The Application of Accelerator Mass Spectrometry to Absolute Bioavailability Studies in Humans: Simultaneous Administration of an Intravenous Microdose of $^{14}$C-Nelfinavir Mesylate Solution and Oral Nelfinavir to Healthy Volunteers

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The absolute bioavailability of nelfinavir was determined in 6 healthy volunteers following simultaneous administration of 1250 mg oral nelfinavir and an intravenous infusion of $^{14}$C-nelfinavir mesylate on day 1 and at steady state. Nelfinavir oral bioavailability decreased from 0.88 to 0.47 over the 11-day study period. The moderate bioavailability of nelfinavir was due to significant first-pass metabolism rather than low absorption, limiting the potential of formulation improvement to decrease pill burden. Human absolute bioavailability studies with accelerator mass spectrometry microdosing, in which an intravenous microdose is given along with a conventional oral dose of the same drug, can differentiate between gastrointestinal absorption and the first-pass metabolism of new drug candidates. Accelerator mass spectrometry allowed a several thousand-fold dose reduction of $^{14}$C-nelfinavir relative to that required for liquid scintillation counting. Accelerator mass spectrometry microdosing reduces potential safety issues around dosing radioactivity to humans and prevents the need to formulate high intravenous doses.

Keywords: Absolute bioavailability; AMS; nelfinavir; human microdosing

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Radotracer studies form a necessary part of the registration dossier for pharmaceuticals, with the most frequently used tracer being $^{14}$C. Detection of radioactivity in absorption, distribution, metabolism, and excretion (ADME) studies is commonly performed using liquid scintillation counting (LSC), whose main drawback is one of sensitivity. Accelerator mass spectrometry (AMS) is an extremely sensitive nuclear physics technique, with around a million times more sensitivity than LSC. It measures the number of $^{12}$C, $^{13}$C, and $^{14}$C atoms in a sample, expressing the result as an isotope ratio. Although the method was developed in 1977 for radiocarbon dating of historical artifacts, it was some 13 years later that it was first applied to the
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Analysis of biological samples containing enriched 14C, in toxicology and cancer research. Since then, AMS has been demonstrated as a useful technique in a diverse number of studies during pharmaceutical research. The most recent biomedical application of the method is in human microdosing, a concept already in use for obtaining early human metabolism information for novel drug candidates. In this approach, very small amounts of 14C-labeled drug in the microgram range are administered to humans. Such trace doses reduce the need for extensive toxicological safety testing in support of human studies but require an ultrasensitive method of detection to enable quantitation of drug and metabolites in plasma and excreta.

The concept of simultaneously dosing with a labeled intravenous (IV) dose and a nonlabeled oral dose to determine absolute bioavailability is well established using stable isotopes such as 13C. Such techniques allow the fate of the IV dose to be distinguished from the oral dose by means of the isotopic tracer. Because the natural abundance of 13C is 1.1%, the limits of detection using this method are experimentally limiting. Absolute bioavailability studies have been attempted with radiolabel such as 14C, which has a much lower natural abundance (ca 10^-10%), but its use is limited by the levels of radioactivity that can be administered to humans. Designing such studies around AMS, however, enables 14C labels to be administered while reducing the levels of radioactivity to such low levels that they are likely to be below those of regulatory concern.

In the present study, oral doses and IV microdoses of nelfinavir, a novel nonpeptidic HIV protease inhibitor, were administered virtually simultaneously to healthy volunteers to determine the absolute bioavailability of the drug. In vitro, multiple cytochrome P-450 enzymes, including CYP3A and CYP2C19, are responsible for the metabolism of nelfinavir. One major (AG1402) and several minor oxidative metabolites of nelfinavir have been identified in humans. The drug shows potent antiviral activity against several laboratory and clinical strains of HIV-1 and a clinical strain of HIV-2 by decreasing plasma HIV RNA levels and increasing CD4 cell counts of patients with HIV infection. Although mortality and morbidity rates have been significantly reduced among HIV patients as a result of highly active antiretroviral therapy (HAART), long-term compliance is still a major factor in virologic failures and drug resistance due to the need for combined treatment with multiple drugs with complicated dosing schedules.

Development of a novel formulation of oral nelfinavir with better bioavailability would reduce the daily dose and pill burden and significantly improve patients’ compliance. Understanding the absolute availability of nelfinavir was crucial for determining the feasibility of the new oral formulation before significant resource was allocated to the effort.

MATERIALS AND METHODS

Study Design and Volunteers

This was an open-label, single- and multiple-dose study in healthy male adult volunteers, aged between 18 and 55 years. Seven subjects were enrolled in the study to provide data from at least 6 volunteers. The study was performed at Pharma Bio-Research Group B.V. (Stationsweg 163, 9470 AE Zuidlaren, the Netherlands). All aspects were approved by the site’s independent ethics committee (Medisch Ethische Toetsings Commissie, Stichting Beoordeling Ethiek Bio-Medisch Onderzoek, Assen, the Netherlands). Informed consent was obtained from all subjects after the nature and possible consequences of participation in the study were explained. Safety was evaluated throughout the study by assessing the results of physical examinations, clinical laboratory tests, vital sign measurements, and electrocardiograms (ECGs) and by monitoring and recording adverse events (AEs) and concomitant medications.

After admission, subjects remained in the study unit on the evening of days 0 and 10 until the morning of days 2 and 12, respectively. They returned to the study unit for all subsequent doses from the evening dose on day 2 until the morning dose on day 10. When confined to the study unit, subjects received standard meals. On days 1 and 11, each subject received a single 30-minute IV infusion of 14C-nelfinavir (~1 mg of nelfinavir and ~50 nCi of 14C-nelfinavir in 5% w/v dextrose solution) immediately after taking a morning oral 1250-mg dose of nelfinavir (VIRACEPT, Pfizer, Inc, New York; five 250-mg tablets) with food and 240 mL water. On days 2 to 10, subjects received oral doses of 1250 mg nelfinavir (five 250-mg tablets every 12 hours) twice daily, with food and 240 mL water. On days 1 and 11, only the morning oral dose of nelfinavir was administered.

Blood samples (5 mL) were collected from each subject within 15 minutes before the morning doses on days 1, 3, 7, and 11 and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24 hours after the morning dose on days 1 and
11. Extra samples were collected at 47.75 and 143.75 hours after the first dose.

**Drugs**

Nelfinavir was supplied as 250-mg tablets. The full chemical name of nelfinavir is \( [3\ S]-[2\ S^*, 3\ S^*], 3(\alpha),4(\beta),8a(\beta)\] -N-(1,1-dimethylethyl) decahydro-2-[(2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinoline carboxamide monomethanesulfonate (salt), MW = 663.90 (567.79 as the free base).

Radiolabeled \(^{14}\mathrm{C}\)-nelfinavir mesylate was supplied as a solid in a borosilicate multidose vial with additional screw cap. Each vial contained 1 mCi. A 100-mL batch of the IV formulation of nelfinavir mesylate was prepared to a target dose of 25 nCi/mL of \(^{14}\mathrm{C}\)-nelfinavir and 0.5 mg/mL of nelfinavir free base in 5% w/v dextrose solution. For IV infusion to each subject, 2 mL of the solution was further diluted to 30 mL (50 nCi, 1.85 KBq, 111 000 dpm).

**Drug Analysis**

Blood samples were centrifuged within 1 hour of collection, split into 2 equal aliquots, and stored at −20°C until analysis. Plasma samples were subject to a single extraction for high-performance liquid chromatography–ultraviolet (HPLC-UV) analysis but required 3 extractions for plasma AMS analysis to ensure maximal quantitative transfer of compounds to the plasma extract sample. Any loss from AMS samples during extraction would lead to errors in the final results. Residues were redissolved in 300 µL of HPLC mobile phase.

An aliquot (100 µL) of sample was analyzed by a validated reverse-phase HPLC-UV method\(^{18}\) for the determination of concentrations of total nelfinavir and its main active metabolite, AG1402. The HPLC system consisted of a Waters Symmetry C-18 250 × 4.6-mm column with an isotropic mobile phase of phosphate buffer/acetoni-trile/methanol (60:32.5:7.5 v/v/v), a particle size of 5 µm, and a flow rate of 1.5 mL/min. This method was validated within a concentration range of 0.05 to 10.0 µg/mL for nelfinavir and AG1402. The standard curves were linear with \( R^2 \) values of >0.997. Nelfinavir and AG1402 were stable (<20% loss from the baseline) for at least 21 months when stored at 20°C. The precision (percent coefficient of variation) of the assay for nelfinavir and AG1402 was 7.2% and 6.6%, respectively. The accuracy (deviation from the nominal concentrations) of the assay for nelfinavir and AG1402 was 16.0% to 6.6% and 5.9% to 1.7%, respectively.

Fractions corresponding to nelfinavir and AG1402 were combined separately, forming 2 sample pools for each injection, with aliquots of about 2000 µL analyzed directly by AMS. Liquid scintillation counting of aliquots (100 µL) of plasma samples was also conducted to ensure that an inappropriately high level of radioactivity was not placed into the AMS instrument. As sample volumes did not always allow the stated volumes to be used, sample volumes or weights of aliquots were determined for AMS analysis and converted to dpm/mL (plasma extracts) or dpm/fraction (pooled HPLC fractions). As AMS provides an isotope ratio that is [\(^{14}\mathrm{C}\)]/[\(^{12}\mathrm{C}\)] and not an absolute value, it was also essential to know the carbon content of the sample. The level of carbon due to the analyte from HPLC fractions, however, is so small that carrier carbon has to be added to be able to perform the analysis. In this case, only the carbon in carrier was used in the calculations.

All sample aliquots for AMS were first converted to graphite in a 2-step oxidation reduction process.\(^{20}\) In the first step, biological carbon in the sample was oxidized to carbon dioxide in the presence of copper oxide at a temperature of around 900°C. In the second stage, carbon dioxide was reduced to graphite in the presence of 2 reductants, zinc powder and titanium hydride, at a temperature of 500°C, with cobalt as a catalyst. The graphite was pressed into small cathodes, each holding about 2 mg of sample. Cathodes were loaded into a sample wheel and inserted into the ion source of the AMS instrument.

The workings of the AMS instrument are outlined in a number of reviews and are summarized here.\(^{21,22}\) Accelerator mass spectrometry determines an isotope ratio, and unlike liquid scintillation counting, it does not directly measure the amount of radioactivity in a sample. To convert an isotope ratio to units of radioactivity...
(dpm), 0.01356 dpm = 98 attomole

Because this value is based on the quantity of carbon in a sample, it is necessary to know what proportion of the sample consists of carbon. In cases in which the carbon content of a sample is extremely low, carrier carbon is added. Carrier carbon is derived from petrochemical sources that, because of their immense age, contain negligible to no

Thus, where carbon carrier is added, the carbon content of the carrier alone is used to calculate the radioactivity concentration from the isotope ratio.

When bombarded with cesium vapor, atoms within the samples on this study formed negative ions that were extracted by a series of plates maintained at several thousand volts more positive than the ion source. The negative carbon ion beam entered an injection magnet where ions were selected according to their mass-to-charge ratio (m/z). For carbon analysis, the magnet resolves to 1 m/z to allow

12C–, 13C–, and 14C– to pass as a series of pulses in sequence. The carbon ion beam was accelerated toward the positive center terminal of the tandem Pelletron accelerator through an Einzel lens. At the center terminal, electrons were stripped from the carbon atom to yield positively charged carbon ions (12,13,14C+1 to C+6). As C4+ ions were the most abundant at this energy, they were accelerated away from the center terminal toward the electrostatic quadrupole triplet and analyzing magnet.

Immediately past the postanalyzing magnet, 12C4+ and 13C4+ ions were measured as an ion current in offset Faraday cups. 14C4+ ions were passed down the high-energy beam line, through an electrostatic quadrupole doublet and a cylindrical electrostatic analyzer. From here, they entered a gas ionization detector to be collected on 4 anodes, which measured the energy loss and total energy of each ion. Other interfering non-14C4+ ions were generally prevented from entering the gas ionization detector by the combinations of electrostatic analyzers, magnets, slits, and charge state separation. The burn-in time and sample analysis time for each graphite sample were 60.4 to 100.7 seconds and 100.7 to 251.75 seconds, respectively.

Calibration curves were constructed for the UV data using peak height and area corrected for internal standard. Both peak area and peak height were used initially, but there was little difference between them, and so peak area was used in the analysis. The internal standard was 6,7-dimethyl-2,3-dif[2-pyridyl]-quinoxaline. The range for the standard curve was approximately 0.01 to 0.26 for nelfinavir and 0.01 to 0.5 for the metabolite (AG1402), based on peak areas. Additional calibration curves were also set up using HPLC mobile phase instead of blank plasma.

Pharmacokinetic Analysis

Plasma concentrations of nelfinavir and AG1402 for each subject were analyzed by noncompartmental pharmacokinetic methods, using the WinNonlin software. Samples with missing values were treated as if the sample had not been scheduled for collection. The following pharmacokinetic parameters were determined following IV administration of nelfinavir mesylate: area under the plasma concentration-time curve (AUC) using the linear trapezoidal method, extrapolated to infinite time (AUC0–∞); maximum (peak) observed plasma drug concentration (Cmax); time to peak concentration (tmax); terminal half-life (t1/2); and clearance (CL).

The following pharmacokinetic parameters were determined following oral administration of nelfinavir: AUC0–τ or AUC0–∞ for a dosing interval, Cmax, predose concentration (Cpre), tmax, t1/2, and bioavailability (F). If the value of F was greater than 1.0, it was set to unity.

The decrease in bioavailability of oral nelfinavir was estimated as 1.0 minus the ratio of AUC0–τ of nelfinavir on day 1.

RESULTS

Safety and Tolerance

Six out of 7 enrolled subjects completed the study. Oral nelfinavir was discontinued in the remaining subject before dosing on day 2 due to elevated triglyceride and cholesterol levels. No clinically significant changes were observed for laboratory results, vital sign measurements, or findings from physical examinations and ECGs. Although 3 subjects reported at least 1 treatment-emergent AE, all were mild in severity. As expected from subacute dosing of oral nelfinavir, diarrhea was the most frequently reported adverse event during 11 days’ treatment. As expected from the extremely small amount of the drug administered, an IV microdose of nelfinavir did not cause any adverse events.

Nelfinavir and AG1402 Pharmacokinetics

Pharmacokinetic parameters for nelfinavir and AG1402 after IV and oral administration of nelfinavir

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are summarized in Tables I through III. Median concentrations of nelfinavir and AG1402 on days 1 and 11 are illustrated on the same graph for IV and oral dosing, respectively (Figures 2-4).

Following a 30-minute intravenous infusion of nelfinavir mesylate, maximal plasma concentrations of nelfinavir on days 1 (24.3 µg/L) and 11 (24.4 µg/L) were observed 0.38 and 0.42 hours after dosing, respectively (Table I). A rapid decline in plasma concentrations of nelfinavir was seen on both days 1 and 11, with t1/2 values of approximately 2.45 and 4.85 hours. After oral administration, Cmax of nelfinavir on days 1 (4.18 mg/L) and 11 (3.82 mg/L) was reached 4.0 and 3.5 hours after dosing (Table II). Terminal half-lives were 3.32 and 2.30 hours, respectively. Nelfinavir oral bioavailability decreased over the study period with values of 0.88 (day 1) and 0.47 (day 11). Autoinduction of metabolism

Table I  Geometric Mean Values (95% Confidence Interval Limits) for Nelfinavir Pharmacokinetic Parameter Estimates After Intravenous Dosing by Study Day

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Day 1</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-∞, µg•h/L</td>
<td>23.6 (16.7-33.4)</td>
<td>29.2 (19.6-43.4)</td>
</tr>
<tr>
<td>Cmax, µg/L</td>
<td>24.3 (19.0-31.1)</td>
<td>24.4 (17.0-35.0)</td>
</tr>
<tr>
<td>tmax, h</td>
<td>0.38 (0.25-0.5)</td>
<td>0.42 (0.25-0.5)</td>
</tr>
<tr>
<td>t1/2, h</td>
<td>2.45 (1.58-3.80)</td>
<td>4.85 (1.96-12.0)</td>
</tr>
<tr>
<td>CL, L/h</td>
<td>37.7 (25.5-55.7)</td>
<td>24.7 (15.9-38.6)</td>
</tr>
</tbody>
</table>

Each subject received a single 30-minute intravenous infusion at a rate of approximately 1 mL/min of 14C-nelfinavir mesylate solution (~1 mg of nelfinavir and ~50 nCi of 14C-nelfinavir).

a. Median and range given.

Table II  Geometric Mean Values (95% Confidence Interval Limits) for Nelfinavir Pharmacokinetic Parameter Estimates After Oral Dosing by Study Day

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Day 1</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-∞, mg•h/L</td>
<td>26.2 (15.9-43.2)</td>
<td>23.7 (15.0-37.5)</td>
</tr>
<tr>
<td>Cmax, mg/L</td>
<td>4.18 (2.66-6.56)</td>
<td>3.82 (2.75-5.31)</td>
</tr>
<tr>
<td>tmax, h</td>
<td>4.0 (3.0-5.0)</td>
<td>3.5 (2.0-4.0)</td>
</tr>
<tr>
<td>F</td>
<td>3.32 (2.62-4.22)</td>
<td>2.30 (1.90-2.78)</td>
</tr>
<tr>
<td>CL, L/h</td>
<td>0.88 (0.75-1.03)</td>
<td>0.47 (0.28-0.78)</td>
</tr>
</tbody>
</table>

The oral dose of nelfinavir on days 1 and 11 was a single dose of 1250 mg (five 250-mg tablets). Oral dose on days 2 to 10 was 1250 mg (five 250-mg tablets) every 12 hours. NA, not applicable.

a. Median and range given.

Table III  Geometric Mean Values (95% Confidence Interval Limits) for AG1402 Pharmacokinetic Parameter Estimates After Oral Dosing of Nelfinavir by Study Day

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Day 1</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-∞, µg•h/L</td>
<td>6.82 (4.38-10.6)</td>
<td>10.9 (7.49-15.9)</td>
</tr>
<tr>
<td>Cmax, µg/L</td>
<td>8.11 (4.64-14.2)</td>
<td>NA</td>
</tr>
<tr>
<td>tmax, h</td>
<td>1.33 (0.89-1.97)</td>
<td>2.10 (1.60-2.74)</td>
</tr>
<tr>
<td>t1/2, h</td>
<td>5.0 (4-6)</td>
<td>4.83 (4.5-4.5)</td>
</tr>
</tbody>
</table>

The oral dose of nelfinavir on days 1 and 11 was a single dose of 1250 mg (five 250-mg tablets). Oral dose on days 2 to 10 was 1250 mg (five 250-mg tablets) every 12 hours. NA, not applicable.

a. Median and range given.

Figure 2. Median nelfinavir plasma concentration versus nominal time after intravenous (IV) dosing on days 1 and 11. Error bars denote ±1 standard deviation.

Figure 3. Median nelfinavir plasma concentration versus nominal time after oral dosing on days 1 and 11. Error bars denote ±1 standard deviation.
was observed for oral nelfinavir because $\text{AUC}_{0-\infty}$ on day 1 was greater than $\text{AUC}_{0-\tau}$ on day 11.

Concentrations of AG1402 were not detectable following intravenous infusion of nelfinavir mesylate. After oral dosing, maximal plasma concentrations of AG1402 were 1.33 and 2.10 mg/L on days 1 and 11, respectively (Table III). $C_{\text{max}}$ of AG1402 was reached by 5.0 and 4.5 hours on days 1 and 11.

**DISCUSSION**

Oral nelfinavir mesylate is currently marketed for the treatment of HIV infection at doses of 750 mg 3 times daily or, more commonly, 1250 mg twice daily.23 However, this study was conducted before the 625-mg tablet was introduced, so the recommended daily dose translated into five 250-mg tablets twice daily (ie, 10 tablets per day). Such high pill burden, coupled with complex multidrug regimens, decreases patients’ compliance and the therapeutic efficacy of nelfinavir. Development of a novel formulation of oral nelfinavir with better bioavailability would reduce the daily pill burden and significantly improve patients’ compliance with this effective antiretroviral drug.

The oral 1250-mg dose of nelfinavir (five 250-mg tablets) represented half of the recommended therapeutic daily dose of 1250 bid, whereas the IV dose (1 mg or 0.014 mg/kg of nelfinavir and ~50 nCi of $^{14}$C-nelfinavir) was far below even the doses used in safety pharmacology and pharmacokinetic studies in animals. In June 2004, the European Medicines Agency (EMEA) issued a regulatory guidance on human microdosing in which a microdose was defined as 1/100 of the predicted therapeutic dose up to a maximum of 100 µg.15 The same dose limit for human microdosing was recently imposed in the Exploratory IND guidance from the US Food and Drug Administration (FDA).24 The 1-mg dose of nelfinavir used in this study was above the EMEA limit of 100 µg. However, the study was performed prior to the issue of the EMEA’s and the FDA’s guidance. Moreover, the microdose was 1250-fold lower than the therapeutic dose. Both doses were observed to be safe and well tolerated in the present study.

The considerable potential of AMS for resolving analytical and drug development issues has already been highlighted in a number of publications.5-11,14,21 Recent application of the method to human microdosing has enabled early absorption, distribution, metabolism, excretion, and pharmacokinetic information to be obtained for small molecules and biotechnology products.12-14 Lowering radioactive exposure in humans not only reduces the need for extensive toxicological safety testing but also has enabled previously unfeasible study designs to be investigated.2 For example, in this study, a radiolabeled IV microdose was administered virtually simultaneously with an unlabeled oral dose, enabling separation of the fate of oral and IV doses in vivo. The application of such a study design provided useful information on the absolute bioavailability of nelfinavir in healthy volunteers.

Nelfinavir showed slow oral absorption but rapid plasma clearance. Based on total radioactivity, elimination from plasma was essentially complete within 5 hours. High-performance liquid chromatography data were consistent with the results from the plasma extracts, but because much of the data were at or below the limit of quantitation, the HPLC-AMS was difficult to interpret.

Understanding the absolute bioavailability and the mechanisms involved in the absorption of nelfinavir was important for understanding the reasons for the moderate oral bioavailability of the drug. Trying to improve the bioavailability of a drug through pharmaceutical/formulation efforts would only make sense if the absolute bioavailability of the drug were not high. If the drug has low or moderate oral bioavailability and it is due to poor absorption, as opposed to the extensive metabolism, then a better formulation could significantly improve the oral bioavailability. Thus, understanding the absolute availability of nelfinavir was crucial for determining the feasibility of the new oral formulation before significant resource was allocated to the effort. Bioavailability of nelfinavir was 0.88 on day 1, suggesting good absorption of nelfinavir in the gastrointestinal tract. Therefore, the decrease observed in nelfinavir bioavailability from 0.88 (day 1) to 0.47 (day 11) was at-
tributed to metabolic enzyme induction after subacute oral dosing. In addition, the differences in the disposition of nelfinavir following IV and oral dosing may reflect the contribution of metabolic enzymes and transporters such as P-glycoprotein in the gastrointestinal tract after oral dosing. For example, AG1402, the major metabolite of nelfinavir, was observed only after oral dosing, suggesting that AG1402 might be produced mainly in the gastrointestinal tract.

In conclusion, this study describes the successful use of AMS in the generation of valuable pharmacokinetic information for nelfinavir in an absolute bioavailability study in healthy volunteers that included the administration of an IV microdose of $^{14}$C-nelfinavir. Because the moderate oral bioavailability of nelfinavir is due primarily to metabolic reasons, the results from this study indicated that traditional formulation improvement aiming at better gastrointestinal absorption would be unlikely to improve the oral bioavailability of nelfinavir in chronic treatment. This allowed the sponsor to make an appropriate cost-benefit decision and avoid allocating significant pharmaceutical science resources in developing a new oral formulation.

Human absolute bioavailability studies with administration of an IV microdose simultaneously with a conventional pharmacological oral dose of the same drug can yield important information about the gastrointestinal absorption versus first-pass metabolic turnover. Ultrasensitive and robust analytical techniques such as AMS can measure parent drug and metabolite concentrations with sufficient precision to characterize the full pharmacokinetic profile of a microdose. Administration of microdoses of $^{14}$C-labeled drugs, in conjunction with the sensitivity of the AMS approach, can reduce potential safety issues arising when dosing radioactivity to humans and prevent the need for formulation of high IV doses for absolute bioavailability studies.

REFERENCES


