Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 11/12, pp. 1349–1352, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Short Communication

Stability problems with taxol in mouse plasma during analysis by liquid chromatography*

J. LESLIE,†‡ J.M. KUJAWA,‡ N. EDDINGTON,‡ M. EGORIN§ and J. EISEMAN§

‡*Pharmacokinetics Biopharmaceutics Laboratory, School of Pharmacy and* §*Cancer Center, University of Maryland at Baltimore, Baltimore, MD 21201, USA*

Keywords: *Taxol; cephalomannine; instability; assay.*

Introduction

Taxol (Fig. 1) is a diterpene derived from the needles and bark of the Western yew, *Taxus brevifolia*. Taxol has received considerable attention recently due to evidence of anti-tumour activity, particularly in patients with ovarian and breast cancer [1]. During development of an assay for taxol in mice plasma obtained during pharmacokinetic studies it was noted that taxol degraded under mild conditions. This paper describes an assay procedure which includes conditions used to stabilize the taxol.

The methods described in the literature for the analysis of taxol in plasma or serum [2–5] involve solvent or solid-phase extraction or protein precipitation, followed by evaporation to prepare and concentrate the sample prior to injection into an LC system. The concentration steps were required to measure the low plasma levels found during studies in humans. However, in the study of the pharmacokinetics of taxol in mice a few pilot experiments indicated that the plasma concentrations were in the range 0.5–140 $\mu g\ ml^{-1}$ which is much higher than the concentrations observed in humans. This is due to the high dose (22.5 $mg\ kg^{-1}$) administered intravenously to mice. This observation led to a consideration of an assay method using a simple protein precipitation step to prepare the samples for HPLC analysis.

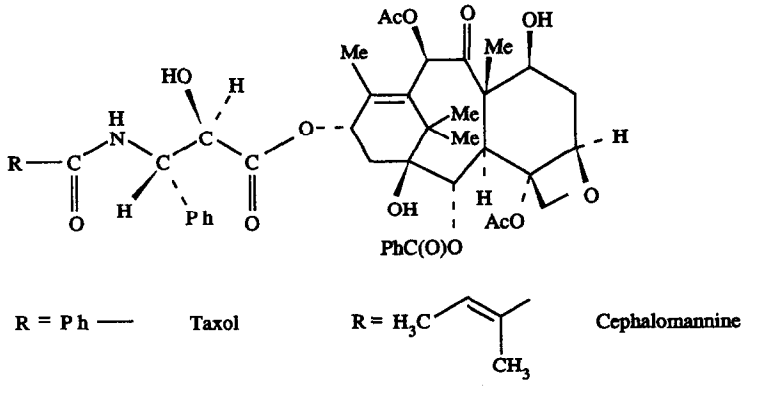

Figure 1
Molecular structures of taxol and cephalomannine.

*Presented at the 'Fourth International Symposium on Pharmaceutical and Biomedical Analysis', April 1993, Baltimore, MD, USA.

†Author to whom correspondence should be addressed.

Experimental

Materials

Taxol and an analogue cephalomannine (Fig. 1) were supplied by the National Cancer Institute. Acetonitrile and acetic acid, HPLC grade, and sodium acetate were obtained from J.T. Baker (Phillipsburg, NJ). The purified water, pH 5.5, used was obtained from a Hydro^{R} Picosystem^{R} Plus system.

Chromatographic conditions

The chromatographic apparatus consisted of a Waters 6000A solvent delivery system, a Perkin–Elmer ISS-100 autoinjector and a Waters 480 detector whose output was recorded on a Hewlett–Packard 3390A integrator set in peak height mode at attenuation 2. The chromatographic conditions used were a modification of those described by C. Jamis and R. Klecker (personal communication, Division of Clinical Pharmacology, Food and Drug Administration, Rockville, MD). A Hewlett–Packard, ODS Hypersil (5 μm, 100 × 4.6 mm i.d.) column was connected to a guard column containing a Supelco LC-318 cartridge. The mobile phase, acetonitrile–water (40:60, v/v), was pumped at 1.8 ml min^{-1}. The injection volume was 40 μl and the column effluent was monitored at 227 nm. The mobile phase was filtered through a 0.45 μm Millipore filter and degassed under vacuum by sonication.

Sample preparation

Stock solutions of taxol and cephalomannine, 1 mg ml^{-1} each in acetonitrile, were stored at −20°C: under these conditions the solutions were stable for several weeks. Standards of taxol in mouse plasma (Biological Specialty, Colmar, PA) in the concentrations 0.5, 1.0, 25.0, 50.0, 100 and 170 μg ml^{-1} were prepared on the day of use. A working internal standard solution at a concentration of 25 μg ml^{-1} cephalomannine in acetonitrile was prepared daily.

Taxol was administered to mice in a Cremophor vehicle by a number of routes including intravenous injection, intraperitoneal injection and oral gavage. The plasma samples obtained from mice in the pharmacokinetics studies were stored at −20°C during the 3 week period prior to analysis; no evidence of instability of the sample during storage was observed. The samples were thawed and thoroughly mixed on a vortex mixer immediately before analysis. Quality control samples of concentrations 4.0, 14.0, 70.0 and 140 μg ml^{-1} were assayed with each batch of mouse samples. To 100 μl of the plasma sample, standard or quality control sample was added 200 μl of the working internal standard solution, the mixture agitated on a vortex mixer for 10 s and then centrifuged for 3–4 min in a Beckman Microfuge E^{TM}. A 200 μl portion of the supernatant was mixed with 200 μl of 0.02 M sodium acetate buffer, pH 4.5, in an autosampler vial and a portion injected into the equilibrated HPLC system.

Results and Discussion

A representative chromatogram using the assay procedure described above is shown in Fig. 2. The taxol and cephalomannine peaks are sharp and well separated. In many samples small late eluting peaks were observed and so the run time for each individual chromatogram was extended to 18 min. It was ascertained that there were no peaks at the retention times of taxol or cephalomannine when Cremophore or processed plasma from mice which had been administered the vehicle without drug were injected into the system. It was noted, however, that two major peaks other than the taxol and cephalomannine appeared in the chromatograms (Fig. 2) of processed samples that had been standing in the autosampler awaiting injection. These additional peaks, which had longer retention times than the drug and internal standard, suggested instability of these compounds in the supernatant. It was also noted that the peak height of the drug and internal standard decreased with the concomitant appearance of the additional peaks. Furthermore, the rates at which the peak heights changed were similar for both compounds. In order, therefore, to use this method for long analytical runs it was necessary to determine the conditions under which degradation occurs and to find conditions to minimize it.

Solutions of taxol and cephalomannine in mobile phase acetonitrile–water (40:60, v/v) were prepared and their chromatograms examined under the conditions (a) after standing at room temperature in light and in the dark, (b) after heating to 37°C in light and in the dark, (c) after addition of sodium hydroxide to a final concentration of 0.004 M and (d) after addition of hydrochloric acid to a final concentration of 0.004 M. The appearance of

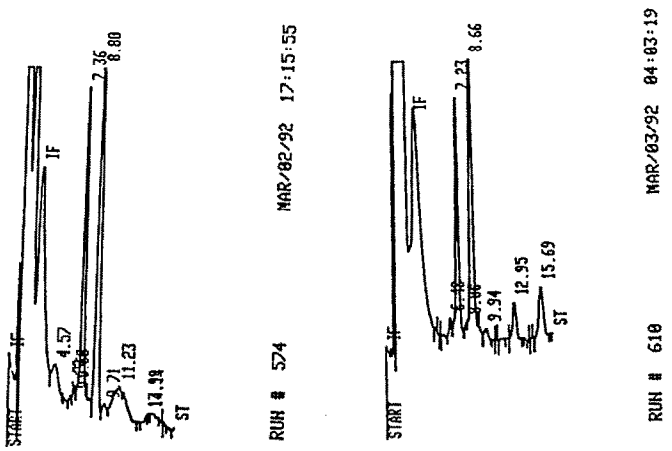

Figure 2
Chromatograms of the supernatants after precipitation of proteins from mouse plasma. Left, immediately after precipitation: taxol peak is at 8.88 min and cephalomannine is at 7.36 min; right, after standing for 11 h. The degradation product from taxol appears at 15.69 min and that from cephalomannine at 12.95 min.

the degradation peaks occurred to a similar extent in the presence and absence of normal fluorescent room light. The degradation peaks appeared five times faster at 37°C compared to room temperature. The degradation peaks were not observed in the presence of acid. However, the degradation of taxol and cephalomannine was greatly increased in the alkaline solutions with both compounds showing similar degradation patterns. The additional peaks for each compound appeared at a retention time of about two, relative to the parent compounds (Fig. 3). Other peaks were observed at shorter retention times suggesting that the degradation process is not a simple one. Similar degradation behaviour was observed when sodium hydroxide was added to supernatants from mouse plasma precipitations, but the degradation rate was lower probably due to the buffering effect of supernatant components. It is apparent that the main factor in the degradation of taxol and cephalomannine is base catalysis. This observation is consistent with the findings of Ringel and Horwitz [6] who identified the degradation products of taxol at pH 9.0 to be 7-epitaxol, which eluted later than taxol, and baccatin III, which eluted earlier than taxol on the HPLC system used.

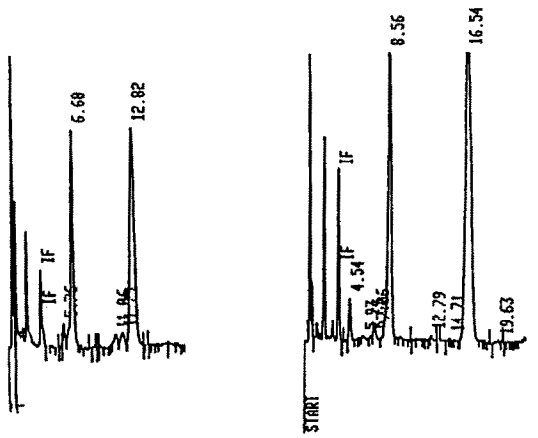

Figure 3
Chromatograms of partially degraded cephalomannine (left) and taxol (right) in alkaline solution. The intact taxol and cephalomannine are at 8.56 and 6.68 min, respectively.

It has been reported that while heating taxol in methanol–water mixtures during an assay development procedure a peak appeared with a slightly longer retention time than taxol [5]. This was attributed to the formation of 7-epitaxol. No other information was reported on the degradation of the drug and no internal standard was used. Other papers [2–4] describing procedures for the analysis of taxol did not mention any evidence of degradation.

With the knowledge that the degradation of taxol and cephalomannine is base catalysed, supernatants after precipitation of mouse plasma proteins were adjusted to pH 4.5 by the addition of acetate buffer and the changes of peak heights with time examined. The results showed that in the absence of buffer the peak heights of the drug and internal standard decreased by 24% after 11 h, whereas the change in the presence of buffer was about 4% and the degradation peaks did not appear (Fig. 4). It was noted that although the absolute

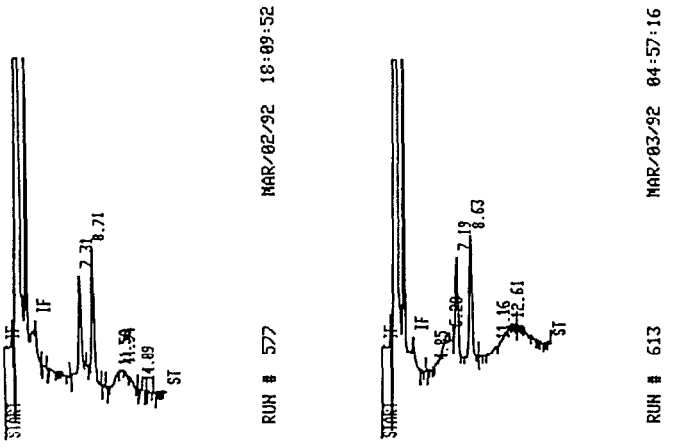

Figure 4
Chromatograms of taxol and cephalomannine in supernatants of mouse plasma after protein precipitation and addition of acetate buffer, pH 4.5, immediately after precipitation (left) and after 9 h (right).

peak heights in the unbuffered solutions changed with time, the rates of degradation of taxol and cephalomannine are so similar that the ratio (mean = 1.30, SD = 0.05) of the peak height of taxol to that of cephalomannine was time independent and was the same in the absence and presence of the buffer, even after 11 h. It is therefore a clear advantage to use cephalomannine as internal standard in the assay of taxol. A method was developed, therefore, for the analysis of taxol in mouse serum using precipitation of plasma proteins by acetonitrile with cephalomannine as internal standard, followed by the stabilization of the drug and internal standard in the supernatant by the addition of acetate buffer.

For the analytical method the data were analysed using the ratio of the peak heights of taxol to that of the internal standard. Peak height ratio versus concentration followed a linear model ($r > 0.99$) for the six standards in the taxol concentration range of 0.5–170 μg ml^{-1}. The intra-run precision during assay validation was found to be 1.1% relative standard deviation (RSD) at 114 μg ml^{-1} to 3.5% at 1.14 μg ml^{-1} ($n = 3$ at each concentration). For 11 calibration lines the back calculated concentrations for the standards using the observed peak height ratios and standard line parameters obtained by weighted (1/conc.) least squares ranged from 3.7% RSD at 160 μg ml^{-1} to 11.5% at 0.5 μg ml^{-1}. Intra-run precision calculated from the values of quality control samples assayed during sample runs ranged from 9.0% RSD at 142 μg ml^{-1} to 18.8% at 4.4 μg ml^{-1}.

In summary, a method that is suitable for the determination of taxol in mouse plasma obtained in pharmacokinetic studies has been presented. The source of the instability observed during analysis was found and the procedure modified to minimize degradation. This knowledge will be useful in evaluating other taxol analytical procedures used in clinical studies.

Acknowledgements — This investigation was performed as part of a study performed under National Cancer Institute Contract N01-CM27711.

References

[1] B.D. Cheson, *Oncology* **6**, 67–70 (1992).
[2] S.M. Longnecker, R.C. Donehower, A.E. Cates, T.-L. Chen, R.B. Brundrett, L.B. Grochow, D.S. Ettinger and M. Colvin, *Cancer Treat. Rep.* **71**, 53–59 (1987).
[3] P.H. Wiernik, E.L. Schwartz, J.J. Strauman, J.P. Dutcher, R.B. Lipton and E. Paietta, *Cancer Res.* **47**, 2486–2493 (1987).
[4] J.L. Grem, K.D. Tutsch, K.J. Simon, D.B. Alberti, J.K.V. Willson, D.C. Tormey, S. Swaminathan and D.L. Trump, *Cancer Treat. Rep.* **71**, 1179–1184 (1987).
[5] J. Rizzo, C. Riley, D. Von Hoff, J. Kuhn, J. Phillips and T. Brown, *J. Pharm. Biomed. Anal.* **8**, 159–164 (1990).
[6] I. Ringel and S.B. Horwitz, *J. Pharm. Exp. Ther.* **242**, 692–698 (1987).

[*Received for review 21 April 1993; revised manuscript received 1 June 1993*]