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In vivo evaluation of rapid release and sustained release Gelucire capsule formulations

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Summary

Ketoprofen was dispersed in a water miscible, highly ethoxylated wax and filled into hard gelatin capsules. This produced drug absorption equivalent in rate and extent to conventional rapid release formulations. Where gastric spreading/dispersion was slow, gastric emptying was delayed and the rate of drug absorption reduced. The use of a slowly hydrating, erodible wax formulation was shown to produce sustained release in vivo, prolonging drug levels relative to conventional formulations. Good in vitro-in vivo correlation was not observed and a relative bioavailability lower than expected was determined. However, comparing the semi-solid ketoprofen capsule (100 mg) plasma data with the comparable product (Oruvail 200 mg) by normalizing for a 200 mg dose, it is apparent that the two formulations are likely to yield similar terminal plasma concentrations. Controlled drug release was not achieved and drug absorption was dependent upon physiological variables such as gastric emptying and intestinal transit as assessed by gamma scintigraphy. In vitro differences in dissolution profiles between freshly manufactured and stored capsules, were shown not to be significant in vivo. Special storage conditions to ensure reproducible release behaviour should therefore be unnecessary.

Introduction

While in vitro dissolution experiments provide information on product uniformity and the effect of formulation changes, they do not necessarily accurately predict the in vivo situation (Alpsten et al., 1976; Dakkuri and Shah, 1982; Davis, 1985; Herman et al., 1988). To demonstrate the clinical efficacy of a dosage form, in vivo assessment is required. This is especially important for prolonged release medication where the programmed rate of release and extended period of absorption are more critical. Volunteer studies are thus essential in order to evaluate bioavailability and to enable assessment of in vitro-in vivo correlation. A clinical investigation of semi-solid formulations has therefore been performed to evaluate the significance of in vitro data. Ketoprofen was selected as the model drug as it requires frequent administration to maintain therapeutic concentrations (Houghton et al., 1984b,c). Elimination half-lives are commonly reported to be between 1 and 2 h (Caille et al., 1978; Sala et al., 1978; Ishizaki et al., 1980; Debruyne et al., 1987), but may be longer in the elderly (Advenier et al., 1983). Ketoprofen can also be analyzed in body fluids using relatively simple HPLC methods (Upton et al., 1980; Kaye

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et al., 1981) with the sensitivity needed for pharmacokinetic analysis.

To derive information on dosage form gastrointestinal transit and physical disposition, gamma scintigraphy was employed. This is a non-invasive technique using radionuclides which emit gamma radiation of suitable energy to enable in vivo imaging of the formulation; for example, tablets (Frier et al., 1982; Davis et al., 1984a, 1986; Sangekar et al., 1987), pellets (Hunter et al., 1982; Davis et al., 1984b, 1987), liquids (Christensen et al., 1985) and osmotic systems (Davis et al., 1984b). Only limited studies have been performed on liquid-filled hard gelatin capsules. Djimbo et al. (1984) reported delayed absorption of acetylsalicylic acid from a high melting point water-dispersible fat, relative to a rapidly water-miscible vehicle. This behaviour was attributed to slow wax spreading. However, drug absorption and gamma scintigraphic studies were not performed simultaneously. In addition, the imaging experiment employed a capsule formulation containing a high proportion of amberlite resin (50%) replacing drug and lactose in the original capsule. Conclusions drawn by comparing the two experiments are therefore of limited value.

Using oily vehicles thickened with silicon dioxide, Francois et al. (1982) demonstrated that thixotropic formulations containing phenylpropanolamine could exhibit prolonged release. At present, there are few other published reports concerning the performance of liquid-filled hard gelatin capsules under physiological conditions. In vivo behaviour is thus only poorly characterized. To allow detailed interpretation of drug absorption in relation to gastrointestinal transit and spreading, selected thermosetting formulations were studied using gamma scintigraphy and simultaneous blood analysis.

The volunteer study was designed: (i) To assess the ability of a selected sustained release formulation to deliver ketoprofen over a 24 h period; (ii) to determine the importance of in vitro ageing for in vivo drug absorption; (iii) to compare the drug absorption and gastrointestinal behaviour of a rapidly water-miscible formulation with the above slowly hydrating matrices, permitting determination of relative bioavailability.

Materials and Methods

Materials

The following were obtained from the indicated sources: ketoprofen powder clinical supply B.N. RM/169/22, Oruvail 100 mg, and Orudis 100 mg capsules (Rhône Poulenc, Dagenham); naproxen, amberlite resin CG-400, and diethylenetriaminepentaacetic acid (Sigma, Poole); Gelucire 50/13, 50/02, and 44/14 (Gattefossé, France); sodium pertechnetate ($^{99m}TcO_4^-$) and indium ($^{113m}InCl_3$) (Amersham International) were eluted from appropriate generators.

Methods and experimental

Capsule formulations (% w/w)

The composition of the formulations was as follows. (a) Rapid release ketoprofen formulation: Gelucire 44/14 (80.50); Amberlite resin (2.50); ketoprofen (17.00). (b) Prolonged release ketoprofen formulation: Gelucire 50/13 (60.37), Gelucire 50/02 (20.13), Amberlite resin (2.50), ketoprofen (17.00).

BIO-DIS method

Capsule dissolution properties were investigated using a BIO-DIS (Caleva, Ascot). The operative conditions were as follows: 1 h in vessel 1 (pH 2.2), 1 h in vessel 2 (pH 4.5), 4 h in vessel 3 (pH 6.9), 4 h in vessel 4 (pH 6.9), 4 h in vessel 5 (pH 7.2) and 8 h in vessel 6 (pH 7.2). After completion of the programme, samples were removed from each vessel and the UV absorbance, corrected for background opalescence, was determined at λ_{max} (260 nm). The percentage drug release in the various buffer solutions over the 22 h period was then calculated.

Radiolabelled aqueous solution

To produce a radiolabelled aqueous solution, an ^{113m}In-diethylenetriaminepentaacetic acid (DTPA) chelate was prepared. This forms a stable non-absorbable moiety suitable for outlining regions of interest (Daly et al., 1982). The complex was prepared by elution of $^{113m}InCl_3$ from a sterile

generator, using 6 ml of sterile eluent (Amersham International). A suitable volume containing a total activity equivalent to 2 MBq at the proposed time of administration was transferred to 1 ml of complexant solution, containing 1.6 mg/ml DTPA and 1.0 mg/ml acetic acid. To the complexant solution was added 1.3 ml of Trizma buffer (0.26 M) and sufficient water to reach a final volume of 200 ml. The solution pH was then adjusted to pH 7.0.

Radiolabelled capsule formulation

Radiolabelling of fats can be achieved by iodination of unsaturated fatty acids (Palin et al., 1982). However, Gelucire excipients contain few sites of unsaturation (Analytical File, Gattefossé) and are therefore unsuited to this technique. Incorporation of a gamma-emitting label by spiking lipid vehicles with iodinated unsaturated fats is an alternative (Palin et al., 1982). This has the disadvantage of changing the physicochemical properties of the matrix, and may increase the radiation burden due to absorption of the radiolabelled fat (Lubran and Pearson, 1958). Amberlite CG-400 resin (mean weight size 140 μm), labelled with ^{99m}Tc, was selected to image the formulations as this forms a stable, inert and non-absorbable marker (Theodorakis et al., 1982).

The labelled resin was prepared by diluting approx. 0.1 ml of sodium pertechnetate equivalent to approx. 30 MBq, with 20 ml of normal saline. After addition of 73.5 mg of amberlite CG-400 resin, the suspension was agitated for 3 min using a vortex mixer (model VM20, Scientific Industries). The preparation was passed through a Buchner funnel using No. 4 Whatman filter paper and allowed to dry for 20 min. The dry, labelled resin was then incorporated into the appropriate fused wax/drug mixture, maintained at 60 °C. Using a 1 ml plastic syringe (Plastipak B-D), warmed to 70 °C, 0.58 ml of hot melt (equivalent to 100 mg ketoprofen) was transferred to size 0 pre-weighed hard gelatin capsules. The filled capsules were allowed to cool at room temperature, weighed and total radioactivity per capsule determined using the gamma camera. Only capsules with a theoretical ketoprofen content of 100 ± 2.5 mg, and a radionuclide disintegration count between 3 and 4 MBq were administered to volunteers.

TABLE 1

In vitro capsule erosion as determined by weighing and isotope procedures

Time (h)	Capsule erosion after BP 1980 dissolution test (50 rpm, water 37 °C)	
	Dry weight remaining (%)	Radioactivity remaining (%)
2	98.7	98.5
4	90.8	88.2
6	72.4	69.1
8	61.3	64.7
10	49.1	47.9
12	20.6	25.2

Materials were weighed in sufficient quantities to manufacture five capsules per batch. Each formulation contained 17% ketoprofen and 2.5% resin in a selected Gelucire vehicle. This amount of resin was sufficient to bind extensively and irreversibly the pertechnetate anion with an efficiency of 98%, of which 96% remained bound after washing in both 0.1 N HCl and pH 7.0 buffer. The resin was distributed uniformly throughout the formulation. This was assessed for both intra-capsule (5.50 ± 0.23 kBq mg^{-1} (mean $\pm$ SD, $n = 6$)) and inter-capsule (5.61 ± 0.09 kBq mg^{-1} (mean $\pm$ S.D.; $n = 6$)) variation. A study of the influence of amberlite CG400 resin on ketoprofen (17% w/w) dissolution rates from a 50/02 (25%) and 50/13 (75%) blend (HLB 10.25) was undertaken and T_{50} values of 180 min (SE 5.1) in the absence of resin and 198 min (SE 3.2) for capsules containing 2.5% resin determined. Using a gamma camera (Maxicamera 400, General Electric), the distribution of radioactivity was shown to provide quantitative data on dosage form position and the state of erosion/dispersion. The latter was achieved by comparing the dry weight of capsule remaining as a function of time with measurement for activity at the one time point as assessed by gamma scintigraphy for capsules subjected to the BP 1980 dissolution test (Table 1).

Volunteers

Seven healthy male volunteers, aged 22–35 years, participated. None were taking medication,

and all were instructed to avoid alcohol for 2 days and products containing caffeine for 1 day prior to dosing. Each subject provided written informed consent.

Protocol

The study protocol was approved by the Joint South Glamorgan Ethics Committee.

Each volunteer was administered one capsule containing 100 mg ketoprofen on three occasions, separated by at least 7 days. Two of these capsules were identical prolonged release formulations. One was administered directly after manufacture, whilst the second was subjected to accelerated aging at 30 °C for 1 month prior to dosing. The third capsule was a rapid release formulation.

After an overnight fast, at 08:30 h, the subjects consumed a light breakfast (1500 kJ). At 09:00 h, each subject swallowed a capsule containing 4 MBq ^{99m}Tc, along with 200 ml of water labelled with 2 MBq of ^{113m}In-DTPA. The 'aged' prolonged release capsules contained no radiolabel and were not followed by gamma scintigraphy.

Scintigraphic images were recorded every 10 min in volunteers receiving the rapid release formulation, until gastric emptying was complete. With the freshly prepared prolonged release formulation, imaging was performed every 20 min for the first 2 h, and at 30-min intervals for the remaining 8 h. During the study, the subjects remained moderately active and were imaged in a standing position.

Blood samples (5 ml) were withdrawn from an indwelling venous cannula. In volunteers receiving the prolonged release capsules, blood was taken immediately prior to dosing and at 30-min intervals for 5 h with additional samples at 6.0, 7.0, 8.0, 10.0 and 24.0 h. After administration of the rapid release formulation, blood samples were taken at 10-min intervals for 1 h with additional samples at 80, 100, 120, 180, 240, 360 and 480 min. The fresh blood was collected in heparinized tubes and centrifuged for 10 min at 3000 rpm. The supernatant plasma was then transferred to plastic screw-top tubes and stored at −20 °C prior to analysis.

A light lunch was provided for the volunteers at 13:30 h and tea at 17:00 h. Each volunteer was permitted to drink water or decaffeinated coffee at will during the study.

Imaging (gamma scintigraphy)

Dual isotope detection is possible with ^{99m}Tc and ^{113m}In (Davis et al., 1984b) and was achieved in this study using energy window settings of 115–160 and 340–443 keV, respectively. However, this requires correction for scatterdown of the higher energy emission from ^{113m}In into the lower energy window. This was estimated by administering the ^{113m}In-DTPA complex to each volunteer alone, and recording in both energy windows. The percentage 'scatterdown' in the lower window can then be calculated, and used in data correction.

Monitoring gastric emptying from only one side of each volunteer has been reported to produce variation in counting efficiency, dependent upon the changing depth of the radioactive source (Tothill et al., 1978; Whalley et al., 1982). Tothill et al. (1978) reported that anterior detection alone underestimated gastric emptying by 26% and recommended calculation of the geometric mean of anterior and posterior views to overcome this problem. In the present study, anterior and posterior frames of 60 s duration were recorded, and the geometric mean calculated. To enable accurate collimator realignment with each volunteer, an external marker containing approx. 0.5 MBq ^{99m}Tc was taped to the skin overlying the liver to the right of the stomach.

The stored scintillation data were analysed using a computer program (Med 256, Link Systems). Gastric emptying was determined by drawing a region of interest around the stomach, outlined by the administered ^{113m}In-DTPA solution. The proportion of total counts present in the region of interest relative to $t = 0$, corrected for background radiation scatterdown and decay provides a quantitative measure of gastric emptying.

In volunteers receiving the prolonged release formulation the time to reach the ileo-cecal junction was also recorded. This was defined as the time to arrive at a region in the lower abdomen, positioned on an imaginary line drawn vertically down from the pyloric sphincter. Determination of this time is facilitated by prolonged residence at the ileo-cecal junction (Wilson and Hardy, 1985;

Fischer et al., 1987), in contrast to the continuous and apparently erratic dosage form movement in the small intestine.

Plasma analysis

Naproxen was used as the internal standard for the assay of ketoprofen. 200 μl of naproxen solution (10 μg/ml) was pipetted into 15 ml screw-cap disposable glass culture tubes (Corning) for samples and standard curves alike. In those tubes used for a standard curve, ketoprofen solution was added to provide standards of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 μg/ml. Additionally, a tube containing no ketoprofen was run. The contents of all tubes were then adjusted to 1.0 ml total volume with 0.01 M phosphate buffer pH 6.0, followed by 1.0 ml of the plasma to be assayed. For standard curves the plasma was derived from drug-free carriers.

Each sample was acidified by the addition of 0.5 ml of 0.1 M hydrochloric acid. After addition of 10 ml diethyl ether, each tube was mechanically shaken for 15 min, followed by centrifugation at 3000 rpm for 3 min. The upper organic layer was transferred by Pasteur pipette to a disposable 15 ml culture tube and evaporated to dryness at 40 °C under a vacuum. The extract was reconstituted in 250 μl of HPLC eluent, vortexed for 15 s and poured into a micro-centrifuge tube. Samples were centrifuged (Model 320a, Mechanika Precyzyjna, Poland) for 4 min and the clear supernatant manually injected into the HPLC column via a 20 μl loop. Chromatography was performed using a Spherisorb 5 μm ODS column, 250 × 4.9 mm (Hichrom), eluted at 1.5 ml/min with 0.05 M Sorensen's phosphate buffer pH 7.0, containing 20% acetonitrile. Peak areas for ketoprofen and naproxen were calculated using an SP 4290 integrator (Spectra Physics).

Quantitation was based on peak area ratios. Since the assay covers almost a 1000-fold spread in concentration, the standard curve was split into a high and a low range, to avoid excessive weight being given to more concentrated standards. A least-squares regression was fitted to each range. Limits were set with an overlap of two calibrators, and samples in this region were quantified using the lower range.

Percentage recovery of ketoprofen was determined by spiking plasma with 5 μg of ketoprofen and comparing the peak area with that of a standard 20 μg/ml ketoprofen solution. Assuming 100% recovery, identical peak areas should be produced. Recovery was therefore calculated from the ratio of plasma and standard integration values.

Pharmacokinetic analysis

Ketoprofen plasma concentration-time data were processed with an Apple II computer using an extended least-squares regression program (MK Model II, N.H.G. Holford, 1983, University of California). Data were fitted to a zero- or first-order absorption model with either one or two compartments. Using the best fit, elimination ($t_{1/2\beta}$) and, where appropriate, distribution ($t_{1/2\alpha}$) half-lives were calculated. Areas under plasma concentration-time curves (AUC) and first moments curves (AUMC) were calculated using the trapezoidal rule. The mean residence time (MRT) was calculated from the plasma concentration data according to the equation:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

Results and Discussion

In vitro dosage form evaluation for clinical study

To facilitate selection of a semi-solid matrix composition likely to produce a desirable in vivo prolonged release profile, experimental formulations were compared to marketed sustained release capsules, Oruvail 100 mg (May and Baker).

Tests were performed using a conventional BP dissolution apparatus with pH switching from 1.0 to 7.2 after 1 h and a BIO-DIS. The BIO-DIS was included as it is claimed that the determination of drug release over a graduated pH range more accurately mimics the changes experienced during dosage form transit through the gastrointestinal tract (Beckett, 1985). This is particularly relevant to drugs with pH-dependent solubility and dosage

forms with pH-sensitive coatings. pH switching was used in the BP test as a simplified comparison. The dissolution profiles of Oruvail (100 mg) using the two test methods produced similar results (Figs 1 and 2). In acidic media release is very slow. At a pH above 4.5, release occurred at 10–15% h^{-1}. If the lag period before ketoprofen liberation commences is taken into account, the profiles are almost superimposable.

Two semi-solid matrix formulations were identified as candidates for investigation. The first, Gelucire 50/13, released ketoprofen too rapidly to expect prolonged release in vivo (Figs 1 and 2). The second vehicle, composed of 50/13 and 50/02, 75 and 25%, respectively (HLB 10.25), demonstrated acceptable prolonged release over 22 h in both tests (Figs 1 and 2). This formulation was selected for clinical investigation. The dissolution data from the wax matrix appeared similar to those for Oruvail, except for the initial rapid release phase. After 22 h complete ketoprofen liberation was recorded using the BIO-DIS (Fig. 2),

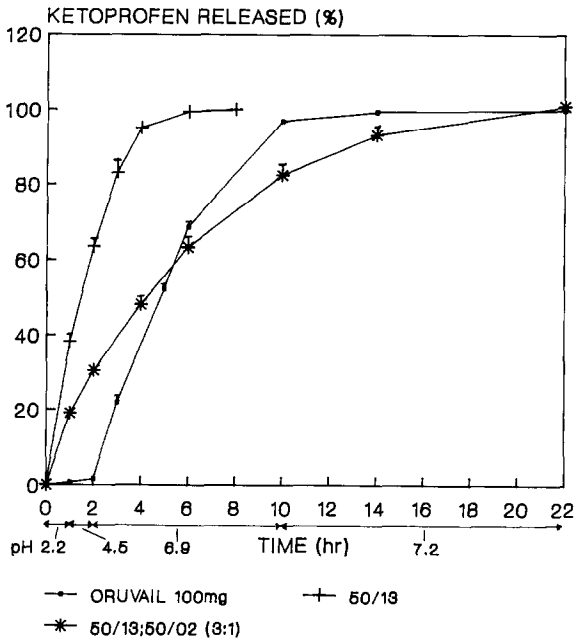

Fig. 2. Ketoprofen (100 mg) dissolution profiles from Oruvail and Gelucire prolonged release capsules using a BIO-DIS apparatus with pH changes at 1.0, 2.0 and 10.0 h. (Dissolution conditions: $t = 0$, pH 2.2; $t = 1.0$ h, pH 4.5; $t = 2.0$ h, pH 6.9; $t = 10$ h, pH 7.2. 37 °C, $n = 4$).

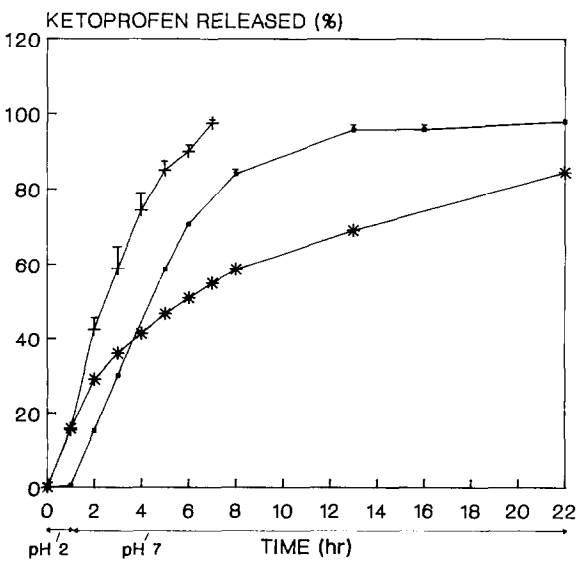

Fig. 1. Ketoprofen (100 mg) dissolution profiles from Oruvail and Gelucire prolonged release capsules using a BP basket apparatus with change from pH 2.0 to pH 7.0 after 1.0 h. (+) Gelucire 50/13; (*) Gelucire 50/13 : Gelucire 50/02 (75 : 25); (■) Oruvail (May and Baker).

but only to the extent of approx. 80% with the BP apparatus (Fig. 1). This difference may be attributed to greater agitation intensity associated with the oscillation in the BIO-DIS basket assembly. Availability may, however, be increased in vivo by the action of digestive enzymes or by compressional forces acting on the soft matrix. Dissolution experiments were also performed in order to confirm that storage-related changes, observed with the individual Gelucire grades (Dennis, 1988), occurred in the formulation selected for clinical study. The prolonged release formulation was shown to exhibit changes in ketoprofen release rate similar to those reported earlier (Dennis, 1988).

The time for 50% drug release was reduced from 253 min (±4 min) directly after manufacture to 161 min (±6 min) after 28 days storage at 30 °C. This formulation was therefore used to assess the importance of in vitro ageing on in vivo release and availability.

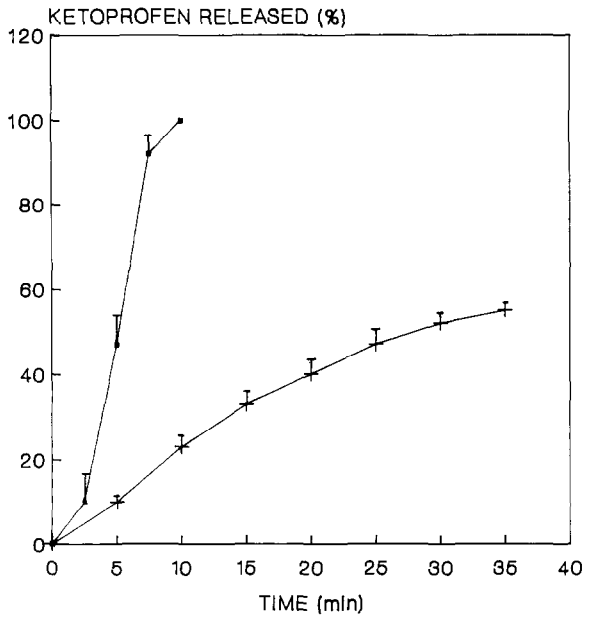

Fig. 3. Ketoprofen release from Orudis (100 mg) and Gelucire 44/14 (100 mg) capsules. (Dissolution conditions: pH 2.2, 37 °C, $n = 4$). (■) Gelucire 44/14 capsule; (+) Orudis capsule (May and Baker).

A rapid release capsule formulation containing a hydrophilic wax ketoprofen dispersion was also investigated.

During in vitro dissolution tests (BP basket method) drug was released as the base (Gelucire 44/14) melted and dispersed/dissolved in the surrounding water. More rapid drug liberation than the conventional powder capsule formulation resulted (Orudis, May and Baker) (Fig. 3). This may be attributed to a combination of properties; improved drug wettability (Sekiguchi and Obi, 1961), molecular dispersion of drug in the vehicle (Hargreaves et al., 1979), and a favourable co-solvent effect produced by dissolved ethoxylated fat (Ford and Rubinstein, 1977).

In vivo dosage form evaluation

Plasma analysis

Excellent linearity was exhibited between ketoprofen : naproxen peak area ratio, and plasma ketoprofen concentration. Using a buffered eluent at pH 7.2, as described by Upton et al. (1980), and assaying at 276 nm, a chromatogram free from extraneous material was produced. This produced superior sensitivity to other methods (Ballerini et al., 1979; Jefferies et al., 1979) using a simple extraction procedure (92% efficiency for 5 μg ml^{-1}) less complicated than that described by Farinotti and Mahuzier (1979). Retention times were 9 and 12 min for naproxen and ketoprofen respectively. Concentration normalized peak areas indicated good linearity with coefficients of variation of normalised peak areas of 6.4 (60–2000 ng ml^{-1}) and 3.4 (1–2 μg ml^{-1}). A lower limit of detection of approx. 50 ng/ml was observed.

In vivo characteristics of Gelucire 44/14 rapid release capsules

Gelucire 44/14-ketoprofen pharmacokinetic data were treated individually. Both mean ± standard deviation for the parameters of highest observed plasma ketoprofen concentration (C_{max}), time of C_{max} (t_{max}), ketoprofen elimination half-life ($t_{1/2\beta}$) and area under the plasma ketoprofen concentration time curve (AUC) were calculated.

Plasma concentration profiles produced after administration of ketoprofen 100 mg (Gelucire 44/14) capsules (Fig. 4) generally indicated rapid absorption (t_{max} 61.7 min), comparable to that of

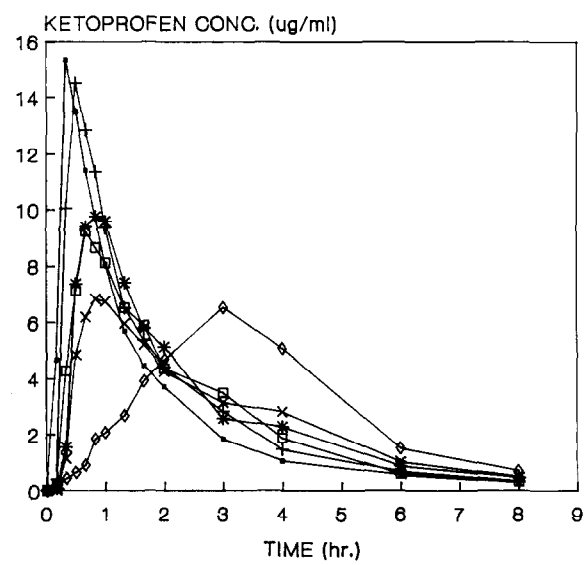

Fig. 4. Individual ketoprofen plasma concentration profiles in six healthy volunteers after administration of Gelucire 44/14 (100 mg) capsules. Volunteers: (■) NA; (+) MA; (*) RW; (□) HA; (×) AD; (◇) GR.

TABLE 2

Area under plasma concentration time profile (AUC) data and derived relative bioavailability for rapid release (formulation II) and prolonged release (formulation I) capsules

Volunteer	Dosage form characteristics				
	Rapid release fresh AUC ($\mu g\ ml^{-1}\ h$)	Prolonged release			
		Fresh		Aged	
		AUC ($\mu g\ ml^{-1}\ h$)	Relative bioavailability (%)	AUC ($\mu g\ ml^{-1}\ h$)	Relative bioavailability (%)
NA	22.15	14.62	66.0	15.40	69.5
GR	26.31	19.00	72.2	11.96	45.5
HA	22.32	13.53	60.6	11.45	51.3
MA	24.86	15.17	61.0	14.69	59.1
RW	24.13	14.76	61.2	15.09	62.5
PA	–	11.02	46.4 [a]	15.55	65.5 [a]
AD	22.65	–	–	–	–
Mean	23.74	14.68	61.2	14.02	58.9
Standard deviation	1.66	2.59	8.5	1.83	9.0

[a] Relative bioavailability calculated using mean rapid release AUC data.

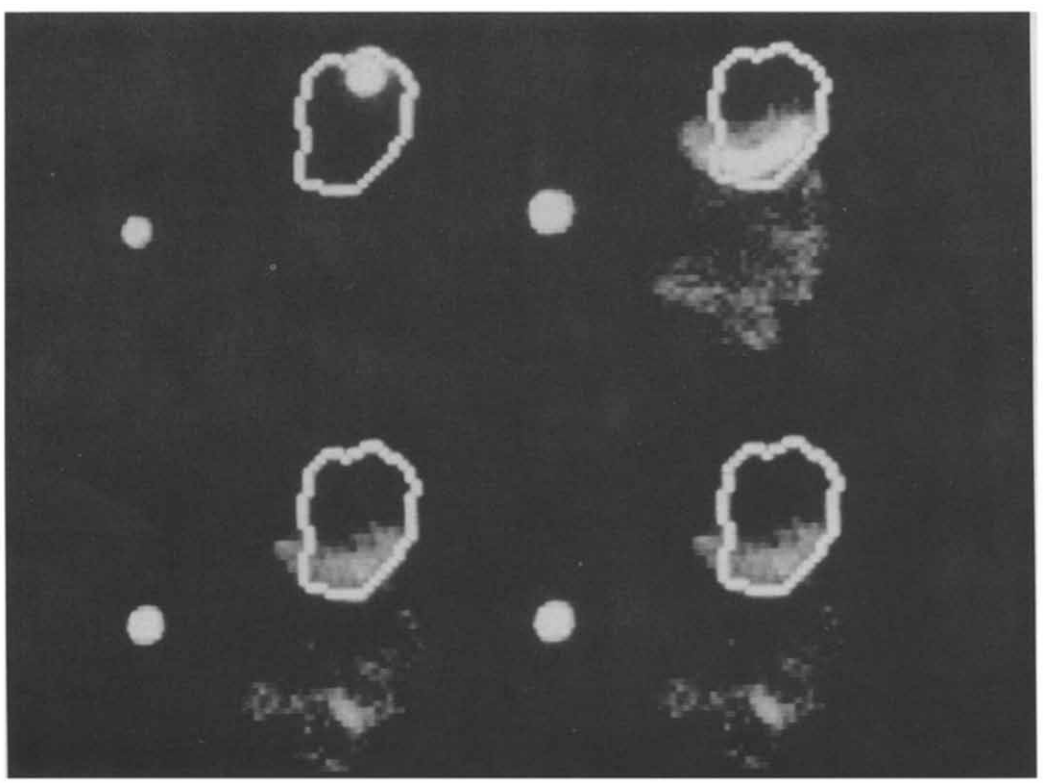

Fig. 5. Gamma scintigraphic images for volunteer NA showing the stomach region of interest and Gelucire 44/14 ketoprofen (100 mg) capsule dispersion/gastric emptying. (Upper left) $t = 0$ min; (upper right) $t = 10$ min; (lower left) $t = 20$ min; (lower right) $t = 30$ min.

conventional oral ketoprofen formulations (Castegnaro et al., 1974; Ishizaki et al., 1980; Borsa et al., 1983; Houghton et al., 1984c). One volunteer (GR) was anomalous, showing a more prolonged absorption phase.

In the two volunteers where absorption was fast ($t_{max} < 30$ min) the best experimental fit was produced using a two-compartment disposition model, as used by Sala et al. (1978) and Debruyne et al. (1987). The distribution phase was very short ($t_{1/2\alpha} = 33.6$ min; $n = 2$). Where absorption was slower, no distribution phase could be identified and data were therefore fitted to a single-compartment disposition model. The mean value of $t_{1/2\beta}$ was 108 ± 15 min ($n = 6$) in agreement with the values reported by Caille et al. (1978), Sala et al. (1978), Ishizaki et al. (1980), Advenier et al. (1983) and Debruyne et al. (1987), but faster than those reported by Houghton et al. (1984b,c).

The reported values of AUC for orally administered ketoprofen, normalized to 100 mg, yield values of 19.4 μg ml^{-1} h (Advenier et al., 1983), 21.9 μg ml^{-1} h (Ishizaki et al., 1980) and 22.0 μg ml^{-1} h (Houghton et al., 1984a), all similar to 20.00 μg ml^{-1} h reported by Debruyne et al. (1987) after intravenous administration. The experimental AUC value of 23.7 μg ml^{-1} h determined after administration of Gelucire 44/14 capsules (Table 2) was slightly higher than that of the other oral formulations and indicative of excellent availability from the dispersible wax.

From the in vitro capsule dissolution findings (Fig. 3), rapid dispersion and drug absorption would be expected. Drug absorption was indeed rapid, provided that spreading and dispersion of the vehicle occurred soon after ingestion. However, two cases appeared to be anomalous. Subject NA produced extremely rapid gastric dispersion

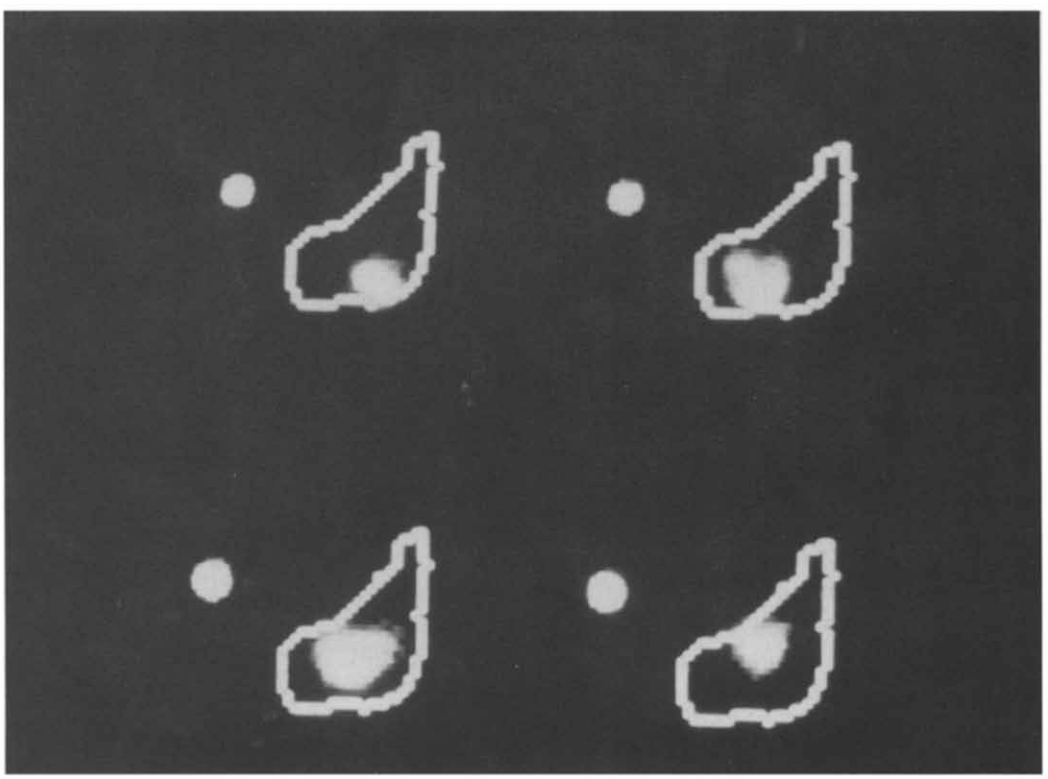

Fig. 6. Gamma scintigraphic images for volunteer GR showing the stomach region of interest and Gelucire 44/14 ketoprofen (100 mg) capsule dispersion/gastric emptying. (Upper left) $t = 0$ min; (upper right) $t = 60$ min; (lower left) $t = 120$ min; (lower right) $t = 180$ min.

and emptying (Fig. 5), whilst subject GR exhibited only minimal dispersion with concomitant prolonged gastric residence (Fig. 6). According to Hunter et al. (1980), in vitro-in vivo correlation is most likely to occur on a full stomach. This may help to explain the anomalous behaviour of volunteer GR. In this case, capsule dosing was delayed until 1 h after food, which may have permitted substantial emptying of gastric contents. Since much of the liquid swallowed with the capsule will be rapidly emptied (Jenkins et al., 1983), only a relative viscous mucus lining will remain, through which the vehicle must spread. This may then account for the slow and limited extent of dispersion in the stomach (Fig. 6), similar to that reported by Hunter et al. (1983b) after administration of thixotropic capsule formulations to fasted volunteers. Alternatively, the dosage form may have lodged between a rugal fold in the stomach (Hey et al., 1979) and so limiting dispersion to the surrounding mucus. The second case, concerning the extremely rapid capsule disintegration observed with NA, may be linked to a higher fluid intake (300 ml) required in this volunteer to wash the capsule down the oesophagus into the stomach. This rapid release of capsule contents has been theorised to involve entry of gastric juices into the capsule interior via a small orifice by release of the soluble components (Casey et al., 1976).

From the percentage activity remaining in the stomach, gastric emptying profiles were constructed (Fig. 7). For hard gelatin capsule formulations these plots have been categorized into five main types (Hunter et al., 1983a). In this study, gastric emptying of the capsule contents occurred quickly and progressed according to an essentially monoexponential relationship. Similar observations were described by Hunter et al. (1983b) with hard gelatin capsules containing thixotropic liquids.

The extent of formulation dispersion was determined by calculating the proportion of the region of interest occupied by the labelled wax. Gastric emptying was quantified using the half-emptying time as described by Datz et al. (1987). By comparing these parameters (Table 3) with those derived from corresponding ketoprofen

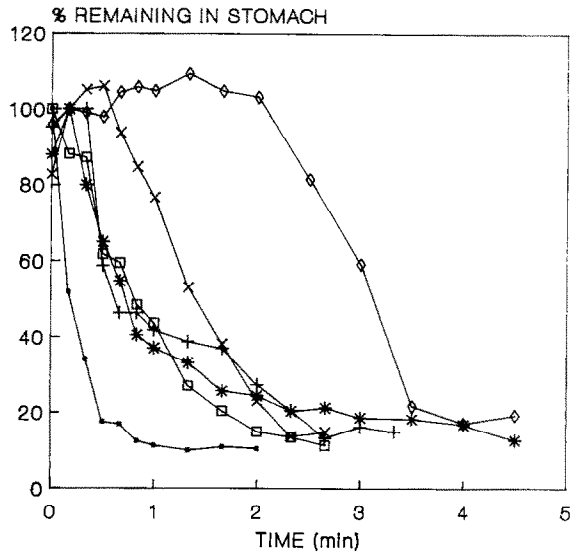

Fig. 7. Gastric emptying profiles for Gelucire 44/14 ketoprofen capsules (100 mg) administered to six healthy volunteers after a light breakfast. Volunteers: (■) NA; (+) MA; (*) AD; (□) RW; (×) HA; (◇) GR.

plasma profiles (Fig. 4), it can be shown that ketoprofen absorption (t_{max}) correlates well with both gastric emptying time ($r = 0.952$) and the rate of gastric dispersion/spreading ($r = 0.976$). Additionally, excellent correlation exists between the time taken for maximum gastric dispersion to occur and the rate of gastric emptying ($r = 0.989$). Taken as a whole, this suggests that the processes leading to ketoprofen absorption principally involve dispersion/spreading in the stomach, followed by emptying of the gastric contents into the small intestine from where the most rapid drug absorption occurs. Thus, capsules which rapidly disperse (e.g. volunteer NA, Fig. 5) empty quickly into the small intestine, a region of high absorptive capacity (Heading et al., 1973), leading to rapid drug absorption (Fig. 4). Where vehicle spreading is only limited (e.g. volunteer GR, Fig. 6), a lag time prior to gastric emptying occurs (Fig. 7) and drug absorption is consequently slower and more prolonged (Fig. 4).

In vivo characteristics of Gelucire (HLB 10.25) sustained release capsules

The mean plasma ketoprofen concentration vs time profile of the freshly prepared sustained re-

TABLE 3

Gamma scintigraphic derived measurements of gastric dispersion and emptying, and corresponding plasma profile data for rapid release ketoprofen capsules

Volunteer	Scintigraphic measurements			Plasma data	
	Time for maximum gastric dispersion (min)	Extent of gastric dispersion (%)	Gastric half emptying time (min)	t_{max} (min)	C_{max} ($\mu g\ ml^{-1}$)
NA	10	41	13	20	15.36
MA	20	37	36	30	14.50
AD	20	70	44	50	6.83
RW	20	91	50	50	9.74
HA	30	45	85	40	9.23
GR	80	28	188	180	6.54
		$r = 0.989$	$r = 0.952$		
		$r = 0.976$			
Mean	30.0	52.0	69.3	61.7	10.37
Standard deviation	25.3	23.7	62.6	59.1	3.76

lease formulation is shown in Fig. 8. Mean peak plasma concentration (1.32 $\mu g\ ml^{-1}$) (Table 4) was reduced relative to the fast release capsule (10.37 $\mu g\ ml^{-1}$; $p < 0.01$), and drug levels were more prolonged. The mean residence time (MRT), and the period over which drug concentration remained above the half peak value (Δt), were increased 3.3- and 2.5-fold, respectively. The post-peak half-life was also increased from 108 to 162 min. However, this was not statistically significant ($p > 0.05$). This probably reflects the omission of some terminal concentration data points (8, 10 and 24 h) which did not fit simple elimination kinetics and were therefore not included in the analysis. The calculated $t_{1/2\beta}$ value therefore represents only a proportion of the elimination phase and is not an accurate indication of drug remaining at times greater than 10 h.

By relating individual plasma concentration profiles to the corresponding gamma scintigraphic data, deductions can be made regarding the performance of the wax matrix in various regions of the digestive tract. As noted with the rapid release capsule, t_{max} correlated with gastric emptying time ($r = 0.916$; Table 4). In this case, however, gastric emptying will be an 'all-or-nothing' process. Monoliths above approx. 4–5 mm in diameter will be retained in the stomach by the pyloric sphincter (Minami and McCallum, 1984; Davis, 1985). Only when the stomach is empty of food will the device be cleared. Beechgaard and Christensen (1982) described this as an essentially random process, determined by the arrival of the 'house keeper' wave (Meyer, 1980) which passes slowly down the digestive tract in cycles of approx. 2 h (Code and

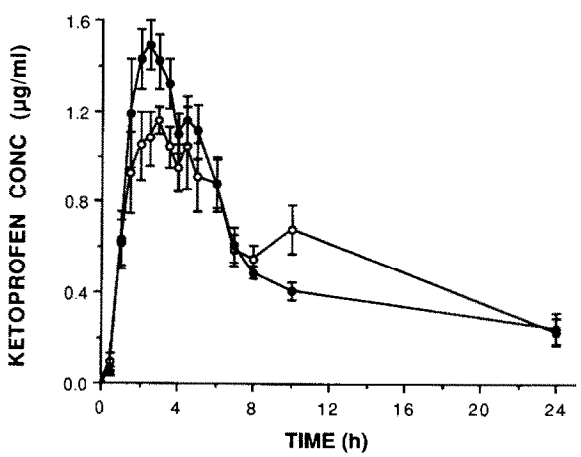

Fig. 8. Mean plasma ketoprofen concentration time profiles of freshly manufactured and 'aged' sustained release ketoprofen (100 mg) capsules administered to six volunteers after a light breakfast. (○) Ketoprofen prolonged release (100 mg) capsules (Gelucire 50/02, 75:25) freshly manufactured. (●) Ketoprofen prolonged release (100 mg) capsules (Gelucire 50/13:Gelucire 50/02, 75:25) stored for 1 month at 30 °C.

TABLE 4

Gamma scintigraphic derived measurements of gastric emptying, small intestinal transit time and corresponding plasma profile data for prolonged release ketoprofen capsules

Volunteer	Scintigraphic measurements			Plasma data		
	Gastric emptying time (min)	Ileo-caecal arrival time (min)	Small intestine transit time (min)	t_{max} (min)	C_{max} ($\mu g\ ml^{-1}$)	Δt (min)
MA	80	210	130	120	1.51	268
NA	80	210	130	120	1.10	252
PA	80	330	250 [a]	90	1.24	370
RW	100	210	110	120	1.11	226
HA	120	270	150	80	1.19	300
GR	270	480	210	270	1.78	253
		$r = 0.916$			$r = 0.751$	
Mean	122	285	163	133.3	1.32	278
Stand. deviation	74	106	55	69.1	0.27	51

[a] Matrix voided after 8 h.

Marlett, 1975). This accounts for the wide range in gastric emptying times (80–270 min), and consequently the variable onset of greatest drug absorption. Gastric emptying was calculated to occur after 122 ± 74 min, which is of a similar order to values reported with other lightly fed volunteers, e.g., 183 and 164 min (Davis et al., 1984a,b).

The freshly prepared sustained release capsules yield a 'steady' ketoprofen plasma concentration between 1 and 5 h (Fig. 8). Directly after this period a significant reduction in plasma concentration occurs. The duration of this steady drug level, as described by Δt, produced a correlation ($r = 0.751$) with small intestinal transit time (Table 4). Since, in all cases, dosage forms did not pass into the colon until after 5 h post administration, it was concluded that optimal release and absorption resides in the small intestine. Poor availability from the colon does not necessarily follow. In five of the six volunteers, secondary maxima occurred on the ketoprofen plasma concentration vs time curves, while the matrices were observed to be resident in the colon. It is unlikely that this observation is due to enterohepatic cycling, since double peaks have not been observed following intravenous or conventional oral doses of ketoprofen (Ishizaki et al., 1980; Advenier et al., 1983; Houghton et al., 1984b,c; Debruyne et al., 1987). This behaviour probably results from wax hydration and softening, causing an increase in erosion/spreading, possibly enhanced by gut contractions exerted on the matrix (Holdstock et al., 1970; Hardy et al., 1985). In one volunteer only (PA) the plasma drug level fell after 5 h, with no secondary peak. This volunteer had a short mouth-to-defecation time, probably due to rapid large bowel transit (Table 4). This provided further evidence in support of the colon as a significant site of drug absorption. Similar secondary maxima and variable transit rates have been reported for an erodible ibuprofen polymer matrix by Parr et al. (1987), and Brufen® by Wilson et al. (1989).

Since the fast release ketoprofen formulation shows excellent bioavailability, a close estimate of the true bioavailability for the sustained release capsule can be achieved by expressing the relevant AUC value as a proportion of the corresponding rapid release capsule. Using this method, the relative bioavailability was calculated to be $61.2 \pm 8.5\%$, lower than that predicted from in vitro experiments (Figs 1 and 2). This suggests that physiological factors which may have acted to increase release rate, such as enzymatic digestion

TABLE 5

Prolonged release ketoprofen capsule matrix erosion in-vivo and in-vitro using gamma scintigraphy

Corrected % activity remaining with dosage form after 10 h (using iso-contour at 10% above background)						
Dissolution test (BP 1980)	Volunteers					
	PA	MA	RW	GR	NA	HA
47.9 (±7.3)	93.1	88.7	76.4	71.0	91.6	83.2

of the wax, or compressional forces exerted by the gut on the hydrated matrix, are not significant. In fact, surface erosion/dissolution of the ketoprofen wax matrix appeared slower in vivo than in vitro (Table 5). However, the extent of in vivo dosage form erosion/spreading is difficult to measure accurately, due to the poor resolving power of the scintigraphic technique. It is therefore possible that radioactivity detected adjacent to the dosage form may not have originated from the intact matrix, but instead from nearby 'debris', therefore accounting for the apparently slow capsule erosion. Alpsten et al. (1976), investigating ferrous sulphate sustained release tablets, similarly reported slow in vivo disintegration and suggested this accounted for the reduced in vivo dissolution rate. Both the BP (1980) method and the BIO-DIS test overestimated release rate and consequently bioavailability. The former method produced the best correlation, predicting 83% release over 22 h as opposed to 61% determined. The poor in vitro/in vivo correlations may have been due to an improper choice of agitation conditions, as suggested by Levy et al. (1965). Alternatively, wax dispersion may have been reduced by 'drier', less fluid conditions, especially within the colon (Powell, 1987). The assumption that the dosage form residence time will be long enough to enable absorption to occur over a full 24 h period is also inappropriate (Table 4). Low bioavailability may therefore have been caused by 'programming' drug release to occur too slowly. This can result in passage of the dosage form completely through the gastrointestinal tract before absorption is complete (Houghton et al., 1984b,c; Wilson and Washington, 1988). Bioavailability will then be dependent upon the speed of gastrointestinal transit. This has been reported with ibuprofen sustained release tablets (Parr et al., 1987) and also occurred in the present study. For example, volunteer PA produced rapid total bowel transit (< 8 h) (Table 4) and a correspondingly low relative bioavailability (46.8%). In contrast, volunteer GR produced slow gastric emptying and an extended period in the upper digestive tract (Table 6). Accordingly, bioavailability was improved (72.2%).

Effect of ageing on in vivo characteristics of Gelucire (HLB 10.25) sustained release capsules

Although capsules stored at 30 °C for 1 month demonstrated a reduction in $t_{50\%}$, from 253 to 161 min in vitro, no statistically significant changes were observed in vivo (Table 6). Administration of either freshly manufactured sustained release capsules or aged capsules produced mean ketoprofen plasma profiles which were almost superim-

TABLE 6

Summary of pharmacokinetic parameters for rapid release and prolonged release freshly manufactured and 'aged' ketoprofen capsules

Pharmacokinetic parameter	Formulation					
	Rapid release		Prolonged release			
	Fresh		Fresh		Aged	
	Mean	SD	Mean [a]	SD	Mean [a]	SD
MRT (min)	162.9	(28.5)	593.2	(146.5)	595.0	(120.5)
Δt (min)	112.2	(64.2)	278.4	(51.0)	279.6	(55.2)
$t_{1/2\beta}$ (min)	108.0	(15.0)	162.0	(58.1)	207.6	(53.4)
C_{max} ($\mu g\ ml^{-1}$)	10.37	(3.76)	1.16	(0.15)	1.50	(0.27)
t_{max} (min)	61.7	(59.1)	133.3	(69.1)	140.0	(49.0)

[a] Pooled estimate of variance Student's t-test indicates no statistical difference between mean values of fresh and aged capsules, $p > 0.05$.

posable (Fig. 8). The aged formulation did produce a higher mean C_{max} (1.50 ± 0.27 μg ml^{-1}) compared to standard capsules (1.16 ± 0.15 μg ml^{-1}), although not statistically significant ($p > 0.05$). T_{max} values were also comparable, and individual maxima appeared more dependent upon gastric emptying than any physicochemical changes occurring in the matrix. Statistical comparison of MRT, Δt and $t_{1/2\beta}$ between batches revealed no statistically significant difference (Table 6). The relative bioavailability of the aged capsules was also unchanged, 61.8 ± 10.9 and $59.1 \pm 7.7\%$ before and after storage, respectively. Overall this indicated that changes occurring in the in vitro dissolution rate during storage were not paralleled by altered blood levels. Similar observations have been reported (Taraszka and Delor, 1969; Slywka et al., 1976), where formulations have been differentiated in vitro but which possess no statistically significant differences in vivo.

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