

21 Bioanalytics for Human Microdosing

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21.1 SUMMARY

Phase 0 human microdosing trials are an approach that can obtain early human data in the drug development process and improve overall efficiency. A microdose of a small molecule is defined to be one-hundredth of the anticipated pharmacological dose or 100 μg , whichever is lower, or <30 nmol of a protein product. The microdose is designed to have cellular effects but not systemic or therapeutic effects and is intended to get basic pharmacokinetic (PK) data in a limited number of volunteers. Early human data can eliminate compounds with poor PK parameters early in the development process before major investments are made in later clinical trials. Better early information in phase 0 improves the planning process and pipeline efficiency, reducing the likelihood of needing to extend or repeat later clinical trials. The European Agency for the Evaluation of Medical Products (EMA) and the US Food and Drug Administration (FDA) support streamlining the drug approval process as long as

safety is not compromised and have issued guidelines on how phase 0 trials are to be conducted.

The low doses used in phase 0 trials present detection and quantitation challenges to conventional analytical instruments, especially when test compounds possess low bioavailability, low systemic distribution, or high potency. Accelerator mass spectrometry (AMS), liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), and positron emission tomography (PET) are bioanalytical techniques used for analysis in phase 0 studies. Each technique possesses strengths and weaknesses. LC-MS/MS is most common and least expensive. It is the easiest to use and can provide structural information on metabolites. It has limited sensitivity, however, and requires analytical standards for all metabolites. AMS has the best sensitivity and quantitative precision of the techniques. AMS quantitates a carbon-14 label but provides no chemical information, enabling detection of unknown or unexpected metabolites that must be identified with other techniques. AMS systems are rare and expensive, but off-site analysis is possible. PET provides unparalleled distribution information and real-time images of whether test compounds accumulate at targets. The positron emitters (carbon-11 or fluorine-18) used in PET have short radioactive half-lives and require an on-site cyclotron for production in addition to a PET scanner. PET is limited to short-term PK measurements and cannot distinguish metabolites from parent compound. Selection of a technique depends on the aims of the study and properties of the test compound. In many cases, combining the distribution information from PET with more quantitative measurements of AMS and LC-MS/MS provides the best picture of the behavior of a candidate compound.

21.2 INTRODUCTION

The current operational paradigm of drug development is time consuming and inefficient. Despite the great advances in biotechnology, computational power and models, analytical techniques, and genomics over the past 20 years, the rate of new compounds reaching the market has not increased. Furthermore, the pipeline lasts 10–20 years and costs of development are estimated at \$800 million to \$1800 million per registered drug [1–3]. As much as 40% of this cost is incurred in phase I trials that fail [4]. Up to 75% of this cost flows into compounds that never reach the market [5]. New approaches to drug development are needed to improve the efficiency of the development pipeline. Getting new compounds into humans earlier should reduce costs by identifying drugs destined to fail because of poor PK profiles before larger investments are made and by improving the planning of trials of those compounds moving ahead. Better informed planning should reduce the frequency of repeated or extended trials and potentially get new drugs to market more quickly. Phase 0 human microdosing trials are an approach that can obtain early human data [5].

A microdose is defined to be one-hundredth of the anticipated pharmacological dose or 100 μg , whichever is lower [6–8]. In the case of protein products, a microdose is limited to 30 nmol [7]. The microdose is designed to have cellular effects but not systemic effects and is intended to get basic PK data in a limited number of healthy volunteers. Phase 0 cancer clinical trials include patients with cancer, but doses of test compounds have no therapeutic effect [9–12]. Patients receive standard of care therapies during the studies.

Regulatory agencies in Europe and the United States support streamlining the drug approval process if safety is not compromised. The EMEA issued its Guideline on Microdosing in 2003 followed by a position paper on supporting nonclinical safety studies needed for microdosing [6]. The FDA followed with its Exploratory and Investigational New Drug Guideline in 2006 [7]. The guideline developed by the FDA Center for Drug Evaluation and Research (CDER) (2006) aimed to clarify the approaches, chemistry, manufacturing, and controls to be considered when planning exploratory studies in humans (also called *phase 0* or *preclinical trials*) [7]. It did not issue new regulations but rather interpreted existing recommendations on drug development. The FDA document describes three types of phase 0 studies: (i) microdose studies evaluating PK or imaging, (ii) studies evaluating pharmacologically relevant doses, and (iii) studies evaluating mechanism of action related to efficacy [7,13]. Multiple compounds can be administered in phase 0 trials allowing evaluation of several related compounds [13–15].

The aim of microdosing studies is to get drugs into humans earlier in the development process so that compounds destined to fail due to poor PK will be dropped before further investment is made [9–17]. The guideline delays full-scale good manufacturing practice (GMP) and some preclinical toxicity studies since microdosing uses very small amounts of material. There is no relaxation of good clinical laboratory practice (GCLP) when conducting phase 0 studies and full phase I studies must still be completed for compounds continuing in development [9,18]. Because very small amounts of test compound are used, quantitation challenges exist for measuring samples provided. In many cases, conventional LC-MS/MS cannot achieve the required sensitivity and either AMS or PET must be used to analyze the samples [14,18–23].

Beyond determining whether to continue development of a compound, early human PK and absorption, distribution, metabolism, and excretion (ADME) data provide insight for later human trials. By conducting phase 0 studies with intravenous (IV) and oral dosing, researchers can quickly assess bioavailability and address formulation before embarking on extensive trials [24]. The starting point and dose range for phase I can be selected with more confidence with early human data. Better early information in phase 0 improves the planning process and pipeline efficiency, reducing the likelihood of needing to extend or repeat later trials. In this case, an individual compound can get to market more quickly by minimizing delays, as it moves through the pipeline. Metabolite safety can also be addressed earlier with smaller doses of labeled drug [25].

Microdosing establishes PK of the subpharmacological dose. Lappin *et al.* [14,26] suggest that microdosing can predict pharmacological PK behavior. Although microdosing is not a perfect predictor of pharmacological PK, performing studies in the target species is often superior to allometry [17,27]. The collective experience of phase 0 trials is not yet sufficiently mature to predict when response will be linear across the entire dose range. It depends on whether receptors become saturated, reversible binding occurs, or first-pass metabolism produces an active metabolite [28]. A microdose is sufficiently small so that threshold, linear, or superlinear behavior cannot be distinguished.

Microdose levels of compounds labeled with short-lived radioisotopes appropriate for PET have been utilized for decades to determine tissue distribution. Isotopes appropriate for PET are produced with cyclotrons and then incorporated into tracer compounds. The chemistry must be done quickly because the isotopes decay quickly

and are very radioactive. The rapid decay of these compounds designed for PET works well for imaging of tissue distribution studies but is often unsuitable for PK studies.

The best way to measure the PK of these small doses is with an unambiguous tracer that can clearly be seen in small biological samples without knowledge of its metabolites. An atomic label that can be detected regardless of its chemical form is ideal. The radioisotope carbon-14 (^{14}C) with its low natural abundance ($^{14}\text{C}/\text{C} \sim 1$ part per trillion) is a fine candidate if the radiological hazards can be minimized. AMS is a rare isotope ratio mass spectrometry technique in which the rare isotope is a long-lived radioisotope such as ^{14}C . The technique is five to six orders of magnitude more sensitive than decay counting because it counts individual ^{14}C atoms in milligram-sized samples rather than waiting for their decay. The typical quantity of ^{14}C used in human metabolism studies is 200–40,000 Bq [29–32], comparable to the amount of ^{14}C in standard man (3700 Bq in 70-kg human) [33]. The small amount of isotope delivers lifetime radiation doses in the order of a couple microsivert, comparable to that received in a commercial airline flight across the United States. The level of radioactivity is so low that generally all samples (blood, urine, feces, and saliva) are nonradioactive, greatly simplifying waste disposal issues.

The strength of AMS is quantitative tracing of specifically labeled compounds. Metabolism studies are also attractive because the elevated ^{14}C provides an unambiguous signal that is clearly quantified. AMS does not provide chemical identification. That must be done by chromatography or other techniques before the tracer molecules are converted to a chemical form suitable for atom counting (either CO_2 or graphite).

Phase 0 trials require the high sensitivity to trace the small amounts of target compounds. Bioanalytical techniques suited for measuring experimental compounds are described below. All three techniques, AMS, LC/MS, and PET, have strengths and weaknesses. Selection of a technique depends on the aims of the study and properties of the test compound. In many cases, combining the distribution information from PET with more quantitative measurements of AMS and LC/MS provides the best picture of the behavior of a candidate.

21.3 BIOLOGICAL ACCELERATOR MASS SPECTROMETRY IN MICRODOSING

21.3.1 Overview of Accelerator Mass Spectrometry

AMS is an extremely sensitive analytical method used for the measurement of rare, long-lived isotopes. This technique was initially applied in isotope dating in the fields of archeology and earth science. More recently, AMS has been applied to the realm of experimental biology, including the areas of pharmacology and toxicology [34]. ^{14}C is the most common radioisotope used with AMS in biological studies, but ^3H [35], ^{41}Ca [36], and ^{10}Be [37] have also been used in biological AMS studies. The traditional analytical method of liquid scintillation counting (LSC) used in biological studies relies on nuclear decay events to quantify radioactive material. In contrast, AMS counts atoms of a rare isotope independent of decay by measuring the mass ratio of the radioisotope of interest relative to a stable isotope of the element. AMS is the most sensitive technique available for measuring ^{14}C , with the ability to quantify labeled material over six orders of magnitude down to the attomole (10^{-18}) level [34,38–40]. Owing to this

exquisite sensitivity, biological experiments using AMS produce negligible radiological exposure to the experimental subjects and do not perturb the normal biology of the system under consideration. The low level of radioactivity used in AMS experiments has enabled the use of human subjects in a number of biomedical tracer studies that would not be possible using other methodologies [34,39].

Studies in the biological sciences utilizing AMS include investigation of tissue turnover rates [41–43]; determination of PK parameters, including ADME, of drugs [14,21,26,44–46], toxicants [29,31], and nutrients [30,32]; measurement of the binding of drugs and reactive metabolites to proteins and DNA [46–48]; as well as risk assessment [49,50]. AMS is especially useful in microdosing studies because of its sensitivity that allows for the accurate measurement of extremely low levels of compound of interest. Measuring disposition and metabolism along with the molecular targets of investigational compounds in phase 0 studies can be done with AMS. Obtaining these data earlier in the drug development pipeline will help determine what compounds warrant further development. Those with unfavorable properties could be eliminated at an earlier stage before more is invested in a drug that has no chance of success. Microdosing experiments have made increasing use of AMS in recent years, introducing investigational compounds into humans earlier than is possible with more traditional techniques [14,26,51]. AMS is also being investigated for microdosing experiments with a growing area in pharmaceutical agents and biopharmaceuticals, which include large molecules such as polypeptides and proteins or macromolecules such as DNA segments [52,53].

21.3.2 Capabilities and Limitations of Accelerator Mass Spectrometry

Long-lived radioisotopes with low natural abundance are best suited for AMS measurement. Low natural abundance is important because the compound containing the radiolabel must be distinguishable from background levels, yet still be a low dose. The stable isotope ^{13}C does not fit in this category because it is naturally present in relatively high abundance, comprising approximately 1% of naturally occurring carbon.

AMS is unlike other forms of mass spectrometry in that it does not provide any structural information because the spectrometer is designed specifically for quantitation of the isotopes of a single element. Therefore, samples for AMS must be properly and adequately defined before analysis in order to ensure that meaningful results are obtained. The actual AMS measurement of a sample can be obtained in a few minutes; however, the sample preparation is a lengthier process and limits the throughput of AMS for ^{14}C analysis on most AMS instruments. AMS samples must be converted to a form that is easily conductive in the ion source and which does not allow for isotopic fractionation. Solid graphite is currently used on a conductive core such as cobalt or iron powder for ^{14}C analysis in most AMS instruments [54,55]. This proper sample definition presents a limitation of the technique, where a single HPLC trace may contain up to one hundred samples for AMS by graphitization [56]. The length of sample preparation and graphitization means several days pass between the time the samples are collected and the time that they are measured by AMS.

When sample material is plentiful and radiological dose is not an important consideration, other techniques such as LSC, isotope ratio mass spectrometry using ^{13}C , ^{13}C -NMR, or in some cases, fluorescent or chemical tagging of the compound or

molecule of interest may be more suitable than AMS. The level of sensitivity, importance of maintaining a low radiological dose, number and complexity of samples to be measured, and the cost of analysis are factors that should be considered in determining the appropriateness of using AMS for a biological study. The sensitive, quantitative, and precise nature of AMS makes it the most appropriate measurement technique for compounds with low bioavailability, low systemic distribution, or high potency. Experiments that require a compound in which only limited amounts can be produced or the tracer must be measured for extended periods of time are also well suited for AMS.

21.3.3 ^{14}C Measurement by Accelerator Mass Spectrometry

The majority of biological AMS experiments use ^{14}C because carbon is the basis of most biological materials and pharmaceutical agents. Recent developments have allowed for sample measurement in the gas phase; however, most AMS instruments measure samples in the solid state, which minimizes experiment-to-experiment carryover of signal in the instrument [57–59]. The solid form for measuring biological containing carbon by AMS is graphite. Samples containing 0.5–1 mg of carbon are optimal for graphitization and AMS, although samples containing 0.3–5 mg of carbon may be successfully measured [54]. In microdose applications, samples containing smaller quantities of carbon can be measured by the addition of a known quantity of carrier carbon that is ^{14}C deficient. The graphitization process consists of combustion of the biological sample to CO_2 , cryogenic isolation of CO_2 , and reduction to graphite in the presence of a cobalt or iron catalyst. The graphitization procedure has been described in greater detail elsewhere [54,55,60].

A schematic of a typical AMS instrument can be seen in Fig. 21.1. A cesium sputter ion source is used to generate negative carbon ions from the graphite sample. The ion beam containing those negative carbon atoms and other molecular isobars is mass selected following passage through a magnet and then introduced to the entrance of a tandem electrostatic accelerator. The ion beam accelerates toward and then collides with either a diffuse argon gas or a thin carbon foil that destroys interfering molecular isobars in velocity-dependent collisions and also removes one or more electrons from the atoms. The resultant positive ion beam is then further accelerated, followed by momentum and energy analysis. The rare ion (^{14}C) is counted using standard particle detection instrumentation. A change to the ion beam energy just after the ion source allows for the transmission of mass-13 ions through the first magnet and tandem accelerator with quantification of the $^{13}\text{C}^+$ ion current using an off-axis Faraday cup. This “bouncing” of the energy of the ion beam occurs several times a second allowing for the near simultaneous measurement of the $^{14}\text{C}/^{13}\text{C}$ isotope ratio of a sample. Absolute quantification of the $^{14}\text{C}/\text{C}$ isotope ratio in a sample comes from comparing the measured ratio of the sample to the measured ratio of similarly prepared standards.

Using the methodologies described, in a typical biological AMS study, a ^{14}C -labeled compound of interest can be routinely detected and quantified with 1–3% precision at levels ranging from ~ 10 pmol ^{14}C to 1 attomol (1×10^{-18} mol) of ^{14}C in samples containing 1 mg of total carbon in 5–10 min [38,54]. This measurement of ^{14}C by AMS can be compared to LSC that requires a gram-sized sample and a measurement time >48 h to obtain a precision of $\leq 1\%$. AMS is 1000 times more sensitive than LSC [61–63]. AMS experiments in humans have used ^{14}C label ranges from 185 Bq/person to 1.85 MBq/person, depending on the duration and type of study being performed

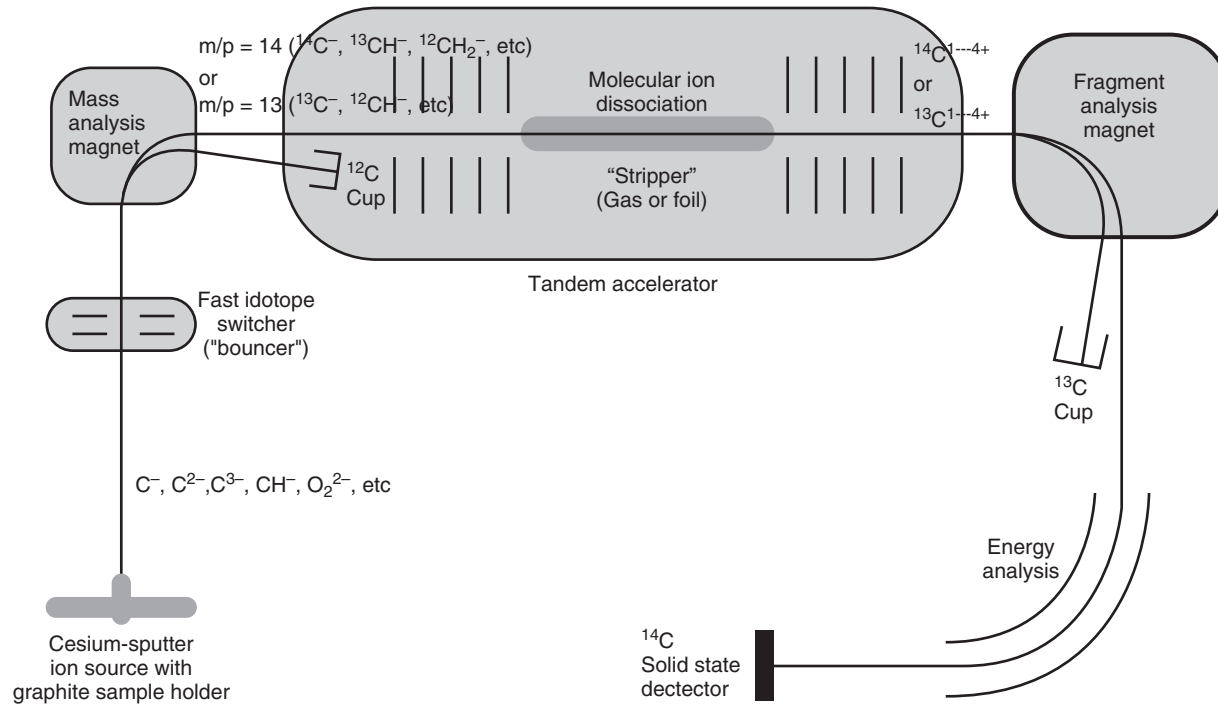


Figure 21.1 Schematic representation of the components of an accelerator mass spectrometer used to measure ^{14}C .

TABLE 21.1 Basic Performance Characteristics for the Biological AMS Instrument at Lawrence Livermore National Laboratory

Instrumental Performance	Approximate Value
Specificity (background signal with no ^{14}C)	Two parts per 10^{15}
Stability (loss of ^{14}C during sample preparation)	CV < 3%
Linear range of measurement	10^{-2} to 10^3 Modern
Instrument carryover between samples	None
Reproducibility	<5% error
Precision	2.5% (varies based on counting)
Lower limit of quantitation	5 amol ^{14}C
Upper limit of quantitation	100 fmol ^{14}C
Signal recovery	98%

[31,38]. The biological AMS instrument at Lawrence Livermore National Laboratory has measured over 70,000 samples during its operation over a period of nearly 10 years [64]. Important performance characteristics of this instrument are listed in Table 21.1.

21.3.4 Applications of Accelerator Mass Spectrometry in Drug Development

21.3.4.1 Basic Principles. Several basic principles can be applied to the unique experiments conducted with biological AMS to ensure that results are valid and informative. The investigator must understand what they are measuring by AMS. This understanding can be achieved by considering these three fundamental requirements for a successful AMS study:

1. absence of contamination in the sample,
2. appropriate amount of labeled compound, and
3. adequate sample definition.

Failure to satisfy one or more of these requirements of the sample can be very costly in time and resources and may entirely negate the value of the AMS experiment.

The absence of contaminating sources of ^{14}C is necessary to ensure that the measured ^{14}C is entirely due to the presence of the labeled compound being examined. Contamination can result in laboratory background levels as low as 5–10 μBq . The amount of labeled compound used in an experiment must result in a sample for AMS that falls within the range of 5 amol to 100 fmol ^{14}C in a milligram-sized sample. Samples with more than 100 fmol ^{14}C may generate a signal that exceeds the linear range of the counting electronics of the detector. Conversely, a sample that contains <5 amol ^{14}C will not produce adequate signal above background to be measured. Finally, the sample must be appropriately characterized by other methods such as HPLC before AMS in order to ensure that the correct and specific material of interest is measured because an AMS measurement provides no structural information. Chemical purity and radiopurity of the starting material should also be well characterized, typically using HPLC/MS and decay counting.

21.3.4.2 Work Area and Chemistry Requirements. The work area for conducting biological AMS experiments and performing sample preparation procedures must be clean and free of sources of ^{14}C -radiolabeled material as well as sources of extraneous

carbon. For AMS sample preparation, a laboratory that has no history of ^{14}C material use should be selected, as even microbecquerel quantities of ^{14}C can contaminate experimental samples [65,66]. The area for preparation of the radiolabeled compound must be separate from the dosing and sample collection areas. Samples to be sent for AMS measurement must also be stored separate from labeled material to prevent the possibility of contamination. The areas should be checked for ^{14}C background before beginning AMS experiments and must be monitored regularly for contamination. LSC is not sufficiently sensitive to detect contamination at low levels that can interfere with correct AMS measurement. Surveys for radioactive contamination should be taken and sent for AMS analysis in any laboratory in which samples are routinely prepared for AMS. The surveying procedure is described in greater detail elsewhere [65].

Chemicals to be used in experiments leading to the biological sample for AMS and the actual AMS sample preparation must be free of interfering radioisotope and should be sampled to determine the background ^{14}C levels by LSC and by AMS. Sodium-containing reagents should be avoided, as sodium can cause the quartz tubes used for graphitization to fail during the sample combustion step, resulting in loss of the sample. In many cases, it is possible to substitute potassium for sodium in reagents.

The major sources of potential errors encountered in AMS are the addition of carbon or ^{14}C from sources that remain unaccounted. This can be a result of contaminated solvents, glassware, laboratory equipment, airborne contamination, or even other researchers. The use of carbon-containing solvents for extractions or separations should be carefully considered in the determination of the total carbon present in the sample. Miscalculations in dosing or dosing of material that has an activity higher or lower than expected can result in excessive or inadequate signal intensity from the AMS sample.

21.3.4.3 Dosing and Collection of Labeled Material. The amount of ^{14}C -labeled compound necessary for adequate recovery and detection of signal by AMS can be approximated using several calculations. It is always advisable to perform initial experiments to optimize dosing, recovery, and detection parameters before beginning the full-scale AMS study. Performing these calculations and initial experiments will greatly increase the probability of obtaining meaningful results in the AMS study.

In estimating the doses of labeled compound that should be administered to achieve a desired degree of labeling in the collected sample, it is helpful to know the approximate bioavailability and volume of distribution of the compound under consideration. Although these parameters are unknown at the start of a phase 0 study, they can be estimated within one to two orders of magnitude for AMS signal-to-noise estimation. The type and quantity of tissue or other material (e.g., urine and feces) to be sampled should also be considered in the dosing calculation. For an experiment with an injected or highly bioavailable compound in which plasma is collected and the labeled compound is measured, the predicted optimal dosing level can be calculated as follows:

$$\text{Dose (in Bq)} = M_{\text{Target}} \times 226 \mu\text{Bq/mg C} \times V_{\text{Distribution}} \times C_{\text{plasma}}$$

where

M_{Target} = target fraction Modern in the collected sample, where
 $1 < M_{\text{Target}} < 100$

$V_{\text{Distribution}}$ = apparent volume of distribution for the compound (L)

C_{plasma} = carbon content of plasma (mg C/ μL).

The dose typically ranges from 300 Bq to 1.8 MBq. In the case of a compound with low bioavailability, the dose must increase according to the following equation:

$$\text{Dose (in Bq)} = M_{\text{Target}} \times 226 \mu\text{Bq/mg C} \times V_{\text{Distribution}} \times C_{\text{plasma}} \times (1/F)$$

where

F = bioavailability of the compound.

Predicted optimal doses can also be calculated for time points after administration of the labeled compound. In this case, the calculation is made based on the target fraction Modern for the final collection time point:

$$\text{Dose (in Bq)} = M_{\text{Final}} \times 226 \mu\text{Bq/mg C} \times V_{\text{Distribution}} \times C_{\text{plasma}} \times (1/F) \times e^N$$

where

N = number of elimination half-lives at the time of final sample collection.

These equations can be used for other materials (e.g., urine) by substituting the carbon content and tissue or fluid volume, as appropriate. When the target dose has been calculated in becquerel, the quantity of labeled compound (e.g., nmol) can be calculated based on the specific activity of the compound (nCi/nmol):

$$\text{Quantity(nmol)} = \text{dose} \times \text{specific activity}$$

The quantity of compound administered is typically in the range of 10–100 nmol.

For novel compounds, where little is known about bioavailability, volume of distribution, and elimination half-life, it may be necessary to perform initial range-finding experiments starting with low doses and working upward before conducting full-scale experiments. All samples with unknown levels of radioactivity should be quantified by LSC before graphitization and AMS analysis. Any sample that can be measured by LSC contains too much label to be measured by AMS.

Sample collection should be carried out using disposable materials, and care should be taken to avoid contamination or mixing of samples during collection, handling, and storage. Owing to the possibility of introducing contamination, separate sets of pipettes should be used for dosing experiments and for preparing samples for AMS analysis. If pipettes are used for dosing, they should not be used for general laboratory work or for preparing samples for AMS submission. Positive displacement pipettes and filter tips should be used at all times in all stages of experimental work and sample preparation to avoid the possibility of label carryover from one sample to the next. It is wise to always use new, disposable laboratory vessels and pipettes for all materials used in AMS experiments. This minimizes the potential for spread of contamination. For all experiments, control samples (predose or undosed) must be generated in order to provide a ^{14}C baseline for each experiment. Samples should be collected individually and sealed.

21.3.4.4 Sample Definition and Preparation. During the process of converting the sample material to graphite for AMS measurement, all chemical information contained in the original sample is lost. Thus, it is vitally important that the sample be adequately characterized before AMS analysis. This characterization will be specific for each type of sample and may in many cases be as simple as collecting and preparing a selected tissue or fluid (plasma, whole blood, urine, etc.). More complicated experiments may require additional separation and characterization techniques, including mass spectrometry, HPLC, flow cytometry, ELISA, isoelectric focusing, immunoblotting, or other analytical techniques, as needed to adequately separate and confirm the identity of the parent compound and metabolites.

It is essential to track all additions of carbon-containing compounds during the biological experiments and during sample preparation in order to produce meaningful data by AMS, since both total carbon and ^{14}C are measured to generate an isotope ratio. With some samples, it may be impossible to achieve the desired carbon amount from the experimental preparation. In this case, it may be necessary to add “carrier carbon” to these ultralow carbon mass samples. The primary source of carrier carbon now in use is tributyrin (glycerol tributanoate). This substance has the desirable characteristics of nonvolatility (bp $>300^\circ\text{C}$), high carbon content, and low ^{14}C content. A typical addition of carrier carbon occurs by adding 1 or 2 μl of tributyrin in capillary tubes to the biological sample. It is always advisable to analyze each new type of sample for carbon content before graphitization to ensure that a proper carbon inventory is maintained.

21.3.4.5 Data Analysis and Interpretation. AMS reports result in Modern, which is a defined unit of $^{14}\text{C}/\text{C}$ isotope concentration equal to

- $1.180 \times 10^{-12} \text{ }^{14}\text{C}/\text{C}$,
- 13.56 mdpm/mg C,
- 226 $\mu\text{Bq}/\text{mg C}$, and
- 97.89 amol/mg C.

Conversion of this number to a meaningful result requires careful inventory of the carbon sources in the sample. For the simplest case in which a sample contains sufficient carbon as not to require the addition of carrier carbon, this conversion is facile. In many experiments, it is necessary to add carrier to make up the total amount of carbon necessary for the graphitization process to occur. In these experiments, the fraction Modern and the carbon mass of both the sample and the carrier must be considered in the calculation. Correctly accounting for all sources of carbon in the analyzed sample is essential, as a low estimate of carbon will artificially increase the apparent quantity of tracer, and an overestimate of carbon will deflate the apparent amount of tracer present.

The measured ratio is a composite value that includes the total amount of ^{14}C and the total carbon present in the measured sample. It is necessary to know the quantity of carbon and ^{14}C in the sample tissue, as well as the amount of carbon due to the tracer, in order to solve for the ^{14}C due to the tracer. If the only sources of carbon in the measured sample are the tracer and the tissue, determining the ^{14}C due to the tracer proceeds as follows:

$$R_{\text{Measured}} = \frac{{}^{14}\text{C}_{\text{Measured}}}{\text{C}_{\text{Measured}}} = \frac{{}^{14}\text{C}_{\text{tracer}} + {}^{14}\text{C}_{\text{tissue}}}{\text{C}_{\text{tracer}} + \text{C}_{\text{tissue}}} \quad (21.1)$$

In most cases, the carbon due to the tissue is much greater than the carbon due to the tracer, so that the tracer carbon can be neglected in the calculation:

$$R_{\text{Measured}} = \frac{{}^{14}\text{C}_{\text{tracer}} + {}^{14}\text{C}_{\text{tissue}}}{\text{C}_{\text{tissue}}} \quad (21.2)$$

Because the measured fraction Modern is a composite of the contribution of the tissue and the contribution of the tracer, rearranging to solve for the tracer ${}^{14}\text{C}$ gives

$${}^{14}\text{C}_{\text{tracer}} = \text{C}_{\text{tissue}} \times (R_{\text{measured}} - R_{\text{tissue}}) \quad (21.3)$$

where

$$R_{\text{tissue}} = \frac{{}^{14}\text{C}_{\text{tissue}}}{\text{C}_{\text{tissue}}} \quad (21.4)$$

If carrier carbon is also present, the general equation is as follows:

$$R_{\text{Measured}} = \frac{{}^{14}\text{C}_{\text{tracer}} + {}^{14}\text{C}_{\text{tissue}} + {}^{14}\text{C}_{\text{carrier}}}{\text{C}_{\text{tracer}} + \text{C}_{\text{tissue}} + \text{C}_{\text{carrier}}} \quad (21.5)$$

In this case, the carrier carbon is usually much greater than the carbon due to the tissue or the tracer, allowing these quantities to be neglected in the calculation:

$$R_{\text{Measured}} = \frac{{}^{14}\text{C}_{\text{tracer}} + {}^{14}\text{C}_{\text{tissue}} + {}^{14}\text{C}_{\text{carrier}}}{\text{C}_{\text{carrier}}} \quad (21.6)$$

which becomes

$${}^{14}\text{C}_{\text{tracer}} = \text{C}_{\text{carrier}} \times [R_{\text{measured}} - (R_{\text{tissue}} + R_{\text{carrier}})] \quad (21.7)$$

21.3.5 Specific Applications of Accelerator Mass Spectrometry

AMS excels at detecting very low levels of labeled compounds of interest and has been applied to a variety of types of experiments applicable to drug development. The common feature of these experiments is the ability to trace the fate of a labeled compound in a biological system. This can include determination of PK parameters, biotransformation of the labeled compound, formation of covalent adducts with cellular macromolecules, drug–ligand interactions, and tissue or subcellular localization of the compound of interest. Such experiments can provide valuable information about the pharmacological and toxicological characteristics of investigational compounds. AMS may likewise be used to investigate metabolism of endogenous, naturally occurring compounds within a biological system without perturbing the normal physiology of the system. The types of experiments listed below are by no means exhaustive but are intended to give the reader a sense of the potential value of AMS in the drug development process.

21.3.5.1 Pharmacokinetics. Several studies have compared the PK profiles of drugs using standard therapeutic doses and microdoses [14,21,26,31,45,51,67,68]. In these microdose experiments, a subtherapeutic level of a ^{14}C -labeled investigational compound is administered to animals or human volunteers, and the plasma levels of the compound are measured over time to determine the PK parameters of the compound. These experiments are essentially the same as traditional “dose and measure” PK experiments, but the investigational compound can be administered and measured at much lower levels than are possible using standard techniques. This allows for human studies to be performed without the risk of harmful effects that could occur with the administration of higher doses. While the utility of such microdosing experiments as a replacement for standard PK techniques remains a matter of discussion, several reports indicate that microdosing may be useful as a screening tool for drug candidates [14,21,26,67,69]. Microdosing may be particularly useful when a drug candidate presents with conflicting *in vitro* and animal PK data [67]. An understanding of the PK parameters of a drug candidate may disqualify the compound for further investigation based on a poor PK profile or may provide guidelines for predicting optimal doses within the therapeutic range. The plasma concentrations following oral and IV therapeutic (50 mg) and microdoses (100 μg) of sumatriptan are shown in Fig. 21.2 [26]. Despite the doses being different by a factor of 500, the dose normalized curves are very similar with the maximum plasma concentration occurring at about an hour. The biological half-life, volume of distribution, and clearance were all similar also [26]. The oral microdose had better bioavailability (20%) than the therapeutic dose (7.6%) [26].

In planning and conducting PK studies using AMS as a measurement technique, it is essential that an appropriate dose of the labeled material be administered to the test subject in order to allow accurate measurement at the selected time points. Assuming that the quantity of drug to be administered is significantly below the level required to elicit a pharmacological or toxic effect, dosing can be calculated based on the level of ^{14}C required to detect an adequate signal in the collected sample. If higher levels of drug are to be administered, the dose should be calculated based on the therapeutic dose

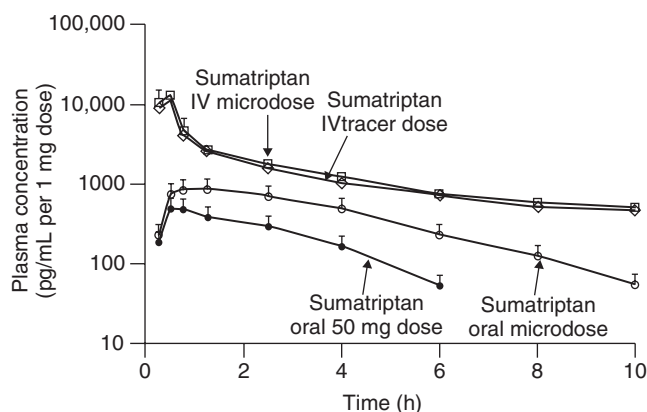


Figure 21.2 Semilog plots of mean plasma sumatriptan concentration–time profiles following a single oral dose of 100 μg (\circ), a 30-min IV infusion of 100 μg (\square), a single oral dose of 50 mg (\blacklozenge), and a 30-min IV infusion of 100 μg with a simultaneous oral dose of 50 mg (\diamond). Data are dose normalized to a 1-mg dose and error bars are +1 standard deviation. *Source:* (a) p. 144 from Lappin *et al.* [26].

range in addition to the radiological dose. The general steps involved in performing a PK study using AMS are as follows:

- Determine the specific activity of the ^{14}C -labeled compound of interest to be used in the PK study.
- Determine the mode(s) of administration of the compound of interest (injection, oral, dermal, subcutaneous, etc.). If appropriate, estimate the bioavailability of the compound.
- Determine the anticipated length of time the compound should be followed, the number of samples to be collected (blood and urine) and the sampling interval.
- Determine the minimum target fraction Modern to be achieved in the sample (blood and urine) at the final time point. In most cases, this level should be at least 10% above 1 Modern in order to allow appropriate resolution.
- Calculate the initial dose based on the estimated number of biological half-lives of the compound to produce the desired ending fraction Modern. Determine the total radiological exposure to be achieved using the calculated initial dose, assuming that the subject will absorb all radioactive decay energy. Consult with a health physicist to ensure that this level of exposure is acceptable based on current regulations and requirements. For purposes of assessing the radiological burden, a human can be assumed to have a normal ^{14}C level of 1 Modern, which for a 70-kg person corresponds to an energy deposit of 110 nJ/h, and assuming that the radiological dose is uniformly distributed throughout the body, this corresponds to 1.6 nSv/h [54].
- Use animal dosimetry data to initially estimate a compound's bioavailability and biological half-life, calculate the initial dose based on the target highest amount of label to be present in the sample, and proceed to calculate the total radiological exposure.
- The initial samples collected should be measured by LSC to ensure that the calculations were correct and that the level of dosing was correct for AMS measurement.

21.3.5.2 Biotransformation and Conjugate Formation. Identification of metabolites of a drug candidate or other molecule of interest can be greatly facilitated by AMS. Tracing of a ^{14}C label allows unequivocal identification of compounds originating from the ^{14}C -labeled parent compound, including metabolites and degradation products, even if the structures of these compounds are not previously known. This type of experiment may be performed by collecting blood and urine and separating components by HPLC or other separation techniques and identifying the parent compound as well as any metabolite or conjugate by measuring ^{14}C in the HPLC fractions.

Appropriate separation techniques are essential for the identification of metabolites. Although the presence of label introduced as the parent compound unambiguously demonstrates the source of the label, it is important to ensure that the collected material is reasonably pure to allow further characterization of novel metabolites. In many instances, the metabolites of a compound of interest may be unknown or incompletely characterized. A typical metabolism study consists of the following components:

- Characterize chemical purity, radiopurity, and specific activity of the ^{14}C -labeled compound of interest.

- Administer the labeled material and collect samples (blood, urine, tissue, etc.).
- Perform mass balance to account for all labeled material.
- Separate metabolites by standard techniques (HPLC, electrophoresis, etc.).
- Remove any solvents used in separations processes and analyze the sample fractions by AMS, adding carrier as necessary. In the case of HPLC fractions, the amount of carbon present in each sample is usually negligible compared to the amount of carrier carbon.
- Analysis occurs by comparison of cochromatography with authentic standards of detector peaks on the HPLC (e.g., UV) with AMS results to identify fractions containing the parent compound, its metabolites, and conjugates.

Glucuronide conjugation occurs with many drugs and it can be the single largest metabolite as shown in Fig. 21.3 [45]. Following a microdose of ^{14}C -acetaminophen, parent compound and major metabolites were separated by LC and fractions were analyzed by AMS [39]. In many studies, unexpected metabolites or conjugates are formed and labeled peaks elute at times different than the available standards [29,56]. Careful analysis of the elution spectrum can help narrow the possible metabolites. The ^{14}C signal clearly distinguishes the peak as deriving from the test compound and not an endogenous compound that happened to elute.

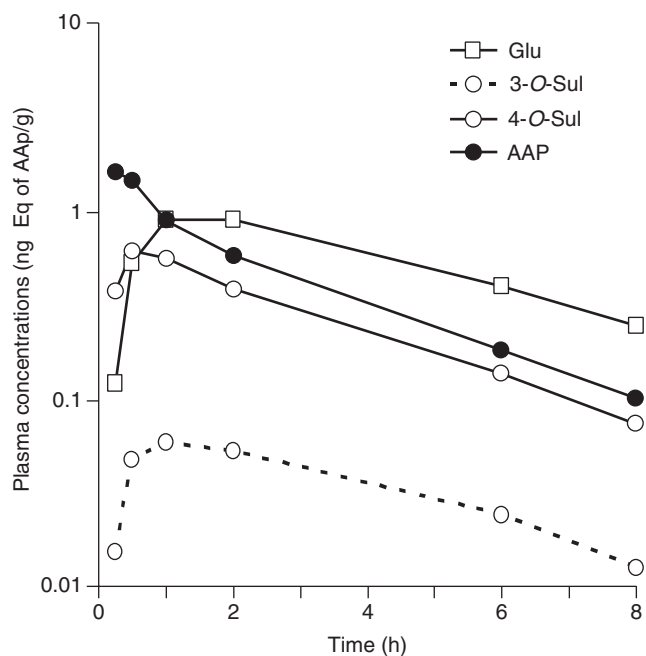


Figure 21.3 Time profiles of acetaminophen (AAP) and its metabolites in the pooled plasma of six subjects after oral administration of ^{14}C -AAP. Pooled plasma specimens collected at 0.25, 0.5, 1, 2, and 8 h were subjected to LC-AMS analysis. 3-*O*-Sul, AAP-3-hydroxysulfate; 4-*O*-Sul, AAP-4-hydroxysulfate; AAP, acetaminophen; Glu, AAP-glucuronide; LC-AMS, liquid chromatography–accelerator mass spectrometry. *Source*: p. 221 from Bauer *et al.* [85].

21.3.5.3 Protein and DNA Adduct Measurement for Determination of Reactive Metabolites. Incorporation of label into protein or DNA is a useful indicator of adduct formation or noncovalent binding of the moiety of interest. This approach has been used very successfully in the evaluation of known or potential carcinogens that exhibit DNA-binding activity [47,48,70,71]. This type of experiment may be particularly valuable in assessing the potential toxicity, mutagenicity, or carcinogenicity of a compound of interest. For example, AMS was used to detect DNA adducts in the colon after humans were exposed to a dietary-relevant dose of the cooked food carcinogen PhIP [47]. It may also be important to determine the quantitative fate of compounds that can form reactive intermediates capable of binding to cellular macromolecules, for example, compounds that can form immunogenic haptens or glutathione conjugates. Experiments of this type can also be designed to distinguish covalent and noncovalent interactions. As in the types of experiments described in previous sections, it is absolutely essential that the sample be thoroughly characterized and as pure as is reasonably achievable to ensure that results are not confounded by the presence of contaminating materials. These experiments include the following parameters:

- Determine the tissue to be sampled.
- Calculate the dose of compound to administer and the quantity of tissue to collect.
- Administer the dose and collect sample material.
- Separate and quantify the biomolecule of interest (protein and DNA).
- Perform AMS measurement on the sample.

21.3.5.4 Drug–Ligand Binding and Subcellular Localization. Tracing a radiolabel compound into tissues, cells, and even subcellular compartments is possible using AMS [69,72,73]. These experiments may be of great importance in determining the site of action and specific molecular target(s) of a drug or other compound of interest. Identifying the site of action or the molecular target of a drug candidate is very important in characterizing its biological activity and elucidating its mechanism of action. Such experiments could be a valuable screening tool to help in the selection of lead compounds for development as investigational drugs or to indicate that a compound has undesirable binding or localization characteristics early in the drug development process. As with other types of experiments, the sample material must be thoroughly characterized before AMS analysis. The steps involved in these types of experiments are similar to those described in the previous section but may include cellular fractionation in addition to isolation of target biomolecules.

21.4 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY IN MICRODOSING

21.4.1 Overview of Liquid Chromatography-Mass Spectrometry

In addition to AMS, liquid chromatography-mass spectrometry (LC-MS) is also used to analyze samples in microdosing studies. Each method provides advantages but also has limitations. The previous section of this chapter described in detail the use of

AMS for microdosing experiments. This section describes the use of LC-MS/MS in microdosing studies, highlighting its advantages and disadvantages compared to AMS.

All microdosing experiments require analytical methods capable of identifying and quantifying the analyte(s) of interest in biological samples at low levels. In some cases, it may only be necessary to measure the administered (parent) compound. More often, the parent compound forms one or more metabolites that must be characterized and measured. Metabolites of interest may be formed at low concentrations relative to the parent compound. This can present significant analytical challenges in microdosing studies, because the parent compound is administered at low doses that may already be near the lower limit of quantitation (LLOQ) for most instruments. Thus, a method that is suitable for measurement of a parent compound in a microdosing study may not provide adequate sensitivity to measure low abundance metabolites, particularly those metabolites with uncharacterized structures [74]. AMS provides unrivaled sensitivity for measurement of ^{14}C -labeled compounds in biological samples and can detect labeled metabolites in an LC elution with *a priori* knowledge, but it provides only quantitation. Samples measured by AMS must be structurally characterized using other analytical methods. In contrast, LC-MS can identify compounds of interest, and can quantify material, but lacks the sensitivity of AMS. The specific questions that need to be answered, and the level of sensitivity required to answer those questions, must guide the researcher's choice of analytical methods to be used in microdosing studies.

21.4.2 Advantages and Limitations of Liquid Chromatography-Mass Spectrometry

Some general considerations can be applied in selecting the appropriate analytical methods for use in microdosing experiments. These considerations include types of instrumentation available and the capabilities of each instrument, the level of sensitivity needed, analytical methods that must be developed to enable microdosing studies, dosing considerations, and cost of performing microdosing studies. It should be noted that many studies have used both LC-MS and AMS to obtain the desired information.

21.4.2.1 Instrumentation. Many research facilities have LC-MS equipment, while very few have AMS instruments in-house. Both types of instrumentation require skilled and knowledgeable operators, and each instrument requires a specific type of sample preparation. AMS instruments measure samples in the form of graphite or CO_2 gas, while LC-MS instruments measure samples that are injected into the instrument in liquid form. Methods for preanalysis sample cleanup and processing will depend on the needs of the particular experiment being performed. In addition to providing quantitative information, LC-MS allows molecular characterization of parent drug and metabolites. Unlike LC-MS, AMS requires the use of a ^{14}C -labeled test compound and gives results in the form of an isotope ratio. AMS provides quantitation and requires that analytes be separated and characterized by other means before AMS analysis. AMS measures analytes that contain the ^{14}C label irrespective of their chemical structures, which can result in measurement of both parent compound and metabolites if adequate separation of these compounds is performed before AMS analysis. LC-MS can distinguish between a parent compound and its known metabolites with different m/z ratios but may not be sufficiently sensitive to detect low abundance metabolites.

Through chromatographic retention time and MS/MS, an observed m/z can be compared to a reference standard of the parent. LC-MS cannot resolve endogenous isobaric interferences, however, so discovering and identifying unexpected metabolites is very difficult. In detecting metabolites by LC-MS, it is helpful for the researcher to know what types of metabolites are likely to occur in order to optimize instrument parameters for the measurement of each metabolite.

21.4.2.2 Sensitivity. The typical analyte concentration range for LC-MS instruments is in the order of nanograms per milliliter sample [74–76], although some instruments can measure analytes in the picogram to femtogram per milliliter range [76,77]. In contrast, AMS instruments provide the highest level of sensitivity currently available and can measure from analytes in the range of femtograms to attograms per milliliter [74,75,78]. While AMS allows greater sensitivity than any other available methods, it is also extremely sensitive to minute quantities of contaminating material, necessitating great care in performing experiments and handling samples. Depending on the design of the study, the utility of AMS may be limited by the fact that all sources of ^{14}C label are measured, so that both the parent compound and the metabolites are measured [77]. Thus, caution must be exercised in interpreting the results of those microdosing studies in which parent compound and metabolites are not adequately separated and characterized. This problem can often be circumvented by the use of HPLC separation of the parent drug and its metabolites, followed by AMS analysis of each collected fraction. However, collection of HPLC fractions can be problematic due to the possibility of peak overlap [77].

21.4.2.3 Analytical Method Development and Sample Preparation. Both AMS and LC-MS methods typically require methods for extracting the test compound or its metabolites from a biological matrix such as plasma or urine. AMS has the advantage of being able to detect and quantify the ^{14}C -labeled test compound in the biological matrix without extraction if quantitation is all that is desired. AMS analysis does not require the use of internal standards or the generation of standard curves for the analyte(s). LC-MS requires the development and validation of an analytical method with a known linear range and lower limit of detection for each analyte and makes use of internal standards for analyte quantitation. MS can be linked directly to chromatography, unlike AMS, thereby mitigating concerns about loss of material as could occur in pre-AMS chromatography and fraction collection. Both AMS and LC-MS may require considerable sample processing before analysis. For AMS analysis, the possibility of contaminating samples with ^{14}C from other sources must be minimized.

21.4.2.4 Dosing Considerations. While both LC-MS and AMS microdosing studies involve the administration of small quantities of a substance to test substance, the test compound in AMS experiments contains a ^{14}C label, which presents extra challenges to ensure that the label is in a structurally stable position. The additional sensitivity provided by the use of a radioisotope-labeled test compound can enable detection of low abundance metabolites, as discussed above, and can allow more frequent sampling than would be possible if LC-MS detection was used. The ability to measure very low levels of analyte by AMS can also enable experiments of greater duration than is possible with LC-MS-based experiments. Conversely, the potentially long washout time for ^{14}C -labeled test compounds may limit the use of multiple dosing experiments or crossover studies.

21.4.2.5 Cost. In addition to the direct sample analysis costs, the time required to prepare test material, design and perform experiments, and complete analyses affects the total cost of microdosing studies. In-house equipment can speed up experiments and give results in a shorter time frame than is typically possible with AMS experiments. The high sensitivity that can be achieved by AMS requires a ^{14}C -labeled test compound, which increases the cost and time necessary to complete microdosing experiments. Additionally, AMS analysis following HPLC separation and fraction collection is limited by the relatively low throughput of this technique. AMS-based microdosing experiments are inherently more expensive than LC-MS-based experiments, but AMS can provide significant advantages that offset the additional costs. In some cases, AMS is the only analytical method available that has the sensitivity to provide the needed information.

21.4.3 Examples of Liquid Chromatography-Mass Spectrometry Microdosing

The ability to simultaneously identify and quantify a circulating drug or metabolite has made LC-MS microdosing an attractive alternative to AMS-based microdosing experiments. While not all drugs are suitable for LC-MS microdosing studies, several proof-of-principle experiments have been performed to demonstrate that LC-MS microdosing can be a viable method. The continuous improvements in instrument sensitivity made in recent years have enabled detection and quantitation of compounds at levels that were not previously possible. While quantitation by mass spectrometry is inherently problematic, it may be adequate in many cases to answer the critical questions in microdosing studies.

Ni *et al.* [68] investigated the utility of microdosing using LC-MS/MS by measuring rat plasma and urine metabolites using the drugs atorvastatin, ofloxacin, omeprazole, and tamoxifen with administered doses ranging from $1.67\ \mu\text{g}/\text{kg}$ (microdose) to $5000\ \mu\text{g}/\text{kg}$ (standard dose). Their analytical instrumentation included a 5500 QTRAP SYSTEM (AB Sciex, Concord, ON, Canada), with chromatographic separation on either a Shimadzu Prominence instrument (Columbia, MD) or an Agilent 1200 system (Palo Alto, CA). Quantitation was obtained using Analyst software (version 1.5, AB Sciex, Concord, ON, Canada), and metabolites were identified using LightSight software, version 2.2 (AB SCIEX). In the microdosing experiments, the researchers were able to characterize the hydroxylation metabolite of omeprazole, the hydration metabolite of tamoxifen, and the glucuronide metabolite of ofloxacin, while additional metabolites were identified at higher doses. These researchers reported that low dose PK parameters were not necessarily predictive of PK parameters at higher doses in the rodent model [74]. Similar experiments using additional drugs provided further support for the use of LC-MS/MS for microdosing experiments [79].

Yamane *et al.* [80] used LC-MS/MS to measure nicarpidine in humans and developed a method capable of measuring in the linear range of $1\text{--}500\ \text{pg}/\text{mL}$ for a microdose and up to $0.2\text{--}100\ \text{ng}/\text{mL}$ plasma for a clinical dose. This experiment used a microdose of $100\ \mu\text{g}$ per patient and a clinical dose of $20\ \text{mg}$. They used mass spectrometry to identify metabolites following the microdose and compared these results with the metabolites identified using the clinical dose [80]. Yamazaki *et al.* [81] performed microdose studies in eight humans with $100\ \mu\text{g}$ of fexofenadine and found that microdose PK measured using LC-MS predicted the PK of a 60-mg therapeutic dose satisfactorily.

Balani *et al.* [75] demonstrated that useful PK data could be obtained for the drugs fluconazole and tolbutamide using a microdose of 1 $\mu\text{g}/\text{kg}$ in rats and compared the linearity of PK with oral doses of 0.002, 0.01, 0.1, and 1 mg/kg of tolbutamide or 0.005, 0.05, and 5 mg/kg of fluconazole. Using LC-MS analysis, they reported 0.1 nM as the LLOQ for fluconazole and 1 nM as the LLOQ for tolbutamide. On the basis of adequate detection of test compounds by LC-MS and linear PK between microdoses and higher doses for both drugs, it was suggested that LC-MS/MS could provide adequate sensitivity for some human microdosing studies [75].

21.4.4 Summary

The decision to use LC-MS/MS or AMS must be informed by knowledge of the advantages and limitations of each method and primarily by the questions the microdosing study need to address. For further guidance in designing microdosing experiments, the interested reader is referred to the excellent review by Ings [77]. As the development of mass spectrometers with improved sensitivity continues, LC-MS is likely to find additional applications in microdosing experiments, but AMS is expected to remain the method of choice for many microdosing applications requiring high sensitivity.

21.5 POSITRON EMISSION TOMOGRAPHY IN MICRODOSING

21.5.1 Overview of Positron Emission Tomography

PET is widely used in medical imaging applications. In PET, a radioisotope that decays by positron emission is used to determine the location of the isotope in the test subject. Positrons react with electrons in tissue to annihilate each other and release two 511-keV photons at 180° from each other. An array of photon detectors located around the subject measure the simultaneous photons, and computers are used to reconstruct the position of the decay within the tissue with spatial resolution of ~ 4 mm [22,82]. In microdosing applications, PET can be used to monitor distribution and clearance of a test compound in real time.

Positron-emitting radioisotopes need to be produced with a cyclotron and are short lived. The common positron-emitting isotopes for microdosing studies are ^{11}C (radioactive half-life $T_{1/2} = 20$ min) and ^{18}F ($T_{1/2} = 110$ min). ^{11}C is most useful since nearly all drugs contain carbon. Because of the short radioactive half-lives, cyclotrons must be located at medical facilities. After production of ^{11}C , it is typically exchanged with ^{12}C in a drug molecule in high activity radiochemistry facility also on-site to produce specific compounds that can be distributed and traced. The short radioactive half-lives require that the chemistry is rapid, and significant shielding is needed to protect chemists synthesizing the compounds. Finally, a PET scanner must be available on-site to measure the photons produced by positron–electron annihilation. After seven radioactive half-lives, an isotope decays to about 0.7% of its initial activity and is usually too weak for useful tracing. For ^{11}C , this is only 2.5 h, limiting PET analyses to initial distribution and rapidly clearing compounds. Since PET depends on the radioactive decay for measurement, it delivers a low to moderate radiation dose to test subjects. PET studies generally describe a normalized tissue deposition of a compound using standardized uptake values (SUVs). SUVs normalize the accumulated compound

in a tissue as measured by PET to the body weight of the subject and the injected dose [83]. Traditional PK parameters can be measured shortly after administration of the labeled tracer before its activity drops below quantitation limits.

21.5.2 Examples of Positron Emission Tomography Microdosing Studies

The advantage of PET over AMS and LC-MS is real-time visualization of distribution and concentration of the test compound in all tissues. PET can provide some PK information, but the rapid decay of the radioisotopes can only assess rapidly cleared compounds and metabolites. In general, PET is ill suited for metabolite analysis because of the time required for analysis and the low levels of compound in the small samples analyzed. The development of robust ultra performance liquid chromatography (UPLC) systems in the past few years with much shorter elution than traditional HPLC system may make PK of positron-emitting isotopes more practical. PET does not provide precise quantitation like AMS or structural information like LC-MS, it tells you if the test compound is concentrating in a particular tissue. The *in vivo* visualization is particularly useful when targeting a specific tissue such as CNS or diseased tissue such as cancer [84].

21.5.2.1 Tracing Across the Blood–Brain Barrier. PET has been used on several occasions to monitor the transport of a compound across the blood–brain barrier (BBB). A microdose study was conducted with the ^{11}C -labeled antiamyloid drug (ST1859) in healthy and patients afflicted with Alzheimer’s disease (AD) [85]. The test compound crossed the BBB relatively quickly and slows to washout in both control and AD groups, good qualities for a compound targeted at amyloid plaques. The study was not designed for detecting differences in the control and AD groups, but it appeared as though differences were present (Fig. 21.4). The AD group had an earlier maximum concentration in most brain regions and higher radioactivity in the brain than the control, suggesting differences in the BBB of the two groups [85].

A comparison of therapeutic dose (80 mg) and microdose (0.05 mg) of verapamil labeled with both ^{11}C and ^{14}C for PET and AMS analyses was conducted to investigate linearity [21]. PET measured similar rate constants across the BBB and distributed volumes for each dose [21]. ^{14}C concentration–time profiles in plasma were also similar for each dose [21]. A much earlier study with [^{11}C]raclopride saw similar cerebellum to blood concentrations with a microdose (1–2 μg) and a pharmacological dose (200–400 μg) [86].

21.5.2.2 Targeting Cancer. The development of new cancer drugs depends on identifying markers of the cancer and concentrating the drug at a tumor site compared to systemic circulation. PET is ideally suited for assessing drug distribution *in vivo* and determining if a cancer drug does indeed target the tumor preferentially compared to healthy tissue. PET microdosing offers the potential to obtain PK parameters in individual patients before commencing a therapeutic dose regimen or a noninvasive way to assess response or stage of a tumor with specific markers [84,87].

Although not strictly a microdose application, small doses of 16α - ^{18}F -fluoro-17 β -estradiol (FES) have been used to assess estrogen receptor (ER) status [88]. ER status of breast cancer can be assessed *in vivo* because FES is a ligand for ER [88]. Additionally, ^{11}C -colchicine has been used in preclinical PET studies to image multidrug resistance

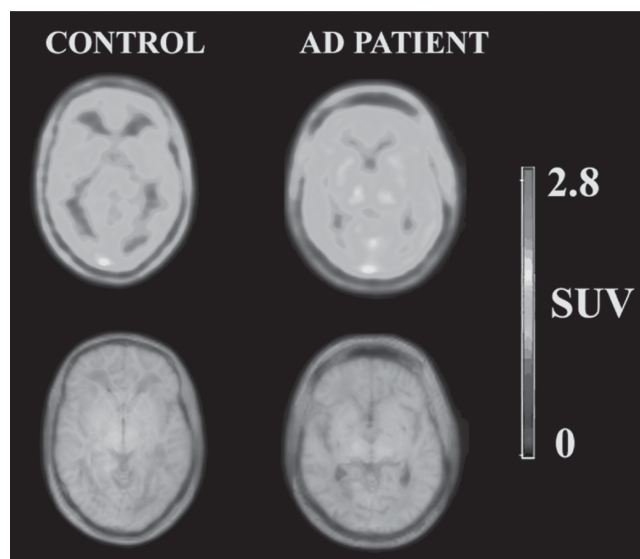


Figure 21.4 Transaxial magnetic resonance–coregistered positron emission tomography (PET) summation images recorded from 20 to 90 min after intravenous injection of [^{11}C]ST1859 into 1 control subject (HV 1) (left) and 1 AD patient (patient 9) (right). The lower row shows the same PET images with the transparency of the superimposed magnetic resonance scan set to 50%. The radioactivity concentration is normalized for injected radioactivity per body weight and expressed as the SUV. *Source:* p. 829 from Tozuka *et al.* [45]. (See color insert.)

and identify tumors that will not respond to some chemotherapy agents [89]. ^{18}F -Fluoride has also been used for bone imaging [90].

An early pre-phase I study that used one-thousandth of the phase I starting dose of ^{11}C -N-[2-(dimethylamino)ethyl]acridine-4-carboxamide showed tissue distribution, tumor concentration, and early time point PK data were the first microdose PET study [91]. Combining PET with microdialysis to assess unbound drug in extracellular spaces has been done and may become more common in the future [92].

21.5.3 Summary

PET provides unparalleled *in vivo* distribution information with very low chemical doses. The short radioactive half-lives of positron emitters prevent acquisition of PK information in many cases.

21.6 CONCLUSION

Each bioanalytical technique employed in microdosing studies has its strengths and weaknesses (Table 21.2). LC-MS/MS is the least expensive and most widely available of the techniques. In most cases, if LC-MS/MS has the sensitivity to measure the test compound accurately, it is the preferred method. LC-MS/MS requires *a priori* knowledge of metabolites, however, and cannot definitively distinguish test compound from other molecules. AMS is most quantitative of the techniques. It is expensive to use and relatively few facilities are in operation. PET is at best semiquantitative, but

TABLE 21.2 Comparison of Bioanalytical Methods Suitable for Microdosing Studies

Attribute	AMS	LC-MS/MS	PET
Sensitivity limit (g)	10^{-18}	10^{-12}	10^{-14}
Linear range	Five to six orders of magnitude	Three to four orders of magnitude	Three to four orders of magnitude
Sample matrix measured	Plasma PK only	Plasma PK only	Plasma and tissue PK
Tissues sampled	Blood, urine, skin, saliva, exhaled CO ₂ , cerebrospinal fluid, feces, biopsy when available	Blood, urine, skin, saliva, cerebrospinal fluid, feces, biopsy when available	Tissues imaged, no sampling required
Radiation dose	Very low, generally microsievert	None	Low to moderate, millisievert
Radiolabel required	Long-lived β -emitter, ¹⁴ C	None	Short-lived β + emitter, ¹¹ C or ¹⁸ F
Chemical separation for metabolite analysis	Chemical separation of drug and metabolites possible. Metabolites quantifiable without analytical standards	Chemical separation of drug and metabolites possible. Structural information of metabolites possible. Metabolite quantification requires analytical standards	No chemical separation of tissue signal
Drug administration	Intravenous or oral usually, dermal and inhalation possible	Intravenous or oral usually, dermal and inhalation possible	Intravenous usually, oral or inhalation possible
Sampling periods	Limited by plasma clearance, minutes to months	Limited by plasma clearance and sensitivity, minutes to days	Limited by short half-life of positron emitters, up to 1.5 h
Costs	High cost, limited availability	Low cost, greater availability	High cost, limited availability
Outsource	Analysis can be contracted	Analysis can be contracted	Analysis must be done in-house

in vivo imaging allows analyses of distribution and accumulation of test compounds at target sites. Combining PET with LC-MS/MS or AMS provides the most information in a microdose study.

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