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PAPER

CRIMINALISTICS

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The Development of Analytical and Interpretational Protocols to Facilitate the Provenance Establishment of Polycarbonate Headlamp Lens Material*

ABSTRACT: Despite the forensic significance of polycarbonate headlamp lenses, robust analytical protocols to facilitate their discrimination are scarce. In this study, laser ablation inductively coupled plasma mass spectrometry has been applied to the analysis of polycarbonate headlamp lenses with multivariate chemometrics techniques utilized to facilitate interpretation of the data. The analytical protocol involves the analysis of 46 analytes on material comprising the exterior surface of the lens. Using this data, it was found that although minor variation exists within a single headlamp lens, discrimination between lenses produced from a single manufacturing plant was still possible using iterative forward stepwise linear discriminant analysis processes. Discrimination between all headlamp lenses, with the exception of some lenses produced on the same day in a single plant, could be achieved using the analytical protocol. Furthermore, an interpretational protocol has been developed that has successfully classified all tested headlamp lens samples, within the discrimination limits of the analytical method.

KEYWORDS: forensic science, trace evidence, polycarbonate headlamp lenses, elemental analysis, laser ablation inductively coupled plasma mass spectrometry, chemometrics

The value of headlamp lens material in forensic investigations has been recognized for *c.* 60 years with its primary application being the identification of a vehicle involved in a hit and run incident (1). Previous studies have investigated the analysis of headlamp lens glass utilizing a wide variety of analytical instrumentation including refractive index (1), spark source mass spectrometry (2), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (3), X-ray fluorescence (XRF) (4,5), laser-induced breakdown spectroscopy (6), inductively coupled plasma mass spectrometry (ICP-MS) (5,7), and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (7).

However, with the exception of a 2001 dissertation (8), there is minimal available literature detailing the analysis of plastic (polycarbonate) headlamp lenses. Although approval to utilize plastic material for headlamp lenses was only granted in Australia and Europe during the early 1990s, plastics have been legally used for this purpose in the U.S.A. since 1979 (9). Consequently, polycarbonate headlamp lenses have been available for many years, with their popularity steadily increasing because of several distinct advantages over glass headlamp lenses. Polycarbonate headlamp lenses are manufactured using an injection molding method, whereby granulated starting material is melted in a heated barrel prior to injection at high pressure into a closed mold. This process leads to an increase in design flexibility and reduction in production costs compared to the manufacture of glass headlamp lenses. Furthermore, an increased durability and reduction in weight resulting from the utilization of polycarbonate material has led to their widespread use throughout the automotive industry (9). Therefore, the development of analytical protocols that would facilitate the unambiguous provenance establishment of polycarbonate headlamp lenses would be of significant benefit to the forensic analyst.

While data for the analysis of polycarbonate headlamp lenses have not appeared extensively in the literature before, the elemental analysis of polymer material has been investigated for many years. Dissolution of a variety of polymers and subsequent analysis using atomic absorption spectroscopy (AAS) (10), ICP-AES (11,12), and ICP-MS (13–15) has been performed. However, the sample preparation techniques required to facilitate the analysis result in the destruction of polymer material, an undesirable characteristic in forensic investigations. Consequently, analytical instrumentation that requires minimal sample preparation and facilitates a relatively non-destructive analysis of solid material is preferred.

Graphite furnace AAS (GFAAS) (16), XRF (17), laser-induced plasma spectrometry (LIPS) (18), and LA-ICP-MS (19) have all been utilized to facilitate the analysis of solid plastic material. These studies typically involved the analysis of between six and 14 elements with the aim being to determine the composition of the polymer. However, none of these studies were designed to investigate the utilization of the elemental composition of the material to facilitate discrimination between samples. Consequently, the purpose of this study was to develop a suitable analytical and interpretational protocol that would enable discrimination between polycarbonate headlamp lenses manufactured from a single

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location. In doing so, the protocols would be examined under the most difficult conditions, ensuring their applicability to real world forensic samples where greater variation in the source of the headlamp lenses would result in greater differences in elemental compositions and subsequently facilitate easier discrimination between samples.

Methods

Sample Preparation

Polycarbonate headlamp lenses from three different vehicle types were collected from Hella Australia Pty. Ltd. (Mentone, Vic., Australia). Sixteen samples of each headlamp type were provided. Samples were also taken on a number of different dates of manufacture (Table 1). Five subsamples, taken from areas across the headlamp lens, were collected for analysis. A power drill was utilized to drill two large holes diagonally opposite to each other, forming two points of a square of size 20×20 mm. A jigsaw was then used to cut the outline of the square and obtain the subsample. The five areas sampled across each headlamp lens are detailed in Fig. 1. These subsamples were then labeled to differentiate between the inner and outer surfaces and stored in labeled plastic sample bags.

Analysis

All polycarbonate headlamp lenses were analyzed using LA-ICP-MS with the New Wave UP-213 laser ablation unit (New Wave Research Inc., Fremont, CA) coupled to an Agilent 7500CS ICP-MS (Agilent Technologies Inc., Santa Clara, CA). Four headlamp lenses from each variety collected (12 headlamp lenses in total) were analyzed with triplicate analyses undertaken at two sites on both sides of the subsample (five subsamples per headlamp lens). The laser operating parameters included a line scan utilizing a 100 μm laser crater spot size. The 213 nm laser was operated at 80% energy (12 mJ fluence), 20 Hz frequency, and 40 μm/sec scan rates with 20 sec of ablation. All 16 headlamp lenses from each variety collected (48 headlamp lenses in total) were analyzed with triplicate analyses undertaken on one site of one side of the five subsamples per headlamp lens using the same laser operating parameters. The raw data resulting from these analyses were processed using Glitter™ (GEOMAC, Macquarie University, Sydney, NSW, Australia) data reduction software to generate counts per second data whereby the resulting intensity is the mean ion count rate over a selected ablation period with the subtraction of a blank analysis period to account for instrument background.

Because of variations in the day-to-day setup characteristics of the laser ablation and ICP-MS systems and the occurrence of some degree of system drift with time, it was necessary to run the NIST 610 Certified Reference Glass Standard throughout all analytical protocols. Triplicate analyses were undertaken on the NIST 610 Glass Standard following every 15 analyses that were performed on the samples. Drift correction of the data associated with each day of analysis was then undertaken for each isotope with reference to the NIST 610 Glass Standard. Furthermore, the analytical data for all samples and standards were cross-normalized with reference to the NIST 610 Glass Standard so that it was possible to compare unbiased data from different days and establish if true isotopic differences existed between different headlamp lenses. Statistical treatment of the final data has then been undertaken utilizing XLSTAT 2008 (Addinsoft, Paris, France).

Results and Discussion

Homogeneity of Headlamp Lenses

Four headlamp lenses from each of the three models were investigated with five subsamples collected from each headlamp lens and analyses performed in triplicate on both sides of the headlamp lens at two sites per side (720 analyses in total). Only the interior and exterior surfaces of the headlamp lenses were analyzed as contamination of the bulk surface would have arisen from the cutting methods utilized during subsampling. However, given the durability of polycarbonate, it is likely that the interior and exterior surfaces of the headlamp lens will be available for analysis with the majority of crime scene debris, negating the need to analyze a bulk surface.

The data for each of the four headlamp lenses from each headlamp model were divided into interior and exterior surface groups prior to normalization to sodium to minimize the effects of variations in the laser to sample coupling efficiency. Following normalization, the initial analyte list of 61 isotopes (Table 2) was refined to exclude multiple isotopes of the same element and remove any analytes that were not present above the detection limits of the instrumentation by excluding those isotopes generating instrument responses of less than 100 net counts per second. The final analyte

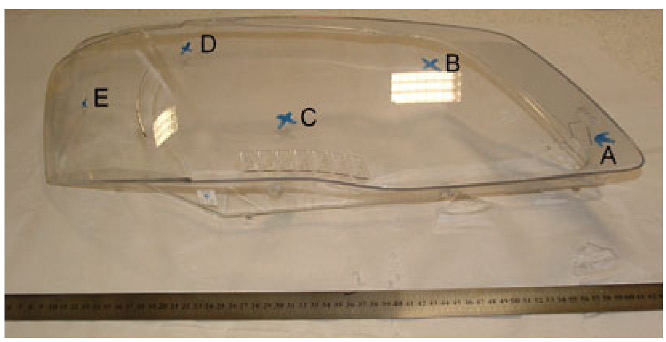

FIG. 1—*Image of polycarbonate headlamp lens detailing areas of subsampling.*

TABLE 1—*List of polycarbonate headlamp samples obtained from Hella Australia (Mentone, Vic., Australia).*

Sample Type	Date of Manufacture, Side of Vehicle, and Quantity
Toyota 042L (Camry) (Makrolon® AL2447 resin)	06/12/2006 RHS (2); 06/12/2006 LHS (2); 11/01/2007 RHS; 15/01/2007 RHS; 15/01/2007 LHS (2);16/01/2007 LHS; 19/01/2007 LHS; 29/01/2007 RHS; 30/01/2007 RHS; 03/02/2007 LHS; 05/02/2007 RHS; 09/02/2007 RHS; 12/02/2007 LHS
Holden WM (Caprice) (Lexan LS2 resin)	13/10/2006 RHS (2); 13/10/2006 LHS (3); 27/11/2006 RHS; 27/11/2006 LHS; 18/01/2007 RHS (2); 18/01/2007 LHS (3); 13/02/2007 RHS (2); 13/02/2007 LHS (2)
Ford Copperhead (Falcon) (Makrolon® AL2647 resin)	01/12/2006 RHS (2); 01/12/2006 LHS (2); 22/01/2007 RHS (2); 22/01/2007 LHS (2); 23/01/2007 LHS; 24/01/2007 LHS; 29/01/2007 RHS (2); 07/02/2007 RHS; 07/02/2007 LHS (2); 08/02/2007 RHS

Number in parentheses represents quantity of each headlamp lens.

TABLE 2—*List of isotopes detected on the polycarbonate headlamp lenses with underlined isotopes observed above detection limits on the exterior surface of the headlamp lenses and bold font style isotopes observed above detection limits on the interior surface of the lenses.*

$\underline{\mathbf{^{7}Li}}$	$\underline{\mathbf{^{23}Na}}$	$\underline{\mathbf{^{24}Mg}}$	$^{25}\mathrm{Mg}$	$\underline{\mathbf{^{27}Al}}$	$\underline{\mathbf{^{39}K}}$	$^{42}\mathrm{Ca}$	$\underline{\mathbf{^{44}Ca}}$	$\underline{\mathbf{^{45}Sc}}$
$^{48}\mathrm{Ti}$	$\underline{\mathbf{^{49}Ti}}$	$\underline{\mathbf{^{51}V}}$	$^{52}\mathrm{Cr}$	$\underline{\mathbf{^{53}Cr}}$	$\underline{\mathbf{^{55}Mn}}$	$\underline{\mathbf{^{57}Fe}}$	$\underline{\mathbf{^{59}Co}}$	$\underline{\mathbf{^{60}Ni}}$
$\underline{\mathbf{^{63}Cu}}$	$^{64}\mathrm{Zn}$	$^{65}\mathrm{Cu}$	$\underline{\mathbf{^{66}Zn}}$	$\underline{\mathbf{^{71}Ga}}$	$\underline{\mathbf{^{72}Ge}}$	$\underline{\mathbf{^{85}Rb}}$	$\underline{\mathbf{^{88}Sr}}$	$\underline{^{89}\mathrm{Y}}$
$\underline{\mathbf{^{90}Zr}}$	$^{91}\mathrm{Zr}$	$\underline{^{93}\mathrm{Nb}}$	$^{96}\mathrm{Mo}$	$\underline{^{98}\mathrm{Mo}}$	$^{112}\mathrm{Cd}$	$^{114}\mathrm{Cd}$	$\underline{\mathbf{^{118}Sn}}$	$^{120}\mathrm{Sn}$
$\underline{\mathbf{^{121}Sb}}$	$^{133}\mathrm{Cs}$	$\underline{\mathbf{^{138}Ba}}$	$\underline{^{139}\mathrm{La}}$	$\underline{^{140}\mathrm{Ce}}$	$\underline{^{141}\mathrm{Pr}}$	$\underline{^{146}\mathrm{Nd}}$	$\underline{^{147}\mathrm{Sm}}$	$\underline{^{153}\mathrm{Eu}}$
$\underline{^{157}\mathrm{Gd}}$	$\underline{^{159}\mathrm{Tb}}$	$\underline{^{163}\mathrm{Dy}}$	$\underline{^{165}\mathrm{Ho}}$	$\underline{^{166}\mathrm{Er}}$	$\underline{^{169}\mathrm{Tm}}$	$\underline{^{172}\mathrm{Yb}}$	$\underline{^{175}\mathrm{Lu}}$	$\underline{^{178}\mathrm{Hf}}$
$^{181}\mathrm{Ta}$	$\underline{\mathbf{^{202}Hg}}$	$^{206}\mathrm{Pb}$	$^{207}\mathrm{Pb}$	$\underline{\mathbf{^{208}Pb}}$	$\underline{^{232}\mathrm{Th}}$	$\underline{^{238}\mathrm{U}}$		

TABLE 3—*Mean variation found within analytes in the three models of headlamps.*

	Interior Surface	Exterior Surface
Copperhead %RSD	81.0	15.0
WM %RSD	93.1	13.2
042L %RSD	127.9	11.7

%RSD, percentage value for the relative standard deviation.

lists included 46 analytes for the exterior surface (underlined in Table 2) and 26 analytes for the interior surface (shown in bold font style in Table 2). Although recent studies (20) have detailed a quantitative approach to the analysis of glass using LA-ICP-MS, no matrix-matched standards are commercially available for polycarbonate headlamp lenses and thus a qualitative approach combined with the application of multivariate statistical techniques is appropriate in this investigation.

The percentage value for the relative standard deviation (%RSD) was determined for each of the analytes by division of the standard deviation by the mean value for each analyte. The mean %RSD values for both the interior and exterior surfaces of the three models of headlamp lenses analyzed are detailed in Table 3. From these results, it can be seen that the variation exhibited by the interior surface is far greater than that of the exterior surface. Consequently, analysis of the exterior surface is preferable for the three models of headlamps.

The lower variation in the analytes on the exterior surface of the headlamp lenses can be attributed to the increased levels of these analytes on this surface. Examination of the inter-element ratios revealed significant ($\alpha = 0.05$) differences between multiple pairs of analytes for the interior and exterior surfaces, such as $^{27}\mathrm{Al}$:$^{90}\mathrm{Zr}$ ($p = 5 \times 10^{-7}$) and $^{49}\mathrm{Ti}$:$^{53}\mathrm{Cr}$ ($p = 2 \times 10^{-29}$), confirming that the increased levels of the analytes were the result of differences in the trace element profile between the two surfaces and not because of variations in coupling efficiency. The differences between the trace element profiles of the interior and exterior surfaces can be attributed to the application of a coating onto the exterior surface of the headlamp lens for improved handling, durability, and optical properties (21). However, despite the mean %RSD values for the exterior surface of headlamp lenses being significantly lower than those of the interior surface, the values were potentially sufficient to facilitate the separation of a single headlamp lens into multiple groups when the data were interpreted if homogeneity issues were not taken into consideration. Therefore, the homogeneity of the exterior surface at the five subsample sites was investigated for the four headlamp lenses from each of the three headlamp models using principal component analysis (PCA) and linear discriminant analysis (LDA).

Ten of the 12 headlamp lenses analyzed exhibited no natural groupings in scores plots generated from PCAs performed on the data. However, two headlamp lenses revealed the natural grouping of some subsamples away from the remainder of the scores. This potentially indicated that these subsamples could be discriminated from the remainder of the headlamp lens from which they originated. Analysis of variance (ANOVA) tests were performed on the data relating to these headlamp lenses and supported the discrimination of these subsamples away from the remainder of the lenses. However, when this separation was investigated further utilizing forward stepwise LDA, no suitable LDA model could be generated to facilitate this discrimination. Therefore, it was concluded that although subtle differences may be present within the trace elemental composition of subsamples from a single polycarbonate headlamp lens, the utilization of the appropriate statistical techniques for interpretation of the data will minimize their impact.

The applicability of the analytical protocol is ultimately dependent upon the ability of the technique to facilitate a match between a subsample and the original headlamp lens (its source). This requires the differences between the mean values of two separate headlamp lenses to be greater than the variation that is observed within a single headlamp lens. Consequently, the separation of the 16 headlamp lenses collected for each of the three headlamp models (48 lenses in total) was investigated to validate the analytical protocol.

Following the performance of LDA in assessing the homogeneity of the headlamp lenses, discrimination between samples in this study has been investigated using LDA, a statistical technique in which linear combinations of variables are utilized to describe differences between two or more groups. It is a supervised statistical technique in that the user must input the initial group classifications upon which the technique generates a suitable model to facilitate discrimination. The analysis assesses the group assignment provided by the user and utilizes the discriminant model to reclassify the data, providing a probability of correct classification. It is this classification, in combination with a scatter plot of the relevant discriminant functions (termed a discriminant plot), that are utilized to assess the separation between sample groups (22).

Automated variable selection procedures may also be implemented to facilitate greater separation between sample groups. In this study, forward stepwise procedures are utilized to add applicable variables to the discriminant model. The procedure utilizes partial F-statistics from a multivariate analysis of variance (MANOVA) to add the variables that contribute most to the discrimination between sample groups. The stepwise selection uses a combination of forward and backward procedures whereby once three variables have been added to the model, the backward procedure is performed on the selected analytes to ascertain whether their addition is essential given the presence of the other variables. A partial F threshold value is set at which the procedure is halted and no more analytes are selected for addition (0.05 in this study) or removal (0.10) (22). In this manner, the most appropriate variables to facilitate discrimination between sample groups may be selected prior to performing LDA.

To ensure that the LDA model generating the data classifications is appropriate, cross-validation procedures are performed to assess the complexity of the model. The most common method of cross-validation involves leaving out samples from the data set utilized to generate the model, prior to fitting these samples to the generated model and assessing the result. Consequently, "estimation" and "validation" sample sets are created from the original data set and the classification rates of these two sets are investigated. In this current study, $\sqrt{n}$ validation samples are randomly selected from each data set, where n is the number of samples in the entire data set. This ensures that a sufficient number of samples are still present in the estimation set to facilitate the generation of a suitable model. Consequently, the identification of overfitting (overly complex) or underfitting (overly simple) models is possible, with any such models discarded by the user as inappropriate to facilitate robust sample discrimination.

The performance of the LDA model can also be assessed for data with only two groups by using the area under the receiver operating characteristic (ROC) curve. This technique is a measure of the distributions of the estimated probability that a sample belongs to a particular group (23). Perfect classification corresponds to an area under the curve (AUC) of 1, with random classification producing an AUC of 0.500. Previous studies have identified an AUC of 0.700 as an appropriate threshold value for a suitable model (24). In this current study, any models generating an AUC of less than 0.700 are classified as performing poorly and disregarded as they are not suitable to facilitate discrimination of the data.

LDA has been utilized within the literature to facilitate discrimination between trace element data with respect to the provenance of ceramic oil lamps (25) and the growing regions of tea (26). It has also been utilized to facilitate the classification of glass types (27) and discrimination between container glass samples manufactured in a single factory over a short time frame (28). In the latter study, an iterative LDA process was utilized whereby all samples within the data set were classified as separate groups prior to undertaking forward stepwise LDA. As groups became separated from the main population, these were extracted from the data set and the remaining data reanalyzed until no further discrimination between sample groups could be achieved. This iterative process of sample discrimination provided a significantly more efficient method of facilitating separation of a data set than pairwise comparisons, particularly when multiple groups of control or recovered samples exist. Consequently, given the ability of this iterative interpretational approach to facilitate discrimination between glass sample groups, a similar iterative approach has been utilized during this current study to facilitate discrimination between polycarbonate headlamp lenses.

Separation of Headlamp Lenses

Forty-eight polycarbonate headlamp lenses were analyzed using LA-ICP-MS to determine their trace elemental composition. As detailed in Table 1, the three varieties of polycarbonate headlamp lenses were all manufactured in a single manufacturing plant over a 4-month period, with the majority of the lenses manufactured within two months of each other. Iterative LDAs were then utilized on the trace elemental data generated from analysis of these lenses to ascertain whether differentiation between polycarbonate headlamp lenses was possible. The iterative process of sample discrimination using forward stepwise LDA is described below and summarized in Table 4, including details of the analytes selected and the outcome of each analysis.

Forward stepwise LDA was performed on the entire data set and an LDA model was generated using 28 analytes, correctly classifying 99.9% of the estimation sample and 100% of the validation sample (LDA 1 in Table 4). The discriminant plot associated with this model is detailed in Fig. 2 and illustrates the separation of data associated with the three models of headlamp lenses, in particular, the separation of the data associated with WM headlamp lenses. Data pertaining to the WM headlamp lenses were then removed from the data set prior to reperforming forward stepwise LDA on the remaining data. The subsequent LDA model utilized 19 analytes, correctly classifying 99.8% of the estimation sample and 100% of the validation sample with an area under the ROC curve of 0.950. Consequently, it was concluded that separation between the three models of headlamp lenses could be easily achieved.

Given that discrimination between models of headlamp lenses was possible, the more challenging task of separating headlamp lenses from the same model of vehicle was investigated. The separation of the majority of the WM model headlamp lens samples is described in detail with the lenses assigned an arbitrary number between #1 and #16 for ease of handling. Forward stepwise LDA was performed on the data with the forward stepwise process selecting 27 analytes with which to generate a model for separation of the data (LDA 2 in Table 4). The discriminant plot resulting from the LDA is included as Fig. 3 with the plot detailing the apparent separation of the data into two separate groups (termed A and B for ease of reference). The generated model confirmed correct classification to their headlamp lens of origin for 96% of the estimation sample and 73% of the validation sample. While such a discrepancy in classification rates between the estimation and validation sets would usually indicate an overfitting model, the misclassifications in this situation are between headlamp lenses within one of the two separated major groups (A or B) and not between the two major groups. Consequently, the model generated by the LDA to facilitate separation of the major group A,

TABLE 4—*Summary of the results of forward stepwise linear discriminant analysis (LDA) performed during study.*

LDA	No. of Analytes	Analytes	Outcome
1	28	^{24}Mg, ^{39}K, ^{44}Ca, ^{45}Sc, ^{49}Ti, ^{51}V, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{118}Sn, ^{121}Sb, ^{139}La, ^{141}Pr, ^{158}Gd, ^{159}Tb, ^{165}Ho, ^{169}Tm, ^{175}Lu, ^{178}Hf, ^{202}Hg	Separates the three models of headlamp lenses
2	27	^{24}Mg, ^{39}K, ^{44}Ca, ^{45}Sc, ^{49}Ti, ^{51}V, ^{53}Cr, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{71}Ga, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{98}Mo, ^{114}Cd, ^{118}Sn, ^{121}Sb, ^{138}Ba, ^{139}La, ^{146}Nd, ^{175}Lu, ^{178}Hf, ^{202}Hg, ^{232}Th	Separates WM #4, #6, #7 and #13 from remainder of WM
3	15	^{24}Mg, ^{39}K, ^{45}Sc, ^{53}Cr, ^{55}Mn, ^{66}Zn, ^{90}Zr, ^{98}Mo, ^{118}Sn, ^{140}Ce, ^{146}Nd, ^{153}Eu, ^{202}Hg, ^{208}Pb, ^{238}U	Separates WM #4 and #7 from #6 and #13
4	5	^{24}Mg, ^{90}Zr, ^{118}Sn, ^{175}Lu, ^{238}U	Cannot separate WM #6 and #13
5	23	^{24}Mg, ^{39}K, ^{44}Ca, ^{45}Sc, ^{51}V, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{71}Ga, ^{85}Rb, ^{90}Zr, ^{118}Sn, ^{121}Sb, ^{140}Ce, ^{152}Sm, ^{178}Hf, ^{202}Hg, ^{208}Pb, ^{232}Th, ^{238}U	Separates data into three groups
6	15	^{24}Mg, ^{44}Ca, ^{45}Sc, ^{51}V, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{118}Sn, ^{121}Sb, ^{139}La, ^{152}Sm, ^{163}Dy, ^{208}Pb	Separates WM #1, #2, #3 and #5
7	16	^{24}Mg, ^{44}Ca, ^{45}Sc, ^{49}Ti, ^{51}V, ^{53}Cr, ^{55}Mn, ^{66}Zn, ^{71}Ga, ^{88}Sr, ^{90}Zr, ^{118}Sn, ^{121}Sb, ^{140}Ce, ^{165}Ho, ^{208}Pb	Separates WM #12
8	13	^{24}Mg, ^{44}Ca, ^{45}Sc, ^{51}V, ^{53}Cr, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{121}Sb, ^{138}Ba, ^{140}Ce, ^{165}Ho, ^{208}Pb	Separates WM #9 and #10
9	2	^{24}Mg, ^{121}Sb	No separation
10	7	^{45}Sc, ^{51}V, ^{55}Mn, ^{140}Ce, ^{172}Yb, ^{178}Hf, ^{208}Pb	Separates WM #15
11	6	^{45}Sc, ^{55}Mn, ^{93}Nb, ^{118}Sn, ^{139}La, ^{238}U	No separation
12	15	^{24}Mg, ^{39}K, ^{45}Sc, ^{51}V, ^{63}Cu, ^{66}Zn, ^{90}Zr, ^{114}Cd, ^{118}Sn, ^{139}La, ^{141}Pr, ^{158}Gd, ^{172}Yb, ^{175}Lu, ^{178}Hf	Correctly classifies group origin of WM08DO12

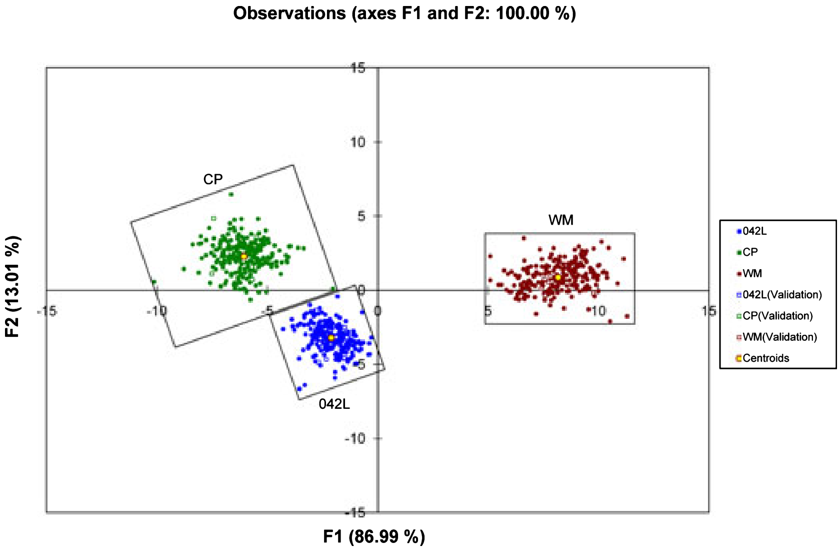

FIG. 2—*Discriminant plot detailing separation of the three models of headlamps lenses.*

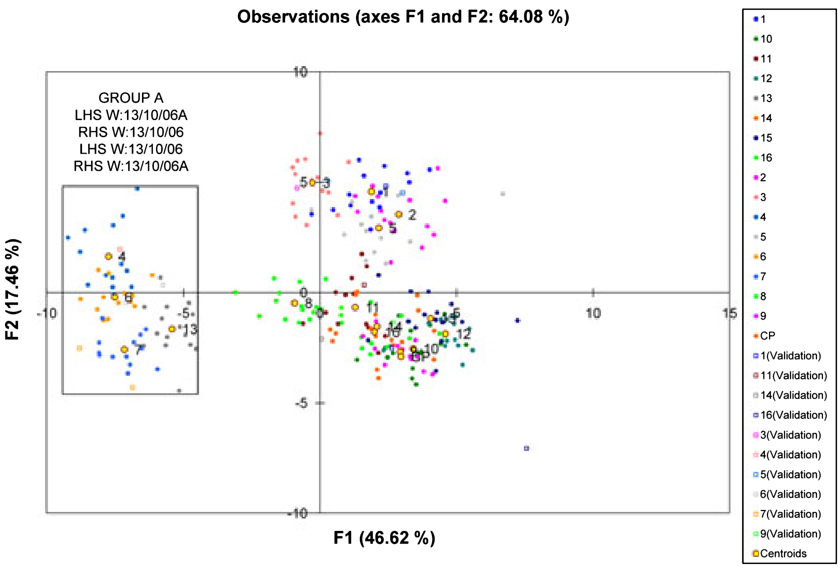

FIG. 3—*Discriminant plot detailing separation of group A, consisting of WM samples #4, #6, #7, and #13 (LHS W:13/10/06A, RHS W:13/10/06, LHS 13/10/06, and RHS W:13/10/06A, respectively).*

consisting of WM samples #4 (LHS W:13/10/06A), #6 (RHS W:13/10/06), #7 (LHS 13/10/06), and #13 (RHS 13/10/06A) is valid and thus these samples were removed from the data set prior to undertaking further analyses.

Having extracted WM samples #4, #6, #7, and #13 (group A) from the data set, a forward stepwise LDA was performed on the data pertaining to these samples to ascertain whether separation could be achieved (LDA 3). The forward stepwise process selected 15 analytes, with the generated LDA model correctly classifying 100% of the estimation sample and 86% of the validation sample. The discriminant plot produced by the LDA is detailed in Fig. 4. Consequently, the separation of WM samples #4 (LHS W:13/10/06A) and #7 (LHS W:13/10/06) from the mixture of samples #6 and #13 was achieved.

An additional forward stepwise LDA was then performed on WM samples #6 and #13 to determine whether discrimination between these samples was possible (LDA 4). The model generated utilized five analytes and while 100% correct classification of the estimation and validation samples was achieved, the area under the ROC curve was 0.692, indicating that the model was not suitable. Consequently, it was concluded that the separation of WM samples #6 (RHS W:13/10/06) and #13 (RHS W:13/10/06A) could not be achieved. This result led to the proposition that headlamps produced on the same day cannot always be discriminated by their trace elemental composition and that this may be the discrimination limit of the analytical protocol.

A forward stepwise LDA was then performed on the remaining data relating to the WM headlamp lens samples (group B). The forward stepwise process selected 23 analytes and the generated model achieved correct classification of almost 95% of the estimation sample and 92% of the validation sample (LDA 5). The discriminant plot relating to this model is included as Fig. 5 and details the separation of the data into three groups. WM sample #8 (RHS W:23/11/06A) separates into its own group while WM samples #1,

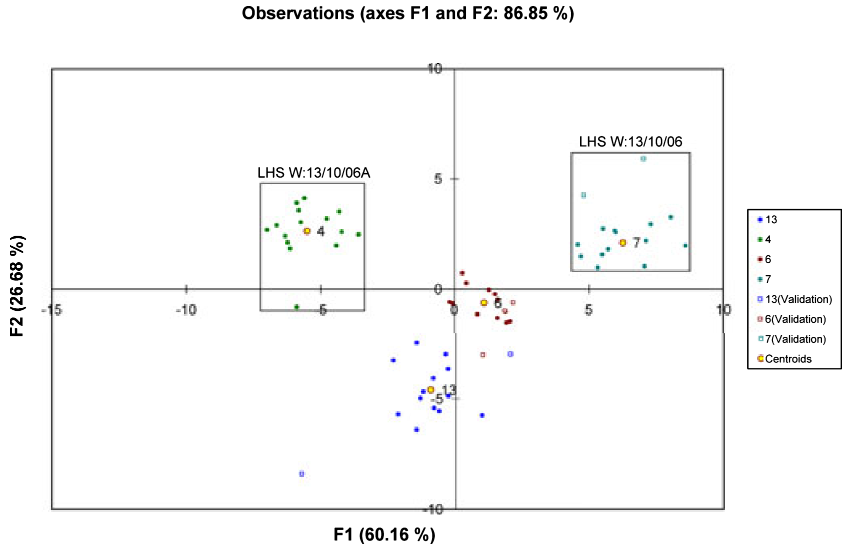

FIG. 4—*Discriminant plot detailing separation of WM samples #4 (LHS W:13/10/06A) and #7 (LHS W:13/10/06).*

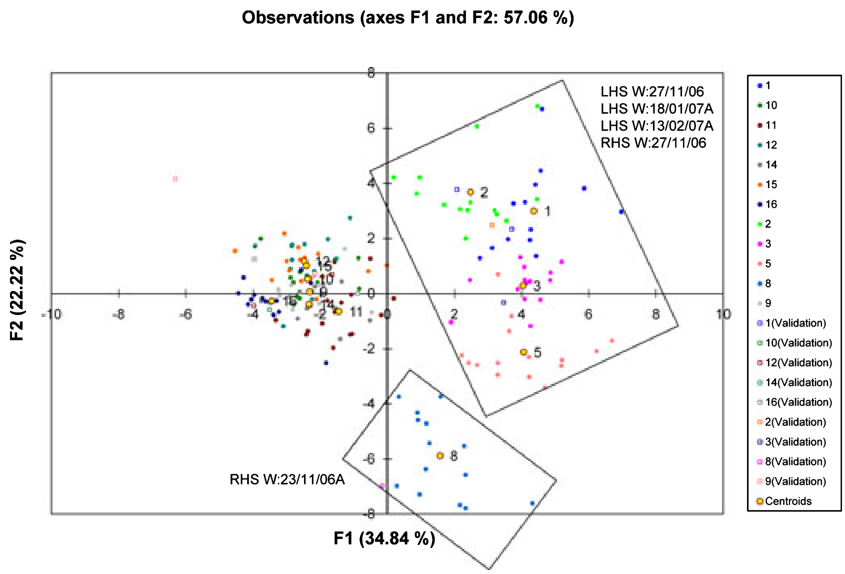

FIG. 5—*Discriminant plot detailing separation of WM sample #8 (RHS W:23/11/06) and the split of the remaining data into two separate groups.*

#2, #3, and #5 form a separate group and the remaining data plots as the third group. These two groups containing multiple headlamp lens samples were then analyzed further to ascertain whether discrimination between all headlamp lenses could be achieved.

WM samples #1, #2, #3, and #5 that had been separated from the main group of WM samples were analyzed using forward stepwise LDA (LDA 6). The forward stepwise process selected 15 analytes with the model generated by the LDA correctly classifying 100% of the estimation and validation samples. The discriminant plot produced by the LDA is detailed in Fig. 6 with clear separation of the four groups of headlamp samples. Consequently, it was concluded that discrimination between WM samples #1 (LHS W:27/11/06), #2 (LHS W:18/01/07A), #3 (LHS W:13/02/07A), and #5 (RHS W:27/11/06) could be achieved and thus these samples were removed from the data set.

Forward stepwise LDA was then performed on the remaining data relating to the WM headlamp samples (LDA 7), utilizing 16 analytes to produce the discriminant plot detailed in Fig. 7. The model generated by the LDA correctly classified 96% of the estimation sample and 70% of the validation sample with the misclassifications occurring for groups other than WM sample #12, which appears to separate from the remaining data. Consequently, WM sample #12 (RHS W:18/01/07A) was concluded to be discriminated from the data set.

Forward stepwise LDA was performed on the reduced data set with the generated model (LDA 8) utilizing 13 analytes and producing the discriminant plot detailed in Fig. 8. The classification rate for the LDA model was 92% for the estimation sample and 78% for the validation sample. As detailed in Fig. 8, the separation of WM samples #9 (RHS W:18/01/07) and #10 (LHS W:18/01/07) from the remainder of the data set was achieved. Consequently, these samples were removed and analyzed separately to ascertain whether discrimination between the samples was possible. When a forward stepwise LDA was performed on this data (LDA 9), the forward stepwise process selected only two analytes and produced a correct classification rate of 84% for the estimation

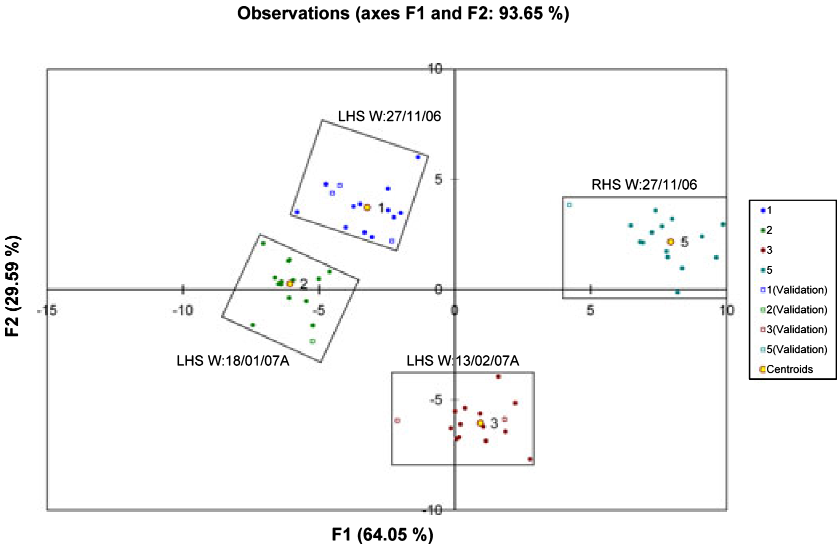

FIG. 6—*Discriminant plot detailing separation of WM samples #1 (LHS W:27/11/06), #2 (LHS W:18/01/07A), #3 (LHS W:13/02/07A), and #5 (RHS W:27/11/06).*

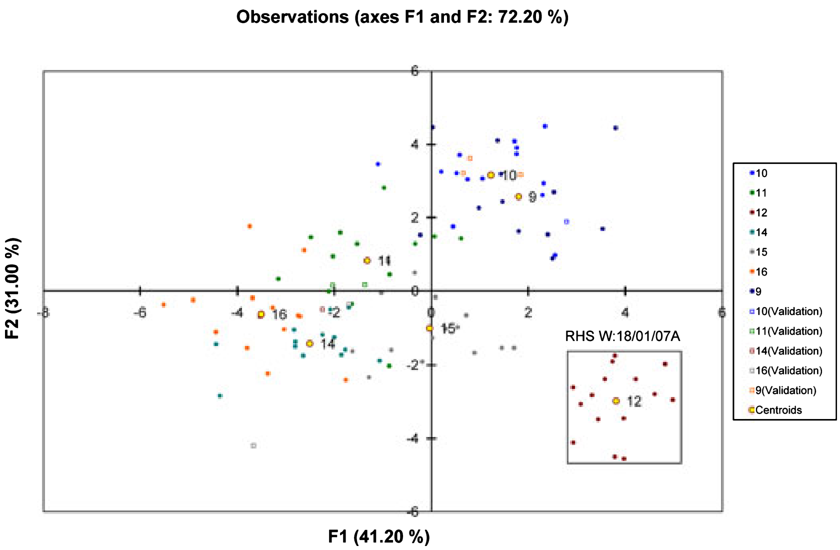

FIG. 7—*Discriminant plot detailing separation of WM sample #12 (RHS W:18/01/07A).*

sample and 60% for the validation sample, indicating an overfitting model. Therefore, the separation of WM samples #9 and #10 from each other was not possible, supporting the previous results with regards to the limit of discrimination of the analytical protocol.

The remaining data were then reanalyzed using a forward stepwise LDA, utilizing seven analytes and correctly classifying 94% of the estimation sample and 100% of the validation sample (LDA 10). The discriminant plot produced from the LDA is included as Fig. 9 and details the separation of WM sample #15 (LHS W:18/01/07) from the remainder of the data set. Once this data had been removed from the data set, the forward stepwise LDA was reperformed, selecting six analytes but only correctly classifying 77% of the estimation sample and 83% of the validation sample (LDA 11). Misclassifications arose between each of the three groups of data and thus it was concluded that discrimination between WM samples #11 (RHS W:13/02/07), #14 (RHS W:13/02/07A), and #16 (LHS W:13/02/07) could not be achieved. Therefore, the only WM headlamps that could not be discriminated based upon their trace elemental compositions were those manufactured on the same day.

When a similar process was undertaken with the Copperhead and 042L model headlamp samples, separation between the majority of the samples was achieved. Eleven forward stepwise LDAs were required with the Copperhead data and ultimately led to discrimination between all samples except for three groups of samples produced on the same day. The 042L model headlamps required 10 forward stepwise LDAs with only a single group of three samples that were produced on the same day unable to be separated. Therefore, variation occurring within a single headlamp does not appear to have adversely affected the discriminating ability of the analytical protocol.

Furthermore, for all three models of polycarbonate headlamp investigated, only lenses that were produced on the same day from the same manufacturing plant could not be discriminated from one another. Despite this apparent limit in the discrimination ability of the analytical protocol, some samples produced on the same day

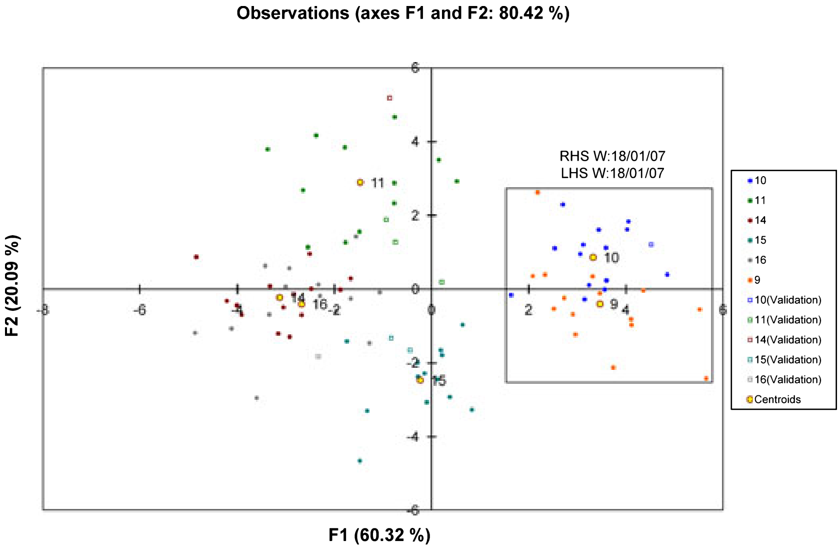

FIG. 8—*Discriminant plot detailing separation of WM samples #9 (RHS W:18/01/07) and #10 (LHS W:18/01/07).*

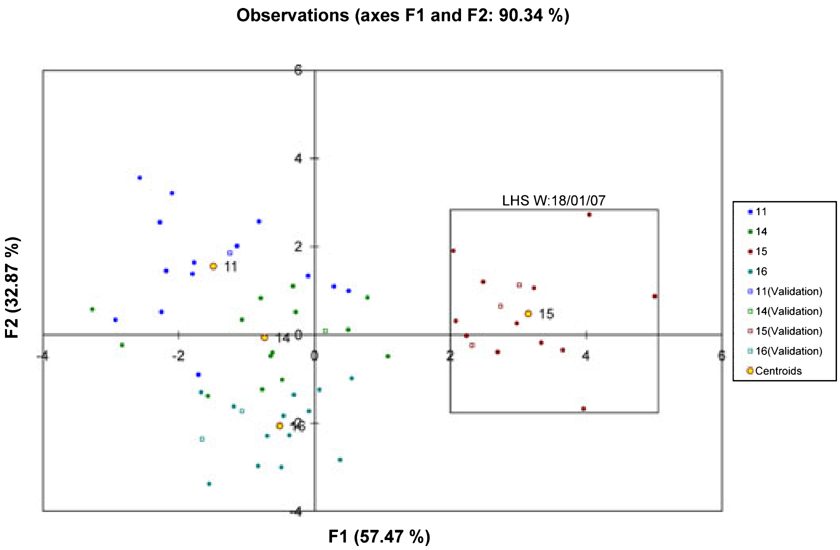

FIG. 9—*Discriminant plot detailing separation of WM sample #15 (LHS W:18/01/07).*

could be discriminated from one another. Therefore, it is likely that the discrimination limit with regards to the date of manufacture of headlamps is less than 1 day and depends upon the exact time of manufacture, though this information was not available during this study. Regardless of this, the analytical protocol developed provides the forensic scientist with a highly discriminatory technique that facilitates comparison between polycarbonate headlamp lens material that had previously not been possible.

Development and Validation of an Interpretational Protocol

Given that discrimination between polycarbonate headlamp lenses produced in a single manufacturing plant over a short period of time can be achieved, a suitable interpretational protocol was developed to facilitate comparison of trace elemental data generated from analysis of a recovered sample to equivalent data generated from analysis of control samples. The interpretational protocol utilizes a combination of the search/match procedure developed by Watling et al. (29), PCA, and forward stepwise LDA to facilitate the comparison. The search/match procedure involves summing the normalized differences between a search reference sample (the unknown/recovered sample) and all other samples that exist within the database (the known/control samples) and represents a mathematical sample grouping protocol equivalent to the graphical grouping conventionally represented in cluster analysis. As the search reference sample is a part of the database, it achieves a 100% fit to itself, establishing a datum point for relative comparison of all other comparability indices that then span between 100% for the best fit and 0% for the worst fit. These are arbitrary values that do not relate to the probability of a match, rather the relative comparison of each sample in the database to the search reference sample (29). The technique is used as a screening procedure to reduce the number of samples in a database that can be said to "match" the reference sample prior to more conventional statistical manipulation of the data.

The 10 best matches to the reference sample are then separated from the data set, and a PCA performed to ascertain whether the match generated by the search/match procedure is suitable or whether the unknown sample potentially belongs to a completely separate population. This is achieved by examining the scores plot generated by the PCA and determining whether the score of the unknown sample performs in a similar manner to an outlier by plotting away from the scores of the potential matches. However, without prior variable selection, the PCA utilizes all analytes and as such is not suitable for discrimination between headlamps of a similar elemental composition because of multiple indiscriminate analytes preventing the separation of the headlamps into sensible groups. Consequently, further analysis using forward stepwise LDA is required to ascertain from which headlamp lens group the unknown sample is likely to have originated.

TABLE 5—*Top 10 matches for search/match procedure performed on sample WM08DO12.*

Match number	Sample	% Comparability
1	WM08DO12	100.00
2	WM08DO13	85.65
3	WM08DO11	84.94
4	WM08EO13	78.17
5	WM06EO12	78.15
6	WM04EO11	77.22
7	WM04AO11	76.16
8	WM08AO11	76.13
9	WM05CO13	74.74
10	WM06DO12	74.17

Having determined that the unknown sample is not an outlier and is likely to belong to one of the headlamp lens groups represented by the top 10 matches of the search/match procedure, the remaining analyses for these headlamp lenses are utilized in addition to the top 10 matches to generate a suitable LDA model that will facilitate discrimination between the lenses. Inclusion of all analyses relating to a headlamp lens is essential to ensure that the generated model is robust and not adversely affected by the minor variations observed within a single headlamp lens. Forward

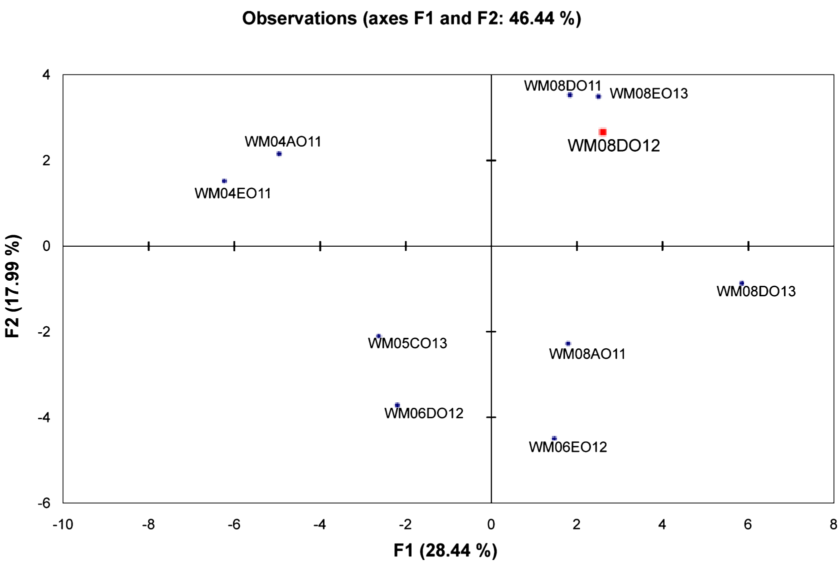

FIG. 10—*Results of principal component analysis conducted on the top 10 matches to sample WM08DO12, as identified by the search/match protocol.*

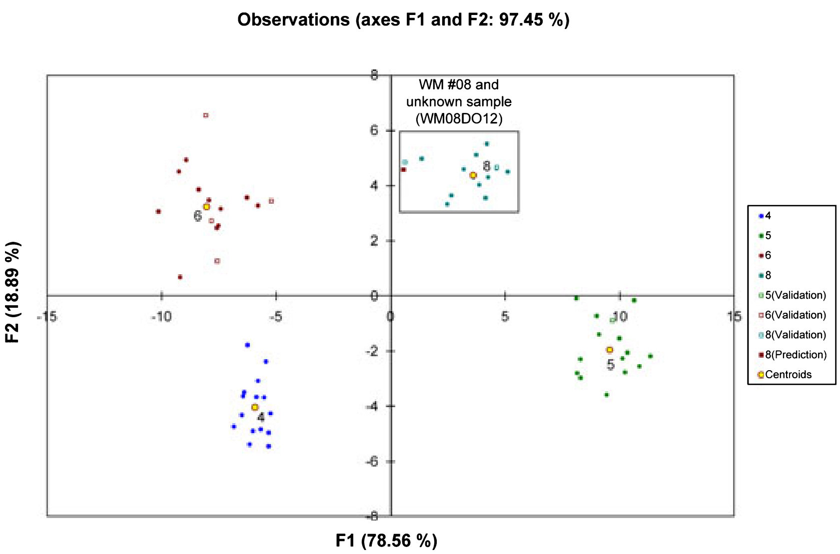

FIG. 11—*Discriminant plot detailing prediction of group assignment for unknown WM headlamp sample.*

stepwise LDA selects the appropriate analytes to facilitate separation and generates a model that does not include the unknown sample. Cross-validation is performed to establish the suitability of the model and assist in determining its ability to discriminate between the groups of data. Once the model is validated, the unknown sample is interpreted using the LDA model and is assigned a group along with a probability that the group assignment is correct. Although the LDA must assign the unknown to one of the groups used to generate the LDA model, it is expected that the initial PCA utilized to remove outliers and the assessment of the discriminant plot to determine whether the distance between the unknown sample and the group centroid is excessive will be sufficient to identify any false positives. Therefore, it is possible to establish from which group of control samples a recovered sample is likely to have originated.

For each variety of headlamp, five blind trials were performed (15 in total) to ascertain the applicability of the interpretational protocol. A random number generator was utilized to select the analysis from the data set that would be classified as the unknown/recovered sample for each trial, removing any potential operator bias. One such trial utilized sample WM08DO12 as the unknown sample. The search/match procedure was performed on the data set, producing the top 10 matches detailed in Table 5. With the blind trials, it was found that the headlamps with the greatest number of top 10 matches were frequently (12/15 trials) the headlamps from which the unknown sample originated. PCA was then performed on the data relating to the top 10 match samples, generating the scores plot detailed in Fig. 10. The data point relating to the unknown sample plots near other samples from the top 10 matches generated by the search/match procedure, indicating that the unknown sample is not an outlier.

Forward stepwise LDA was then performed on all data pertaining to WM headlamp samples #4, #5, #6, and #8, with the exception of the unknown sample data. The forward stepwise process selected 15 analytes (LDA 12 in Table 4) upon which an LDA model was generated. The LDA model facilitated separation of the data relating to the four headlamps, achieving 100% correct classification of both the estimation and validation samples. The model was then used to predict the headlamp sample group from which the unknown sample originated. The discriminant plot illustrating the prediction of the unknown sample using the LDA model is detailed in Fig. 11. The LDA correctly predicted with 100% certainty that the unknown sample, WM08DO12, originated from WM headlamp sample #8, supporting the validity of the interpretational protocol. Similarly for the other 14 blind trials, correct assignment of the unknown sample could be achieved, so long as it did not exceed the discrimination limit of the analytical protocol whereby some headlamp lenses produced on the same day could not be discriminated from each other.

Conclusion

It has been found that the trace elemental analysis of polycarbonate headlamp lenses is possible using LA-ICP-MS. Sixteen lenses from each of three varieties of vehicular headlamps were investigated, resulting in 48 lenses in total. The analysis of the exterior surface of the lenses was determined to be preferable, producing average results of ±13%RSD. Although minor variation was observed in the trace elemental profile of a small number of headlamp lenses, this variation did not prevent the utilization of iterative LDAs to facilitate discrimination between the majority of the headlamp lenses analyzed. The discrimination limit of the developed analytical protocol was found to be between polycarbonate headlamp lenses produced on a single day from the same manufacturing plant.

An interpretational protocol has also been proposed to be utilized with the trace elemental data generated from the analysis of the headlamp lenses. The protocol utilizes a search/match comparability index followed by PCA and forward stepwise LDA to facilitate discrimination of all headlamp lens samples, within the discrimination limits of the analytical protocol. Consequently, the analysis of polycarbonate headlamps lenses recovered as crime scene debris and the subsequent comparison of the data to equivalent data generated from the analysis of control samples are now possible.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

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