DEPARTMENT OF FORENSIC SCIENCE
METHOD VALIDATION SUMMARY FORM

Section: Trace Evidence

Method: Micro X-ray Fluorescence (μ-XRF) Spectrometry for the Forensic
Comparison of Glass

Results recorded? ☑

Procedure documented? ☑

Method fit for use? ☑

Approved by: Robyn Weimer Date: 03/25/2026

Executive Verification Summary: “Micro X-ray Fluorescence (µ-XRF) Spectrometry for the Forensic Comparison of Glass”

Recommended Uses:

This verification indicates that the µ-XRF screen and semi-quantitative methods, in accordance with ASTM E2926, *Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (µ-XRF) Spectrometry,* are viable options within an analytical scheme for the forensic analysis of glass. The method also verified the use of dual detectors or a single detector with twice the collection time is suitable for analysis.

Limitations:

Glass sample size has a great impact on the sampling method and elemental ratio results, requiring similar sizes/thicknesses of samples to be compared.

Information Provided:

- Summary of Results and Conclusions
- References

Studies Performed:

- A – Method Optimization
- B – Single vs. Dual Detector(s)
- C – Sample Preparation
- D – Intra-day versus Inter-day Repeatability (previously referred to as “Reproducibility & Repeatability”)
- E – Precision
- F – Accuracy (referred to in the verification plan as “Calculation and Interpretation of Data Collected from Glass Fragments with Similar Refractive Index Values”)

RBW
03/25/2026

µXRF Method Verification Summary

Virginia Department of Forensic Science
Trace Evidence Section

Memo To:	Robyn Weimer, Chemistry Program Manager
From:	Michelle Drake, Karissa Gorr, Emily Langlois
CC:	Alka Lohmann, Director of Technical Services, Rebecca Wagner, PhD, Chemistry Research Section Supervisor
Date:	March 15, 2026
Re:	Verification Summary: Micro X-ray Fluorescence (µ-XRF) Spectrometry for the Forensic Comparison of Glass

Verification Summary – Micro X-ray Fluorescence (µ-XRF) Spectrometry for the Forensic Comparison of Glass

The verification of the Bruker M4 Tornado µ-XRF at the Virginia Department of Forensic Science (VA DFS) Central Laboratory pursuant to the verification plan, included the following studies:

- A – Method Optimization
- B – Single vs. Dual Detector(s)
- C – Sample Preparation
- D – Intra-day versus Inter-day Repeatability (referred to in the verification plan as "Reproducibility & Repeatability")
- E – Precision
- F – Accuracy (referred to in the verification plan as "Calculation and Interpretation of Data Collected from Glass Fragments with Similar Refractive Index Values")

These studies were performed in accordance with ASTM E2926, *Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (µ-XRF) Spectrometry*. During the verification, ASTM E2926 was updated from E2926-17 to E2926-25e1. The studies completed were appropriately adapted to meet the specifications of the new revision. The data obtained during the verification is stored on the instrument (including the Trace Instrument Network) and in DFSFile1\Shared\Trace\Trace Shared\Glass\uXRF\Bruker uXRF Verification.

µ-XRF is capable of identifying elements with an atomic number ≥11. Although data is collected for all elements during analysis, the elements primarily used to evaluate the verification were those listed in ASTM E2926-25e1 Table X2.1. ASTM E2926-25e1 lists both the elements and concentrations obtained for NIST Standard Reference Material (SRM) #1831. In addition to these elements, zinc was also included as it is commonly observed in glass. The elements to be evaluated during the verification are delineated in Table 1.

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Table 1: Elements utilized for evaluation

Element	**Abbreviation**	**Concentration (ppm)***	**Peak Channels****	**Pre-Peak Channels****	**Post-Peak Channels****
Sodium	Na	98820 [A]	95-113	92-96	112-116
Magnesium	Mg	21200	116-134	113-117	133-137
Aluminum	Al	6380	142-156	138-142	155-157
Potassium	K	2700	325-340	319-322	341-344
Calcium	Ca	58600	349-389	317-321	424-428
Titanium	Ti	110	436-466	422-431	471-480
Manganese	Mn	15.0 [A]	574-604	559-569	609-619
Iron	Fe	610	620-660	605-615	665-675
Rubidium	Rb	6.11 [A]	1321-1351	833-843	883-893
Strontium	Sr	89.1 [A]	1394-1434	1379-1389	1439-1449
Zirconium	Zr	43.4 [A]	1554-1594	1539-1549	1599-1609
Zinc	Zn	---	848-878	833-843	883-893

* As reported by ASTM E2926-25e1 *Table X2.1*

** Adapted from ASTM E2926-25e1 *Table X5.1*

[A] Discrepancy from certificate of analysis of NIST SRM #1831

The concentration of elements from the NIST SRM #1831 Certificate of Analysis were compared to the concentrations delineated in ASTME E2926-25e1. When comparing the concentration of Na from the Certificate of Analysis to the standard test method, a difference in concentration was noted with the concentration in ASTM E2926-25e1 being 1000 ppm greater than the Certificate of Analysis. Upon consultation with the authors of ASTM E2926-25e1, it was confirmed that the concentration for Na was a typographical error and should be 98820 ppm. Additionally, Mn, Rb, Sr, and Zr are not listed on the Certificate of Analysis but are reported with ASTM E2926-25e1. The elements and concentrations delineated in ASTM E2926-25e1 were obtained from studies performed during the development of ASTM E2330-19, *Standard Test Method for Determination of Concentrations of Elements in Glass Samples Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Forensic Comparisons*. The concentrations delineated in ASTM E2926-25e1 were utilized during the verification as it dictates requirements for performing the standard test method.

Analysis of µ-XRF data utilizes both individual elemental responses and ratios between elements. The responses associated with individual elements can be used to calculate the signal-to-noise ratio (SNR), limit of detection (LOD), and limit of quantitation (LOQ). Additionally, ASTM E2926-25e1 states that the elemental composition can be used as a preliminary screen to demonstrate the absence or presence of exclusionary differences between samples. If the preliminary screen does not provide exclusionary differences, samples shall be further analyzed by collecting replicate spectra to adequately characterize samples. As per ASTM E2926-25e1, a minimum of three replicates shall be evaluated for a questioned sample and nine replicates for a known glass source. This evaluation is considered a full-length method that is semi-quantitative in nature. ASTM E2926-25e1 provides typical ratios that can be utilized if the elements have a SNR ≥10 and are present above the limit of quantitation. In addition to the ratios provided by the standard test method, customized ratios may be used. ASTM E2926-25e1 suggests using iron as the denominator for elements with an atomic number ≥20, or an element close in atomic number. The elemental ratios utilized in the verification were derived from ASTM E2926-25e1 and are listed in Table 2.

Table 2: Elemental ratios evaluated during verification

Ratio of Element 'X' to Element 'Y'
Ca/Mg
Ca/Ti
Ca/Fe
Sr/Zr
Fe/Zr
Ca/K
Na/Mg
Ti/Fe
Mn/Fe
Sr/Fe

Throughout the verification, multiple replicates were collected for sample analysis to ensure adequate representation of the sample. Each replicate corresponds to a different location on the sample to ensure consistency across verification studies. The number of replicates for each experiment are delineated in their respective sections of the verification summary.

Prior to data collection, quality assurance checks were performed in accordance with the requirements delineated in ASTM E2926-25e1§8.2. In addition to the standard test method requirement, manufacturer specifications were also utilized for calibration. The calibration was evaluated on a weekly basis. Calibration deviation shall be within ±5 eV of the previous calibration for each detector. If the deviation was >±5 eV, the detector was calibrated and verified to be within ±5 eV after calibration. The detector counts of a copper standard were confirmed to be <10% of the previous reading. The factor value of the scatter spectrum was confirmed to be within 0.9 – 1.1 as compared to the initial scatter spectrum. The x-ray spot check verified that the spot circle was within the brighter fluorescence region at a magnification of 100x and within the length of the crosshair arms at a magnification of 10x. If outside of these specifications, the coordinates were adjusted. The Ti and Sr peaks were confirmed to have an LOD ≤75 ppm.

A. Method Optimization

To begin verification, optimal method conditions for the semi-quantitative method were determined using ASTM E2926-17§6, ASTM E2926-25e1§6, and manufacturer recommendations. Method variables selected for evaluation were collection time (Lsec), x-ray tube current (µA), and maximum pulse throughput (kcps). Spot size (20 µm), x-ray voltage (50 KV), maximum energy (40 keV), and use of both silicon drift detectors (SDD) were held constant throughout optimization. To assess all permutations of instrumental conditions, a total of 30 parameter sets were established. Each parameter set is delineated in Table 3.

Table 3: Tested variables of the parameter sets

Parameter Set #	Collection Time (Lsec)	X-ray Tube Current (µA)	Max. Pulse Throughput (kcps)
1	30	200	130
2	120		
3	300		
4	600		
5	1200		
6	30	300	
7	120		
8	300		
9	600		
10	1200		
11	30	600	
12	120		
13	300		
14	600		
15	1200		
16	30	200	275
17	120		
18	300		
19	600		
20	1200		
21	30	300	
22	120		
23	300		
24	600		
25	1200		
26	30	600	
27	120		
28	300		
29	600		
30	1200		

For each parameter set, nine replicates were collected across the NIST SRM #1831. The parameter sets were evaluated to determine which set(s) achieved the best spectral quality (e.g., Gaussian peak shape, resolution of peaks, background noise) and peak intensity (e.g., SNR). The SNR for identification of an element shall be ≥3. Further, for ratio comparisons, the SNR shall be ≥10. SNR was calculated for each replicate using Equation 1 and then averaged.

Equation 1:

$$SNR = \frac{P - \left(H \times \left(\frac{\bar{R} + \bar{S}}{2}\right)\right)}{\sqrt{H \times \left(\frac{\bar{R} + \bar{S}}{2}\right)}}$$

where (P) is the total counts of the peak range for the element,
(H) is the number of channels comprising the peak range of the element,
(R) is the average counts of the pre − peak range of the element,
(S)is the average counts of the post − peak range of the element

In addition to SNR, the LOD and LOQ were calculated for each replicate using Equation 2 and Equation 3, respectively. The values were then averaged for the replicates of the parameter set. A summary is shown in Table 4.

Equation 2:

$$LOD = \frac{3 \times C}{SNR}$$

where (C) is the concentration of the element

Equation 3:

$$LOQ = \frac{10 \times C}{SNR}$$

where (C) is the concentration of the element

Table 4: Averaged SNR, LOD (ppm), and LOQ (ppm) for each parameter set for elements

Parameter Set #	**Na**			**Mg**			**Al**			**K**			**Ca**			**Ti**		
	SNR	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*
1	51	5814	19380	49	1312	4374	36	535	1784	126	64	214	4180	42	140	17	20	67
2	104	2853	9509	101	632	2106	72	268	893	246	33	110	8287	21	71	31	11	36
3	165	1803	6011	155	412	1372	112	171	571	390	21	69	13201	13	44	49	6.7	22
4	232	1279	4263	223	286	952	159	120	401	554	15	49	18644	9.4	31	70	4.7	16
5	328	905	3017	314	203	676	226	85	282	789	10	34	26409	6.7	22	99	3.3	11
6	61	4857	16191	60	1066	3555	43	446	1485	151	54	179	5206	34	113	20	17	55
7	127	2342	7807	122	524	1746	87	220	733	300	27	90	10192	17	58	37	8.9	30
8	199	1467	4956	192	332	1107	138	139	462	479	17	56	16177	11	36	61	5.5	18
9	282	1053	3509	271	235	783	195	98	328	676	12	40	22826	7.7	26	85	3.9	13
10	397	747	2491	383	166	554	275	70	232	954	8.5	28	32395	5.4	18	120	2.7	9.2
11	87	3429	11431	85	753	2509	62	310	1032	208	39	130	7176	25	82	26	13	42
12	178	1669	5564	170	374	1245	123	156	520	417	19	65	14331	12	41	53	6.2	21
13	281	1056	3520	272	234	778	194	98	328	659	12	41	22760	7.7	26	83	4.0	13
14	401	740	2467	383	166	554	275	70	232	931	8.7	29	32134	5.5	18	120	2.8	9.2
15	564	526	1752	539	118	393	387	49	165	1315	6.2	21	45391	3.9	13	169	1.9	6.5
16	51	5862	19541	48	1336	4453	33	590	1966	120	68	226	4127	43	142	15	22	74
17	102	2899	9662	96	665	2217	68	282	939	232	35	117	8189	22	72	31	11	36
18	159	1865	6217	153	417	1390	105	183	609	367	22	74	12999	14	45	50	6.6	22
19	224	1322	4407	215	296	988	150	128	426	518	16	52	18315	9.6	32	69	4.8	16
20	316	938	3128	302	211	702	210	91	303	731	11	37	26001	6.8	23	99	3.3	11
21	60	4980	16601	58	1090	3633	40	477	1590	140	58	194	5060	25	116	20	17	55
22	123	2414	8045	116	549	1829	81	235	784	283	29	96	10107	17	58	38	8.7	29
23	194	1526	5086	186	343	1143	128	149	479	447	18	60	15949	11	37	60	5.5	18
24	274	1083	3610	261	243	811	183	105	349	632	13	43	22589	7.8	26	86	3.8	13
25	387	767	2555	371	172	572	258	74	247	892	9.1	30	31984	5.5	18	120	2.7	9.1
26	86	3455	11516	82	773	2578	59	324	1081	196	41	138	7137	25	82	27	12	41
27	171	1739	5795	165	385	1284	116	165	550	388	21	70	14141	12	41	56	5.9	20
28	273	1086	3619	262	243	811	182	105	350	614	13	44	22448	7.8	26	85	3.9	13
29	386	767	2558	371	172	572	260	74	246	872	9.3	31	31877	5.5	18	122	2.7	9.0
30	546	543	1810	523	122	406	366	52	174	1234	6.6	22	45037	3.9	13	171	1.9	6.4

Table 4: (continued)

Parameter Set #	Mn			Fe			Rb			Sr			Zr		
	SNR	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*	*SNR*	*LOD*	*LOQ*
1	3.5	15	51	132	14	46	2.0	38	127	24	11	37	16	8.3	28
2	7.6	6.1	20	264	6.9	23	3.6	5.7	19	47.8	5.6	19	30	4.4	15
3	13	3.4	11	420	4.4	15	6.2	3.3	11	75	3.6	12	47	2.8	9.3
4	20	2.3	7.8	593	3.1	10	9.2	2.1	7.0	106	2.5	8.4	67	1.9	6.4
5	27	1.7	5.7	841	2.2	7.3	12	1.5	5.1	151	1.8	5.9	94	1.4	4.6
6	4.5	11	37	163	11	38	2.7	3.8	13	30	9.0	30	18	7.2	24
7	12	3.9	13	328	5.6	19	4.7	4.6	15	60	4.5	15	37	3.5	12
8	16	2.8	9.3	517	3.5	12	7.9	2.4	8.1	93	2.9	9.6	59	2.2	7.4
9	23	1.9	6.5	731	2.5	8.3	9.2	2.0	6.8	133	2.0	6.7	83	1.6	5.2
10	34	1.3	4.4	1035	1.8	5.9	14	2.0	6.8	190	1.4	4.7	119	1.1	3.7
11	8.2	5.7	19	230	8.0	27	2.8	1.9	6.2	42	6.3	21	26	5.0	17
12	14	3.1	11	462	4.0	13	6.3	3.1	10	84	3.2	11	52	2.5	8.4
13	25	1.8	6.0	727	2.5	8.4	11	1.8	5.9	134	2.0	6.6	83	1.6	5.2
14	35	1.3	4.3	1027	1.8	5.9	15	1.3	4.2	189	1.4	4.7	119	1.1	3.7
15	48	0.9	3.1	1455	1.3	4.2	20	0.9	3.0	266	1.0	3.4	167	0.8	2.6
16	4.1	18	61	133	14	46	2.1	8.0	27	24	11	37	15	8.5	28
17	8.4	5.6	19	266	6.9	23	4.1	9.0	30	48	5.6	19	30	4.3	14
18	14	3.1	10	421	4.3	15	5.8	3.5	12	74	3.6	12	48	2.7	9.1
19	19	2.4	8.1	593	3.1	10	7.6	2.6	8.5	107	2.5	8.4	67	2.0	6.5
20	27	1.7	5.5	842	2.2	7.2	11	1.7	5.7	151	1.8	5.9	95	1.4	4.6
21	5.1	11	35	164	11	37	3.6^{A}	6.8^{A}	23^{A}	29	9.2	31	19	7.0	23
22	10	4.5	15	329	5.6	19	4.8	4.6	15	59	4.6	15	38	3.4	11
23	17	2.7	8.9	520	3.5	12	7.4	2.6	8.6	94	2.8	9.5	59	2.2	7.4
24	25	1.8	6.1	733	2.5	8.3	11	1.7	5.6	134	2.0	6.7	84	1.5	5.2
25	33	1.4	4.6	1040	1.8	5.9	14	1.3	4.5	188	1.4	4.7	118	1.1	3.7
26	7.8	6.0	20	231	7.9	26	2.7	22	72	43	6.3	21	27	4.9	16
27	15	3.0	10	465	3.9	13	7.7	2.6	8.6	84	3.2	11	53	2.5	8.2
28	25	1.8	6.1	731	2.5	8.3	11	2	5.8	133	2.0	6.7	84	1.5	5.1
29	34	1.3	4.5	1034	1.8	5.9	15	1.2	4.1	188	1.4	4.7	117	1.1	3.7
30	48	0.9	3.1	1465	1.2	4.2	22	0.8	2.8	268	1.0	3.3	169	0.8	2.6

* Shaded cells indicate SNR failures (SNR ≥3 for LOD and/or ≥10 for LOQ)

^{A}Replicates 1 and 5 were omitted due to having a background larger than total counts within the peak range, resulting in negative LOD and LOQ values (n=6)

ASTM E2926-25e1 requires an SNR ≥3 for elemental identification. For semi-quantitative analysis using elemental ratios, an SNR of ≥10 is required. Parameter sets in which elements present in NIST SRM #1831 did not meet an SNR of ≥10 were eliminated, leaving 11 parameter sets as potential optimized method settings.

According to ASTME2926-25e1, Ti and Sr shall have an LOD of ≤75 ppm in a soda-lime glass matrix (NIST SRM #1831). All parameter sets met this requirement, with an LOD <25 ppm for both elements. The elemental LODs obtained from parameter sets 15 and 30 were compared to the LODs obtained from similar instrument configurations listed in ASTM E2926-25e1 *Table X4.3*. The LODs from Laboratories A, B1, and B2 were used for comparison and found to be comparable to those observed. No parameter sets were eliminated based on the calculated LOD.

It was observed that the spectral quality improved with a higher x-ray tube current. The higher tube current increased the instrumental response, subsequently increasing the SNR. This also resulted in a decrease in the LOD and LOQ. Therefore, parameter sets with a tube current less than 600 µA were eliminated. An example spectrum is shown in Figure 1. The increase in tube current resulted in an increase in dead time, or the amount of time in which the signal channel is closed during signal processing. All parameter sets had dead times less than 16%, which was within the 50% maximum required by ASTM E2926-25e1. The remaining parameter sets, and their dead times, are listed in Table 5.

Figure 1. Enlarged spectra of parameter set 13

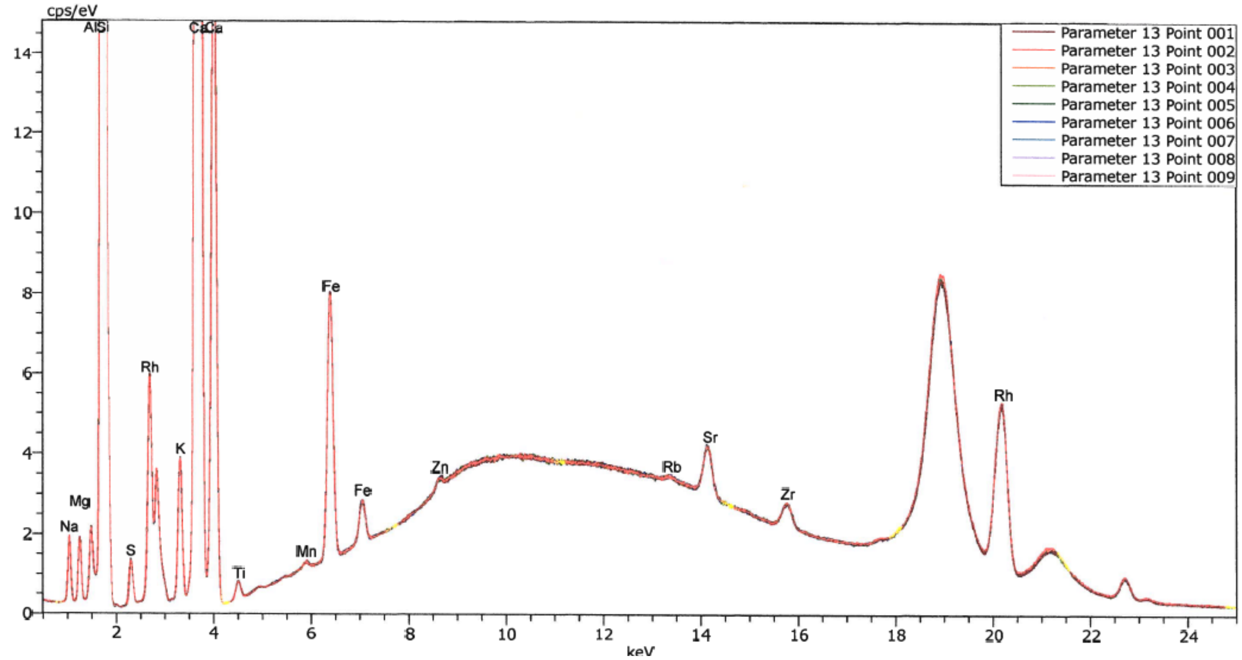


Table 5: Remaining parameter set dead time and conditions

Parameter Set #	Dead Time (%)	Collection Time (Lsec)	X-ray Tube Current (µA)	Max. Pulse Throughput (kcps)
13	15	300	600	130
14	15	600		
15	15	1200		
28	8	300	600	275
29	8	600		
30	8	1200		

The spectral quality also improved with longer collection times due to the increase in observed instrumental response of the element being evaluated. With this increased response, a subsequent decrease in background noise was also observed. Although a longer collection time can result in higher quality spectra, the relationship is not directly proportional, and a shorter collection was determined to be optimal to reduce some of the wear of the detectors. When placing an emphasis on analysis time, parameter sets 13 and 28 are the two parameters sets that remain.

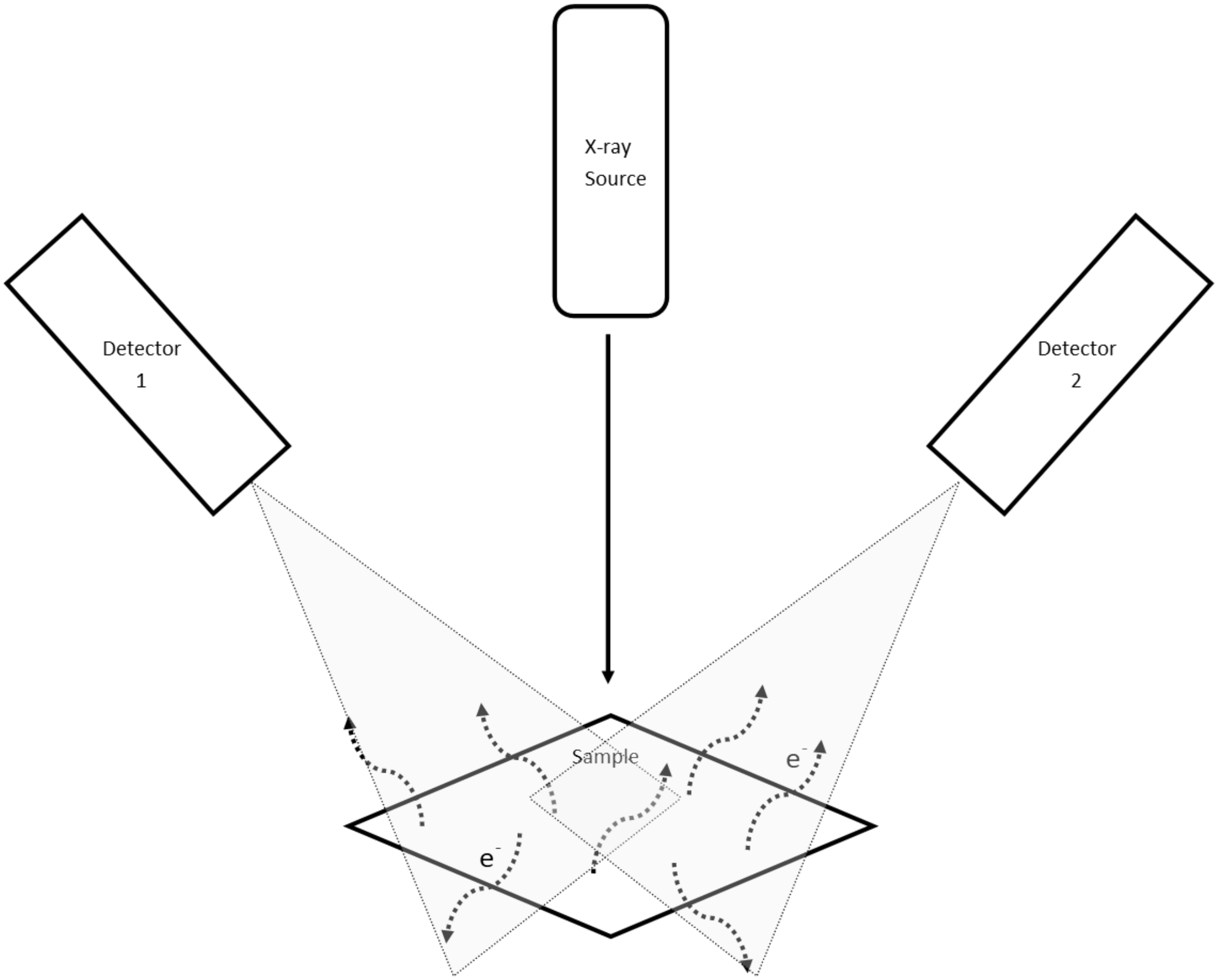
The maximum pulse throughput is the only variable that differs between these parameter sets. It was observed that lighter elements (i.e., Na to Ca) had better resolution at 130 kcps while heavier elements (i.e., Ti to Zr) had similar resolution at either throughput. Manufacturer recommendations suggest a pulse throughput of 275 kcps but experimentally the 130 kcps pulse throughput provided better resolution. Additionally, ASTM E2926-25e1 provides flexibility in the pulse throughput as long as it remains constant between compared samples. Therefore, the variables of parameter set 13 are considered optimal and will be utilized.

ASTM E2926-25e1 states that a screening method to discriminate samples based solely on large visual peak intensity differences (preliminary screen) may reduce analysis times and can utilize fewer replicates than the ratio method (semi-quantitative analysis). As with the semi-quantitative method, to evaluate exclusionary differences between samples while using the screening method, the elements identified as exclusionary shall have a SNR of ≥3 in at least one of the samples being compared. If the SNR ratio is not met by either sample within the comparison, the element cannot be used for exclusionary purposes. In accordance with the standard test method, the sample may be excluded based on other elements with acceptable SNRs or analyzed using the semi-quantitative method. The parameter set with the shortest collection time (30 Lsec) but comparable x-ray tube current and maximum pulse throughputs to the optimal method parameters, is parameter set 11. Although Rb does not meet the SNR of ≥3, in the screening method it is acceptable for elements to not meet this criterion as long as they are not used for exclusionary purposes.

B. Single vs Dual Detector(s)

The aim of this study was to evaluate the difference between the utilization of a single detector versus both instrumental detectors. In the µXRF, the use of two detectors collects more data with respect to time while covering a wider area of emission. When a sample fluoresces, electrons emit at all angles. By having two detectors, more angles of emission are within detection range (Figure 2). Each detector subsequently detects the data from different spatial orientations. When using dual detectors, the data collected from each individual detector is combined to generate the final data.

Figure 2. Box diagram of a dual detector µXRF



To assess single versus dual detector use, each configuration was assessed for its ability to meet quality assurance checks and produce repeatable data. Using the optimal parameter sets established during method optimization for semi-quantitative analysis, six detector configurations were established (Table 6). Maximum pulse throughputs of both 130 kcps and 275 kcps were evaluated as the manufacturer recommends 275 kcps with its newer detector however the forensic community frequently recommends 130 kcps. The intent was to determine whether the 275 kcps improved the data while maximizing data output and preserving the x-ray source. When evaluating a single detector, the collection time was doubled compared to the dual detector collection time to assess the consistency of the signal response. The spot size (20 µm), x-ray voltage (50 kV), x-ray tube current (600 µA), and maximum energy (40 keV) were held constant.

Table 6: Differing conditions of detector parameters

Parameter #	Max. Pulse Throughput (kcps)	Detector(s) #	Collection Time (Lsec)
1	130	1	600
2	275		
3	130	2	
4	275		
5	130	1 & 2	300
6	275		

For each parameter, with the exception of one, nine replicates were collected across NIST SRM #1831. During the third replicate of parameter 5, no data was obtained due to an electronic error. Based on the ASTM E2926-25e1 standard test method requirements for Ti and Sr to have an LOD of ≤75 ppm, the LOD was calculated for each. Additionally, the SNR was ≥3 for both elements (Table 7). The LODs of Ti and Sr were <75 ppm, regardless of the detector configuration. This indicates that either one or both detectors may be used for analysis upon completion of quality assurance checks.

Table 7: Averaged SNR and LOD for Ti and Sr

Parameter Set #	Ti		Sr	
	SNR	*LOD*	*SNR*	*LOD*
1	85.0	3.9	133	2.0
2	86.3	3.8	133	2.0
3	85.4	3.9	133	2.0
4	84.6	3.9	133	2.0
5 [A]	85.0	3.9	134	2.0
6	85.6	3.9	133	2.0

[A] No data collected for Replicate 3 (n=8)

To assess the repeatability of each detector configuration, the relative standard deviation (RSD) for each element was calculated by dividing the standard deviation by the average value (Table 8). In accordance with the values mentioned in ASTM E2926-25e1, an RSD ≤10% was considered acceptable repeatability. All elements had acceptable RSD values for each detector configuration. This indicates that a single detector with a longer runtime or a dual detector configuration can be used interchangeably.

Table 8: Relative standard deviation per element at each detector configuration

Parameter Set #	Na	Mg	Al	K	Ca	Ti	Mn	Fe	Sr	Zr
1	0.47%	0.53%	1.03%	0.61%	0.05%	2.10%	6.34%	0.40%	0.70%	1.78%
2	0.74%	0.96%	0.90%	0.44%	0.08%	2.27%	7.17%	0.28%	0.80%	1.98%
3	0.84%	0.84%	0.87%	0.40%	0.06%	1.50%	4.97%	0.24%	1.52%	1.43%
4	0.75%	0.72%	0.75%	0.38%	0.05%	2.38%	4.42%	0.31%	1.04%	1.97%
5[A]	0.73%	0.35%	0.94%	0.48%	0.03%	2.01%	3.77%	0.16%	0.73%	2.46%
6	0.63%	0.44%	0.83%	0.48%	0.06%	1.06%	5.07%	0.36%	1.23%	2.06%

[A] No data collected for Replicate 3 (n=8)

Although the use of dual detectors is preferred to reduce collection time, it was adequately demonstrated that the use of one detector is acceptable. For either detector configuration, day of use quality assurance requirements shall be met and known and questioned samples shall be analyzed under the same instrumental conditions.

C. Sample Preparation

The purpose of this study was to determine the optimal mounting methods for sample analysis. Since mounting media can contain the elements present in glass samples, the elemental compositions of fourteen different media (Table 9) were determined using the method variables of parameter set 13. The preferred combination of mounting media and methods for different sized fragments was evaluated. Due to the angle of the x-ray beam and placement of the detectors, the optimal placement area of the sample was also investigated.

Table 9: Mounting method summary

	Type	Manufacturer	Model Number	Designation
Sample Holder	XRF Sample Cups	FluXana	SC-4231	FluXana Small Cup
			SC-4240	FluXana Large Sup
		Chemplex	1940	Chemplex Cup
	Transparency Film Sheet	Apollo	VPP100C	Transparent Sheet
Supportive Film	Polypropylene Film	FluXana	TF-260-255	Polypropylene Film
	MYLAR X-Ray Film	FluXana	TF-160	MYLAR Film Roll
			TF-160-355	MYLAR Film Circle
			TF-160-345	MYLAR Film Sheet
Adhesive	Carbon Adhesive Tabs	-----	545T	Carbon Tape
	Liquid Adhesive Mountant	SPI Supplies	04982-AB	SPI-Tac Thin Layer
				SPI-Tac Thick Layer
				SPI-Tac Adhesive Ball
	Blu Tack	Bostik	-----	Blu Tack Adhesive
	Double-Sided Tape	Scotch 3M	34-8702-1856-6	Double-Sided Tape

Samples were mounted for analysis using a combination of sample holder (e.g., sample cup, transparent sheet), supportive film, and/or adhesive. ASTM E2926-25e1 recommends that samples under 1.5 mm of thickness are raised using a sample cup to help reduce spectral noise created by the acrylic stage. When using a sample cup, a supportive film is stretched across the top of the cup to provide a flat surface for the sample. An adhesive is placed on the supportive film or a transparent sheet to fix the sample in place. Thicker samples may be placed directly on a transparent sheet on top of the acrylic stage.

To determine which mounting media combinations were optimal, a background spectrum of the acrylic stage was first generated. The background spectrum was generated from eight replicates collected from the front left quadrant of the stage. The net count of each element identified was calculated by the µXRF program using the "Oxide with Zr" quantitation method. When evaluating a medium, elements with an average net count greater than those observed from the stage were considered present in the medium. A lower average net count from the medium indicates a reduction in observed background from the acrylic stage. To assess each sample cup, eight replicates were collected and the average net count for each element present was evaluated against the elements present in the acrylic stage. The average net counts for elements observed from the acrylic stage and sample cups are shown in Table 10.

Table 10: Average net counts of elements identified in acrylic stage and sample cups

Sample	Al	Si	P	S	Ca	Ti	Zn
Stage	*2770*			*9537*			
Chemplex Sample Cup	2693	2933	3609	3717	55375	22845	
FluXana Large Sample Cup	3425	1720		2687		3643	306171
FluXana Small Sample Cup	3704	3512		2383	5634	2264	370922

*Shaded cells indicate elemental contribution by inherent background
**Italicized cells denote inherent background
***Silicon (Si), Phosphorus (P), Sulfur (S)

The presence of sulfur can be attributed to the acrylic stage due to the reduction in the net counts in the sample cups, indicating that the element is not present in any of the sample cups. Aluminum on the other hand, was not present in the Chemplex sample cup, but the increase in net counts indicates its presence in the FluXana sample cups. Since all cups have four elements present, the specific elements and their counts were considered. Of the elements identified in the sample cups, calcium, titanium, and zinc are the only components in the elemental ratios for glass analysis (Table 2). Of these, calcium is utilized in four of the eleven elemental ratios, titanium is utilized in two elemental ratios, and zinc is utilized in one. Given the lower calcium contribution, the FluXana large sample cups are considered optimal for the analysis of glass.

To determine the optimal supportive film, each film was placed on top of each sample cup (e.g., Chemplex, FluXana). It was hypothesized that the MYLAR films (circle, roll, and sheet) would produce consistent results regardless of whether the film was precut upon purchase. It was noted that the application of even pressure to the outer ring of the FluXana cup with a preparation tool (provided with the sample cups) resulted in a flatter film surface compared to other sample cup types. This further supports its designation as the optimal sample cup. Five replicates were collected from the center of the supportive film to determine the elemental impact on the background, now comprised of the acrylic stage and the applicable sample cup (Table 11a-11c).

Table 11a: Average net counts of elements identified in supportive films on Chemplex cups

Sample	Al	Si	P	S	Ca	Ti	Fe	Zn
Stage	*2770*			*9537*				
Chemplex Sample Cup	*2693*	*2933*	*3609*	*3717*	*55375*	*22845*		
Polypropylene Film	963		260		7425		4060	
MYLAR Film Circle	816	3	31358		66819	2830	3357	10849
MYLAR Film Roll	780		34938		77266	3121	3185	11731
MYLAR Film Sheet	698		36417		80054	2892	3237	10695

*Shaded cells indicate elemental contribution by inherent background
**Italicized cells denote inherent background

Table 11b: Average net counts of elements identified in supportive films on FluXana large cups

Sample	Al	Si	P	S	Ca	Ti	Fe	Zn
Stage	*2770*			*9537*				
FluXana Large Sample Cup	*3425*	*1720*		*2687*		*3643*		*306171*
Polypropylene Film	673						3257	21332
MYLAR Film Circle	780	6	33213		68000	2076	3865	32951
MYLAR Film Roll	605		35753		73029	1783	3494	21156
MYLAR Film Sheet	776		36446		76140	1788	3851	29787

*Shaded cells indicate elemental contribution by inherent background

**Italicized cells denote inherent background

Table 11c: Average net counts of elements identified in supportive films on FluXana small cups

Sample	Al	Si	P	S	Ca	Ti	Fe	Zn
Stage	*2770*			*9537*				
FluXana Small Sample Cup	*3704*	*3512*		*2383*	*5634*	*2264*		*370922*
Polypropylene Film	1032						6096	67182
MYLAR Film Circle	888		32726		66881	2231	5464	56628
MYLAR Film Roll	872		33728		69481	2098	5363	58064
MYLAR Film Sheet	921		35480		75480	2315	5744	58728

*Shaded cells indicate elemental contribution by inherent background

**Italicized cells denote inherent background

The instrumental response associated with aluminum and silicon from the acrylic stage are reduced in the presence of support films on top of the three sample cups. When evaluating the polypropylene film, the reduction of the instrumental response associated with presence of phosphorus, calcium, zinc was indicative of the presence in the sample cup and not from the film itself based on the decreased instrumental response. It was noted that the polypropylene film contained iron.

When assessing the MYLAR films, the instrumental response of present elements was consistent between film types regardless of shape. Further, the MYLAR film indicated the presence of phosphorus, calcium, and iron. The presence of both aluminum and zinc was attributed to the sample cup contributions while the presence of iron was from the MYLAR film itself. Polypropylene film is the optimal support film due to the limited number of elements identified.

Although the polypropylene film was determined to be the optimal support film, analysis of adhesives was performed using the MYLAR film roll on a large FluXana sample cup. The three SPI-Tac samples were different preparations of the same material, a single application of the mountant (SPI-Tac Thin Layer), multiple applications in the same location (SPI-Tac Thick Layer), and application of the mountant to a microscope slide which was allowed to dry prior to removal and formation of a ball (SPI-Tac Adhesive Ball). The Blu Tack adhesive was an anomaly in that it was placed directly on the acrylic stage due to its reusability and ease of removal. Five replicates were collected from the center of each adhesive to determine elements present and effect on background (Table 12a and 12b).

Table 12a: Average net counts of elements identified in acrylic stage, FluXana large sample cup, MYLAR film roll, and adhesives on MYLAR film roll and FluXana large sample cup

Sample	**Na**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Ti**	**Fe**	**Zn**	**Br**
Stage		*2770*			*9537*						
FluXana Large Sample Cup		*3425*	*1720*		*2687*			*3643*		*306171*	
MYLAR Film Roll		*605*		*35753*			*73029*	*1783*	*3494*	*21156*	
SPI-Tac Thin Layer		787	289	30291			69390	1931	4559	31954	
SPI-Tac Thick Layer		817	164	12178			61809	1780	6401	37320	
SPI-Tac Adhesive Ball		1298	249	2			15292	3	17741	44190	
Carbon Tape	3062	1370	15662	26351	563360		2898	24907	12700	31500	
Double-Sided Tape		4388		1272	151147	29655	38976		7016	26864	37017

*Shaded cells indicate elemental contribution by inherent background
**Italicized cells denote inherent background
***Bromine (Br)

Table 12b: Average net counts of elements identified in acrylic stage and Blu Tack adhesive

Sample	**Mg**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Ti**	**Mn**	**Fe**	**Sr**
Stage		*2770*			*9537*						
Blu Tack Adhesive	2479	17824	122322	10171	37004	22710	55201921	11871	82744	249300	417180

*Shaded cells indicate elemental contribution by inherent background
**Italicized cells denote inherent background

All elements detected in the SPI-Tac adhesive can be attributed to the acrylic stage, sample cup, and/or supportive film. While a layer of mountant generally resulted in a greater background reduction, there are some instances in which a ball may be necessary to affix the sample in a flat plane depending on the sample's size. The carbon tape, double sided tape, and Blu Tack adhesives introduced elements not previously observed in the other mounting medium configurations. Carbon tape and double-sided tape each contain five elements not attributable to the instrumental background (i.e., acrylic stage, sample cup). Large, full thickness glass fragments tend to adhere better to tape than other adhesives. Since the x-ray penetration depth is less than the thickness of the glass fragment, the elements present in the double-sided tape would not interfere with the elemental composition of the glass, if the glass fully covers the tape. Given the possible interferences indicated for both adhesives, the more cost effective, double-sided tape, is recommended for mounting full thickness glass samples. The Blu Tack adhesive, however, contained eleven different elements and should not be used as an adhesive for mounting glass samples.

The potential interference caused by a transparent sheet was evaluated by placing a transparent sheet on a FluXana large sample cup and collecting five replicates from the center of the sheet. The aluminum and zinc detected could be attributed to the acrylic stage and sample cup. Silicon and iron were the only elements detected in the transparent sheet (Table 13). Although iron is used in several elemental ratios, silicon is not. With only one element having the potential of interference, the use of a transparent sheet is considered acceptable.

Table 13: Average net counts of elements identified in acrylic stage, FluXana large sample cup, and transparent sheet on a FluXana large sample cup

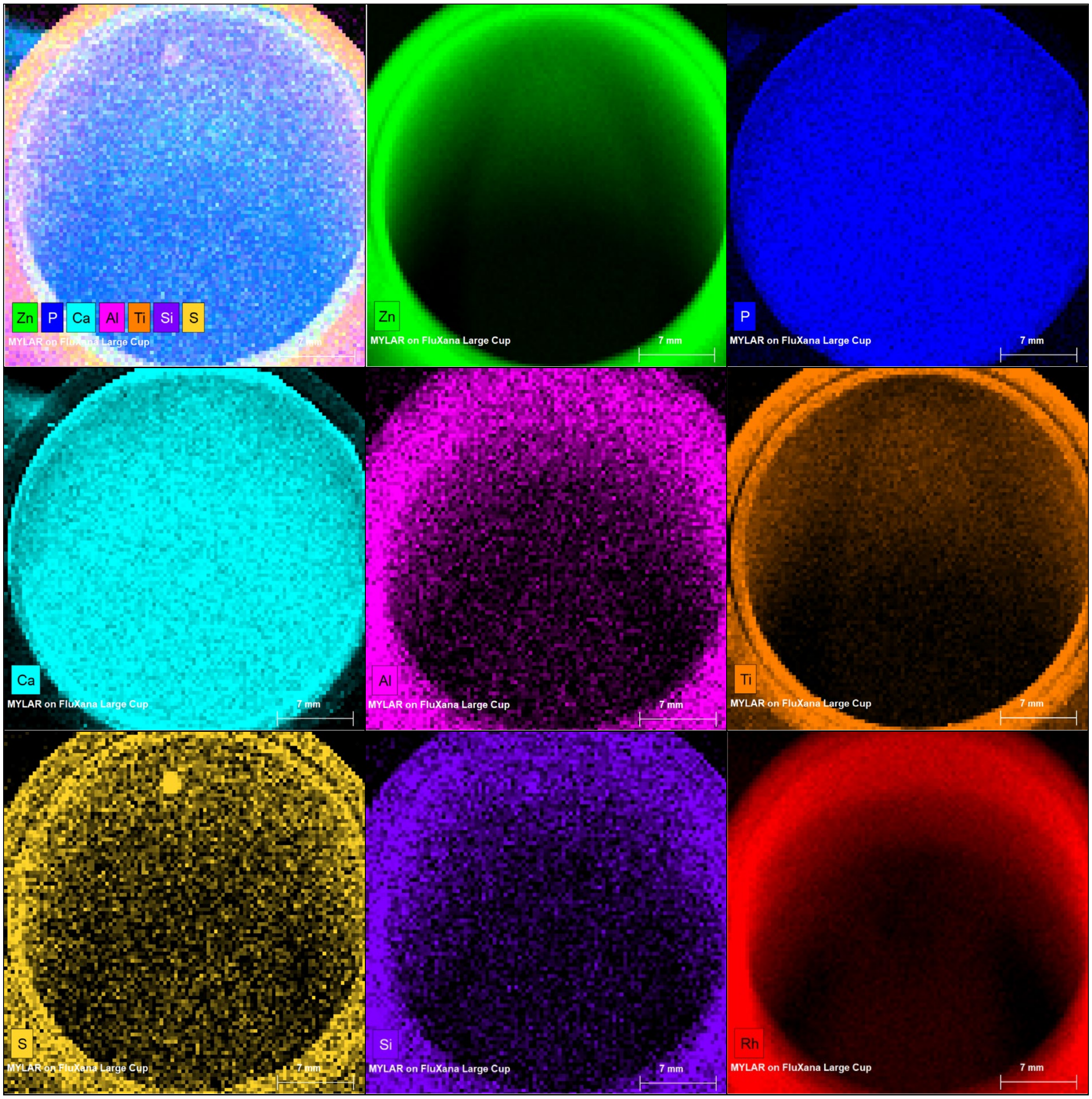
Sample	Al	Si	S	Ti	Fe	Zn
Stage	*2770*		*9537*			
FluXana Large Sample Cup	*3425*	*1720*	*2687*	*3643*		*306171*
Transparent Sheet	820	9310			5638	22529

*Shaded cells indicate elemental contribution by inherent background
**Italicized cells denote inherent background

The suitability of different combinations of mounting materials varies depending on the sample's size. For thin glass fragments or microscopic glass particles, ASTM E2926-25e1 recommends the use of a sample cup to reduce instrumental background. Therefore, the FluXana large sample cup, polypropylene film, and SPI-Tac adhesive should be utilized for thin glass fragments. For full thickness glass fragments, a transparent sheet with double-sided tape can be utilized to better affix the sample to the sheet. In instances where double-sided tape would be exposed, such as small pieces of full thickness glass, it is recommended to use the SPI-Tac adhesive on the transparent sheet. Any of the mounting media tested, apart from the Blu Tack adhesive, can be utilized for sample mounting, provided an adequate background was collected and the same media is used for both known and questioned samples.

The final consideration was the placement area of the sample on the sample cup. Due to the angle of the x-ray beam and orientation of the detectors, it was anticipated that different locations on the sample cup may be prone to different amounts of background. Elemental area maps were obtained from the MYLAR film roll on each of the three sample cups. These area maps show where specific elements are emitted from the sample with colored pixels that can be overlaid or separated by individual elements. Since similar results were noted for all three sample cups, the data obtained from the FluXana large sample cup is used as a representative image (Figure 3).

Figure 3. Elemental area maps for MYLAR film roll on FluXana large sample cup



*Rhodium (Rh)

The area maps for P and Ca show emission across the entire cup due to its presence in the MYLAR film. Given their presence in the sample cup, Al, S, and Si emissions span the majority of the area, but the color has higher density towards the top edge of the cup. Of the remaining elements, Zn and Ti, there is a void of emission in the bottom third of the sample cup. It should also be noted that Rh is present due to the Rh x-ray tube utilized to create the x-ray beam. Similar to other elements, there is less Rh at the bottom edge of the cup. When utilizing a sample cup, it is recommended to attach the sample near the bottom edge of the cup to minimize the amount of spectral interference in the sample.

D. Intra-day versus Inter-day Repeatability

The goal of this study was to confirm repeatability in the data collected by the instrument across multiple analyses of the same sample. This was accomplished by calculating the relative standard deviation of the net counts, calculated by the µXRF program using the "Oxide with Zr" quantitation method, across a series of analyses as a percentage. In accordance with the requirements delineated in ASTM E2926-25e1, a %RSD ≤10% was considered acceptable repeatability. To establish intra-day repeatability, NIST SRM #1831 was analyzed twice within the same day, with 7.5 hours separating analyses. Each analysis comprised of nine replicates for a total of 18 replicates between the two timepoints. The net counts of each replicate were averaged for each element, at each timepoint. The average and standard deviation were then calculated to determine the %RSD as shown in Table 14.

Table 14: Intra-day repeatability (n=18)

Day	**Na**	**Mg**	**Al**	**K**	**Ca**	**Ti**	**Mn**	**Fe**	**Sr**	**Zr**	**Zn**
3a	39444	39339	41632	133691	5632859	20979	8298	372503	108451	38400	24237
3b	39378	39239	41362	133468	5637144	20995	8132	373483	108718	39012	24670
Average	39411	39289	41497	133580	5635001	20987	8215	372993	108585	38706	24453
Std Dev	47	71	191	158	3030	11	117	693	189	433	306
RSD	0.1%	0.2%	0.5%	0.1%	0.1%	0.1%	1.4%	0.2%	0.2%	1.1%	1.3%

To establish inter-day repeatability, NIST SRM #1831 was analyzed across five days, with nine replicates comprising each analysis. The net counts of each replicate were averaged for each element, each day. The average net counts for each day where then used to calculate the average and standard deviation. The %RSD was then calculated for each element (Table 15).

Table 15: Inter-day repeatability (n=54)

Day	**Na**	**Mg**	**Al**	**K**	**Ca**	**Ti**	**Mn**	**Fe**	**Sr**	**Zr**	**Zn**
1	39455	39347	41643	133563	5632678	20953	8192	373205	107980	37879	24472
2	39519	39442	41763	133756	5635447	20943	8197	372548	108337	38224	24507
*3**	39411	39289	41497	133580	5635001	20987	8215	372993	108585	38706	24453
4	39058	39077	41528	133275	5622732	20884	8317	372663	107611	38464	24577
5	39201	38973	41358	132978	5621414	20808	8136	372180	107929	37999	24575
Average	39329	39226	41558	133430	5629454	20915	8211	372718	108088	38254	24517
Std Dev	193	195	153	306	6836	70	66	398	379	337	57
RSD	0.5%	0.5%	0.4%	0.2%	0.1%	0.3%	0.8%	0.1%	0.4%	0.9%	0.2%

*An average of 3a and 3b

Both intra-day and inter-day repeatability meet the predetermined acceptance criteria of RSD ≤10% confirming the repeatability of the instrument.

E. Precision

To establish the precision of the instrument, the study referenced in ASTM E2926-25e1 for "Precision for Instruments with SDD" (*§11.2*) was replicated. Seven replicates were collected from Float Glass Standards (FGS) #1 and #2, soda-lime-silicate glasses manufactured by Scott for Bundeskriminalamt. To normalize the data obtained for FGS #1 and FGS #2 and allow comparison to the ASTM E2926-25e1 ratios, seven replicates were collected from NIST SRM #1831. The net count from each FGS was divided by the net count of the NIST SRM #1831 for each elemental ratio. The net count of each element was calculated by the µXRF program using the “Oxide with Zr” quantitation method. The SNRs of the elements used in the ratios were confirmed to be ≥10 before determining the average net count of each element from the replicates (Table 16). To compare the results to ASTM E2926-25e1 *Table X3.2*, the data was normalized (Equation 4) and the %RSD calculated (Table 17 and Table 18).

Table 16: Average SNR and net counts

	Na		**Mg**		**K**		**Ca**		**Ti**	
	SNR	*Net Count*	*SNR*	*Net Count*	*SNR*	*Net Count*	*SNR*	*Net Count*	*SNR*	*Net Count*
FGS 1	255	41072	270	44300	258	43095	24286	5849192	56	14215
FGS 2	239	39783	255	42879	1024	253399	20569	5631208	262	71119
SRM 1831	275	38947	264	38833	647	131659	22337	5584241	82	20556

Table 16: (continued)

	Mn		**Fe**		**Sr**		**Zr**	
	SNR	*Net Count*	*SNR*	*Net Count*	*SNR*	*Net Count*	*SNR*	*Net Count*
FGS 1	63	24228	717	367967	90	74909	86	48236
FGS 2	260	107740	3376	1757358	390	325160	401	258330
SRM 1831	25	8482	716	369033	131	107734	82	37957

Equation 4:

$$R_{normalized} = \frac{\left(\frac{E_x}{E_y}\right)_{sample}}{\left(\frac{E_x}{E_y}\right)_{standard}}$$

where (R) is the normalized elemental ratio and (E) is the net count of element (x) or (y)

Table 17: Normalized elemental ratios with RSD for FGS #1

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Fe/Zr**	**Ca/K**	**Na/Mg**	**Ti/Fe**	**Mn/Fe**	**Sr/Fe**
1	0.91	1.50	1.05	0.53	0.76	3.19	0.93	0.70	2.99	0.70
2	0.92	1.52	1.05	0.53	0.78	3.23	0.92	0.69	2.57	0.68
3	0.91	1.51	1.05	0.54	0.77	3.20	0.92	0.70	2.91	0.70
4	0.91	1.53	1.05	0.56	0.81	3.17	0.92	0.69	2.90	0.69
5	0.93	1.54	1.04	0.57	0.81	3.19	0.93	0.68	2.95	0.71
6	0.93	1.53	1.06	0.55	0.79	3.25	0.92	0.69	2.70	0.69
7	0.93	1.47	1.05	0.55	0.78	3.17	0.93	0.71	3.09	0.71
Average	0.92	1.51	1.05	0.55	0.78	3.20	0.92	0.69	2.87	0.70
Std Dev	0.009	0.024	0.004	0.015	0.020	0.029	0.005	0.012	0.178	0.010
RSD (%)	0.96	1.59	0.43	2.83	2.58	0.91	0.52	1.72	6.21	1.42

Table 18: Normalized elemental ratios with RSD for FGS #2

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Fe/Zr**	**Ca/K**	**Na/Mg**	**Ti/Fe**	**Mn/Fe**	**Sr/Fe**
1	0.91	0.30	0.21	0.44	0.65	0.52	0.93	0.71	2.75	0.67
2	0.93	0.29	0.21	0.44	0.67	0.53	0.93	0.73	2.45	0.65
3	0.92	0.29	0.21	0.44	0.70	0.52	0.93	0.73	2.66	0.63
4	0.90	0.29	0.21	0.44	0.70	0.52	0.92	0.72	2.78	0.63
5	0.91	0.29	0.21	0.45	0.72	0.52	0.93	0.73	2.74	0.62
6	0.91	0.29	0.21	0.45	0.74	0.52	0.91	0.73	2.48	0.61
7	0.92	0.29	0.21	0.45	0.73	0.52	0.93	0.74	2.87	0.62
Average	0.91	0.29	0.21	0.44	0.70	0.52	0.93	0.73	2.68	0.63
Std Dev	0.009	0.002	0.001	0.006	0.031	0.003	0.008	0.008	0.156	0.021
RSD (%)	1.01	0.80	0.40	1.45	4.45	0.48	0.89	1.06	5.85	3.37

All of the %RSDs were ≤10%, indicating acceptable precision. The average elemental ratios and the %RSDs were compared to the values reported in ASTM E2926-25e1 *Table X3.2* and the percent differences calculated (Table 19).

Table 19: Comparison of precision data

		Average			**RSD (%)**		
		VA DFS	*ASTM**	*% Diff.*	*VA DFS*	*ASTM**	*Within Range?*
FGS #1	Ca/Mg	0.92	0.92	0.0%	0.96	2 ± 6	Yes
	Ca/Ti	1.51	1.43	5.4%	1.59	5 ± 13	Yes
	Ca/Fe	1.05	1.06	0.9%	0.43	3 ± 9	Yes
	Sr/Zr	0.55	0.57	3.6%	2.83	5 ± 14	Yes
	Fe/Zr	0.78	0.82	5.0%	2.58	6 ± 16	Yes
	Ca/K	3.20	3.06	4.5%	0.91	5 ± 14	Yes
	Na/Mg	0.92	0.94	2.2%	0.52	3 ± 8	Yes
	Ti/Fe	0.69	0.74	7.0%	1.72	5 ± 13	Yes
	Mn/Fe	2.87	2.79	2.8%	6.21	5 ± 14	Yes
	Sr/Fe	0.70	0.70	0.0%	1.42	6 ± 16	Yes
FGS #2	Ca/Mg	0.91	0.93	2.2%	1.01	2 ± 6	Yes
	Ca/Ti	0.29	0.35	18.8%	0.80	4 ± 12	Yes
	Ca/Fe	0.21	0.22	4.7%	0.40	1 ± 3	Yes
	Sr/Zr	0.44	0.51	14.7%	1.45	4 ± 11	Yes
	Fe/Zr	0.70	0.83	17.0%	4.45	5 ± 13	Yes
	Ca/K	0.52	0.52	0.0%	0.48	1 ± 4	Yes
	Na/Mg	0.93	0.94	1.1%	0.89	3 ± 8	Yes
	Ti/Fe	0.73	0.62	16.3%	1.06	4 ± 12	Yes
	Mn/Fe	2.68	2.57	4.2%	5.85	1 ± 4	No
	Sr/Fe	0.63	0.62	1.6%	3.37	2 ± 6	Yes

* As reported by ASTM E2926-25e1 *Table X3.2*

The percent difference between the averages observed in the verification and those reported by ASTM E2926-25e1 are within 20%, indicating similar detection performance as other instruments. The instrument precision is considered acceptable as all %RSDs observed in the verification fall within the ranges provided for FGS #1 and FGS #2 in ASTM E2926-25e1 with the exception of the Mn/Fe ratio in FGS #2. The ratio was outside of the range provided by ASTM E2926-25e1.

F. Accuracy

The accuracy of conclusions drawn from the µXRF data on samples of known sources was evaluated. A total of eight sets, each containing a ‘known’ sample (*B*) and a ‘questioned’ sample (*A*) were analyzed. In each set, the samples could not be source discriminated by other glass analysis methods such as visual comparison, physical properties, or refractive index. Five of the sets contained samples known to originate from different sources. Three of the sets contained samples known to originate from the same source. All samples were full thickness glass fragments and therefore three of the sets were crushed to generate large glass particles (approximately 1-2 millimeters in length) and small glass particles (less than 900 micrometers in length). As recommended by ASTM E2926-25e1, particles within these two sets were similar in size and thickness.

Prior to mounting, samples were cleaned according to recommendation in ASTM E2926-25e1 and the VA DFS Trace Evidence Procedures Manual. Individually, full thickness fragments were sonicated in a beaker with 5% micro 90 solution for seven minutes. They were then placed in a beaker with deionized water and sonicated for an additional five minutes. Finally, they were placed in a beaker with methanol and manually swirled for ten seconds prior to being removed and allowed to air dry. A summary of the sets and the mounting method are listed in Table 20.

Table 20: Sets of samples analyzed for accuracy (Questioned = A, Known = B)

Set	Sample Name*	Size	Sources	Mounting
1	A	Full thickness	Different	Double-sided tape on transparent sheet, float side down if applicable
	B			
2	A	Full thickness	Different	Double-sided tape on transparent sheet, float side down if applicable
	B			
3	A	Full thickness	Different	Double-sided tape on transparent sheet, float side down if applicable
	B			
	A1	Large particles	Different	FluXana Large Sample Cup, with MYLAR film, and thin layer of SPI Tac
	B1			
	A2	Small particles	Different	FluXana Large Sample Cup, with MYLAR film, and thin layer of SPI Tac
	B2			
4	A	Full thickness	Different	Double-sided tape on transparent sheet, float side down if applicable
	B			
	A1	Large particles	Different	FluXana Large Sample Cup, with MYLAR film, and thin layer of SPI Tac
	B1			
	A2	Small particles	Different	FluXana Large Sample Cup, with MYLAR film, and thin layer of SPI Tac
	B2			
5	A	Full thickness	Different	Double-sided tape on transparent sheet, float side down if applicable
	B			
6	A	Full thickness	Same	Double-sided tape on transparent sheet, float side down if applicable
	B			
7	A	Full thickness	Same	Double-sided tape on transparent sheet, float side down if applicable
	B			
	A1	Large particles	Same	FluXana Large Sample Cup, with MYLAR film, and thin layer of SPI Tac
	B1			
	A2	Small particles	Same	FluXana Large Sample Cup, with MYLAR film, and thin layer of SPI Tac
	B2			
8	A	Full thickness	Same	Double-sided tape on transparent sheet, float side down if applicable
	B			

Nine replicates were collected from each sample. A preliminary screen was performed by overlaying the spectra from each sample in the set to allow for discrimination based on distinct visual differences, such as differing element composition or readily apparent differences in relative peak heights. Based on spectral differences (Supplemental Figures S1 – S9) sets 1 through 5 were source discriminated. Sets 6 through 8 were not source discriminated based on spectral comparison (Supplement Figures S10 – S14). These observations were consistent with the ground truth results. Despite being able to discriminate some sets based solely on spectral differences (preliminary screen), semi-quantitative analysis was performed evaluating elemental ratios for all sets. First, samples were evaluated for their SNR for each element present ensuring that the SNR was ≥10. The response and SNR are shown for each element identified per sample in Table 21.

Table 21: Average response and SNR for each sample

	Na		Mg		K		Ca		Ti		Fe		Sr		Zr		Mn	
Sample	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**	**Resp**	**SNR**
1A	40725	244	48406	285	14586	100	5902589	24782	41590	170	359174	692	54258	61	75844	113	10077	29
1B	2717	16	7133	45	---	-858	4577847	20186	---	98	350962	741	29062	31	74650	110	11422	11
2A	35187	230	39020	247	415551	1453	5617756	18791	31316	18	2195178	4097	84947	103	40066	79	6951	7
2B	35768	239	36902	238	161570	720	5755293	22080	36404	146	2100819	3973	37833	45	67132	111	18348	43
3A	33951	217	41966	262	284902	1115	5169422	13790	422504	-133	2442704	3530	58731	74	51738	91	20033	-244
3B	36345	219	34737	207	14283	97	6589346	26726	276687	1105	3600758	6719	53494	62	99594	147	14616	28
3A1	36545	250	39914	269	264971	1077	4936324	13577	395346	-135	2359939	3503	55626	67	44331	79	18749	-232
3B1	37991	263	30673	211	12600	97	6020038	25776	247556	1034	3345186	6468	46751	55	80719	129	13326	27
3A2	39021	285	42176	299	290771	1133	5398175	14301	427720	-132	2189064	3237	12265	17	5871	16	18291	-232
3B2	39061	292	31223	231	12101	97	5799547	24894	228859	963	2414595	4799	8819	11	8983	20	10638	23
4A	34647	234	40513	264	53763	286	5900765	23932	48094	193	3791228	6997	89411	111	33244	72	8887	14
4B	36151	240	42682	274	6062	54	5894523	24726	15887	64	3909741	7214	41797	50	53001	91	8167	11
4A1	42189	290	38825	264	53611	308	5815682	23935	49203	204	3788129	7015	48696	54	19458	40	9358	13
4B1	37485	267	36788	260	6224	63	5258757	23316	13958	58	3523210	6755	22062	23	18337	35	7548	9
4A2	27667	211	25884	196	42630	266	4443547	20059	39148	176	3119590	6287	26777	35	9417	25	7477	9
4B2	35158	262	34672	256	5707	61	5052073	22824	13742	58	3460279	6662	15380	19	13080	27	7230	8
5A	37197	236	34264	214	14922	103	6478094	26590	275913	1113	2779741	5252	42819	52	91946	142	9012	18
5B	37449	226	42340	251	---	-5	5967317	25296	11379	46	2439473	4600	52587	61	31611	56	5937	9
6A	35023	215	22761	139	40596	212	9592252	36969	53870	248	1009894	2005	62167	77	68477	113	24163	67
6B	35776	225	23131	146	40611	212	9644745	37177	54609	250	1019280	2022	63754	80	69976	117	24542	68
7A	38283	226	42770	249	14323	99	6114841	24937	51056	1204	5108897	9301	84135	95	75630	119	8864	7
7B	38075	243	42661	266	14046	99	6097867	25050	50852	205	5076692	9269	78602	95	72318	122	8520	7
7A1	45255	312	44876	302	17068	116	6693917	27037	55057	218	5595545	9861	32432	37	20538	40	9185	4
7B1	42032	285	41533	279	15077	108	6166051	25664	50756	206	5302519	9653	49396	54	34962	61	8873	7
7A2	41713	302	41439	294	15993	111	6391683	26401	52537	211	4731416	8639	17558	24	9577	23	8501	8
7B2	36553	273	35740	262	13216	104	5557997	23789	44878	189	3825915	7341	11941	17	6984[A]	17	8474	18
8A	35966	232	32929	209	20269	128	6472183	26424	273632	1094	2866707	5394	67445	81	113087	176	6612	11
8B	35843	242	32910	215	20454	129	6468533	26501	273500	1098	2860248	5382	66015	81	112349	182	6355	12

*Shaded cells indicate the failure of SNR ≥10

[A] Replicate 1 omitted as an outlier, no response collected

If an element failed the SNR acceptance criteria in either of the samples in a set, ratios using that element were omitted. Following recommendations from ASTM E2926-25e1, the modified ±3s interval was used to statistically assess the association or discrimination of samples. In the modified ±3s interval, the standard deviation of the known measurements (measured standard deviation) and 3% of the average of the known measurements (minimum standard deviation) are calculated for each ratio. Whichever value is larger, the measured or minimum standard deviation is used as, *s,* in ±3s to establish the range of the ratios in the known sample. Ratios from questioned samples are compared to the ranges of the known sample. If all ratios are within the range, the samples are considered associated. If any ratios are outside of the range, the samples are considered discriminated (Supplemental Tables S1 – S14). Statistical assessment of elemental ratios correctly associated and discriminated the samples within each of the sets (Table 22).

Table 22: Summary of results for each sample set

Set	Sample	Sources	Spectral Comparison	Elemental Ratios	Correct µXRF Determination?
1	A B	Different	Discriminates	Discriminates, 6 of 7 ratios out of range	Yes
2	A B	Different	Discriminates	Discriminates, 4 of 9 ratios out of range	Yes
3	A B	Different	Discriminates	Discriminates, 7 of 7 ratios out of range	Yes
	A1 B1	Different	Discriminates	Discriminates, 7 of 7 ratios out of range	Yes
	A2 B2	Different	Discriminates	Discriminates, 3 of 7 ratios out of range	Yes
4	A B	Different	Discriminates	Discriminates, 6 of 10 ratios out of range	Yes
	A1 B1	Different	Discriminates	Discriminates, 5 of 9 ratios out of range	Yes
	A2 B2	Different	Discriminates	Discriminates, 6 of 9 ratios out of range	Yes
5	A B	Different	Discriminates	Discriminates, 7 of 8 ratios out of range	Yes
6	A B	Same	Indeterminate	Associates	Yes
7	A B	Same	Indeterminate	Associates	Yes
	A1 B1	Same	Indeterminate	Associates	Yes
	A2 B2	Same	Indeterminate	Associates	Yes
8	A B	Same	Indeterminate	Associates	Yes

While comparing particles of similar sizes was demonstrated to produce accurate conclusions, the comparison of particles of differing sizes from the same source was also evaluated. From sets 3, 4, and 7, samples A, A1, and A2 were compared, and sample B, B1, and B2 were compared. The full thickness samples were considered the knowns while the particles were considered questioned. Based on spectral differences (Supplemental Figures S15 – S20) the small glass particles would likely be considered source discriminated despite originating from the same source. If elemental ratios are calculated and statistically compared, both the large particles and the small particles are incorrectly discriminated from their full thickness glass counterpart (Supplemental Tables S15 – S20). The inability to accurately associate samples when their size varies, highlights the need for similar size and thickness of known and questioned samples when analyzing by µXRF (Table 23).

Table 23: Summary of results for same source samples of varying sizes

Set	Sample	Spectral Comparison	Correct Determination?	Elemental Ratios	Correct Determination?
3	A1	Indeterminate	N/A	Discriminates, 3 of 7 ratios out of range	No
	A				
	A2	Discriminates	No	Discriminates, 5 of 7 ratios out of range	No
	A				
	B1	Indeterminate	N/A	Discriminates, 1 of 7 ratios out of range	No
	B				
	B2	Discriminates	No	Discriminates, 5 of 7 ratios out of range	No
	B				
4	A1	Indeterminate	N/A	Discriminates, 3 of 9 ratios out of range	No
	A				
	A2	Discriminates	No	Discriminates, 7 of 9 ratios out of range	No
	A				
	B1	Indeterminate	N/A	Discriminates, 5 of 9 ratios out of range	No
	B				
	B2	Discriminates	No	Discriminates, 4 of 9 ratios out of range	No
	B				
7	A1	Indeterminate	N/A	Discriminates, 4 of 9 ratios out of range	No
	A				
	A2	Discriminates	No	Discriminates, 5 of 9 ratios out of range	No
	A				
	B1	Indeterminate	N/A	Discriminates, 4 of 9 ratios out of range	No
	B				
	B2	Discriminates	No	Discriminates, 6 of 9 ratios out of range	No
	B				

The final component of the assessment was to investigate the accuracy of the screening method. Although the screening method would be utilized prior to the semi-quantitative method, as it is an optional addition to the analytical scheme, it was investigated after the semi-quantitative method. Fragments 1A and 1B and Fragments 3A and 3B were analyzed using parameter set 11. Spectral comparison of Fragments 1A and 1B indicate spectral differences which would result in the discrimination of the samples (Figure 4). Since the samples are known to be of different sources, this would be considered an accurate determination made using the screening method. The same was observed for Fragments 3A and 3B (Figure 5). This demonstrates that the screening method developed produces a suitable quality spectrum that can accurately discriminate based on large visual differences.

Figure 4. Spectra of glass fragments 1A and 1B with screening method

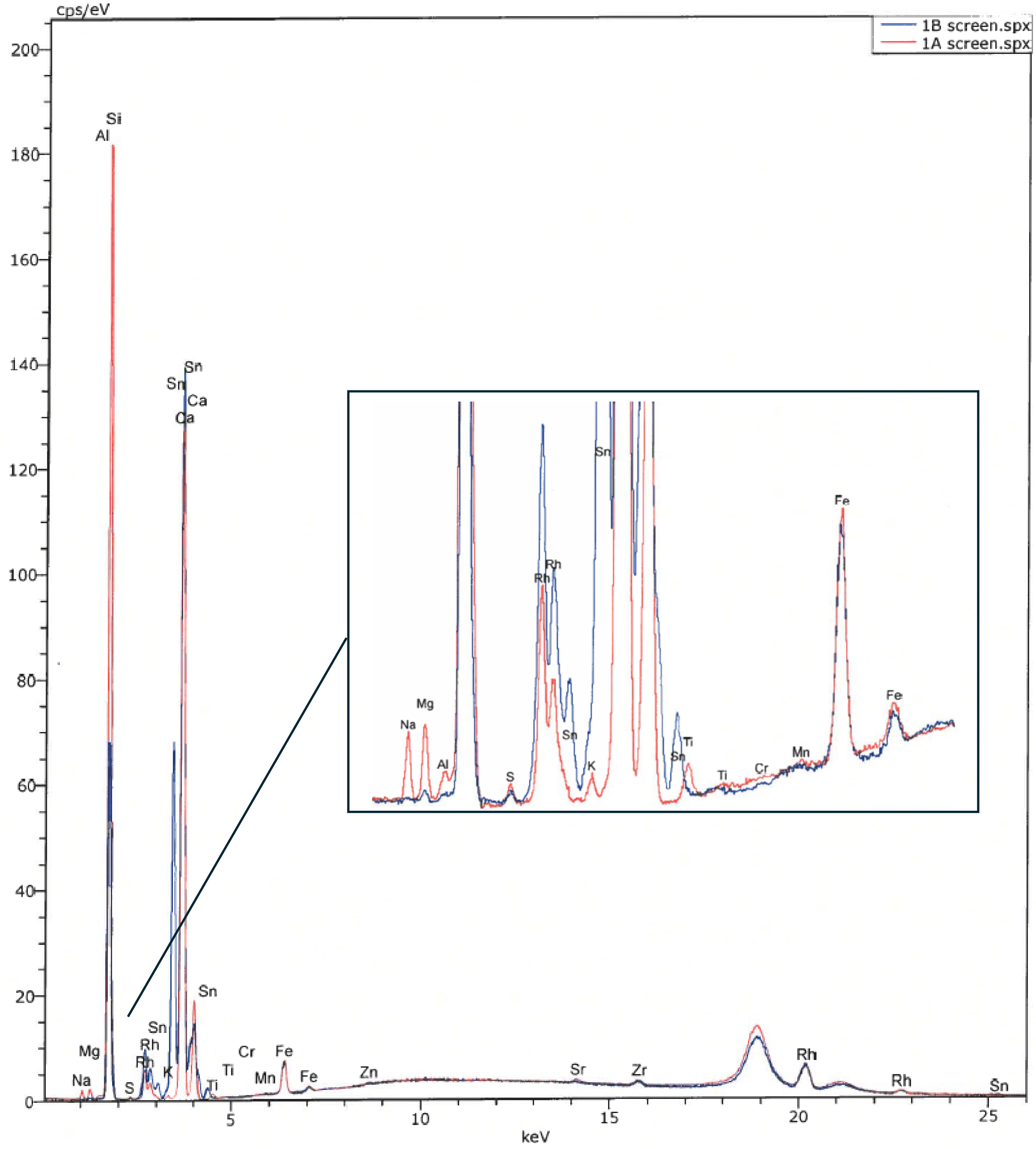
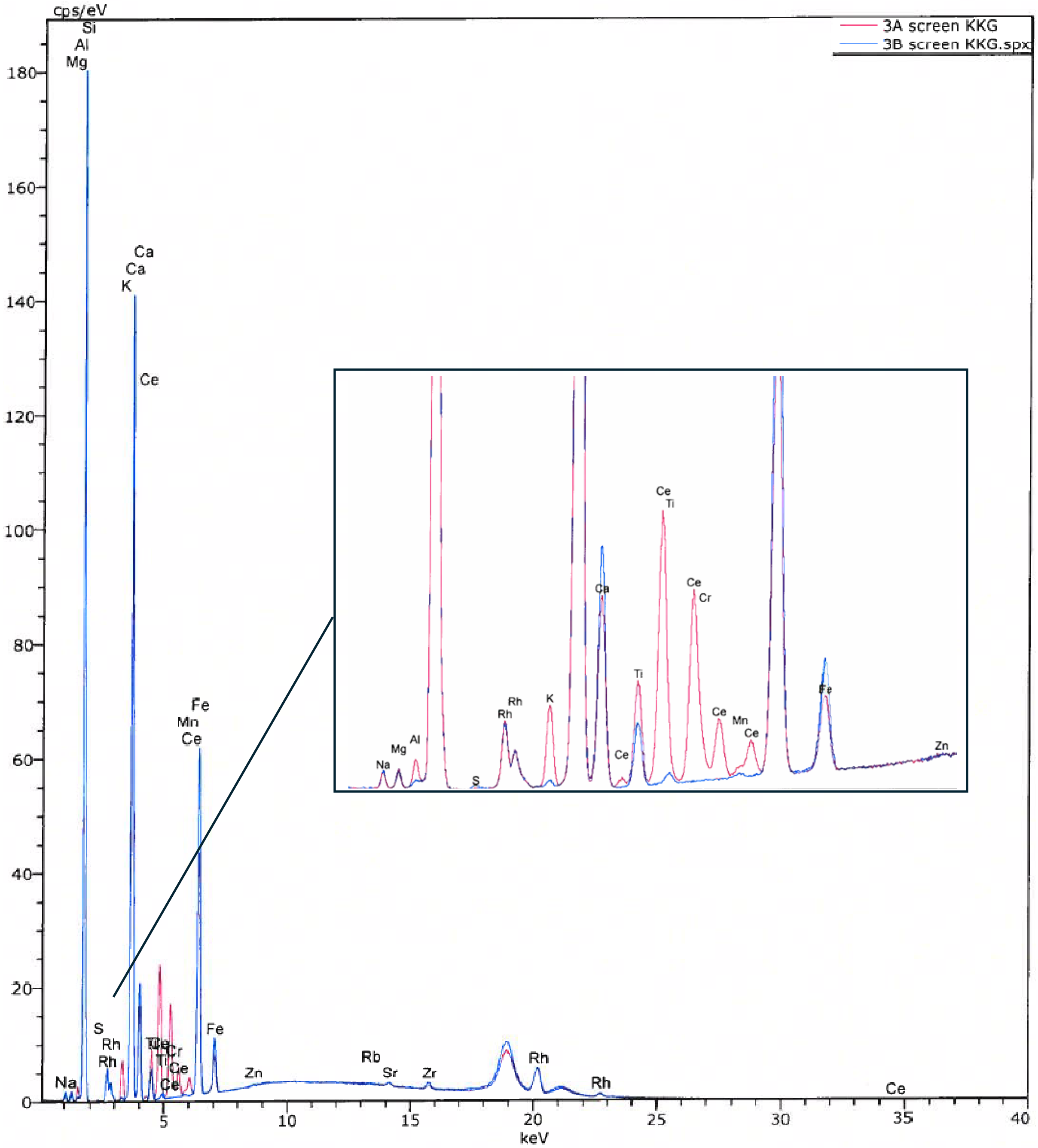


Figure 5. Spectra of glass fragments 3A and 3B with screening method



G. Summary

In summary, this verification demonstrated that the Bruker M4 Tornado Micro XRF is fit for use for the comparison of glass samples. Optimal method parameters were established and verified. In addition to the semi-quantitative method, a screening method was also established as noted in ASTM E2926-25e1. It was confirmed that either one or both detectors can provide accurate data. It is important to note that the quality assurance checks must pass, and the known and questioned samples are analyzed under the same instrumental conditions.

The recommended sample preparation for varying sample types was determined to minimize spectral interferences. The intra-day repeatability, inter-day repeatability, and precision were confirmed to be within recommended %RSD of ≤10%. Precision data was also found to be comparable to other instruments as listed in ASTM E2926-25e1 *Table X3.2*. The instrument was able to generate data that accurately allowed source discrimination between samples of known origins, both using the semi-quantitative method and the screening method. It was confirmed that known and questioned samples must be of similar size to accurately discriminate between sources. Based on these criteria, the Bruker M4 Tornado µXRF is a viable option within an analytical scheme for the forensic analysis of glass.

H. References

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Figure S1. Spectra of glass fragments 1A and 1B

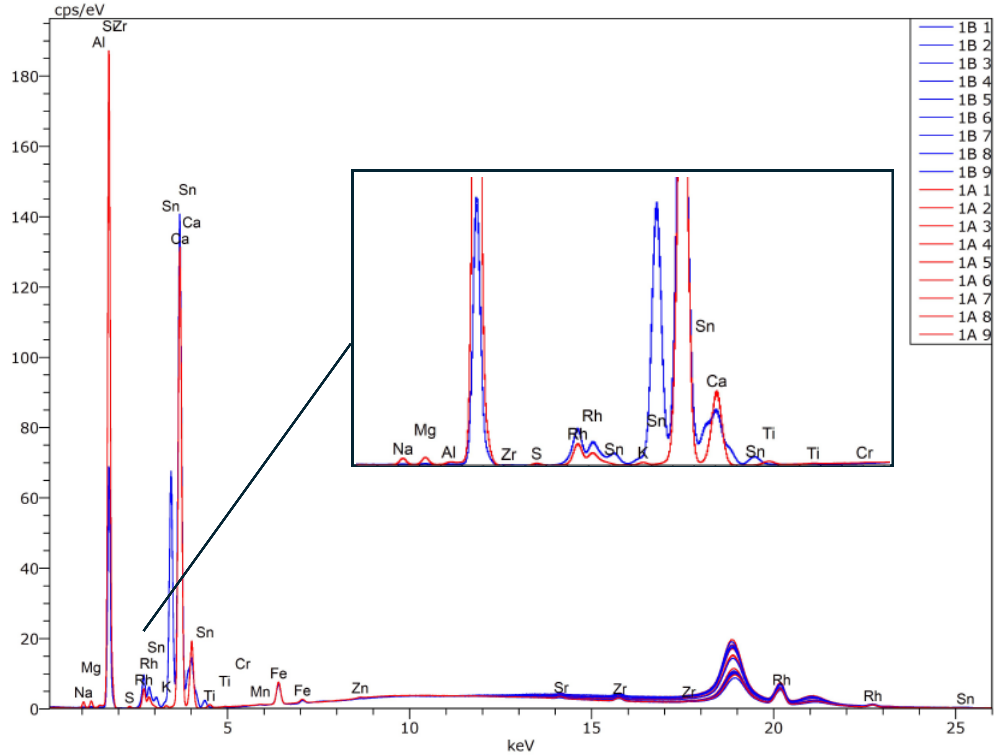


Figure S2. Spectra of glass fragments 2A and 2B

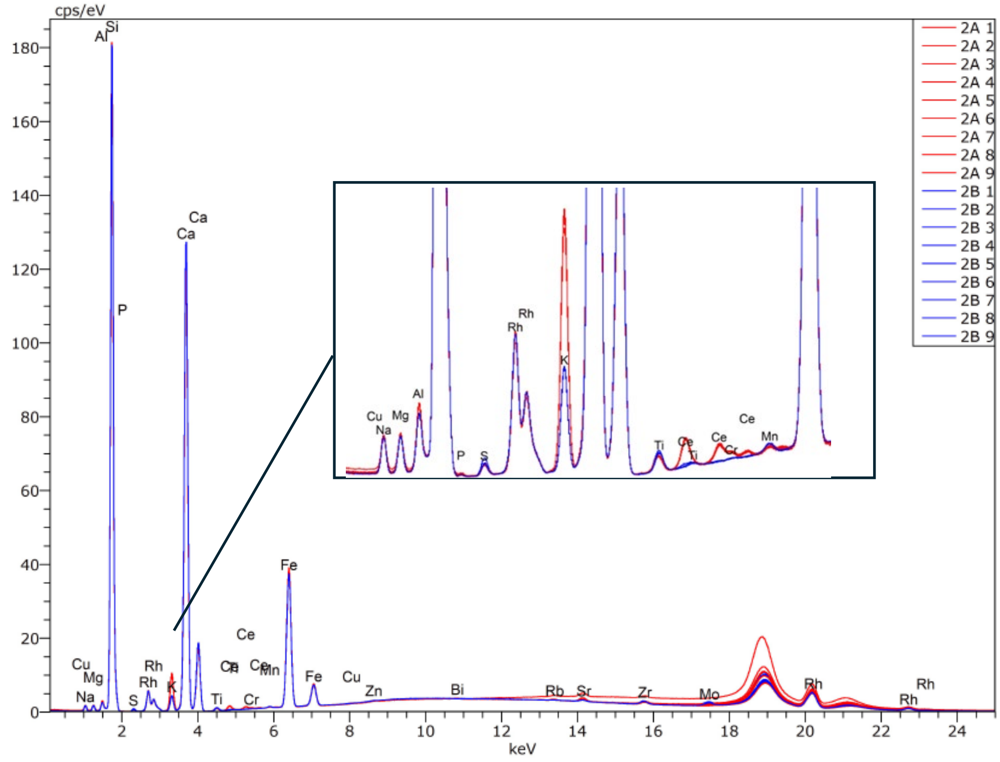


Figure S3. Spectra of glass fragments 3A and 3B

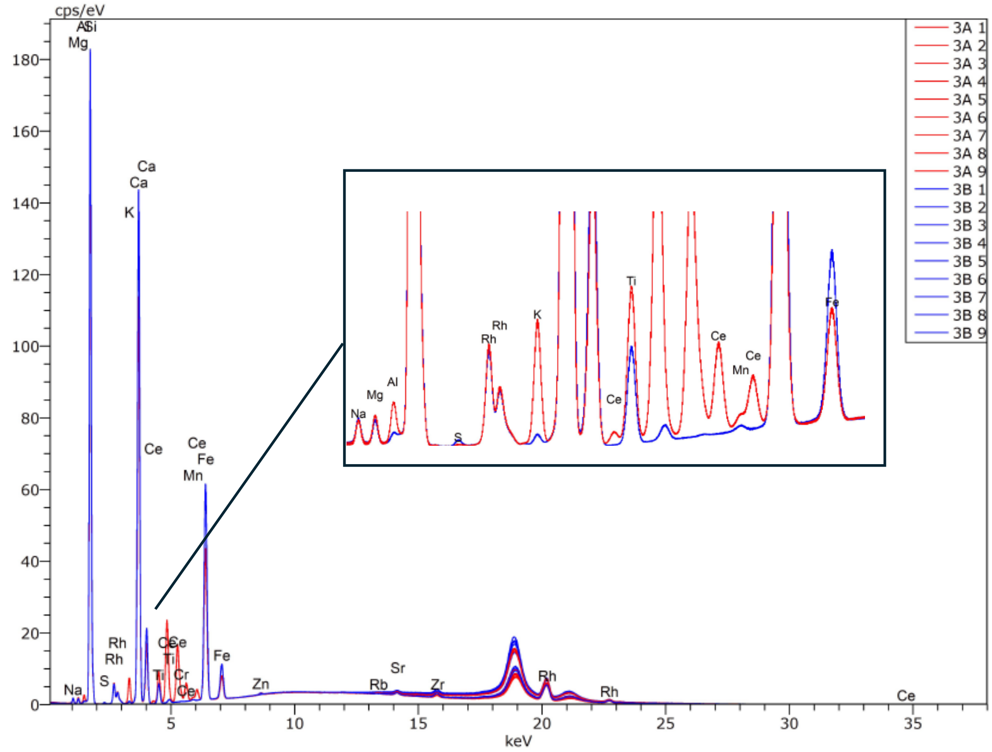


Figure S4. Spectra of glass fragments 3A1 and 3B1

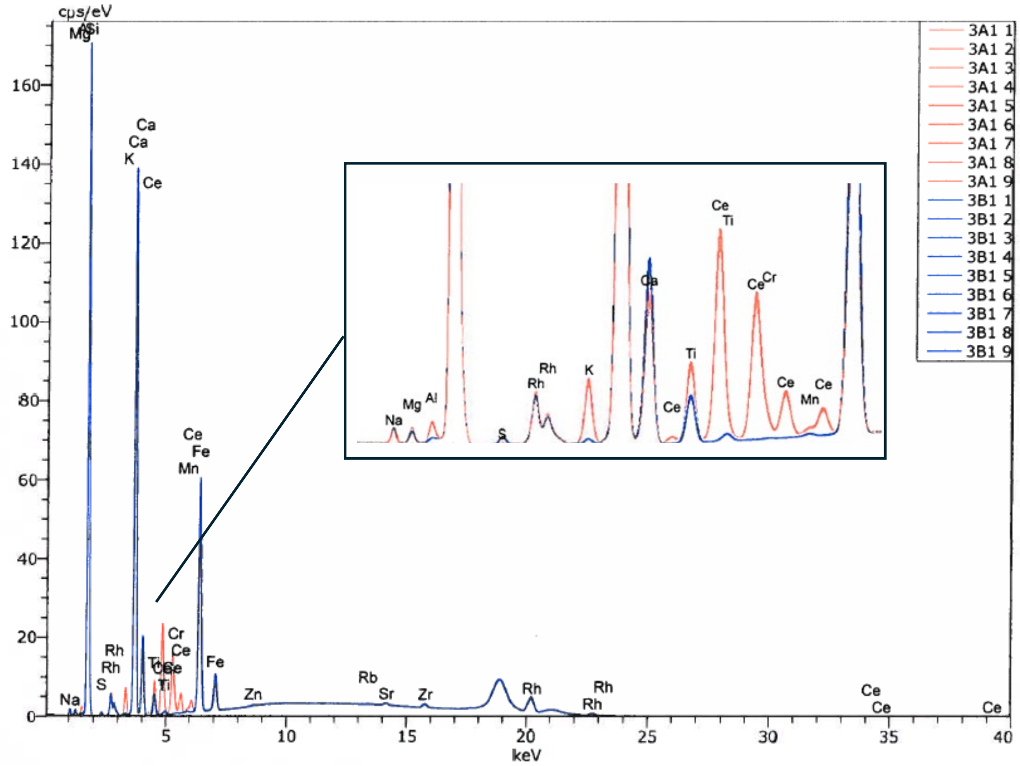


Figure S5. Spectra of glass fragments 3A2 and 3B2

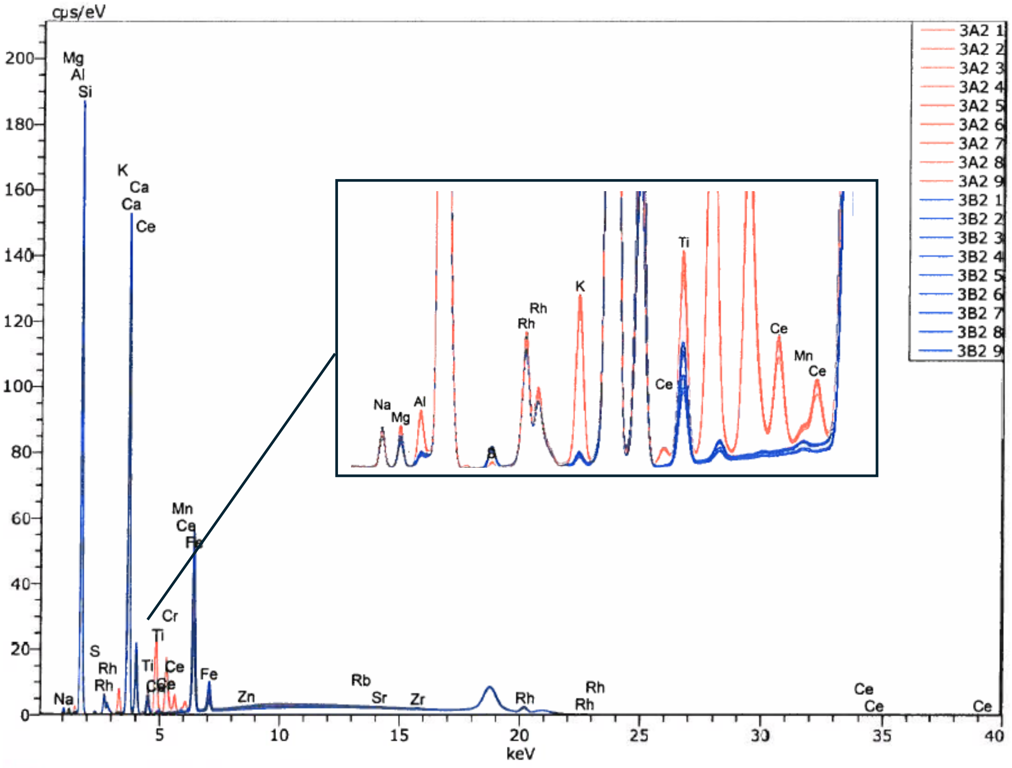


Figure S6. Spectra of glass fragments 4A and 4B

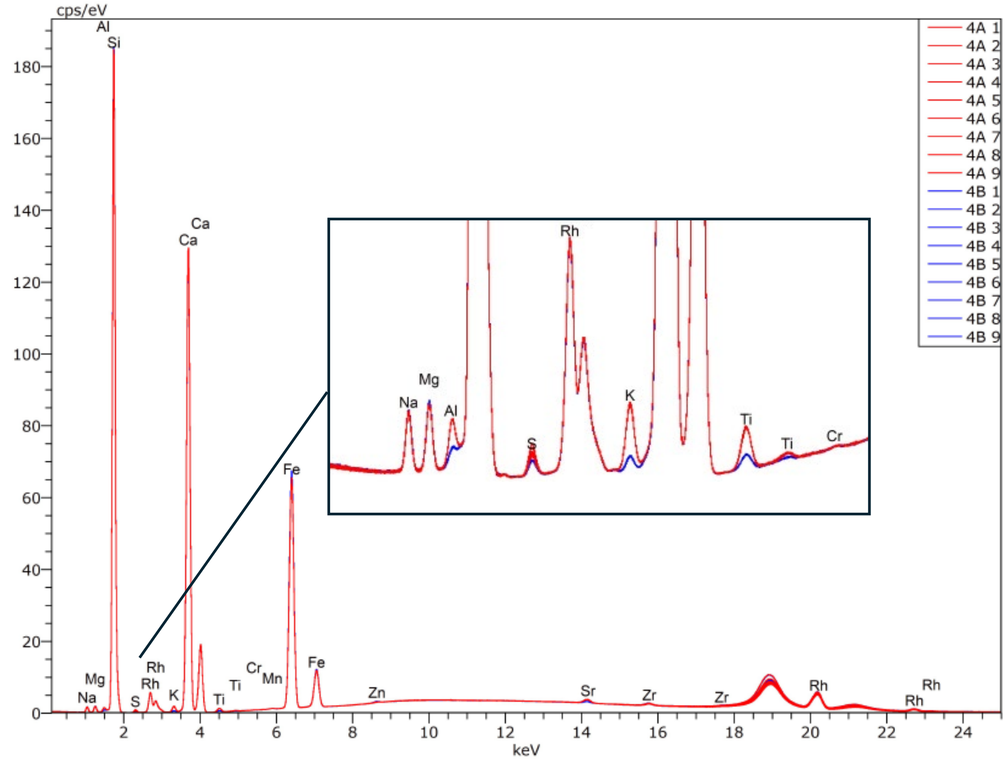


Figure S7. Spectra of glass fragments 4A1 and 4B1

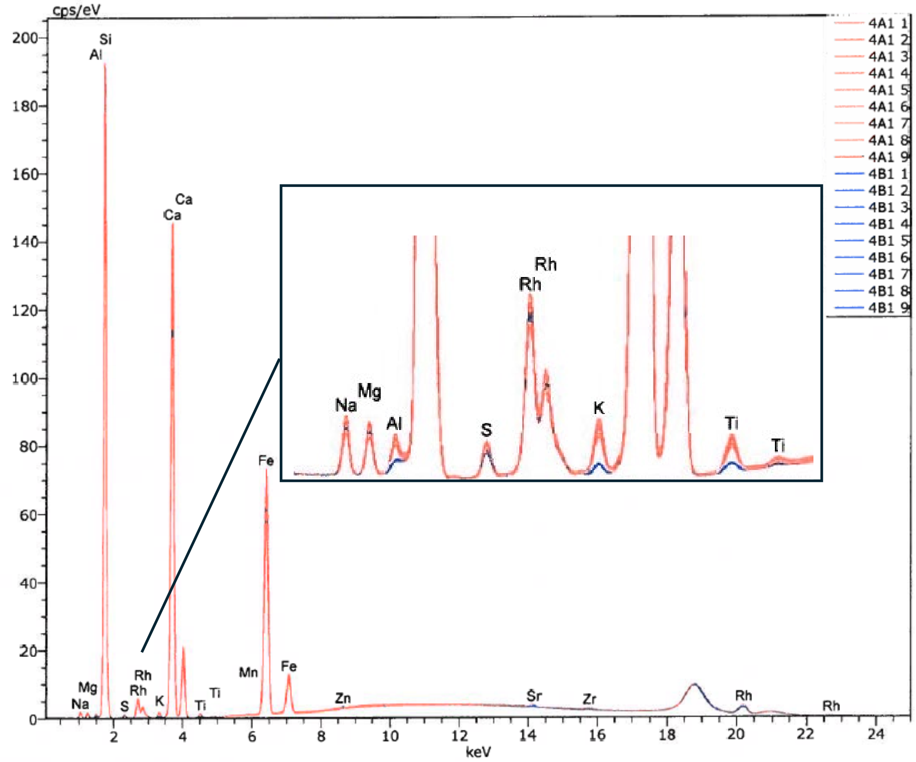


Figure S8. Spectra of glass fragments 4A2 and 4B2

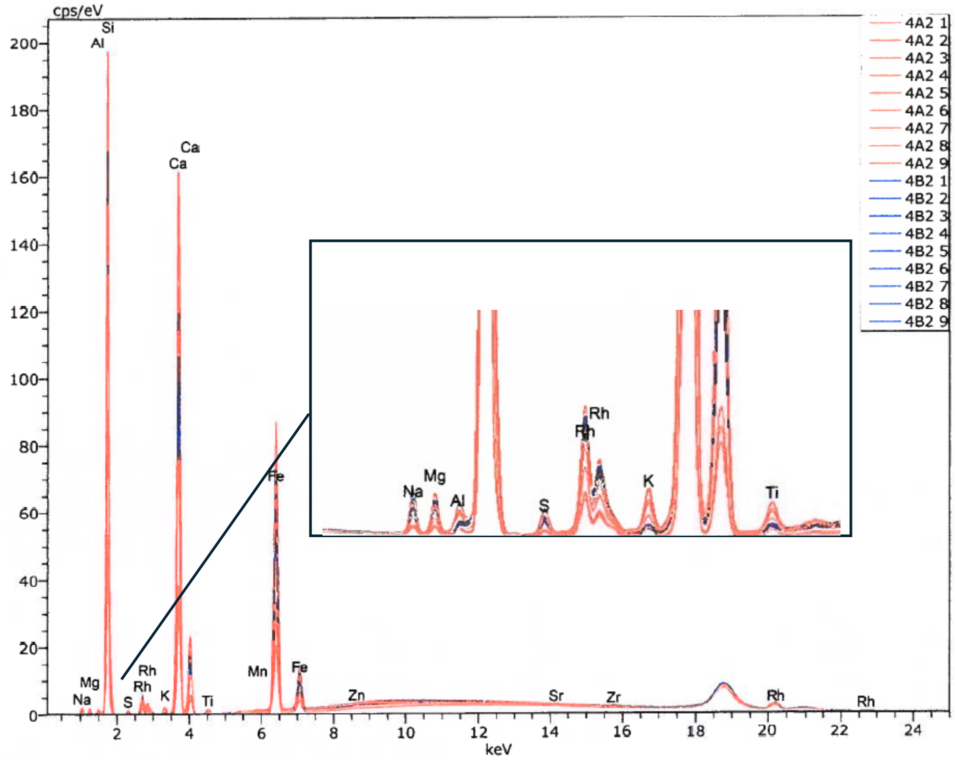


Figure S9. Spectra of glass fragments 5A and 5B

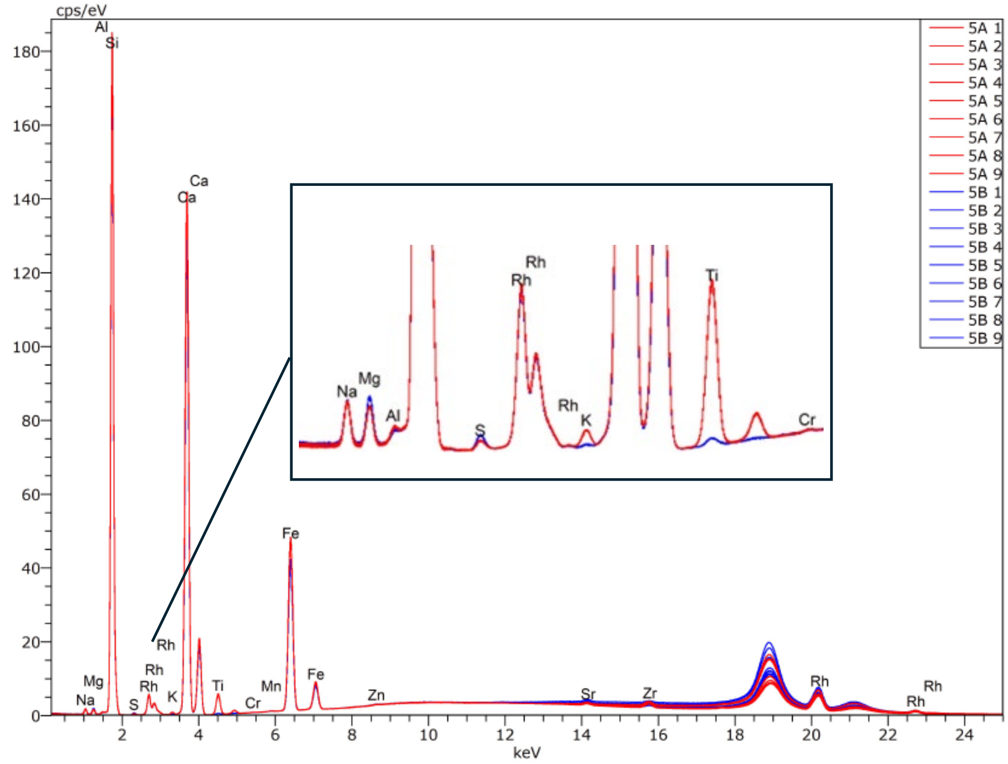


Figure S10. Spectra of glass fragments 6A and 6B

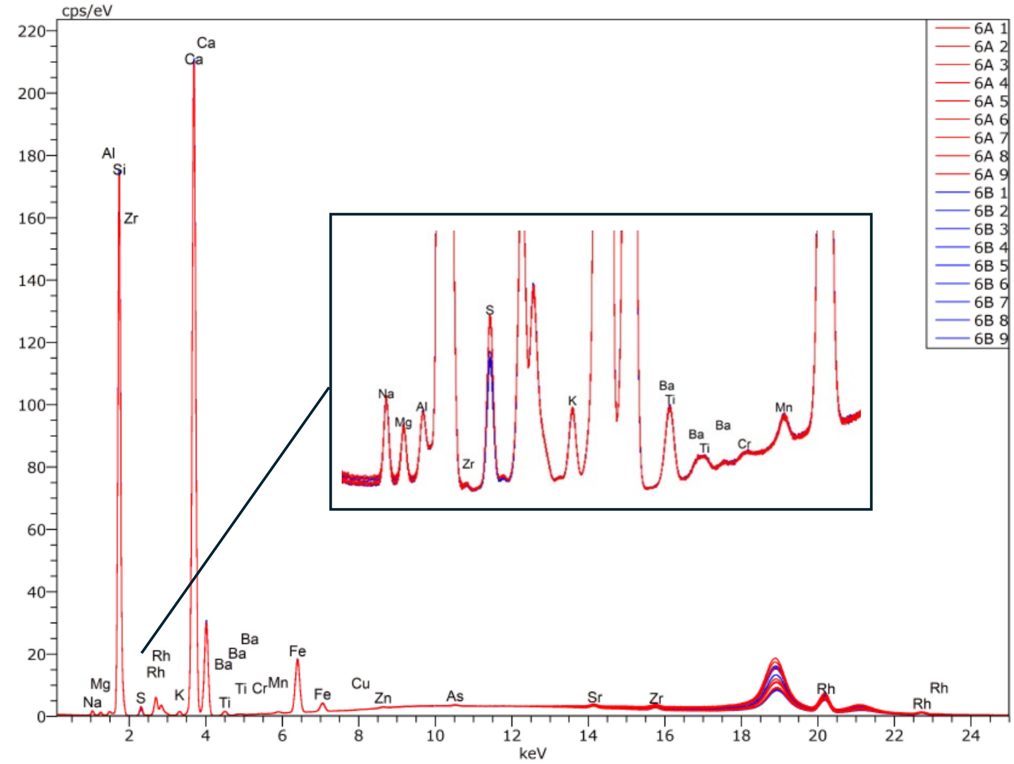


Figure S11. Spectra of glass fragments 7A and 7B

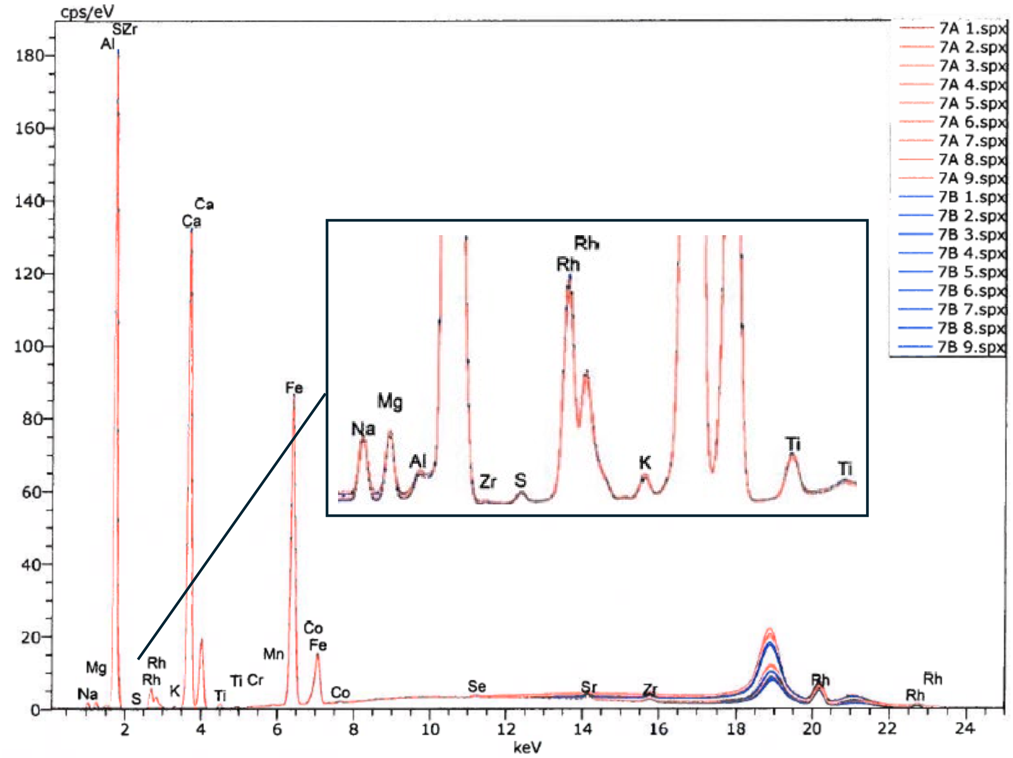


Figure S12. Spectra of glass fragments 7A1 and 7B1

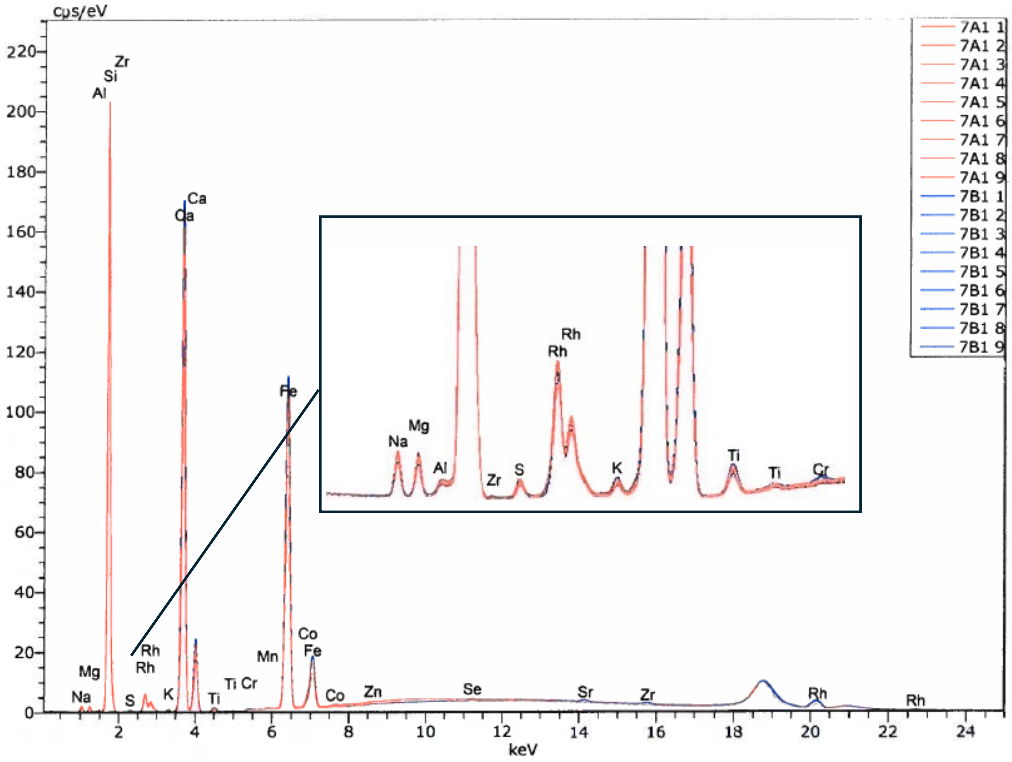


Figure S13. Spectra of glass fragments 7A2 and 7B2

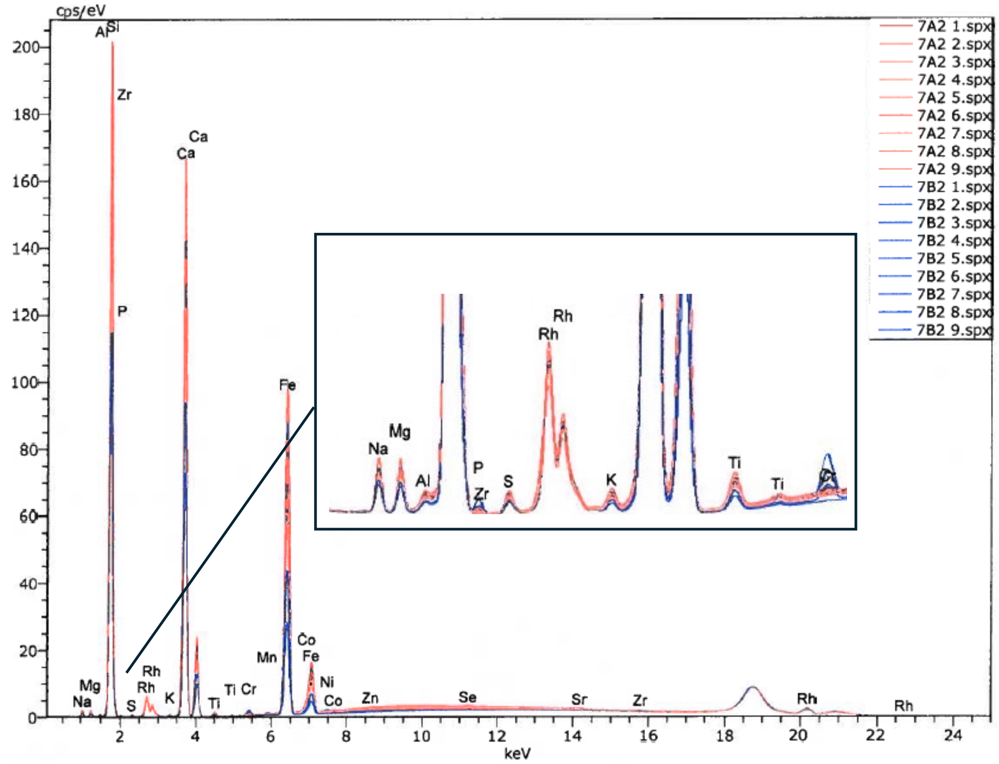
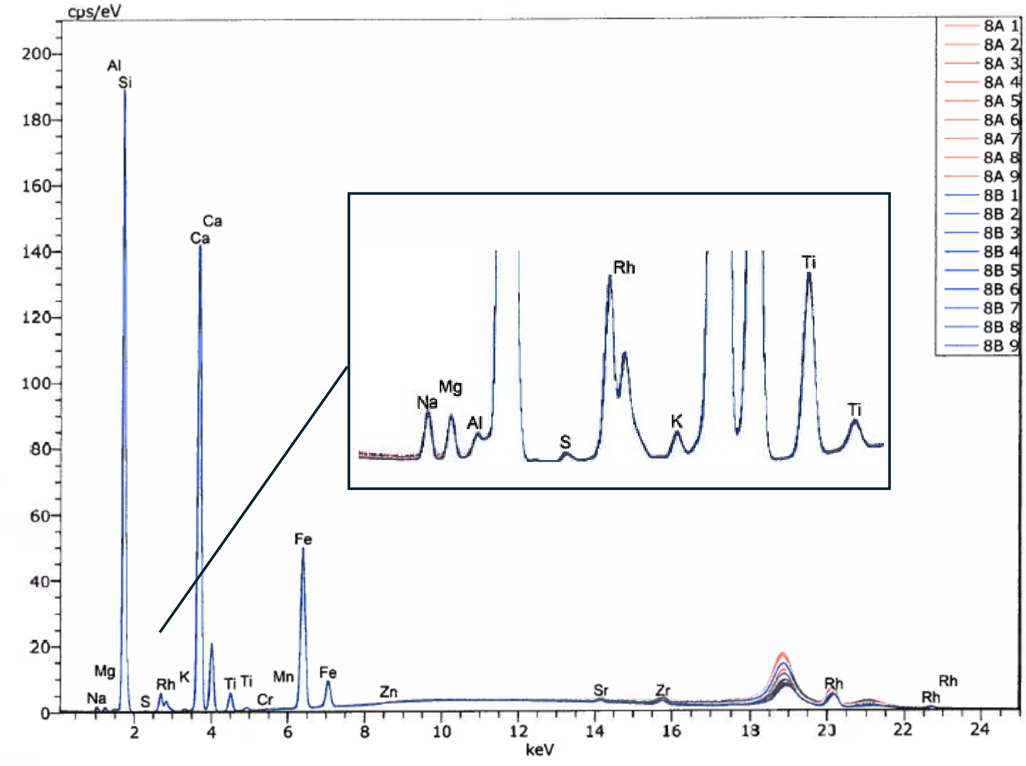


Figure S14. Spectra of glass fragments 8A and 8B



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Supplemental Table S1: Comparison of samples A (questioned) and B (known) in set 1

	Ca/Mg	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**Na/Mg**	**Mn/Fe**	**Sr/Fe**
K_{avg}	642	13.0	0.39	0.21	0.38	0.033	0.08
Measured SD * 3	**63**	0.1	**0.12**	**0.03**	**0.13**	0.0025	**0.02**
Minimum SD * 3	58	**1.2**	0.04	0.02	0.03	**0.0029**	0.01
K_{avg} -3s	579	11.9	0.27	0.18	0.25	0.030	0.07
K_{avg} +3s	705	14.2	0.51	0.24	0.51	0.035	0.10
Q_{avg}	122	16.4	0.72	0.21	0.84	0.028	0.15
Result	Discriminated						

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S2: Comparison of samples A (questioned) and B (known) in set 2

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Ti/Fe**	**Sr/Fe**
K_{avg}	156	159	2.74	0.56	0.032	0.0281	0.969	0.017	0.018
Measured SD * 3	4	**25**	0.01	0.03	0.002	0.0004	0.036	**0.003**	0.001
Minimum SD * 3	**14**	14	**0.25**	**0.05**	**0.003**	**0.0025**	**0.087**	0.002	**0.002**
K_{avg} -3s	142	133	2.49	0.51	0.029	0.0255	0.882	0.014	0.016
K_{avg} +3s	170	184	2.99	0.61	0.035	0.0306	1.056	0.020	0.020
Q_{avg}	144	179	2.56	2.13	0.018	0.0740	0.902	0.014	0.039
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S3: Comparison of samples A (questioned) and B (known) in set 3

	Ca/Mg	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Sr/Fe**
K_{avg}	190	1.83	0.54	0.028	0.0022	1.05	0.015
Measured SD * 3	7	0.04	0.03	**0.004**	**0.0003**	0.02	**0.002**
Minimum SD * 3	**17**	**0.16**	**0.05**	0.002	0.0002	**0.09**	0.001
K_{avg} -3s	173	1.67	0.49	0.024	0.0019	0.95	0.013
K_{avg} +3s	207	1.99	0.59	0.032	0.0024	1.14	0.017
Q_{avg}	123	2.12	1.14	0.021	0.0551	0.81	0.024
Result	Discriminated						

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S4: Comparison of samples A1 (questioned) and B1 (known) in set 3

	Ca/Mg	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Sr/Fe**
K_{avg}	196	1.80	0.580	0.024	0.0021	1.24	0.0140
Measured SD * 3	9	0.03	0.049	**0.003**	0.0001	0.05	0.0010
Minimum SD * 3	**18**	**0.16**	**0.052**	0.002	**0.0002**	**0.11**	**0.0013**
K_{avg} -3s	179	1.64	0.527	0.021	0.0019	1.13	0.0127
K_{avg} +3s	214	1.96	0.632	0.027	0.0023	1.35	0.0152
Q_{avg}	124	2.09	1.257	0.019	0.0537	0.92	0.0236
Result	Discriminated						

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S5: Comparison of samples A2 (questioned) and B2 (known) in set 3

	Ca/Mg	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Sr/Fe**
K_{avg}	185	2.6	1.4	0.0033	0.00208	1.25	0.0035
Measured SD * 3	**58**	**2.0**	**2.2**	0.0047	**0.00020**	0.03	**0.0020**
Minimum SD * 3	17	0.2	0.1	0.0003	0.00019	**0.11**	0.0003
K_{avg}-3s	127	0.6	-0.9	-0.0014	0.00188	1.14	0.0016
K_{avg}+3s	243	4.6	3.6	0.0080	0.00228	1.36	0.0055
Q_{avg}	128	2.5	2.2	0.0027	0.05386	0.93	0.0056
Result	Discriminated						

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S6: Comparison of samples A (questioned) and B (known) in set 4

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Ti/Fe**	**Mn/Fe**	**Sr/Fe**
K_{avg}	138	371	1.508	0.79	0.0136	0.00103	0.85	0.0041	0.0021	0.0107
Measured SD * 3	4	12	0.004	0.04	0.0008	**0.00015**	0.03	0.0001	**0.0005**	0.0006
Minimum SD * 3	**12**	**33**	**0.136**	**0.07**	**0.0012**	0.00009	**0.08**	**0.0004**	0.0002	**0.0010**
K_{avg}-3s	126	338	1.372	0.72	0.0123	0.00088	0.77	0.0037	0.0015	0.0097
K_{avg}+3s	151	404	1.643	0.86	0.0148	0.00118	0.92	0.0044	0.0026	0.0117
Q_{avg}	146	123	1.556	2.70	0.0088	0.00911	0.86	0.0127	0.0023	0.0236
Result	Discriminated									

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S7: Comparison of samples A1 (questioned) and B1 (known) in set 4

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Ti/Fe**	**Sr/Fe**
K_{avg}	143	377	1.49	1.2	0.0052	0.0012	1.02	0.0040	0.006
Measured SD * 3	6	20	0.02	**0.2**	**0.0018**	**0.0002**	0.02	0.0002	**0.002**
Minimum SD * 3	**13**	**34**	**0.13**	0.1	0.0005	0.0001	**0.09**	**0.0004**	0.001
K_{avg}-3s	130	343	1.36	1.0	0.0034	0.0010	0.93	0.0036	0.005
K_{avg}+3s	156	411	1.63	1.4	0.0070	0.0014	1.11	0.0043	0.008
Q_{avg}	150	118	1.53	2.5	0.0052	0.0092	1.09	0.0130	0.013
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S8: Comparison of samples A2 (questioned) and B2 (known) in set 4

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Ti/Fe**	**Sr/Fe**
K_{avg}	145.7	368	1.46	1.2	0.0038	0.0011	1.01	0.0040	0.0044
Measured SD * 3	**13.3**	28	**0.20**	**0.4**	**0.0010**	**0.0003**	0.03	**0.0005**	**0.0016**
Minimum SD * 3	13.1	**33**	0.13	0.1	0.0003	0.0001	**0.09**	0.0004	0.0004
K_{avg}-3s	132.4	335	1.26	0.8	0.0028	0.0008	0.92	0.0034	0.0029
K_{avg}+3s	159.1	402	1.66	1.5	0.0047	0.0014	1.11	0.0045	0.0060
Q_{avg}	170.2	111	1.33	2.9	0.0037	0.0096	1.04	0.0119	0.0104
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S9: Comparison of samples A (questioned) and B (known) in set 5

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	Na/Mg	Ti/Fe	Sr/Fe
K_{avg}	141	525	2.45	1.7	0.013	0.88	0.0047	0.022
Measured SD * 3	4	37	0.02	**0.2**	**0.002**	0.02	0.0003	**0.003**
Minimum SD * 3	**13**	**47**	**0.22**	0.1	0.001	**0.08**	**0.0004**	0.002
K_{avg}-3s	128	477	2.23	1.5	0.011	0.80	0.0042	0.018
K_{avg}+3s	154	572	2.67	1.9	0.015	0.96	0.0051	0.025
Q_{avg}	189	23	2.33	0.5	0.033	1.09	0.0993	0.015
Result	Discriminated							

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S10: Comparison of samples A (questioned) and B (known) in set 6

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Mn/Fe	Sr/Fe
K_{avg}	417	177	9.5	0.91	0.069	0.0042	1.55	0.054	0.024	0.063
Measured SD * 3	9	4	0.1	0.05	**0.009**	0.0001	0.04	0.001	0.001	**0.007**
Minimum SD * 3	**38**	**16**	**0.9**	**0.08**	0.006	**0.0004**	**0.14**	**0.005**	**0.002**	0.006
K_{avg}-3s	379	161	8.6	0.83	0.060	0.0038	1.41	0.049	0.022	0.055
K_{avg}+3s	455	193	10.3	0.99	0.078	0.0046	1.69	0.058	0.026	0.070
Q_{avg}	422	178	9.5	0.91	0.068	0.0042	1.54	0.053	0.024	0.062
Result	Associated									

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S11: Comparison of samples A (questioned) and B (known) in set 7

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Sr/Fe
K_{avg}	143	120	1.201	1.09	0.0142	0.00230	0.89	0.0100	0.0155
Measured SD * 3	3	3	0.003	0.05	0.0010	0.00015	0.03	0.0002	0.0007
Minimum SD * 3	**13**	**11**	**0.108**	**0.10**	**0.0013**	**0.00021**	**0.08**	**0.0009**	**0.0014**
K_{avg}-3s	130	109	1.093	0.99	0.0130	0.00210	0.81	0.0091	0.0141
K_{avg}+3s	156	131	1.309	1.19	0.0155	0.00251	0.97	0.0109	0.0169
Q_{avg}	143	120	1.197	1.11	0.0148	0.00234	0.90	0.0100	0.0165
Result	Associated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S12: Comparison of samples A1 (questioned) and B1 (known) in set 7

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Sr/Fe
K_{avg}	148	122	1.16	1.42	0.007	0.00244	1.01	0.00956	0.009
Measured SD * 3	9	3	0.09	**0.21**	**0.004**	0.00017	0.02	**0.00088**	**0.005**
Minimum SD * 3	**13**	**11**	**0.10**	0.13	0.001	**0.00022**	**0.09**	0.00086	0.001
K_{avg}-3s	135	111	1.06	1.21	0.003	0.00222	0.92	0.00868	0.004
K_{avg}+3s	162	132	1.27	1.62	0.010	0.00266	1.10	0.01044	0.014
Q_{avg}	149	122	1.20	1.60	0.004	0.00254	1.01	0.00983	0.006
Result	Associated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S13: Comparison of samples A2 (questioned) and B2 (known) in set 7

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Sr/Fe
K_{avg}	154	124	1.5	2.1^{A}	0.0016^{A}	0.0023	1.02	0.012	0.0030
Measured SD * 3	**33**	10	**0.7**	**1.8^{A}**	**0.0015^{A}**	**0.0007**	0.04	**0.005**	**0.0011**
Minimum SD * 3	14	**11**	0.1	0.2^{A}	0.0001^{A}	0.0002	**0.09**	0.001	0.0003
K_{avg}-3s	122	113	0.8	0.3^{A}	0.0002^{A}	0.0016	0.93	0.007	0.0019
K_{avg}+3s	187	136	2.3	3.8^{A}	0.0031^{A}	0.0031	1.12	0.017	0.0042
Q_{avg}	154	122	1.4	1.9	0.0020	0.0025	1.01	0.011	0.0037
Result	Associated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

[A] Replicate 1 omitted as an outlier

Supplemental Table S14: Comparison of samples A (questioned) and B (known) in set 8

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Mn/Fe	Sr/Fe
K_{avg}	197	23.7	2.26	0.59	0.039	0.0032	1.09	0.096	0.0022	0.023
Measured SD * 3	4	0.2	0.01	0.03	**0.006**	0.0002	0.03	0.001	**0.0006**	**0.003**
Minimum SD * 3	**18**	**2.1**	**0.20**	**0.05**	0.004	**0.0003**	**0.10**	**0.009**	0.0002	0.002
K_{avg}-3s	179	21.5	2.06	0.54	0.033	0.0029	0.99	0.087	0.0016	0.020
K_{avg}+3s	214	25.8	2.47	0.64	0.046	0.0034	1.19	0.104	0.0028	0.026
Q_{avg}	197	23.7	2.26	0.60	0.039	0.0031	1.09	0.095	0.0023	0.024
Result	Associated									

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Figure S15. Spectra of glass fragments 3A, 3A1 and 3A2

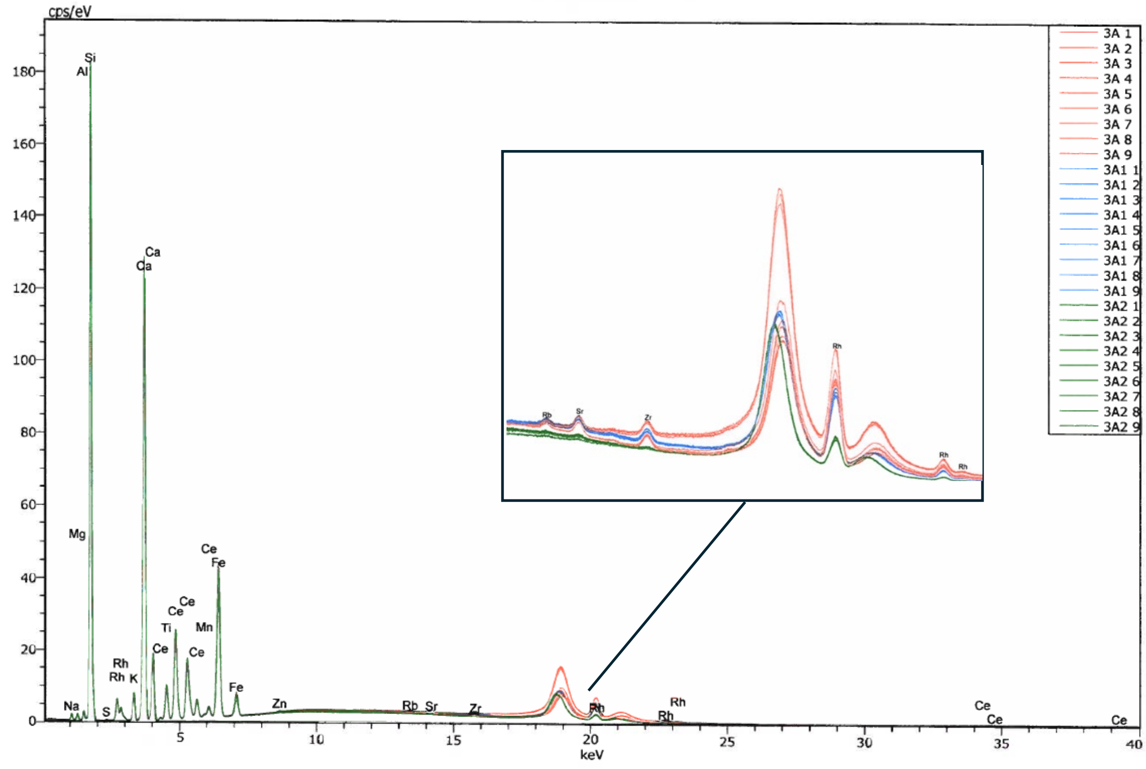


Figure S16. Spectra of glass fragments 3B, 3B1, and 3B2

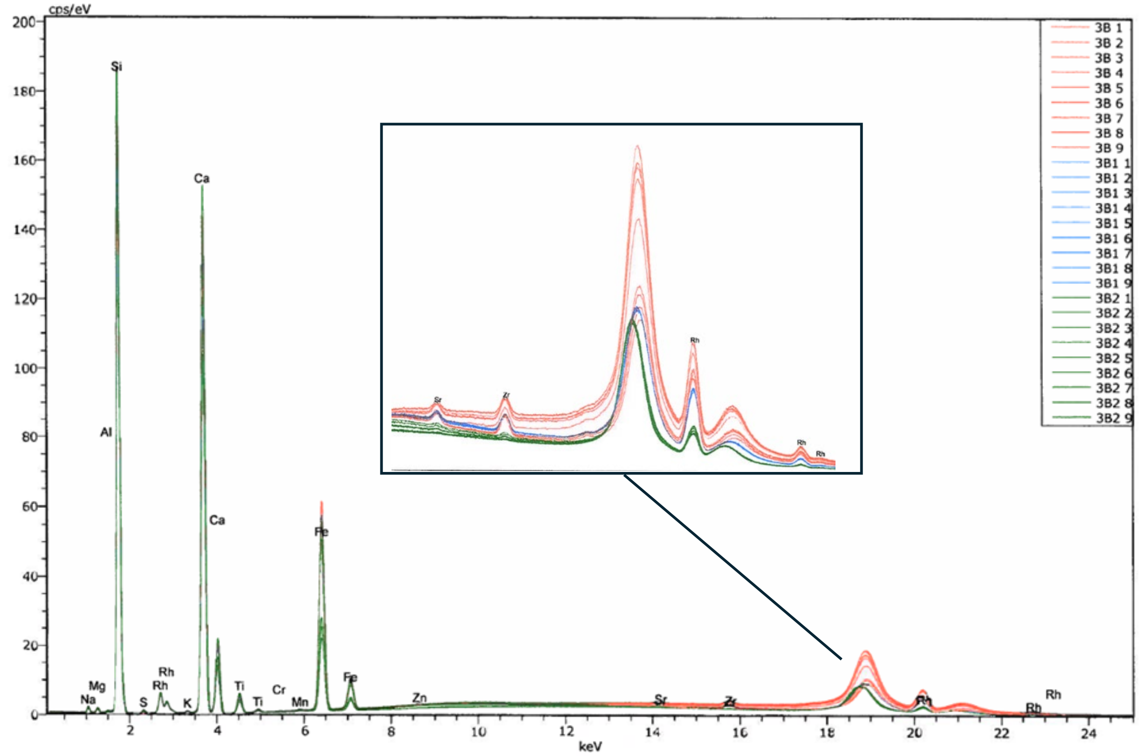


Figure S17. Spectra of glass fragments 4A, 4A1 and 4A2

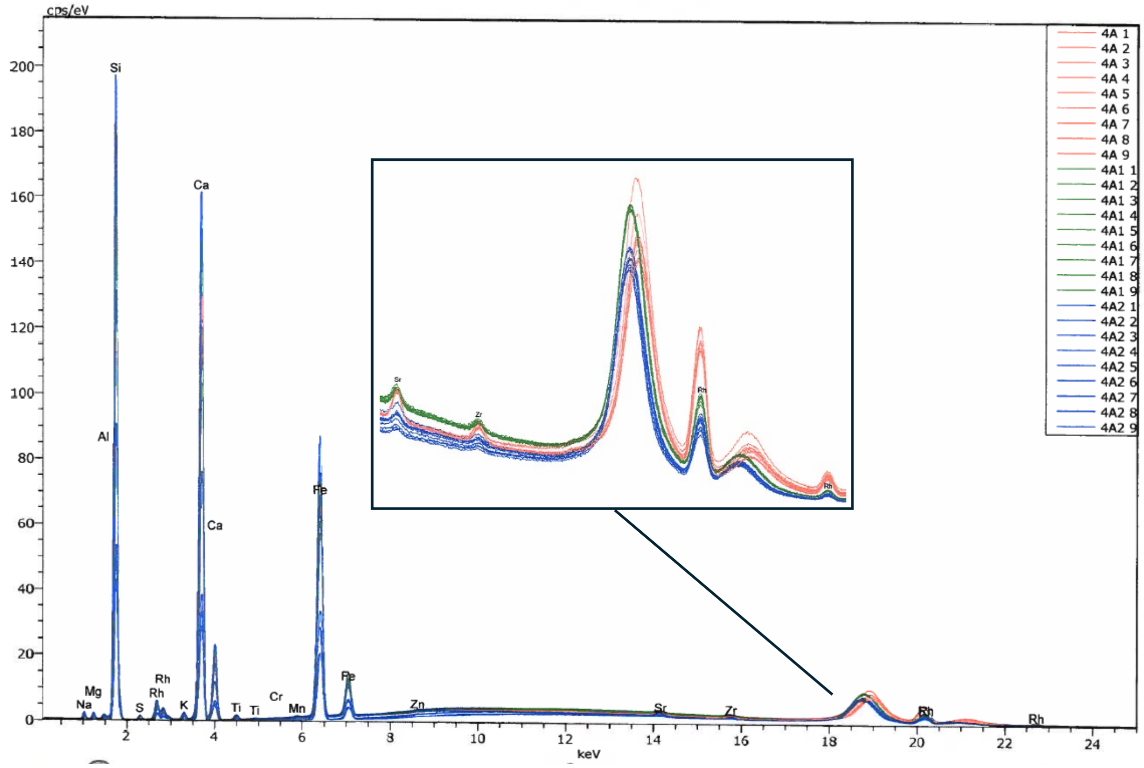


Figure S18. Spectra of glass fragments 4B, 4B1, and 4B2

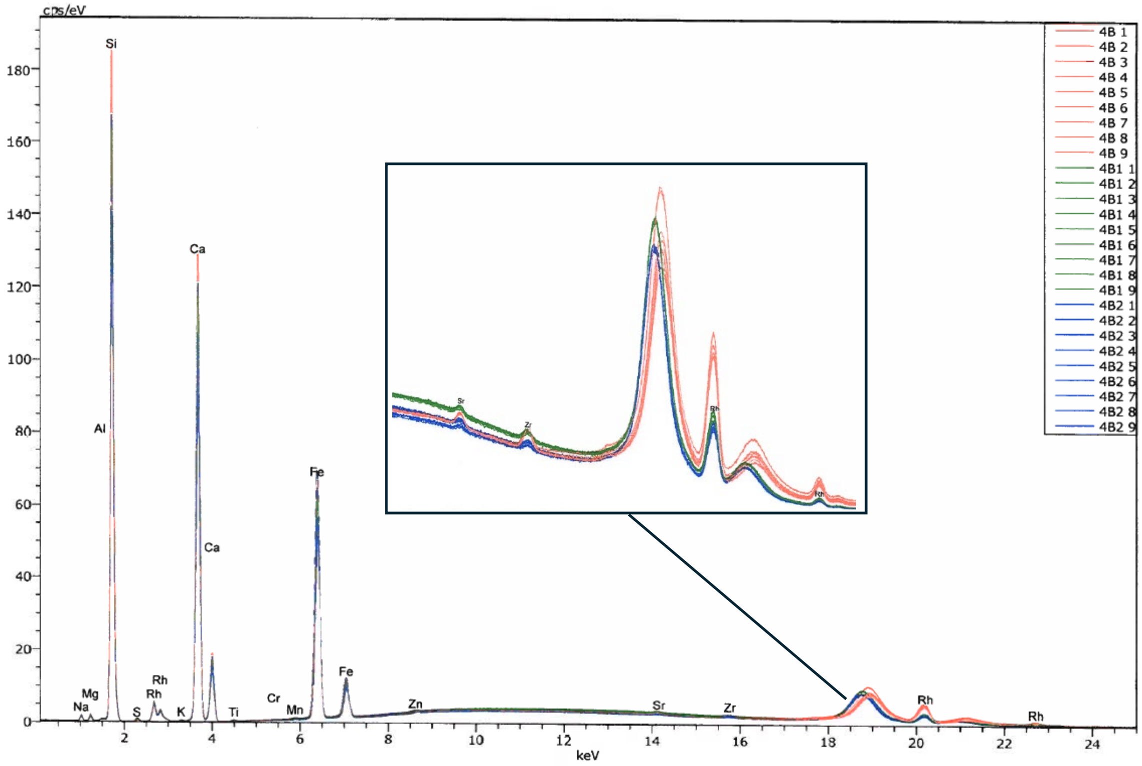


Figure S19. Spectra of glass fragments 7A, 7A1 and 7A2

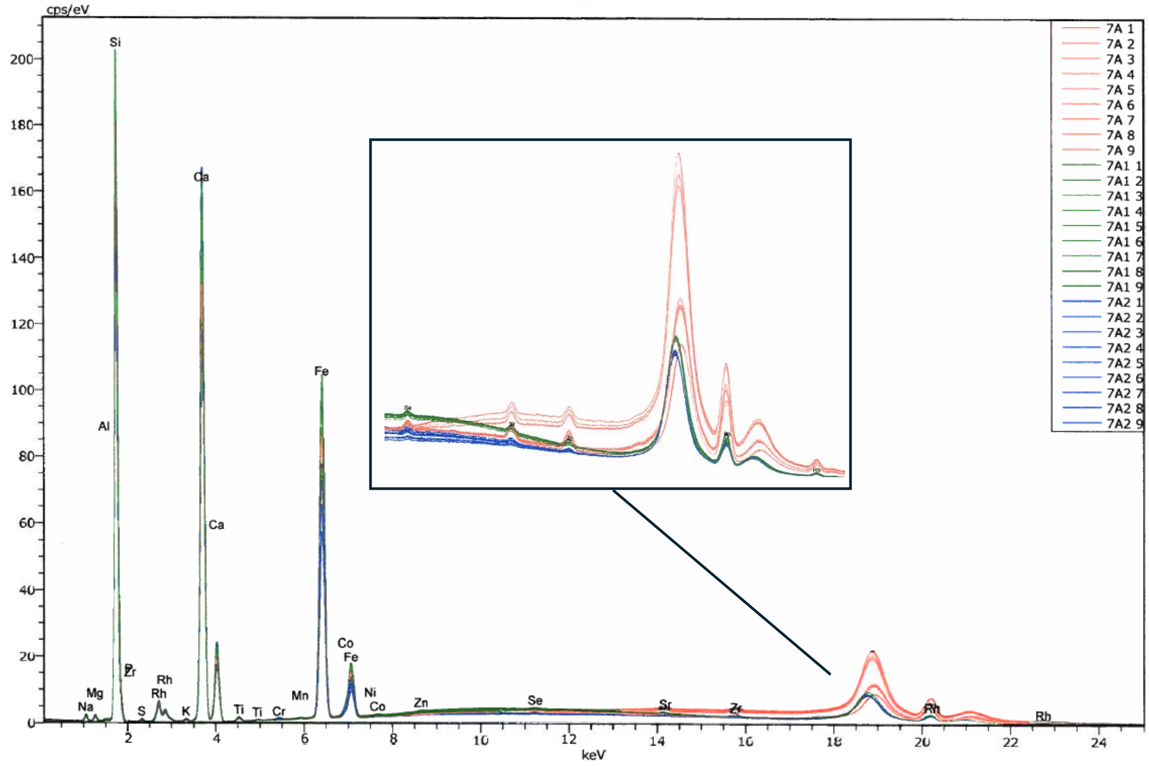
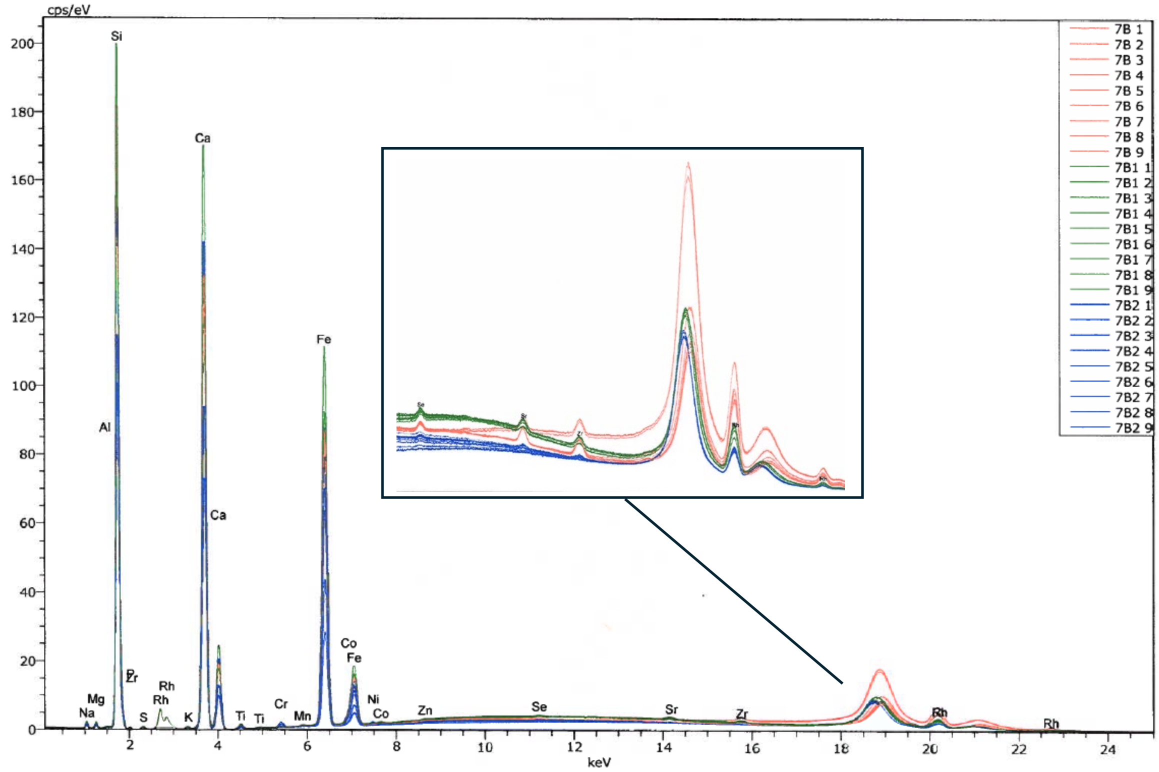


Figure S20. Spectra of glass fragments 7B, 7B1, and 7B2



Supplemental Table S15: Comparison of samples A, A1, and A2 in set 3

	Ca/Mg	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Sr/Fe
A_{avg}	123	2.12	1.14	0.0212	0.0551	0.81	0.024
Measured SD * 3	2	0.01	**0.11**	**0.0020**	0.0005	0.01	0.001
Minimum SD * 3	**11**	**0.19**	0.10	0.0019	**0.0050**	**0.07**	**0.002**
A_{avg} -3s	112	1.93	1.03	0.0191	0.0502	0.74	0.022
A_{avg} +3s	134	2.31	1.25	0.0232	0.0601	0.88	0.026
$A1_{avg}$	124	2.09	1.26	0.0188	0.0537	0.92	0.024
Result	Discriminated						
$A2_{avg}$	128	2.48	2.19	0.0027	0.0539	0.93	0.006
Result	Discriminated						

*Bolded cells indicate the larger standard deviation and the value used as ‘s’

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S16: Comparison of samples B, B1, and B2 in set 3

	Ca/Mg	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Sr/Fe
B_{avg}	190	1.83	0.54	0.0277	0.0022	1.05	0.015
Measured SD * 3	7	0.04	0.03	**0.0041**	**0.0003**	0.02	**0.0002**
Minimum SD * 3	**17**	**0.16**	**0.05**	0.0025	0.0002	**0.09**	0.001
B_{avg} -3s	173	1.67	0.49	0.0236	0.0019	0.95	0.013
B_{avg} +3s	207	1.99	0.59	0.0317	0.0024	1.14	0.017
$B1_{avg}$	196	1.80	0.58	0.0241	0.0021	1.24	0.014
Result	Discriminated						
$B2_{avg}$	185	2.62	1.35	0.0033	0.0021	1.25	0.004
Result	Discriminated						

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S17: Comparison of samples A, A1, and A2 in set 4

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Sr/Fe
A_{avg}	146	123	1.556	2.70	0.009	0.0091	0.86	0.0127	0.0236
Measured SD * 3	2	8	0.003	**0.39**	**0.002**	0.0002	0.03	0.0009	0.0020
Minimum SD * 3	**13**	**11**	**0.140**	0.24	0.001	**0.0008**	**0.08**	**0.0011**	**0.0021**
A_{avg} -3s	133	112	1.416	2.31	0.007	0.0083	0.78	0.0115	0.0215
A_{avg} +3s	159	134	1.697	3.08	0.011	0.0099	0.93	0.0138	0.0257
$A1_{avg}$	150	118	1.533	2.51	0.005	0.0092	1.09	0.0130	0.0129
Result	Discriminated								
$A2_{avg}$	170	111	1.326	2.91	0.004	0.0096	1.04	0.0119	0.0104
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S18: Comparison of samples B, B1, and B2 in set 4

	Ca/Mg	Ca/Ti	Ca/Fe	Sr/Zr	Zr/Fe	K/Ca	Na/Mg	Ti/Fe	Sr/Fe
B_{avg}	138	371	1.508	0.79	0.0136	0.001028	0.85	0.0041	0.0107
Measured SD * 3	4	12	0.004	0.04	0.0008	**0.000150**	0.03	0.0001	0.0006
Minimum SD * 3	**12**	**33**	**0.136**	**0.07**	**0.0012**	0.000093	**0.08**	**0.0004**	**0.0010**
B_{avg} -3s	126	338	1.372	0.72	0.0123	0.000879	0.77	0.0037	0.0097
B_{avg} +3s	151	404	1.643	0.86	0.0148	0.001178	0.92	0.0044	0.0117
$B1_{avg}$	143	377	1.493	1.21	0.0052	0.001183	1.02	0.0040	0.0063
Result	Discriminated								
$B2_{avg}$	146	368	1.462	1.18	0.0038	0.001123	1.01	0.0040	0.0044
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S19: Comparison of samples A, A1, and A2 in set 7

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Ti/Fe**	**Sr/Fe**
A_{avg}	143	120	1.20	1.1	0.015	0.0023	0.90	0.0100	0.016
Measured SD * 3	4	2	0.03	**0.2**	**0.004**	**0.0003**	0.04	0.0003	**0.004**
Minimum SD * 3	**13**	**11**	**0.11**	0.1	0.001	0.0002	**0.08**	**0.0009**	0.001
A_{avg}-3s	130	109	1.09	0.9	0.011	0.0020	0.81	0.0091	0.013
A_{avg}+3s	156	131	1.30	1.3	0.018	0.0027	0.98	0.0109	0.020
$A1_{avg}$	149	122	1.20	1.6	0.004	0.0025	1.01	0.0098	0.006
Result	Discriminated								
$A2_{avg}$	154	122	1.37	1.9	0.002	0.0025	1.01	0.0112	0.004
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

Supplemental Table S20: Comparison of samples B, B1, and B2 in set 7

	Ca/Mg	**Ca/Ti**	**Ca/Fe**	**Sr/Zr**	**Zr/Fe**	**K/Ca**	**Na/Mg**	**Ti/Fe**	**Sr/Fe**
B_{avg}	143	120	1.201	1.09	0.0142	0.00230	0.89	0.0100	0.0155
Measured SD * 3	3	3	0.003	0.05	0.0010	0.00015	0.03	0.0002	0.0007
Minimum SD * 3	**13**	**11**	**0.108**	**0.10**	**0.0013**	**0.00021**	**0.08**	**0.0009**	**0.0014**
B_{avg}-3s	130	109	1.093	0.99	0.0130	0.00210	0.81	0.0091	0.0141
B_{avg}+3s	156	131	1.309	1.19	0.0155	0.00251	0.97	0.0109	0.0169
$B1_{avg}$	148	122	1.162	1.42	0.0066	0.00244	1.01	0.0096	0.0093
Result	Discriminated								
$B2_{avg}$	154	124	1.522	2.08[A]	0.0016[A]	0.00233	1.02	0.0122	0.0030
Result	Discriminated								

*Bolded cells indicate the larger standard deviation and the value used as 's'

** Shaded cells indicate an average questioned sample ratio outside the interval of the known sample ratio

[A] Replicate 1 omitted as an outlier

MEMORANDUM FOR RECORD

From: Michelle Drake

Handwritten Initials:

Date: 4/11/25

Subject: Verification Summary for the Bruker M4 Tornado Micro XRF

DISTRIBUTION: (mark with an X; mark distribution of original with XX)

	Director		FS Lab #:			
	Deputy Director		Laboratory Director:			
	DTS	XX	Program Manager:	Robyn Weimer		
	HR		Supervisor, Section:		Laboratory:	
	Counsel		Other:			

The Bruker M4 Tornado Micro XRF (µ-XRF), located in the Trace Evidence Section of the Central Laboratory, was installed. While this instrument is new for use in glass analysis, the methodology is not new and is documented in ASTM E2926 Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (µ-XRF) Spectrometry.

In addition to the verification of the µ-XRF to ASTM E2926, the Trace Evidence Standard Operating Procedures Section 1.16 Instrumentation and Equipment - General Guidelines were also followed:

1.16.3.1 Obtain documentation from the instrument service representative that demonstrates that the instrument performs to the manufacturer's specification.

See documentation from the manufacturer.

1.16.3.2 Load methods, macros, libraries, or other software as appropriate and test their functionality.

Throughout the verification process, the methods, libraries, macros, and software were tested frequently and found to function as described by the manufacturer.

1.16.3.3 Verify that the instrument is fit for use by analyzing QA samples appropriate for that instrument. Ensure the instrument response is appropriate before placing the instrument in service for casework.

See the verification plan and summary.

1.16.3.4 Ensure that examiners using the replacement instrument have been trained and authorized to use the instrumentation prior to use in casework.

While this is a new instrument and not a replacement instrument, this step is still necessary. Anthony Brown, Michelle Drake, Karissa Gorr, and Chad Schennum received training from Bruker and training from Troy Ernst (Michigan State Police Forensic Science Division, Trace Evidence Unit) on the instrument with regards to µ-XRF for glass analysis in 2023 (November and December respectively). Each examiner ran samples on the instrument.

MEMORANDUM FOR RECORD

From: Michelle Drake

Handwritten Initials:

Date: 4/11/25

Subject: Approval Request for the Bruker M4 Tornado Micro XRF

DISTRIBUTION: (mark with an X; mark distribution of original with XX)

	Director		FS Lab #:			
	Deputy Director		Laboratory Director:			
	DTS	XX	Program Manager:	Robyn Weimer		
	HR		Supervisor, Section:		Laboratory:	
	Counsel		Other:			

The Bruker M4 Tornado Micro XRF (μ-XRF), located in the Trace Evidence Section of the Central Laboratory, was installed. While this instrument is new for use in glass analysis, the methodology is not new and is documented in the ASTM standard E2926 Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (μ-XRF) Spectrometry. The μ-XRF was evaluated using the ASTM standard E2926.

Through the verification process, the Bruker M4 Tornado μ-XRF performed as expected for the elemental analysis of glass samples using the ASTM E2926 method, and the instrument demonstrated that it meets the conditions and requirements outlined in ASTM E2926. It was determined that the Bruker M4 Tornado μ-XRF produced reliable, reproducible, precise, and accurate data for glass ranging from full thickness glass to microscopic particles. It was also determined that, while the use of the double detectors is preferred, a single detector could be used if only one detector was available. The instrument was able to correctly discriminate between glass samples that were indistinguishable from each other by refractive index but were from different sources. The instrument was able to perform as expected by being unable to distinguish glass samples from each other that were from the same glass sources.

Based on the results of the verification study using ASTM E2926, this instrument has demonstrated it is fit for purpose.