ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 1257–1265

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

High-performance liquid chromatographic analysis of the investigational anticancer drug 9-aminocamptothecin, as the lactone form and as the total of the lactone and the hydroxycarboxylate forms, in micro-volumes of human plasma

R. van Gijn [a,*], V.M.M. Herben [b], M.J.X. Hillebrand [b], W.W. ten Bokkel Huinink [b], A. Bult [c], J.H. Beijnen [a,b,c]

[a] *Department of Pharmacy and Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute, Louwesweg 6, 1066 EC Amsterdam, The Netherlands*
[b] *Department of Medical Oncology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands*
[c] *Department of Pharmaceutical Analysis and Toxicology, Faculty of Pharmacy, Utrecht University, P.O. Box 80,082, 3508 TB Utrecht, The Netherlands*

Received 3 July 1997; received in revised form 3 November 1997; accepted 10 November 1997

Abstract

A high performance liquid chromatographic (HPLC) assay is described for the determination of the investigational anticancer drug 9 aminocamptothecin (9-AC) as the lactone form (9AC(lac)) and as the total of the lactone and hydroxycarboxylate forms (9AC-(tot)), in micro volumes of plasma. The analytical methodology reported here involves a protein precipitation step with cold methanol (− 30°C) as sample pretreatment procedure. The methanolic extract is used for the determination of 9AC-(tot). The intact (active) lactone form of 9-AC is separated from the hydroxycarboxylate form in the methanolic plasma extract by solid phase extraction within 48 h after sampling and deproteination. After evaporation to dryness (nitrogen, 40°C) the extract can be stored at − 70°C for at least 3 weeks. The drug is analysed by reversed-phase liquid chromatography on a Zorbax SB RP-18 column, using methanol–water eluent (pH 2.2) and fluorescence detection. The presented assay is linear over a concentration range 0.2–100 ng·ml^{-1} with a detection limit and a limit of quantitation of 0.05 and 0.2 ng·ml^{-1}, respectively, for both 9-AC(tot) and 9-AC(lac) using a 100 ml plasma sample. The proposed method has been implemented in a phase I clinical trial for pharmacokinetic evaluation of this potential new drug.

Keywords: 9 Aminocamptothecin; 9-AC; Camptothecin analogues; Plasma assay; HPLC; Fluorescence detection

1. Introduction

9-Amino-20(*S*)-camptothecin (9-AC)(NSC 603071, FCE 280044) (Fig. 1) is an analogue of

* Corresponding author.

0731-7085/98/$ - see front matter
PII S0731-7085(97)00257-4

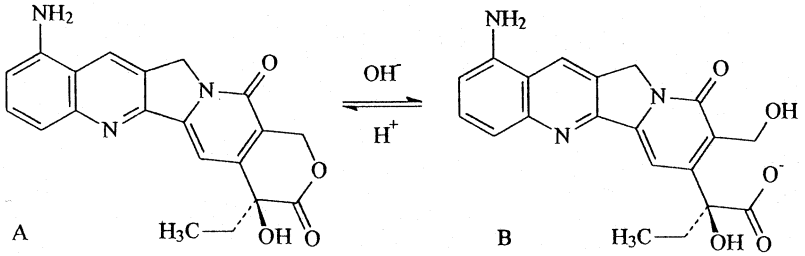

Fig. 1. Chemical structures and equilibrium reactions between the lactone (A) and hydroxycarboxylate (B) forms of 9-AC.

the alkaloid camptothecin, with an amino group substituted at the C_9 position of the A-ring. 9-AC acts as a specific inhibitor of the DNA unwinding enzyme topoisomerase I. It has a wide spectrum of activity in animal tumour models including rapidly proliferating murine leukaemia and slowly growing transplantable solid tumours. Like all camptothecins, 9-AC is present in biological systems in equilibrium between its active lactone and inactive hydroxycarboxylated (ring-opened) form (Fig. 1). In acidic medium (pH < 4) the lactone structure predominates, while at alkaline pH's, including physiological pH, the formation of the carboxylate form is favoured [1–5]. Unlike most other camptothecins (topotecan, CPT-11, GG211), 9-AC is poorly water soluble and requires a lipophilic pharmaceutical formulation. In preclinical studies, 9-AC antitumour activity and toxicity were investigated by using mixed solvent formulations. To improve solubility, 9-AC is formulated as a colloidal dispersion and this formulation is now undergoing clinical evaluation in Europe and the USA. In our hospital, a phase I study with 9-AC, administered by daily intravenous infusion over 30 min for 5 consecutive days, is ongoing. One of the objectives of this phase I trial is to study the pharmacokinetics of the drug. High performance liquid chromatography (HPLC) methodologies for the analysis of 9-AC in biological matrices have been described [6,7] which are, however, laborious and require post column acidification [7] or extensive sample pretreatment immediate after blood sampling on the ward [6]. We describe here a validated, easy to use, selective and sensitive HPLC assay for the determination of 9-AC as the lactone form (9-AC(lac)) and as the total of the lactone and hydroxycarboxylate form (9-AC(tot)) in micro volumes of human plasma; the difference between 9-AC(tot) and 9-AC(lac) levels provide concentrations of the hydroxycarboxylate form (9-AC(hydroxyacid)).

2. Experimental

2.1. Materials

9-AC (delivered as the base; SL39300192–0) originated from the National Cancer Institute (MD, USA). Methanol (ChromAR, HPLC) was obtained from Prochem (Wesel, Germany). Perchloric acid (70%) and dimethylsulfoxide (DMSO), all analytical grade, were from Merck (Darmstadt, Germany). Home-made distilled water was used throughout. A water solution of pH 2.2 was prepared by adding approximately 1 ml perchloric acid to 1000 ml distilled water to a final pH of 2.2. Drug-free heparinized human plasma was obtained from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands).

2.2. Equipment

The chromatographic system consisted of a type 510 solvent delivery system (Waters, Milford, MA), an automatic injection device model SP 8880 (Thermo Separation Products, Fremont, CA) and an FP920 intelligent fluorescence detector (Jasco, Tokyo, Japan). Separation was performed on a Zorbax SB C-18 column (75 × 4.6 mm i.d., particle size: 3.5 μm, Rockland Technocologies, Newport, DE) which was protected with

a pre-packed reversed phase guard column (10 × 3 mm i.d., Chrompack, Bergen op Zoom, The Netherlands).

Retention times and peak areas were measured with an SP 4270 integrator which is part of a PC1000 data system (both from Thermo Separation Products).

2.3. Stock, standard and quality control solutions

Stock solutions of 9-AC (1000 $\mu g \cdot ml^{-1}$) were prepared by dissolving the appropriate amount of the drug, accurately weighed, in DMSO. The stock solution was diluted with DMSO–methanol (1:9, v/v) to give standard solutions with concentrations of 4, 2, 1, 0.4, 0.2, 0.04, 0.02 and 0.008 $\mu g \cdot ml^{-1}$ 9-AC. Another stock solution, with separate weighing of 9-AC, was diluted with DMSO–methanol (1:9, v/v) to give quality control (QC) solutions with concentrations of 2, 0.2, and 0.02 $\mu g \cdot ml^{-1}$ 9-AC. The stock, standard and QC solutions were stored, prior to use, at −30°C, and were found to be stable for at least 3 months under these conditions.

2.4. Calibration and QC samples

Calibration samples were prepared by adding 25 μl of each standard solution to 975 μl drug-free plasma in 2.0 ml polypropylene eppendorf cups. In this way, calibration samples were obtained with concentrations of 100, 50, 25, 10, 5, 1, 0.5 and 0.2 $ng \cdot ml^{-1}$ 9-AC in plasma. QC samples were prepared by adding 25 μl of each QC solution to 975 μl drug-free plasma in 2.0 ml polypropylene eppendorf cups, yielding QC samples with a concentration of 50, 5 and 0.5 $ng \cdot ml^{-1}$ 9-AC in plasma. All solutions (including the drug-free plasma) were placed in ice-water at 0°C before use and during preparation of calibration and QC samples. The calibration and QC samples were prepared freshly for each run.

2.5. Sample pretreatment

To a 2.0 ml polypropylene eppendorf cup containing 200 μl cold methanol (−30°C) 100 μl of sample (patient, calibration or QC) was added. The cup was vortex-mixed for 10 s and centrifuged for 3 min at 9500 × *g*. Next, for the determination of 9-AC(tot) 100 μl of the methanolic extract was transferred to a clean eppendorf cup and stored at −30°C until analysis. For the determination of 9-AC(lac), an aliquot of 150 μl of supernatant was transferred to a clean eppendorf cup and stored at −30°C, for at most 24 h, and transferred within this period to −70°C. Within 48 h the samples must be processed further with a solid phase extraction.

2.6. Solid phase extraction

To the eppendorf cups containing the 150 μl of the supernatant (methanolic extract) for the determination of 9-AC(lac), 450 μl distilled water (0°C) was added and vortex mixed. Next, 500 μl of the mixture was immediately processed on pre-conditioned C18 Bakerbond, bonded phase (3 ml, 500 mg) solid phase extraction columns (J.T. Baker, Deventer, The Netherlands). The solid phase extraction (SPE) was performed on a SPE 24-G vacuum manifold column processor (J.T. Baker). The columns were conditioned with 6 ml methanol and 6 ml distilled water. The analyte solution was loaded with a flow rate of approximately 1 $ml \cdot min^{-1}$ and washed with 6 ml methanol–water (25:75, v/v). Finally, the analyte was eluted from the column into a clean polypropylene tube with 750 μl methanol at a flow rate of approximately 1 $ml \cdot min^{-1}$. The eluate was dried under a gentle stream of nitrogen at 40°C and stored, prior to analysis, at −30°C. In order to investigate and to validate the capability of the SPE procedure to separate the lactone form from the hydroxycarboxylate form of 9-AC, drug free plasma samples were spiked in triplicate with 50 $ng \cdot ml^{-1}$ of 9-AC. The samples were exposed for 0, 35 and 300 min to a temperature of 37°C ($t = 0$ min, percentage hydroxycarboxylate form of 9-AC $\ll 1\%$; $t = 35$ min, percentage hydroxycarboxylate form of 9-AC approximately 60%; $t = 300$ min, percentage hydroxycarboxylate form of 9-AC approximately 90%) [5] and then extracted with methanol (−30°C). The methanolic plasma extracts were further processed as described under Section 2.5 and Section 2.6 and

analyzed with HPLC. The concentrations of 9-AC found were compared with unextracted standards made by diluting 9-AC standard solutions in the mobile phase.

2.7. Reconstitution

A total of 100 μl of water (pH 2.2) was added to the polypropylene eppendorf cups containing the methanolic plasma extracts (100 μl) for the determination of 9-AC(tot). The cups were then vortex-mixed for 10 s and the mixture was transferred to an autosampler vial with insert. Dry extracts after SPE for the determination of 9-AC(lac) were reconstituted with 200 μl eluent, vortex-mixed for 10 s and transferred to an autosampler vial with insert.

2.8. Chromatography

Chromatographic analysis was performed at ambient temperature with a mobile phase which consisted of methanol–water (pH 2.2)(30:60 w/w). Prior to use, the mobile phase was filtered through an 0.22 μm filter. The flow-rate was maintained at 1.0 $ml \cdot min^{-1}$. The column effluent was monitored spectrofluorimetrically with excitation and emission wavelengths set at 370 and 450 nm, respectively. Aliquots of 50 μl were injected into the chromatograph. 9-AC eluted at a retention time of 4.2 min.

2.9. Absolute recovery, accuracy, precision, limit of detection and lower limit of quantitation

A full validation (three analytical runs) for the analysis of 9-AC(tot) and 9-AC(lac) in human plasma was completed. The absolute recoveries of 9-AC(tot) and 9-AC(lac) were determined by comparing the slopes of the processed human plasma standard curves to standard curves prepared in methanol–water pH 2.2 (30/60 w/w). The applicability of this assay to determine 9-AC(tot) from samples containing 9-AC in its hydroxycarboxylate form was determined by analysis of QC 5 and QC 50 $ng \cdot ml^{-1}$ samples, which were exposed for 2 h at 37°C (conversion into hydroxycarboxylate form $\gg 95\%$ [6]). Eight calibration standards were prepared and analyzed in duplicate. The linear regression of the ratio of the areas of the analyte versus the concentration were weighted by $1/x$ (reciprocal of the concentration). The F test for lack of fit ($\alpha = 0.05$) was used to evaluate the linearity of the calibration curves. The accuracy and precision of the method were determined by replicate analysis ($n = 5$) of the quality control samples in three individual analytical runs. The accuracy was calculated at each test concentration by dividing measured concentration by the nominal concentration and multiplying by 100%. An estimate of the between-day precision for the 9-AC(lac) and 9-AC(tot) assays was obtained by one-way analysis of variance (ANOVA) for each test concentration using the run day as the classification variable. The day mean square (DayMS), error mean square (ErrMS) and the grand mean (GM) of the observed concentrations across run days were obtained. The between-day precision (pr.) was calculated for each quality-control concentration:

$$\text{Between} - \text{day pr.} = \frac{((\text{DayMS} - \text{ErrMS})/n)^{0.5}}{\text{GM}} \times 100\%$$

where n = the number of replicates within each run.

The estimate of the within-day precision was also calculated for each test concentration:

$$\text{Within} - \text{day pr.} = \frac{(\text{ErrMS})^{0.5}}{\text{GM}} \times 100\%$$

The lower limit of the detection (LOD) of the assay was established as the concentration where a significant difference could be seen between a blank and a spiked sample (paired Student's t-test, $P > 0.05$). The lower limit of quantitation was defined as the concentration of the lowest standard in the analytical run which was quantitated with a definite level of certainty (precision $< 20\%$) [8–10].

2.10. Separation from endogenous plasma constituents and co-medication

Six batches of human plasma control were processed and analyzed to determine whether endogenous plasma constituents co-eluted with 9-AC.

To investigate the potential interference of the co-medication with the analytical method, the drugs were added to control plasma in therapeutic concentrations. These samples were processed and analyzed according to the described method. The choice of the tested concomitant drugs was based on an inventory of relevant co-medication to the patients in our phase I study.

2.11. Stability

The chemical stability of 9-AC(tot) has been studied in plasma and methanolic plasma extracts at −30°C. The chemical stability of 9-AC(lac) has been studied in the methanolic plasma extracts at −30 and −70°C and as dry extracts after SPE at −30°C. To investigate the stability of the 9-AC(tot) in plasma, drug-free plasma was spiked with 5 $ng \cdot ml^{-1}$ 9-AC and stored at −30°C. Methanolic plasma extracts were made at a concentration of 10 $ng \cdot ml^{-1}$ 9-AC and were stored at −30°C. The drug concentration in the plasma samples and the methanolic plasma extracts were determined periodically.

To investigate the stability of 9-AC(lac) in methanolic plasma extracts in different 9-AC(lac) and 9-AC(hydroxyacid) ratios, drug free plasma was spiked with 50 $ng \cdot ml^{-1}$ 9-AC. To transform the closed lactone form into the open hydroxycarboxylate form, the samples were exposed for 0, 35 and 300 min to a temperature of 37°C ($t = 0$, 9-AC(lac) $\gg$ 99%; $t = 35$ min, 9-AC(lac) approximately 70%; $t = 300$ min, 9-AC(lac) approximately 10%) [6], and then extracted with methanol. The methanolic plasma extracts were stored at −30 and −70°C and processed, prior to HPLC analysis, on SPE columns at periodic time intervals. The stability of 9-AC(lac) in the dry extract after SPE was studied at a concentration of 50 $ng \cdot ml^{-1}$ 9-AC.

2.12. Pharmacokinetics

A 69 year-old patient suffering from colorectal cancer was treated with 0.4 $mg \cdot m^{-2}$ 9-AC (formulated as a colloidal dispersion) dissolved in 50 ml 20% dextrose in 0.9% sodium chloride by intravenous infusion over 30 min. Periodically, blood samples were collected from an indwelling intravenous cannula placed in the arm contralateral to that receiving the drug. Samples were taken in heparinized tubes prior to the start of the infusion, during infusion and at specific times post-infusion. Samples were placed immediately in ice-water and centrifuged for 5 min at 4°C and $2500 \times g$. Plasma was isolated and treated as described in Section 2.5 and Section 2.6.

3. Results and discussion

An intact lactone form of 9-AC is of paramount importance for the biological activity of this antitumour agent. At physiological pH, 9-AC is not stable and hydrolysis of the lactone moiety leads to the formation of the inactive hydroxycarboxylate form of 9-AC. Therefore, a bio-analytical method is required for the selective quantification of the intact lactone form of 9-AC. The presence of the 9 amino group in the molecule decreases the fluorescence properties dramatically when compared to other camptothecins. However, protonation of the 9-amino function in the molecule restores the typical high fluorescence intensity of this class of compounds. In the HPLC method developed by Supko et al. [7], post column acidification was used for the on-line protonation of the 9-amino function. This laborious method requires a second HPLC pump, a switching valve and a 90° metal free mixing tee for on-line acidification. In the HPLC method described by Takimoto et al. [6], the protonation of the 9-amino function of 9-AC was established by using a mobile phase at pH 2.55. However, immediate SPE was necessary to separate the lactone form of 9-AC from its hydroxycarboxylate form. The disadvantages of this method include: (a) the necessity of a SPE procedure which should be performed as soon as possible after

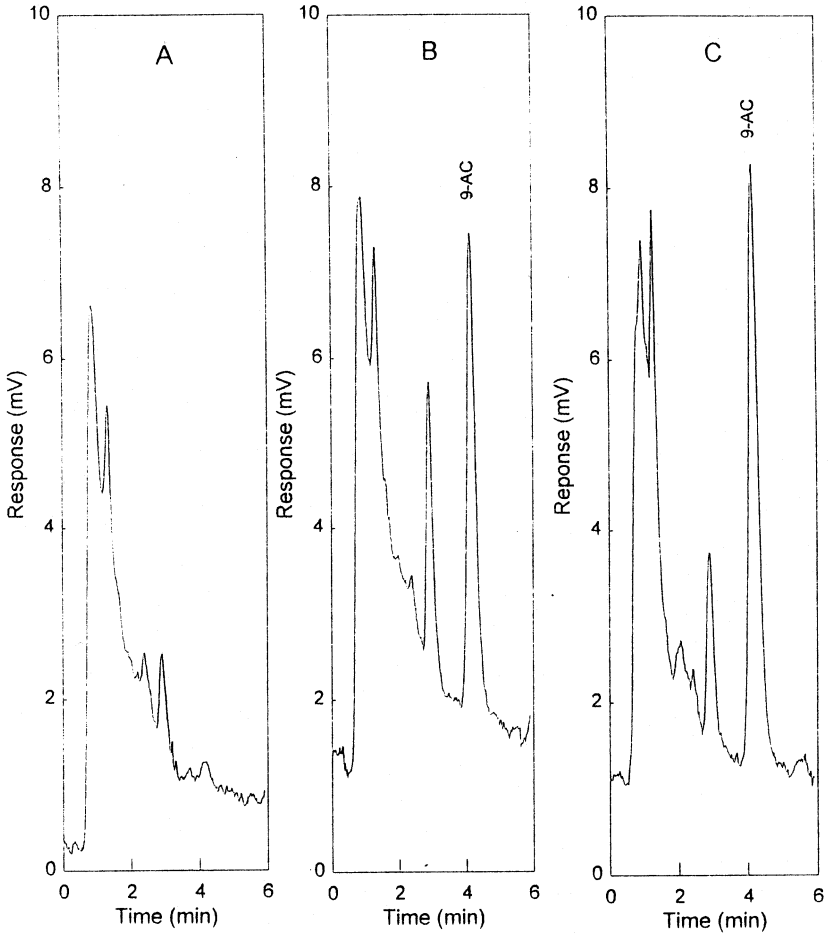

Fig. 2. HPLC chromatograms of blank human plasma (A), a spiked human plasma (B) (concentration of 9-AC: 1.0 $ng \cdot ml^{-1}$) and a patient plasma sample (C) collected 12 h after administration of 0.4 $mg \cdot m^{-2}$ 9-AC as a 30 min intravenous infusion (concentration of 9-AC: 1.3 $ng \cdot ml^{-1}$).

blood sampling on the ward; (b) the use of an HPLC system with an eluent at pH 2.55 which is outside the pH range (1.7–2.3) of optimal fluorescence behaviour of 9-AC; and (c) the need for relatively large plasma volumes (1000 μl).

The assay presented here processes micro-volumes of plasma samples (100 μl) with cold methanol (− 30°C) for fixation of the equilibrium between the lactone form and the hydroxycarboxylate form. Deproteination can be carried out easily on the ward and the resulting methanolic plasma extract is stable at − 30°C for 24 h and for at least 48 h when stored at − 70°C. Within this period, the methanolic plasma extracts can be processed on SPE in the laboratory. HPLC analysis was improved by using reversed phase C18 stable bond column material, which is proof against an eluent with pH 2.2.

3.1. Chromatography and detection

We have tested various chromatographic systems, including the systems we used earlier, for the bio-analysis of camptothecin and analogues [1,4], the published HPLC assays for the determination of 9-AC in its lactone form [6] and a post-column acidification method of 9-AC [7], with the aim of finding the optimal conditions for

Table 1
Assay performance data for the determination of 9-AC(tot) and 9-AC(lac)

Theoretical concentration $ng \cdot ml^{-1}$	Mean measured concentration $ng \cdot ml^{-1}$	Accuracy ± RSD[a] (%)	Within-run precision (%)	Between-run precision (%)	n
9-AC(tot)					
0.5	0.473	94.6 ± 0.8	9.3	0.8	3
5	4.87	97.3 ± 2.5	2.7	2.5	3
50	50.8	101.5 ± 2.6	2.7	2.6	3
9-AC(lac)					
0.5	0.501	100.1 ± 6.1	8.7	5.4	3
5	4.93	98.6 ± 12.7	3.2	14.0	3
50	46.1	92.1 ± 5.4	14.2	5.6	3

n, number of replicates.
[a] Mean relative standard deviation of the accuracy found at replicate analysis ($n = 5$) of the quality control samples in three individual analytical runs.

the determination of 9-AC. Systems describing the analysis of camptothecin and topotecan were found to be unsuitable for the bio-analysis of 9-AC. The drug shows relatively poor fluorescence in the mobile phases at neutral pH's used for the other camptothecins. The native fluorescence properties of 9-AC are mainly a function of pH, which should be lower than 3 with a maximum at pH 2.2 for maximal fluorescence yield [7]. One complication is that, at pH 2.2, the life-time of most reversed phase C18 columns is very limited. The use of stable bond C18 columns in combination with a mobile phase composition of water (pH 2.2) and methanol (60:30, w/w) made it possible to determine 9-AC under optimum conditions. Thus far (after two years of use), we have not noted any deterioration of the columns under these conditions. The selection of fluorescence wavelengths was based on scanning experiments with the drug in the mobile phase, and optimum excitation and emission wavelengths were found at 370 and 450 nm, respectively. Representative HPLC chromatograms are depicted in Fig. 2.

3.2. Sample pretreatment and solid phase extraction

Deproteination with cold methanol (− 30°C) is a simple and effective method [1] for fixation of the lactone to hydroxycarboxylate ratio of camptothecins including 9-AC in plasma. Due to the poor fluorescence behaviour of the hydroxycarboxylate form of 9-AC and the unstable equilibrium between 9-AC(lac) and 9-AC(hydroxacid) in matrices other than cold methanol, determination of the (active) lactone form and the (inactive) hydroxycarboxylate form of 9-AC in one analytical system proved impossible. Furthermore, sensitive detection necessitated the use of an acidic mobile phase in which the hydroxycarboxylate form of 9-AC exhibited immediate on-column conversion into the lactone form. To circumvent this, we separated the lactone form from the hydroxycarboxylate form of 9-AC prior to HPLC analysis. Separation was accomplished in the methanolic plasma extract, after addition of water, on C18 solid phase extraction columns.

3.3. Validation

The analytical methodology was validated in terms of recovery, detection limit, precision, accuracy and linearity, as agreed during the consensus meeting on 'Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies' [9]. The extraction efficiencies of 9-AC(tot) and 9-AC(lac) were 92.8 ± 6.1 and 76.3 ± 2.5%, respectively. The extraction recovery of 9-AC(tot) in samples stored at 37°C for 2 h (conversion into hydroxycarboxylate form ≫

Table 2
Equations of calibration lines for the analysis of 9-AC(lac) and 9-AC(tot) in plasma

Concentration range ($ng \cdot ml^{-1}$)	Equation[a,b]	r^2	n[c]
9-AC (lac)			
0.2–100	$Y = 0.1297(\pm 0.0017)X - 6.1(\pm 8.2)$	0.9988	3
9-AC (tot)			
0.2–100	$Y = 0.1276(\pm 0.0091)X - 3.9(\pm 7.1)$	0.9967	3

[a] X is the concentration of 9-AC in $ng \cdot ml^{-1}$ and Y are integrator peak area units.
[b] SD between parenthesis.
[c] Number of replicate calibration lines.

95%), was 89.5 ± 4.1 and $96.7 \pm 6.5\%$ for concentrations of 5 and 50 $ng \cdot ml^{-1}$, respectively. The SPE procedure was capable of separating 9-AC(lac) from plasma with different ratios of lactone:hydroxycarboxylate forms of 9-AC. The detection limit and limit of quantitation for the presented assay is 0.05 $ng \cdot ml^{-1}$ (signal to noise ratio > 3) and 0.2 $ng \cdot ml^{-1}$ (precision -19.5–3.8%), respectively, for both 9-AC(tot) and 9-AC(lac) using a 100 μl plasma sample. Accuracy, within-day and between-day precisions are shown in Table 1. The assay was linear over a concentration range of 0.2 to 100 $ng \cdot ml^{-1}$ using 100 μl of plasma for both 9-AC(lac) and 9-AC(tot) in human plasma as determined by the F test for lack of fit ($\alpha = 0.05$). (Table 2). No interfering peaks were observed in any of the biological samples used in our study, or in any of the tested batches of drug free plasma.

Possible co-medication drugs (phenprocoumon, dipyridamole, metoprolol), at therapeutic concentrations, were extracted from plasma with methanol and injected into the HPLC system and showed no interferences.

Table 3
Stability of 9-AC at different ratios between lactone and hydroxycarboxylate forms in plasma methanol extracts at −70°C

Percentage of 9-AC(lac) ($\pm$CV) at the indicated time[a]			
$t = 0$	1 Day	2 Days	8 Days
103.5–96.5	100.9–98.2	100.0–100.0	91.0–92.3
46.9–45.9	47.1–45.0	46.5–42.7	43.7–43.1
10.1–10.1	11.5–11.6	13.3–11.9	14.7–13.0

[a] Values of duplicate determinations.

3.4. Stability

9-AC(tot) is stable in plasma methanol extracts at −30°C for at least 3 weeks. 9-AC(lac) (in different lactone to hydroxycarboxylate ratios) is stable in plasma methanol extracts at −30 and −70°C for 24 and 48 h, respectively (Table 3). This implies that methanolic extracts stored at −70°C for 9-AC(lac) analysis must be subjected within 48 h to SPE to separate lactone and hydroxycarboxylate forms. Following this, 9-AC(lac), as dry extract, is stable for at least three weeks at −30°C. Storage of 9-AC methanolic

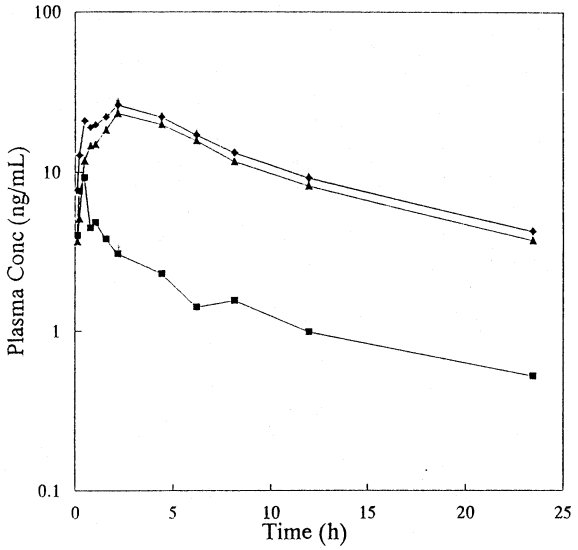

Fig. 3. Log concentration–time curves of 9-AC(lac) (■), 9-AC(tot) (◆) and 9-AC(hydroxyacid) (▲) in plasma of a patient receiving 0.4 $mg \cdot m^{-2}$ as a 30 min intravenous infusion.

plasma extracts at −70°C for prolonged times results in a shift of the lactone–hydroxycarboxylate equilibrium towards the ring-opened hydroxycarboxylate congener.

3.5. Clinical pharmacokinetics

Log concentration–time curves of 9-AC(tot) and 9-AC(lac) in plasma of a patient receiving 0.4 $mg \cdot m^{-2}$ of 9-AC as a 30 min intravenous infusion are shown in Fig. 3 and demonstrate the applicability of the assay for complete pharmacokinetic evaluation of the drug. Subtraction of the 9-AC(lac) curves from the 9-AC(tot) curves was used to establish the curves of the hydroxycarboxylate form of 9-AC.

4. Conclusions

A simple, highly sensitive and validated HPLC method for the analysis of the investigational drug 9-aminocamptothecin as the total of the lactone and the carboxylate forms and also as the lactone form in micro volumes of human plasma, has been developed which can be used for phase I pharmacokinetic studies. A deproteination step with cold methanol for the fixation of the equilibrium between the lactone and hydroxycarboxylate forms of 9-aminocamptothecin made direct SPE after blood sampling, as advocated in other methods, superfluous.

References

[1] J.H. Beijnen, B.R. Smith, W.J. Keijer, et al., J. Pharm. Biomed. Anal. 8 (1990) 789–794.
[2] J. Fassberg, V.J. Stella, J. Pharm. Sci. 81 (1992) 676–684.
[3] W.J.M. Underberg, R.J.M. Goossen, B.R. Smith, J.H. Beijnen, J. Pharm. Biomed. Anal. 8 (1990) 681–683.
[4] J.H. Beijnen, H. Rosing, W.W. ten Bokkel Huinink, H.M. Pinedo, J. Chromatogr. 617 (1993) 111–117.
[5] V.M.M. Herben, W.W. ten Bokkel Huinink, J.H. Beijnen, Clin. Pharmacokinet. 31 (1996) 85–102.
[6] C.H. Takimoto, R.W. Klecker, W.L. Dahut, et al., J. Chromatogr. B. 655 (1994) 97–104.
[7] J.G. Supko, L. Malspeis, J. Liq. Chromatogr. 15 (1992) 3261–3283.
[8] H.T. Karnes, C. March, J. Pharm. Biomed. Anal. 9 (1991) 911–918.
[9] V.P. Shah, K.K. Midha, S. Dighe, et al., J. Pharm. Sci. 81 (1992) 309–312.
[10] H.T. Karnes, C. March, Pharm. Res. 10 (1993) 1420–1426.