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Development and validation of a liquid chromatography–tandem mass spectrometry method for the determination of ST1926, a novel oral antitumor agent, adamantyl retinoid derivative, in plasma of patients in a Phase I study

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ABSTRACT

E-3-(4′-Hydroxy-3′-adamantylbiphenyl-4-yl) acrylic acid (ST1926) is a novel oral synthetic adamantyl retinoid derivative, now under early clinical investigation in patients with ovarian cancer. To investigate the pharmacokinetics of this new antitumor agent in human plasma, we developed and validated a high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method based on the addition of ST2222 as internal standard and simple protein precipitation with methanol. The method requires a small volume of sample (100 μL); it is rapid and selective, allowing a good resolution of peaks from the plasma matrix in 9 min. The method offers high recovery (>90%), it is sensitive, precise and accurate with overall precision expressed as CV% less than 8.2%. We set the lower limit of quantitation at 20.0 ng/mL and validated the assay up to the concentration of 1000.0 ng/mL. The present method has been successfully applied to study ST1926 pharmacokinetics in patients with advanced ovarian cancer in a Phase I trial, administering the drug orally for five consecutive days. During analysis of the study samples, it became evident the presence of glucuroconjugates of the parent compound, established by LC-Orbitrap MS. Preliminary results show low and variable drug absorption in patients, with extensive glucuroconjugation influencing the bioavailability of ST1926.

1. Introduction

ST1926 (Fig. 1, part A), an adamantyl retinoid derivative, belongs to a new class of retinoid-related molecules with apoptogenic properties [1,2]. Although it shares with all-trans-retinoic acid compounds the property of specific binding to retinoic receptors, its spectrum of activity is not confined to promyelocytic leukemias, but also to other leukemias and solid neoplasms. Its mechanism of action has not been fully elucidated yet, but its strong pro-apoptotic properties seem to be associated with the ability of induction of DNA damage [3–6].

ST1926 is active when administered by the oral route possessing potent antitumor activity in several pre-clinical tumor models, particularly ovarian cancer and various leukemias [2,7]. The compound is now under clinical investigation, and the purpose of the work presented here was to set up and validate a method to measure ST1926 concentrations in plasma of patients. Several methods are currently employed for the determination of retinoids in plasma, mainly based on mass spectrometry that achieves sensitivity in the order of 5–20 ng/mL [8–12], but to our knowledge no method is yet available to measure adamantyl retinoid derivatives.

In order to obtain a sensitive, specific and rapid method to quantify ST1926 in human plasma, we developed and validated a high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method that requires a small volume of plasma sample (100 μL), only simple treatment with methanol and a reasonable time of analysis. High selectivity and sensitivity are guaranteed by working in the Selected Reaction Monitoring (SRM) mode. The present method is successfully applied in a pharmacokinetic study in patients with ovarian cancer in a Phase I study of ST1926. We investigated a range of concentrations, from 20.0 to 1000.0 ng/mL, that we expected to find in the patients' plasma.

2. Materials and methods

2.1. Standards and chemicals

Analytical reference standard of ST1926 (lot N0500982) was supplied by Sigma-tau (Pomezia, Rome, Italy). The internal stan-

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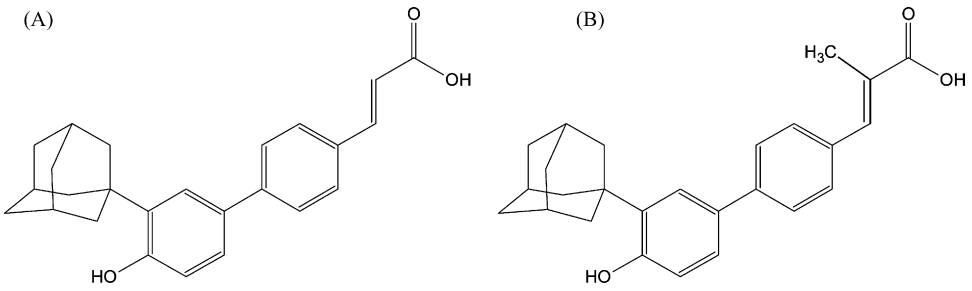

Fig. 1. Chemical structure of ST1926 (A) and ST2222 (B).

dard, ST2222 (lot RN34) (Fig. 1, part B) was obtained from the University of Milan. Analytical grade methanol and acetonitrile were purchased from Carlo Erba (Milan, Italy). Triethylamine (TEA) was obtained from Fluka BioChemika (Buchs, Switzerland). Filtered and deionized water was obtained from a Milli-Q Plus system (Millipore, Billerica, MA, USA). Control human plasma from heparinated blood, used to prepare daily standard calibration curves and quality control samples (QCs), was obtained from volunteers.

2.2. Standard and quality control solutions

ST1926 stock solution was prepared at the concentration of 100.0 μg/mL for standards and at 90.0 μg/mL for quality controls (QCs). These stock solutions were prepared in methanol considering the ST1926 purity factor of 98.1% and stored at −20 °C. The stock solution for the internal standard (IS), was prepared at 100.0 μg/mL in acetonitrile and stored at −20 °C.

Working solutions (A–F) necessary to prepare the standard points of the calibration curve and those (L, M and H) necessary to prepare the plasma quality control samples (QCs) were obtained by diluting different amounts of the stock solution with methanol to obtain ST1926 at the final concentrations reported below:

Standard and quality control	ST1926 concentration (μg/mL)
A	10.0
B	5.0
C	2.5
D	1.0
E	0.5
F	0.2
H	9.0
M	4.5
L	0.45

The IS working solution was prepared at 0.5 μg/mL by diluting the stock solution with acetonitrile.

2.3. Preparation of standards and quality control samples

Control human plasma (90 μL) was spiked with 10 μL of each working solution (A–F) to obtain a final dilution of 1:10, giving six calibration standards in the range 20.0–1000.0 ng/mL. The calibration curve included a blank and a zero standard plasma sample (processed with IS).

To prepare QC samples, three fractions of human plasma were mixed with an appropriate amount of QC solutions (L, M and H) obtaining QC plasma samples at the final concentration of 45.0, 450.0 and 900.0 ng/mL. Several 100 μL aliquots of the three fractions were stored at −20 °C, as controls for future assays, to check the stability under storage conditions and after two freeze/thaw cycles.

2.3.1. Samples extraction

Plasma samples (100 μL) were mixed with 10 μL (5.0 ng) of the IS working solution and with 500 μL of cold methanol (4 °C). After vortexing for 30 s, the plasma mixture was centrifuged at 4 °C for 10 min at 15,000 × *g*. Then the supernatant was recovered, transferred to an autosampler vial maintained at 4 °C and 10 μL were injected into the HPLC–MS/MS system.

2.4. Chromatographic conditions

The HPLC system consisted of an Alliance separation module 2695 (Waters, Milford, MA, USA).

Samples were separated on a chromatographic column C18 X-Bridge 3.5 μm, 150 mm × 2.1 mm, coupled with a Guard column (10 mm × 2.1 mm) of the same material (Waters), held at 30 °C. The mobile phases (MP) used for the chromatographic separation were composed of 0.05% TEA in bidistilled water (MP A) and 0.05% TEA in methanol (MP B).

The HPLC system was set up to operate with a flow rate of 0.2 mL/min under the following linear gradient conditions: step 1—from 99% MP A to 10% over 2 min; step 2—constant condition for 6 min; step 3—from 10% MP A to the initial condition over 1 min; the initial condition was held for 6 min.

2.5. Mass spectrometry

The HPLC system was coupled with a Micromass Quattro Ultima Pt triple quadrupole mass spectrometer (Waters). The instrument was equipped with an electrospray ionization source and operated in negative ion mode, with the ion-spray needle set at −3500 V and the cone voltage at 100 V. Argon was used as collision gas in the collision cell. The mass spectra (MS^1) and the product ion spectra (MS^2) of ST1926 and ST2222 were acquired and used to optimize the instrument operating conditions. For the analysis of biological samples, the mass spectrometer operated in the selected reaction monitoring mode, allowing the $[M-H]^-$ of ST1926 and ST2222 (*m*/*z* 373.4 and 387.2, respectively) to pass through the first quadrupole into the collision cell. After fragmentation, the characteristic product ions of the two compounds were monitored in the third quadrupole at *m*/*z* 329.6, 303.6 and 271.5 for ST1926 and at *m*/*z* 343.4 and 327.5 for ST2222.

During the analysis of clinical samples a different type of instrumentation was available. A HPLC system Series 200 autosampler and micropump (Perkin Elmer, Waltham, Massachusetts, USA) were used coupled with an API 4000 (Applied Biosystem, Foster City, CA, USA) triple quadrupole mass spectrometer. To verify the reproducibility of the method with this instrumentation, we evaluated inter-day precision and accuracy through a single run over one working day, consisting of one calibration curve and three replicates of Low, Medium and High QC plasma samples.

For qualitative analyses and metabolite characterization a high-resolution hybrid instrument was used. The instrument consisted of a 1200 Series capillary LC pump and autosampler (Agilent Technologies, Santa Clara, CA) coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). The ion source was a desorption electrospray ionization Omni Spray

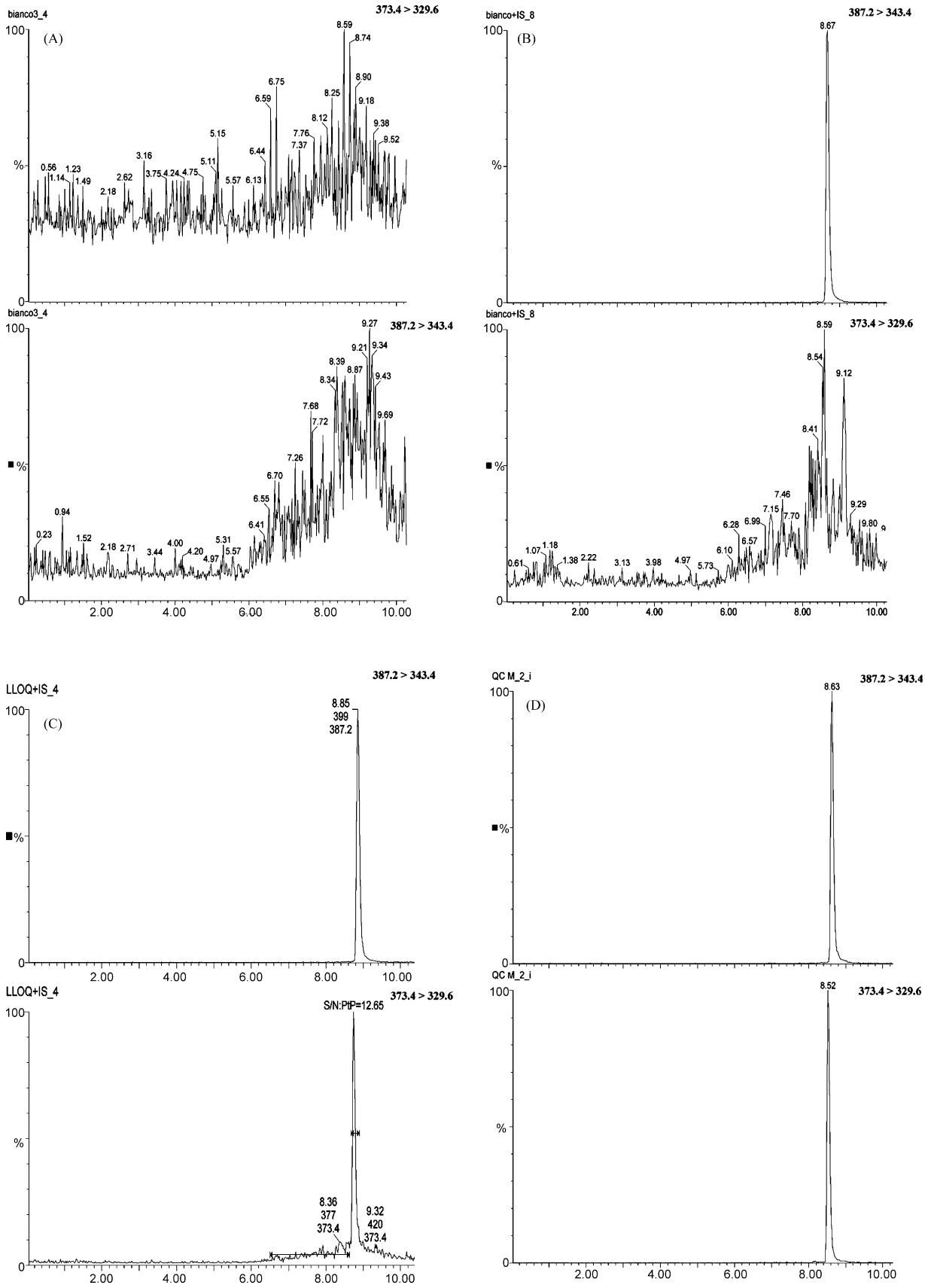

Fig. 2. (A) SRM chromatograms of a human blank plasma sample; (B) SRM chromatograms of a human zero standard plasma sample; (C) signal-to-noise ratio of ST1926 at LLOQ (20 ng/mL); (D) SRM chromatograms of QCM sample (450.0 ng/mL).

Table 1
Recovery of ST1926 from human plasma ($n = 5$).

Concentration ng/mL	Recovery ratio (%) ±SD	CV%
45.0	99.1 ± 5.8	5.9
450.0	95.7 ± 2.1	2.2
900.0	90.2 ± 6.2	6.9

(Prosolia, Indianapolis, IN), used in the nanoelectrospray mode for negative ions. HPLC separation was obtained using an Aquasil C18 column, 100 mm × 0.5 mm ID, 5 μm (Thermo Fisher Scientific), with an elution solvent composed of 0.05% acetic acid in water and acetonitrile (gradient from 20 to 100% of acetonitrile in 30 min) at a flow rate of 10 μL/min. Data were acquired with the Orbitrap analyzer at 60,000 resolution for full scan MS spectra and with the LTQ analyzer (Linear Ion Trap) at unit resolution for MS^2 spectra. MS^2 spectra were automatically obtained in a single chromatographic run, using real time data dependent acquisition, based on the characteristics of the previously acquired MS spectra.

2.6. Validation study

2.6.1. Recovery

The percentage extraction recovery of ST1926 was calculated at three different plasma concentrations (45.0, 450.0 and 900.0 ng/mL) in quintuplicate. The peak area of the analyte obtained from extracted samples was compared to that obtained from external standards prepared in methanol. The recovery of IS was evaluated in the same way.

2.6.2. Calibration curves

The linearity of calibration curves was validated over 7 days and calculated as the ratio of the HPLC–MS/MS peak areas of ST1926/IS versus the nominal amount of ST1926 in the sample. The linearity of the standard curves was determined by a regression model (weighted by the reciprocal of the concentration) calculating the Pearson's determination coefficient (R^2) and by comparison of the true and back-calculated concentrations of the calibration standards.

2.6.3. Precision, accuracy and LLOQ

Precision and accuracy were evaluated on five different days by determining the analyte in three replicates of three QC samples at the nominal concentration of 45.0, 450.0 and 900.0 ng/mL. To analyse the QCs, five different standard calibration curves were prepared and processed each day.

The precision of the method at each concentration was reported as a coefficient of variation (CV%), expressing the standard deviation as a percentage of the mean calculated concentration; while the accuracy of the measure was determined by expressing the percentage ratio of the absolute difference of the determined concentration from the theoretical and the theoretical value (deviation of accuracy%).

The detection limit (LOD) and the limit of quantitation (LOQ) were defined as the concentration at which the signal-to-noise ratio was 3 and 10, respectively. The lower limit of quantitation (LLOQ) was defined as the lowest amount of the analyte that can be determined in a sample with sufficient degree of precision and accuracy (within 20% for both parameters). The quantitation method for ST1926 was set up in order to investigate its pharmacokinetics in human plasma, so we chose an appropriate concentration interval, fixing the lowest one of the calibration curve as the LLOQ.

Furthermore, as indicated at the 3rd AAPS/FDA Bioanalytical Workshop, evaluation of bioanalytical methods through reanalysis of "incurred" or study samples is recommended and can be taken as one additional measure of assay reproducibility [13]. Particularly when incurred samples from drug studies may have metabolites that neither the standards nor the quality control samples contain. It could be that the drug metabolite reverts to its parent drug *in vitro*, causing non-reproducible results. Therefore, we assessed the accuracy of the present method by re-analyzing, in a further analytical session, several incurred plasma samples of two patients from the pharmacokinetic study. The analyses can be considered equivalent if the variability in accuracy is within the range 85–115%.

Table 2
Accuracy and precision data for calibration curves of ST1926.

Concentration ng/mL		Precision (%) between runs	Dev. of accuracy (%)
Theoretical	Found (mean ± SD, 7 days)		
20.0	22.2 ± 1.1	5.0	11.1
50.0	47.7 ± 1.5	3.2	4.7
100.0	93.2 ± 3.4	3.7	6.8
250.0	249.3 ± 4.7	1.9	0.3
500.0	499.1 ± 15.7	3.1	0.2
1000.0	1008.5 ± 14.8	1.5	0.9
Calibration curve parameters			
R^2		0.9988 ± 0.0007	
X-coefficient		0.0068 ± 0.0013	
Y-intercept		0.0110 ± 0.0025	

2.6.4. Stability

The stability of ST1926 in plasma was assessed by analyzing QC samples at concentrations of 45.0, 450.0 and 900.0 ng/mL during the sample storage and handling. Bench-top stability was determined after 4 h at room temperature and autosampler stability was evaluated by re-analyzing the processed QC samples 72 h after the first injection. To check the freeze/thaw stability an aliquot of each QC sample concentration was processed and analyzed fresh, and after the first and the second freeze/thaw cycle. Furthermore, long-term stability was assessed in plasma and in working solutions after storage at −20 °C for 6 months. ST1926 was considered stable at each concentration when the differences between the freshly prepared samples and the stability testing samples were found to be not exceeding 15% deviation of the nominal concentration.

2.7. Application of the method to clinical samples

The present method was used to evaluate the pharmacokinetics of ST1926 in three women with advanced epithelial ovarian cancer participating in an open-label, monocentric, Phase I dose escalation study in sequential cohorts. Patients received the drug orally twice a day for five consecutive days (starting in the evening on day 1 and finishing in the morning on day 6), every 28 days. To determine the plasma concentration of ST1926 and to study the pharmacokinetic profile, blood samples were collected at the following time points:

Day 1 (in the evening), before the 1st administration (pre-dose).
Day 2 (in the morning), before the 2nd administration (pre-dose) and 0.5, 1, 2, 4, 8 and 12 h post-dosing.
Day 6 (in the morning), before the 10th administration (pre-dose) and 0.5, 1, 2, 4, 8, 12 h, in the interval 13–23 h (two samples) and 24 h post-dosing.

Samples were collected in heparinized tubes and immediately centrifuged at 4000 × *g* at 4 °C for 10 min. After centrifugation, the plasma was separated, collected and divided as volume of 1.5 mL in two polypropylene tubes that were stored at −20 °C until analysis.

Table 3
Intra- and inter-day validation of the method for quantitative determination of ST1926.

	Theoretical concentrations (ng/mL)		
	45.0	450.0	900.0
Intra-day			
Mean (N = 3)	50.8	505.2	1003.9
SD%	0.4	8.4	31.5
Precision%	0.8	1.7	3.1
Dev. of accuracy%	13.0	12.3	11.5
Inter-day			
Mean (N = 15)	47.6	490.8	987.8
SD%	3.9	21.0	38.1
Precision%	8.2	4.3	3.9
Dev. of accuracy%	5.8	9.1	9.8

Table 4
LLOQ of ST1926 in human plasma.

Concentration ng/mL	Concentration found ng/mL	Dev. of accuracy%
20.0	19.6	2.0
	19.7	1.5
	20.2	1.0
	20.7	3.5
	20.5	2.5
	20.6	3.0
Mean (N = 6)	20.2	2.3
SD	0.5	0.9
CV%	2.3	

3. Results and discussion

3.1. HPLC–MS/MS

Fig. 2 (part C) shows the SRM chromatograms, with the quantifier transitions of ST1926 and ST2222 of a standard sample at the LLOQ, containing ST1926 and IS at 20.0 and 50.0 ng/mL, respectively, in comparison with a chromatogram of a blank plasma sample (part A) and a zero standard plasma sample (part B). Part D of Fig. 2 shows SRM chromatograms of a QCM sample (450.0 ng/mL).

The elution of the analyte was selective with retention times for ST1926 and IS of 8.5 and 8.6 min, respectively. No interfering peaks were present at these retention times, the peak of the analyte had a good shape and was completely resolved from the plasma matrix as can be seen from the signal-to-noise ratio (S/N) of 12.65 calculated at LLOQ (Fig. 2, part C). The $[M-H]^-$ of the analyte was selected as the precursor ion, and the collision energy was optimized to obtain the product ions with the highest signal. Three SRM transitions were selected, and the quantification of ST1926 and IS was performed using respectively the transition m/z 373.4 > 329.6 and m/z 387.2 > 343.4, corresponding to the loss of the carboxylic group.

The method revealed its ruggedness because it was possible to apply it to another HPLC–MS/MS system for the analysis of patients' plasma samples.

3.2. Validation study

3.2.1. Recovery

ST1926 was extracted from plasma by simple protein precipitation with methanol, a method which satisfactory recovery. The recovery was evaluated for three concentrations, each in quintuplicate. The matrix effect was assessed at each concentration level by comparing the mean area of the analyte obtained from plasma samples with the same amount of the analyte added to the biological matrix after recovery. The variation of the analyte area was ≤15%, excluding any matrix effect of ion suppression or enhancement. As shown in Table 1, the mean extraction recovery for ST1926 at 45.0, 450.0 and 900.0 ng/mL was >90.0%, with good reproducibility in the range 2.2–6.9% (expressed as CV%). The recovery of the IS was 75.9 ± 3.2%.

3.2.2. Calibration curves

Table 2 reports the accuracy and the precision for the analyte in a 7-day validation study.

The ratios of peak areas of ST1926 over the IS were plotted against the concentration, and least-squares linear regression weighted by the reciprocal of the concentration was applied to generate calibration curves. The calibration curves, prepared on seven different days, were linear over the range of concentrations tested (20.0–1000.0 ng/mL), with a mean coefficient of determination (R^2) of 0.9988 (range: 0.9978–0.9999). Mean accuracy was in the range 0.2–11.1% and precision was in the range 1.5–5.0% (5.0% at LLOQ) when expressed as CV%.

3.2.3. Precision, accuracy and LLOQ

The precision and accuracy of the method were evaluated by analyzing three replicates of the QC samples at 45.0, 450.0 and 900.0 ng/mL, within a single run analysis for intra-day study and over five consecutive runs of analysis for inter-day study. The precision (CV%) and accuracy obtained are shown in Table 3. The method was precise, with intra- and inter-day CVs ≤3.1% and 8.2%, respectively, and accuracy in the range of 5.8–13.0%.

Table 5
Stability of ST1926 in plasma and in working solutions.

Matrix	Conditions	Initial concentration (ng/mL)	Concentration found (ng/mL)	Concentration recovered (%)	Dev (%)
Plasma	Room temperature, 4 h	51.5	50.3	97.7	−2.3
		503.0	504.2	100.2	0.2
		991.3	990.6	99.9	−0.1
Plasma	Autosampler, 4 °C, 72 h	46.1	47.7	103.5	3.5
		501.3	489.8	97.7	−2.3
		990.7	939.8	94.9	−5.1
Plasma	2 freeze (−20 °C)/thaw cycles	51.5	45.5	88.3	−11.7
		503.0	456.6	90.8	−9.2
		991.3	897.3	90.5	−9.5
Plasma	−20 °C, 6 months	49.3	43.0	87.2	−12.8
		466.8	412.5	88.4	−11.6
		825.1	818.2	99.2	−0.8
CH_3OH	−20 °C, 6 months	38.8	39.2	101.0	1.0
		387.0	390.1	100.8	0.8
		787.1	769.0	97.7	−2.3

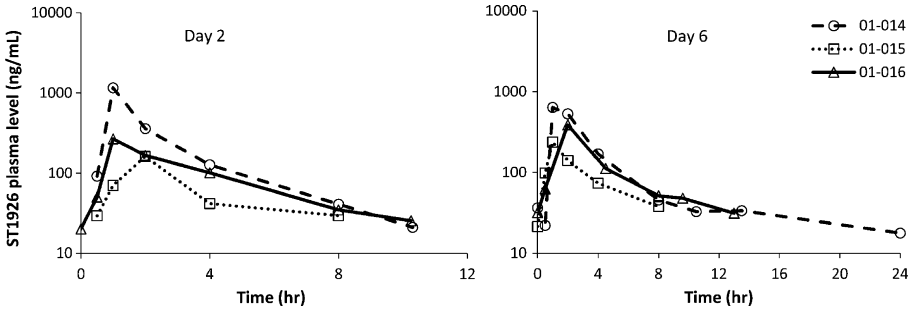

Fig. 3. Plasma levels of ST1926 in three patients receiving 210 mg of the drug by oral route.

The limit of detection of ST1926 was approximately 5 ng/mL; the LLOQ was fixed at 20.0 ng/mL and validated by six replicates of different samples of human plasma spiked with ST1926. The precision and deviation of accuracy were both 2.3%. Single values are shown in Table 4.

A further demonstration of the high reproducibility and accuracy of the method was obtained by re-analyzing several incurred plasma samples of two patients at C_{max}, at concentrations close to the C_{max} and at concentrations in the drug elimination phase, close to the LLOQ. We found that the concentrations of ST1926 determined in these two occasions are practically identical in all samples analyzed, being the values of accuracy in the ranges 87.6–97.7% and 86.8–112.2% for the two patients. This range encompasses the accepted variability in accuracy of the analytical method, so the two measurements can be considered equivalent.

3.2.4. Stability

Stability data are presented in Table 5. ST1926 was stable in fresh human plasma maintained at room temperature for 4 h, the amount left corresponding to more than 95% of the initial concentration. The drug was stable in human plasma extracts kept in the autosampler at 4 °C for 72 h. ST1926 was stable in human plasma over two freeze/thaw cycles as demonstrated by suitable values shown in Table 5. The drug was found stable in frozen human plasma; more than 85% of the initial concentrations were found in QC samples after 6 months of storage at −20 °C.

The stability of ST1926 QC stock solutions prepared in methanol and stored at −20 °C was evaluated after 6 months and the found concentrations were more than 95% of the initial values.

3.3. Clinical samples

Fig. 3 shows the representative curves of the plasma concentration versus time of ST1926 determined by the present method in three patients treated with ST1926 at 210 mg on days 2 and 6. The mean C_{max} and AUC were characterized by high inter-patient variability on both days, as shown by the following data:

Day 2: C_{max} 528.7 ± 546.6 ng/mL (CV 103.4%),
AUC 1133.3 ± 746.5 ng/mL h (CV 67.8%).
Day 6: C_{max} 420.8 ± 200.7 ng/mL (CV 47.7%),
AUC 1537.0 ± 821.6 ng/mL h (CV 53.5%).

Considering the high dose administered, the plasma concentrations of ST1926 were lower than expected, based on comparison with results obtained for the starting dose of 30 mg (Day 2: C_{max} 239.5 ± 141.7 ng/mL; Day 6: C_{max} 297.2 ± 176.5 ng/mL). At time points beyond the C_{max}, ST1926 disappeared from plasma with a variable elimination half-life ranging from 2 to 7 h. It was measurable in only one patient up to 24 h after the last dose on day 6, at concentrations close to the LLOQ.

In case of plasma samples with a concentration of ST1926 above the highest point of the calibration curve, they were suitably diluted with control human plasma and reanalyzed.

As shown in Fig. 4 (parts A and B), the HPLC–MS/MS trace from patients' samples, revealed the presence of the analyte (in boldface at the retention time of 5.9 min) in addition to other peaks with retention times of 5.2 and 5.4 min, showing the same transitions as ST1926. In parts C and D, the clean trace of blank plasma sample from the same patients suggests the absence of matrix constituents which interfere with the analysis. The chromatographic profile of LLOQ and a QC sample allowed us to exclude the presence of contaminants or degradation products (Fig. 2, parts C and D). Therefore, we surmised that these other peaks might be metabolites of ST1926 which could undergo fragmentation during ionization of the eluate, becoming detectable at the same transitions as ST1926. To verify this hypothesis, we analyzed plasma samples with the LC-LTQ-Orbitrap XL mass spectrometer, a hybrid instrument offering superior mass accuracy and resolution, for reliable identification of compounds in complex matrices. Based on the available *in vitro* and *in vivo* pre-clinical data on ST1926 metabolism (Sigma-tau Study Report 0120-2005), we searched for the presence of glucuronide conjugates (m/z 549.2). The chromatographic trace in Fig. 5 (part A) shows three glucuronide conjugates, at retention times of 9.5, 11.0 and 14.6 min. Part B gives the full mass spectra and ms/ms spectra of these three metabolites. Even though the chromatographic conditions during the analysis by LC-LTQ-Orbitrap XL mass spectrometer were different from those used for the quantitation of ST1926, we assume that the unknown peaks correspond to glucuroconjugates. This is supported by the same order of elution in both the reversed-phase chromatographic runs and by the fact that the fragmentation pattern of the glucuroconjugates shows ST1926 as main fragment.

To confirm the presence of the glucuroconjugates, the plasma samples of patients, who received ST1926, were incubated with β-glucuronidase and reanalyzed by HPLC–MS/MS. The unknown peaks in the chromatographic trace related to ST1926 transitions disappeared (data not shown). More than 80% of ST1926, as a mean of all subjects analysed on either day 2 or 6, was present in plasma as glucuroconjugated metabolites. The C_{max} of ST1926 measured after hydrolysis with β-glucuronidase rose 8–18-fold and the plasma AUC 9–54-fold.

There are two different sites in the ST1926 molecule (Fig. 1, part A) which are conceivably susceptible to glucuronidation, one phenol hydroxyl group and one acyl group. So formation of two glucuronide conjugates with the same m/z is easy to explain. The third is perhaps a *C*-glucuronide formed by reaction of the electrophilic double bond in C3 position of ST1926 with activated glucuronic acid, as previously reported for other compounds (sulfinpyrazone, ethchlorvynol and tetrahydrocannabinol) [14–16].

As a final consideration, it is interesting to note that with this method, it was possible to draw attention to the presence of

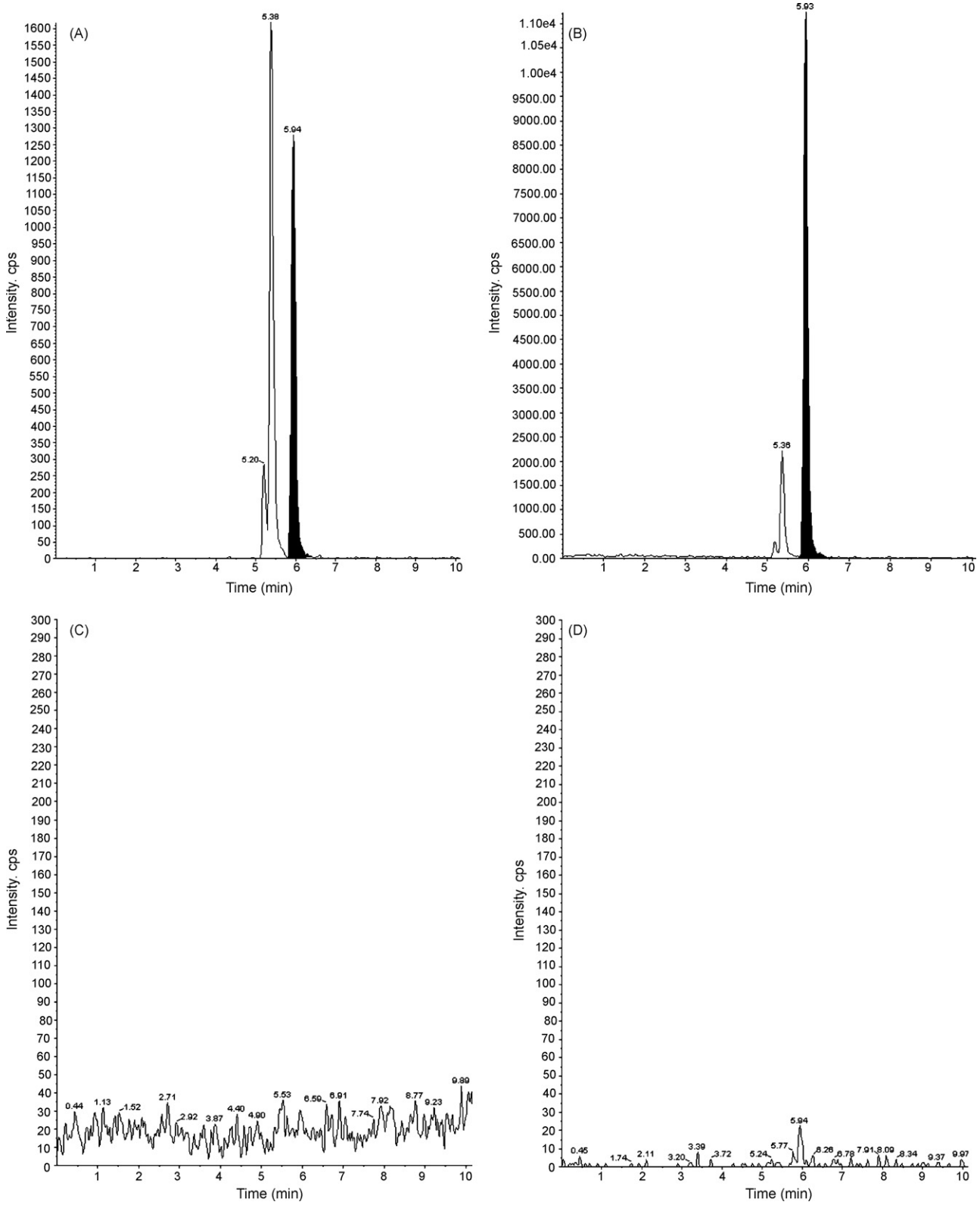

Fig. 4. SRM chromatograms of two patients receiving 210 mg of ST1926: parts C and D are related to the pre-dose sample and parts A and B to the sample collected 2 h (A) and 1 h (B) after the 2nd dose.

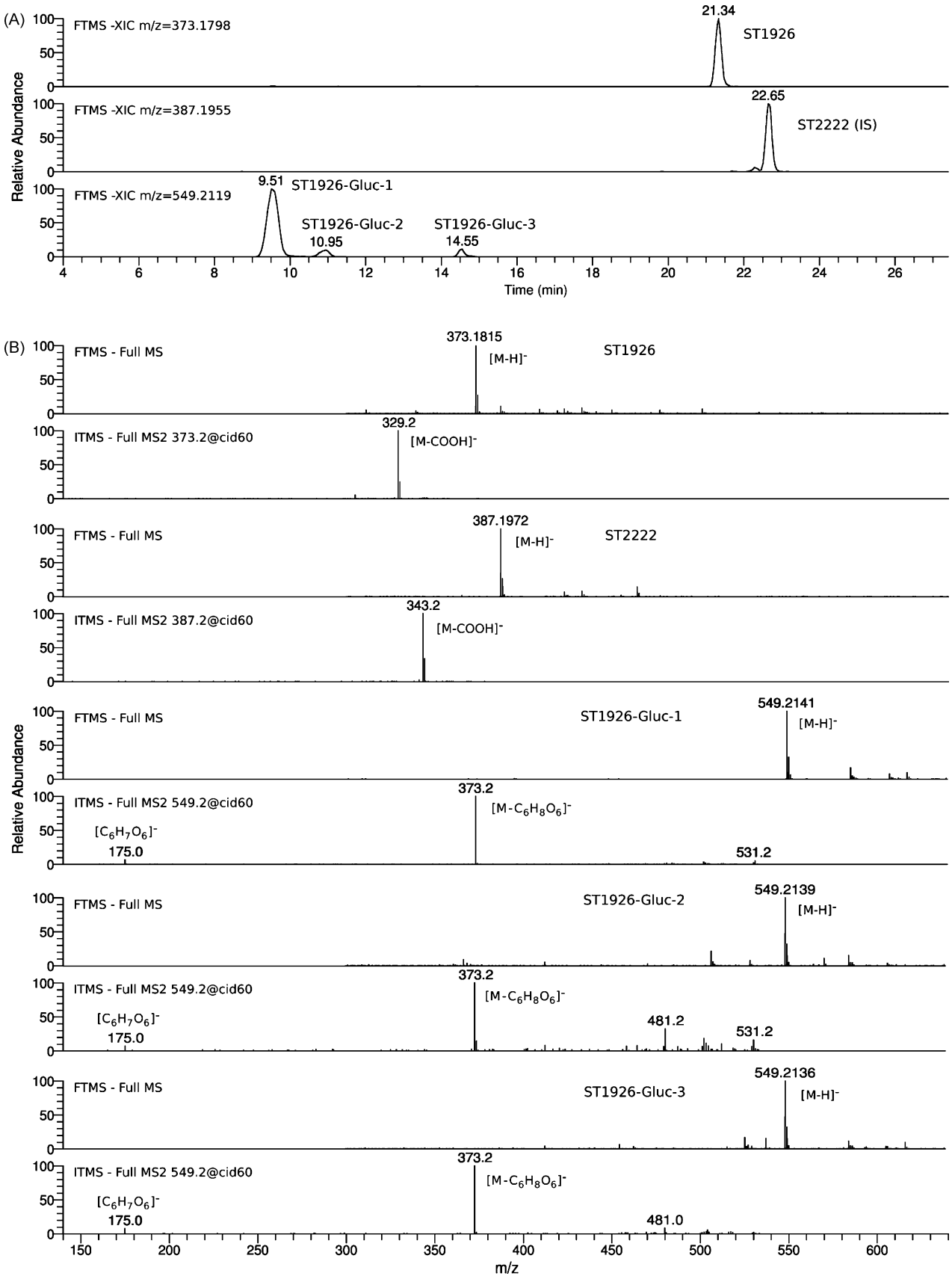

Fig. 5. (A) Chromatographic traces of ST1926, ST2222 (IS) and glucuronide conjugates (ST1926-Gluc-1, ST1926-Gluc-2, ST1926-Gluc-3) obtained with the LTQ-Orbitrap XL mass spectrometer; (B) Full mass scan and MS/MS scan of the analyte, IS and glucuronide conjugates.

glucuronides thanks to satisfactory chromatographic separation, avoiding coelution with the parent drug.

4. Conclusion

The analytical procedure described, based on simple protein precipitation and HPLC–MS/MS analysis, allows the determination of ST1926 in human plasma. The method requires only a small volume of sample, it is selective, sensitive, precise and accurate. It is currently in use to measure plasma concentrations of ST1926 in samples from ovarian cancer patients participating in a Phase I trial who have received the drug by the oral route. The preliminary pharmacokinetic results show variable absorption and lower plasma concentrations than expected. More than 80% of the drug underwent glucuroconjugation, dramatically lowering its bioavailability.

We noticed that ST1926 glucuroconjugates underwent fragmentation during ionization of the eluate, becoming detectable at the same transitions as ST1926, thus possibly affecting correct quantitation of the parent drug. This finding highlights the importance of satisfactory chromatographic conditions to avoid the coelution of peaks.

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