ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1521-1530

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

# Determination of GI147211 in human blood by HPLC with fluorescence detection

Krzysztof Selinger*, Glenn Smith, Scott Depee, Cathy Aureche

*Glaxo Inc. Research Institute, Five Moore Drive, Research Triangle Park, NC 27709, USA*

Received for review 13 December 1994

**Abstract**

GI147211 (GG211) is a camptothecin analogue, which exhibits antileukemic and antitumor activity by blocking DNA synthesis. The drug stability considerations and specimen handling were important aspects in method development and validation. This method involves collection of blood at the clinical site, immediate freezing, and storage at −70°C. The lactone form is extracted from blood at physiological pH with a mixture of *n*-butyl chloride and acetonitrile (4:1); the carboxylate is not extracted under these conditions. After evaporation the extract is injected into an HPLC system with a fluorescence detector set at 378/420 nm. The internal standard used is 6,7-dimethoxy-4-methylcoumarin. The main advantages of the procedure are the separation of lactone and carboxylate by means of extraction, simplified specimen collection at clinical sites and the ability to inject almost all of the extracted material (extraction recovery, 60%) into an HPLC system. The method has been validated over the range 0.15–100 ng ml$^{-1}$ with sufficient precision and accuracy (coefficient of variation below 10%) to support pharmacokinetic studies. Under the conditions of this procedure, the drug is stable in human blood at −70°C for at least 93 days, as well as through two additional freeze-thaw cycles.

*Keywords:* Camptothecin analogue; Fluorescence detection; GI147211; High-performance liquid chromatography; Human blood

## 1. Introduction

In 1966, Wall and co-workers [1] discovered a new antieoplastic agent, a natural alkaloid, camptothecin, isolated from the Chinese tree *Camptotheca acuminata*. Camptothecin is a topoisomerase I inhibitor, and in its presence supercoiled duplex DNA is prevented from relaxing and consequently from replicating and transcribing. Camptothecin and its congeners show antineoplastic activity against melanoma, non-small-cell lung cancer, adenocarcinoma, and leukemia. The crucial element of the structure is the presence of the intact α-hydroxy-δ-lactone ring moiety, which is essential for both passive diffusion into cancer cells and for drug interaction with topoisomerase I. In acidic medium, this ring remains intact; in the basic medium it opens, yielding an inactive carboxylate form. At physiological pH around 7.4, the two forms remain in equilibrium shifted towards the carboxylate.

Despite its promise, camptothecin did not become a successful drug due to its poor water solubility and toxic side effects (myelosuppression, hemorrhagic cystitis and severe diarrhea). However, a number of its more soluble analogues have been synthesized, and are undergo-

* Corresponding author.


*SSDI* 0731-7085(95)01592-2

ing clinical trials — topotecan (SKF 104864), irenotecan (CPT-11), 9-aminocamptothecin. The lactone–carboxylate equilibrium is common for this group, and their mechanism of action is the same as that of camptothecin. The analytical literature on camptothecin and analogues in biological fluids is quite extensive. There are a number of analytical problems common to this group of compounds. The most important is whether to measure the lactone and carboxylate in the same assay; the alternative is to measure lactone only in one assay and total lactone in another, after acidification of the sample and conversion of the carboxylate. A number of researchers opted for the former [2–5], and an almost equal number for the latter [6–10].

Another issue is sample preparation. Here the method of choice is protein precipitation with organic solvents [2,3,5,9,10], followed by solid-phase extraction [4,6,7], and lastly, protein precipitation combined with liquid-phase extraction [8].

The final and most important issue is the preservation of sample integrity and the ratio of lactone to carboxylate. This is quite uniformly solved by the rapid harvesting of plasma from blood, followed by quick plasma processing to obtain organic extracts, as camptothecin analogues are stable in organic solvents at low temperature.

GI147211 [11] is a new camptothecin analogue developed as an anticancer drug by Glaxo Inc. The structure is shown in Fig. 1. The aim of this project was to develop and validate an analytical procedure, including sample collection and handling, of sufficient sensitivity (subnanogram per milliliter), precision and accuracy to support effectively phase I of drug development and beyond. This is the first communication in the literature on the determination of GI147211 in biofluids.

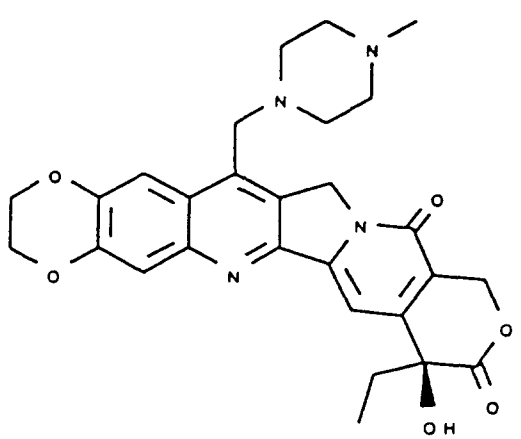


Fig. 1. Structure of GI147211.

## 2. Experimental

### 2.1. Materials

GI147211 dihydrochloride dihydrate (molecular weight, 609.5; free base, 518.5) was synthesized at Glaxo Inc. Research Institute. The internal standard 6,7-dimethoxy-4-methylcoumarin was obtained from the Aldrich Chemical Company (Milwaukee, WI). Human serum albumin fraction V (HSA) was purchased from Sigma (St. Louis, MO). All the other reagents and solvents were of HPLC grade, obtained from local suppliers and used without further purification.

### 2.2. Chromatographic conditions

Chromatography was performed with use of a system consisting of a model LC-10A pump, an autoinjector SIL-10A, a system controller SCL-10A, and a fluorescence detector RF-10A (all from Shimadzu, Columbia, MD) set at an excitation wavelength 378 nm and an emission wavelength 420 nm. The column (250 × 4.6 mm i.d.) was packed with BDS Hypersil C8, 5 μm particle size (Keystone Scientific), and equipped with an RP18 Newguard guard column (15 × 3.2 mm i.d.; Brownlee, ABI Applied Biosystems, San Jose, CA). The column was kept at 30°C in a column heater. The flow rate of the mobile phase was 1.7 ml $min^{-1}$ with a resulting back pressure of about 150 kg $cm^{-2}$. The data were collected by a Hewlett-Packard-based Laboratory Automation System (LAS). Linear regression calculations with $1/x^2$ were made by in-house-developed software (PRANBAS, version 2.03.00).

### 2.3. Mobile phase

The mobile phase was prepared by adding 500 ml of acetonitrile, and 200 ml of ammonium acetate buffer (pH 5.5; 1 m) to a 2 l volumetric flask. The flask was then brought to volume with water, and the solution was stirred and filtered through a 0.45 μm Nylon filter.

### 2.4. Calibration of standards preparation

The calibration standards in human blood were prepared by adding appropriate volumes of GI147211 spiking solutions in 0.01 M HCl to freshly collected heparinized human blood, mixing and storing on ice until extraction. The standards were prepared fresh daily; the spiking solutions were diluted also daily; amber glass was used to store the solutions. The quality control samples (QCs) were prepared to simulate the conditions in clinical samples, where both the lactone and acid exist at different ratios, generally close to 1:1. The quality control samples were spiked at equal concentrations with lactone and also with the acid form of the drug, which was obtained by diluting initial acetonitrile-solvated stock solutions (20 μg ml$^{-1}$) with 0.05 M NaOH. After taking aliquots, 1-ml fractions were stored at −70°C (±5°C) and thawed on the day of analysis.

### 2.5. Clinical sample collection

Blood samples were collected in heparinized glass tubes, and were placed immediately in an ice–water bath for no longer than 30 min, then frozen at −70°C until analysis. Upon arrival at the Clinical Pharmacology Laboratory at the Glaxo Research Institute, the samples were thawed in an ice–water bath, divided into two or three 1-ml aliquots, one of which was usually extracted the same day. The remaining sample and aliquots were refrozen for future analyses.

### 2.6. Extraction procedure

To a 1 ml aliquot of the standard, quality control blood or a clinical sample stored on ice, the internal standard was added (100 μl of 6,7-dimethyl-4-methylcoumarin, 200 ng ml$^{-1}$ in water). After mixing, 5 ml of a *n*-butyl chloride–acetonitrile (4:1, v/v) mixture were added and the tubes were mixed for 15 min on a rotomixer at about 60 rev min$^{-1}$. After extraction, the samples were centrifuged at 1000*g* for 10 min at −10°C, and the upper organic layer was transferred to a glass tube and evaporated to dryness at 37°C. The residue was dissolved in 200 μl of an acetonitrile–0.1 M sodium phosphate monobasic (1:4, v/v) mixture. A volume of 100 μl was injected into the chromatographic system.

### 2.7. Kinetic experiments

Kinetic experiments were carried out on plasma, human serum albumin (HSA) solution (50 mg ml$^{-1}$) in phosphate-buffered saline (PBS), and on blood. The pH of the matrices was monitored by means of a pH meter and/or adjusted to the required pH by adding small volumes of 2 M HCl or NaOH. The experiments on the plasma and the HSA involved spiking GI147211 at 40.0 ng ml$^{-1}$ into the medium, the addition of the internal standard at 100 ng ml$^{-1}$, and incubation at 0, 22, and/or 37°C. At specified sampling times, 20 μl of the mixture were injected directly into the chromatographic system described above; a shorter 150 × 4.6 mm C8 column was used. The experiments on blood involved spiking GI147211 at 475 ng ml$^{-1}$, addition of the internal standard at 2380 ng ml$^{-1}$ and incubation. At specified sampling times, 50 μl of the blood were diluted with 0.5 ml of PBS (pH 7.33), and 50 μl of the mixture were injected into the system. The procedure described here was, of course, harmful to the column, yet by minimizing the volume injected we were able to complete the experiment while maintaining satisfactory chromatography.

The pseudo first-order hydrolysis rate constant, $k$, was calculated as the slope of the plot of the natural logarithm of the difference between the lactone concentration at a time $t$ and at equilibrium versus the incubation time. The half-life, $t_{1/2}$, was calculated as $0.693/k$.

## 3. Results

### 3.1. Precision and accuracy

A set of seven calibration standards, a zero, a drug-free blood sample (all in duplicate), quality control samples at three concentration levels (in duplicate) prepared by a primary analyst, and a set of quality control samples formulated by a secondary analyst were analyzed on each of four validation days. The calibration curve covered the range from 0.15 to 100.0 ng ml$^{-1}$. The peak height ratio was used for quantitation. A linear regression analysis using a least-squares fit was performed with the squared reciprocal of the drug concentration as weight. The correlation coefficient was 0.9926 or better. The between-run precision of the method, expressed as the coefficient

Table 1
Precision and accuracy

| Statistical parameter | QC sample | | | | | |
|---|---|---|---|---|---|---|
| | 0.40A[a] | 0.40B[a] | 10.00A[a] | 10.00B[a] | 70.00A[a] | 70.00B[a] |
| Between-run | | | | | | |
| Mean | 0.430 | 0.427 | 10.644 | 10.383 | 68.294 | 69.631 |
| SD | 0.0366 | 0.0170 | 0.4888 | 0.5441 | 4.4770 | 4.2442 |
| CV (%) | 8.6 | 4.0 | 4.6 | 5.2 | 6.6 | 6.1 |
| % Nominal | 107.5 | 106.2 | 106.4 | 103.8 | 97.5 | 99.5 |
| N | 8 | 7 | 8 | 8 | 7 | 8 |
| Within-run | | | | | | |
| Mean | 0.421 | – | 9.320 | – | 63.934 | – |
| SD | 0.0155 | – | 0.3052 | – | 1.3902 | – |
| CV (%) | 3.7 | – | 3.3 | – | 2.2 | – |
| % Nominal | 105.3 | – | 93.2 | – | 91.3 | – |
| N | 8 | – | 8 | – | 7 | – |

[a] The number is the concentration of GI147211 in nanograms per milliliter, and A denotes the primary analyst and B the secondary analyst.

of variation (CV), was 4.0–8.6%, while the accuracy, expressed as a per cent of the nominal concentration, was 97.5–107.5%. The within-run precision of the method was 2.2–3.7%, while the accuracy was 91.3–105.3%. Both the precision and accuracy were calculated using the back-calculated QC concentrations. The between-run precision and accuracy were computed using data obtained separately by the primary and secondary analysts. The complete data are shown in Table 1.

### 3.2. Recovery

The recovery was calculated by comparing the peak heights in the extracted quality control samples with an unextracted calibration curve representing 100% recovery prepared by mixing of the appropriate stock solutions. All the potential losses due to sample transfer were factored in during the calibration curve preparation. The mean recovery of GI147211 extracted from human blood was 60.7% at 0.40 ng ml$^{-1}$ (CV; 3.9%; $n = 6$), 59.1% at 10.00 ng ml$^{-1}$ (CV; 7.8; $n = 6$), and 59.4% at 70.00 ng ml$^{-1}$ (CV; 9.6; $n = 6$). The recovery of the internal standard at the concentration used (20 ng per sample) was 90.2%.

### 3.3. Limit of quantitation

The limit of quantitation (LOQ) was estimated on the basis of comparing a signal at the LOQ to the noise rather than following the Washington Conference recommendations [12]. The limit of quantitation has been evaluated in the first four validation runs. The average background noise in both blank blood samples was measured by integrating three peaks/oscillations of baseline around the retention time of the GI147211 (±0.4 min.). The signal-to-noise ratio (S/N) at the LOQ has been calculated by dividing the mean peak height of the lowest standards by the mean of the noise. Another way of calculating the LOQ is to compare the peak height of the lowest standards with the sum of noise plus six standard deviations of this noise. The results are presented in Table 2. Standard 0.15 ng ml$^{-1}$ can be considered as an adequate LOQ, as the S/N ratio is always greater than 4, as well as the fact that the mean peak height of this standard is greater than sum of the noise plus six standard deviations (SD). Additionally, the coefficient of variation calculated on intrapolated concentrations at 0.15 ng ml$^{-1}$ is 9.4%.

### 3.4. Chromatography

Fig. 2A shows a chromatogram of the calibration standard 0.15 ng ml$^{-1}$, which is followed by a patient's sample at 0.51 ng ml$^{-1}$ (Fig. 2B), and a drug-free blood sample to which the internal standard has been added (Fig. 2C).

The chromatographic run time is about 11 min. The retention times of GI147211 and the internal standard are 4.1 min and 8.2 min,

Table 2
Limit of quantitation and signal-to-noise ratio (S/N)

| Batch no. | Noise | Signal | | | | | |
|---|---|---|---|---|---|---|---|
| | Peak height[a] | Mean | SD | $x + 6SD$ | Std 0.15 ng ml$^{-1}$ | Mean | S/N |
| 1 | 89, 143, 132, 99, 103, 72 | 106.3 | 26.6 | 266 | 409, 479 | 444 | 4.2 |
| 2 | 122, 61, 71, 120, 44, 87 | 84.2 | 31.8 | 275 | 627, 743 | 685 | 8.1 |
| 3 | 107, 130, 59, 195, 106, 172 | 128.2 | 49.2 | 423 | 577, 804 | 690 | 5.4 |
| 4 | 201, 123, 163, 71, 124 | 114.2 | 74.7 | 562 | 639, 560 | 600 | 5.3 |

[a] Peak heights are expressed in peak height units.

respectively. The prominent peak at 5.2 min is unknown and is always seen in human blood. The specificity of the assay was investigated by extracting six different blood samples from healthy volunteers not taking any medication. No interfering peaks were seen. The analytical system throughput is approximately 100 samples every 24 h. The extracts injected are rather cloudy from lipids and/or proteins and gradually increase the column back pressure. This could be easily remedied by changing the guard column every 2–3 runs. The cloudiness of the extracts could be removed by adding one more cleaning step to the extraction procedure, but this idea was rejected as no harmful effect on column efficiency was seen; they last at least 1000 injections. A practical advice is to use Teflon liners (or aluminium foil) underneath the screw caps in the extraction tubes; unknown interferences seen in the chromatograms have been traced to rubber and polymer liners used in the extraction tubes.

### 3.5. Choice of the internal standard

Although other GI147211 analogues were available, it was decided not to use any of them as a potential internal standard in order to avoid one more variable in the system. The internal standard chosen, 6,7-dimethoxy-4-methylcoumarin is not chemically related to camptothecin. It is a very stable, easily available, pure, strongly fluorescing, well-extractable lactone with an appropriate retention time in the system studied.

### 3.6. Stability

The issue of GI147211 stability was of key importance in the design of the assay as well as in the sample handling procedure. The method development and stability assessment were carried out in parallel as the stability results influenced the method development and vice versa. In the first experiment, we defined the sample temperature as a function of the time that this sample spends in the ice–water mixture. A 10 ml blood tube with an inserted digital thermometer was brought to 37°C and then placed in the ice–water mixture at 0°. The temperature decrease was monitored over 30 min. The results are presented in Fig. 3. It takes 1 min for the blood tube to reach the room temperature of 22°C, 10 min to reach 4°C, and 20 min to cool down to below 1°C.

Acceptable stability for GI147211 (including two cycles of freezing and thawing) has been demonstrated. The results are presented in Table 3. Samples cycled through freezing and thawing twice before analysis showed 110.0, 110.1, and 98.6% of the nominal concentration as compared to the freshly spiked standards. Samples kept for 30 min on ice after thawing showed 105.0, 108.6, and 103.9% of the nominal concentration.

The long-term storage stability at −70°C was evaluated while executing the first clinical study over a period of 93 days. Quality control samples were analyzed several times against freshly spiked calibration curves. The results are presented in Table 3; the QC values shown there represent a mean of two. Out of 33 sets of data, 3 only are outside the respective acceptance criteria. There is neither a trend visible in the data nor can lactone disappearance be observed.

### 3.7. Kinetics experiments

There is quite an extensive body of data on the kinetics of hydrolysis of camptothecin analogues [13–20]. Our kinetics experiments were undertaken to assess the stability of GI147211 in various matrices, at various pH values and temperatures, to compare it with other camptothecin analogues, and to evaluate blood/

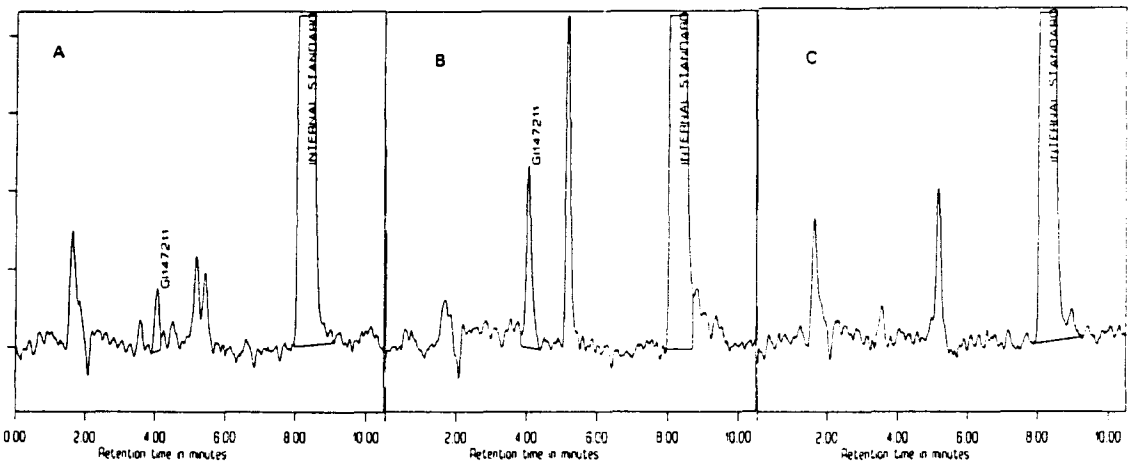


Fig. 2. Representative chromatography. (A) Calibration standard 0.15 ng ml$^{-1}$; (B) patient's sample at 0.51 ng ml$^{-1}$; (C) drug-free blood to which the internal standard has been added.

plasma interactions with the drug. The results expressed as half-lives are presented in Table 4, while Fig. 4 represents the kinetics of lactone ring opening.

The GI147211 stability seems to be similar to that of other camptothecin drugs, of which the half-lives in PBS are in the range 18–35 min, and in HSA, are 12–35 min [18]. The kinetics experiments sometimes may be difficult to evaluate, as the pH values of biological fluids may change during storage or incubation. Thawed and frozen plasma may even reach pH above 8.5 [6], the addition of HSA to PBS lowers the pH by approximately 0.2 units, and the addition of lipid dispersions to PBS decreases the pH by 0.6 [20]. The lactone–carboxylate equilibrium is very pH-sensitive around the physiological pH, as can be seen in Table 4. To obtain reliable and reproducible data, kinetics experiments should be performed in a pH stat, particularily if the incubation times are long.

### 3.8. Pharmacokinetic data

The assay was used to support a phase I study. Cancer patients were dosed either by a 30 min infusion or a 72 h continuous infusion of GI147211 at 0.25–1.75 mg m$^{-2}$ of body surface. An example of a pharmacokinetics profile is presented in Fig. 5. The patient received 0.6 mg m$^{-2}$, and the GI147211 concentration in his blood reached 11.46 ng ml$^{-1}$ of the free lactone.

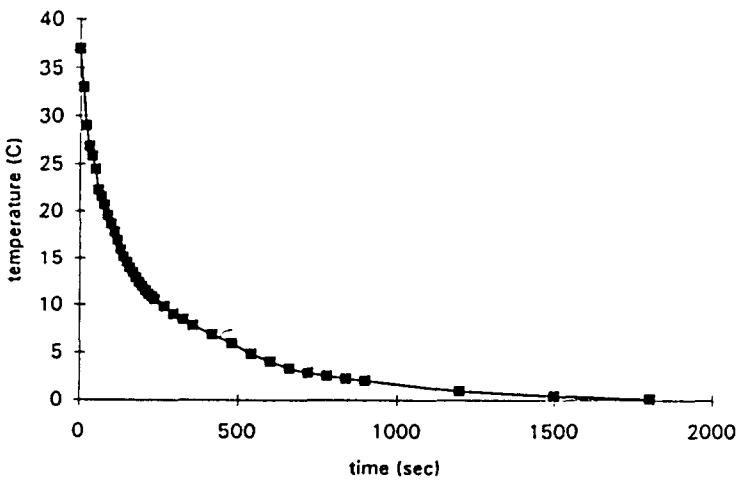


Fig. 3. Temperature decrease of the sample as a function of time. See the text for experimental details. The temperature $T$ at a given time $t$ (s) can be calculated from the empirical equation $T = \exp(3.1898 - 0.002882t)$, which back-calculates $T$ well at $t > 50$ s.

Table 3
Stability of GI147211 in human blood

| Statistical parameters | QC sample | | |
|---|---|---|---|
| | 0.40 ng ml$^{-1}$ | 10.00 ng ml$^{-1}$ | 70.00 ng ml$^{-1}$ |
| Processing stability[a] | | | |
| Mean (ng ml$^{-1}$) | 0.420 | 10.86 | 72.710 |
| SD | 0.0436 | 0.3108 | 2.4479 |
| CV (%) | 10.4 | 2.9 | 3.4 |
| % Nominal | 105.0 | 108.6 | 103.9 |
| N | 3 | 4 | 4 |
| Freeze–thaw stability[b] | | | |
| Mean (ng ml$^{-1}$) | 0.440 | 11.018 | 69.015 |
| SD | 0.0668 | 0.3188 | 2.6559 |
| CV (%) | 15.2 | 2.9 | 3.9 |
| % Nominal | 110.0 | 110.1 | 98.6 |
| N | 4 | 4 | 4 |
| Storage stability[c] | | | |
| Storage time (Days) | QC sample | | |
| | 40 ng ml$^{-1}$ (ng ml$^{-1}$) | 10.00 ng ml$^{-1}$ (ng ml$^{-1}$) | 70.00 ng ml$^{-1}$ (ng ml$^{-1}$) |
| 1 | 0.44 | 9.47 | 68.77 |
| 16 | 0.44 | 9.20 | 72.30 |
| 26 | 0.42 | 8.99 | 67.78 |
| 29 | 0.45 | 10.86 | 83.30 |
| 36 | 0.38 | 10.15 | 76.26 |
| 57 | 0.38 | 9.10 | 68.75 |
| 63 | 0.42 | 10.25 | 80.83 |
| 65 | 0.44 | 9.83 | 73.51 |
| 75 | 0.42 | 9.92 | 75.08 |
| 79 | 0.43 | 9.90 | 77.33 |
| 93 | 0.43 | 10.15 | 75.40 |

[a] Samples were thawed in an ice–water bath and kept there for 30 min; compared to a calibration curve extracted immediately after spiking.
[b] Samples were cycled twice before extracting and comparing to a fresh calibration curve.
[c] Samples were stored at −70°C before use; the results represent the mean of two.

## 4. Discussion

One cannot develop a bioanalytical method considering only the physical and chemical properties of a molecule and the analytical state of art; one has also to consider the question of the ultimate purpose of this method and who will be its potential user. Hence, the analytical issues must be influenced by pragmatic, economic, or even ethical considerations. In this particular case, an analytical method was needed to support a phase I drug development (first time in man) of an anticancer drug candidate, which is unstable under physiological conditions. Anticancer drugs tend to be toxic and it would be unacceptable to administer healthy volunteers with doses believed to be therapeutic. Although a particular drug will be equally toxic to a cancer patient, the chances are that the benefits may outweigh the potential disadvantages.

Programmes of this nature require the dosing of patients and sampling in a clinical setting, where specialized analytical skills and equipment may not be always available and where speed and simplicity of the procedure are at a premium and may contribute positively to the progress of a drug development programme.

Two issues of the greatest importance were the type of matrix sampled (blood, plasma, or serum) and how to preserve the integrity of the sample in the simplest and easiest way. Campthotecin analogues were usually mea-

Table 4
Half-life of GI147211 in various matrices, at various pH values and temperatures

| Matrix | 37°C | | | 22°C | | | 0°C | |
|---|---|---|---|---|---|---|---|---|
| | Half-life (min) | pH | Acid lactone ratio | Half-life (min) | pH | Acid lactone ratio | Half-life (min) | pH |
| PBS | 25.4 | 7.3 | 74.6/25.4 | 118.7 | 7.0 | 75.0/25.0 | 1780[a] | 7.3 |
| | | | | 107.1 | 7.3 | 87.8/12.2 | | |
| | | | | 42.8 | 7.7 | 91.4/8.6 | | |
| | | | | 26.3 | 8.1 | 95.1/4.9 | | |
| HSA | 31.7 | 7.3 | 79.3/20.7 | 120.5 | 7.0 | 73.2/26.8 | – | – |
| | | | | 88.7 | 7.3 | 87.5/12.5 | | |
| | | | | 45.5 | 7.7 | 96.2/3.8 | | |
| | | | | 31.6 | 8.1 | 99.5/0.5 | | |
| Plasma | 30.9 | 7.3 | 95.5/4.5 | 118.1 | 7.3 | 95.0/5.0 | | |
| | 12.7 | 7.6 | 97.9/2.1 | | | | | |
| Blood | 44.1 | 7.3 | 79.0/21.0 | 161.9 | 7.3 | 84.0/16.0 | 1100[a] | 7.3 |

[a] Half-life estimated assuming the acid/lactone ratio 85/15; experiment terminated after 48 h without achieving an equilibrium.
Stability determinations were made using HPLC methodology as described in the Experimental section. Peak height ratios of GI147211 to the internal standard were used to calculate the rate constants and carboxylate/lactone ratios

sured in plasma [2–10], which adds one more step together with time to the procedure as compared to whole blood; serum was entirely out of the question as its harvesting requires a further 1 h of incubation at room temperature. In some cases, plasma samples were analyzed immediately after collection using protein precipitation with acetonitrile, which is not practical at clinical sites.

Several conclusions related to the drug stability could be drawn from the kinetics experiment. There are rather minor differences between the half-lives in PBS and HSA (see Table 4), and hence potential GI147211 binding to HSA does not contribute to the stability of the drug; the lactone ring is probably not involved in this interaction. At the same pH, the half-life increases in blood, suggesting that

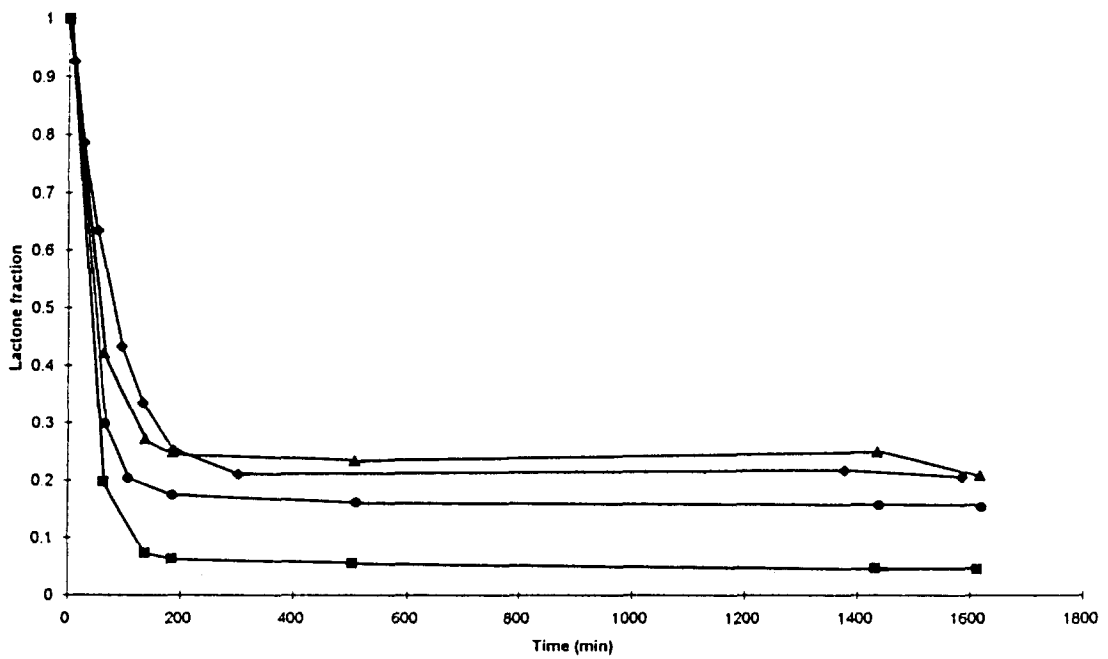


Fig. 4. Kinetics of the GI147211 lactone ring opening in various matrices at pH 7.3 and 37°C. ◆, Blood; ■, plasma; ▲, HSA in PBS (50 mg ml$^{-1}$); ●, PBS.

other elements may bind the lactone ring and protect it from hydrolysis. Burke et al. [19,20] demonstrated that lipid bilayer partitioning increases greatly the stability of camptothecin drugs. Out of four types of media investigated, whole blood is the one containing most lipids and hence having the greatest chance to benefit from the process. The GI147211 half-life in blood is 30% longer than that in plasma and HSA, and 40% longer than the half-life in PBS. It was calculated, using the data presented in Table 4 and Fig. 3, that approximately less than 3% of lactone should convert into carboxylate under the recommended conditions for clinical sample handling (i.e. a starting sample temperature of 37°C; a maximum of 30 min storage in the ice–water bath).

For reasons of having the longest half-life and maximum simplicity of processing, a decision was made to use whole blood as the matrix in this programme. The ratio of the concentration in red blood cells to that in plasma after 40 min incubation in the ice–water bath was 1.60 at a starting GI147211 concentration of 78 ng ml$^{-1}$. It was also decided to quantify the carboxylate in the indirect way by measuring initially the lactone concentration only, and the total concentration after the sample acidification. This approach, although less efficient, was deemed to be the safest approach to obtain reliable data on the lactone and the lactone/carboxylate ratio. The method of determination of total GI147211 will be a subject of a separate communication.

Additionallly, GI147211 does not fluoresce as strongly as other camptothecin analogues. In fact, the fluorescence intensity varies greatly in this family of compounds, as was shown by Burke et al. [20]. This forced us to extract and concentrate the drug from blood in order to achieve subnanogram per milliliter concentrations; something that came relatively easy by protein precipitation in the case of camptothecin or topotecan. The extraction developed proved to be lactone-selective. Having removed the carboxylate from the medium, the extracts were kept later on in slightly acidic medium preventing the lactone from back-conversion into carboxylate.

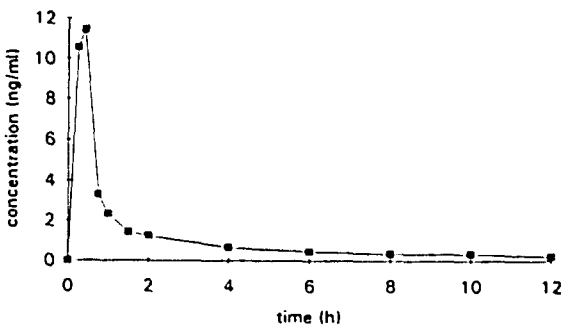


Fig. 5. Pharmacokinetis profile of GI147211 in a patient after the 30 min intravenous infusion of the drug at 0.6 mg m$^{-2}$.

## 5. Conclusions

The described method is simple, accurate, precise, and sensitive enough to be used in pharmacokinetics studies. The element of novelty is the use of blood as the medium, as the stability of GI147211 in human blood is better than in plasma. Another novelty is the development of a collection and storage procedure, which permits us to carry out the assay entirely in a bioanalytical laboratory, without the need to carry out a partial sample preparation in a clincial setting.

## References

[1] M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmer, A.T. McPhail and G.A. Sim, J. Am. Chem. Soc., 88 (1966) 3888–3890.

[2] J.H. Beijnen, B.R. Smith, W.J. Keijer, R. Van Gijn, W.W. ten Bokkel Huinink, L.Th. Vlasveld, S. Rodenhuis and W.J.M. Underberg, J. Pharm. Biomed. Anal., 8 (1990) 789–794.

[3] J.H. Beijnen, H. Rosing, W.W. ten Bokkel Huinink and H.M. Pinedo, J. Chromatogr., 617 (1993) 111–117.

[4] I. Barilero, D. Gandia, J-P. Armand, A. Mathieu-Boue, M. Re, A. Gouyette and G.G. Chabot, J. Chromatogr., 575 (1992) 275–280.

[5] D.O. Scott, D.S. Bindra and V.J. Stella, Pharm. Res., 10 (1993) 1451–1457.

[6] C.H. Takimoto, R.W. Klecker, W.L. Dahut, L.K. Yee, J.M. Strong, C.J. Allegra and J.L. Grem, J. Chromatogr., 655 (1994) 97–104.

[7] E. Araki, M. Ishikawa, M. Iigo, T. Koide, M. Itabashi and A. Hoshi, Jpn. J. Cancer Res., 84 (1993) 697–702.

[8] J. Loh and A.E. Ahmed, J. Chromatogr., 530 (1990) 367–376.

[9] J.G. Supko and L. Malspeis, J. Liq. Chrom., 19 (1991) 1779–1803.

[10] J.G. Supko and L. Malspeis, J. Liq. Chrom., 15 (1992) 3261–3283.

[11] M.J. Luzzio, J.M. Besterman, G.M. Evans and P.L. Myers, Eur. Patent 0540 099 A1, 1992.

[12] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman and S. Spector, J. Pharm. Sci., 81 (1992) 309–312.

[13] J. Fassberg and V.J. Stella, J. Pharm. Sci., 81 (1992) 676-684.
[14] Y. Kurono, M. Miyajima and K. Ikeda, Yakugaku Zasshi, 113 (1993) 167-175.
[15] W.J.M. Underberg, R.M.J. Goossen, B.R. Smith and J.H. Beijnen, J. Pharm. Biomed. Anal., 8 (1990) 681-683.
[16] T.G. Burke and Z. Mi, Anal. Biochem., 212 (1993) 285-287.
[17] Z. Mi and T.G. Burke, Biochemistry, 33 (1994) 10325-10336.
[18] T.G. Burke and Z. Mi, J. Med. Chem., 37 (1994) 40-46.
[19] T.G. Burke, A.E. Staubus, A.K. Mishra and H. Malak, J. Am. Chem. Soc., 114 (1992) 8318-8319.
[20] T.G. Burke, A.K. Mishra, M.C. Wani and M.E. Wall, Biochemistry, 32 (1993) 5352-5364.