



Challenge

Automated direct analysis of nitrogen in highly viscous oil samples without dilution and highly reproducible results at minimum operation and maintenance effort.

Solution

Elemental analyzer with temperature-controlled sample supply and flame sensor technology for combustion control

Intended audience

Industrial quality control labs in edible oil production, biofuel and oleochemical production, independent control labs and authorities

Fully Automated Determination of Total Nitrogen in Different Edible Oils by Means of Elemental Analysis and Chemiluminescence Detection

Introduction

Monitoring edible oil along the value chain for quality and safety parameters is a key task for food production. Whether for food preparation, in the production of processed foods, or for cosmetics, biofuels, oleochemicals and pharmaceuticals, edible oils are irreplaceable. Regulations and directives for the protection of consumers and the environment ensure that high production standards are maintained and quality and safety parameters required therein are continuously monitored along the value chain. Given the sheer volume of edible oil to be analyzed – in 2022, more than 220 millions of tons of edible oils were produced worldwide. Industrial laboratories of food and drug manufacturers as well as laboratories of government agencies face the challenge of obtaining reliable analytical data for large sample volumes in the shortest possible time while managing complex and dynamic supply chains.

In addition to metal and heavy metal traces, non-metals such as sulfur, nitrogen and chlorine often need to be determined. Mostly, these elements do not occur naturally in edible oils, but enter as impurities that can affect the production process and negatively affect the quality of the final product in terms of odor, color, taste and toxicity. To prevent these hazards and mitigate the necessary measures, these parameters are monitored. Not every element is subject to strict regulations, but must still be monitored regularly. One such element in edible oils is nitrogen. Combined nitrogen can occur in many forms: Some compounds are natural components of the edible oil, while others are unwanted contaminants from the environment or the production process. For example, many fertilizers and pesticides contain nitrogen compounds. When they accumulate in the soil or water, these are absorbed

by the plants and thus enter the vegetable oil. Nitrogen compounds of artificial origin are considered dangerous and are therefore not desirable in edible oils. They may indicate, for example, the use of glyphosate and paraquat, pesticides whose use is strictly regulated or prohibited. The situation is quite different with natural nitrogenous substances: they may have positive effects on human health. The most prominent representatives are the B vitamins and cyanogenic glycosides.

Regardless of whether the limits for undesirable nitrogen compounds or the presence of desirable nitrogen compounds are to be monitored: The determination of Total Nitrogen (TN), a sum parameter that reflects the amount of all nitrogen compounds in the sample, is the easiest way. The best way to do this is by combustion-based elemental

analysis in conjunction with sensitive chemiluminescence detection. The instrument-based analytical technique is easy to perform, fully automatable, and can be used to obtain matrix-independent, sensitive results over a wide range of concentrations. This makes it a valuable tool for the rapid testing of bulk material that has proven its value already in industries such as oil and gas. Here, numerous standards have been established in recent decades. For challenging matrices such as oil and related hydrocarbons, ASTM D4629 and D5762 are frequently cited. Since edible oils have many features in common with mineral oil and related samples - high viscosity, high boiling points, and composition of very long-chain or saturated hydrocarbons - these methods can be applied to edible oils with slight adjustments. If compound-specific information is required, the more complex chromatographic methods should be used.

Materials and Methods

Samples and reagents

- 14 edible oil samples
 - different stages of the production process: feedstock, intermediates, final products
 - different production approaches: cold-pressed, solvent extracted, refined, hydrogenated
- One ultra-pure reference oil (paraffine oil)

The sample matrices are a representative spectrum of edible oils derived from plant seeds. Due to their main components – long-chain hydrocarbons, so-called fatty acids – they have a high viscosity.

- Kit calibration solutions, c: 0.1 – 25 mg/L N, Analytik Jena GmbH+Co.KG, 402-889.075
- Kit calibration solutions, c: 10 – 100 mg/L N, Analytik Jena GmbH+Co.KG, 402-889.165

Sample preparation

The samples and standards were analyzed directly without sample preparation. The use of a heated sample supply system, e.g. a temperature-controlled liquid sampler, allowed direct injection with a syringe. Heating to 75 °C significantly reduced the viscosity of the samples. This is possible when dealing with homogeneous liquids with increased viscosity. Another approach is to dilute the samples with a suitable solvent to reduce viscosity and allow automated sample introduction without a dedicated sampler. This strategy is not part of the experiments described here.

Calibration

Prior to sample measurement, the multi EA 5100 was calibrated for nitrogen determination. Liquid standards based on pyridine in isooctane in the concentration range from 0.1 to 100 mg/L N were used for this purpose. The obtained calibration was verified with certified reference standards. 40 µL injection volumes were used for the calibration and verification measurements. Figure 1 shows the calibration curve for the ultratrace range as an example. In table 1, the performance data of the calibration are summarized.

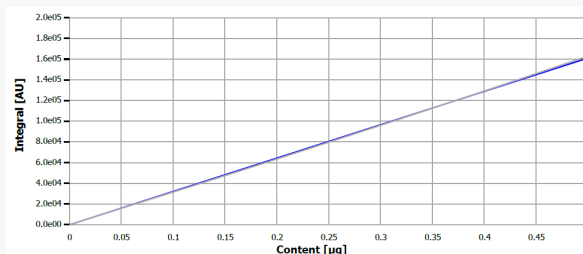


Fig. 1: Example calibration curve for the nitrogen determination, ultra-trace range

Tab. 1: Statistical data of the calibration

| Parameter | Value |
|-------------------------|----------------------|
| Injection volume | 40 µL |
| Concentration range | 0 - 1.0 µg N absolut |
| Limit of detection | 1.98 µg/L |
| Linearity | linear |
| Correlation coefficient | 0.9996 |

Instrumentation

For the determination of nitrogen, a multi EA 5100 with HiPerSens CLD was used in horizontal operation mode. For automated sample introduction and transfer to the analyzer, the system was equipped with an automatic boat drive with flame sensor technology and the MMS-T, a temperature-controlled Multi-Matrix Sampler. The MMS-T can either be cooled to allow automated dosing even for extremely volatile liquids or, as in this case, heated to allow direct injection of highly viscous homogeneous materials without prior dilution. For the determination of nitrogen, the MMS-T was used to inject 40 μL of sample or standard into a quartz sample boat, which was then automatically transferred to the combustion furnace. The flame sensor ensured trouble-free and matrix-optimized vaporization and combustion of different materials. Sample digestion was performed by

Instrument settings and Method Parameters

For analysis of the liquid standards and oil samples a standard method from the method library was used. The method parameters are summarized in Table 2. The injection volume for the TN determination was set to 40 μL . A temperature-controlled syringe was used for direct injection of pure samples. Both the syringe and the sample (sample tray) were maintained at a constant temperature of 75 $^{\circ}\text{C}$ throughout the analysis period. This allows direct injection independent of the initial sample viscosity, thus avoiding time-consuming and error-prone dilution steps.

Evaluation Parameters

The evaluation parameters for the detection of nitrogen (TN) by chemiluminescence are summarized in Table 3.

efficient catalyst-free high-temperature combustion in a quartz tube. This process is fully automatically controlled by flame sensor and adapted to the specific needs of each matrix component, ensuring matrix-independent, optimal results in the shortest possible time. The process is divided into two phases. In the first process phase, the volatile sample components are vaporized in an inert gas stream, followed by combustion of the gaseous products formed in an oxygen-rich atmosphere. In the second phase, the heavier, non-volatile sample components and the pyrolysis products formed are quantitatively oxidized in pure oxygen. The implemented auto protection system guarantees highest operational safety (particle and aerosol trap) and a complete transfer of the formed NO_x to the chemiluminescence detector. The multi EA 5100 allows a detection limit of 10 $\mu\text{g}/\text{L N}$.

Table 2: Process parameters multi EA 5100 – horizontal operation

| Parameter | Setting |
|---|----------------------------|
| Furnace temperature | 1050 $^{\circ}\text{C}$ |
| 2 nd combustion | 60 s |
| Ar flow (1 st phase) | 200 mL/min |
| O ₂ main flow | 200 mL/min |
| O ₂ flow (2 nd phase) | 200 mL/min |
| Draw up speed | 2.0 $\mu\text{L}/\text{s}$ |
| Injection speed | 2.0 $\mu\text{L}/\text{s}$ |
| ABD mode | automatic* |

* flame sensor controlled automatically optimized combustion

Table 3: Detection parameters for total nitrogen determination (CLD)

| Parameter | Setting |
|-----------------------|---------|
| Max. integration time | 720 s |
| Start | 0.5 ppb |
| Stop | 0.5 ppb |
| Block | 7 |

Results and Discussion

The analytical results for samples and standard solutions are summarized in Table 4. These are average values from three replicate analyses. The edible oil samples could be analyzed without any problems. Despite their higher viscosity, no memory effect was observed. The high repeatability is confirmed by the low RSD values. This is comparable to the quality of the analysis of the standard solutions. Even for samples such as almond oil, where nitrogen is present only as an impurity in the ultratrace range, the quality of the results is reasonable, indirectly confirming the repeatability of the injection and the optimal digestion process. After analysis, the combustion tube and boat drive were found to be free of contamination. In addition to the analysis of edible oil, this method could also be applied to other homogeneous oils and comparable matrices (e.g. UCO (used cooking oil), tall oil or cod liver oil, glycerol). Linseed oil has a very high TN value, but in this case it is not a serious impurity, but a natural component of the oil. Linseed contains a remarkable amount of amygdalin, a cyanogenic glycoside. Other oils with natural nitrogen sources include mustard

and canola oils with N-containing allyl isothiocyanates and glucosinolates, respectively; rice bran and sesame oils with thiamine as an N-carrier, better known as vitamin B1; and walnut oil with pyridoxine - better known as vitamin B6. The rice bran oil analyzed is a refined oil. During refinement all other components are removed, except the fatty acids. The additional blank sample of paraffin oil analyzed shows traces of nitrogen in the sub-ppm range. It is obtained from mineral oil, and is therefore free of natural products. Palm oil does not naturally contain bound nitrogen such as B vitamins or cyanogenic glycosides, so here the nitrogen source must be different. Most likely, it comes from a phase of the production process or contamination from a previous phase (e.g., agriculture) in which various nitrogen-containing pesticides are used in oil palm plantations. If such an unexpectedly high TN value is detected, further investigation using compound-specific techniques is required to obtain certainty.

Table 4: Results of Total Nitrogen (TN) determination for the edible oil samples and standards

| Sample | C _N [mg/L] | RSD [%] |
|------------------------------|-----------------------|---------|
| Linseed oil** | 159 | 0.34 |
| Mustard oil, pure** | 96.8 | 0.96 |
| Mustard oil 2 | 32.81 | 0.64 |
| Mustard oil* | 2.71 | 0.72 |
| Rapeseed oil (canola), crude | 3.32 | 1.38 |
| Walnut oil** | 13.2 | 0.61 |
| Sesame oil** | 9.82 | 0.45 |
| Palm oil, bleached | 4.13 | 0.26 |
| Rice bran oil* | 3.13 | 2.62 |
| Coconut oil, crude | 2.05 | 0.48 |
| Peanut oil** | 1.74 | 0.94 |
| Olive oil** | 1.53 | 4.90 |
| Almond oil* | 1.23 | 0.04 |
| Paraffin oil SA (blank) | 0.39 | 0.51 |
| Standard 5.00 mg/L N | 5.00 | 1.30 |
| Standard 100 mg/L N | 100 | 0.56 |

* refined oil, ** cold-pressed

In the following, typical measurement curves for selected oil samples and standard solutions are shown.

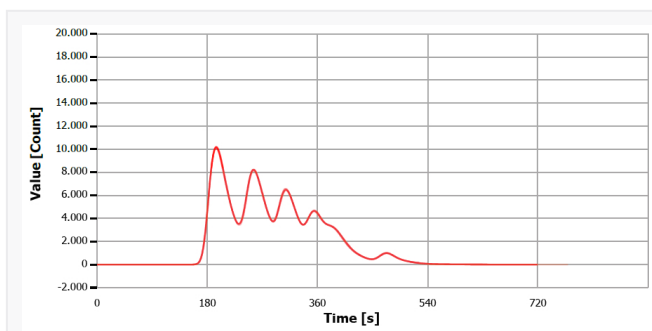


Figure 2: TN measuring curve for sample "linseed oil"

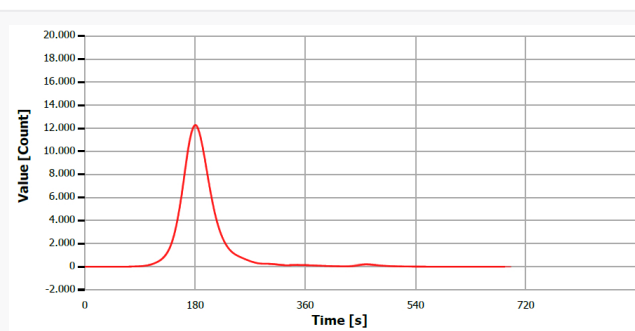


Figure 3: TN measuring curve for sample "mustard oil native"

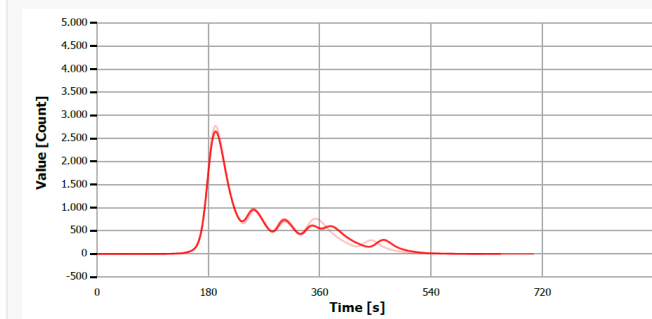


Figure 4: TN measuring curve for sample "mustard oil 2"

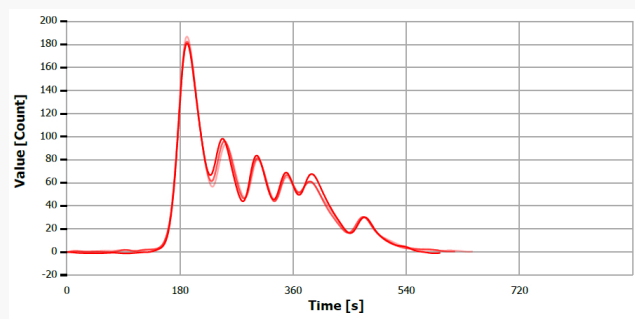


Figure 5: TN measuring curve for sample "mustard oil"

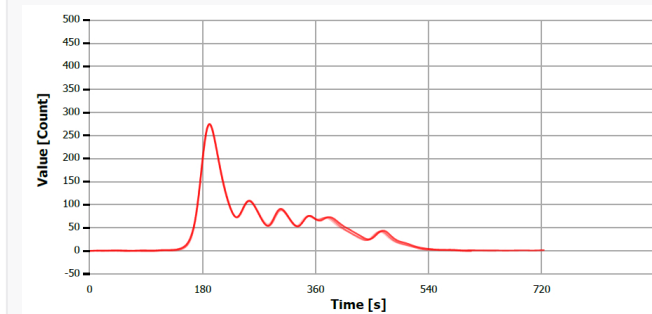


Figure 6: TN measuring curve for sample "rapeseed oil"

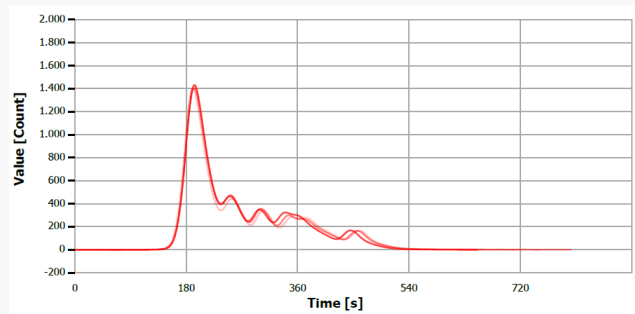


Figure 7: TN measuring curve for sample "walnut oil"

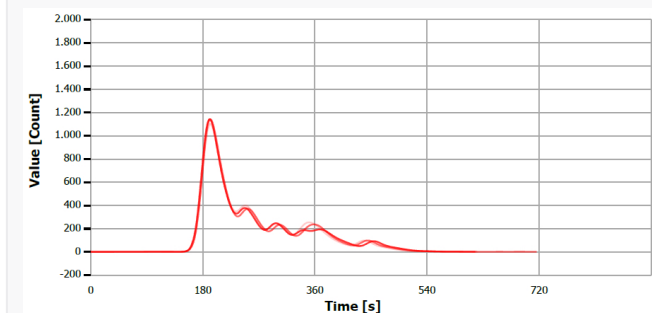


Figure 8: TN measuring curve for sample "sesame oil"

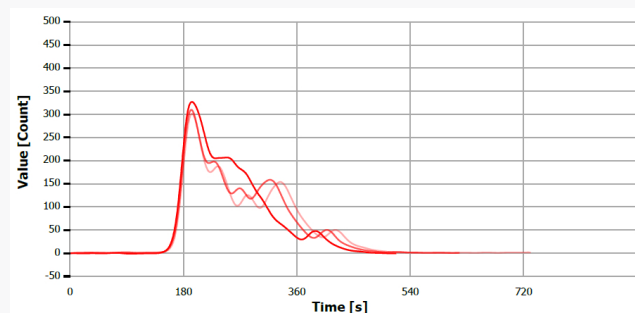


Figure 9: TN measuring curve for sample "palm oil"

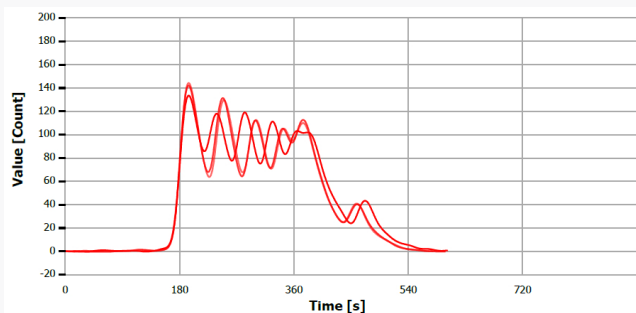


Figure 10: TN measuring curve for sample "rice bran oil"

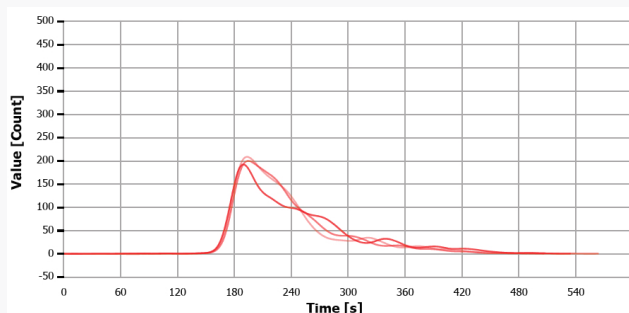


Figure 11: TN measuring curve for sample "coconut oil"

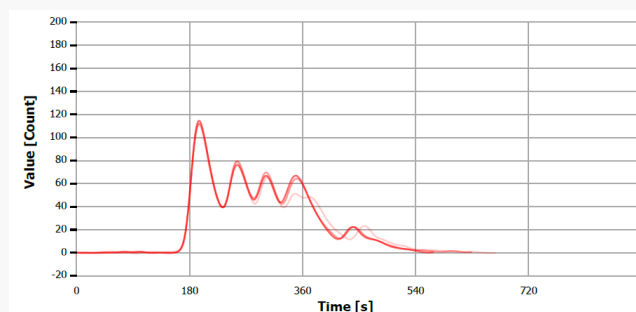


Figure 12: TN measuring curve for sample "peanut oil"

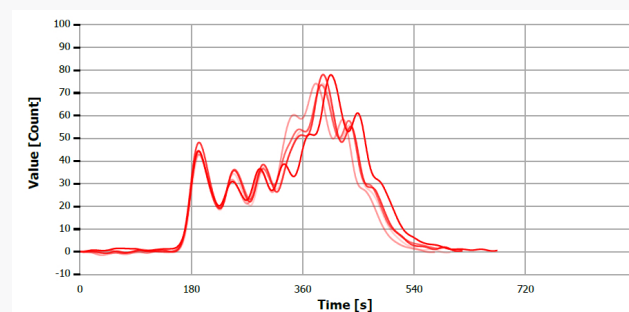


Figure 13: TN measuring curve for sample "olive oil"

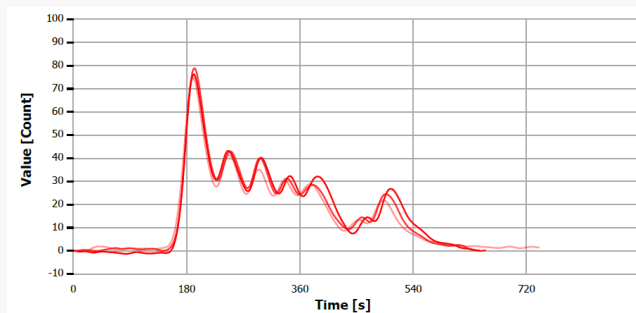


Figure 14: TN measuring curve for sample "almond oil"

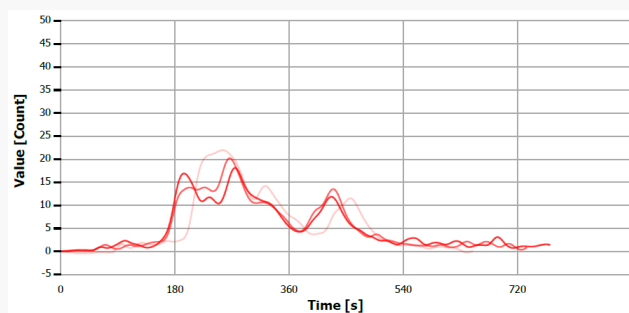


Figure 15: TN measuring curve for sample "paraffin oil SA blank"

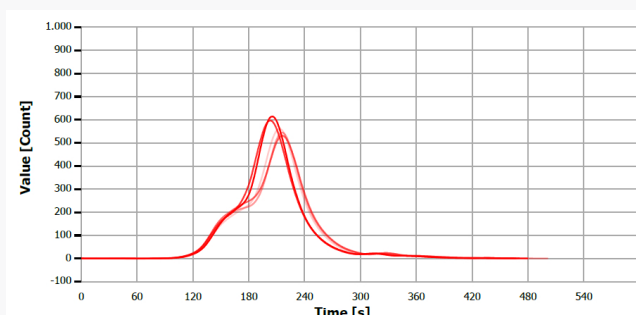


Figure 16: TN measuring curve for standard "5 mg/L N"

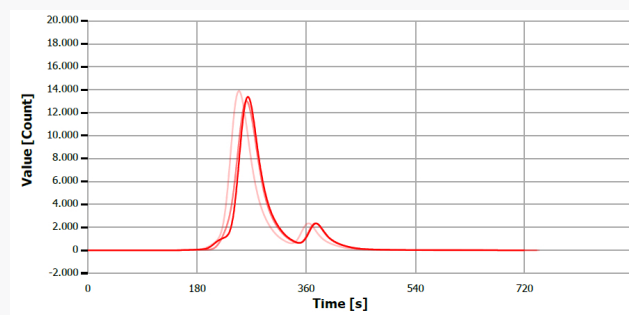


Figure 17: TN measuring curve for standard "100 mg/L N"

Summary

Unlike other methods, combustion-based elemental analysis (C-EA) determines total nitrogen as a sum parameter (TN), which can be used as a screening parameter for all types of organically bound nitrogen. Thus, C-EA can provide a quick and accurate picture of the total nitrogen content before performing a targeted analysis to identify individual substances.

Perfectly suited for this purpose is the multi EA 5100 with MMS-T in horizontal mode, which allows direct, time-optimized analysis of viscous sample matrices, such as edible oils. Thanks to the efficient heating of the sample tray and dosing syringe of this liquid autosampler, viscous matrices can also be dosed without a prior dilution step. This saves valuable working time, reduces the consumption of solvents, and minimizes the risk of user errors. Especially when determining very low element contents close to the detection limit, this feature is essential to ensure reliable results. The combustion process is fully automatically optimized with the aid of a flame sensor, creating ideal

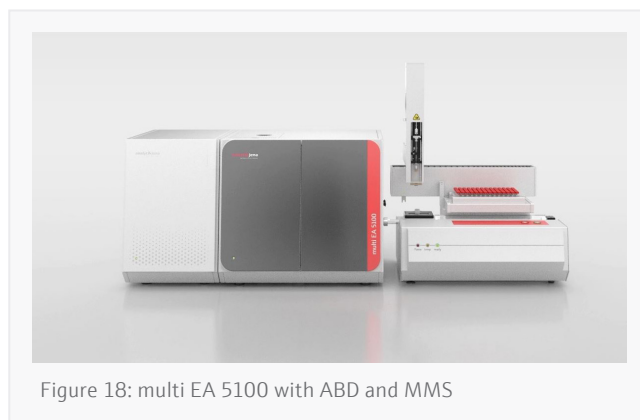


Figure 18: multi EA 5100 with ABD and MMS

digestion conditions for any organic matrix. This avoids incomplete combustion of samples as well as system contamination without the need for manual adjustments or special boat programs. A single method is sufficient to analyze the full range of liquid matrices, regardless of their viscosity, volatility, combustibility, and concentration. This eliminates the need for matrix-specific method development and ensures best comparability of results.

Recommended device configuration

Table 4: Overview of devices and accessories

| Article | Article number | Description |
|----------------------------------|----------------|---|
| multi EA 5100 | 450-300.011 | multi EA 5100 – elemental analyzer for sulfur, nitrogen, chlorine and carbon analysis in solids, liquids, gases |
| C/N/S High Performance drier kit | 450-300.012 | High Performance reaction gas drier for analysis of carbon, nitrogen and sulfur with multi EA 5100 |
| N module | 450-300.022 | Extension of multi EA 5100 for nitrogen determination with chemiluminescence |
| ABD | 450-300.013 | Automatic Boat Drive with flame sensor technology – horizontal operation of multi EA 5100 |
| MMS-T | 450-900.453 | Temperature-controlled autosampler – heating /cooling option for liquids, extendable for solids analysis |
| multiWin software | 450-011.803 | Operation and data evaluation software for multi EA 5100 |

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