

WINNOWING OUT DRUG CANDIDATES

Accelerator mass spectrometry may help identify earlier than ever which drugs are destined to fail



NATURAL EVOLUTION Now a hot tool in drug development, AMS was first used for biological tracing by researchers at this facility at Lawrence Livermore National Laboratory.

LAWRENCE LIVERMORE NATIONAL LAB PHOTO

It's not every day that archaeologists can claim provenance of a valuable drug discovery tool. But it was archaeologists' quest for more accurate dating of old bones that led to the development of accelerator mass spectrometry (AMS), a technique that's now attracting the interest of drugmakers who are hoping to better understand the way drug candidates are taken up, broken down, and excreted from the body.

"Archaeologists drove the development of AMS," says [John Vogel](#), a physicist at Lawrence Livermore National Laboratory who helped develop the technique. Previously, radiocarbon dating methods relied on scintillation counting to measure the radiation emitted from decaying ^{14}C in a given

bone, he notes. "But archaeologists had to destroy a whole bone to get enough of this long-lived radioisotope to get a good date."

So when AMS was first developed in the 1970s, it quickly won the hearts of archaeologists. Instead of measuring the radiation emitted from decaying ^{14}C , AMS allows direct, quantitative detection of ^{14}C atoms themselves. In practice, this means that AMS-based carbon dating requires samples containing only attomole (10^{18} mole) to zeptomole (10^{21} mole) quantities of ^{14}C . A tiny sample of a precious old bone often will suffice.

Vogel's team later pioneered the use of AMS for biological tracing, using the technique to track the fate of DNA-damaging carcinogens and pollutants and to follow vitamin metabolism. Now, AMS has begun to earn converts in the pharmaceutical industry. "AMS may reduce the cost of bringing a new drug to market by winnowing out failures early on," reasons Colin Garner, chief executive officer of [Xceleron Ltd.](#), a York, England-based AMS services company.

"Too often, animal models fail to predict how humans will absorb and metabolize drug candidates," Garner explains. Pharmaceutical companies routinely use inbred animals, human tissue, or computational methods to define a drug candidate's absorption, distribution, metabolism, and excretion (ADME) characteristics. But it's notoriously difficult to extrapolate how the drug will behave in humans from these studies, notes James W. Harris, chief scientific officer of [Bioavailability Systems](#), Cocoa Beach, Fla. In fact, animal models--the most popular of the three--fail to predict human pharmacokinetics and metabolism for more than a third of the new chemical entities that enter clinical trials, Garner estimates.

AMS, however, allows collection of human ADME data before costly Phase I clinical trials, Garner notes. Patients or volunteers are given a trace dose of a drug candidate that has been isotopically labeled with ^{14}C . The carbon in small samples of the volunteer's blood, urine, and feces (and in some rare cases, biopsy samples) is then subjected to AMS

analysis. The quantity and distribution of ^{14}C in these samples gives information about how the candidate is absorbed, distributed, and excreted in humans. Separating the samples by chromatography prior to AMS analysis can provide information about the extent to which the candidate is metabolized.

"Humans are the best model for humans," Harris says. So-called microdosing studies could prove invaluable to the pharmaceutical industry, if proponents can prove that AMS can indeed predict ADME properties for a range of different molecules and if regulatory agencies give their seal of approval, he adds.

These hurdles are already falling, says Ali Arjomand, chief operating officer of Seattle-based [Accium Biosciences](#), a small company that offers a range of AMS services. The amount of radiation that human volunteers are exposed to during such studies is negligible, he points out, noting that the typical microdose delivers just 100 nanocuries of radiation, the amount you'd get from eating 100 bananas or flying in a plane at 30,000 feet for several minutes.

In 2003, European regulators encouraged exploration of microdosing, allowing drug candidates to be administered to humans in microdosing studies at doses less than 1/100th of the dose calculated to yield a pharmacological effect (maximum of 100 μg). The [Food & Drug Administration](#) issued similar draft guidance this spring, a move widely seen to give the green light to microdosing in the U.S.

To test the technique's worth, an international team led by Xceleron conducted a trial in Europe in which human subjects were given microdoses of five molecules for which animal studies had failed to predict human pharmacokinetics. Fluid and fecal samples were taken and analyzed by AMS. The results of the trial were released last month.

OVERALL, microdosing trumped traditional methods in providing more accurate information about human pharmacokinetics at pharmacological

doses, the results show. Microdosing accurately predicted that diazepam, a benzodiazepine sedative, is poorly excreted in humans and that midazolam, another benzodiazepine sedative, is rapidly degraded in humans. The technique also accurately predicted that humans do not absorb ZK253, a proprietary [Schering AG](#) compound that was abandoned during clinical trials because of poor bioavailability.

The trial also revealed several potential limitations of the technique. Although microdosing accurately predicted that warfarin, an anticoagulant, would stick around for a long time in the body, the kinetics of this process at microdoses proved very different from the kinetics at pharmacological doses. "This nonlinearity may be a result of the high dose overloading enzymes or pathways, where the low dose does not," Vogel says. "Although there may be differences in pharmacokinetics as you change dose, the differences between animals and humans are likely to be even bigger."

With another compound (erythromycin), the ^{14}C label proved to be acid labile, and no useful data were obtained. This disappointment serves as a reminder of the importance of planning synthetic schemes for radiolabeling, Vogel notes. "Many times, you can't make the ^{14}C -labeled compound the same way you make the unlabeled compound." But because AMS is so sensitive, a low-yielding route often will suffice, he adds.

Despite these caveats, the trial's chair, distinguished pharmacologist Malcolm Rowland of the University of Manchester, in England, comments that the results suggest that "microdosing coupled with AMS offers a promising additional tool to facilitate decisions at an early stage in candidate selection. More data with a wider range of compounds will help to further clarify the uses and limitations of the approach."

A handful of companies are already applying AMS microdosing to help them select or discard drug candidates. [Speedel](#), a small Swiss biopharmaceutical company, has used the technique

"to quickly assess bioavailability in humans of three second-generation renin-inhibitor candidates for hypertension," says J. Chris Jensen, the company's director of pharmacology. This microdosing study, he notes, allowed the company to focus its resources on its most promising candidate.

Similarly, positive bioavailability information obtained from a 2004 microdose study gave Swedish biotechnology firm [Tripep](#) the confidence to advance its anti-HIV compound alphaHGA to Phase II clinical trials, which are currently under way in Thailand. The microdose study was performed by [Vitalea Science](#), a small Davis, Calif.-based company that offers a host of AMS-based services, and Nottingham, England-based [Pharmaceutical Profiles Group](#).

AMS IS ALSO likely to find many other uses in the pharmaceutical industry, notes Stephen R. Dueker, Vitalea's president and CEO. The highly sensitive technique is ideal for dissecting the slow elimination of drugs from tissues and cells over long periods of time, he points out. His company recently began an AMS microdosing study of AZT, a DNA nucleoside mimic used to treat HIV.

"AZT has some unusual tissue uptake and distribution properties because it can be incorporated into organ genetic material," Dueker says. The team hopes to use AMS to track the movement of AZT in and out of white blood cells and other tissues. The technique can track the fate of a single microdose for months, he notes.

AMS can also be used to profile drug metabolites when coupled with separation techniques such as ultra-high-pressure liquid chromatography, Dueker points out. Likewise, Accium has helped a large pharmaceutical company identify all the metabolites produced for each member of a family of compounds currently undergoing Phase I clinical trials, Arjomand says. "AMS allows detection of metabolites too scarce to be detected by other methods," he notes. "But it does not provide any direct structural information."

[GlaxoSmithKline](#) also has used AMS to track the slow excretion of a drug candidate in humans and to identify all of the metabolites produced. The company recently demonstrated its continuing commitment to the power of this analytical tool by purchasing its own AMS instruments.

The technique can also be used as an alternative to scintillation counting for detecting radiolabeled compounds in traditional animal ADME /pharmacokinetic studies, Arjomand says. AMS's high sensitivity requires far less ^{14}C -labeled compound, making it cheaper than traditional radioactive tracer studies, he comments.

Vogel notes that AMS is also ideally suited for tracking drug delivery from nasal sprays and dermal patches. "I don't think we yet know all of the potential applications of AMS in drug development," Vogel says. He notes that ^{14}C is just one of many isotopes, including ^3H , ^{26}Al , ^{36}Cl , and ^{41}Ca , that can be detected and quantified with exquisite sensitivity by AMS.

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