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Research Article

Use of Stable Isotopes for Evaluation of Drug Delivery Systems: Comparison of Ibuprofen Release *in Vivo* and *in Vitro* from Two Biphasic Release Formulations Utilizing Different Rate-Controlling Polymers

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Certain delivery systems are intended to release the active ingredient in different phases to obtain the desired therapeutic effect. For these formulations, such as a bilayer tablet, it is desirable to distinguish and measure the release of drug from the different phases simultaneously. Mass spectrometric methods were developed to measure three ibuprofen isotopomers in serum and two in dissolution fluid. The analytical methods were linear ($r \geq 0.992$) over the concentration range of interest and recovery was greater than 99.2% for all isotopomers. Coadministration of [$^2H_0$]ibuprofen, [$^2H_4$]ibuprofen, and [$^2H_7$]ibuprofen to male beagles demonstrated that the isotopomers were bioequivalent and verified the absence of any kinetic isotope effect due to deuterium incorporation ($p = 0.286$). These methods were then used to evaluate a bilayer tablet formulation composed of an immediate release layer of 100 mg [$^2H_4$]ibuprofen and a sustained release layer with a drug load of 300 mg [$^2H_0$]ibuprofen. Two different rate-controlling polymer matrices that provided similar *in vitro* dissolution profiles were compared in the sustained release phase, while the immediate release formulation remained the same. In male beagles, the HPMC matrix delivered a significantly greater amount of ibuprofen ($p < 0.05$). The AUC was threefold greater for HPMC (1067 ± 437 nmole * h/ml) versus EUDRAGIT® (320 ± 51), and C_{max} was nearly four times greater (145 ± 62.1 nmole/ml for HPMC versus 37.9 ± 14.4 for EUDRAGIT®). Although T_{max} for HPMC (3.4 ± 1.9 h) lagged behind EUDRAGIT® (2.0 ± 0.82 h), the difference was not significant ($p > 0.05$). The immediate release layer was absorbed to the same extent as an oral solution (containing [$^2H_7$]ibuprofen) that was administered concomitantly with the bilayer tablet. Using the stable isotope markers also demonstrated that the release rates of the two layers were independent of each other, both *in vivo* and *in vitro*. Stable isotope techniques are a useful tool in the development of biphasic release formulations since they can be used to determine proper drug load of each phase as well as the appropriate rate of release.

KEY WORDS: bioequivalence; deuterium; drug delivery; ibuprofen; isotopes; mass spectrometry; polymers.

INTRODUCTION

Dosage forms that deliver a biphasic release of active ingredient can provide rapid attainment and sustenance of effective serum levels within a single dosage interval. This would be the case for some analgesics and antihistamines that provide a rapid therapeutic effect, but possess a relatively short elimination half-life. Such biphasic dosage forms include multiparticulate systems and bilayer tablets. These typically contain an immediate release (IR) component that provides a certain fraction of the total dose for initial absorption, as well as a sustained release (SR) component that delivers the remainder at a rate necessary to maintain appropriate serum levels over the desired dosage interval.

Conventional dissolution and pharmacokinetic techniques that measure cumulative drug release and absorption are sufficient for formulation screening of delivery systems in which the drug is released via a single mechanism. To adequately evaluate biphasic release formulations, however, it would be advantageous to determine the individual rates of dissolution and absorption attributable to the IR and SR components. Such information would be useful to determine the proper drug load for each component and the appropriate rate of release from the SR portion to obtain the desired pharmacokinetic profile.

The objective of this work was to demonstrate the use of

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stable isotopes as an approach to address these needs. Different isotopomers (compounds varying only in their isotopic makeup) of the same compound can be incorporated into the various phases of drug release, and their dissolution and pharmacokinetic profiles independently determined. In pharmacokinetic studies, by administering yet another isotopomer intravenously or as an oral solution, the control treatment can also be administered concomitantly with the experimental treatment.

Ibuprofen (Figure 1) was selected as the model compound because its therapeutic indications (analgesic and anti-inflammatory) and short elimination half-life make it a suitable candidate for a biphasic release formulation. The delivery system was a bilayer tablet composed of an IR layer containing 100 mg [$^2H_4$]ibuprofen and a SR layer with a drug load of 300 mg [$^2H_0$]ibuprofen. An oral solution of [$^2H_7$]ibuprofen served as the control treatment. Hence, one of the prerequisites for the *in vivo* studies required that the different isotopomers exhibit equivalent kinetics *in vivo* (1–8). *In vivo* and *in vitro* analytical techniques which could differentiate and quantify three different stable isotopomers of ibuprofen were also required.

A second objective was to compare the *in vivo* performance of two rate-controlling polymer matrices that exhibited similar *in vitro* dissolution profiles. Cellulose ethers (9,10) and acrylic resins (11,12) are two classes of polymers popularly used for matrix-based, controlled release formulations. Both are commercially available and safe for human consumption. Two different bilayer tablet formulations of ibuprofen were prepared that differed only in their SR component. One utilized cellulose ethers to form the matrix for the SR layer and the other incorporated an acrylic resin. Using stable isotope techniques, the pharmacokinetic profile of each polymer system was determined in male beagles.

MATERIALS AND METHODS

Reagents and Materials

To attain the objectives of this study, three different isotopomers of ibuprofen were required: one for each component of the bilayer tablet (IR and SR layers), and one that would serve as a control (oral solution) in the pharmacokinetic study. Additionally, the three ibuprofen isotopomers required sufficient mass differences so that they could be simultaneously detected without overlap caused by varied labeling or natural isotopic abundance. Figure 1 shows the chemical structures of the three isotopomers.

The [$^2H_0$]ibuprofen used for the SR layer was recrystallized from methanol (13). [ar-$^2H_4$]ibuprofen, [ar,3,3,3,-$^2H_7$]ibuprofen and 2-(4-isopropylphenyl)propionic acid (IPPA) were prepared as previously described (14–15). The chemical purities of the three isotopomers were all greater than 99.7% as determined by standard HPLC techniques (16). Deuterium incorporations, as determined by mass spectral analysis, were 97 and 98 atom % for [$^2H_4$]ibuprofen and [$^2H_7$]ibuprofen, respectively (15). All other reagents were obtained through standard sources and used as received.

The rate-controlling polymers used in the SR formulations were hydroxypropyl methylcellulose 2208 USP 100cP (HPMC) and a copolymer of acrylic and methacrylic acid esters containing a low content of quaternary ammonium groups. The latter was utilized in an acrylic resin product that contained 0.5% talc to promote flow (EUDRAGIT® RL/PM, hereafter referred to as EUDRAGIT®). The polymer products were purchased from the Dow Chemical Company (Midland, MI) and Rohm Tech Inc. (Malden, MA), respectively. All other excipients used in the bilayer formulations were NF grade.

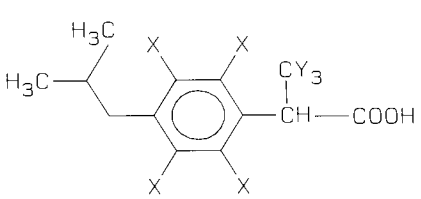

Figure 1. Structures of the ibuprofen isotopomers where [^{1}H] = hydrogen and [^{2}H] = deuterium: [$^2H_0$]ibuprofen (X = [^{1}H], Y = [^{1}H]); [$^2H_4$]ibuprofen (X = [^{2}H], Y = [^{1}H]); [$^2H_7$]ibuprofen (X = [^{2}H], Y = [^{2}H]).

Bilayer Formulations/Tablet Manufacture

Tables I and II list the IR and SR formulations used to manufacture the bilayer tablets. The IR formulation incorporated 0.48 mmole (100 mg) of [$^2H_4$]ibuprofen while the two SR formulations contained 1.4 mmole (300 mg) of [$^2H_0$]ibuprofen. The two SR formulations differed with respect to concentration of the rate-controlling polymer as well as excipient composition. These particular formulations were selected because both exhibited similar *in vitro* time to 90% dissolved ($T_{90\%}$) values. The formulations were prepared using the direct compression method by screening drug and excipients through a #20 U.S. Standard sieve and mixing in a twin-shell blender for fifteen minutes (Model LB-7347, Patterson-Kelly, East Stroudsberg, PA). The bilayer tablets were manufactured using a laboratory scale press (Model C, Carver Press, Menomonee Falls, WI). The SR layer was loaded into the die and tamped to 900 pounds after which the IR layer was added and compressed to 2500 pounds. All tablets were prepared using 16/32″ full-oval tooling.

In Vitro Dissolution

Dissolution testing of the bilayer tablets was conducted using USP Apparatus No. 1 with 900 ml of 0.05M pH 7.2 phosphate buffer (37°C). The rotational speed of the basket was 150 rpm. Samples were taken at the following time points: 0, 0.25, 1, 2, 4, 6, 8, and 12 hours. The fluid was filtered through a polypropylene filter (5 micron), an aliquot (0.1 ml) was transferred to a 12 × 75 mm test tube and internal standard (0.1 ml) was added. Internal standard was

Table I. Immediate Release Formulation

Ingredient	mg	%
[$^2H_4$]ibuprofen	100	42.3
microcrystalline cellulose NF, coarse powder	110	46.7
croscarmellose sodium NF, Type A	25	10.6
colloidal silicon dioxide NF	1	0.4
total	236	100

Table II. Sustained Release Formulations

Ingredient	HPMC mg	HPMC %	EUDRAGIT® mg	EUDRAGIT® %
[$^2H_0$]ibuprofen	300	63.0	300	80.0
hydroxylpropyl methylcellulose 2208 USP 100cP	125	26.3	—	—
EUDRAGIT® RL/PM	—	—	18.8	5.0
lactose NF hydrous spray process standard	—	—	27.6	7.4
microcrystalline cellulose NF, coarse powder	50.0	10.5	27.6	7.4
colloidal silicon dioxide NF	1.0	0.2	1.0	0.3
total	476	100	375	100

prepared by dissolving [$^2H_7$]ibuprofen in a small amount of ethanol (2.5 mg in 0.25 ml) prior to diluting in buffer (final concentration 100 μg/ml). After mixing by vortex, the samples were dried *in vacuo* with centrifugation using a Speed-Vac (Salvant Instruments, Farmingdale, NY). The residue was dissolved in chloroform (2–4 ml) prior to injection into the gas chromatographic-mass spectrometric (GC-MS) system described below. Working standards at concentrations covering the expected range were prepared similarly. The concentrations of the samples were determined by iterative linear regression of the standard curve.

Linearity was determined by analyzing chloroform solutions containing varying amounts of [$^2H_0$]ibuprofen (0–350 μg/ml) and [$^2H_4$]ibuprofen (0–120 μg/ml). For recovery studies, excipients from each formulation were added to buffer solutions (0.05 M pH 7.2) containing various amounts of [$^2H_0$]ibuprofen (0–350 μg/ml) and [$^2H_4$]ibuprofen (0–120 μg/ml).

Total ibuprofen levels were also determined by a non-discriminating method using reverse phase chromatography and UV detection at 254 nm.

In Vivo Serum Analysis

A previously reported serum method for ibuprofen was adapted for the work here (14). Instead of [$^2H_7$]ibuprofen as the internal standard, IPPA was used (0.1 ml of a 300 μg IPPA/ml acetonitrile:water 40:60 solution). Serum samples were extracted using solid phase extraction with a DuPont PREP I automated sample processor (DuPont, Wilmington, DE). Standard curves were generated by preparing acetonitrile:water solutions containing the ibuprofen isotopomers at concentrations covering the desired range. Standards were prepared in a similar fashion to the samples, substituting standard solution for the serum. Samples and standards were dissolved in chloroform (4 ml) prior to injection into the GC-MS system described below. The concentrations of the samples were determined by iterative linear regression of the standard curve. Linearity was determined by analyzing standards over the range from 0 to 70 μg/ml. Recovery studies were performed by spiking ibuprofen isotopomers into blank canine serum at similar levels. Samples were prepared as above.

GC-MS Method for Isotopomer Quantitation

The GC-MS system used to measure the ibuprofen isotopomers was similar to that reported earlier (14) with the following exceptions. The Hewlett Packard (HP) GC-MS system was now controlled by a Model 5990C ChemStation and sample introduction was accomplished using a Model 7673B Autosampler. Selected ion monitoring (SIM) was performed at the following m/z ratios for the ibuprofen isotopomers: 206 ([$^2H_0$]ibuprofen), 210 ([$^2H_4$]ibuprofen), 213 ([$^2H_7$]ibuprofen). The internal standard for serum samples (IPPA) was monitored at an m/z of 192, while for dissolution samples, the internal standard ([$^2H_7$]ibuprofen) was monitored at 213.

The initial oven temperature was held at 100°C for 1 minute followed by a ramp to 250°C at 20°C/min for serum samples, and 25°C/min for the dissolution samples. The oven was maintained at 250°C for 10 minutes after which the oven was returned to 100°C. The temperatures for the injector, detector, and transfer line were 250, 250 and 280°C, respectively.

In Vivo Studies

The subjects in the *in vivo* studies were male beagles weighing between 15 and 20 kg. Subjects were fasted for twelve hours prior to dosing and water was available *ad libitum*. At eight hours after dosing, the subjects were fed their normal meal and routine feeding schedules resumed.

Blood samples were obtained from the antebrachial section of the cephalic vein in the foreleg at predetermined intervals: 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 hours. Blood was collected in red stoppered tubes (Vacutainer, Becton-Dickinson, Rutherford, NJ) containing no preservative or anticoagulant. After clotting and centrifugation, serum was frozen until further preparation.

Bioequivalence of the Isotopomers

The ibuprofen isotopomers were administered to three male beagles as oral solutions containing equal molar amounts (0.97 mmole, 200 mg) of [$^2H_0$]ibuprofen, [$^2H_4$]ibuprofen, and [$^2H_7$]ibuprofen. Solutions were prepared by dissolving the drug in 3 ml of 0.2 M sodium hydroxide with sonication. Sufficient 0.2 M monobasic potassium phosphate (5 ml) was used to adjust the pH to 7 and water was added to yield a 0.05 M phosphate solution. The solutions were administered to the beagles via gastric intubation using a plastic catheter.

The area under the serum concentration versus time curve (AUC) was determined by the trapezoidal rule using the Program RSTRIP (Version 5, MicroMath Scientific Software, Salt Lake City, UT). The maximum concentration of drug in the serum (C_{max}) and the time of that maximum concentration (T_{max}) were determined by examining each subject's data. These *in vivo* data were treated as a repeated measures design and the serum profiles statistically compared using JMP Statistical Visualization Software (Version 2.0.5, SAS Institute, Cary, NC).

HPMC Versus EUDRAGIT® Study

A crossover study design with two treatments was used to evaluate the *in vivo* performance of bilayer tablet formulations. The dosing schedule was randomized and a one-

week washout period was allowed between administration of the two treatments. One treatment consisted of a bilayer tablet incorporating HPMC in the SR layer, and an oral solution of [$^2H_7$]ibuprofen that provided a control for the study. The other treatment was a bilayer tablet containing EUDRAGIT® in the SR layer and the [$^2H_7$]ibuprofen solution. The solution contained 0.48 mmole (100 mg) of [$^2H_7$]ibuprofen dissolved in a phosphate buffer (pH 7). Dosing was performed by first administering the solution by gastric intubation using a plastic catheter. Immediately after catheter removal, the tablet was administered orally.

Statistical analysis was applied to the pharmacokinetic data to specifically address interactions such as the relationship between the *in vivo* performance of the IR layer and the SR layer to which it was attached. Consequently, the pharmacokinetic parameters (C_{max}, T_{max}, and AUC) were analyzed using a two-way treatment structure in the variables *Release* (SR, IR, or Solution) and *Polymer* (HPMC or EUDRAGIT®). The design structure was a randomized complete block design where the blocking variable was *Subjects*. Treating the data set (n = 24 for each response) in this manner provided fifteen degrees of freedom for error, and was followed by significance testing of the pairwise comparisons of interest using the procedure recommended by Milliken and Johnson (17). If the p value for *Release* * *Polymer* interaction was significant ($p < 0.05$), comparisons were tested using the LSD (least significant difference) method. If the interaction was not significant, the Bonferroni method was applied.

The analysis of variance models of the pharmacokinetic parameters were done using a Box-Cox transformation of the response variables. This power transformation was used because the residual from the untransformed data analysis showed that the variance depended on the magnitude of the response. The statistical evaluation was performed using JMP Statistical Visualization Software and Table V includes the value of the power parameter (λ) used to transform the data for each pharmacokinetic parameter.

RESULTS AND DISCUSSION

Methods Validation

The GC-MS method of detecting the ibuprofen isotopomers was linear over the range of 0–350 μg/ml for [$^2H_0$]ibuprofen and 0–120 μg/ml for [$^2H_4$]ibuprofen. Complete dissolution of the bilayer tablet gave a concentration of 333 μg [$^2H_0$]ibuprofen/ml for the SR layer and 111 μg [$^2H_4$]ibuprofen/ml for the IR layer. The regression lines for the response versus concentration data were linear (correlation coefficient, $r \geq 0.992$) and the corresponding intercepts were not statistically different from zero at the 95% confidence level. Recovery data of isotopomers from dissolution fluid and formulation excipients showed the method to be accurate. The average amount of [$^2H_0$]ibuprofen recovered was 100.2% over the range tested. For [$^2H_4$]ibuprofen, the average was 99.2% over a similar percentage range. Regression analysis of the amount found versus amount added curves showed that the slopes and intercepts were not significantly different from one and zero, respectively, with good correlation ($r \geq 0.998$).

GC-MS detection of the three ibuprofen isotopomers in serum was equally accurate. The average amounts of [$^2H_0$]ibuprofen, [$^2H_4$]ibuprofen, and [$^2H_7$]ibuprofen recovered from serum were 99.5%, 101.2%, and 100.3%, respectively. The regression analysis of the amount found versus amount added curves showed slopes and intercepts not significantly different from one and zero, respectively, with good correlation ($r \geq 0.999$). Figure 2 is a representative SIM chromatogram of a serum sample showing the chromatographic separation of IPPA from the ibuprofen isotopomers.

In Vitro Dissolution

Figures 3 and 4 show the dissolution profiles for HPMC and EUDRAGIT® bilayer tablets, respectively. The GC-MS method differentially detects the ibuprofen isotopomers. Using this approach, the profiles of the two phases (IR and SR) were delineated. The [$^2H_4$]ibuprofen incorporated in the IR layer of both bilayer tablets dissolved within fifteen minutes. The SR components utilizing either HPMC or EUDRAGIT® showed a sustained release of [$^2H_0$]ibuprofen over a twelve hour period. Although the EUDRAGIT® formulation provided an initially faster release, the two profiles intersected at six hours after which the HPMC formulation dissolved more quickly. At eight hours, ibuprofen release from the HPMC and EUDRAGIT® formulations were 92% and 87%, respectively. The variability in drug release at each time point was quite acceptable and verifies the uniformity of tablet manufacture and reproducibility of polymer performance in the sustained release matrices.

In contrast, the dissolution curve generated by the conventional method utilizing UV detection does not delineate the individual contributions of each release phase (Figures 3 and 4 also). Comparing composite dissolution profiles, showing total drug release, demonstrates good correlation between the UV and GC-MS methods.

To determine if compressing the SR formulations into a bilayer tablet affected drug release, the SR formulations were compressed individually using the same combination of

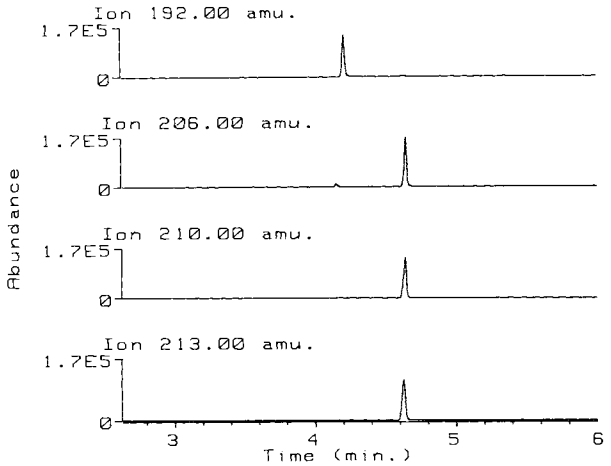

Figure 2. SIM chromatogram of a serum sample preparation at the 4 hour timepoint from one subject. From top to bottom: IPPA internal standard (ion 192): [$^2H_0$]ibuprofen (ion 206); [$^2H_4$]ibuprofen (ion 210); [$^2H_7$]ibuprofen (ion 213). Concentrations of each ibuprofen isotopomer are approximately 27 μg/ml.

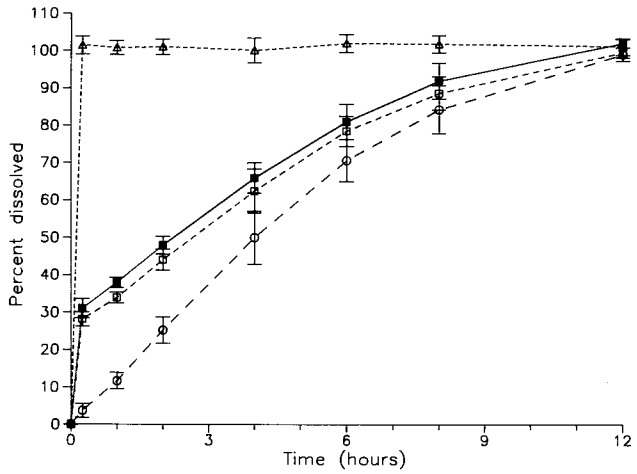

Figure 3. Dissolution curves for the HPMC bilayer tablets as generated by the GC-MS method (n = 6): [$^2H_0$]ibuprofen from the SR layer (○); [$^2H_4$]ibuprofen from the IR layer (△); total ibuprofen released ([$^2H_0$] + [$^2H_4$]) (□), and by the UV method (n = 15): total ibuprofen released (■).

compressional forces (900 lbs/2500 lbs) used to prepare the bilayer tablets. Their dissolution profiles were determined using the UV method of detection and compared to their profiles when incorporated into a bilayer tablet (and determined by the GC-MS method of drug release). Drug release from the respective matrices was largely unaffected by their incorporation into a bilayer dosage form. This was somewhat unexpected since it was anticipated that the matrix surface attached to the IR layer would be disrupted upon the rapid dissolution of the latter, resulting in a faster initial release of drug.

In Vivo Studies

Bioequivalence of the Isotopomers

Incorporating stable isotopes into a biphasic release formulation to distinguish differential release rates requires that each isotope behaves equivalently *in vivo*. In the case of ibuprofen, this was expected since few metabolites are found in the serum and the major metabolites that are found in urine are those involving the isobutyl side-chain (18). This portion of the molecule is not affected by isotopic substitution (Figure 1). Although ibuprofen undergoes chiral inversion at the C-2 carbon, it has been shown that the process does not result in a kinetic isotope effect (19,20). Hence, the substitution of deuterium at the hydrogens of the β-methyl group (C-3) to yield the isotopomers used in this study would not produce a significant effect kinetically. However, because the difference in the number of deuterium atoms between the isotopomers could affect the lipophilicity of the molecule, a difference in absorption rate needed to be evaluated. For example, one study found that [$^2H_0$]terbutaline was absorbed faster than [$^2H_6$]terbutaline in humans (8). This isotope effect was attributed to the lower lipophilicity of the [$^2H_6$]-isotopomer compared to [$^2H_0$]terbutaline.

Figure 5 shows the serum profile of the three ibuprofen isotopomers in male beagles following a dose of 1 mmole of each isotopomer, given as an oral aqueous solution. These curves and the parameters listed in Table III show the equivalence of the three isotopomers. AUC, C_{max}, and T_{max} were nearly identical in each case.

Multivariate analysis of variance of the serum profiles verified that there were no differences in the pharmacokinetic parameters for the three isotopomers ($p = 0.286$). No interaction was detected between isotopomer and sampling time ($p = 0.855$), while sampling time, as expected, significantly affected serum concentration ($p < 0.0001$). The test for significance due to isotopomer was the multivariate Wilks' Lamba test. Both sampling time and the interaction of (isotopomer × time) were tested using the Greenhouse-Geiser adjusted method.

HPMC Versus EUDRAGIT® Study

Comparison Between the SR Phases. Figures 6 and 7 show the average serum levels of the three ibuprofen iso-

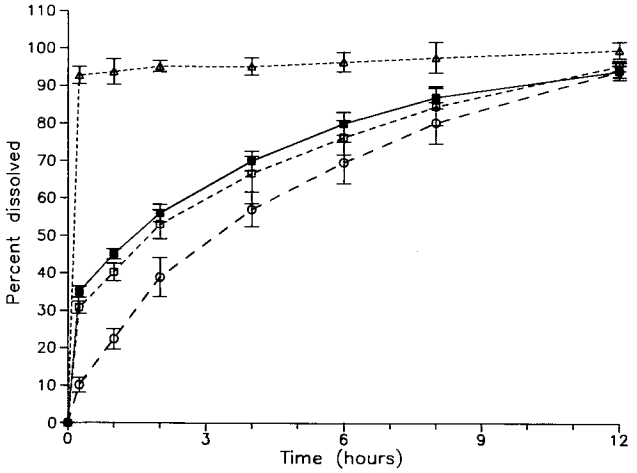

Figure 4. Dissolution curves for the EUDRAGIT® bilayer tablets as generated by the GC-MS method (n = 6): [$^2H_0$]ibuprofen from the SR layer (○); [$^2H_4$]ibuprofen from the IR layer (△); total ibuprofen released ([$^2H_0$] + [$^2H_4$]) (□), and by the UV method (n = 15): total ibuprofen released (■).

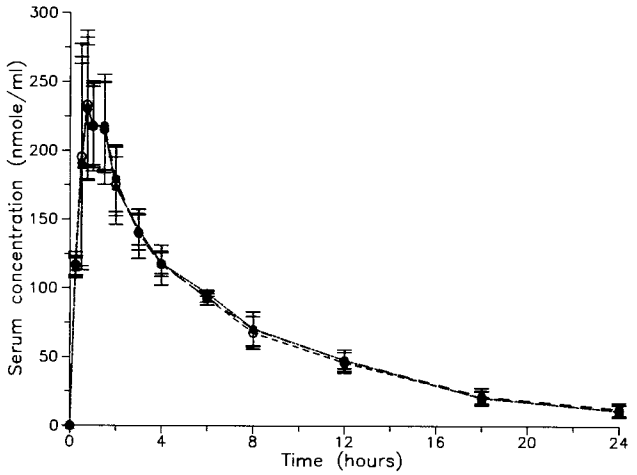

Figure 5. Serum ibuprofen concentration versus time curve after dosing with equal molar amounts of three deuterium labeled isotopomers. Means of three subjects ± 1 sd are plotted. [$^2H_0$]ibuprofen (○); [$^2H_4$]ibuprofen (△); [$^2H_7$]ibuprofen (●).

Table III. Pharmacokinetic Data for the Ibuprofen Isotopomer Bioequivalency Study. For Each Isotopomer, the Average of the Subjects (n = 3) ± 1 Standard Deviation is Reported.

Parameter	$[^2H_0]$Ibuprofen	$[^2H_4]$Ibuprofen	$[^2H_7]$Ibuprofen
AUC[a]	1550 ± 157	1570 ± 140	1580 ± 172
C_{max}[b]	244 ± 49	244 ± 38	247 ± 37
T_{max}[c]	1.00 ± 0.433	1.00 ± 0.433	1.00 ± 0.433

[a] AUC, Area under the serum versus time curve corrected for molecular weight, units are nmole * h/ml.
[b] C_{max}, Maximum concentration corrected for molecular weight, units are nmole/ml.
[c] T_{max}, Time of the maximum serum concentration, in hours.

topomers for the two treatments (HPMC and EUDRAGIT®, respectively) as determined by the GC-MS method. While the two formulations were chosen based on the similarities of their *in vitro* drug release, there was a significant difference in their *in vivo* performance as indicated in Table IV which lists the pharmacokinetic parameters obtained from these profiles.

AUC was significantly affected by both *Polymer* in the SR layer ($p = 0.0007$) and the *Release* * *Polymer* interaction ($p = 0.0002$) [Table V]. Further analysis of the comparisons of interest showed that the polymer in the SR layer (the $SR_{EUDRAGIT®}$ versus SR_{HPMC} comparison in Table VI) significantly affected AUC ($p = 0.0000$) with HPMC (1067 ± 437 nmol * h/ml) delivering an approximately threefold greater amount than EUDRAGIT® (320 ± 51 nmole * h/ml).

Likewise, C_{max} was nearly four times greater for the HPMC versus EUDRAGIT® matrix. Both the *Release* and *Polymer* variables, as well as their interaction, significantly affected this response ($p < 0.05$). Analyzing the *Release* * *Polymer* interactions showed that only the SR polymer comparison was significant ($p = 0.0002$) with HPMC (145 ± 62.1 nmole/ml) providing much greater serum concentrations than EUDRAGIT® (37.9 ± 14.4 nmole/ml).

Although T_{max} for HPMC (3.4 ± 1.9 h) lagged behind the EUDRAGIT® matrix (2.0 ± 0.82 h), the difference was not significant ($p > 0.05$) as determined by a Bonferroni comparison (Table VI).

Comparison Between the IR Phase and the Attached SR Layer. The same immediate release formulation was used for both bilayer tablets and the *in vivo* profile of the IR layer was the same regardless of the SR layer to which it was attached (Table IV and Figures 6 and 7). No significant differences were found between the IR layers (the $IR_{EUDRAGIT®}$ versus IR_{HPMC} comparison in Table VI) for AUC ($p = 0.8092$), C_{max} ($p = 0.4434$), or T_{max} ($p > 0.05$).

Comparison Between the IR Phase and the Oral Solution. Both the IR layer and the oral solution contained 100 mg of ibuprofen (although in different isotopic states) and the extent of absorption was equivalent for both. Although the *Release* * *Polymer* interaction was significant for both AUC ($p = 0.0002$) and C_{max} ($p = 0.0025$), investigating the pairwise comparisons indicated no significant differences between the IR layer and the oral solution for either AUC or C_{max}. This was true regardless of the SR layer to which the IR was attached (Table VI). T_{max} was significantly longer for the IR layer ($p < 0.05$) and reflected the slower rate of absorption when ibuprofen is administered in tablet form versus oral solution.

Comparison Between the Overall Bilayer Tablet Formulations. Figure 8 compares the composite (IR + SR) *in vivo*

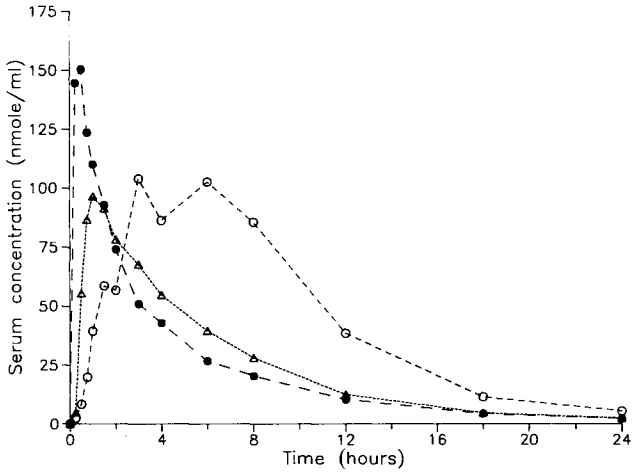

Figure 6. Average serum level (n = 4) versus time curves for the HPMC bilayer treatment, *in vivo:* $[^2H_0]$ibuprofen from the SR layer (○); $[^2H_4]$ibuprofen from the IR layer (△); $[^2H_7]$ibuprofen from the oral solution (●).

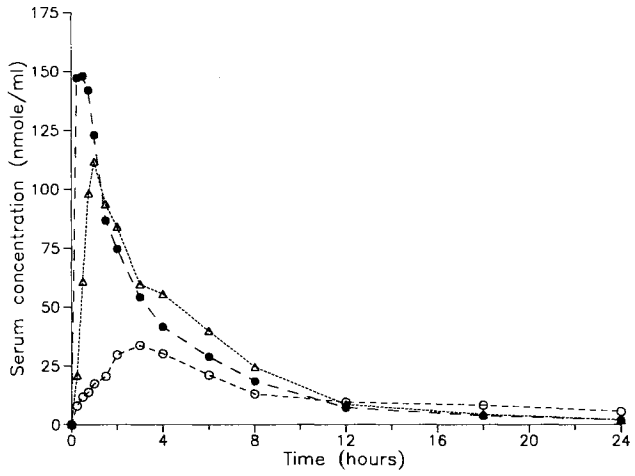

Figure 7. Average serum level (n = 4) versus time curves for the EUDRAGIT® bilayer treatment, *in vivo:* $[^2H_0]$ibuprofen from the SR layer (○); $[^2H_4]$ibuprofen from the IR layer (△); $[^2H_7]$ibuprofen from the oral solution (●).

Table IV. Pharmacokinetic Data for the HPMC and EUDRAGIT® Treatments. Average Parameters for all Subjects (n = 4) ± 1 Standard Deviation are Reported.

HPMC			
Parameter	SR ($^2H_0$)Ibuprofen	IR ($^2H_4$)Ibuprofen	Solution ($^2H_7$)Ibuprofen
C_{max} (nmole/ml)	145 ± 62.1	103 ± 38.1	158 ± 26.8
T_{max} (h)	3.4 ± 1.9	1.4 ± 1.0	0.44 ± 0.12
AUC (nmole * h/ml)	1067 ± 437	585 ± 140	563 ± 103
EUDRAGIT®			
Parameter	SR ($^2H_0$)Ibuprofen	IR ($^2H_4$)Ibuprofen	Solution ($^2H_7$)Ibuprofen
C_{max} (nmole/ml)	37.9 ± 14.4	131 ± 56.6	157 ± 12.2
T_{max} (h)	2.0 ± 0.82	1.8 ± 1.6	0.44 ± 0.24
AUC (nmole * h/ml)	320 ± 50.9	566 ± 124	549 ± 87.3

release profiles of the two bilayer tablet formulations. This figure graphically shows the greater ibuprofen absorption provided by the HPMC matrix after the first hour following initial absorption of the 100 mg of ibuprofen from the IR layer. Without the use of stable isotopes to define the individual contribution of each release phase in the two formulations, this would not necessarily be obvious. An alternative interpretation based on the composite profile might be that the EUDRAGIT® profile was similar to HPMC for the first hour due to the faster initial drug release from the EUDRAGIT® matrix (as suggested by the *in vitro* profile). This compensated for an interaction between the IR and SR phases that prevented total, rapid absorption of ibuprofen from the IR layer in the first hour. Similar interpretations could also be offered to justify the HPMC profile. (Note: Nonlinear dose pharmacokinetics is a consideration which should be considered in designing any *in vivo* study, and is also the case with ibuprofen (21).)

CONCLUSIONS

The analytical techniques described in this report differentiated and measured three ibuprofen isotopomers in serum and two in dissolution fluid. A bioequivalence study in male beagles showed that the three ibuprofen isotopomers behaved similarly *in vivo* and displayed no significant isotope effects due to deuterium incorporation. Together, these enabled the simultaneous determination of drug release from each phase of a biphasic release formulation, namely, a bilayer tablet of ibuprofen composed of an IR and SR layer.

These methods were then used in this study to evaluate the performance of two rate-controlling polymers (HPMC and EUDRAGIT®) in a bilayer tablet. They were also used to determine if there was any interaction between the IR layer (containing [$^2H_4$]ibuprofen) and the SR layer (containing [$^2H_0$]ibuprofen) during drug dissolution.

Although, the SR formulations had similar *in vitro* release profiles, the HPMC formulation proved superior *in vivo*. The AUC was threefold greater for HPMC versus EUDRAGIT®, and C_{max} was nearly four times greater. While T_{max} for HPMC lagged behind EUDRAGIT®, the difference was not significant ($p > 0.05$).

The same IR layer was used in both bilayer formulations and behaved the same regardless of the SR layer to which it was attached. Drug from the IR layer was absorbed to the same extent as an oral solution administered concomitantly (and containing [$^2H_7$]ibuprofen). No significant differences existed for either AUC or C_{max}. The longer T_{max} for the IR layer was indicative of slower oral absorption from a tablet dosage compared to a solution. These results verified *in vitro* testing in which the release rates of the two layers were independent of each other.

These methods can be applied to other compounds and multiphasic release formulations (e.g., multiparticulate dosage forms). They can provide the independent determination of the release rates of the phases both *in vivo* and *in vitro*. Knowing the contributions of each phase provides the necessary information to rationally adjust the drug load between

Table V. ANOVA Table for Pharmacokinetic Parameters.

Source	df[a]	T_{max}	C_{max}	AUC
Subject	3	0.2922[b]	0.4634	0.8502
Release	2	0.0000	0.0030	0.5018
Polymer	1	0.5115	0.0359	0.0007
Release * Polymer	2	0.7276	0.0025	0.0002
λ[c]	—	−0.2	0.2	−0.8

[a] Degrees of freedom.
[b] p-values.
[c] Power parameter used to transform pharmacokinetic data for ANOVA.

Table VI. Pairwise Comparisons of Interest. P-Values for C_{max} and AUC were Determined Using the LSD Method. P-Values for T_{max} were Determined Using the Bonferroni Method (17).

Comparison of interest	T_{max}	C_{max}	AUC
$SR_{EUDRAGIT®}$ vs SR_{HPMC}	>0.05	0.0002	0.0000
$IR_{EUDRAGIT®}$ vs IR_{HPMC}	>0.05	0.4434	0.8092
$IR_{EUDRAGIT®}$ vs $Solution_{EUDRAGIT®}$	<0.05	0.3057	0.9487
IR_{HPMC} vs $Solution_{HPMC}$	<0.05	0.0879	0.8566

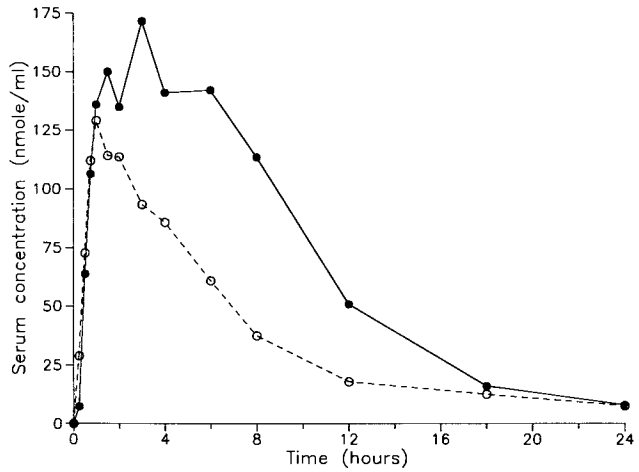

Figure 8. Average serum levels of total ibuprofen released from the bilayer tablet formulations determined by summing the IR ($^2H_4$) and SR ($^2H_0$) values from the GC-MS determination: HPMC (●); EUDRAGIT® (○). (n = 4).

the two layers, as well as determine the suitability of the rate of drug release from the SR layer to maintain suitable serum levels. Additionally, stable isotopes that exhibit no kinetic effects *in vivo* can serve as their own control. Consequently, the control treatment can be coadministered with the experimental treatment, thereby eliminating a separate dosing interval in a crossover design.

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