefore, the linearity of the magnitude of the signal of the instrument chosen for this research as a function of analyte concentration was evaluated. This was performed for the samples RJF and GO by spiking them with different amounts of n-hexadecane-d_{34} (0–10 w/w%; typical amounts of n-hexadecane for these sample types). Mass spectra were collected over the entire GC method run time of 8 min (Section 2.2.1.2). The recorded data were represented as a total ion chromatogram (TIC) which shows the sum of all ion abundances as a function of retention time. An extracted ion chromatograph (XIC) was then constructed that showed the abundance of fragment ions of *m/z*-value 66.10 ± 0.5 of the dopant as a function of time. Next, the XIC peak area was normalized to the peak area

of the TIC to obtain XIC/TIC. The XIC/TIC was plotted as a function of the weight percent (w/w%) of n-hexadecane-d_{34} doped into each sample (supplementary data: Figures S1a and b). The resulting unweighted, linear regression lines were not forced to zero. The plots obtained for both RJF and GO samples showed strong linearity with R^2 values over 0.999. Linearity was further evaluated using the residuals (ε), which are the differences between the experimental peak area ratios of XIC/TIC (y_i) and the peak area ratios obtained from the linear regression curves of XIC/TIC (v_i) (Eq. (1)). Equations from the regression fit line (Figures S1a and b; Equations S1a and b) were used to solve for v_i, where x is the n-hexadecane-d_{34} mass percent and y is v_i. The residual was determined for each sample and the residuals were plotted as a function of mass percent of n-hexadecane-d_{34} (Fig. 3). Based on these plots, the linear regression model was considered valid for both sample matrices on the instrument used as no correlation was observed between the residuals and the n-hexadecane-d_{34} mass percent for either RJF or GO. As no systematic errors were found, the magnitude of the MS signal should correctly reflect the weight percent of the different hydrocarbon types.

$$\varepsilon = y_i - v_i \qquad (1)$$

3.1.3. Instrument ruggedness

The ruggedness (or robustness) of a procedure is a "measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters. Ruggedness provides an indication of the methodś reliability during normal usage [6]." Tuning the mass spectrometer was predicted to be the major parameter affecting the intra-laboratory method performance. Tuning is usually conducted on a regular basis and a change in tuning creates small but deliberate variations in the MS instrument parameters. An internal standard, PFTBA, was used to automatically tune the MS. This process adjusted internal MS voltage settings so that the targeted abundance ratios were achieved for six ions derived from PFTBA. The target ion abundances are rarely reached for all six ions and some vary by over 25% (Table 1). Thus, a range of ion abundances was tested to evaluate method ruggedness, as described below.

The mass spectrometer was automatically tuned five times and the resulting tune files were used to set instrument parameters for each tuning test. A tuning test involved measuring mass spectra for five identical saturated hydrocarbon mixtures and five identical aromatic hydrocarbon mixtures created by separating the GO sample into saturated hydrocarbons and aromatic hydrocarbons, as described in the methods and materials section. The measured ion abundance ratios were then utilized in the ASTM D2425 method [1], for which the output is a weight percent for the following hydrocarbon types: cycloparaffins, aromatic compounds, saturated compounds, paraffins, mononaphthenes, dinaphthenes, trinaphthenes, alkylbenzenes, indane and tetralin, indene (C_nH_{2n-10}), naphthalene, acetylnaphthene (C_nH_{2n-14}), acetylnaphthylene (C_nH_{2n-16}), and tricyclic aromatic compounds (where n is any whole number).

The Dixon Q-test was used for the identification and removal of any outliers in the data sets. All data points within a single tune and between the five different tunes passed the Dixon Q-test and therefore, no outliers were identified for removal. Homogeneity of variance (HOV) was tested to determine whether any specific tune file resulted in a deviating data set. The Brown-Forsythe (BF) HOV test was chosen over other HOV test because this data set is small and non-normal (moderately skewed and platykurtic overall), as identified by the specific kurtosis and skewness values (Tables S2 and S3) [8–11]. The BF HOV test utilizes the median of a data set to test whether samples have equal variances. If the calculated *p*-value is greater than α then the variances in the different groups are the same. For each sample, the calculated *p*-value for every hydrocarbon type was found to be greater than α, for an α equal to
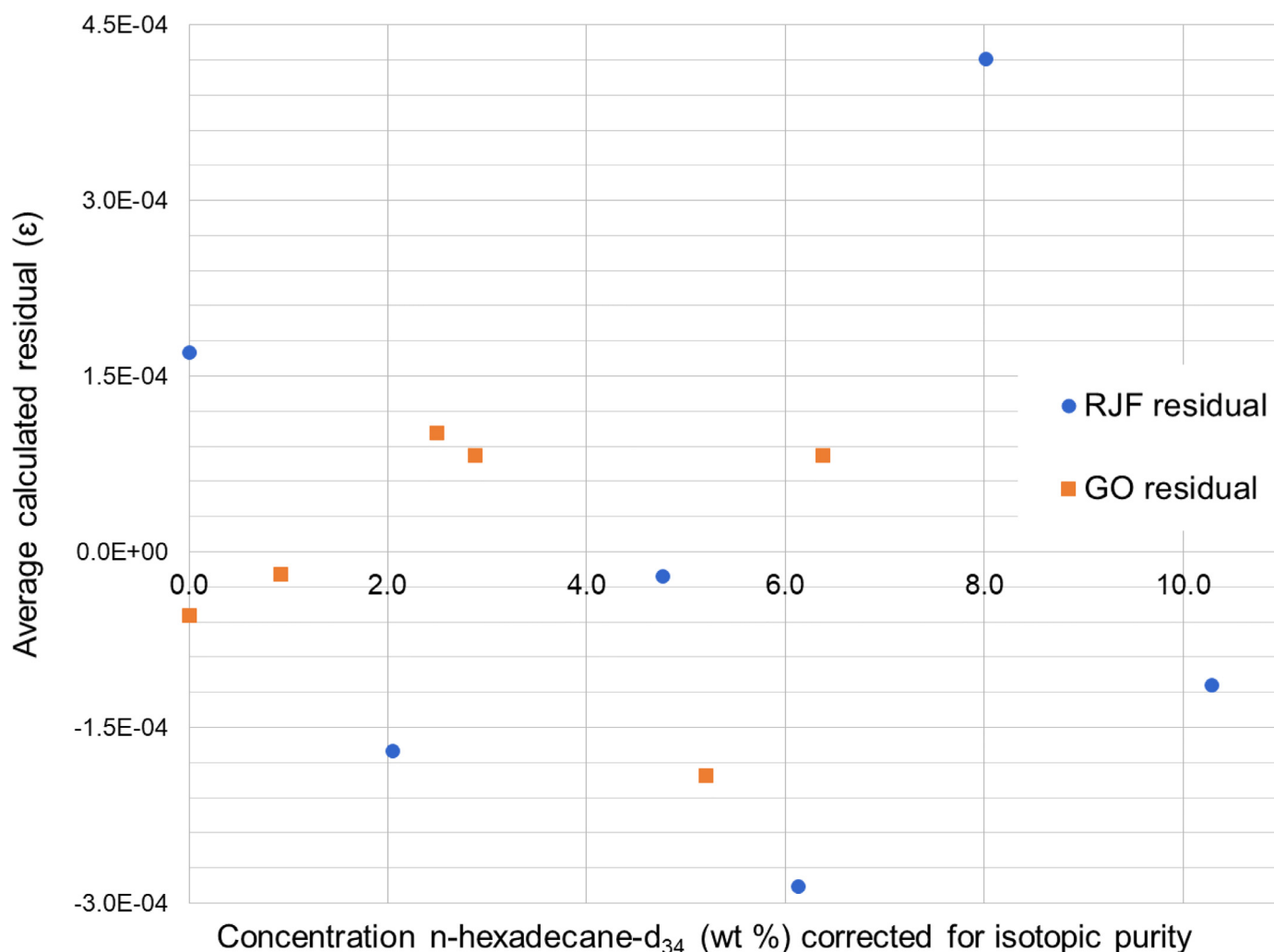
Fig. 3. Residual plots for renewable jet fuel (RJF) and gas oil (GO) samples showing no obvious correlation between the average residuals and the mass percent of n-hexadecane-d$_{34}$.

0.01 (Table S4). Therefore, the data sets were all homogeneous in variance, given an α of 0.01, and the developed method and chosen instrument type was concluded to provide robust results and repeatable data. All tune files resulted in data sets that were regularly varied, and no tune file resulted in a deviating data set. Therefore, the instrument type chosen is considered rugged with respect to tuning of the MS.

3.2. Method evaluation

The 2019 updated ASTM D2425 method was evaluated for limit of detection, limit of quantitation, and bias. They are discussed below in this order.

3.2.1. Limit of detection

To establish the lowest concentration of an analyte that can be detected (limit of detection, LOD), eight samples of RJF and GO were taken through the entire measurement procedure dictated by the 2019 updated method ASTM D2425 (outlined in Fig. 1). The MS instrument was automatically tuned for the ion abundance ratio ranges listed in Table 1, and the same tune file was used for all samples. After MS analysis, the hydrocarbon compositions were used to determine the adjusted standard deviation. The adjusted standard deviation is the standard deviation for a hydrocar-

bon type divided by the number of replicate samples, in this case eight. For validation purposes, an approximation of the LOD by using the "3s" approach can be applied, in which the adjusted standard deviation is multiplied by three [6]. Due to the large number of hydrocarbon types included in ASTM D2425, no test samples or reference sample matrices are available that would contain all the hydrocarbon types at low concentrations. Low concentrations are necessary to determine the lowest quantity of a substance that can be distinguished from the absence of that substance. However, dilution is not possible in this case because the solvent used for dilution should be the matrix blank, which can not be purchased. For this reason, LOD/LOQ values were determined for the least abundant hydrocarbon types in the RJF and GO samples and assumed to be valid for all hydrocarbon types in the respective sample matrix. Because the LOD should be determined for the least abundant analyte, the acetylnaphthylene hydrocarbon type was used for RJF LOD determination and the tricyclic aromatic compound type was used for GO LOD determination. The adjusted standard deviation for acetylnaphthylenes in RJF was 0.001 and the LOD was 0.004 w/w%. The adjusted standard deviation for tricyclic aromatic compounds in GO was found to be 0.002 and the LOD was found to be 0.01 w/w%.

3.2.2. Limit of quantitation

To establish the lowest concentration of an analyte at which quantitation is acceptable (limit of quantitation, LOQ), eight samples of RJF and GO were taken through the entire measurement procedure dictated by the updated 2019 ASTM D2425 (outlined in Fig. 1). The instrument was tuned, and the same tune file was used for all samples. After MS analysis, the hydrocarbon type compositions were used to determine the adjusted standard deviation. The LOQ was calculated by multiplying the adjusted standard deviation by a factor, k_Q, which IUPAC defines as 10 in this case [7]. Because the LOQ should be calculated for the least abundant analyte, the acetylnaphthylene hydrocarbon type was used for RJF while the tricyclic aromatic compound type was used for GO. The adjusted standard deviation for acetylnaphthylenes in RJF was found to be 0.001 and the LOQ was found to be 0.01 w/w%. The adjusted standard deviation for tricyclic aromatic compounds in GO was found to be 0.002 and the LOQ was found to be 0.02 w/w%. While the 2019 version of ASTM D2425 offers no specific LOD or LOQ values for comparison, the reporting accuracy of the 2019 update of ASTM D2425 is listed as 0.1 w/w%, which is much greater than the LOD and LOQ values obtained in this study. Therefore, the chosen instrumentation type and sample types in this study surpass the 2019 reported ASTM D2425 limits.

3.2.3. Bias

There are three general approaches for determining bias: analysis of reference materials, recovery experiments using spiked samples, and comparison with results obtained via another method [6]. As stated in the 2019 version of ASTM D2425, "there is no acceptable reference material suitable for determining the bias for this test method [1]." However, an estimation of the bias can be obtained using recovery experiments with spiked sample sets.

The RJF and GO samples were spiked with monocycloparaffins and alkylbenzenes, and paraffins and alkylbenzenes, respectively. Bias for the measurement of the abundances of these hydrocarbon types was determined by calculating a recovery percentage by using the measured (determined by the updated 2019 ASTM D2425 method) and actual (weighed amount) hydrocarbon content (w/w%) of the average value obtained for the eight spiked analyte sample sets. The relative recovery percentage was obtained by using Eq. (2). The RJF cycloparaffin content was determined to have 140% recovery and the alkylbenzene content had 63% recovery. The GO paraffin content was determined to have 117% recovery while the alkylbenzene content had 90% recovery. Both sample sets are positively biased for paraffin content (recovery percentage over 100%) and negatively biased for alkylbenzene content (recovery percentage less than 100%).

$$R'(\%) = \frac{\bar{x}' - \bar{x}}{x_{spiked}} * 100 \tag{2}$$

In Eq. (2): $\bar{x}'$ = average weight percent (monocycloparaffins and alkylbenzenes for RJF and paraffins and alkylbenzenes for GO) measured using the updated 2019 ASTM D2425 method; $\bar{x}$ = average weight (monocycloparaffins and alkylbenzenes for RJF and paraffins and alkylbenzenes for GO) for neat (non-spiked) RJF and GO samples measured using the 2019 updated ASTM D2425 method, and x_{spiked} = the actual weight percent of monocycloparaffins and alkylbenzenes added into RJF and paraffins and alkylbenzenes added into GO.

The 2019 version of ASTM D2425 updated the sample types covered by this standard test method to include RJF samples. However, the bias estimation performed here for RJF suggests that this test method may not be appropriate for RJF samples. The cycloparaffin content in RJF was determined to have 140% recovery while the alkylbenzene content had only 63% recovery. This deviates from trueness (100% recovery) quite significantly when compared to the traditional sample type (GO), where the paraffin content was determined to have 117% recovery and alkylbenzene content 90% recovery. Spiking RJF with monocycloparaffins and alkylbenzenes and GO with paraffins and alkylbenzenes was an attempt to mimic natural compounds; however, in reality, there is no acceptable reference material for determining the bias for the test method as a whole. Rather, the spiking method used in this study provides an estimation of the bias for the specific hydrocarbons added into the sample matrices. However, without a valid reference material to evaluate the whole method, this cannot be fully confirmed. For future D2425 standard versions, the bias should be assessed using a reference material, if possible. If a suitable reference material is found, the following actions should be considered: the bias should be eliminated/corrected, or the bias should be reported. If the latter approach is chosen, it should be acknowledged that the method is empirical rather than truly quantitative [12].

5. Conclusions

The limit of detection (LOD), limit of quantitation (LOQ), and bias were evaluated for Procedure B of the ASTM D2425 updated in 2019 for both sample matrices covered by this method: conventional (GO) and synthesized (RJF) hydrocarbons. First, the gas chromatograph/electron ionization quadrupole mass spectrometer (GC/(EI) Q MS) chosen for use in the procedure was evaluated. The MS tuning met the requirements given in the updated 2019 ASTM D2425. Further, the magnitude of the instrument signal as a function of analyte concentrations was linear within the relevant mass percent range. The chosen MS was also shown to be rugged for tuning; regular tuning of the instrument, which is commonly performed on all MS instruments, resulted in data sets that were regularly varied, and no deviating data sets were found. Therefore, since the instrument chosen satisfied all the test parameters given in the updated 2019 version of ASTM D2425, the GC/(EI) Q MS instrument used in this study has been proven suitable for use in Procedure B.

The measured LOD and LOQ values were 0.01 and 0.02 w/w% for GO and 0.004 and 0.01 w/w% for RJF, respectively. For RJF, bias values determined by recovery experiments were 140% and 63% for monocycloparaffins and alkylbenzenes, respectively. GO recovery values were 117% and 90% for paraffins and alkylbenzenes, respectively. Based on these results, this method may not work well for RJF samples.

For future D2425 updates, bias should be evaluated using suitable reference materials and significant measures should be made to eliminate or correct the bias for RJF. Alternatively, the bias should be reported. In general, to ensure the validity of a standard method for the intended use, it is advisable that the organization responsible of the method carefully assess and publish all relevant quantitative measures for all relevant method performance characteristics, particularly if used as a mandatory test in product certification.

Declaration of Competing Interest

The authors declare that they have no know competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

K. Elias Ikonen: Conceptualization, Formal analysis, Resources, Investigation, Writing - original draft, Supervision, Funding acquisition. **Katherine E. Wehde:** Validation, Formal analysis, Investiga-

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2019.460705.

References

[1] ASTM Standard D2425, Standard Test Method For Hydrocarbon Types in Middle Distillates By Mass Spectrometry, ASTM International, West Conshohocken, PA, 2019 www.astm.org.

[2] ASTM Standard D7566, Standard Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbons, ASTM International, West Conshohocken, PA, 2018 www.astm.org.

[3] ASTM Standard D2549, Standard Test Method For Separation of Representative Aromatics and Nonaromatics Fractions of High-Boiling Oils by Elution Chromatography, ASTM International, West Conshohocken, PA, 2017 www.astm.org.

[4] ASTM Standard D1319, Standard Test Method For Hydrocarbon Types in Liquid Petroleum Products by Fluorescent Indicator Adsorption, ASTM International, West Conshohocken, PA, 2018 www.astm.org.

[5] ASTM Standard D6379, Standard Test Method For Determination of Aromatic Hydrocarbon Types in Aviation Fuels and Petroleum Distillates – High Performance Liquid Chromatography Method with Refractive Index Detection, ASTM International, West Conshohocken, PA, 2011 www.astm.org.

[6] , Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, in: B. Magnusson, U. Örnemark (Eds.), Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2014 Available from. www.eurachem.org .

[7] L.A. Currie, International Union of Pure and Applied Chemistry (IUPAC) by, Nomenclature in evaluation of analytical methods including detection and quantification capabilities, Pure Appl. Chem. 67 (10) (1995) 1699–1723.

[8] Y. Wang, P. Rodriguez de Gil, Y.-H. Chen, J.D. Kromrey, E.S. Kim, T. Pham, D. Nguyen, J.L. Romano, Comparing the performance of approaches for testing the homogeneity of variance assumption in one-factor Anova models, Educ. Psychol. Meas. 77 (2) (2017) 305–329.

[9] S. Zhang, Fourteen homogenity of varience tests: when and how to use them, Annual Meeting of the American Educational Research Association, San Diego, CA, 1998.

[10] H.B. Lee, G.S. Katz, A.F. Restori, A Monte Carlo study of seven homogeneity of variance tests, J. Math. Stat. 6 (3) (2010) 359–366.

[11] C.S. Wright, Testing Homogeneity of Variance Master's Thesis, University of Newcastle, Australia, 2009.

[12] , Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, in: S.L.R. Ellison, A. Williams (Eds.), Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, 2012 Available from www.eurachem.org .