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RESEARCH ARTICLE

Rapid Quadrupole-Time-of-Flight Mass Spectrometry Method Quantifies Oxygen-Rich Lignin Compound in Complex Mixtures

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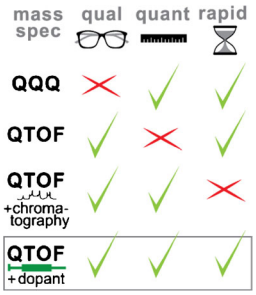

Abstract. Complex mixture analysis is a costly and time-consuming task facing researchers with foci as varied as food science and fuel analysis. When faced with the task of quantifying oxygen-rich bio-oil molecules in a complex diesel mixture, we asked whether complex mixtures could be qualitatively and quantitatively analyzed on a single mass spectrometer with mid-range resolving power without the use of lengthy separations. To answer this question, we developed and evaluated a quantitation method that eliminated chromatography steps and expanded the use of quadrupole-time-of-flight mass spectrometry from primarily qualitative to quantitative as well. To account for mixture complexity, the method employed an ionization dopant, targeted tandem mass spectrometry, and an internal standard. This combination of three techniques achieved reliable quantitation of oxygen-rich eugenol in diesel from 300 to 2500 ng/mL with sufficient linearity (R^2 = 0.97 ± 0.01) and excellent accuracy (percent error = 0% ± 5). To understand the limitations of the method, it was compared to quantitation attained on a triple quadrupole mass spectrometer, the gold standard for quantitation. The triple quadrupole quantified eugenol from 50 to 2500 ng/mL with stronger linearity (R^2 = 0.996 ± 0.003) than the quadrupole-time-of-flight and comparable accuracy (percent error = 4% ± 5). This demonstrates that a quadrupole-time-of-flight can be used for not only qualitative analysis but also targeted quantitation of oxygen-rich lignin molecules in complex mixtures without extensive sample preparation. The rapid and cost-effective method presented here offers new possibilities for bio-oil research, including: (1) allowing for bio-oil studies that demand repetitive analysis as process parameters are changed and (2) making this research accessible to more laboratories.

Keywords: Quantitation, Complex mixtures, Quadrupole-time-of-flight, QTOF, Triple quadrupole, Electrospray ionization, Diesel, Bio-oil, Tandem mass spectrometry, Internal standard

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Introduction

From food chemistry to forensic science to fuel research, complex mixture analysis by mass spectrometry (MS) is costly and time-consuming. Whether a chemist is seeking to analyze the small aromatic compounds in a flavor profile or a forensic analyst is trying to pinpoint a contaminant in a crime scene sample, ion suppression abounds. Ion suppression can cause the target molecule to falsely appear at a lower abundance or disappear from the spectrum altogether [1]. This obstacle to quantitation is caused by a decrease in ionization efficiency due to the interference of matrix ions. Current methods seek to avoid this pitfall by minimizing the complexity of the sample as it enters the ionization source [2, 3]. Extractions are often used first to fractionate complex mixtures into simpler ones [4, 5]. Sample components are further separated using lengthy chromatography methods, such as two-dimensional gas chromatography [6, 7] and high performance liquid chromatography (HPLC) [8]. Simplified samples are then analyzed using a state-of-the-art mass spectrometer with high resolving power capabilities, such as Fourier transform-ion cyclotron resonance (FT-ICR) [9, 10]. However, long analysis times and high costs limit the scope of analyses as

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well as which laboratories can perform them. Laboratories with limited means must often choose between two less expensive instruments—the triple quadrupole (QQQ) which offers low resolving power quantitative analysis or the quadrupole-time-of-flight (QTOF) which offers mid-range resolving power qualitative analysis. The question arises: can one instrument perform both qualitative analysis and targeted quantitation of a complex mixture without the use of lengthy separations?

Rapid quantitation is of particular interest in the study of renewable replacements and additives for transport fuels. One performance-enhancing diesel fuel additive that shows promise is fast pyrolysis bio-oil. Bio-oil is the liquid product of anaerobically degrading plant matter by heat and is primarily composed of carbohydrate derivatives and oxygen-rich aromatic lignin derivatives [11]. The aromatic lignin derivatives can disrupt intermolecular bonding in diesel, increasing combustion efficiency and decreasing hazardous hydrocarbon emissions [12]. Current research is focusing on which compounds can be extracted from bio-oil into diesel and in what quantities. This is of interest to the United States Department of Energy, which is studying the co-optimization of bio-oil production and engine performance and emissions. It is critically important to analyze these mixtures for co-optimization [13]. Unfortunately, due to the complexity of both bio-oil and diesel, this is not an easy task. Existing separation and analysis methods are prohibitively expensive for many bio-oil research programs and disallow the rapid tailoring and testing of bio-oil process parameters to optimize the performance-enhancing additives. Recently, researchers have sought to limit time-consuming sample preparation by implementing ambient ionization techniques, but not only are these ionization sources an added expense, they also do not mitigate ion suppression, which limits their quantitative precision [14].

These issues with complex mixture analysis can be alleviated by the method introduced in this paper, which employs the widely available QTOF mass spectrometer equipped with ESI to quantify a known small molecule in diesel. High throughput of samples is made possible by directly injecting unseparated mixtures into the instrument, minimizing sample preparation and eliminating lengthy chromatography. The limitations associated with mid-range resolving power and lack of prior chromatography are overcome by a combination of three techniques—ionization dopants, product-ion monitoring, and internal standards. The ionization dopant sodium hydroxide (NaOH) minimizes concerns of ion suppression for the oxygen-rich compound of interest [15, 16]. Targeted tandem mass spectrometry (MS/MS) alleviates the problem of isobaric matrix compounds. An internal standard compensates for any remaining fluctuations in peak height that hinder repeatable and reproducible quantitation. The utility of this method is demonstrated by quantifying the bio-oil compound eugenol in diesel fuel. By harnessing the combination of these ESI-QTOF-MS techniques, reproducible quantitation of a low-mass oxygen-rich aromatic compound was achieved in the complex mixture of diesel.

Materials and Methods

Raw Materials

Pure (>99.0%) eugenol and pure (>98.0%) 2-methoxy-4-methylphenol were purchased from TCI America. All solvents, including isopropyl alcohol (IPA), were LC-MS grade and obtained from VWR. Diesel was purchased at the local gas station. To prepare bio-oil extraction in diesel, bio-oil was produced via fast pyrolysis at 500 °C using loblolly pine biomass. Fifteen mL of pyrolysis bio-oil was mixed with 15 mL of diesel and shaken at 250 rpm for 30 min. Diesel portion was recovered by decanting.

Sample Preparation

An internal standard stock solution was prepared by diluting 2-methoxy-4-methylphenol (138.0681 *m/z*) in 9:1 (v/v) IPA/diesel to a concentration of 10 mg/mL. Standard solutions were prepared by mixing 100 μL of stock internal standard solution with adequate volumes of eugenol and 9:1 (v/v) IPA/diesel to reach a final volume of 1 mL and the following concentrations of eugenol: 50, 100, 250, 300, 350, 400, 500, 1000, 1500, 2000, and 2500 ng/mL. Quality control standards were prepared similarly on a separate day to the following concentrations: 750 ng/mL and 1750 ng/mL. The bio-oil extraction in diesel was prepared similarly to achieve a 100-fold dilution. To 10 μL of extraction solution were added 100 μL of internal standard solution and 890 μL of IPA. Eugenol (163.0837 *m/z*) was chosen as a characteristic low-mass lignin-derivative molecule for method development because of its aromatic backbone with ortho hydroxyl and methoxyl groups, comparable to those found on the building blocks of lignin, the monolignols.

Ionization Dopant

The main hurdles to overcome are spectrum complexity and ion suppression. The peaks of interest are either swallowed by a neighboring isobaric peak that has a greater abundance or do not appear in the spectrum at all because they are not efficiently ionized [2]. Addition of the ionization dopant sodium hydroxide alleviates these concerns. Hydroxide minimizes ion suppression by selectively enhancing the ionization efficiency of the bio-oil compounds of interest, causing all of those ions to appear and rise in abundance. The bio-oil compounds become easy to identify and analyze.

For uniform ionization enhancement, ionization dopant must be added equally to all quantitation standards. Adding individually to each sample would increase sample preparation time and irreversibly alter samples. A T-junction was implemented to facilitate in-line dopant injection between the autosampler and the ionization source, as seen in Figure 1. Ionization dopant is added to the mobile phase via syringe pump at a constant flow rate so that consistent deprotonation chemistry occurs in the line prior to ionization.

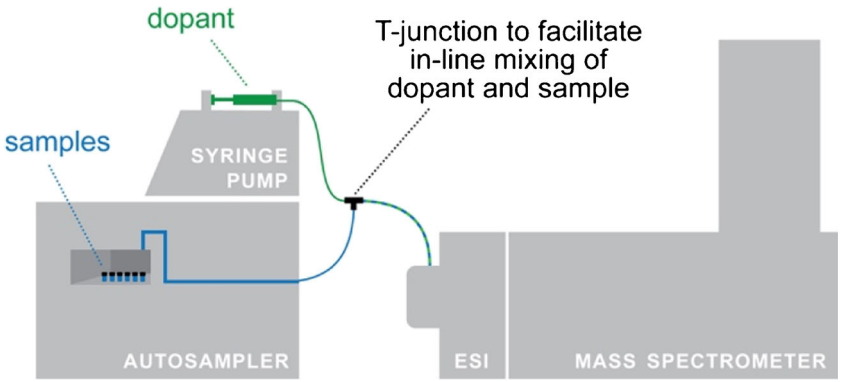

Figure 1. T-junction combines flow of mobile phase from autosampler with flow of ionization dopant from syringe pump to provide in-line chemistry for uniform ionization enhancement

Internal Standard

Although ion suppression is reduced by the addition of hydroxide, it is not completely eliminated. Peak heights change due to variations in ionization and fluctuations in the instrument, hindering a perfectly linear relationship between concentration and peak height. To ameliorate this, an internal standard is employed. The structural analogue 2-methoxy-4-methylphenol (138.07 *m/z*) was chosen as an internal standard for eugenol because of its lower cost compared with isotopically labeled eugenol. This corrects for remaining fluctuations and provides a linear relationship between concentration and peak height.

Targeted Tandem Mass Spectrometry

Although ion suppression has been accounted for, spectrum complexity remains an issue. Owing to the mid-range resolving power of the quadrupole-time-of-flight, analyte and internal standard ions may coincide with matrix ions from the diesel. Targeted MS/MS is utilized to select and fragment the analyte and internal standard ions and coinciding isobaric matrix ions. The matrix ions fragment differently than the analyte and internal standard ions, since they are a different class of compounds. It is straightforward then to monitor only the product ions of the analyte and internal standard, which are typically formed by methyl loss ($[M–H–CH_3]^-$). Product ion monitoring differentiates the analyte and internal standard from surrounding matrix ions, offering a simpler spectrum with higher sensitivity.

Validation

This method was validated by comparing the linearity, sensitivity, accuracy, and precision of the ESI-QTOF-MS method to FDA quantitation standards [17]. Once validated, the ESI-QTOF-MS method was compared to quantitation of the same samples on a QQQ-MS to assess the extent to which a laboratory would be compromising its quantitative research by only purchasing a QTOF. No ionization dopant was used with the QQQ because the QQQ was equipped with a dual-ionization source (DUIS) that combines ESI and atmospheric pressure chemical ionization (APCI), which efficiently ionized all ions of interest without the use of dopant. Since the DUIS-QQQ-MS was the setup available for this research, all the QQQ experiments were run under optimized conditions with the DUIS to allow for ionization of the analyte and internal standard. Accuracy and precision were evaluated with both inter-day and intra-day studies, where experiments were repeated on three separate days a minimum of three times each day.

Instrument Specifications

Samples were analyzed using an Agilent Technologies 6520 Accurate-Mass QTOF LC/MS (Agilent, Santa Barbara, CA, USA) equipped with 1200 series HPLC and an ESI source, operated in negative ion mode. The QTOF was operated in high resolution mode (4 GHz) with a resolving power ranging from 10,450 at 112 *m/z* to 22,842 at 1633 *m/z* and a mass range of 100 to 1700 *m/z*. The sample solutions were injected into the ESI source using the HPLC auto-sampler with the column bypassed. An injection volume of 10.00 µL was used with a mobile phase of 95:5 (v/v) ACN/water at a flow rate of 0.200 mL/min

Sodium hydroxide was injected into the line by a Harvard PhD 2000 Infusion syringe pump at a rate of 4 µL/min and a concentration of 100 mg/L. The operating conditions for optimized ion formation in the ESI source consisted of nitrogen drying gas at a temperature of 303 °C and a rate of 8 L/min, 30 psig nebulizer, 70 V fragmentor voltage, 65 V skimmer voltage, 750 V octopole voltage, 3500 V Vcap voltage, and 0.046 µA capillary current.

Target ions were subjected to collision-activated dissociation (CAD) for the purpose of product-ion monitoring. CAD experiments involved isolation of the anion by using a narrow (~1.3 *m/z*) window and acceleration of the anion to collide with nitrogen gas with collision energy of 10 as defined by the MassHunter LC/MS Data Acquisition Workstation software ver. B.05.01 for 6200 series interface. Spectra were analyzed

in profile mode with MassHunter Qualitative Analysis Workstation software ver. B.06.00 interface.

Comparative quantitation was performed on a Shimadzu Scientific Instruments LCMS-8060 Liquid Chromatograph Mass Spectrometer (Shimadzu, Kyoto, Japan) equipped with a Nexera X2 LC-30AD pump, DGU-20A 5R degassing unit, Nexera X2 SIL-30AC autosampler, CBM-20A communication bus module and a DUIS, operated in negative-ion mode. The QQQ was operated using multiple reaction monitoring (MRM) with a range 50 to 1000 *m/z*, 1500 u/s scan speed, a 0.075 s event time, and unit resolution in Q1 and Q3. The sample solutions were injected into the DUIS using the HPLC auto-sampler with the column bypassed. An injection volume of 10.00 µL was used with a mobile phase of 95:5 (v/v) ACN/water at a flow rate of 0.500 mL/min. The operating conditions for optimized ion formation in the DUIS source consisted of an APCI needle position of 5 mm, 10 L/min nitrogen drying gas flow, 10 L/min zero air heating gas flow, 3 L/min nitrogen nebulizer gas flow, 300 °C interface temperature, 200 °C DL temperature, 400 °C heatblock temperature, 3.0 kV interface voltage, and 0.3 µA interface current. Ions were fragmented in Q2 with argon gas at a pressure of 270 kPa. The instrument was controlled using Shimadzu Corporation Lab Solutions ver. 5.80 software, and spectra were analyzed in this software.

Results and Discussion

Method Validation

By combining base-assisted electrospray ionization, QTOF tandem mass spectrometry, and an internal standard, quantitation of eugenol was reliably achieved down to 300 ng/mL. The basic ionization dopant sodium hydroxide increased the relative abundance of the eugenol and 2-methoxy-4-methylphenol 40- and 10-fold, respectively (Figure 2). Although this shows that the chosen structural analogue does not ionize as similarly to the analyte as an isotopically-labeled analogue might, statistical validation (Table 1) demonstrates that the internal standard chosen still allows for good quantitation. A quantitation curve was constructed, and a linear regression was fitted to the data. The peak height of the product ion of eugenol was divided by that of the product ion of the internal standard for each concentration. The product ions utilized for both eugenol and 2-methoxy-4-methylphenol were the result of methyl-loss, as seen in Figure 3. Sufficient linearity was achieved (R^2 = 0.97

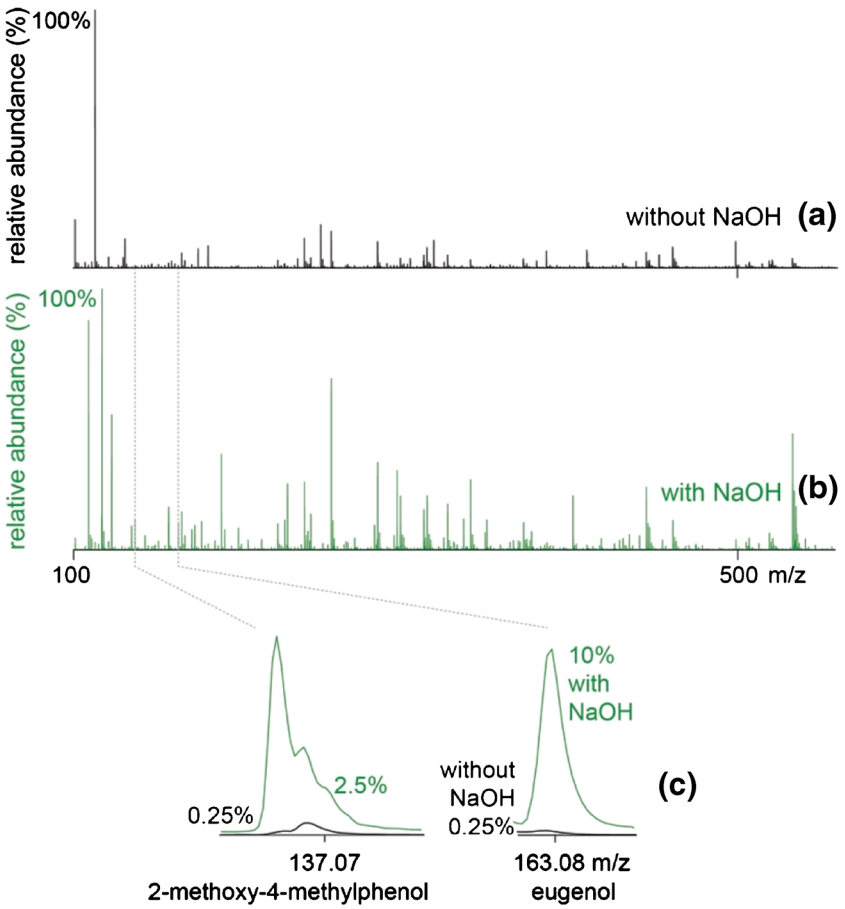

Figure 2. Addition of sodium hydroxide (NaOH) to bio-oil diesel extraction increases the intensity of bio-oil ions in ESI-QTOF-MS spectra **(b)** compared with the same sample with no NaOH added **(a)**. Magnifying and overlaying spectra A (black) and B (green) for deprotonated eugenol and 2-methoxy-4-methylphenol shows this increase in intensity explicitly **(c)**

Table 1. Accuracy and precision comparison between QTOF and QQQ

Concentration (ng/mL)	QTOF				QQQ			
	Intra-day		Inter-day		Intra-day		Inter-day	
	Precision (% CV)	Accuracy (% error)	Precision (% CV)	Accuracy (% error)	Precision (% CV)	Accuracy (% error)	Precision (% CV)	Accuracy (% error)
50	*	*	*	*	0.486	2.56 (51.3 ng/mL)	2.70	6.50 (53.3 ng/mL)
100	*	*	*	*	0.858	–1.33 (98.7 ng/mL)	1.98	–2.83 (97.2 ng/mL)
250	*	*	*	*	0.911	–2.30 (244 ng/mL)	1.95	–7.25 (232 ng/mL)
300	9.59	6.60 (320 ng/mL)	8.70	–1.18 (296 ng/mL)	†	†	†	†
350	14.0	2.84 (360 ng/mL)	10.8	–8.21 (321 ng/mL)	†	†	†	†
400	11.0	13.5 (454 ng/mL)	9.31	8.24 (432 ng/mL)	†	†	†	†
500	7.25	0.887 (504 ng/mL)	3.26	4.89 (526 ng/mL)	2.0	–0.133 (499 ng/mL)	2.2	–7.52 (462 ng/mL)
1000	15.3	–13.6 (864 ng/mL)	8.12	–5.19 (959 ng/mL)	1.2	1.55 (1020 ng/mL)	5.7	–4.26 (957 ng/mL)
1500	10.6	0.746 (1510 ng/mL)	5.30	–0.772 (1484 ng/mL)	2.3	–1.19 (1480 ng/mL)	6.5	–2.61 (1460 ng/mL)
2000	10.9	–3.5 (1930 ng/mL)	4.75	0.607 (2035 ng/mL)	2.3	2.34 (2050 ng/mL)	4	–4.68 (1910 ng/mL)
2500	6.08	–1.8 (2460 ng/mL)	3.26	–0.568 (2490 ng/mL)	3	–1.53 (2460 ng/mL)	4	–11.5 (2210 ng/mL)

Values reported to three significant figures, CV = coefficient of variation, *below LLOQ of QTOF, †these concentrations were added to QTOF quantitation standard curve in order to have eight data points but were not required for QQQ.

± 0.01, see Supplementary Figure S1 and Supplementary Table S2), suggesting success in developing a more rapid and accessible method for quantitation of small oxygen-rich molecules in complex mixtures.

The method was further evaluated in terms of sensitivity, accuracy, and precision. Sensitivity was evaluated according to the average lower limit-of-detection (LLOQ) across 3 d, which was determined to be 281 ng/mL. Originally, quantitation standards with the following concentrations were run: 50, 100, 250, 500, 1000, 1500, 2000, and 2500 ng/mL. However, due to the variability in the response of the QTOF at and below 250 ng/mL, the LLOQ was found to be above 250 ng/mL. This was calculated considering LLOQ = $10\sigma/b$ where σ is the standard error of y-intercept of the linear regressions and b is

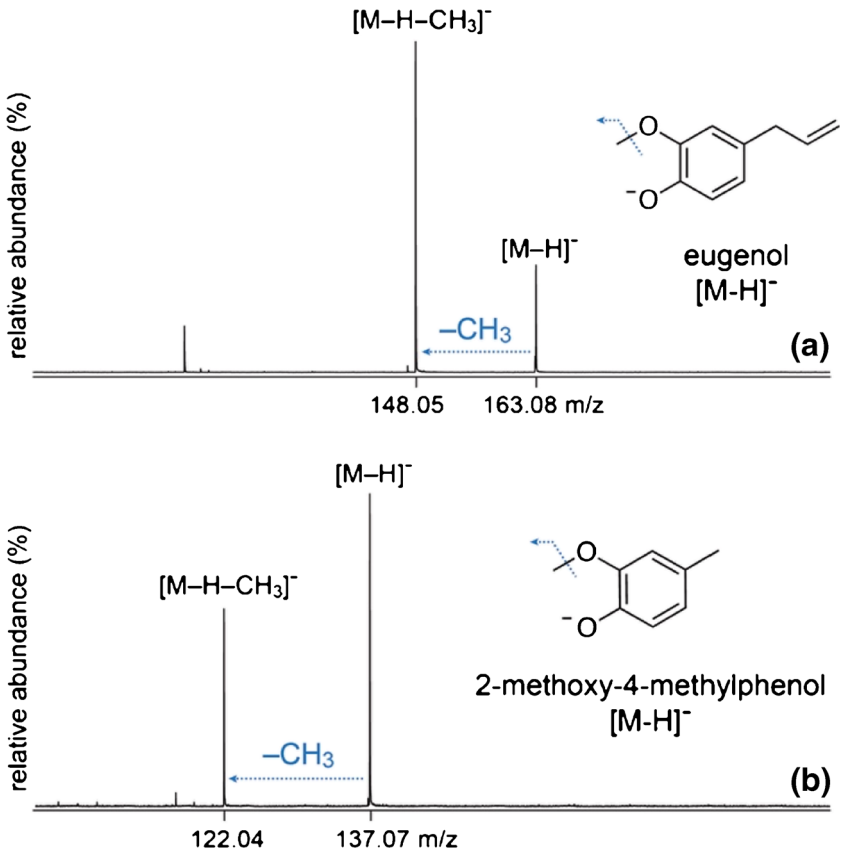

Figure 3. Stable product-ions $[M–H–CH_3]^-$ are monitored for **(a)** eugenol (163.07 *m/z* > 148.05 *m/z*), and **(b)** 2-methoxy-4-methylphenol (137.07 *m/z* > 122.04 *m/z*)

the average slope of the linear regressions [18]. Additional standards were made with mid-range concentrations (300, 350, and 400 ng/mL). When the mid-range standards were added and the low-concentration standards (50, 100, and 250 ng/mL) were removed, the linearity and precision improved and provided a calculated LLOQ of 281 ng/mL.

Accuracy was evaluated in two ways: (1) comparing experimental abundances of each standard to theoretical values on 1 d (intra-day) and across 3 d (inter-day), and (2) quantifying two quality control standards of known concentrations (750 and 1750 ng/mL). The intra-day and inter-day accuracy of the experimental abundances were well within the acceptable ±15% (Table 1) [17]. Quantitation of the two quality control standards similarly demonstrated an accuracy within the accepted range of ±15% from the theoretical value (Table 2). Owing to variation in the ionization of the complex mixture and the pulsed nature of the data collection, it is necessary to run the standards and unknown at least three times and average the data to achieve accurate results. Although this extends data collection time to 1.5 h, it allows for the use of a QTOF for quantitation of oxygen-rich compounds in complex mixtures.

Precision was evaluated according to intra- and inter-day coefficients of variation (CV%), as seen in Table 1. The intra-day and inter-day CV% also fell well within the acceptable ±15% [17]. It was found that precision was reliant on consistent ionization, as well as absence of contaminants in the system. The linearity, sensitivity, accuracy, and precision achieved with the QTOF-MS demonstrate that a QTOF can perform quantitation to FDA standards.

Comparison to Triple Quadrupole

The goal was to develop a method that not only eliminated sample preparation but also performed quantitation well enough that a second quantitation-specific mass spectrometer was not needed. After confirming that the QTOF could perform quantitation to FDA standards, it was compared with the gold standard of mass spectrometric quantitation: the triple quadrupole (QQQ). This comparison was performed to better understand the strengths and limitations of the QTOF quantitation method. Since the QQQ was equipped with a DUIS, it did not require an ionization dopant. We anticipate that the DUIS would also eliminate the need for an ionization dopant if it was used with the QTOF. Furthermore, if the QQQ is instead equipped with an ESI, it requires an ionization dopant. More quantitative comparisons of the methods were also considered.

The method in this paper achieved lower linearity ($R^2 = 0.97 \pm 0.01$) than that of the gold standard ($R^2 = 0.996 \pm 0.003$, see Supplementary Table S3). The method is also less sensitive than the gold standard (LLOQ: QTOF = 281 ng/mL, QQQ = 50 ng/mL), although the narrower dynamic range of the method is acceptable for the task at hand. It is worthwhile to note that although the QQQ is known for its excellent sensitivity, its unit resolving power could not handle the spectrum complexity as easily as the higher resolving power QTOF. Unlike in the QTOF, a matrix ion was isobaric to eugenol and had a product ion that could not be differentiated from eugenol's product ion by the QQQ. This affected the LLOQ for the QQQ. The calculated LLOQ for the QQQ was 37.2 ng/mL, but the isobaric product ion caused concentrations lower than 50 ng/mL to be indistinguishable, deeming 50 ng/mL the LLOQ. The QTOF's higher resolving power allowed it to differentiate between the eugenol product ion peak and the matrix product ion peak, so the matrix ion did not affect the LLOQ for the QTOF. In conclusion, although the QQQ offers greater sensitivity and therefore a greater dynamic range than the QTOF, it is mitigated by the QQQ's lower resolving power. In terms of accuracy, the method presented in this paper performed comparably to the gold standard, the QQQ (Table 2). In terms of precision, the method performed acceptably (mean CV%: intra-day = 10.6% ± 3.1, inter-day = 6.69 % ± 0.88), albeit not quite as well as the gold standard (mean CV%: intra-day = 1.63% ± 0.88, inter-day = 3.64 % ± 1.63). This is expected because the QTOF performs pulsed targeted MS/MS, whereas the QQQ can perform scanning multiple reaction monitoring. Overall, it was determined that the method presented in this paper performed with lower sensitivity and linearity but similar accuracy and precision.

Table 2. Validation parameter comparison between QTOF and QQQ

	QTOF	QQQ
Linearity (R^2)	0.97±0.01	0.996±0.003
Sensitivity (LLOQ)	281 ng/mL	50 ng/mL
Accuracy (mean % error)		
Quantitation standards		
Intra-day	1±8	0±2
Inter-day	0±5	–4±5
Quality control standards		
750	–4±3	3±3
1750	5±8	–4±2
Precision (mean CV%)		
Intra-day	11±3	1.6±0.9
Inter-day	6.7±0.9	4±2

Accuracy and precision data is reported as the average across all quantitation standards.

Analysis of Bio-oil Extraction in Diesel

Once the QTOF method was validated using standard solutions, a bio-oil extraction in diesel was prepared for quantitation (diluted 100-fold and doped with internal standard). The extraction solution was quantified on the QTOF along with the quantitation standards ranging from 350 to 2500 ng/mL. The concentration of eugenol in the dilute extraction solution was determined to be 900 ± 50 ng/mL by averaging the quantitative results ($R^2 = 0.965 \pm 0.009$) of three runs on the same day. This extrapolates to a concentration of 9.0 ± 0.5 mg/mL for the undiluted extraction solution. This demonstrates that our

method had the necessary sensitivity and mass range to quantify eugenol in a bio-oil extraction in diesel.

Scope of Method

The method presented in this paper was developed for targeted quantitation of a low-mass oxygen-rich lignin compound in the complex mixture of diesel. Lignin analysis using ESI with the QTOF requires an ionization dopant for consistent ionization. Compounds that contain phenolic hydrogens should be similarly enhanced by the ionization dopant sodium hydroxide and comparably quantified using this method. This has been demonstrated in diesel as well as lignocellulosic mixtures, such as fast pyrolysis bio-oils and autohydrolyzate [16]. Using a DUIS, such as was available with the QQQ, alleviates the need for an ionization dopant for low-mass oxygen-rich lignin compounds. In order to apply this ESI method to other types of compounds and complex mixtures, a preliminary qualitative study would need to be performed in order to determine the best ionization dopant for efficient ionization of the targeted compound. When using a structural analogue rather than an isotopically labeled analogue as an internal standard, large variations in the complexity of the diesel matrix may affect the robustness of the method. Since a structural analogue will not ionize identically to the analyte, accurate quantitation can be confirmed by verifying that the internal standard has the same response in the standard matrix as in the sample matrix.

Conclusion

A method was developed to analyze low-mass bio-oil compounds in an unseparated complex diesel mixture using cost-effective and widely available instrumentation. Reliable targeted quantitation was achieved using ESI-QTOF-MS by employing a combination of ionization dopant, targeted MS/MS, and an internal standard. These three techniques eliminated the need for time-consuming separations and chromatography prior to injection. By developing the method on a QTOF and eliminating the chromatography step, we answer the question asked in the introduction: it is possible for one instrument, the QTOF, to perform both qualitative analysis and targeted quantitation in complex mixtures without the use of lengthy separations. Although the QQQ is still the gold standard for quantitation, this paper demonstrates that a QTOF can also perform quantitation to FDA standards. This opens the door for laboratories to tackle research involving complex sample analysis using only one mass spectrometer. The rapid nature of the method also enables research that demands recursive analysis of complex samples, such as that required for tailoring bio-oil products by varying parameters. Although the method was developed for bio-oil compounds in diesel, it could be leveraged for targeted quantitation of oxygen-rich aromatic compounds in other complex mixtures, such as food and forensic samples.

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