Forensic glass analysis by LA-ICP-MS: Assessing the feasibility of correlating windshield composition and supplier

Award No: 2004-IJ-CX-K007

FINAL TECHNICAL REPORT

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Literature Review

Introduction

Glass fragments represent a valuable class of trace evidence. Like other traces materials, they are easily transferred from source to suspect, and are easily unnoticed by the suspect bearing them; further, glass fragments are particularly durable. Most glass products readily shatter when broken, distributing glass fragments to objects and persons in their path. Because there is a limited radius of distribution, glass transfers generally represent primary transfers resulting from contact or close proximity with the broken glass product (1). While secondary and environmental transfers do occur, they are rare (2-4); this suggests that most individuals bearing glass fragments were near the glass product(s) distributed on their person when the breaking event occurred. The persistence of these transfers is largely dependent on the retention of the material to which the transfer is deposited (1), whether the transfer was passive or forcible, and the ability of glass to withstand environmental effects. Common items of clothing (cotton and woolen materials) show a high retention for glass, passively or forcibly transferred. Certain materials, such as wood, soft polymers and metals, retain glass transfers only if forcible contact is made between the material and the glass source. Because glass fragments are often minute and transparent, it is usually difficult for a suspect to see the evidence and remove it. Glass fragments persist on a suspect's clothing or in soft materials for extended periods of time since glass is resistant to environmental degradation.

Classical methods of forensic glass examination are based primarily on variation within the physical properties of glass. Color, density, surface characteristics and optical properties have been relied upon for the comparison of unknown glass fragments with

control glasses (5-7). Surface characteristics and optical properties deserve attention in particular. Similarities in peculiar surface contamination or patterns of erosion between reference and questioned items are highly associative; like fracture edge matching, however, comparing original surfaces requires recovery of abnormally large questioned fragments.

The most common technique for comparing reference and questioned items is by refractive index (RI) comparison (5-7). RI measurement is excellent for distinguishing glasses by type and limited sample is required for multiple measurements. Historically speaking, RI measurement had limited application for classifying glass fragments because RI varied greatly among and within the traditional classes of glass (e.g., tableware, architectural glass, automotive glass, etc). Modern improvements in glass manufacture have decreased the RI variation within a particular class of glass (8). Modern glass has been observed to have a fairly consistent RI that corresponds to the type of glass in question; this makes RI an excellent tool for classifying glass but limits the utility of RI measurement for forensic individualization (7, 9-11). Some have proposed the measurement of RI at multiple wavelengths (called “dispersion analysis”) to enhance individualization by RI. It is rare that dispersion analysis enhances the discriminating power of RI (6).

The first reports of chemical analysis for the forensic discrimination of glass were published in the early 1970s. Initial analyses were made with the intention of classifying glass by type, using a wide variety of instrumental techniques including: neutron activation analysis (NAA), direct current arc source atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), spark-source mass spectrometry, and x-ray

fluorescence (10-18). The inorganic constituents targeted by these methods were in the part-per-million (or μg g^{-1}) to part-per-hundred (or dg g^{-1}) concentration levels. Over twenty elements were shown to have application in differentiating between sheet, container and tableware classes of glass (10).

Due to the cumbersome operation of NAA, the semi-quantitative nature of spark source mass spectrometry and x-ray fluorescence, and the limitation of single element quantitation inherent to AA, forensic researchers incorporated inductively coupled plasma source AES in the late 1970s and 1980s. Using this technology, Catterick and Hickman showed the potential to discriminate glasses by type having sample sizes of 500 μg or less (9). They also reported that no correlation existed between chemical composition and RI, indicating that chemical data can be used in conjunction with RI for increased distinction of glasses by type. The increased discrimination of elemental data used in conjunction with RI data was also suggested by Koons, Peters and Rebbert (17). In a separate report, Koons and Buscaglia (19) estimated the random occurrence of two fragments being indistinguishable in RI and elemental composition to be 10^{-13} – 10^{-15}. This would indicate that chemical data in tandem with RI measurement could facilitate individualization of glass with a high degree of certainty.

Discrimination among glasses using chemical data alone was first suggested by the results of Catterick and Hickman; they showed the discrimination potential of certain elements in discriminating glass samples that fell into the same general class (9). Koons, Fiedler and Rawalt (20) demonstrated the differentiation of sheet glass produced by separate manufacturing plants using six elements. Zurhaar and Mullings (21) argue that the quantitation of 15 – 25 elements provides a unique elemental profile for a particular

glass sample. They further report that 85 – 95% of window glass samples manufactured in the US and Australia are easily distinguished when a greater suite of elements are analyzed. While the uniqueness of a particular elemental profile can be argued (22), there has been a marked increase in the discrimination of glasses within a class by increasing the number of elements quantified and targeting trace elements ($\leq \mu g\ g^{-1}$ in concentration, 23).

Until the advent of ICP-source mass spectrometry (ICP-MS), the ability to perform simultaneous, multielement quantitation was not available for the forensic analysis of glass. While ICP-AES is capable of performing multielement quantitation, this technique does not offer simultaneous, multielement data collection. Nor is ICP-AES able to detect low-level elements, especially following acid digestion and sample dilution. Zurhaar and Mullings (21) were the first to apply ICP-MS to the glass matrix for forensic analysis. Parouchais, *et al* (24) used the principles of analysis set forth by Zurhaar and Mullings to propose improved sample preparation protocol for glass analysis by ICP-MS. In the work following, Suzuki, *et al* (25) were able to show the superior discriminatory capabilities of elemental data collected by ICP-MS for bottle glass; Montero, *et al* (26) similarly showed a high level of discrimination available for vehicle float glass using ICP-MS.

Forensic Glass Analysis by ICP-MS

The ICP-MS is a highly sensitive instrument capable of performing rapid, simultaneous, multielement analysis. This technique offers exceptionally low detection limits ($< pg\ mL^{-1}$) compared to other techniques for elemental analysis, and can be used

to detect over 70 isotopes. The ICP-MS has three main components: (1) the sample introduction mechanism, which is variable to accommodate gaseous, liquid, and solid samples, (2) the plasma and MS interface region, and (3) the mass analyzer and detector. Following sample introduction, the sample is injected into the plasma and undergoes desolvation and atomization. The resulting atoms are then ionized in the high-energy environment of the plasma; ions are transported through the MS interface region due to a sequential decrease in pressure. They are then mass-filtered and detected by a quadrupole or time-of-flight (TOF) mass spectrometer. Most commercial instruments are equipped with a quadrupole MS.

One of the many benefits of ICP-MS is the number of sample types that may be accommodated. The ICP-MS has been adapted for gaseous, liquid and solid samples, though the original design was intended for liquid sample introduction (called solution nebulization, SN). Early applications of ICP-MS to glass analysis involved lengthy dissolution protocols so that glass could be introduced using SN (21, 23-27). Advances in solid sampling for ICP-MS have been realized in the past decade, and now several reports regarding solid sampling for ICP-MS exist. The majority of forensic applications involve the use of laser ablation (LA) sample introduction (8, 28-30).

SN is the most common sample introduction technique for ICP-MS (31-35) and has wide application in forensic science (21, 36-40). It was the first method of introduction for the forensic analysis of glass (21, 23, 24). In fact, it is the only sampling technique for which an American Standards for Materials and Testing (ASTM) method exists (27). A multitude of sample types are appropriate for SN; matrix-matched calibration and quality control standards are easily obtained. One practical benefit of SN

is the decreased cost over other introduction techniques. Further, current instrumental configurations facilitate highly automated and rapid analysis. Automated instrument optimization and analysis are available, enabling high sample throughput with little analyst intervention. By comparison to solid sampling, less instrument maintenance is required using liquid sample introduction. This is because liquid samples leave fewer deposits on the sampler and skimmer cones and ion lens. While there are a few exceptions, liquid samples tend be "cleaner" overall (31).

The main drawback of SN introduction is that it is difficult to adapt to solid sample types (31-35). This is especially true of the glass matrix. For the forensic analysis of glass, a costly, time-intensive and potentially hazardous digestion using hydrofluoric acid (HF) is required. This digestion is open-vessel and is followed by two days of sample preparation (27). This process provides many opportunities for contamination and dilution errors; worse, it is a destructive technique.

The existing digestion protocol facilitates only a narrow range of sample masses because the final dilution volumes are relatively small. The minimal suggested sample size, 500 μg, is atypical of glass fragments received as evidence. Glass fragments of only several micrograms in mass are more frequently recovered than those of several hundred micrograms. Having trace elements at 1 – 100 parts-per-million, routine casework fragments push the lower sample size limits for SN-ICP-MS using the established forensic methodology. Finally, the resulting sample volumes might prevent the analyst from performing replicate analyses with certain devices for SN sample introduction. Nonetheless, SN introduction remains the most frequently used sample introduction technique for forensic glass analysis by ICP-MS.

Only a handful of forensic applications exist for LA-ICP-MS, including methods for glass, paint, and bulk metal materials (8, 28-30). Glass analysis stands out as the prominent and best-developed forensic application of both ICP-MS and LA-ICP-MS. Since the application of the LA sampling technique to ICP-MS, forensic analysts have been able to push the lower limits regarding sample size while providing comparable or greater statistical information than was previously available (29, 30).

Due to the diminutive sample size requirements for LA sampling, typical casework sample sizes are easily accommodated. For example, to perform triplicate analyses of a single questioned glass fragment an optimal sample volume of 3×10^6 μm^3 is preferred; this corresponds to a fragment 300 μm in length, 100 μm in width and 100 μm in depth. In terms of mass, such a fragment is approximately 7 μg. To examine glass by SN-ICP-MS, many would argue that the minimum sample mass is 500 μg but some agencies require 2000 μg – of which 100% is consumed by digestion and analysis. If a traditional-flow (1 mL min^{-1}) nebulizer is used, only one analysis can be performed for a particular digest. This presents a limitation in that statistics cannot be applied to the result. Alternatively, only 0.9 μg glass is consumed during a triplicate analysis using LA. This is roughly 12% of a 7-μg fragment. The limited sample consumption of this technique enables the analyst to perform replicate analyses while preserving the majority of the sample. A benefit to the minimal sample consumed during LA analysis is that when fragments larger than the minimum are recovered (15 μg or more), there is enough sample for additional analyses to be conducted indepdently. To analyze fragments by SN-ICP-MS, the criminalist performing elemental analysis would be required to obtain permission prior to digestion and analysis; additional scientific experts would be then

able to view the analysis conducted by the criminalist but they would not be able to conduct an independent analysis.

Sample preparation requirements for LA introduction are greatly reduced over those required for SN introduction. For glass analysis by LA-ICP-MS, the samples need only to be cleaned and mounted on a glass slide. There is little opportunity for contamination and no dilution error – both advantages of analyzing the sample "as it is." This benefit was much lauded by Lundell in 1933 (41), who asserts that valuable analyte information can be lost when removed from the original sample matrix. The field of criminalistics similarly fosters the ideology of *in situ* analysis – physical evidence examinations are always performed in such a manner as to provide highly discriminating data while preserving the character and quantity of the sample to the extent possible.

Currently, the primary analytical limitation of LA is calibration; for some applications, internal standardization is an equally limiting factor. The issue of calibration stems from the fact that well characterized matrix-matched calibration standards are not readily available for many sample matrices. To overcome this issue many have attempted liquid calibration by SN or LA, while many others use the solid National Institute of Standards (NIST) standard reference glasses 610 and 612 for single point calibration (31, 42-46). The use of a single calibrant has been done following the validation of linear response in analyte: internal standard. Alternatively, it is done under the assumption that LA sampling does not alter the many decades of linear response available from ICP-MS. However, this assumption may not be valid for some analytes in certain matrices (47). As Akbar Montaser aptly put, calibration remains the "Achilles' heel of the laser ablation technique" (31).

Internal standardization is not easily incorporated into solid sampling techniques for ICP-MS. Unlike samples in solution, internal standard spikes are not easily added to solid samples. Instead, it is desired to use an isotope for which analytical response is representative of most or all analytes, that is naturally occurring in the sample, and that is easy to quantify. Generally speaking, this entails the use of a mid-mass isotope (31, 42). Isotopes of silicon have been used for analytes in a variety of matrices including glass (30, 48).

The FBI and forensic research groups have been the primary users of ICP-MS for forensic glass analysis in recent years. To date, less than a dozen state-run crime laboratories own an ICP-MS and among these, the majority makes use of SN sample introduction. The central reason behind the slow integration of ICP-MS to routine casework is the cost of purchasing and maintaining this instrumentation. Modern ICP-MS instruments cost approximately $300,000 and consume high purity argon at a rate costing operators anywhere from $12,000 to $15,000 per year. When the additional cost of a LA unit is considered (approximately $125,000) criminalists are skeptical of such a purchase. Many crime labs struggle to make instrument purchases equal to that of the LA system alone. Nonetheless, the criminalistics community is becoming aware of the discriminating potential of glass analysis by ICP-MS. This has created a demand for increased collaboration among the more and less equipped forensic labs: forensic research groups often consult for local crime labs, while larger crime labs (such as the FBI and some state crime labs) will similarly consult for less well-funded labs.

Since a variety of techniques are available for the forensic analysis of glass by ICP-MS, it is important to provide a direct comparison of the figures of merit achievable

using each technique. One report exists describing the application of both SN-ICP-MS and LA-ICP-MS (30) to forensic casework samples; a multitude of publications exist describing some aspects of the analytical performance of SN and LA (8, 21, 24, 27, 30, 31, 42-46, 48-51). However, none were dedicated to the comparison between traditional flow SN, micro-flow SN and LA sampling in terms of classical figures of merit. Here, the relative figures of merit for SN using a traditional concentric nebulizer (CN), a microflow concentric nebulizer (or microconcentric nebulizer, MCN), and LA using a 213-nm Nd:YAG laser ablation unit are compared. These are among the most common methods of SN and LA introduction available; it is likely that members of the forensic community will encounter these sample introduction techniques before others.

The Forensic Significance of Elemental Variation in Glass

The face of forensic glass examination has changed dramatically with the advent of trace elemental analysis by ICP-MS. Classical methods of forensic glass analysis, such as color comparison, density measurement and RI comparison, do not offer the individualizing potential that quantitative trace elemental analysis promises. While the discriminating potential of trace elemental analysis has been demonstrated, the individualizing power of this technique has not been well characterized.

Technically, glass is an amorphous, super cooled liquid formed by fusion (52). Glass is manufactured from inorganic oxides, which when melted together chemically react to form the final glassy product. The primary component of glass is silica sand (SiO_2). Because the fusion temperature of pure silica is too high for most commercial furnaces, sodium oxide (Na_2O) is added to reduce the fusion temperature. Such an

additive is a "fluxing agent." Glass is classified by the chemical "modifiers" added to produce the glass end product having certain performance characteristics. Common classes of glass are soda-lime-silicates, alumino-silicates, and borosilicates. Respectively, these are silicate glasses with limestone (CaO), alumina (Al_2O_3) and boric oxide (B_2O_3) modifiers. The major inorganic constituents of common consumer glasses are described in Table 1.

Table 1. Major constituents of common consumer glasses (52).

Glass type	Use	Major constituents (0.02 – 80 % by weight)
Container		
White *Amber* *Green*	Bottles, jars	SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO (except Amber), Na_2O, K_2O (except Green) and Cr_2O_3 (Green only)
Float	Windows, windshields	SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, K_2O
Borosilicate	Kitchenware, labware	SiO_2, Al_2O_3, Fe_2O_3, CaO, Na_2O, K_2O, B_2O_3
Lead crystal	Decorative	SiO_2, Al_2O_3, Fe_2O_3, Na_2O, K_2O, PbO, B_2O_3, As_2O_5

The process of glass manufacture can be summarized by the following steps: (1) weighing and mixing of raw materials, (2) glass melting, (3) glass forming, (4) annealing, and (5) secondary processing (52, 53). The process of weighing and mixing of raw materials for the manufacture of consumer glass is highly automated, making use of conveyer belts and computer-operated chutes that dispense specified amounts of raw

materials. The glass is melted in brick furnaces, wherein temperatures rise to approximately 2000°C. During this process, the glass is mixed to ensure adequate homogenization. Mixing during melting also facilitates the release of gaseous carbon dioxide created during chemical reactions of inorganic constituents. Glass formation is progressive; as melted material moves forward in the furnace (which is continuously fed with raw materials), the temperature is manipulated such that fusion occurs at the rate appropriate for the glass forming method used. The annealing process involves slow cooling of the glass, so that stress points are minimized. Finally, depending on the glass end product desired, the glass may require secondary treatments such as tempering, coating or decorating.

The most common type of glass encountered in the forensic context is "float glass," so named for the bed of molten tin on which glass is floated during forming. After exiting the melting furnace, glass is floated in melted tin in a controlled environment. The glass forms a sheet, called a "ribbon," that exits the tin chamber and is progressively cooled on lehrs. This method was first introduced by the Pilkingtons of Great Britain in the late 1950s. The float process produces glass of exceptional quality over other methods of glass manufacture (52). Over the years, glass produced by the float process has begun to vary less and less in physical properties, including RI (8). As a result, RI measurement alone has limited utility for discriminating float glass, especially modern float glass. The success of trace element analysis in discriminating float glass products of different origin, yet having similar physical properties, is related to the detection of trace constituents. These constituents are unintentional components of the batch, and their amounts are uncontrolled at the manufacturing stage.

The amounts and sources of major batch constituents, such as silica sand (SiO_2), soda ash (Na_2O) and limestone (CaO), are never modified (Personal communication, Chris Miller of Pilkington-Libbey Owens Ford). However, the amounts of colorants and recycled glass, or cullet, are varied depending on the desired characteristics of the glass end product (52, 53). Colorants are added in predictable amounts; like the major constituents, these chemicals are of little forensic utility since their amounts are consistent among manufacturers producing similar glass products. Cullet introduces the greatest potential for individualizing otherwise similar glass, since the amount of cullet that is incorporated in the batch can change daily. Further, there is no federal or state-mandated minimum for cullet consumption in the manufacture of float glass. The amount of cullet that is incorporated is left to the judgment of the manufacturer alone. An additional source of compositional variation is the potential for micro-impurities in the batch constituents. Such low-level impurities are not likely to be made manifest in color changes and probably vary by source of the raw material in question.

While trace elemental analysis is currently the most distinguishing technique available for the forensic analysis of glass, it is difficult to assign the significance of trace elemental data because the potential for natural variation in glass composition has not been adequately addressed. Little has been published regarding the potential for compositional variation within a single glass product, or within a class of glass products.

Trejos and Almirall conducted a study aimed at evaluating the potential for micro-heterogeneity in glasses pertinent to forensic casework, targeting compositional variations at the micro-scale. It was shown that the typical ablation parameters used for bulk analysis by LA-ICP-MS did not result in misrepresentative sampling (22). A similar

conclusion was reached by Kempanaers, *et al* in a study describing the micro-heterogeneities found in glass standard reference materials (54). Duckworth, *et al* published a report describing significant elemental variation in a population of 72 automotive side windows produced by 19 separate manufacturers (55); the large variations observed within this class of glass facilitated a high degree of individualization, especially when RI data was included with elemental data. Duckworth, *et al*, however, failed to report whether within-sample variation was observed in these automotive side windows.

It is reasonable to hypothesize that some compositional variation exists in glass, since it is continuously manufactured with the addition of new raw materials and cullet in variable amounts. This is particularly true of automotive windshields, which are composed of two relatively large discrete panes of glass joined by a layer of lamination (53). If compositional heterogeneity in glass exists at the trace level, it is reasonable to postulate that such heterogeneity would become apparent in larger glass products. Further heterogeneity is possible in automotive windshields, since they are not always composed of panes that are manufactured sequentially. In fact, certain manufacturers assemble windshields from panes of glass produced from completely separate batches, which differ in thickness and color.

While potential micro-heterogeneities in glass have been shown to have no effect on the quality of forensic glass analysis by LA-ICP-MS, the potential impact of macro-heterogeneity has not been assessed, especially with respect to automotive windshield glass. The potential for elemental variation between the two panes of glass comprising automotive windshields has been shown (22), but this variation has not been determined

for a large population of windshields. Without confidence in the homogeneity of automotive windshields, it is difficult to establish the suitable number of reference samples to be collected for forensic comparisons by LA-ICP-MS. Further, it is difficult to assign the significance of similar or dissimilar elemental profiles when the potential for heterogeneity has not been excluded.

Trace elemental profiling has, thus far, been used exclusively as a comparative tool. That is, as a means to compare questioned and reference glass fragments to establish whether the questioned fragments could have originated from the reference glass. The potential for elemental profiling to provide investigative information has not been explored. Typically, individual glass manufacturing plants are dedicated facilities that specialize in the production of a single type of consumer glass product. Since the major batch constituents used to produce various glass products are rarely changed, the trace impurities present in the raw materials may serve as a unique fingerprint for a specific manufacturing facility. Such impurities would necessarily exhibit larger variation in a broad population of windshields from various manufacturers than the variation observed between windshields produced by the same manufacturer, to successfully fingerprint glass.

The elemental variation of automotive windshields was investigated in three ways: within-pane variation, within-sample variation and population variation. This was done to supplement the existing body of knowledge regarding the discriminatory potential of elemental analysis by LA-ICP-MS, since this potential has not been fully evaluated for automotive windshield glass exclusively.

Research Purpose

With the advent of forensic trace element detection by ICP-MS comes the unique opportunity to evaluate the variability in the elemental composition of automotive windshield glass. Of particular interest to the Sacramento County District Attorney's Laboratory of Forensic Services is the utility of trace elemental profiling for fingerprinting automotive windshields produced by a specific manufacturer. The impetus for this research is a long-standing case. Recently, we became involved in a homicide case wherein a vehicle was used as the murder weapon. Glass fragments were the only recovered evidence from the victim. Though a vehicle was seen running the victim down, a suspect was not immediately identified. After several months of investigation, a man who had argued with the victim became the primary suspect in the case. However, when investigators examined the suspect's car, there was no apparent damage. None of the windows were broken, and the vehicle's paint appeared flawless. Upon further examination, investigators were able to locate glass fragments inside the engine compartment of the vehicle. These were collected and compared to the glass fragments recovered from the victim. The techniques used to analyze these glass samples included RI measurement and elemental analysis by SEM-EDS and LA-ICP-MS. In terms of RI and major elemental composition, the questioned and reference fragments appeared similar. The results of trace elemental analysis by LA-ICP-MS corroborate this finding, however, it is difficult to assign significance to this finding as the uniqueness of a given trace elemental profile is unknown. Further, the homogeneity of automotive windshields is unknown.

The goal of this study was to provide forensic caseworkers with sufficient context regarding within-sample variation and population variation in the compositions of automotive windshields so that the appropriate significance can be assigned to trace elemental data. To do so, we collected and analyzed 50 automotive windshields representing 17 separate manufacturers and one unknown manufacturer. We examined the within-pane variation of the interior and exterior panes of automotive windshields using a subset of 10 windshields, the total variation in elemental composition exhibited by all 50 windshields (100 panes of glass all together), and the variation observed within groups of manufacturers. The elemental data for all 50 windshields was organized into a Microsoft Access database. Qualitatively, we examined the variation in glass production by analyzing quality control samples collected directly from float glass manufacturers' stocks. This was done to determine whether other types of float glass products could exhibit the patterns of variation observed in automotive windshields.

We observed that some, not all, windshield panes exhibited heterogeneity and found that about half the windshields we analyzed were composed of significantly different panes of glass. We also found that the compositional variation of windshields from individual manufacturers was much smaller than that observed in the total population of automotive windshields. This finding suggests the potential for fingerprinting glass produced by specific manufacturers; additional research is required to fully evaluate this potential. Should this prove to be a feasible means of correlating consumer glass products with their manufacturers, investigative information may be gained from analyzing questioned fragments in the absence of reference samples. Using elemental data to provide investigators with putative sources of evidentiary glass

fragments is an attractive possibility, since this is a common occurrence in hit-and-run offenses.

Prior to commencing the above research, we validated the use of SN- and LA-ICP-MS for forensic casework. As a public service forensic laboratory, it is our priority to make new techniques immediately available for casework analysis. Once we have tested the validity of new techniques, it is our mission to make these techniques available to other public service forensic laboratories that, for whatever reason, do not otherwise have access to them. We found that SN- and LA-ICP-MS can be confidently applied to casework analyses, as long as the relative shortcomings of each technique are acknowledged. However, we found that LA-ICP-MS emerges as the superior technique for forensic glass analysis when the performance characteristics of SN- and LA-ICP-MS are compared.

At the close of this research, we were able to establish the validity of trace elemental analysis for the forensic analysis of casework samples and determine what level of significance to apply to trace elemental data.

Materials and Methods

SN-ICP-MS: Figures of Merit

We characterized the figures of merit achievable using SN-ICP-MS and the ASTM method E 2330-04 (with some modifications) for two different nebulizers alternatively joined to the same quartz conical spray chamber. The common concentric nebulizer (CN) and the microconcentric nebulizer (MCN) were investigated. The MCN operates on the same principles as the CN, but is designed for low sample consumption.

The CN used in this study was operated at a nominal sample uptake rate of 1 mL min^{-1} while the MCN was operated a nominal sample uptake rate of 0.1 mL min^{-1}.

The figures of merit established for each nebulizer included: method detection limits (MDLs), limits of quantitation (LOQs), analytical sensitivity, accuracy, precision, and bias as well as short- and long-term reproducibility.

Analyte Selection and Sample Preparation. The element menu used for this comparison was based on ASTM E 2330-04 (Table 2).

Table 2. Table of analytes.

Element, Symbol	*m/z*
Magnesium, Mg	25, 26
Titanium, Ti	47
Manganese, Mn	55
Gallium, Ga	69, 71
Rubidium, Rb	85
Strontium, Sr	86, 88
Zirconium, Zr	90, 91, 92, 94
Antimony, Sb	121
Barium, Ba	137, 138
Lanthanum, La	139
Cerium, Ce	140
Hafnium, Hf	178
Lead, Pb	206, 207, 208

Two Standard Reference Materials (SRMs) from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) were selected to examine the accuracy, precision and bias of SN-ICP-MS: NIST SRMs 610 (nominally 500 μg g^{-1} in selected trace elements) and 612 (nominally 50 μg g^{-1} in selected trace elements). These SRMs were selected over other available standard glasses because they are well characterized (56).

A single wafer of each SRM was broken from which ten fragments were sampled spanning a mass range of 0.1 to 0.5 mg (Table 3). These fragments were washed in methanol (VWR, West Chester, PA, USA), and soaked in 10% ultra pure nitric acid (HNO_3, OmniTrace *Ultra*; EM Sciences, Merck KgaA, Darmstadt, Germany) for no less than 30 minutes. Each was rinsed deionized water between washings (Resistivity = 18 MΩ • cm; Barnstead, Dubuque, IA, USA). Every sample was allowed to air-dry. Masses for each fragment were recorded (±0.002 mg) before each was transferred to a 15-mL metal free polyethylene sample tube (CPI International, Santa Rosa, CA, USA).

Table 3. Masses of selected NIST SRM fragments.

NIST SRM 610		**NIST SRM 612**	
Designation	***Mass, mg***	***Designation***	***Mass, mg***
1	0.306	1	0.279
2	0.180	2	0.273
3	0.195	3	0.194
4	0.422	4	0.230
5	0.189	5	0.307
6	0.409	6	0.269
7	0.095	7	0.309
8	0.182	8	0.235
9	0.374	9	0.191
10	0.549	10	0.504

Samples were digested using 600 μL of a 3:1:1 concentrated hydrofluoric acid (HF, from OmniTrace; EM Sciences, Merck KgaA, Darmstadt, Germany), concentrated HNO_3, and concentrated hydrochloric acid (HCl, from OmniTrace; EM Sciences, Merck KgaA, Darmstadt, Germany) mixture. The sample tubes were capped, vortex mixed and placed in a sonicating bath for at least two hours until completely dissolved. The samples

were then uncapped and placed on a heating block at 80°C for approximately 36 hours or until dry. Samples were stored dry, at room temperature.

Samples were reconstituted with 4 mL 10%-HNO_3 for at least 24 hours but not longer than 48 hours. Finally, each sample was internally standardized by adding 25 µL of a 10-µg mL^{-1} rhodium (Rh) stock solution (CPI International, Santa Rosa, CA, USA) and diluted to a final volume of 5 mL using 18-MΩ H_2O. The final concentration of Rh in each of the samples was 50 ng mL^{-1}. NIST SRMs 610 and 612 prepared in this way yielded approximately 30 – 50 ng mL^{-1} and 1 – 5 ng mL^{-1} trace elements, respectively. Procedural blanks were prepared by adding the acid mixture to a clean, empty sample tube. These samples were digested, reconstituted and diluted as above.

For reproducibility testing, a single fragment of SRM 610 was selected (mass = 6.963 mg). This fragment was chosen to serve as a stock from which multiple dilutions could be made, to eliminate the digestion procedure as a potential source of variation. Following digestion (as described above), this solution was reconstituted in 5 mL 10%-HNO_3 for 24 hours. To prepare reproducibility solutions, a 500-µL aliquot of this stock solution (approximately 700 ng mL^{-1} in concentration) was transferred to a metal free sample tube; the aliquot was diluted with 25 µL 10-µg mL^{-1} Rh and 4.750 mL 18-MΩ H_2O to obtain solutions that were approximately 70 ng mL^{-1} in concentration.

Instrumentation and Analytical Methods. A Perkin Elmer Elan DRC II ICP-QMS (Boston, MA, USA), equipped with a Cetac Autosampler/Autodilutor (Omaha, NE, USA), was used for this study. The instrument was operated in normal mode (i.e., without reaction gas), using default mathematical corrections for common isobaric interferents (corrected elements are shown in Table 4). Prior to analysis, instrument

optimization was performed using standard solutions. Table 5 summarizes the typical instrumental parameters used during this study.

Table 4. Default interferent correction.

Isotopes	Interferents	Correction
^{86}Sr	$^{86}Kr^{+}$	$-1.505657 \times S(^{83}Kr^{+})$
^{92}Zr	$^{92}Mo^{+}$	$-0.932161 \times S(^{95}Mo^{+})$
^{94}Zr	$^{94}Mo^{+}$	$-0.581030 \times S(^{95}Mo^{+})$
^{138}Ba	$^{138}La^{+}$, $^{138}Ce^{+}$	$-0.000901 \times S(^{138}La^{+}) - 0.002838 \times S(^{138}Ce^{+})$

Key: $S(^{83}Kr^{+})$ refers to the signal due to $^{83}Kr^{+}$

For all solution analyses, the same quartz cyclonic spray chamber (Perkin Elmer, Boston, MA, USA) was alternately joined to a quartz concentric nebulizer (CN; Perkin Elmer, Boston, MA, USA) or a MicroMist quartz microconcentric nebulizer (MCN; Glass Expansion, West Melbourne, Victoria, AU). The quartz CN had a typical sample uptake rate of approximately 1 mL min^{-1}; the quartz MCN had a typical sample uptake rate of approximately 0.1 mL min^{-1}.

Table 5. Typical ICP-MS operating parameters.

Parameter	Value
Nebulizer Gas Flow	
CN	1.0 L min^{-1}
MCN	1.1 L min^{-1}
Auxiliary Gas Flow	1.2 L min^{-1}
Plasma Gas Flow	15 L min^{-1}
RF Power	1350 W
MS Analytical Settings	20 sweeps / reading 1 reading / replicate 3 replicates

External calibration was performed using a simple linear model with the multielement calibration standards described in Table 6. Each was internally standardized with 50 ng mL^{-1} Rh. To prepare these standards, multielement standard stock solutions obtained from Spex CertiPrep (Metuchen, NJ, USA) were used. A quality control (QC) sample was prepared using the same standard stocks to monitor calibration drift. The QC sample was a multielement solution, containing all analytes of interest prepared at 60 ng mL^{-1} with 50 ng mL^{-1} Rh. A 10% tolerance was applied to the QC sample.

Table 6. Calibration and QC standard concentrations.

Calibration Level	Final Concentration, ng mL^{-1}
Blank, S(0)	0
Level 1, S(1)	1
Level 2, S(2)	10
Level 3, S(3)	50
Level 4, S(4)	75
Level 5, S(5)	150
Quality Control	60

To determine method detection limits, three procedural blanks were analyzed on two nonconsecutive days. To fairly compare the CN and MCN, these were analyzed on the same days to eliminate potential interday variation in instrument performance. Sensitivity was determined by averaging calibration data for two nonconsecutive days. For accuracy and precision testing, ten fragments of NIST SRMs 610 and 612 were digested, reconstituted and prepared as above. Single replicates were performed on these samples using both the CN and MCN. Bias determinations were made from these results. Within-run reproducibility could be established for MCN introduction only; this was accomplished by analyzing the first five digests of NIST SRMs 610 and 612 in quadruplicate. Within-day reproducibility was determined by single replicates of three

reproducibility samples (dilutions from a single stock, as described above) separated by no less than 90 minutes using the CN. Triplicate analyses were run on the same solutions at similar time intervals using the MCN. Long-term reproducibility was determined by analyzing freshly prepared reproducibility samples using CN and MCN for four nonconsecutive days. Each day, the same solution was analyzed using the CN and MCN. Single replicates were performed using the CN; triplicate analyses were performed using the MCN.

Data Analysis and Calculations. Method detection limits (MDLs) and limits of quantitation (LOQs) were then calculated using the method described by Miller and Miller (57), modified to account for internal standardization:

$$MDL = \frac{3s_b}{(Rh_s)(m)}$$

$$LOQ = \frac{10s_b}{(Rh_s)(m)}$$

Where s_b is the standard deviation in the blank measurement in counts per second (CPS), Rh_s refers to the internal standard signal in CPS and m refers to the slope of the calibration curve with units of:

$$m = \frac{[A, ng\ mL^{-1}] \cdot CPS_A(background\ subtracted)}{CPS_{IS}}$$

Where *[A, ng mL^{-1}]* is the concentration of analyte *A* in units of ng mL^{-1}; CPS_A refers to the background-subtracted analyte signal in CPS; and CPS_{IS} refers to the internal standard signal in CPS.

Absolute detection limits (ADLs) were calculated by multiplying MDLs, in units of ng mL^{-1}, by the average sample volume consumed per analysis (approximately 1.2 mL using a CN and 0.12 mL using a MCN).

To establish the accuracy of SN-ICP-MS, experimental results for NIST SRMs 610 and 612 were compared to previously published values (56) using the Student's *t*-test (57). When:

$$\left\| \bar{x}_{Exp} - \bar{x}_P \right\| > t \cdot S_D$$

The difference in the experimental ($\bar{x}_{Exp}$) and published ($\bar{x}_P$) values were considered significant, given t = Student's t at 95% confidence and S_D, the standard deviation in the difference between the two means, equals:

$$S_D = S_{pooled} \cdot \sqrt{\frac{N_1 + N_2}{N_1 N_2}}$$

Where N_1 and N_2 respectively refer to the number of replicate measurements in the experimental and published data sets. S_{pooled} is expressed as:

$$S_{pooled} = \sqrt{\frac{s_1^{\,2}(N_1 - 1) + s_2^{\,2}(N_2 - 1)}{N_1 + N_2 - 2}}$$

Where s_1^2 *and* s_2^2 refer to the variance in the experimental and published data sets, respectively. These calculations were performed for results obtained using each nebulizer.

The precision of the method was established by determining the percent relative standard deviations (%-RSDs) in replicate measurements of each SRM using both nebulizers (N = 10, for both). The bias of SN-ICP-MS using either a CN or MCN was calculated using the following relationship:

$$Bias = \mu - x_t$$

Where μ is the experimentally determined population mean and x_t is the true value for a particular element. In this case, the NIST certified values were used as the true value. It was determined that ten replicates were good estimates of the true population means, given the difficulty in sample preparation.

Within-day and long-term reproducibility results using the CN were compared using an arbitrary 3%-tolerance of the result. This value was used because it is the maximum %-RSD tolerated during instrument optimization. Mean isotopic concentrations and standard deviations were calculated for results obtained using the MCN. Confidence intervals at 95% confidence (p = 0.05) were used as a measure of analysis error.

LA-ICP-MS: Figures of Merit

Sample Selection and Preparation. NIST SRMs 610, 612 and 614 (Table 7) were chosen to evaluate the linearity of LA-ICP-MS. NIST SRMs 610 and 612 were then used to evaluate the accuracy and precision of this technique. Bias was calculated using accuracy results. Finally, NIST SRM 610 was used to evaluate the short- and long-term reproducibility of this method.

Standard glass wafers were washed in methanol (VWR, West Chester, PA, USA) and soaked in 10 % by volume trace metal grade nitric acid for approximately 30 minutes. Each wafer was rinsed with 18-MΩ H_2O following the methanol and acid washes. They were allowed to air dry prior to ablation.

Table 7. Nominal composition of NIST SRMs.

Designation	Nominal concentration of trace metals
610	500 mg kg^{-1}
612	50 mg kg^{-1}
614	1 mg kg^{-1}

Instrumentation and Analytical Methods. A 213-nm neodymium-yttrium aluminum garnet (Nd:YAG) laser ablation unit (New Wave Research; Fremont, CA USA) was connected to the same Perkin Elmer ELAN DRC II ICP-MS described above. Helium was used as the ablation gas (flow = 1 L min^{-1}). The sample line exiting the ablation cell was connected to the nebulizer argon flow with a t-connector approximately one foot in front of the ICP torch entrance. The ICP-MS was optimized and tuned for the best possible performance using solution standards, introduced to the instrument by way

of the traditional quartz CN used above and quartz cyclonic spray chamber. Following solution optimization, the helium carrier gas flow rate was optimized using an external mass flow controller with NIST SRM 612. Refer to Table 4 for instrumental interference corrections.

Elements of interest included those described in Table 2, with one exception: ^{49}Ti was used to quantify titanium. Calibration for accuracy, precision, bias and reproducibility testing was accomplished using a single point calibrant, primarily SRM 612. SRM 610 was used as a calibrant when obtaining quantitative data for NIST SRM 612. A quality control sample, NIST SRM 1831 (soda lime sheet glass), was analyzed following calibration to ensure accurate quantitation and monitor instrument drift. Ablation of samples was accomplished using a 60-μm spot at 100% laser power (providing ~0.4 mJ output energy), 10-Hz repetition rate and 50-sec dwell time. Approximately 300 ng sample was introduced to the plasma during a single ablation.

Glitter Time Resolved Software (marketed by New Wave Research; Fremont, CA USA) was used to convert raw instrumental signal in counts per second (CPS) to quantitative data. Compositional information for each calibrant was taken from Pearce, *et al* (56); ^{29}Si was used as the internal standard for all elements.

Because the signal generated during a laser ablation experiment is transient (Figure 1), background and signal were manually selected for integration. The ablation burst (Figure 1), where apparent, was not included for signal integration. This signal characteristic corresponds to the initial burst of material ejected during the ablation event (called an "eruption"). It is routinely excluded from the signal to ensure sample

equilibration in the sample transfer line and as an added measure to avoid potential signal contributions from surface contaminants.

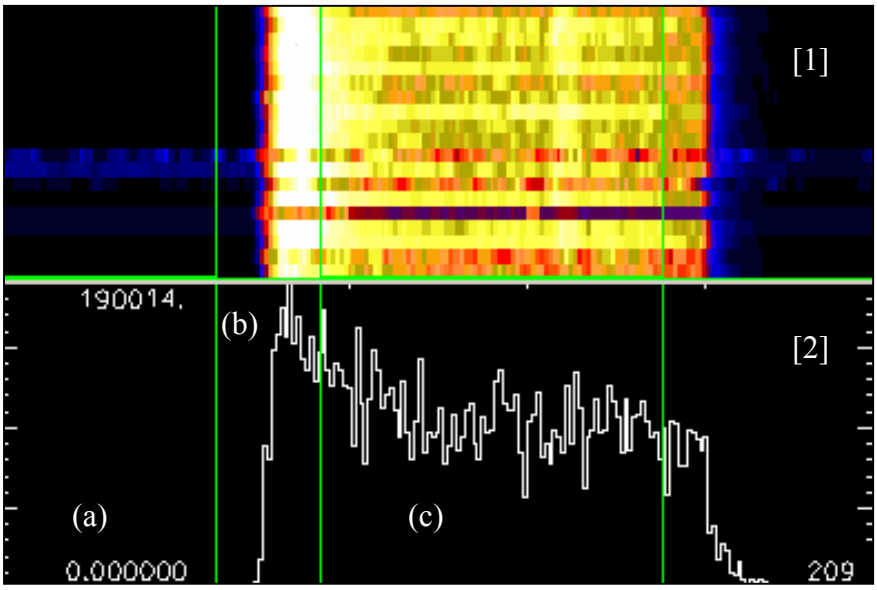

Figure 1. Screen shot of *Signal Selection Window*: Glitter Time Resolved Software. [1]: Three-dimensional representation of time-dependent signal intensity data. Time is represented by the *x*-plane, the isotope examined is represented by the *y*-axis, and signal intensity is represented by the *z*-plane. [2]: ^{7}Li signal intensity as a function of time, NIST SRM 612). *Key*: (a) Selected background signal, (b) ablation "burst," and (c) selected steady state signal.

Two ablations were performed for calibration, once before QC analysis and again following sample analysis. Instrumental drift was corrected by bracketing samples with calibrants. For linearity testing, SRMs 614, 612 and 610 were each ablated four times, without calibration. Twenty consecutive ablations were performed for accuracy, precision and bias testing using SRMs 610 and 612. For reproducibility testing, four to five ablations were performed on SRM 610 at three time points, which were separated by no

less than 90 minutes on four nonconsecutive days. No more than 25 ablations were performed between calibration standard analyses.

Data Analysis and Calculations. To verify that a linear response in analyte: internal standard is obtained over the analytical mass range used during LA sample introduction, the raw signals of ^{49}Ti, ^{85}Rb, and ^{206,207,208}Pb in NIST SRMs 610, 612 and 614 were divided by the raw signal due to ^{29}Si during each run. Average analyte: internal standard signal values for four ablations were plotted as a function of NIST reported concentrations. Linear regression using Microsoft Excel was applied to determine the slope, *y*-intercept, and correlation coefficient (R^2) as well as the errors in the slope and *y*-intercept. ^{49}Ti, ^{85}Rb and $^{206,\ 207,208}$Pb were selected as representatives of the relevant isotopic mass range and because NIST reported values[1] for these isotopes in all three glasses.

Method detection limits were reported by Glitter Time Resolved Software (58) on the basis of the following relationship:

$$MDL = 2.3\sqrt{2 \cdot B}$$

Where *B* refers to the mean background signal of a particular isotope, obtained during a given ablation experiment. Absolute detection limits (ADLs) were calculated by multiplying MDLs (in units of $\mu g\ g^{-1}$) by the approximate mass of ablated material during a single run (300 ng or 3.0×10^{-7} g).

[1] Concentration values of rubidium and lead are NIST-certified. Titanium concentrations are provided for "information-only."

As before, the accuracy of this technique was determined by comparing quantitative results for NIST SRMs 610 and 612 (N = 20, each) to previously published data using a Student's *t* test (57) for each element. Precision was determined by calculating percent %-RSDs for replicate analyses. Bias was calculated as before. Since numerous data points could be collected (N = 20), it was assumed that the experimental data collected for both NIST SRMs 610 and 612 were good estimates of true population means.

For reproducibility testing, mean isotopic concentrations and standard deviations were calculated and compared using confidence intervals at 95% confidence (p = 0.05) as a measure of analytical error.

Evaluation of SN and LA for Forensic Casework

To determine what effects the relative figures of merit of SN and LA might have on casework analyses, a blind test was designed to simulate a case. The sample amounts used for this study were such that enough sample would be available for chemical analysis by three methods and refractive index (RI) measurement. Thus, in terms of sample size this study did not accurately mimic a case.

An individual having knowledge of the glass sources chose three samples of several fragments each. Each was submitted in a plastic dish respectively labeled, "Reference Glass #1," "Questioned Glass #1," "Questioned Glass #2," and "Questioned Glass #3." Hereafter to be referred to as "K-1," "Q-1," "Q-2," and "Q-3."

K-1, Q-1, and Q-2 were transparent and green in color; Q-3 was transparent and clear. At first observation, it was noted that Q-3 could be excluded from sharing a

common origin as K-1 on the basis of color. K-1, Q-1, and Q-2 could not be distinguished on the basis of color; these samples were then subjected to RI measurement and chemical analysis.

For RI measurement, one fragment of each glass sample was selected and scraped with a diamond-tipped scribe. Glass particles were transferred to Standard B oil (Locke Scientific Limited; Tadley, Hampshire, UK) on a glass slide and covered with a standard glass cover slip. A Glass Refractive Index Measurement System II (Foster and Freeman; Evesham, Worcestershire, UK) was used for RI measurement. Five measurements of RI were taken for the reference glass, while four were taken for each questioned fragment.

Additional samples were taken for chemical analysis by SN-ICP-MS and LA-ICP-MS. Both the CN and MCN were used during SN introduction. Fragment masses selected for digestion are described in Table 8. Multiple fragments of the reference were digested and analyzed via single and quadruplicate analyses with a CN and MCN respectively. Questioned fragments were analyzed similarly, with the exception that single fragments were removed for acid digestion. This was done to simulate a case. Fragments subjected to LA were not weighed; four ablations were performed on the reference glass fragment while three were performed on questioned glass fragments. Sample preparation, analysis and data analysis was conducted as before.

Table 8. Sample masses dissolved for SN-ICP-MS analysis.

Sample name	Mass, mg (± 0.002)
Reference Glass (K)	
Subsample 1	0.594
Subsample 2	0.313
Subsample 3	0.324
Subsample 4	0.235
Subsample 5	0.817
Subsample 6	0.242
Questioned Glass #1 (Q-1)	0.423
Questioned Glass #2 (Q-2)	0.515

Elemental Variation in Automotive Windshield Glass

Analyte Selection and Sample Preparation. The analytes chosen for this portion of the study included ^{25,26}Mg, ^{47}Ti or ^{49}Ti, ^{55}Mn, ^{57}Fe, ^{85}Rb, ^{88}Sr, ^{71}Ga, ^{90}Zr, ^{121}Sb, ^{137}Ba, ^{139}La, ^{140}Ce, ^{178}Hf, and ^{208}Pb. For comparison purposes, elemental ratios from these analytes were determined for each sample with the exception of ^{25,26}Mg and ^{121}Sb (Table 9). ^{25,26}Mg was not used for comparison purposes because magnesium is a major batch constituent; as a result, the amounts of magnesium were the same in every sample. ^{121}Sb was not used for comparison purposes because the antimony concentration in every windshield sample was at or below the detection limit of LA-ICP-MS. Elemental ratios were used to correct for instrumental drift typical of LA-ICP-MS (30, 59).

Table 9. Table of analytes and element ratios.

Elemental Ratio
Titanium (^{49}Ti) : Iron (^{57}Fe)
Manganese (^{55}Mn) : Strontium (^{88}Sr)
Rubidium (^{85}Rb) : ^{88}Sr
Gallium (^{71}Ga) : ^{85}Rb
Zirconium (^{90}Zr) : Barium (^{137}Ba)
Lanthanum (^{139}La) : Cerium (^{140}Ce)
Hafnium (^{178}Hf) : Lead (^{206}Pb)

Fifty automotive windshields and windshield samples were donated by Mygrant Glass Company (Sacramento, CA USA), Central Valley Tow (Sacramento, CA USA) and Pilkington/ Libbey Owens Ford Company (Lathrop, CA USA; Table 10). These windshields represented 17 separate manufacturers and one unknown manufacturer (60) and approximately one decade of glass manufacture (ca 1995 – 2005). Mygrant Glass Company and Pilkington/ Libbey Owens Ford Company donated whole windshields that were not suitable for retail sale because they were broken in stock or during shipping. Central Valley Tow allowed collection of windshield samples directly from totaled vehicles.

Where possible, the logo of each windshield was photographed for later identification. Windshields having identical markings were grouped together as multiple samples representing the same manufacturing lot. For example, Lamisafe windshields 1a and 1b had identical bugs as did Lamisafe windshields 2a and 2b; Lamisafe windshield 3 had a distinct bug (Table 10).

Ten of these automotive windshields were selected to evaluate the homogeneity of trace elements in automotive float glass: Lamishield 1, PLOF 1a, PLOF 1b, Sekurit 1a, Sekurit 1b, Vitro Flex/ Carlite 1a, Vitro Flex/ Carlite 1b, Vitro Flex/ Carlite 2 and Xyg 1. Core subsamples of each windshield were taken from six locations: top left, top right, top

center, bottom left, bottom center, and bottom right. Core samples were removed by chiseling or drilling; in the latter case, a 1-inch diameter diamond tipped coring drill bit (Advantage Drills Incorporated; Winter Park, FL USA) was used.

Core subsamples from the remaining 40 windshields were similarly collected. As some windshield samples were taken directly from vehicles, only one location in the windshield could be subsampled; whole windshields were subsampled in three places: left, right and center. The exterior pane of each subsample was marked so that the origin of each glass sample could be known. Hereafter, "Pane 1" refers to the outer pane of a windshield; "Pane 2" refers to the inner pane of a windshield.

Subsamples that were removed by chiseling were too large to fit into the LA sample cell chamber. Thus, fragments from each pane of each subsample were picked off using a diamond tipped scribe. These fragments were washed in methanol, soaked in 10% ultra pure nitric acid for no less than 30 minutes, and rinsed with 18-MΩ H_2O. Each was then allowed to air-dry. Subsamples that were removed by drilling were cut in half using wire cutters; they were cleaned as described above and allowed to air dry.

Table 10. Windshield sample set.

Manufacturer, Brand	Manufacturer, Brand
AP Technoglass Corporation Bellefontaine, OH USA Lamisafe Brand • Lamisafe 1a, 1b • Lamisafe 2a, 2b • Lamisafe 3 • Lamisafe 4 *Lamisafe for Honda Brand* • Lamisafe 5 ***Carlex Glass Company*** Vonore, TN USA *Carlex Brand* • Carlex 1 • Carlex 2 ***Cristales Inastillables de Mexico*** Xalostoc EDO, Mexico *Crinamex Brand* • Crinamex 1 • Crinamex 2a, 2b, 2c ***Ford Motor Company*** Dearborn, MI USA *Carlite Brand* • Ford 1 *Not branded* • Ford 2 ***Fox Fire Incorporated*** Pontiac, MI USA *Not branded* • Fox 1	***L-N Safety Glass SA de CV of Mexicali*** Toledo, OH USA *Pilkington Brand* • L-N 1 ***Pittsburgh Plate Glass (PPG) Industries*** Pittsburgh, PA USA *PPG for Toyota Brand* • PPG 1 *PPG Brand* • PPG 2 • PPG 3 ***Sekurit Saint-Gobain Cuautla*** Cludad de Ayala, Estado de Moreles Mexico *Sekurit Brand* • Sekurit 1a, 1b ***Shenzhen Benxun Auto-Glass Co., Ltd.*** Shekou, Shenzhen China *Lamishield Brand* • Lamishield 1 ***Societa Italiana Vetro*** San Salvo (Chieto) Italy *Sicursiv Brand* • Sicursiv 1 ***United L-N Glass Incorporated*** Versailles, KY USA *Toyota Brand (from Pilkington)* • Toyota 1a, 1b, 1c, 1d, 1e

Table 10, continued: Windshield sample set.

Manufacturer, Brand	Manufacturer, Brand
Fujian Yanhua Glass Industry, Co., Ltd. Honglu Town, Fujian Province China *Not branded* • Fy 1 • Fy 2 ***Industrias Venezolanas Automotrices*** Caracas, Venezuela *Not branded* • Iva 1 ***Pilkington,*** ***Libbey Owens Ford (LOF) Company*** USA *LOF Brand* • LOF 1 • LOF 2 • LOF 3 *Pilkington/ Pilkington-LOF Brand* • PLOF 1a, 1b • PLOF 2a, 2b • PLOF 3 *PPG Brand* • PLOF/ PPG 1	***Viracon Incorporated*** Owatonna ME USA *PPG Brand* • Viracon/ PPG 1a, 1b ***Vitro Flex SA*** Monterrey, Mexico *Carlite Brand* • Vitro Flex/ Carlite 1a, 1b • Vitro Flex/ Carlite 2 *Ford Brand* • Vitro Flex/ Ford 1 • Vitro Flex/ Ford 2 ***Xinyi Automobile Glass Company*** Shenzhen City, Guangdong Province China *Not branded* • Xyg 1 ***Unknown Manufacturer*** *Carlite Brand* • Unknown/ Carlite 1

Instrumentation and Analytical Methods. LA-ICP-MS was used to analyze these fifty windshields. The instrument parameters used are described in Tables 4 and 5, with helium flow set to 1 L min^{-1} as before. Each sample was analyzed in quadruplicate. NIST SRM 612 was analyzed before and after sample analysis, and converted to calibration data using Glitter Time Resolved Software. The calibrant was analyzed before and after sample analysis to correct for instrumental drift. The quality of the calibration was tested

by analyzing NIST SRM 1831 prior to sample analysis. A 10%-tolerance was used for QC values.

Data Analysis and Calculations. Elemental variation in automotive windshield glass was evaluated by examining the variation in composition within a single pane, within a single windshield and within a population of 50 windshields. Elemental variation within a single pane and within a single windshield was determined using the 10-windshield subset described above. The analytical results for each of the six subsamples were compared to establish the variation within a single pane. The results for each of the six subsamples were then compiled so that the overall composition of each pane within a windshield could be compared. Finally, the variation within a population of 50 windshields was determined by compiling and comparing all analytical results for a given windshield pane (N = 3 to 24 for each of 100 panes).

Within-pane variation was characterized in three ways. Firstly, the percent relative standard deviations (%-RSDs) associated with quantifying a given elemental ratio was calculated for each of the 20 panes analyzed in the 10-windshield subset.

Secondly, the analytical results of each elemental ratio from each of the six subsamples were compared using a univariate Student's *t*-test (57, 61). This was done to determine whether significantly different amounts of each elemental ratio were present in each of the subsamples. The results of each subsample from each pane were systematically compared; in total, there were fifteen possible comparisons per pane (top left to top center, top left to top right, etc.). Every elemental ratio was treated as an independent variable; the Student's *t*-test used here was a two-tailed test assuming unequal variance. Unequal variance was assumed because the LA sampling technique is

very sensitive to laser focusing, sample placement within the sample cell, and small changes in the flow of helium (as when the sample cell is opened and shut between analyses). Because these conditions cannot be exactly reproduced from sample to sample, subsequent datasets do not have the same variance. When:

$$t_{Calculated} > t_{Critical}$$

The difference between two means was considered significant at 95% confidence. Since the two datasets had unequal variances, $t_{Calculated}$ was determined by the following:

$$t_{Calculated} = \frac{\bar{x} - \bar{y}}{\sqrt{\left(s_x^{\,2}/n\right) + \left(s_y^{\,2}/m\right)}}$$

Where $\bar{x}$ was the mean value of the first dataset to be compared, $\bar{y}$ was the mean value of the second dataset to be compared, s_x and s_y refer to the standard deviations of the first and second datasets respectively; n and m refer to the number of trials in the first and second datasets, respectively. The degrees of freedom, df, for this comparison was determined by:

$$df = \left\{ \frac{\left[\left(s_1^{\,2}/n_1\right) + \left(s_2^{\,2}/n_2\right)\right]^2}{\frac{\left(s_1^{\,2}/n_1\right)^2}{n_1 + 1} + \frac{\left(s_2^{\,2}/n_2\right)^2}{n_2 + 1}} \right\} - 2$$

The resultant was nonintegral. To use traditional charts of $t_{Critical}$, the value was rounded up to the next integer and the decimal portion was truncated. The final value, *int(df + 1)*, was then used as the degrees of freedom so that the appropriate $t_{Critical}$ at 95% confidence could be found using a table. All univariate t-tests were conducted with a user-defined macro in Microsoft Excel.

Thirdly, each subsample of one windshield (Vitro Flex/ Carlite 2) was compared using a multivariate *t*-test to determine whether small differences in individual elemental ratios were significant within a single pane of glass if the total variation of all elemental ratios was simultaneously considered. A multivariate analog to the Student's *t*-test was used, called Hotelling's T^2 (61). This test statistic facilitates the comparison of two samples in terms of all available variables, which may or may not be covariant. As before, each subsample was systematically compared to the remaining subsamples for a total of 15 comparisons. Vitro Flex/ Carlite 2 was chosen as a model to represent the remaining windshields.

When *x* and *y* represent the following multivariate datasets:

$$x = \begin{bmatrix} x_{11} & x_{21} & x_{31} \cdots x_{n1} \\ x_{12} & x_{22} & x_{32} \cdots x_{n2} \\ \vdots & \vdots & \vdots \quad \vdots \\ x_{1p} & x_{2p} & x_{3p} \cdots x_{np} \end{bmatrix} \qquad y = \begin{bmatrix} y_{11} & y_{21} & y_{31} \cdots y_{m1} \\ y_{12} & y_{22} & y_{32} \cdots y_{m2} \\ \vdots & \vdots & \vdots \quad \vdots \\ y_{1p} & y_{2p} & y_{3p} \cdots y_{mp} \end{bmatrix}$$

Where x_{11} refers to the first analysis of the first variable, when *p* variables are considered, the test statistic, $T^2_{Calculated}$, was determined by the following:

$$T^2{}_{Caclulated} = (\bar{x} - \bar{y})^T \left[\left(\frac{1}{n} + \frac{1}{m} \right) S_{Pooled} \right]^{-1} (\bar{x} - \bar{y})$$

Where $\bar{x}$ and $\bar{y}$ are vectors describing the average values for two multivariate datasets, *x* and *y*, and S_{Pooled} is the covariance matrix of the two multivariate data sets. The quantity $(\bar{x} - \bar{y})^T$ is simply the transpose of column vector $(\bar{x} - \bar{y})$. The mean vectors, $\bar{x}$ and $\bar{y}$, are described by:

$$\bar{x} = \begin{bmatrix} \bar{x}_{n1} \\ \bar{x}_{n2} \\ \bar{x}_{n3} \\ \vdots \\ \bar{x}_{np} \end{bmatrix} \qquad \bar{y} = \begin{bmatrix} \bar{y}_{m1} \\ \bar{y}_{m2} \\ \bar{y}_{m3} \\ \vdots \\ \bar{y}_{mp} \end{bmatrix}$$

The pooled variance matrix, S_{Pooled} was then estimated by:

$$S_{Pooled} = \frac{(n-1)S_x + (m-1)S_y}{n + m - 2}$$

Where the variance matrices, S_x and S_y, are:

$$S_x = \frac{1}{n} \sum_{j=1}^{n} (x_j - \bar{x})(x_j - \bar{x})^T \qquad \text{and} \qquad S_y = \frac{1}{m} \sum_{j=1}^{m} (y_j - \bar{y})(y_j - \bar{y})^T$$

Hotelling's T^2 distribution has the same general shape as the F-distribution. Therefore, the critical value, $T^2_{Critical}$, was calculated by the following:

$$T^2_{Critical} = \frac{(n+m-2)p}{(n+m-p-1)} F_{p,n+m-p-1}$$

Where $F_{p,n+m-p-1}$ is the value of the F distribution for p variables, and $n+m-p-1$ degrees of freedom. Hotelling's T^2 test has the condition that $n + m > p + 1$; thus, there must be at least two more data points than there are variables. The null hypothesis was rejected when $T^2_{Calculated}$ was greater than $T^2_{Critical}$. Because there must be two more data points than there are variables, only four elemental ratios could be considered when comparing subsamples due to the limited number of replicate analyses performed on each. To make the most conservative statistical comparisons, then, the four most variable elemental ratios from Vitro Flex/ Carlite 2 Pane 1 and Pane 2 were selected. For the comparisons made between subsamples of Pane 1, the elemental ratios ^{49}Ti/ ^{57}Fe, ^{85}Rb/ ^{88}Sr, ^{139}La/ ^{140}Ce, and ^{178}Hf/ ^{208}Pb were used. For Pane 2, ^{55}Mn/ ^{88}Sr, ^{85}Rb/ ^{88}Sr, ^{90}Zr/ ^{137}Ba, and ^{178}Hf/ ^{208}Pb were used. All multivariate T^2 tests were conducted using MatLab 6.5 (The MathWorks Incorporated; Natick, MA USA).

To establish the variation within a single windshield, the analytical results of each subsample that represented a single pane were compiled (N = 18 – 24). The overall results for each pane within a given windshield were compared at 95% confidence.

Within the 10-windshield subset selected for sample homogeneity testing, there were three sets of windshields produced within the same lot: PLOF 1a and 1b; Sekurit 1a and 1b; and Vitro Flex/ Carlite 1a and 1b. The panes of each set were compared using

Hotelling's T^2 test at 95% confidence to determine whether each pane within a set could be distinguished from the others manufactured at or around the same time.

To test the "fingerprinting" capability of trace elemental profiling, the population variation of all 50 windshields was compared to the variation observed within single groups of manufacturers. The concentration ranges of trace elements in the population of all 50 windshields was inter-compared to the concentration ranges observed in Lamisafe windshields, Pilkington-LOF windshields, and Vitro Flex windshields at 95% confidence. The average trace elemental profile of each of these manufacturers' windshields was also inter-compared at 95% confidence. Lamisafe, Pilkington-LOF and Vitro Flex windshields were selected for these comparisons because these groups contained five or more windshields.

Finally, the discrimination of trace elemental profiling for automotive windshield glass was examined by comparing compiled results from all panes of windshield glass at 95% confidence. Due to the number of samples (100 panes), a preliminary grouping method was employed. The samples were first grouped according to $^{85}Rb/\ ^{88}Sr$ values, because this elemental ratio exhibited the greatest variation within the total population. Each windshield pane was placed into one of four groups. Windshield panes with $^{85}Rb/\ ^{88}Sr$ of 0.01 or less were placed into Group 1; those with $^{85}Rb/\ ^{88}Sr$ of 0.01 – 0.1 were placed into Group 2. Windshield panes having $^{85}Rb/\ ^{88}Sr$ of 0.1 – 1 were placed into Group 3 and those with $^{85}Rb/\ ^{88}Sr$ greater than 1 were placed into Group 4. Each group was then further divided into subgroups by comparing the remaining elemental ratios of each pane within a group at 95% confidence. Each division was made on the basis of the next most variable elemental ratio. For these 100 panes, the order of elemental ratios used

to subdivide groups after $^{85}Rb/\ ^{88}Sr$ was $^{55}Mn/\ ^{88}Sr$, $^{90}Zr/\ ^{137}Ba$, $^{71}Ga/\ ^{85}Rb$, $^{49}Ti/\ ^{57}Fe$, $^{178}Hf/\ ^{208}Pb$, and $^{139}La/\ ^{140}Ce$.

Elemental Variation in Float Glass

Quality control (QC) samples donated by Pittsburgh Plate Glass Company (PPG; Fresno, CA USA), Libbey Owens Ford (LOF; Lathrop CA USA) and Pilkington-Libbey Owens Ford (Lathrop, CA USA)[2] were used for this study. These QC samples were collected by staff at predetermined locations in the ribbon and specified times in order to monitor the batch color and thickness over time. PPG donated QC samples collected between May and June 2004. These were collected from three different locations in the ribbon (left, right and center) at three times (0700 h, 1500 h and 2300 h). The batch samples donated by LOF were originally given to the Sacramento California Department of Justice Crime Lab in 1997; the Department of Justice laboratory then donated these samples for this study. This set contained QC samples manufactured by LOF in November and December of 1997. These QC samples were collected from a single location in the ribbon at 0500 h of each day. The Pilkington-LOF batch samples were manufactured in May of 2005; these were also collected from a single location in the ribbon at 0500 h of each day. Only a subset of each group was analyzed to establish the short- and long-term variation typical of these manufacturing plants.

Spatial variations in float glass composition were determined by analyzing the left, center and right batch samples each collected at 0700 h, 1500h and 2300 h from the PPG float glass ribbon for three consecutive days. Daily variations were examined using

[2] Pilkington acquired LOF in the late 1990s, after the LOF batch samples were donated.

the center QC samples collected at each time point for these three days, as well. The short-term variation in this manufacturer's batch was examined by comparing the analytical results for the center QC samples each collected 1500 h. The long-term variation in this manufacturer's product was determined by analyzing center samples collected weekly at 1500 h, for four weeks.

The short-term variation in float glass manufactured by LOF was determined by analyzing QC samples collected at 0500 h for three consecutive days; long-term variation was determined by analyzing QC samples collected at 0500 h on the fifth day, the 17th day, and the 24th days of November as well as the sixth day of December in 1997.

The short-term variation in float glass manufactured by Pilkington LOF was determined by analyzing QC samples collected at 0500 h for three consecutive days; the long-term variation was determined by analyzing QC samples collected at 0500 h weekly for three weeks.

Each QC sample was approximately four inches long and two inches wide with variable thickness. Each was broken into smaller fragments using a chisel and hammer. Smaller fragments of each QC sample were washed in methanol, soaked in 10% ultra pure nitric acid, and rinsed with deionized water. Each was then allowed to air-dry. Samples were mounted on glass slides with blue putty.

Analysis was conducted as described above (p. 38). The elemental composition of each QC sample (represented by elemental ratios described in Table 9) was compared using 95% confidence limits.

Findings

SN-ICP-MS: Figures of Merit

Detection Limits and Sensitivity. MDLs ranged from 0.005 – 0.2 ng mL^{-1} using either a CN or MCN (Table 11). In general, MDLs determined using the MCN were greater than that determined by using a CN. In some cases, this difference was as large as an order of magnitude. It is of interest that MDLs for ^{25}Mg and ^{47}Ti were similar using either nebulizer, suggesting that the variation in signal due to ^{25}Mg and ^{47}Ti is independent of the total amount of sample delivered to the instrument. Both have relatively low isotopic abundances (10% and 7.3%, respectively), which may explain the variations observed since lower abundance ions are less effectively transferred to the mass spectrometer than higher abundance ions. ADLs ranged from 0.001 – 0.1 picograms, the MCN providing lower ADLs by as great as a factor of 10 for certain isotopes. Sub-ng mL^{-1} LOQs were achievable using either a CN or MCN.

Table 11. Summary of MDLs, ADLs, and LOQs.

	MDLs, ng mL^{-1}		*ADLs, ng*		*LOQs, ng mL^{-1}*	
Analyte	**CN**	**MCN**	**CN**	**MCN**	**CN**	**MCN**
^{25}Mg	0.11	0.18	0.13	0.022	0.37	0.59
^{26}Mg	0.053	0.20	0.064	0.024	0.18	0.65
^{47}Ti	0.028	0.026	0.034	0.0031	0.096	0.085
^{55}Mn	0.0099	0.070	0.012	0.0084	0.033	0.23
^{69}Ga	0.0047	0.065	0.0056	0.0078	0.016	0.22
^{71}Ga	0.0074	0.060	0.0089	0.0072	0.025	0.20
^{85}Rb	0.0061	0.051	0.0073	0.0061	0.020	0.17
^{86}Sr	0.014	0.057	0.017	0.0068	0.047	0.19
^{88}Sr	0.0073	0.044	0.0088	0.0053	0.024	0.15
^{90}Zr	0.017	0.091	0.020	0.011	0.057	0.30
^{91}Zr	0.017	0.073	0.020	0.0088	0.058	0.24
^{92}Zr	0.017	0.083	0.020	0.0010	0.056	0.28
^{94}Zr	0.015	0.092	0.018	0.011	0.049	0.31
^{121}Sb	0.0076	0.058	0.0091	0.0070	0.025	0.19
^{137}Ba	0.011	0.050	0.013	0.0060	0.037	0.17
^{138}Ba	0.0054	0.052	0.0065	0.0062	0.018	0.17
^{139}La	0.0055	0.046	0.0066	0.0055	0.018	0.15
^{140}Ce	0.0051	0.046	0.0061	0.0055	0.017	0.15
^{147}Sm	0.0063	0.045	0.0076	0.0054	0.021	0.15
^{178}Hf	0.013	0.097	0.016	0.012	0.044	0.32
^{206}Pb	0.019	0.15	0.023	0.018	0.064	0.77
^{207}Pb	0.034	0.12	0.041	0.014	0.11	0.75
^{208}Pb	0.024	0.11	0.029	0.013	0.080	0.78

Analytical sensitivity was calculated by averaging the slopes of two calibrations performed on nonconsecutive days using either a CN or MCN (Table 12). The average error in the slope determination made by linear regression was no greater than $\pm 1 \times 10^{-6}$ for any particular isotope.

Generally speaking, there was a decrease in sensitivity using MCN over CN with the exception of ^{55}Mn and ^{25,26}Mg. No difference was observed in the sensitivity for ^{55}Mn; there was a 4 – 6 % increase in sensitivity for ^{25,26}Mg. All other isotopes showed 0.3 – 9% decreases in sensitivity. This effect seemed to be mass dependent. Mid-mass isotopes (*m/z* 85 – 94) showed only 2 – 3% reductions in sensitivity, whereas the higher mass isotopes showed decreases of > 5%. The magnitude of this sensitivity reduction increased with mass, which may be due to mass-related differences in response of analyte: internal standard.

Internal standardization is a common practice in plasma spectrometry due to well-known occurrence of instrumental drift inherent to plasma spectrometry, as well as certain types of noise and various matrix effects. Matrix effects are minimized by internal standardization if the sample matrix equally affects the internal standard and analyte. In many cases, matrix effects are mass dependent. For example, space charge effects are known to bias ion sampling in favor of larger ions (higher mass analytes). Matrix effects can also predictably alter instrument response as a function of ionization potential. For example, if solvent loading cools the plasma, elements with higher first ionization potentials are not as easily ionized. This situation creates a sampling bias in favor of analytes with lower first ionization potentials. It should be noted that instrument optimization could have an impact on internal standardization, as well. Modern ICP-MS

instruments make use of an "autolens" to selectively focus ions within a certain *m/z* range prior to mass selection and detection. The autolens increases voltage to select for higher *m/z* analytes; the voltage increase is calibrated for maximum sensitivity using a mixture of low, middle and high mass elements. Over time, the optimum voltage that provides the greatest sensitivity for each of the low, middle and high mass elements changes. Low and high mass elements are selected against when this occurs. It is difficult to assign the apparent mass-dependent decrease in sensitivity to a result of matrix or instrumental effects. However, these known effects make mass-dependent differences in instrumental response not unexpected.

Finally, it was observed that isotopic differences in sensitivity for each nebulizer type correlated well with isotopic abundance. This is a strong indication that a negligible amount of isobaric interference is occurring.