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## Characterization of Trace Toxic Chemical Species: $[\text{Cr}^{3+}/\text{Cr}^{6+}]$ and $[\text{As}^{3+}/\text{As}^{5+}]$ In Alternative Fuel and Isolated By-Products Using HPLC-ICP-MS

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**Abstract:** A reformed transesterification process for biofuel production using crown ethers was developed and proved to be successful in restricting accumulation of toxic species in the biodiesel phase. Combining crown ethers (18-crown-6; 15-crown-5) with conventional catalysts (KOH/NaOH) in generating neem biodiesel resulted in reduction in the levels of  $\text{Cr}^{6+}$  and  $\text{As}^{3+}$  in the biodiesel fraction. Converse results were obtained for  $\text{Cr}^{3+}$  and  $\text{As}^{5+}$  under the same conditions. Toxicity of biofuels is receiving widespread attention, and suppressing hazardous chemical toxins in biodiesel and glycerol waste products is beneficial to sustainable development. A Perkin Elmer SCIEX DRC-e ICP-MS was coupled with a liquid chromatographic system (HPLC) for high performance studies. Elution of the toxic species followed by mass spectrometry for simultaneous detection was conducted for convenient resolution of all four species. A C-8 reinforced silica column formed the stationary phase (150 mm in length; internal diameter: 4.6 mm; particle size: 5.0  $\mu\text{m}$ ). The mobile phase was a special mixture of tetrabutylammonium hydroxide (TBAH) blended with the potassium salt of ethylenediaminetetraacetic acid (EDTA) in 5% methanol and water to a pH of 7.2. The elution rate was ~1.2 mL/min; and associated retention times (min) were:  $\text{As}^{3+}$ : 1.61;  $\text{As}^{5+}$ : 4.06;  $\text{Cr}^{3+}$ : 3.71; and  $\text{Cr}^{6+}$ : 5.80. The system is equipped with a dynamic reaction cell (DRC) to facilitate mass detection. The oxygen flow in the DRC was controlled to give a rate of ~ 4.0 mL/min. Following mass detection  $m/z$  values of 52 characterized the  $\text{Cr}^{3+}/\text{Cr}^{6+}$  species; while the arsenic components were detected as adduct ions,  $\text{AsO}^+$ ,  $m/z$  91, in affiliation with oxygen. Certified reference materials were used for purposes of calibration. In the biodiesel phase  $\text{Cr}^{3+}$  levels were most pronounced reaching levels of ~2000  $\mu\text{g/L}$ ;  $\text{Cr}^{6+}$  and  $\text{As}^{3+}$  occurred mainly at ~5  $\mu\text{g/L}$ ; whereas  $\text{As}^{5+}$  reflected minimal levels at 0.8  $\mu\text{g/L}$ . Certain trends in the results were observed on comparing levels in the glycerol and biodiesel fractions. For instance, retention of  $\text{Cr}^{3+}$  in the glycerol phase was consistently lower, while for  $\text{As}^{3+}$  it was relatively higher. In the other two cases of  $\text{Cr}^{6+}$  and  $\text{As}^{5+}$  the results varied and showed no obvious trend. The study revealed that definite environmental hazards accompanying biofuel production could be curbed by combining crown ethers with conventional catalytic treatment.

**Keywords:**  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$ , HPLC/ICP-MS, neem biodiesel, glycerol.

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### 1. INTRODUCTION

Toxic speciation in biofuel is an unexplored area of study. Characterisation, therefore, of trace toxic species  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$  in neem biodiesel breaks new ground in biofuels research. In addition curbing the levels of these species in biodiesel by deploying crown ethers has not been previously reported. These two factors encapsulate the basis of our work and mark their contribution to environmental science and research in alternative fuels.

The growing use of biodiesel worldwide [1]-[5] necessitates advanced knowledge of its toxicity [6]. Toxic components in biofuels could pose a threat to the environment and create unwanted pollution [7]-[10]. Undesirable chemical species can also inflict damage to parts of machinery and vehicles that employ biofuel [11]. An ultra-sensitive hyphenated facility comprising affiliated liquid chromatography and mass spectrometry annexed to an inductively coupled plasma unit (HPLC-ICP-MS) was deployed for toxic speciation studies of chromium and arsenic. The facility is equipped with a dynamic reaction cell (DRC) for enhanced results, and has the unique capability of separating all four species  $[\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}]$  in a single elution with high resolution mass detection [12]. Retention times tend to overlap in the HPLC segment of the process but complete separation is achieved when components are dispatched via quadrupole selection to the mass detector. The sensitivity of the technique is exceptional, reaching levels in the sub-ppb domain (ng/L); with minimal

sample pre-treatment or pre-concentration procedures. Few other contemporary techniques [13]-[17] can rival the detection limits of hyphenated plasma mass spectrometry.

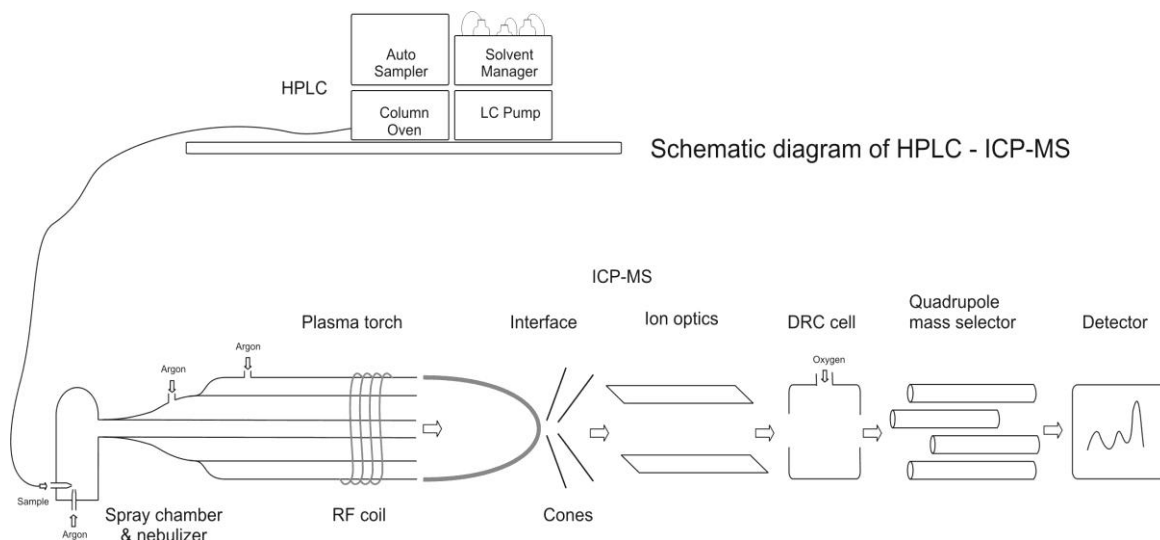
Neem biodiesel was prepared in our laboratory from neem oil feedstock with standard catalysts NaOH and KOH [18]-[20]. Crown ethers were introduced to the transesterification process and their effect was monitored [21]. We found that samples treated with crown ethers in combination with KOH and NaOH showed limited retention of  $\text{Cr}^{6+}$  and  $\text{As}^{3+}$  in the biodiesel fraction, respectively. Untreated samples gave mixed results, with NaOH treatment resulting in minimal levels of  $\text{As}^{5+}$  in both isolated fractions. Interestingly, the waste by-product (glycerol) associated with the treated and untreated samples revealed inverse trends for  $\text{Cr}^{3+}$  and  $\text{As}^{3+}$ . An interpretation of these phenomena is presented and the outcome of our results is discussed. The impact of our work on the future of biofuel engineering is considered.

## 2. MATERIALS AND METHODS

### 2.1. Sample Treatment/Mobile-Stationary Phases (HPLC)

Samples of neem oil were procured from local outlets and converted to biodiesel via transesterification using conventional catalytic treatment with NaOH and KOH. Crown ethers 18-crown-6 (CE/6) and 15-crown-5 (CE/5) were deployed *in vivo* to control retention of chemical species, as described in a previous report by the authors [21]. A Perkin Elmer quaternary LC pump in combination with a solvent manager (Fig 1) was implemented for elution of all four species -  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$ . The stationary phase consisted of a 150 mm column (C8) - internal diameter 4.6 mm, dispersed with deactivated silica, pore size: 5  $\mu\text{m}$ . The mobile phase consisted of a blended mixture of pH 7.2 (adjusted with dilute aqueous  $\text{HNO}_3/\text{NH}_4\text{OH}$ ), made up of  $1 \times 10^{-3}$  M TBAH,  $0.5 \times 10^{-3}$  M EDTA and 5% aqueous solution of methanol. The injection volume was 50  $\mu\text{L}$ , and an acceptable elution rate of 1.2 mL/min was attained. Specifications for the mobile and stationary phases are documented in Perkin Elmer reports on the subject [22] and were adapted for favourable peak symmetry and retention time. The column was prepared by running the mobile phase through the silica base before use and briefly immersing it in aqueous methanol solution following each run to preserve its integrity. The system was standardized by using certified standards procured from VHG Labs, UK.

### 2.2. Dynamic Reaction Cell (DRC)/Hyphenated Mass Spectrometry



**Fig1.** Schematic of the HPLC-DRC-ICP-MS system

Mass differentiation of the eluted species was achieved with a quadrupole selector in a Perkin Elmer SCIEX DRC-e ICP-MS (Fig 1). The gas flow originating from the nebuliser was 0.80 L/min. The species in question were transported to a dynamic reaction cell (DRC) for interaction with oxygen gas (flow rate 0.4 mL/min) to facilitate mass detection in the absence of interferences. For instance  $\text{ArC}^+$  interferences with  $\text{Cr}^+$  at  $m/z$  52 were minimized by use of the DRC. And  $\text{As}^+$  coalesced with oxygen in the cell to form an adduct ion,  $\text{AsO}^+$ ,  $m/z$  91, to suppress interferences with unwanted species such as  $\text{ArCl}^+$  and  $\text{CaCl}^+$  at  $m/z$  75 [22]. The ICP-MS was calibrated with suitable standards (Fluka 70007;

10.00 ppb per element); and repeatability studies produced relative standard deviations <5% indicating that the performance of the system was acceptable (Table1). Minor aberrations in instrumental performance were adjusted by use of an internal standard. Samples and standards were all made up in aqueous solution consistent with the mobile phase. The software corrected for random matrix effects.

### 3. RESULTS AND DISCUSSION

#### 3.1. Retention Times/Collision Cell Technology

The primary objective of employing a hyphenated facility combining HPLC with plasma mass spectrometry is to bypass the problem of overlapping retention times. Inspection of the standard chromatograms in Fig 2 shows that under conventional HPLC operation all four species cannot be distinctly resolved. The recorded retention times (min) are as follows: As<sup>3+</sup>: 1.61; As<sup>5+</sup>: 4.06; Cr<sup>3+</sup>: 3.71; Cr<sup>6+</sup>: 5.80. It is evident from the experimental data (Fig 2) that As<sup>5+</sup> overlaps with Cr<sup>3+</sup>; and its tail could interfere with Cr<sup>6+</sup>. These retention times can be offset by controlling the elution flow rate, but their proximity to one another is seldom altered unless the properties of the stationary and mobile phases are changed. Asymmetry due to tailing could also contribute to overlap, but in our study the level of tailing was found to be acceptable and in line with standard operating procedures [22]. It is necessary to underscore that the phenomenon of overlapping retention times does not particularly create a setback in analysis mainly because the convenience of the technique is based on mass distinction, which is fulfilled by transporting the eluted species to the mass detector for well-defined resolution. A quadrupole selector plays a vital role as mass separator by guiding the isolated eluents and distinguishing the species based on their masses. Our technique implemented collision cell technology, which is well documented [12]. The dynamic reaction cell (DRC) or collision cell attached to the system plays a pivotal role and its significance is worth mentioning. Certain species created within the instrument at the time of detection could interfere with the analysis [6]. For example Cr<sup>+</sup> occurs at m/z 52 and ArC<sup>+</sup> (also present at m/z 52) interferes with it. Use of oxygen in the DRC dissociates ArC<sup>+</sup> and thus removes the interference. Similarly, As<sup>+</sup> occurs at m/z 75 and instrumental species ArCl<sup>+</sup> and CaCl<sup>+</sup> at m/z 75 creates an interference. Within the cell As<sup>+</sup> complexes with oxygen to form an adduct-ion, AsO<sup>+</sup>, detected at m/z 91, thus bypassing the interfering species. Sample injection volumes are low (50 µg/L), which is particularly useful for repeatability studies. The entire facility is a specially designed unit that is ultra-sensitive, copes with high sample throughput and is adapted for high performance.

**Table1.** Repeatability test of the ICP-MS for various elements (µg/L) in an aqueous standard showing the mean, standard deviation (SD) and relative standard deviation (SD%)

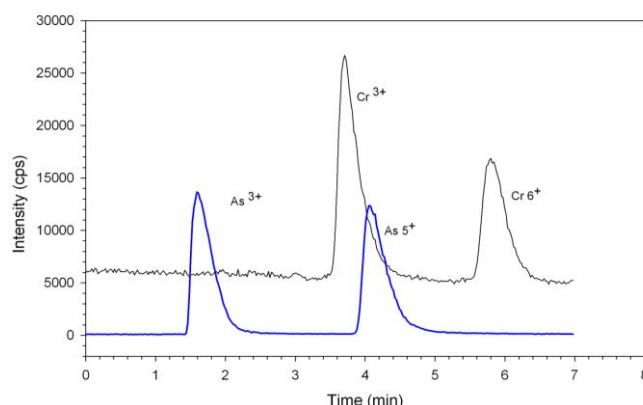
Element	Trial #1	Trial #2	Trial #3	Mean	SD	SD%
V	10.01	9.36	9.82	9.73	0.33	3.43
Cr	9.51	10.04	10.07	9.87	0.32	3.19
Mn	9.59	10.06	10.21	9.95	0.32	3.25
Co	10.54	9.82	10.12	10.16	0.36	3.56
Ni	10.08	9.46	9.56	9.70	0.33	3.43
As	9.82	10.21	9.74	9.92	0.25	2.53
Se	9.78	9.59	9.61	9.66	0.10	1.08
Mo	9.89	9.14	9.72	9.58	0.39	4.10
Cd	9.51	10.06	10.09	9.89	0.33	3.30
Tl	9.59	9.65	10.21	9.82	0.34	3.48
Th	9.85	10.28	9.42	9.85	0.43	4.37
U	10.08	9.65	10.12	9.95	0.26	2.62

#### 3.2. Characterization of Toxic Species

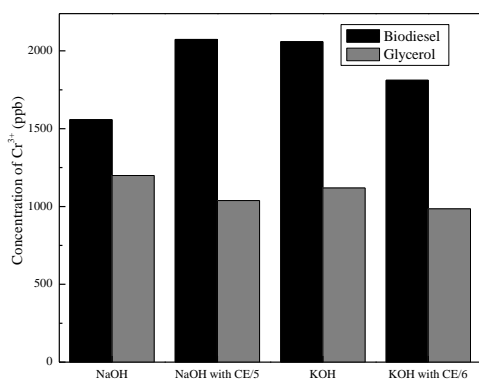
The transesterification process produced two phases: the biodiesel phase and the glycerol waste product. The toxic species of interest originated from the neem oil feedstock are linked to organic or inorganic chemical compounds, which could either migrate into the biodiesel phase, or into the glycerol fraction. The level of migration would depend on several factors [23] such as the reaction conditions, completion of the transesterification process, water contamination and the physical and chemical properties of the chemical compounds affiliated with these species and their affinity for either phase. Plots for all four species showing levels of retention in both phases appear in Figs 3-6.

3.2.1.  $Cr^{3+}/Cr^{6+}$ 

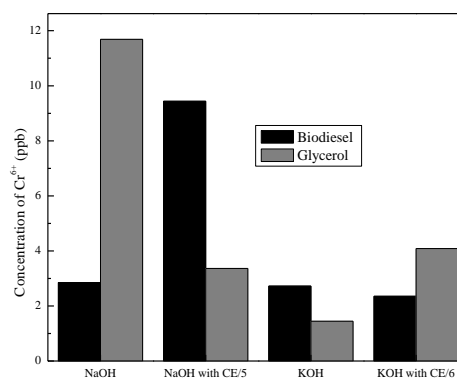
In general the plot for  $Cr^{3+}$  compared to  $Cr^{6+}$  shows that  $Cr^{3+}$  was present at considerably higher levels in both fractions (Figs 3 and 4), suggesting a stronger affinity of  $Cr^{3+}$  for the biodiesel phase. Interestingly, a distinct pattern is observed for  $Cr^{3+}$  (Fig 3) reflecting elevated retention in the biodiesel fraction in general. Comparatively diminished levels - by more than a factor of two in some cases - were found in the corresponding glycerol by-product. The application of crown ethers had little effect on relegating  $Cr^{3+}$  to the glycerol phase. This could possibly be attributed to strong attraction of organic  $Cr(III)$  compounds to the biodiesel phase [24], which, according to the data, tends to serve as a powerful medium of solubility for  $Cr^{3+}$  species. On the other hand, the case of  $Cr^{6+}$  depicted a mixed trend (Fig 4) with varying levels of migration in the biodiesel and glycerol phases.



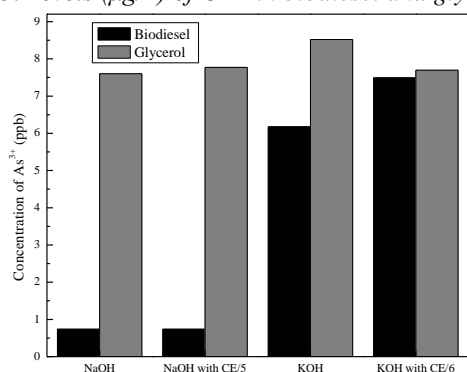
**Fig2.** Typical HPLC chromatograms showing retention times for  $As^{3+}/As^{5+}$  and  $Cr^{3+}/Cr^{6+}$



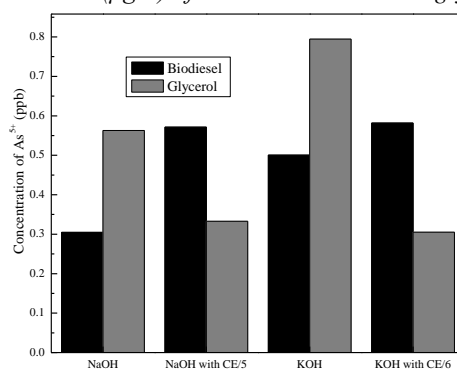
**Fig3.** Levels ( $\mu\text{g/L}$ ) of  $Cr^{3+}$  in biodiesel and glycerol



**Fig4.** Levels ( $\mu\text{g/L}$ ) of  $Cr^{6+}$  in biodiesel and glycerol



**Fig5.** Levels ( $\mu\text{g/L}$ ) of  $As^{3+}$  in biodiesel and glycerol



**Fig6.** Levels ( $\mu\text{g/L}$ ) of  $As^{5+}$  in biodiesel and glycerol

An interesting feature is that treatment with KOH/(CE/6) produced a comparatively reduced level of retention for  $Cr^{6+}$  in the biodiesel fraction ( $\sim 2 \mu\text{g/L}$ ), which sharply contrasts with  $Cr^{3+}$ , present in the biodiesel phase at levels of  $\sim 2000 \mu\text{g/L}$  - more than a factor of 900 (Figs 3 and 4). It is not clear why the results are reversed in this particular case, and suggests a decidedly weak alliance of  $Cr^{6+}$  compounds with the biodiesel phase.

### 3.2.2. $\text{As}^{3+}/\text{As}^{5+}$

The  $\text{As}^{3+}$  plot delineated a reverse trend to that for  $\text{Cr}^{3+}$  (Figs 5 and 3). In the case of  $\text{As}^{3+}$  the glycerol fraction showed greater retention for this particular species, due probably to the presence of inorganic  $\text{As}_2\text{S}_3$  and  $\text{H}_3\text{AsO}_3$  in neem oil feedstock (which is derived from the neem plant) [25]. Treatment with NaOH and NaOH/(CE/5) produced highly diminished levels of  $\text{As}^{3+}$  in the biodiesel fraction. This contrasts to a large degree with the corresponding  $\text{Cr}^{3+}/\text{Cr}^{6+}$  plots (Figs 3 and 4) and could be attributed to the metal/metalloid nature of chromium and arsenic [6]. The  $\text{As}^{5+}$  plot somewhat resembles the  $\text{Cr}^{6+}$  plot and depicted a mixed trend.

The levels of  $\text{As}^{5+}$  are the lowest among the four species in both phases suggesting that As(V) complexes are not prolific in neem oil feedstock. An inspection of the  $\text{As}^{3+}$  and  $\text{As}^{5+}$  plots (Figs 5 and 6) reveals that catalytic treatment with NaOH and NaOH/(CE/5) produces comparable levels of both species in the biodiesel phase. This is not the case with corresponding treatment with KOH and KOH/(CE/6). The inference is that it is difficult to predict the migratory tendencies of the arsenic species (and those of chromium) based on the performance of the catalysts. As aforementioned, reaction conditions, the presence of water and completion of the transesterification process all play a strong role in determining the presence of these species in either phase.

### 3.3. Impact of the Study/Environmental Implications

The study brings new information to the field of biofuels research. In particular, the noxious nature of  $\text{Cr}^{6+}$  and  $\text{As}^{3+}$  should not be minimised. A significant feature of this study is that retention of these species in the glycerol by-product is equally important for purposes of environmental protection. Sustainable living relates directly to environmental safety and there could be a hazard if these species find their way to the ecosystem [23]. It is known that  $\text{Cr}^{6+}$  has a higher toxicity rating than  $\text{Cr}^{3+}$  [6] and is responsible for serious disorders in the human body at elevated levels. On the other hand the lower oxidation state of arsenic,  $\text{As}^{3+}$ , tends to be more toxic than  $\text{As}^{5+}$ , and could be equally detrimental to living organisms at elevated levels [26]. Permissible levels of all four species appear elsewhere [6] and our study has shown that for neem biodiesel in particular the  $\text{Cr}^{3+}$  levels are exceedingly elevated producing a maximum level of  $\sim 2000 \mu\text{g/L}$ . In general  $\text{Cr}^{6+}$  and  $\text{As}^{5+}$  levels (Figs 4 and 6) are comparable with environmentally accepted levels [6]. Of significance though is that  $\text{As}^{3+}$  is elevated by about factors between 6-8 in some cases (Fig 5) indicating that it could be present at toxic levels and there is a definite need to remove the hazard by treating the biodiesel product chemically.

A point to note that is these species are not ordinarily determined unless the technique employed is particularly unique. Conventional HPLC fails to resolve all four species discretely. The hyphenated facility, however, of HPLC-ICP-MS provides both the sensitivity and means for facile determination of these species. A major impact of this study is undoubtedly the fact that crown ethers played a role in selective retention of these species in the biodiesel fraction. Treatment with NaOH/(CE/5) minimized retention of  $\text{As}^{3+}$  in biodiesel (Fig 5); while KOH/(CE/6) diminished  $\text{Cr}^{6+}$  (Fig 4). The data suggest that NaOH catalyst performs more effectively than KOH for suppression of these species in the biodiesel fraction. Although the yield may not be as favorable with NaOH [5] it is recommended in the interest of environmental safety that NaOH be employed as a catalyst for general biodiesel production. One of the prime motives of “detoxifying” biofuels is to improve sustainable living. It is important to bear in mind that biofuels are considered to be a source of clean energy, but their use is not of much value if unnecessary pollution is created.

## 4. CONCLUSION

The investigation of  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$  in biofuel has not been previously documented and breaks new ground. Our study showed clearly that addition of crown ethers to the transesterification process with standard catalysts NaOH and KOH tends to suppress retention of  $\text{Cr}^{6+}$  and  $\text{As}^{3+}$  in the biodiesel fraction (respectively). It is important to stress, however, that results could vary depending on experimental conditions. Factors such as incomplete reactions, water contamination and the presence of impurities could affect the results. The instrumental technique combining liquid chromatography with high resolution ICP-MS provides a convenient method for characterisation of these species. Detection of chemical toxins in biofuels constitutes an important body of research linked to environmental science and sustainable living. Waste products such as glycerol are usually

dumped and there is a need, therefore, for characterisation of toxins in such by-products. This study could be extended to the determination of other toxic species, including those associated with mercury and antimony.

#### ACKNOWLEDGEMENTS

The authors wish to thank the Petroleum Institute for financial support

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