Automated Combustion Accelerator Mass Spectrometry for the Analysis of Biomedical Samples in the Low Attomole Range

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Supporting Information

ABSTRACT: The increasing role of accelerator mass spectrometry (AMS) in biomedical research necessitates modernization of the traditional sample handling process. AMS was originally developed and used for carbon dating, therefore focusing on a very high precision but with a comparably low sample throughput. Here, we describe the combination of automated sample combustion with an elemental analyzer (EA) online coupled to an AMS via a dedicated interface. This setup allows direct radiocarbon measurements for over 70 samples daily by AMS. No sample processing is required apart from the pipetting of the sample into a tin foil cup, which is placed in the carousel of the EA. In our system, up to 200 AMS analyses are performed automatically without the need for manual interventions. We present results on the direct total $^{14}$C count measurements in $<2 \mu$L human plasma samples. The method shows linearity over a range of 0.65–821 mBq/mL, with a lower limit of quantification of 0.65 mBq/mL (corresponding to 0.67 amol for acetaminophen). At these extremely low levels of activity, it becomes important to quantify plasma specific carbon percentages. This carbon percentage is automatically generated upon combustion of a sample on the EA. Apparent advantages of the present approach include complete omission of sample preparation (reduced hands-on time) and fully automated sample analysis. These improvements clearly stimulate the standard incorporation of microtracer research in the drug development process. In combination with the particularly low sample volumes required and extreme sensitivity, AMS strongly improves its position as a bioanalysis method.

Microdosing/microtracer research is a clinical investigational technique to dose experimental drugs (e.g., $^{14}$C-labeled) to humans at extremely low concentrations that have negligible radioactive risk, while providing robust quantitative data. These studies enable one to establish the pharmacokinetic profile of new drugs in humans at a much earlier point in pharmaceutical development than ever possible. A microtracer can be incorporated in the first-in-man (Phase 1) studies relatively easy at low additional costs. However, it provides direct information on absolute bioavailability of the compound and can generate a mass balance profile. Thus, information gained with microdosing and microtracer studies allows industry to focus only on new investigational drugs with acceptable absorption, distribution, metabolism, and excretion (ADME) characteristics and thereby displays a huge benefit for drug developmental processes. In order to perform successful microdosing or microtracer experiments, extremely sensitive analytical techniques are required that can analyze the biological samples. Accelerator mass spectrometry (AMS) is an isotope ratio technique and well-established in the field of radiocarbon dating. AMS can measure the ratio between carbon-14 and carbon-12 ($^{14}$C/$^{12}$C) at attomole to zeptomole sensitivity. Obviously, this technique was not developed for high throughput screening of samples and requires optimization in order to be more routinely applied in biomedical research. Especially, the laborious sample preparation procedure in combination with the required sample amounts is a limiting factor.

Over the past few years, biomedical AMS has become an active research area, and important advances in sample preparation have already been achieved. It has become general knowledge that especially the acceptance of CO$_2$ by the AMS
ion source, instead of solid graphite material, would significantly simplify the overall procedure. Several successful applications have been shown, including the miniaturized carbon dating system (MICADAS) developed by Ruff et al. The MICADAS system accepts CO$_2$ supplied in a glass ampule and releases it in an evacuated compartment. Next, CO$_2$ is mixed with helium (carrier gas) and directly infused into the ion source. In general, lower currents are obtained for gaseous samples, but still, reliable and very stable measurements are obtained, with the additional advantage that much less material is required (<100 μg instead of 1 mg of material for solid graphite analysis). Despite these major improvements, sample throughput is limiting when considering AMS as a standard bioanalytical method during drug development. One has to realize that an average early clinical study will generate at least 200 samples, and the gas ampules have to be applied to MICADAS one by one.

Thomas et al. demonstrated a potentially higher throughput method, whereby a liquid chromatography system is coupled to a moving wire combustion interface. At the moment that a compound elutes from the column, it will be dried and combusted to CO$_2$. This can subsequently be placed in line with an AMS. While it reduces the total AMS analysis time, this methodology is always combined with LC. Consequently, sample extraction is a prerequisite, there is a limited choice in eluents compatible with the AMS, and when a total count analysis is required, LC separation becomes superfluous. Another possibility to increase sample throughput is by using an automated laser gas interface. Samples are loaded into a 96-well plate (stainless steel wells), which contains copper oxide. After the liquid samples are dried, CO$_2$ is released after laser ablation. This method requires approximately 300 μg of carbon, 5 μBq, and 10 min for a single sample to be analyzed.

A few years ago, the combination of an elemental analyzer (EA) for sample combustion coupled to an AMS was introduced. Wacker et al. used this setup to generate CO$_2$ by the EA which was subsequently transferred to a zeolite trap. By thermal release, the CO$_2$ was led to a reactor, containing an iron catalyst, and mixed with hydrogen to start graphitization. As soon as graphitization was complete, samples were pressed to the targets and analyzed by AMS. On the other hand, Bronk Ramsey et al. and Uhl et al. directly coupled the EA to the AMS; however, here the interface between both instruments contained a cryogenic trap to concentrate the generated CO$_2$. These individual developments were nicely combined by Ruff et al. to result in an online EA system coupled to the AMS via a zeolite containing interface, which runs a sequence of samples automatically. The major focus in their work was on minimizing sample volumes to values below ≤10 μg of carbon, and their method was demonstrated using various reference materials.

Here, we show an automated sample combustion system online coupled to the AMS that is specifically applied to analyze biomedical samples. Our approach is based on the system developed by ETH, whereby solid samples are introduced in the EA and combusted to CO$_2$. CO$_2$, mixed with helium, is transferred to an interface, containing a zeolite trap and syringe as the main components. At room temperature, the CO$_2$ is adsorbed on the trap, while helium is allowed to pass. By heating the trap, CO$_2$ is released and transferred into a vacuum syringe. The pressure of CO$_2$ is registered, and a mixture of CO$_2$ with helium is generated, which is directly infused in the AMS ion source. Thus, this interface enables the online coupling of the EA with the AMS. Using this approach, routine application of microdosing and microtracer approaches in drug development becomes feasible.

### EXPERIMENTAL SECTION

#### Elementar Analyzer-Interface-AMS

The automated CO$_2$ combustion system is composed of three different modules, the EA, the interface, and the AMS (Figure 1). The individual elements are described below in detail, and timing of the entire method is described in Supporting Information Figure S1).

**Figure 1. Schematic of the operational automated CO$_2$ combustion AMS system.**

**Sample Combustion and Carbon Concentration.** All AMS samples are combusted using a Vario Micro elemental analyzer (Elementar, Hanau, Germany). Samples are accepted in tin foil cups and placed in the carousel. The EA combusts one sample at the time and separates the generated N$_2$, CO$_2$, and H$_2$O. The EA uses microreduction and combustion tubes. The generated gases are guided by the carrier gas (helium) and upon detection detected by thermo-conductivity; CO$_2$ is directed to the zeolite trap, while the other gases go to waste. To quantify the total amount of carbon, originating from CO$_2$, the EA was calibrated using standards of acetanilide. 25, 50, 100, 250, and 500 μg of acetanilide were combusted on the EA and in duplicate, corresponding to 18, 36, 71, 178, and 355 μg of carbon. From the area under the curve (AUC) of the CO$_2$ peaks, the amount of carbon present in pooled plasma was determined (Supporting Information Tables S1 and S2).

#### Interface Description

The central part of the interface consists of a trap from stainless steel tubing (type 316; 30 mm × 1/8 in. o.d. × 2.1 mm i.d.), filled with Supelco Zeolyte X-10 (Sigma-Aldrich, Zwijndrecht, The Netherlands), similar to the material used by ETH. This trap absorbs CO$_2$ at room temperature and will release the carbon dioxide at elevated temperatures. The exhaust from the EA and inlet of the trap are connected to a six-port electrical actuated valve (VICI Valco, Shenkon, Switzerland) enabling one to direct the combustion products from the EA to waste or to the trap. The position of the valve is software controlled. The remaining ports are coupled to an air actuated on/off valve (Valco), a helium mass flow controller (Bronckhorst, Veenendaal, The Netherlands), and a connection valve of the same type as mentioned previously. The connection valve is coupled to an air actuated purge valve (Valco), from which one port is connected to an electrically actuated and RS-232 controlled four-port valve (Valco) and another to a high vacuum system (Pfeiffer). One of the adjacent ports of the four-port valve is connected to a 2500 μL syringe (Hamilton). This syringe is fitted in a syringe pump with a stepper motor (New Era Pump Systems, Inc.,
Farmingdale, USA) and electrically driven. A pressure sensor with a range from 0 to 5 bar absolute (Kistler, Winterthur, Switzerland) is fitted between the valve and the syringe. Another port of the four-port valve is connected to a solenoid valve, enabling the supply of helium while the remaining port is connected with a peek capillary (2 m × 1/16 in. o.d. × 60 μm i.d.) to the source of the AMS. All components were interconnected with stainless steel tubing (30 mm × 1/16 in. o.d. × 0.5 mm i.d.).

**Electronics Interface.** Air valves are activated by solenoid driven valves. The trap is heated by short-circuiting the capillary. For that purpose, the trap is connected to a remote controlled power supply of 60 A (Voltcraft, Hirschau, Germany). The temperature is measured by a J-type thermocouple, fastened to the trap. The air controlled valves and sensors are connected to a National Instruments 6212 MIO-DAQ controlled by software written with LabView 8.51.

**Flow System of the EA-Interface-AMS.** The system is composed of three different modules, the EA, the interface, and the AMS (Figure 1). In the initiation phase, the interface applies a vacuum on the trap and the syringe. The temperature of the trap is raised to 350 °C, and it is flushed with helium (1 mL/min). The insertion of a target into the ion source of the AMS automatically provides the start signal to the interface, which in turn triggers the EA. As a result, all three modules start simultaneously. The EA will combust the sample as described previously; the interface starts with a cleaning procedure, and the AMS presuppresses the target. The cleaning of the interface starts by heating the trap to 450 °C while the helium flow is kept constant (1 mL/min). In order to purge the syringe, the plunger is retracted and forwarded twice to 10% (250 μL) of the syringe volume. After purging, the helium flow is stopped and the trap is air-cooled to below 40 °C. The syringe is retracted completely, and a vacuum is applied on the trap and the syringe. Just before the CO2 is leaving the EA, the six port valve is switched, and the gas stream is conducted through the trap. CO2 adsorbs to the trap while helium and potentially other contaminating gases are removed by the vacuum. After total elution of the peak, the remaining gas stream from the EA is directed to waste. At this time, the trap and the syringe are again connected. The system is kept under vacuum to remove the excess of helium, after which the vacuum is closed and the temperature of the interface trap is raised to 350 °C. The released carbon dioxide is divided in volume ratio over the trap and the syringe. The pressure in the system is measured, and a gas mixture of 10% CO2 in helium is generated, by addition of helium via the zeolite trap to the syringe. Next, the connection valve between the trap and the syringe is closed. If the total pressure of the system is below the entered infusion pressure due to a low carbon content of the sample, the plunger of the syringe is forwarded until the correct pressure (1 bar) is reached. Next, the four-way valve is opened and the 10% CO2 mixture is infused into the AMS at a flow rate of 60 μL/min. After the total AMS analysis time has elapsed, the four-way valve returns to its original position and the syringe is emptied as the vacuum valve is opened again. At this moment, the interface starts its regeneration procedure. After completion of the regeneration procedure, which ensures minimal carryover, the interface is waiting for a new sample. The insertion of a new target into the AMS triggers the entire workflow again.

**Accelerator Mass Spectrometry.** A 1 MV multielement AMS, model 4110Bo (High Voltage Engineering, Amersfoort, The Netherlands), is used for the AMS analysis.23 The layout of the 1 MV AMS system has been described in detail previously.33 The hybrid ion source accepts both solid and gaseous samples. A gaseous mixture of CO2 and helium is guided through a capillary onto the titanium insert of the aluminum target. CO2 adsorbs to the surface and is sputtered by cesium, resulting in negatively charged carbon ions. Each sample is analyzed on a new target, and in total, 200 target positions are available in the storage carousel. Ions with an injection energy of 35 kV are generated and sequentially injected into the Tandetron accelerator using a 90° analyzing magnet with fast bouncing system. The ions traverse through the accelerator toward the positive potential (1 MV). In the center of the accelerator, electrons are stripped off the ions by interaction with argon gas. The resulting positive ions are further accelerated to the end of the accelerator, where they pass a 90° analyzing magnet. The instrument is programmed to pass doubly charged carbon ions. 12C2+ and 13C2+ ion currents are analyzed in Faraday cups, while 14C2+ ions are counted in a dual anode gas ionization detector. Average high energy 12C currents were approximately 1.5 μA. The transmission efficiency of the instrument is about 30%.

**Chemicals and Biological Products.** 4-Acetamidophenol [ring-14C(U)] was obtained from American Radiolabeled Chemicals (St Louis, USA). The compound had a specific activity of 48.7 Ci/mol (1.80 TBq/mol). Acetaminophen was purchased at Sigma-Aldrich (Zwijndrecht, The Netherlands) and acetanilide at VWR (Amsterdam, The Netherlands). Human plasma and sodium ethylenediaminetetraacetic acid (Na-EDTA) were obtained from Bioreclamation (New York, USA). Oxalic acid II (OXII, Standard Reference Material 4990C) and Australian National University (ANU, IAEA-CH-6, Reference Material 8542) sucrose were obtained from the National Institute of Standards and Technology. Antracite (ANT) was used as a zero-reference sample (Certified Reference Material, BCR-460 Coal).

**Sample Preparation.** Pooled blank plasma (human, Na-EDTA) was generated by mixing equal volumes of plasma originating from six individuals. The pooled plasma was used to prepare spiked calibration standards. A 4-acetamidophenol [ring-14C(U)] (14C-acetaminophen) solution with a specific activity of 4106 Bq/100 μg was used to spike the first calibration standard or quality control sample (CAL1 and QC1) at an activity level of 821 mBq/mL. This calibration sample was sequentially diluted in pooled plasma, down to a level of 0.65 mBq/mL. 1.8 μL of plasma is pipetted into a tin foil cup, using positive displacement pipets, and the liquid is evaporated to dryness. The tin foil cups are placed into the carousel of the EA, which accepts one sample at the time.

**Method Validation.** In accordance with the EBF recommendation, pooled blank plasma was analyzed 5-fold to determine the selectivity of the method.29 In addition, the blank plasma samples from the six individuals were also analyzed in duplicate. Each calibration standard was analyzed 6-fold. The carryover of the method is characterized by the analysis of three pooled blank plasma samples before and after CAL1 (821 mBq/mL). Analyte stability in human plasma was determined by the triplicate analysis of QC1 (821 mBq/mL), QC2 (12.83 mBq/mL), and QC3 (3.24 mBq/mL) after three freeze and thaw cycles. For QC1, QC2, and QC3 standards, their stability was also determined by their triplicate analysis after storage at 2–10 °C and −18 °C for 1 week and storage at −70 °C for approximately 3 months.
aminophenol (APAP), acetaminophen) has been used as the
measured $^{14}$C/$^{12}$C ratio for OXII was 1.3148
8 ANT, and 8 ANU samples were analyzed. The average
Throughout the run, consisting of 150 samples, in total 8 OXII,
sucrose control samples were included in the sample list.

The solution was prepared in blank pooled human
acetaminophen with an appropriate amount of$^{12}$C-acetamino-
phen. The solution was prepared in blank pooled human
plasma. The calibration standards were also prepared in blank
pooled plasma.

To check the instrument performance standard reference
material, OXII and ANU sucrose were analyzed several times
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sucrose were analyzed several times
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Instrument Performance Control Samples. Isotope
ratios were directly measured for each sample on the AMS.
To check the instrument performance standard reference
material, OXII and ANU sucrose were analyzed several times
during one run. The certified ratio for OXII is $3.614 \times 10^{-12}$
and $1.5061 \times 10^{-12}$ for ANU sucrose.$^{30,31}$ All values reported
are uncorrected for isotope fractionation.

RESULTS AND DISCUSSION

The well-known analgesic drug acetaminophen (N-acetyl-p-
aminophenol (APAP), acetaminophen) has been used as the
$^{14}$C-labeled molecule drug to spike blank human plasma at
various concentration levels. Acetaminophen at a specific
activity of 4106 Bq/100 μg was prepared by dilution of $^{14}$C-
acetaminophen with an appropriate amount of$^{12}$C-acetamino-
phen. The solution was prepared in blank pooled human
plasma. The calibration standards were also prepared in blank
pooled plasma.

To check the AMS instrument performance, OXII and ANU
sucrose control samples were included in the sample list.
Throughout the run, consisting of 150 samples, in total 8 OXII,
8 ANT, and 8 ANU samples were analyzed. The average
measured $^{14}$C/$^{12}$C ratio for OXII was $3.148 \times 10^{-12}$
(coefficient of variation (CV) 3.4%), a deviation of $-5.7\%$
from the reference value. The average measured $^{14}$C/$^{12}$C ratio
for ANU sucrose was $1.4578 \times 10^{-12}$ (CV 1.2%), a deviation of
$-3.2\%$ from the reference value.

The selectivity of the $^{14}$C total count method was
investigated by the 5-fold analysis of blank pooled plasma. The
plasma originating from six individuals was analyzed in
duplicate. For each sample, 1.8 μL of plasma was placed in a tin
foil cup and evaporated to dryness under nitrogen. The mean
result for the pooled plasma was 8.43 ± 0.05 mBq/mL, with a
standard deviation of 0.19 and CV of 2.3%. The EBF
requirement is easily met.

To convert a $^{14}$C/$^{12}$C ratio to mBq/mL, eq 1 is used,
whereby $1.3614 \times 10^{-12}$ corresponds to the certified ratio for
OXII and 0.3077 to the amount of Bq/g for OXII.

$$\text{activity} \left( \frac{\text{mBq}}{\text{mL}} \right) = \frac{\text{measured ratio}}{1.3614 \times 10^{-12}} \times 0.3077 \times \left[ \frac{\text{C}_{\text{sample}}}{\text{mg}} \left( \frac{\text{mg}}{\text{mL}} \right) \right]$$

(1)

In literature, the average plasma carbon concentration is
reported as 44 mg carbon/mL,$^{34}$ and the natural abundance of
$^{14}$C is $1.17 \times 10^{-12}$. Consequently, the theoretical background
level of activity in plasma can be calculated using eq 1 and
equals 11.73 mBq/mL. For the analysis of samples down to the
low attomole range, it becomes important to use exact sample
specific carbon percentages. Since there is a direct relation

![Figure 2. Current of $^{12}$C (black) and $^{14}$C/$^{12}$C ratio (gray) plotted versus time during subsequent cycles of automated CO$_2$ combustion-AMS analysis. Traces shown correspond to calibration standards 4, 2, and 1, respectively.](image-url)
between the carbon concentration of the sample and the calculated activity, a relatively small difference in carbon concentration can significantly influence the calculated value in mBq/mL.

The EA was calibrated using acetanilide. The AUC of the CO₂ peak that is obtained for each sample after combustion on the EA allows the calculation of the sample specific carbon concentration. For pooled plasma, this value was 39.14 mg/mL, with a standard deviation of 4.27 and a coefficient of variation of 10.9% (Tables S1 and S2 in the Supporting Information). Using this value, the theoretical background level of activity in blank plasma was 10.35 mBq/mL. The experimental value (8.56 ± 0.17 mBq/mL) is in accordance with the theoretical value. As previously mentioned, also our measured values for OXII and ANU sucrose were slightly lower but very reproducible.

Next, all calibration standards were analyzed in 6-fold (1.8 μL per analysis). In Figure 2, the carbon current and ¹⁴C/¹²C ratios are shown versus time for the subsequent analysis of different samples. It is clear that a very constant current is obtained over time for different samples, each measured on a new AMS target. Table 1 summarizes the results. Background subtraction (8.56 mBq/mL) has been applied to the calibration standards. Values are converted to mBq/mL, using a carbon concentration of 39.14 mg/mL, as all calibration standards were prepared in pooled blank plasma. Figure 3 shows the calibration curve as a function of the log nominal activity versus the log mean measured activity.

![Figure 3. Calibration curve of ¹⁴C total count analysis. The log of the nominal activity for all calibration standards is shown versus the log of the mean measured activity in mBq/mL.](image)

According to the EBF guideline for AMS analysis, the accuracy of the measurements should be within 20% for all calibration standards, except for the lower limit of quantification (LLOQ) that can deviate up to 25%. Similarly, regarding the precision of the analysis, the CV for all calibration standards should be within 20% and within 25% for the LLOQ sample. The signal for the LLOQ sample should be generally at least three times higher than the results obtained from running blank plasma extracts. Consequently, the signal of the LLOQ sample, after background subtraction, should at least be 0.51 mBq/mL (3× standard deviation of the pooled plasma); in the current method, the LLOQ corresponds to CAL14 (0.65 mBq/mL). This calibration standard also meets the requirements for accuracy and precision and can therefore be accepted as the LLOQ. For this particular pharmaceutical drug (¹³C-acetaminophen), 0.65 mBq/mL corresponds to a low atomole amount, i.e., 0.67 amol, and approximately 100 ag per analysis, taking into account that only 1.8 μL of plasma is analyzed. A detection limit of 0.65 mBq/mL (only 1.2 μBq in 1.8 μL plasma) can be reached for each component with a ¹³C label incorporated, without the need for any method development. Together, the impressive results indicate the superior sensitivity of the automated CO₂ combustion AMS system.

During the method validation, also the proportion of carryover was quantified. Pooled blank plasma samples were analyzed directly after the highest calibration standard (CAL1, 821 mBq/mL). The percentage of carryover determined in the blank sample, related to the activity level of CAL1, corresponded to 0.56%. During an analytical run, samples are analyzed in sequence from low to high activity levels, but of course, this requires prior knowledge of the sample composition. Our experiences are that often an appropriate sample sequence can be predicted, especially for ¹⁴C total count analysis samples, whereby normally in time the activity level decreases. However, accidentally it can occur that a sample with a high activity is followed by a sample with a lower activity. To guarantee the quality of the results, our procedure, based on an approach for estimated carryover influence, is to schedule a sample for reanalysis when a sample with high activity is followed by a sample with a lower activity and the percentage of carryover contributes ≥5% to the total activity present in the lower sample.

Finally, the stability of the calibration standards under various conditions was investigated. For this purpose, QC1 (821 mBq/mL), QC2 (12.83 mBq/mL) and QC3 (3.24 mBq/mL) were analyzed in triplicate after three freeze and thaw cycles, after storage at 2−10 °C and −18 °C for 1 week and storage at −70 °C for approximately 3 months. Table 2 summarizes these results. The deviation and CV for all calibration standards are well below 20%.

Figure 2 also illustrates that, while the ¹³C currents and ¹⁴C counts stabilize in about 2−3 min, the ¹³C/¹²C ratio is immediately constant, opening possibilities to significantly shorten the total measurement time. In the current setup, the sample is analyzed for 1100 s, which is relatively long but allows us to reach an LLOQ of 0.65 mBq/mL. Shortening the analysis time to 300 s results in an LLOQ of 1.30 mBq/mL, which is still extremely low (as there is only 2.5 mBq present per sample that is analyzed). This increased LLOQ is a result of a slightly higher standard deviation in the background samples (0.34, allowing a minimal LLOQ of 1.03 mBq/mL; Table S3 in the

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Table 2. Mean Results for Calibration Standards CAL1, CAL7, and CAL10 (Triplicate Analysis) Under Various Conditions

<table>
<thead>
<tr>
<th>stability test condition</th>
<th>CAL1 (deviation %/CV %)</th>
<th>CAL7 (deviation %/CV %)</th>
<th>CAL10 (deviation %/CV %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3X freeze/thaw</td>
<td>749.52 ± 6.06 (−8.73/0.81)</td>
<td>11.73 ± 0.40 (−8.56/3.38)</td>
<td>3.07 ± 0.06 (−5.32/2.04)</td>
</tr>
<tr>
<td>1 week storage 4−10 °C</td>
<td>727.23 ± 4.52 (−11.44/6.22)</td>
<td>12.33 ± 0.37 (−3.93/2.97)</td>
<td>3.47 ± 0.31 (7.01/9.04)</td>
</tr>
<tr>
<td>1 week storage &lt;−18 °C</td>
<td>758.89 ± 5.11 (−7.59/0.67)</td>
<td>12.34 ± 0.22 (−3.80/1.78)</td>
<td>3.61 ± 0.18 (11.36/4.90)</td>
</tr>
<tr>
<td>3 months storage &lt;−70 °C</td>
<td>738.64 ± 2.98 (−10.05/0.40)</td>
<td>12.63 ± 0.09 (−1.57/0.71)</td>
<td>3.39 ± 0.32 (4.63/3.96)</td>
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The deviation from the nominal value, as well as the coefficient of variation (CV), is given for each calibration standard.
CONCLUSIONS

An automated CO₂ combustion AMS system has been developed that can quantify samples of biological origin in the low attomole range. We present the first high-throughput fully automatic sample analysis method by AMS, with the greater advantage of complete omission of sample preparation in combination with the self-directed analysis of up to 200 samples. As the carrousel of the EA holds place for 119 samples, the extreme sensitivity is unrivalled by any other analysis method. In the current example, we were able to reach a limit of quantification of 0.65 mBq/mL, which corresponds to 0.67 attomol and 100 ag for acetaminophen quantification, which requires sever sample processing and the standard graphitization procedure followed by AMS analysis,⁶⁰ which requires sever sample processing and significantly larger sample volumes.

In plasma, a standard background of 8 to 10 mBq/mL is present, depending on the carbon concentration of the plasma sample. Depletion of the background, by protein precipitation, will lower the LLOQ level even further. However, this requires sample processing, such as plasma extraction. If information about parent drug and metabolite levels in time is requested, additional separation of the plasma extract by liquid chromatography (LC) is optional.

Currently, we aim to further improve the technology, by increasing the throughput of samples. As mentioned previously, it is relatively straightforward to decrease the total sample measurement time to ~300 s without major consequences, as still an LLOQ of 1.30 mBq/mL can be achieved under these conditions. However, the overall time for the analysis of a single sample is largely determined by the combustion and regeneration procedure, taking up to 12 min per sample. At the moment, a dual interface is under construction, allowing the daily analysis of >100 samples. The actual AMS sample measurement is followed by a system regeneration step. This can occur simultaneously with another sample being combusted in the second line of the interface. The current interface, as well as the dual interface, is compatible with any AMS instrument that is equipped with a gas-accepting ion source.

Altogether, this development finally allows the more routine application of this technology during biomedical research. Due to significantly reduced hands-on time and minimal sample requirements, the costs of AMS analysis obviously decrease and are close to those of the standard LC-MS bioanalysis. Now, AMS provides a true option for microtracer incorporation in early clinical phase programs. It will have a clear impact on the drug development process, as it allows fast compound selection and makes it possible to obtain early pharmacokinetic data in humans.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are very grateful to Prof. Dr. H.A. Synal (ETH Zürich, Switzerland) for sharing his expertise on automated CO₂ combustion systems directly coupled to AMS instruments. We would like to thank Dr. F. Cuyckens and Dr. G. Mannens (Janssen R&D, Belgium) for their helpful discussions and for providing an industrial view on the application of AMS in biomedical research. We would like to thank Dr. M. Klein (High Voltage Engineering Europa B.V., The Netherlands) for his expertise on accelerator mass spectrometry and the many useful discussions and insights. This study was conducted with support of the ministry of economic affairs.

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