APPLICATION NOTE 42516

# Elemental analysis: CHNS characterization of forensic samples by the Flash*Smart* Elemental Analyzer

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## Goal

To assess the performance of the elemental analyzer for forensic samples in terms of the accuracy, precision, and repeatability.

## Introduction

For contributing forensic investigations with essential information, a variety of materials (including illegal drugs, explosives, food, beverages, animal tissues), and human remains are analyzed by elemental analysis.

In human investigations, elemental analysis is used for several matrices (hair, nails, blood, urine, feces among others) providing important information about an individual's diet, drug consumption and health conditions.

Forensic investigations also deal with environmental and ecological research, investigating, for example, sources of pollution: by using elemental analysis, levels of acid rains can be monitored (by determining sulfur in leaves), the particulate matter in water and air can be measured and pollution in soils and sediments and water can be analyzed.

Petrochemical investigations are another important aspect of forensic studies: to protect our soil, air and water from contamination, elemental analyses are carried out to assess the risks that petroleum exploration, storage, and transport carries.

The application of elemental analysis in forensics brings unique capabilities to the laboratory and to the forensics field that increasingly demands a quantitative empirical evidence base that is reproducible and easy to validate.

For these reasons, the use of an accurate instrumental analytical techniques is required. As the demand for improved sample throughput, reduction of operational costs and minimization of human errors is becoming every day more notable, a simple and automated technique which allows fast analysis with an excellent reproducibility is the key.

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility and high sample throughput, while offering an automated approach and ease-of-use for end-users.

**Figure 1. Thermo Scientific Flash*Smart* Elemental Analyzer.**

## Methods

The Flash*Smart* EA operates according to the dynamic flash combustion of the sample.

For CHNS determination, samples are weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler alongside a pulse of oxygen. After combustion, the produced gases are carried in a helium carrier gas to a layer filled with copper. The analyte then enters the GC column, which separates the produced gases before detection by a Thermal Conductivity Detector (TCD) (Figure 2). For weight percent determination a complete report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis.

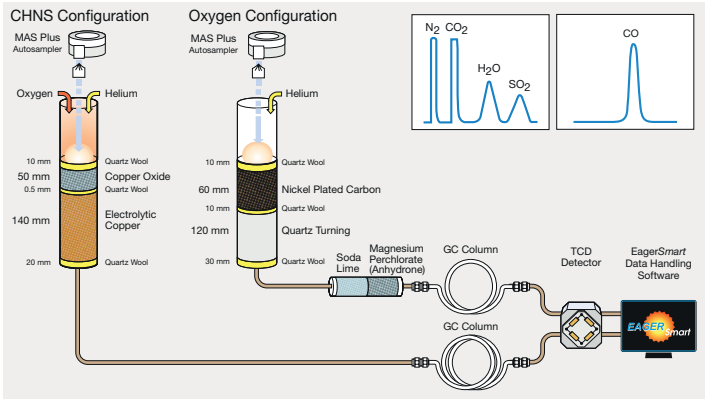


**Figure 2. CHNS/O configuration.**

For S (single determination) or simultaneous NCS configuration, after combustion of the sample the resultant gases are carried by a helium flow to a layer filled with copper, then through a water trap, a GC column and finally, detected by the Thermal Conductivity Detector (TCD) (Figure 3).

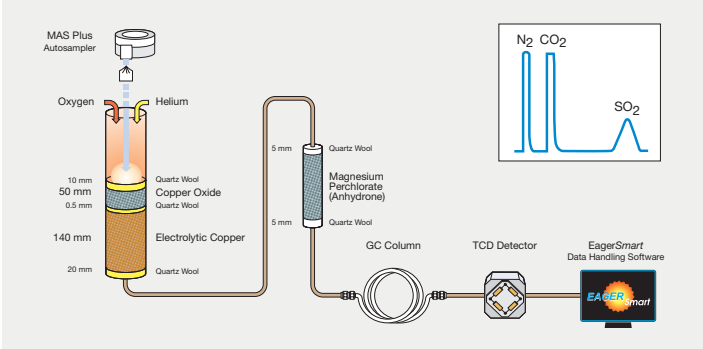


**Figure 3. Single Sulfur or NCS configuration.**

For single N determination, after combustion, the produced gases are carried by a helium flow to a second reactor filled with copper, then swept through $CO_2$ and $H_2O$ traps, a GC column and finally detected by a Thermal Conductivity Detector (TCD) (Figure 4).

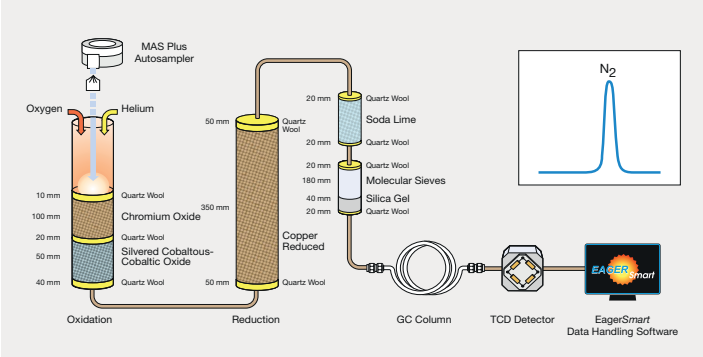


**Figure 4. Nitrogen configuration.**

For oxygen determination, the system operates in pyrolysis mode. Samples are weighed in silver containers and introduced into the pyrolysis chamber (right furnace) via the MAS Plus Autosampler. The reactor contains nickel coated carbon maintained at 1,060 ˚C. The oxygen present in the sample, combined with the carbon, forms carbon monoxide which is then gas chromatographically separated from other products and detected by the TCD Detector (Figure 2).

For weight percent determination a complete report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis.

## Results

The analysis of several samples matrices with different element concentration were performed to demonstrate the performance of the Flash*Smart* EA.

Human and animal samples were analyzed using CHNS/O, NCS, only sulfur and only nitrogen configurations. For CHNS/O abundance determination, the calibration curve was produced by analyzing 2–3 mg BBOT (2,5-Bis (5-ter-butyl-benzoxazol-2-yl) thiophene) while for oxygen determination, 1–1.5 mg of benzoic acid and sulfanilic acid were used for calibration; K factor was used as the calibration method. The samples were analyzed 5 times to evaluate the repeatability, sample weight was 2–3 mg for CHNS and 1–1.5 mg for oxygen analysis. Table 1 shows the CHNS/O data of animal hair and keratine samples.

In NCS configuration two samples of animal feces were analyzed in triplicate. In single sulfur configuration, blood, nails, hairs, serum, and urine were analyzed in duplicate. The calibration curve was produced by analyzing 2–3 mg BBOT, K factor was used as the calibration method and sample weight 3–4 mg. At last, feces and urine samples were analyzed duplicate and quadruplicate times respectively also using nitrogen configuration. For feces, the calibration curve was produced by analyzing 5–10 mg aspartic acid, K factor was used as the calibration method and sample weight 50–150 mg. For urine samples, the calibration was performed with urea water solution, K factor was used as the calibration method and the volume injected 100 ul, standard and samples were injected directly by the liquid autosampler. Table 2 shows the NCS data of samples of feces, Table 3 shows the sulfur data of blood, nails, hairs, serum and urine and Table 4 shows the nitrogen data of feces and urine samples.

**Table 2. NCS data of animal feces samples.**

| Sample | N% | RSD% | C% | RSD% | S% | RSD% |
|---|---|---|---|---|---|---|
| Animal faeces 1 | 3.20<br>3.10<br>3.13 | 1.63 | 40.47<br>41.41<br>40.87 | 1.15 | 0.343<br>0.339<br>0.336 | 1.03 |
| Animal faeces 2 | 2.40<br>2.42<br>2.41 | 0.41 | 40.19<br>40.07<br>40.17 | 0.16 | 0.247<br>0.249<br>0.248 | 0.40 |

**Table 1. CHNS/O data of animal hair and keratine samples.**

| Sample | N% | RSD% | C% | RSD% | H% | RSD% | S% | RSD% | O% | RSD% |
|---|---|---|---|---|---|---|---|---|---|---|
| Animal hair | 14.43<br>14.37<br>14.32<br>14.39<br>14.38 | 0.28 | 42.96<br>43.06<br>42.98<br>42.93<br>43.09 | 0.16 | 6.30<br>6.24<br>6.31<br>6.31<br>6.27 | 0.49 | 4.69<br>4.74<br>4.70<br>4.72<br>4.68 | 0.51 | 27.05<br>26.94<br>26.91<br>26.95<br>27.00 | 0.20 |
| Keratin | 15.26<br>15.28<br>15.26<br>15.23<br>15.23 | 0.14 | 45.90<br>45.93<br>45.83<br>45.78<br>45.81 | 0.14 | 6.57<br>6.58<br>6.47<br>6.47<br>6.49 | 0.84 | 4.73<br>4.73<br>4.72<br>4.74<br>4.73 | 0.15 | 25.12<br>25.19<br>25.05<br>25.16<br>25.15 | 0.21 |

Table 3. Sulfur data of blood, nails, hairs, serum, and urine samples.

| Origin | Sample | S% | RSD% |
|---|---|---|---|
| Healthy subject | Hairs | 4.20–4.22 | 0.33 |
| | Nails | 2.20–2.21 | 0.32 |
| | Blood | 0.147–0.160 | 5.99 |
| | Serum | 0.0775–0.0850 | 6.53 |
| | Urine | 0.0650–0.0780 | 8.68 |
| Children | Nails | 2.90–3.00 | 2.39 |
| | Hairs | 4.34–4.40 | 0.97 |

Table 4. Nitrogen data of feces and urine samples.

| Sample | N% | RSD% | Sample | N% | RSD% |
|---|---|---|---|---|---|
| Feces A | 0.013 | 2.95 | Urine A | 0.78 | 2.01 |
| Feces B | 0.009 | 1.42 | Urine B | 0.53 | 1.24 |
| Feces C | 0.021 | 0.35 | Urine C | 0.43 | 0.77 |
| Feces D | 0.017 | 2.63 | Urine D | 0.69 | 0.61 |
| Feces E | 0.019 | 0.19 | Urine E | 0.74 | 0.56 |
| Feces F | 0.009 | 2.04 | Urine F | 1.34 | 0.66 |

For food, two samples were analyzed animal gelatin used as additive in animal feed production and hazelnuts used in human food. The gelatin samples are from different animals and the hazelnut samples come from different countries. The difference in the element content is proven. For animal gelatin, the calibration was performed with 2–3 mg BBOT and nicotinamide using K factor as calibration method, the sample weight was 2–3 mg. For the hazelnut samples, the calibration was performed with 2–3 mg BBOT for CHNS determination using K factor as calibration method and the sample weight was 2–3 mg, while for oxygen determination 1–2 mg methionine was used to calibrate the system, K factor as calibration method and sample weight 1–2 mg. Table 5 shows the data of animal gelatin and Table 6 shows the data of hazelnuts. Samples were homogenized by a ball mill.

Table 5. CHNS data of animal gelatin samples.

| Sample | N% | RSD% | C% | RSD% | H% | RSD% | S% | RSD% |
|---|---|---|---|---|---|---|---|---|
| Fish gelatine | 16.25<br>16.21<br>16.19 | 0.18 | 43.02<br>43.10<br>43.05 | 0.09 | 6.90<br>6.61<br>6.59 | 2.63 | 0.394<br>0.408<br>0.408 | 2.00 |
| Bovine gelatine | 15.80<br>15.83<br>15.84 | 0.15 | 44.61<br>44.65<br>44.62 | 0.04 | 6.62<br>6.65<br>6.62 | 0.31 | 0.531<br>0.536<br>0.537 | 0.60 |
| Porcine gelatine | 16.09<br>16.02<br>16.04 | 0.23 | 44.46<br>44.40<br>44.38 | 0.10 | 6.63<br>6.66<br>6.58 | 0.58 | 0.670<br>0.675<br>0.683 | 0.97 |

Table 6. CHNS/O data of hazelnut samples.

| Sample | N% | RSD% | C% | RSD% | H% | RSD% | S% | RSD% | O% | RSD% |
|---|---|---|---|---|---|---|---|---|---|---|
| Hazelnuts 1 | 2.02<br>2.08<br>2.01<br>2.03<br>2.09 | 1.77 | 66.39<br>66.95<br>67.16<br>66.89<br>66.43 | 0.50 | 10.09<br>10.19<br>10.26<br>10.21<br>10.06 | 0.84 | 0.0885<br>0.0879<br>0.0845<br>0.0847<br>0.0886 | 2.38 | 19.96<br>20.04<br>20.17<br>19.85<br>19.82 | 0.73 |
| Hazelnuts 2 | 2.34<br>2.34<br>2.39<br>2.37<br>2.39 | 1.05 | 64.22<br>64.50<br>64.58<br>64.35<br>64.57 | 0.24 | 9.95<br>9.86<br>9.93<br>9.82<br>9.84 | 0.57 | 0.1150<br>0.1171<br>0.1178<br>0.1173<br>0.1187 | 1.17 | 21.22<br>21.25<br>21.42<br>21.32<br>21.36 | 0.37 |
| Hazelnuts 3 | 2.44<br>2.42<br>2.45<br>2.42<br>2.42 | 0.64 | 65.91<br>65.83<br>65.55<br>65.92<br>65.55 | 0.62 | 10.11<br>10.20<br>10.14<br>10.00<br>10.19 | 0.79 | 0.1193<br>0.1163<br>0.1171<br>0.1182<br>0.1159 | 1.19 | 20.07<br>20.13<br>20.43<br>19.95<br>19.94 | 0.99 |

For environmental, leaves, soils, sediments, and water were analyzed using the NCS configuration. Through the leaves is possible to monitor the acid rain by the determination of sulfur. The solid samples were homogenized by a ball mill while the water was injected directly by the liquid autosampler. The calibration was performed with 2–3 mg BBOT for the solid samples and 100 ul urea water solution for water samples. The trace sulfur content in water samples was determined by the FPD Detector (Flame Photometric Detector). The weight of the solid samples was 3–4 mg leaves and 10–15 mg for soils and sediments. Table 7 shows the average data obtained of the triplicates.

At last, some explosives samples were analyzed in N, NC and CHNS configurations. For nitrogen determination, the calibration was performed with 15–20 mg of potassium nitrate, K factor as calibration method and sample weight 30–50 mg. Table 8 shows the nitrogen data obtained. For NC determination, the calibration was performed with 4–5 mg of cyclohexanone-2,4-dinitrophenylhydrazone, K factor as calibration method and sample weight 4–5 mg. Table 9 shows the NC data obtained and the $CO_2/N_2$ area ratio useful index for QC. For CHNS determination, the calibration was performed with 2–3 mg of BBOT, K factor as calibration method and sample weight 2–3 mg. Table 10 shows the CHNS data obtained.

Table 7. NCS data of environmental samples.

| Sample | N% | RSD% | C% | RSD% | S% | RSD% |
|---|---|---|---|---|---|---|
| Pine needle | 1.18 | 1.03 | 48.15 | 0.31 | 0.0317 | 2.62 |
| Birch leaves | 1.19 | 0.80 | 46.89 | 0.13 | 0.2450 | 1.24 |
| Soil 1 | 1.05 | 0.67 | 13.39 | 0.78 | 0.5273 | 0.37 |
| Soil 2 | 0.0583 | 0.26 | 0.8717 | 0.24 | 0.0102 | 1.49 |
| Sediment 1 | 0.0668 | 0.52 | 0.7606 | 0.35 | 0.0372 | 1.38 |
| Sediment 2 | 0.8965 | 0.22 | 9.88 | 0.35 | 1.18 | 0.72 |
| Water 1 | 0.0116 | 1.67 | 0.0050 | 0.91 | 0.0092 | 3.07 |
| Water 2 | 0.0019 | 2.04 | 0.0009 | 3.05 | 0.0047 | 3.01 |

Table 8. Nitrogen data of explosives samples.

| Sample | N% | RSD% | Sample | N% | RSD% |
|---|---|---|---|---|---|
| Cellulose nitrate A | 12.59<br>12.54<br>12.56 | 0.20 | Guncotton A | 13.57<br>13.58<br>13.61 | 0.15 |
| Cellulose nitrate B | 12.30<br>12.25<br>12.28 | 0.20 | Guncotton B | 13.14<br>13.17<br>13.13 | 0.16 |

Table 9. NC data of explosives samples.

| Sample | N% | RSD% | C% | RSD% | $CO_2/N_2$ area ratio |
|---|---|---|---|---|---|
| Nitrocellulose | 13.47<br>13.45<br>13.49 | 0.15 | 25.19<br>25.14<br>25.25 | 0.22 | 5.30<br>5.29<br>5.30 |
| Mix 227 | 13.06<br>13.13<br>13.04 | 0.36 | 25.55<br>25.58<br>25.52 | 0.12 | 5.54<br>5.52<br>5.54 |
| 34 Lot 327 | 12.07<br>12.04<br>12.06 | 0.13 | 27.04<br>26.72<br>26.87 | 0.60 | 6.34<br>6.28<br>6.32 |
| FL 593 | 13.59<br>13.55<br>13.57 | 0.15 | 25.00<br>24.98<br>24.97 | 0.06 | 5.20<br>5.21<br>5.19 |

Table 10. CHNS data of explosives samples.

| Sample | N% | RSD% | C% | RSD% | H% | RSD% | S% | RSD% |
|---|---|---|---|---|---|---|---|---|
| TNT | 18.47<br>18.19<br>18.43 | 0.83 | 37.42<br>37.35<br>37.26 | 0.21 | 2.23<br>2.21<br>2.24 | 0.84 | - | - |
| Nitrocellulose | 8.30<br>8.35<br>8.39 | 0.60 | 17.26<br>17.07<br>17.23 | 0.57 | 3.54<br>3.36<br>3.60 | 3.55 | - | - |
| Mix A | 12.02<br>11.91<br>11.86 | 0.68 | 26.52<br>26.53<br>26.59 | 0.15 | 2.97<br>2.96<br>2.97 | 0.14 | 0.0098<br>0.0109<br>0.0096 | 6.93 |
| Mix B | 11.76<br>11.95<br>11.88 | 0.84 | 26.54<br>26.62<br>26.59 | 0.16 | 2.95<br>2.97<br>2.98 | 0.47 | 0.0168<br>0.0187<br>0.0168 | 6.29 |

## Conclusions

For the different applications of forensic investigations the Flash*Smart* EA, based on the combustion method (Dumas) determines N, NC, CHNS by combustion and oxygen by pyrolysis for the analysis of solid and samples in a wide range from low to high content and without the use of sample digestion or toxic chemicals, which is normally required by traditional methods. The need for analysis productivity and sample throughput is met, by performing simultaneous CHNS or NCS determination in a single run and analyzing sulfur only with minor modifications of the analytical conditions. Nitrogen only or NC determinations can be performed by increasing the sample weight and changing the configuration.

Specifically, the Analyzer demonstrates excellent repeatability, reproducibility, accuracy, and precision, as automation, speed of analysis and cost per analysis.

No memory effect was observed when changing the sample type, indicating the complete conversion and detection of all elements.

Thanks to the modularity of the Flash*Smart* Analyzer, the same hardware, autosamplers and software can be readily used for other configurations such as CHN/O, CHN/S, CHNS/O, CHNS/CHNS, CHN/CHN, NC (single reactor)/S, N-Protein (single reactor)/S and more. This can mainly be realized by changing the consumables as the Analyzer and software are complete, illustrating the all-in-one nature of the Analyzer.

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