

Verification of a Greener Method of Sex Determination Through the Multi-Elemental Analysis of Hair Using Electrothermal Vaporization Coupled to Inductively Coupled Plasma Optical Emission Spectrometry

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ABSTRACT: In forensic science, trace evidence establishes links between victims, suspects, and crime scenes. Head hair commonly serves as a reliable biomarker because it contains a permanent record of elements from an individual's body. By substituting CF₄ reaction gas for polytetrafluoroethylene powder, this work modifies and re-optimizes a previous method for determining the sex of humans by directly analyzing hair samples via electrothermal vaporization with detection by inductively coupled plasma optical emission spectrometry. Hair samples were washed in portions of doubly deionized water, dried, and ground into a fine powder before analysis. Data processing included the use of an Ar emission line to compensate for sample loading effects on the plasma via point-by-point internal standardization. The peak areas were then integrated before multivariate analysis by principal component analysis (PCA) and linear discriminant analysis (LDA). Using the same predictor elements as the original method (Mg, S, Sr, and Zn), LDA and PCA remained effective at accurately predicting the sex of female and male samples despite using a different chemical modifier and instrumental operating conditions, indicating method robustness. This work thus offers an alternative method that uses a more affordable and accessible carrier agent at no cost to the original method's reliability.

INTRODUCTION

Trace evidence refers to fragments of physical evidence that can be collected from a crime scene. These are often used to identify suspects or victims connected to a crime or to explain the events that took place during that crime. Trace evidence, such as fibers, hair, gunshot residue, or paints, may be transferred between people, objects, or the environment during a crime. A common example is head hair that can provide crucial information about an individual as it serves as a reliable biomarker containing a permanent record of elements from an individual's body, which can come from either endogenous or exogenous sources.¹

Endogenous sources are the biological surroundings of developing hair. The most predominant endogenous sources are the matrix cells at the base of the hair follicle, which are the centre

of metabolic activity responsible for hair formation and growth.¹ In addition to matrix cells, the tissue papilla and circulating lymph serve as a "metabolic milieu" that contains trace elements. Several studies propose that eccrine sweat glands are another likely source of trace elements (Cu, Cr, Zn, and Fe) and salts of Na and K in hair.¹ Other potential endogenous sources include the apocrine sweat glands, the sebaceous glands, and the epidermis.¹ Conversely, exogenous sources describe external sources of elements such as diet or the environment.¹ Elements originating from external sources can include pollutants, potentially toxic elements (PTEs), and chemicals present as particulates in the air, water, soil, food, hair treatments or dyes, and personal care products. Understanding this information can be beneficial in identifying and understanding an individual's lifestyle.

One of the main roles of forensic chemistry in investigations is

the identification of suspects and victims. Sex determination is often the priority in this process, which can be accomplished through deoxyribonucleic acid (DNA) analysis of biological evidence (e.g., blood, semen, saliva, hair). DNA analysis of hair, however, depends on the presence of DNA, which requires that the hair has the root attached. Upon extracting DNA, polymerase chain reaction is often used to amplify genetic markers on the sex chromosomes. These are analyzed with analytical techniques like gel electrophoresis or DNA sequencing. The sex of the individual is determined based on the presence or absence of X and Y alleles.² This method of sex determination is beneficial in forensics applications but often requires full hair containing the root, which is not always feasible in this type of application. In addition, the fragments of DNA extracted from hair are often too small to yield accurate results useful in forensic profiling. Furthermore, while the hair will last a long time, once hair has shed, the DNA begins to undergo degradation quickly when subject to excessive environmental conditions, making it difficult to obtain information.

As a result of this, Huang and Beauchemin developed a method that can successfully determine the sex of humans with only a few mg of hair, even rootless hair, using electrothermal vaporization (ETV) with C_2F_2 as chemical modifier and detection by inductively coupled plasma optical emission spectrometry (ICPOES).³ Using Mg, S, Sr and Zn as predictor elements, both principal component analysis (PCA) and linear discriminant analysis (LDA) correctly predicted sex.³ When C_2F_2 was banned in Canada, MacConnachie *et al.*⁴ verified the method using CF_4 as an alternative chemical modifier. Only replacing C_2F_2 by CF_4 was sufficient, the remaining operating conditions being identical to those in the original method. MacConnachie *et al.* then had to replace Mg by Na in the set of four predictor elements to correctly infer the sex of 500-year-old Peruvian mummies using human hair from North America for the model. This change was required because of the Mg-rich diet of Peruvians at the time, which resulted in some males being incorrectly predicted as female when using Mg, S, Sr and Zn as predictor elements.⁴

Multi-elemental analysis using ETV-ICPOES is a technique used for time-efficient, direct determination of trace and major elements in a broad range of samples (solids, liquids, and slurries) with minimal sample requirement (1-5 mg).⁵ It offers multi-element capability with a high sensitivity, low detection limit, and good reproducibility, which has been proven efficient for the analysis of hair samples.⁶ By replacing the conventional nebulization system of ICPOES with an ETV furnace, transport efficiency into the plasma is increased to around 80% (versus around 5% with conventional nebulization systems),⁶ and direct solid sample analysis becomes possible. This eliminates the need for sample digestion, which would require much more than a few milligrams of hair (up to 200 mg), making it impractical in forensic scenarios where samples may be scarce. Eliminating the digestion step reduces potential sources of contamination, sample

requirements, and sample preparation time, in turn reducing the overall analysis time.

When using ETV-ICPOES, a chemical modifier is often used to increase analyte volatility, improve matrix separation during pyrolysis, and improve transport efficiency. The latest method⁴ relies on CF_4 as a reaction gas as it can form volatile fluorides, which enhances the volatilization efficiency of certain elements. However, this is an expensive gas to obtain and dispose of, and it is a potent greenhouse gas. Indeed, it can contribute to ozone depletion in the upper atmosphere as ultraviolet radiation breaks it down and releases fluorine atoms that participate in ozone-depleting reactions.⁷ CF_4 also has a long atmospheric lifetime, which allows the gas to persist in the atmosphere.⁸ In addition to environmental concerns, the thermal decomposition of CF_4 produces the toxic gases carbonyl fluoride and carbon monoxide. In inadequately ventilated areas, these gases can displace air and create an asphyxiation hazard.^{9,10} As CF_4 is stored as a compressed gas, accidental release could cause physical hazards, such as an explosion or fire.¹¹ At the end of CF_4 's life, it is released into the atmosphere, which can contribute to global warming.¹²

Polytetrafluoroethylene (PTFE) powder is an alternative, more affordable (no disposal costs associated with compressed gas cylinders and regulatory compliance like with CF_4 gas), and thermally stable chemical modifier that poses less concern to human health or the environment because of its solid properties. PTFE powder does not partition into the atmosphere, so there is no resistance to degradation when oxygen is present, and thus it poses no concern regarding contribution to global warming. Furthermore, PTFE powder has been proven to improve transport efficiency and vaporization of analytes.^{13,14} As with CF_4 gas, the fluorides produced are easily vaporized because their boiling point is much lower than those of carbides and oxides. At this elevated temperature, the fluorination reaction results in the complete evaporation of the analytes without residues during the heating cycle.¹⁴ According to a study by Peng *et al.*¹⁴, the signal intensity of various elements such as Al, Cr, Cu, Fe, and V was weak when PTFE powder was absent. However, with PTFE powder, a sharper and more intense peak resulted without any tailing or memory effects.¹⁴ This halogenating agent works by coating the sample to prevent interaction with the surroundings in the ETV furnace, thus reducing memory effects and enhancing analytical precision.¹¹ It has been successfully applied to the analysis of clays¹⁵ and of insects,¹⁶ but has never been tested for the analysis of hair.

The objective of this work is to replace CF_4 by PTFE powder in the recent ETV-ICPOES method for sex determination and verify the method using this more environmentally friendly, more affordable, and more accessible chemical modifier. PTFE is expected to enhance analyte volatility, improve matrix separation, and improve transport efficiency, in a similar way as CF_4 . It also satisfies several Principles of Green Chemistry, which is the design

of a chemical process that either reduces or eliminates the use of hazardous substances.¹⁷ Developing a greener method, to some degree, is crucial to reduce the negative impacts of chemical processes on human health and the environment. Because of its solid properties, switching to PTFE powder follows several of the 12 Principles of Green Chemistry: #3 Less Hazardous Chemical Syntheses, #6 Design for Energy Efficiency, #10 Design for Degradation, and #12 Inherently Safer Chemistry for Accident Prevention.¹⁷

EXPERIMENTAL

Instrumentation. Sample analysis was performed using a lateral plasma view ARCOS ICPOES instrument (SPECTRO Analytical Instruments, Kleve, Germany) coupled to an ETV system (ETV 4000C, Spectral Systems, Fürstfeldbruck, Germany) to achieve direct solid sample introduction. Table 1 summarizes the operating conditions and the ETV temperature programs that resulted from optimization. The cleaning program was used between each analysis of sample to clean the graphite boat using a high temperature to minimize contamination or potential memory effect. Samples were weighed into pyrolytically coated graphite boats (coated using chemical vapour deposition of carbon, usually from methane gas, onto graphite boats) and manually inserted into the ETV furnace using tweezers. A 1 m-long Teflon tube connected the outlet of the ETV system to the ICP torch, as was used in previous studies, to enable direct comparison of the results to those previously obtained.

The following emission lines (in nm) were monitored: Ar I (430.010), Ca II (396.847), Mg II (279.553), Mg II (280.270), Na I (588.995), S I (180.731), S I (182.034), Sr II (407.771), Sr II (421.552), and Zn II (213.856). Atomic emission lines, denoted I, come from excited neutral atoms returning to the ground state, while ionic emission lines, denoted II, result from excited singly ionized atoms relaxing to the ground state.¹⁸ Analyte emission lines were selected from those monitored by MacConnachie *et al.*,⁴ prioritizing the lines with good sensitivity and minimal spectroscopic interferences. Additional emission lines were selected for elements demonstrating consistent variation upon optimization and high abundance in hair samples. Internal standardization using the Ar emission line was done to correct for variations due to sample loading effects on the plasma.

The emission lines (in nm) Mg II (279.553), Mg II (280.270), S I (180.731), S I (182.034), Sr II (407.771), Sr II (421.552) and Zn II (213.856) were used for PCA and LDA analysis based on the model developed by MacConnachie *et al.*,⁴ and on the influences of each peak area normalized to sample mass on the principal components. Alternative emission lines and combinations of emission lines for the predictor elements were evaluated; yet none demonstrated the ability to differentiate between the two sexes.

Table 1. Instrumental operating conditions for ETV-ICPOES

ICP Parameter	Setting	
Ar plasma gas flow rate (L min ⁻¹)	14	
Ar auxiliary gas flow rate (L min ⁻¹)	3.0	
RF power (kW)	1.4	
Ar bypass gas flow rate (L min ⁻¹)	0.6	
Ar carrier gas flow rate (L min ⁻¹)	0.6	
ETV Temperature Program – Analysis of Sample		
Step	Temperature (°C)	Time (s)
Pyrolysis	300	20
Cooling	No heating	15
Vaporization	2200	30
ETV Temperature Program – Cleaning		
Step	Temperature (°C)	Time (s)
Initial Temperature	21	-
Cleaning	2300	45
Cooling	No heating	15

Table 2. Rootless hair samples used in the statistical training set and for further method verification

Training set		Model verification	
Sample	Sex	Sample	Sex
T1	F	V1	F
T2	F	V2	M
T3	F	V3	M
T4	F	/	/
T5	F	/	/
T6	F	/	/
T7	F	/	/
T8	F	/	/
T9	M	/	/
T10	M	/	/
T11	M	/	/
T12	M	/	/
T13	M	/	/

Samples and reagents. All rootless and untreated hair shaft cut samples were randomly collected in Kingston, Ontario, Canada from adults and placed in Ziplock bags. All samples collected were 10-15 cm long and their classifications are shown in Table 2. Doubly deionized water (DDW) with a resistivity of 18 MΩ cm (Arium Pro UV/DI System, Sartorius Stedim Biotech, Göttingen, Germany) was used to wash the hair samples. PTFE powder, 1 micron, M-Clarity™ quality level = MQ100 (Sigma-Aldrich, Saint Louis, MO, USA) was used as chemical modifier during ETV analyses.

Sample preparation. Prior to analysis, hair samples are washed to remove any possible contamination, external dirt, or build up that may alter the results. Each sample was washed with three 30-mL portions of DDW as described by Williams *et al.*,¹⁹ air dried on tissue, and ground into a fine powder using a pre-cleaned mortar and pestle to ensure homogeneity. Samples were washed using this method to avoid organic solvent waste and to increase precision. Indeed, MacConnachie *et al.*⁴ reported that washing in DDW caused no significant difference in the measured analyte concentrations at the 95% confidence level while resulting in better precision than washing in hexanes and DDW or washing in

Table 3. Comparison of the original and proposed methods applying the AGREE approach, comparing the principles of green analytical chemistry

Principle of Green Analytical Chemistry ²²	Original Method	Proposed Method
Principle 1. Direct analytical techniques should be applied to avoid sample treatment	Direct analysis Score: 0.85	Direct analysis Score: 0.85
Principle 2. Minimal sample size and minimal number of samples are goals	2 mg of sample required per analysis (microanalysis, 1-10 mg) Score: 1.00	2 mg of sample required per analysis (microanalysis, 1-10 mg) Score: 1.00
Principle 3. <i>In Situ</i> measurements should be performed	No <i>In Situ</i> measurements Score: 0.0	No <i>In Situ</i> measurements Score: 0.0
Principle 4. Integration of analytical processes and operations saves energy and reduces the use of reagents	Procedure involves three or fewer steps. Score: 1.00	Procedure involves three or fewer steps. Score: 1.00
Principle 5. Automated and miniaturized methods should be selected	Manual sample introduction, no miniaturization Score: 0.0	Manual sample introduction, no miniaturization Score: 0.0
Principle 6. Derivatization should be avoided	Derivatization Unable to calculate score.	Derivatization Unable to calculate score.
Principle 7. Generation of a large volume of analytical waste should be avoided and proper management of analytical waste should be provided	Little to no waste. Score: 1.00	Little to no waste. Score: 1.00
Principle 8. Multianalyte or multiparameter methods are preferred versus methods using one analyte at a time	Multielement determination. Score: 1.00	Multielement determination. Score: 1.00
Principle 9. The use of energy should be minimized	ICPOES analytical system consumes 0.1-1.5 kWh per sample as stated by the AGREE approach. ²² Score: 0.5	ICPOES analytical system consumes 0.1-1.5 kWh per sample according to the AGREE approach. ²² Score: 0.5. Although unknown, a decrease in energy required for the pyrolysis step (500°C to 300°C) would increase the points in correspondence with this principle.
Principle 10. Reagents obtained from renewable source should be preferred	CF ₄ is produced as a by-product of industrial processes that rely on non-renewable resources. ²³ Score: 0.0	PTFE powder is a synthetic polymer derived from petroleum-based resources. ²⁴ Score: 0.0
Principle 11. Toxic reagents should be eliminated or replaced	CF ₄ gas is toxic via inhalation and toxic to aquatic life. Score calculated based on the CF ₄ reaction gas flow rate and approximate analysis duration. Score: 0.31	Since PTFE can be potentially toxic to the lungs following pyrolysis, ²⁵ the score is calculated according to eq. 5 ²² based on amount used, 0.002 g. Score: 1.56
Principle 12. The safety of the operator should be increased	5+ threats: toxic by inhalation, can cause CNS depression and cardiac arrhythmias, explosive, highly persistent, potent greenhouse gas, toxic to aquatic life. Score: 0.0	1 known threat: potential toxic lung effects. ²⁵ Score: 0.8
Total Score	5.66	7.71

acetone and DDW. Similarly, Raposo *et al.*²⁰ reported that ultra-pure water effectively cleaned surface dirt and grease without decreasing endogenous element concentrations. Prepared samples were stored in small glass vials at room temperature. For analysis by ETV-ICPOES, five replicates containing 2.0 ± 0.2 mg of the sample were weighed directly into the graphite boats that already contained 2.0 mg of PTFE powder before being mixed with the PTFE powder using a stainless-steel spatula.

Data Processing. Point-by-point internal standardization was performed by calculating the ratio of the analyte's emission intensity to the internal standard's emission intensity, Ar I 430.010 nm emission line, for each point. This approach was previously

demonstrated to compensate for signal drift and sample loading effects on the plasma and improve the linearity of calibration curves.²¹ Background correction was then done: the average signal before and after the analyte peak was calculated and subtracted from each point of the peak. Peak areas were finally integrated across the vaporization step, except for sulfur where signals from both the pyrolysis and vaporization step were integrated, as this element was reported to be vaporized during the pyrolysis and vaporization step during hair analysis.⁴ The resulting peak areas were used for multivariate analysis with LDA and PCA using Minitab Statistical SoftwareTM (V.21) for dimensionality reduction and to simplify the data while retaining patterns in elemental concentrations.

RESULTS AND DISCUSSION

AGREE – Analytical GREENness Metric Approach. Green analytical chemistry aims to make analytical processes greener and safer for human health.²² To assess the greenness of a method, various factors are considered, such as the toxicity of the reagents, the amount of waste produced, the energy consumed, the complexity of the procedure, etc. The AGREE approach allows for a simple evaluation and comparison of methods, offering a clear and informative assessment that is easy to interpret.²² Table 3 compares the original method⁴ to the proposed method using this approach. In this assessment, the greater the score per principle, the greener the assessed procedure.

Differences in the methods correspond with principles 9 and 12 in which the performance is lower for the original method by MacConnachie et al.⁴ Overall, although not a drastic difference in the score, this proposed method using PTFE powder as opposed to CF₄ reaction gas, has a greater overall score from the AGREE analysis when the 12 principles are evaluated. Although not explicitly included in the assessment, economic criteria are also considered in principle 8. It costs less than 50\$ for a bottle of PTFE, which lasts for a long time, whereas a cylinder of CF₄ gas costs thousands of dollars. When the cylinder is empty, it then costs an additional couple of thousand dollars to dispose of it, as opposed to recycling an empty bottle of PTFE powder.

Other noteworthy points include the global warming potential (GWP), human toxicity potential by inhalation and ingestion (refer to Table 3), and the end of life. The GWP of CF₄ is a significant concern when considering the greenness of a method. CF₄ is a potent greenhouse gas, with a GWP of 7380 over a 100-year time horizon and has an estimated atmospheric lifetime ranging from 2300 to 50,000 years.²⁶ This gas is released into the atmosphere and is not expected to biodegrade rapidly, contributing to the greenhouse effect. Conversely, PTFE powder does not partition into the atmosphere, so there is no resistance to degradation when oxygen is present, and it thus poses no concern regarding its contribution to global warming.

Optimization of ETV operating conditions. The initial step upon switching from a gaseous carrier agent to a powder involved determining the optimal ratio of PTFE powder to the sample amount. Various ratios of PTFE to sample were tested, including 1.0:2.0, 1.5:3.0, 2.0:2.0, and 2.5:2.5 (in mg weight ratio). Fig. 1 presents the results for Mg, S, Zn, and Ca. With the exception of Mg, whose peak area appears independent of the sample:PTFE ratio used, statistical analysis using a Student's t-test (Table 4) indicated that a ratio of 2.0:2.0 of PTFE to sample yielded the optimal peak area and that a 1.0:2.0 ratio could be used interchangeably. This suggests that 1.0-2.0 mg of PTFE will give similar results with 2-mg sample aliquots. For consistency in this work, a ratio of 2.0:2.0 PTFE to sample was used herein. It is worth

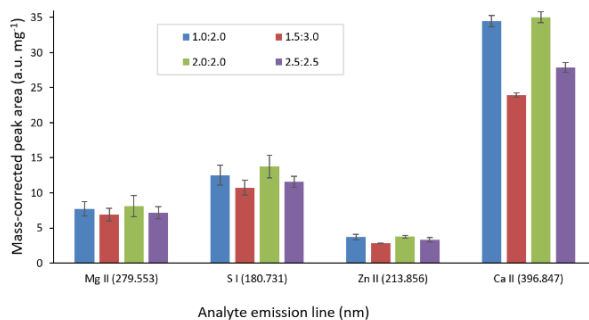


Fig. 1 Average mass-corrected peak areas using different ratios of PTFE powder mixed with sample (mg) (n=5).

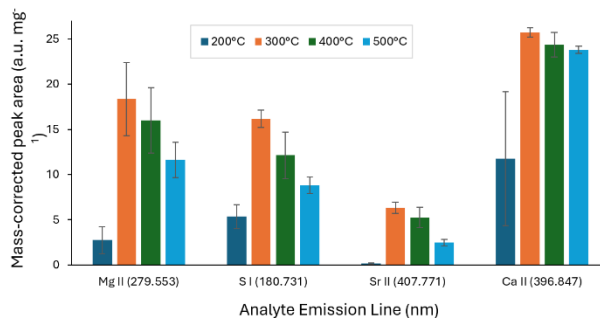


Fig. 2 Average mass-corrected peak areas using ETV methods with different pyrolysis temperatures (°C) (n=5).

Table 4. Comparison of PTFE:sample ratios via comparison of the resulting analyte mass-corrected peak areas using an unpaired Student's t-test at the 95% confidence interval

PTFE:sample (mg:mg)	Statistically significant (p<0.05)	Statistically insignificant (p>0.05)
1.0:2.0 vs. 1.5:3.0	S, Zn, Ca	Mg
1.5:3.0 vs. 2.0:2.0	S, Zn, Ca	Mg
2.0:2.0 vs. 2.5:2.5	S, Zn, Ca	Mg
1.0:2.0 vs. 2.0:2.0		Mg, S, Zn, Ca
1.5:3.0 vs. 2.5:2.5	Zn, Ca	Mg, S

Table 5. Comparison of pyrolysis temperatures via comparison of the resulting analyte mass-corrected peak areas using an unpaired Student's t-test at the 95% confidence interval

Temperature (°C)	Statistically significant (p<0.05)	Statistically insignificant (p>0.05)
200 vs. 300	Mg, S, Sr, Ca	
300 vs. 400	Mg, S	Sr, Ca
400 vs. 500	S, Sr	Mg, Ca
300 vs. 500	Mg, S, Sr, Ca	

worth noting that, although the ratios of 2.0:2.0 and 2.5:2.5 are equal, the ratio of 2.5:2.5 resulted in a lower signal for some elements monitored. Homogenizing a larger sample size can indeed be more challenging, which could lead to more variability and affect the accuracy of the analysis.

Sample was mixed with PTFE in the graphite boat as opposed to pre-mixing as there is less transfer of sample so less risk of sample

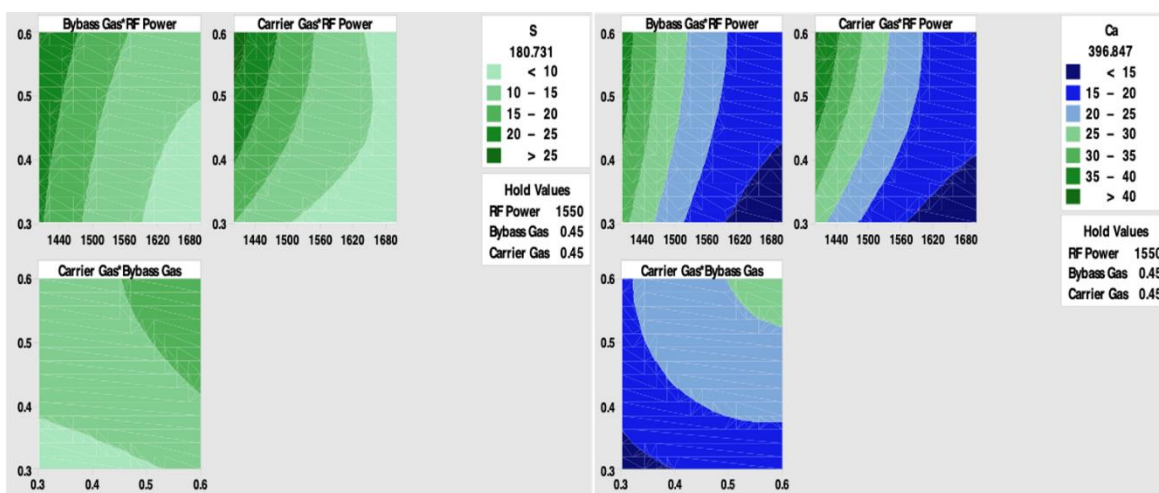


Fig. 3 Contour plots of S I (180.731) nm and Ca II (396.847) nm resulting from the multivariate optimization of plasma RF power, bypass gas flow rate, and carrier gas flow rate.

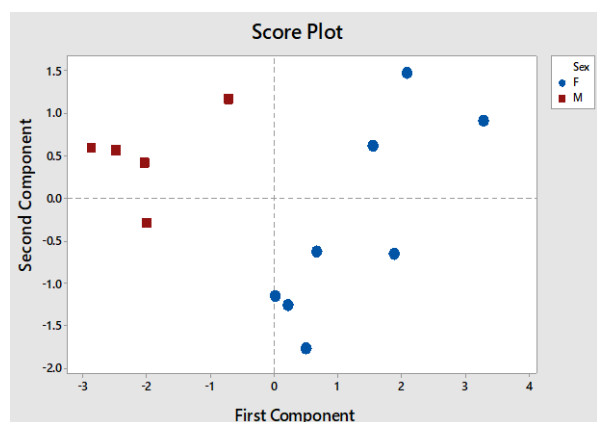


Fig. 4 PCA score plot using the following emission lines Mg II (279.553), Mg II (280.270), S I (180.731), S I (182.034), Sr II (407.771), Sr II (421.552), and Zn II (213.856).

loss and contamination. In this way, the exact mass measured is the exact amount of sample analyzed, which is crucial for mass correction. Pre-mixing was attempted for a few sample analyses to compare analyte signal and relative standard deviation (RSD) values, but the signals obtained were much lower. Furthermore, pre-mixing in a separate vial does not guarantee that quantitative mass transfer to the graphite boat can be achieved.

The pyrolysis temperature required optimization because PTFE powder begins to degrade at temperatures around 260°C and fully decomposes above 350°C.²⁷ To prevent full degradation of the PTFE powder and enable the formation of volatile fluorides while preventing the formation of refractory carbides, different pyrolysis temperatures (200°C, 300°C, 400°C, and 500°C) were tested with the vaporization temperature kept at 2200°C for each analysis (see

Table 1). Fig. 2 and Table 5 indicate that 300°C provided the greatest signal and overall mass-corrected peak area across the elements. This is a decrease in pyrolysis temperature compared to the previous methods that used 500°C.^{3,4} The temperatures 200°C and 500°C provided the lowest signal and greatest RSD values.

Multivariate optimization of the plasma RF power, bypass gas flow rate to minimize solid deposition on the walls of the transport tube, and carrier gas flow rate was conducted using Minitab over a 3-day randomized analysis sequence to account for potential fluctuations in samples, memory effects, and/or experimental errors. A central composite design detailed in Table S1 was used to explore the optimal settings and the relationships between multiple factors and their linear and quadratic effects.²⁸ Based on contour plots, exemplified by those for S and Ca in Fig. 3, an optimized peak area resulted when the carrier and bypass gas flow rates were around 0.6 L min⁻¹, with an RF power around 1400 W (see the top left corner of Fig. 3). Similar results were indicated by the contour plots for Zn, Na, Sr, and Mg (not shown). Hence, 0.6 L min⁻¹ was adopted for each of the carrier and bypass gas flow rates along with 1400 W of RF power.

Method verification using PTFE powder. Like the original method on inferring sex from human rootless hair samples using ETV-ICPOES with C₂F₂ chemical modifier,³ the PCA score plot obtained for this model, despite its small sample population, was successful at grouping correlating points together based on the variables provided. In Fig. 4, all male data points lie in the left two quadrants and all the female points are in the right two quadrants, as reported with the original method.³

Eigenvalues in Table S2 quantify the variance along each eigenvector. As per the Kaiser rule, all components with eigenvalues under 1.0 are dropped in the score plot and in the data

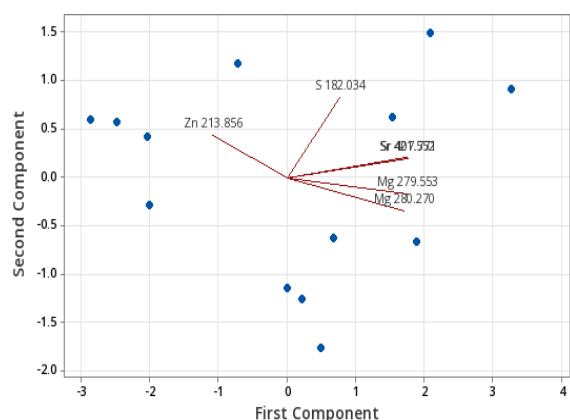


Fig. 5 PCA biplot of Mg II (279.553), Mg II (280.270), S I (180.731), S I (182.034), Sr II (407.771), Sr II (421.552), and Zn II (213.856).

Table 6. LDA classification using leave-one-out cross-validation for method verification using Mg, S, Sr, and Zn as predictor elements

Sample Classification	# samples predicted as male	# samples predicted as female	% correctly predicted
Male	5	0	100%
Female	0	8	100%

in Table S3. The two components that have eigenvalues greater than 1.0 explain 79.6% of the variation in the dataset, meaning that that percentage of information of the main dataset is preserved. Principal component 1 explains 62.2% of the variability in the data, whereas principal component 2 explains 17.4% of the variability.

Eigenvectors in Table S3 indicate the direction of the principal components. Clearly, the first principal component has large positive associations with emission lines Mg II (279.553), Mg II (280.270), Sr II (407.771), and Sr II (421.552). While the second component has large negative associations with the Mg emission lines, S I (182.034) and Zn II (213.856) have large positive loadings on that component. This can be seen visually in the biplot in Fig. 5.

LDA was also used for sample discrimination. Leave-one-out cross-validation was first used to validate the different operating conditions in this work. This cross-validation method provides an unbiased estimate of test error and involves splitting data into a training set containing all observations except one and validation set containing the remaining observation. This is repeated until each sample has been validated. As can be seen in Table 6, all thirteen samples were correctly predicted with 100% accuracy. Although 100% accuracy suggests confidence in the model, population size does influence the outcome. The addition of more samples could change the outcome of the accuracy, being something to be investigated upon model expansion.

To further verify the method, the model was applied to three

samples (samples V1-V3) outside of the training set (consisting of samples T1-T13). The method was able to correctly assign the three samples with 100% accuracy. Hence, changing the ETV chemical modifier from CF₄ and C₂F₂ to PTFE and re-optimizing the operating conditions did not impact the discriminatory ability of the original method. This greener method can now be expanded in future work to include exogenous sources such as dyed hair or samples from people of different race and ethnicity to broaden the diversity of the samples used in the model and thus improve the robustness of this model.

CONCLUSION

The work presented demonstrates that changing the ETV chemical modifier from CF₄ gas to PTFE powder and reoptimizing the operating conditions did not have an impact on the discriminatory powers of the original method,³ indicating method robustness. The change in chemical modifier did not introduce bias into the LDA training set. Using the original set of four predictor elements (Mg, S, Sr, and Zn), both PCA and LDA accurately predicted sex from analysis of human hair samples with 100% accuracy. As PTFE showed no adverse effects on the model or its outcomes, it is thus a viable alternative at a lower cost.

Future work will involve expanding the method to include dyed hair and the addition of hair from people of different races and ethnicities. A previous study using CF₄ as chemical modifier found that hair dye acts as an exogenous source, altering the elemental composition in hair.²⁹ Knowing this, there is a strong probability that the analysis of a dyed hair sample using PTFE as chemical modifier might also result in mispredictions. This expansion is crucial in forensic applications, given the widespread use of hair dye among individuals and will thus be investigated. Additionally, concentrations of elements may also be influenced by ethnicity and race due to factors such as diet and environmental conditions. Hence, ensuring the model's impartiality across these factors is essential. This is particularly important for applications in Canada, where 26.5% of the population identifies as racialized.³⁰ Therefore, developing a method that can correctly infer sex and ethnicity from head hair, irrespective of whether it is dyed, will significantly increase the robustness of this model in forensic contexts.

ASSOCIATED CONTENT

Supporting information (Tables S1-S3) is available at www.at-spectrosc.com/as/home

AUTHOR INFORMATION



Diane Beauchemin received her Ph.D. in 1984 from Université de Montréal. She is a professor (Full) at Queen's University. Her research efforts are focused on inductively coupled plasma mass spectrometry (ICPMS) and ICP optical emission spectrometry (OES) from both fundamental and application perspectives and expanding the range of application of ICPMS/OES to geochemical exploration, risk assessment of food safety, characterization of nanoparticles, and forensic analysis. She is on the editorial board for *Atomic Spectroscopy*. Diane Beauchemin won the Alan Date Memorial Award (1988) from VG Elemental, the Distinguished Service Award (2001) from Spectroscopy Society of Canada, the Maxxam Award (2017) and Clara Benson Award (2019) from Canadian Society for Chemistry, the Gerhard Herzberg Award (2018) from the Canadian Society for Analytical Sciences and Spectroscopy, and the Environment Division Research and Development Dima Award from the Chemical Institute of Canada (2024). She is author or co-author of over 183 articles published in peer-reviewed scientific journals.

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Notes

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REFERENCES

1. G. Chittleborough, *Sci. Total Environ.* 1980, **14**, 53–75. [https://doi.org/10.1016/0048-9697\(80\)90126-6](https://doi.org/10.1016/0048-9697(80)90126-6)
2. B. A. Álvarez-Sandoval, L. R. Manzanilla, and R. Montiel, *PloS One* 2014, **9**, e104629. <https://doi.org/10.1371/journal.pone.0104629>
3. L. Huang and D. Beauchemin, *J. Anal. At. Spectrom.*, 2014, **29**, 1228–1232. <https://doi.org/10.1039/c4ja00071d>
4. M. MacConnachie, S. Lu, Y. Wang, J. Williams, and D. Beauchemin, *RSC Adv.*, 2022, **12**, 2764–2771. <https://doi.org/10.1039/d2ra05654b>
5. F. Kaveh and D. Beauchemin, *J. Anal. At. Spectrom.*, 2014, **29**, 1371–1377. <https://doi.org/10.1039/c4ja00041b>
6. D. M. Hughes, C. L. Chakrabarti, D. M. Goltz, D. C. Grégoire, R. E. Sturgeon, and J. P. Byrne, *Spectrochim. Acta B*, 1995, **50**, 425–440. [https://doi.org/10.1016/0584-8547\(94\)00149-P](https://doi.org/10.1016/0584-8547(94)00149-P)
7. R. E. Weston, *Atoms. Environ.*, 1996, **30**, 2901–2910. [https://doi.org/10.1016/1352-2310\(95\)00499-8](https://doi.org/10.1016/1352-2310(95)00499-8)
8. P. Artaxo, T. Berntsen, R. Betts, D. W. Fahey, J. Haywood, J. Lean, D. C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. van Dorland, February 2018. Intergovernmental Panel on Climate Change. p. 212.
9. P. Gupta, *Toxicologic Hazards of Solvents, Gases, Vapors, and Other Chemicals*. In: Concepts and Applications in Veterinary Toxicology. Springer, Cham, 2019. <https://doi.org/10.1007/978-3-030-22250-5>
10. Air Liquid Company. (2018). Safety Data Sheet (Tetrafluoromethane). <https://www.airgas.com/msds/001051.pdf>
11. Compressed Gas Association. *Handbook of Compressed Gases*, 1st ed.; Springer: New York, NY, 1990; pp 1-660. <https://doi.org/10.1007/978-1-4613-0673-3>
12. United States Environmental Protection Agency. (2024). Understanding Global Warming Potentials. <https://www.epa.gov/ghgemissions/understanding-global-warming-potentials>
13. F. Y. Wang, Z. C. Jiang, H. Bin, and T. Y. Peng, *J. Anal. At. Spectrom.*, 1999, **14**, 1619–1624. <https://doi.org/10.1039/a904639i>
14. T. Peng, X. Sheng, B. Hu, and Z. Jiang, *Analyst*, 2000, **125**, 2089–2093. <https://doi.org/10.1039/b006441f>
15. A. S. Masquelin, F. Kaveh, A. Asfaw, C. J. Oates, and D. Beauchemin, *Geochem. Explor. Environ. Anal.*, 2013, **13**, 11–20. <https://doi.org/10.1144/geochem2012-129>
16. Y. Holowaty and D. Beauchemin, *Atom. Spectrosc.* 2024, **45**, 226–232. <https://www.at-spectrosc.com/as/article/pdf/2024104>
17. A. DeVieno Kreuder, T. House-Knight, J. Whitford, E. Ponnusamy, P. Miller, N. Jesse, R. Rodenborn, S. Sayag, M. Gebel, I. Aped, I. Sharfstein, E. Manaster, I. Ergaz, A. Harris, and L. Nelowet Grice, *ACS sustainable Chem. Eng.*, 2017, **5**, 2927–2935. <https://doi.org/10.1021/acssuschemeng.6b02399>
18. J. Michael Hollas, "Modern Spectroscopy", 4th Edition, Wiley, 2004. <https://www.wiley.com/en-us/Modern+Spectroscopy%2C+4th+Edition-p-9780470844168>
19. J. S. Williams and M. A. Katzenberg, *J. Archaeol. Sci.*, 2012, **39**, 41–57. <https://doi.org/10.1016/j.jas.2011.07.013>
20. J. C. Raposo, P. Navarro, A. Sarmiento, E. Arribas, M. Irazola, and R. M. Alonso, *Microchem. J.*, 2014, **116**, 125–134. <https://doi.org/10.1016/j.microc.2014.04.012>
21. N. Sadiq and D. Beauchemin, *Anal. Chim. Acta*, 2014, **851**, 23–29. <https://doi.org/10.1016/j.aca.2014.09.017>
22. F. Pena-Pereira, W. Wojnowski, and M. Tobiszewski, *Anal. Chem.*, 2020, **92**, 10076–10082. <https://doi.org/10.1021/acs.analchem.0c01887>
23. S. C. Teixeira, N. O. Gomes, T. Veloso de Oliveira, P. Fortes-Da-Silva, N. de Fátima Ferreira Soares, and P. A. Raymundo-Pereira, *Biosensors and Bioelectronics: X*, 2023, **14**, 100371. <https://doi.org/10.1016/j.biosx.2023.100371>
24. M. Lloyd-Smith. *Encyclopedia of Toxicology*, Third Edition, 353–65. Oxford: Academic Press, 2014. <https://doi.org/10.1016/B978-0-12-386454-3.01052-6>

25. Federal Institute for Occupational Safety and Health, Bureau REACH, Swedish Chemicals Agency, Norwegian Environment Agency, The Danish Environmental Protection Agency. 2023. ANNEX XV Restriction Report – Per- and polyfluoroalkyl substances (PFASs). (accessed September 18, 2024).
 26. EPA. (2024). Understanding Global Warming Potentials. <https://www.epa.gov/ghgemissions/understanding-global-warming-potentials>
 27. J. Zhang, J. Huang, Y. Li, Q. Liu, Z. Yu, J. Wu, Z. Gao, S. Wu, J. Kui, and J. Song, *Polymers* 2019, **11**, 1469. <https://doi.org/10.3390/polym11091469>
 28. Minitab™ Support. <https://support.minitab.com/en-us/minitab/help-and-how-to/statistical-modeling/doe/supporting-topics/response-surface-designs/response-surface-central-composite-and-box-behnken-designs/>
 29. M. MacConnachie, and D. Beauchemin, *J. Anal. At. Spectrom.* 2023, **38**, 1394–141. <https://doi.org/10.1039/d3ja00165b>
 30. F. Hou, C. Schimmele, and M. Stick. Changing Demographics of Racialized People in Canada. Statistics Canada, 2023. <https://doi.org/10.25318/36280001202300800001-eng>
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