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High-performance liquid chromatographic assay for the determination of the novel C-Seco-taxane derivative (IDN 5390) in mouse plasma

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Abstract

A HPLC assay was developed to determine IDN 5390, a new paclitaxel analogue, in mouse plasma. The method involves solid-phase extraction from cyano cartridges (recovery >80%), HPLC separation on Symmetry C_{18} (4.6×150 mm), on isocratic mobile phase of water–acetonitrile–acetic acid (49:50:1) and detection at 227 nm. Retention times of IDN 5390 and IDN 5517 (internal standard, I.S.) were 9.1 and 10.5 min, respectively. The assay was linear from 0.05 to 5 μg/ml ($r^2 \geq 0.995$), showed intra- and inter-day precision within 1.0 and 6.2%, and accuracy of 94.7–106.8%. LOQ was 0.050 μg/ml. Using this method IDN 5390 pharmacokinetics was determined in mice.

Keywords: IDN 5390; C-Seco-taxane derivatives

1. Introduction

IDN 5390, 13-(*N*-Boc-β-isobutylisoserinoyl)-10-dehydro-10-deacetyl-C-secobaccatin (Fig. 1), is a proto-type of a new class of synthetic taxane-derivatives, C-Seco paclitaxel analogues, which have anti-angiogenic properties, and are under preclinical evaluation [1,2].

The compound has shown high antitumor activity against a variety of human tumor xenografts, includ-

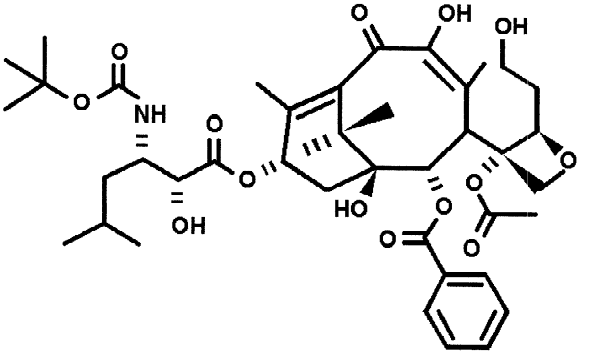

Fig. 1. Chemical structure of IDN 5390.

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ing ovarian and colon carcinoma and glioblastoma [3], when administered as protracted subcutaneous or oral dosage schedules.

Several reports describe the development and validation of HPLC assay to determine taxanes in biological fluids [4–6] to evaluate the preclinical and clinical pharmacokinetic profile of these drugs. Here we report a novel HPLC method suitable to measure IDN 5390 in plasma, which was applied to perform the first pharmacokinetic study in the mouse.

2. Experimental

2.1. Chemicals

IDN 5390 (lot # 553/16) and its 9-methylether derivative (IDN 5517, lot # 572/1/A), that will be used as internal standard (I.S.), were obtained from Indena (Settala (MI), Italy). Control mouse plasma was obtained from IFFA (Credo, France). Methanol, of HPLC grade was obtained from J.T. Baker (Deventer, The Netherlands). Glacial acetic acid for analysis and acetonitrile of HPLC grade were obtained from Carlo Erba (Milan, Italy). Triethylamine was obtained from Fluka (Buchs, Switzerland). Water of HPLC grade was obtained from a Milli Ro 60 Water System (Millipore, Milford, MA, USA).

2.2. Instrumentation and materials

A Bench Mate Workstation system for solid-phase extraction from Zymark (Hopkinton, MA, USA) was used to extract the analytes from plasma.

The HPLC system consisted of a Model 717 WISP autosampler, a Model 510 pump, a detector Model W2487 at variable-wavelength UV–Vis, and an acquisition system Millennium 32 Software Chromatography Manager from Waters Associates (Milford, MA, USA).

Sep-pak-CN cartridges (100 mg, 1 ml) for solid-phase extraction, Symmetry C_{18} HPLC column (3.5 μm, 4.6×150 mm) and pre-column (5 μm, 20×4.6 mm), vials and limited volume inserts for the WISP 717 autosampler were obtained from Waters.

The HPLC filter, 0.4 μm Nucleopore PC membrane filter, was obtained from Nucleopore Italia (Milan, Italy).

Disposable borosilicate glass tubes, 16×100 mm, were from Corning (Corning, NY, USA).

2.3. Animals

The experiment was performed with female CDF1 mice (body weight 20±2 g) obtained from Charles River Italia (Calco, Italy). They were housed and handled according to the institutional guidelines.

2.4. Drug

Vials of IDN 5390 formulated in Polysorbate 80 (Montanox 80 VG DF) were provided by Indena.

2.5. Preparation of stock and working solutions

IDN 5390 stock solution was prepared in methanol at a concentration of 1 mg/ml. The stock solution was further diluted with methanol to obtain working solutions at concentrations of 100, 10 and 1 μg/ml.

I.S. stock solution was prepared in methanol at a concentration of 1 mg/ml. The stock solution was further diluted with methanol to obtain working solutions at concentrations of 20 μg/ml.

2.6. Preparation of plasma standards

Six calibration standards of 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 μg/ml were prepared. Aliquots of 0.4 ml of control mouse plasma were combined with 20 and 40 μl of IDN 5390 working solution at the concentration of 1 μg/ml, 10, 20 and 40 μl of IDN 5390 working solution at 10 μg/ml and 8 μl of IDN 5390 working solution at 100 μg/ml.

Each point was prepared in duplicate.

2.7. Preparation of quality control samples

Plasma was divided into three fractions of 20 ml each (A, B and C) to prepare quality control (QC) samples for IDN 5390. A, B and C were combined with 15, 150 and 300 μl of IDN 5390 100 μg/ml in methanol to obtain a final plasma concentration of 0.075 μg/ml, 0.750 μg/ml and 1.500 μg/ml, respectively.

Several aliquots of 400 μl of the three fractions

were stored at −20 °C, as a control for future assay and to check the stability under storage conditions.

2.8. Extraction procedure

Four hundred μl of plasma standard samples or QC samples or animal samples were spiked with 50 μl of IDN 5517 (20 μg/ml in methanol) as internal standard (I.S.) and with 0.7 ml of 0.2 *M* ammonium acetate buffer at pH 4.5. After vortex mixing for 10 s, the samples were kept at 4 °C for 30 min. After centrifugation at 3000 rev./min for 10 min, the samples were processed automatically by the use of the Bench Mate Workstation, with Sep-pak-CN cartridges for solid-phase extraction (SPE).

The SPE columns were preconditioned at a flow-rate of 0.26 ml/s with 4 ml of methanol and 3 ml of water, then with 2 ml of 0.01 *M* ammonium acetate buffer (pH 4.5). A volume of 1.15 ml of the plasma mixture was loaded (flow-rate 0.05 ml/s) on the cartridge, then washed at a flow-rate of 0.05 ml/s with 2 ml of 0.01 *M* ammonium acetate buffer and with 1.2 ml of ultrapure water.

After drying the cartridge for 120 s with nitrogen, the final elution (flow-rate 0.05 ml/s) was performed with 2.5 ml of acetonitrile–triethylamine (1000:1, v/v) into borosilicate tubes.

The eluted solutions were dried under nitrogen and the residue dissolved in 120 μl of mobile-phase.

The reconstituted samples were vortexed for 1 min, centrifuged at 14,000 rev./min for 10 min and a volume of 80 μl of the supernatant was injected onto the HPLC column for quantitative analysis.

2.9. Chromatographic conditions

HPLC analyses were carried out using a Symmetry C_{18} (3.5 μm 4.6×150 mm) column and a mobile phase of acetonitrile–water–acetic acid glacial (50:49:1) previously filtered through 0.45 μm filters and degassed. The flow-rate was 1.2 ml/min and peaks were monitored at 227 nm (i.e. λ_{max}).

2.10. Validation study

Precision and accuracy were evaluated by determining IDN 5390 in three replicates of three QC samples at the nominal concentration of 0.075, 0.750 and 1.500 μg/ml (prepared as shown in Section 2.6) on 3 different days. To quantify the QCs, three different standard calibration curves (see Section 2.5) of six plasma concentrations (0.05, 0.1, 0.25, 0.5, 1.0, 2.0 μg/ml) of IDN 5390 were prepared in duplicate and processed as described in Section 2.7.

Chromatograms were evaluated for the peak heights of IDN 5390 and I.S.

At the end of the daily analyses, the HPLC column was washed with acetonitrile–water (1:1) 30 min at the flow-rate of 1.0 ml/min.

To check the linearity of the standard curves, the peak height ratios (expressed as detector response in volts) for IDN 5390/I.S. was plotted versus nominal concentrations. The linearity of the standard curves was determined by the correlation coefficient (r^2) and by comparison of the nominal and back-calculated concentrations of the calibration standards.

The precision of the method at each concentration was expressed as a coefficient of variation (C.V.%) by expressing the standard deviation as a percentage of the mean calculated concentration, while the accuracy of the measure was determined by expressing the mean calculated concentration as percentage of the nominal concentration.

The percentage extraction recovery of IDN 5390 was determined at two different plasma concentrations (0.20 and 1.0 μg/ml) in triplicate. Peak-height ratios of analyte/I.S. of chromatograms obtained from extracted plasma samples, with I.S. added after the extraction at the moment of drying, were compared to those of external standards prepared in acetonitrile–triethylamine.

The detection limit (LOD) was defined as the concentration at which the signal-to-noise ratio was 3. The quantification limit (LOQ) was defined as the lowest amount of the analyte which can be determined in a sample with a precision expressed as intra-day C.V.<20% and an accuracy of 100±20%.

To prepare samples at LOQ, an aliquot of 1 ml of control plasma was combined with 50 μl of a 1 μg/ml methanolic solution of IDN 5390 to produce a nominal plasma concentration of 50 ng/ml of the analytes. Five replicates of the obtained LOQ samples were processed and analyzed by HPLC according to the previously described procedure together with a freshly prepared standard curve with a blank control in duplicate.

The stability of plasma samples under storage conditions was also checked for analyzing five replicates of QCs, prepared on day 1 of the validation study, after 1 or 6 weeks of storage at −20 °C.

2.11. Application of the method

Mice were treated intravenously with 90 mg/kg of IDN 5390 in normal saline solution. Blood samples were collected at selected times during the 4 h after the IDN 5390 administration. Four animals were used for each time point. Blood was obtained from the retro-orbital plexus under diethylether anesthesia and collected in heparinized tubes. The animals were sacrificed by cervical dislocation. The plasma fraction was immediately separated by centrifugation (10 min, 2000 *g*, 4 °C) and stored at −20 °C until analysis for IDN 5390.

The concentration data at each time point represented the mean±SD obtained from four animals. Plasma samples, in which the measured concentration was above the highest standard point of the calibration curve, were diluted and re-assayed.

The experimental area under the curve of the concentration vs. time points (AUC) of IDN 5390 was calculated by the linear trapezoidal rule. Pharmacokinetic parameters (clearance, Cl, and volume of distribution, V_β) were calculated by using a non-linear fitting program [7] according to the following formulas: Cl=dose/AUC; $V_\beta = Cl/K_\beta$, where K_β is the constant of elimination of the drug obtained by the fitting.

3. Results and discussion

3.1. Chromatography

Fig. 2A shows a typical chromatogram of an extracted control mouse plasma. No interfering substances were present at the retention time of IDN 5390 and I.S.

Fig. 2B shows a plasma blank spiked with 1 μg of I.S. Fig. 2C shows a chromatogram of a plasma sample containing IDN 5390 at the concentration of 50 ng/ml (i.e. LOQ). Fig. 2D shows a chromatogram of plasma sample taken 1 h after i.v. treatment with 30 mg/kg of IDN 5390 and corresponds to an amount of 0.90 μg/ml of IDN 5390. The retention times of IDN 5390 and I.S. were 9.1 and 10.5 min, respectively. The chromatographic separation and

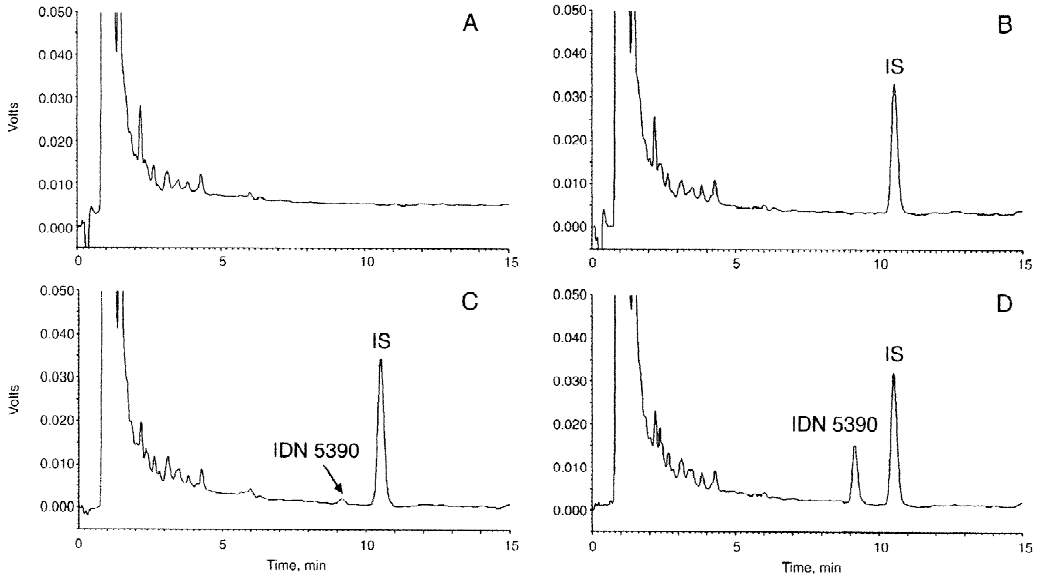

Fig. 2. Chromatograms of blank plasma sample (A); a sample with 1 μg of I.S. (B); a sample with 50 ng/ml of IDN 5390 (LOQ) (C) and a plasma sample taken 1 h after an i.v. administration of IDN 5390 (calculated concentration, 0.90 μg/ml) (D).

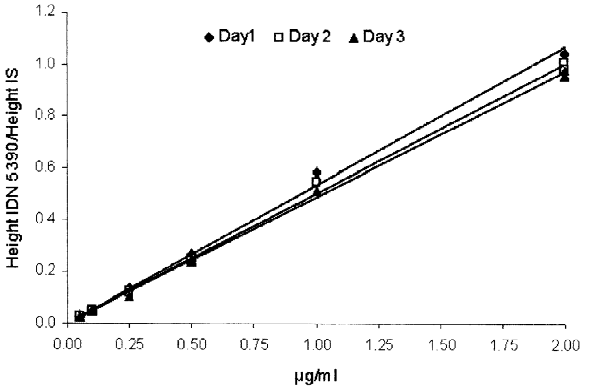

Fig. 3. Regression curves obtained for plasma on 3 different days relating peak response to the added concentrations of IDN 5390.

resolution of the two peaks from the plasma matrix is good.

3.2. Validation study

Fig. 3 shows the multi-point calibration graph of the standard curves for IDN 5390 in the concentration range of 0.05–2.0 μg/ml. They were linear, with a r^2 always more than 0.995 and a slope of 0.507±0.024 (Table 1). Table 1 also reports the results of the calibration curve accuracy during the 3 days of the validation study. Mean accuracy values were always around 100%, and the C.V. was in the range of 1.0–6.2%. On the day of the analysis of the sample from the pharmacokinetic study a standard sample at 5 μg/ml was added; the calibration curve maintained linearity ($r^2=0.999$) and was superimposable on those obtained in the 3 days of the validation study.

Table 2
Summary of intra- and inter-assay precision and accuracy data for IDN 5390 in quality control samples

Day	*N*	Mean observed	C.V.%	% Accuracy [a]
1	3	0.065	12.7	86.7
	3	0.781	4.4	104.1
	3	1.529	6.5	101.9
2	3	0.067	7.6	89.3
	3	0.809	2.9	107.9
	3	1.574	6.1	104.9
3	3	0.072	10.6	96.0
	3	0.802	3.3	106.9
	3	1.411	2.3	94.1
Overall	3	0.068	5.3	90.7
	3	0.797	1.8	106.3
	3	1.536	2.3	102.4

[a] % Accuracy at nominal QC concentration: (calculated/nominal)×100%.

The reproducibility of the method was evaluated analyzing three replicates of three QC samples containing IDN 5390 at the nominal concentrations of 0.075, 0.750 and 1.500 μg/ml on 3 different days. The intra- and inter-day precision and accuracy are reported in Table 2. The method was found to be precise, with a C.V.=12.7% and accuracy in the range of 86.7–107.9% for each of the concentrations tested.

As shown in Table 3, the mean extraction re-

Table 3
Recovery of IDN 5390 from mouse plasma

Added conc. (μg/ml)	% Recovery[a]	C.V.%
0.2	83.5	2.8
1.0	87.6	3.8

[a] Each value is the mean of three determinations.

Table 1
Correlation coefficients (r^2) and comparison of nominal and back-calculated concentration standards for IDN 5390 in mouse plasma

Calibration curve accuracy (%)[a]								
Days	0.05[b]	0.1	0.25	0.5	1.0	2.0	r^2	Slope
1	99.7	87.9	97.0	95.0	106.2	97.6	0.9958	0.534
2	101.3	98.2	93.1	98.4	108.8	99.1	0.9977	0.503
3	107.4	107.9	92.8	98.8	101.4	99.8	0.9999	0.486
Mean	98.3	94.7	95.1	97.9	106.8	98.7		0.507
SD	3.9	5.9	2.0	2.6	1.8	1.0		0.024
C.V.%	4.0	6.2	2.1	2.7	1.7	1.0		4.9

[a] Accuracy: (calculated conc./nominal conc.)×100%.
[b] Nominal concentration of standards (μg/ml).

covery of IDN 5390 performed at two representative concentrations (0.2 and 1.0 μg/ml) is >80% with a C.V.<4%.

The LOD was defined as the concentration at which the signal-to-noise ratio was 3. The mean of the noise recorded in the intervals between 8 and 12 min (comprising the retention times of both the analytes and I.S.) was equivalent to an amount of 15 ng/ml of analyte, the resulting LOD is 45 ng/ml.

The LOQ was fixed at 50 ng/ml, at this concentration the within-day C.V. and accuracy were 18.9% and 95.4%.

The drug appears stable in frozen mouse plasma; >85% of the original concentration of IDN 5390 was found in the QC samples after 6 weeks at −20 °C.

3.3. Application of the method

Fig. 4 shows the pharmacokinetic profile of IDN 5390 in plasma of CDF1 mice after i.v. administration of 30 mg/kg of the drug. Samples taken at 5 and 15 min, in which the concentration of IDN 5390 was superior to the highest standard point of the calibration curve, were re-analyzed diluted 10 times with control mouse plasma.

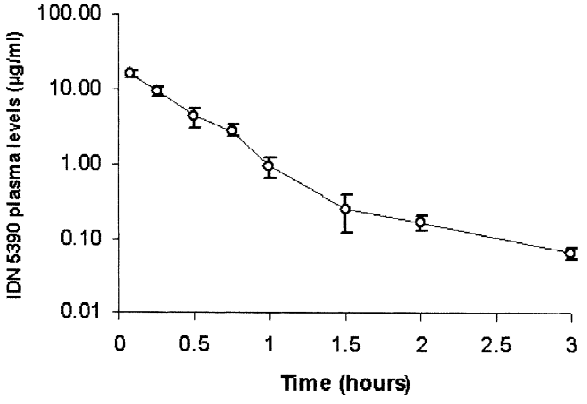

Fig. 4. Plasma concentrations versus time profile after an intravenous dose of 30 mg/kg of drug.

IDN 5390 was rapidly distributed and was cleared from plasma according to a two-compartment model with a terminal half-life of 0.6 h. The clearance and volume of distribution were 4.5 l/h/kg and 5.2 l/kg, respectively.

IDN 5390 was still quantifiable at 3 h.

4. Conclusion

This report describes a method to measure IDN 5390 in mouse plasma.

The method is sensitive, selective, precise and accurate. It should be noted that the method is a rapid and automated SPE procedure with HPLC analysis. The method is suitable for use in determining plasma levels in preclinical investigations and will prove useful for evaluating the pharmacokinetic properties of this antitumor agent.

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