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# Pharmacokinetic screening for the selection of new drug discovery candidates is greatly enhanced through the use of liquid chromatography–atmospheric pressure ionization tandem mass spectrometry

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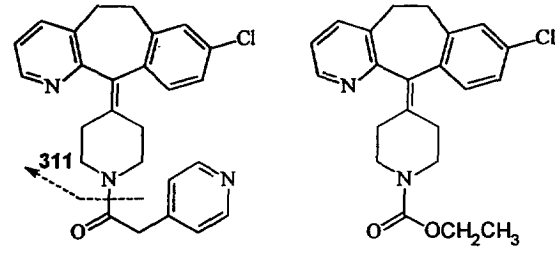
**Abstract**

Selection of a new drug discovery candidate from a series of compounds requires a means of performing rapid analytical method development and sensitive quantitation of each drug in serum, plasma or other biological matrices. Information on serum/plasma concentration, bioavailability and half-life can often aid the discovery process by selecting those candidates with the desired pharmacokinetic parameters. In one series of farnesyl protein transferase (FPT) inhibitors, gas chromatography with nitrogen–phosphorus detection (NPD) was initially used to analyze samples from pharmacokinetic studies in mice and monkeys. Typical turnaround times using this technique approached 2–4 weeks for method development, quantitation of study samples and calculation of pharmacokinetic parameters. Once LC–atmospheric pressure ionization (API) MS–MS analysis was implemented in these same studies, they could be completed in less than one week. The advantages of using LC–API-MS–MS to aid in the drug candidate selection process is demonstrated for one compound (SCH 44342) in this series of FPT inhibitors. © 1997 Elsevier Science B.V.

*Keywords:* Pharmaceutical analysis; Enzyme inhibitors; SCH 44342; Piperidines

## 1. Introduction

SCH 44342 4-(8-chloro-5,6-dihydro-11H-benzo-[5,6]cyclohepta[1,2 - *b*]pyridin -11- ylidene) -1- (4 - pyridinylacetyl)-piperidine (Fig. 1) [1] is a potent inhibitor of farnesyl protein transferase (FPT). FPT catalyzes the initial step in the post-translational processing of the ras oncogene, transfer of the 15-carbon farnesyl group onto a cysteine residue in the carboxy terminal of all ras proteins. This farnesylation step appears to be essential for ras proteins to




SCH 44342 SCH 29851

Fig. 1. Structures of SCH 44342, its major CID fragment ion and the internal standard for the GC assay (SCH 29851).

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become membrane-associated and transform cells [2]. Therefore, inhibition of this step has been speculated to be a potential target for development of compounds with antitumour activity [3]. This therapy area has enormous potential since nearly one-third of all human cancers appear to contain mutations in the ras gene [4].

The process of selecting a potent FPT inhibitor compound involves a number of steps. First, new compounds are evaluated for their intrinsic potency in several biological assays. The FPT $IC_{50}$ is a measure of the compound's ability to inhibit farnesylation of H-ras in vitro. The cos $IC_{50}$ is determined in a cell-based system to measure post-translational processing of H-ras in cos monkey kidney cells. The FPT and cos $IC_{50}$ values for SCH 44342 are 0.25 $\mu M$ and 3 $\mu M$, respectively [3,5]. Compounds with the lowest $IC_{50}$ values have the highest intrinsic potency. Nonetheless, the pharmacokinetic characteristics of the compound must also be determined to evaluate whether the compound persists in the circulation for a sufficient time and at a concentration to be effective. Presumably, those compounds which have circulating levels greater than its $IC_{50}$ values would be able to inhibit farnesylation in vivo. Thus, many compounds would be synthesized by chemical research and then tested for their potency using these and other biological activity screens. Only after showing certain levels of intrinsic potency, would they be evaluated in a pharmacokinetic study in mice, the pharmacological species. After demonstrating acceptable pharmacokinetics, the compound would be further tested in a tumor growth inhibition model, which requires a significant amount of time and resources. This tiered approach to selection of the best compounds is relatively common in modern new drug discovery research. In order to carry out an efficient pharmacokinetic evaluation program, sensitive analytical methods are required for the candidate compounds in biological matrices such as serum or plasma.

This study describes an analytical method for the determination of one compound in this series of FPT inhibitors (SCH 44342) using capillary gas chromatography which was used in a pharmacokinetic evaluation program. A much more powerful analytical method using high-performance liquid chromatography atmospheric pressure ionization tandem mass spectrometry (HPLC–API-MS–MS) is also described, along with its many advantages to more rapidly select candidate compounds in a drug discovery program.

## 2. Experimental

### 2.1. Drug administration and sample collection

Mice were administered a single dose of SCH 44342 at 25 mg/kg by oral or intravenous administration as the HCl salt dissolved in saline. Blood samples were obtained by cardiac puncture under methoxyflurane anesthesia at specified time points up to 2 h post-dosing. After clotting on ice, serum was isolated by centrifugation and stored frozen until analysis.

### 2.2. Sample preparation

#### 2.2.1. GC–thermionic specific detection

A 0.1-ml aliquot of serum was added to a PTFE-lined screw cap test tube and spiked with an internal standard (400 ng of SCH 29851) and 0.5 ml water. After vortexing to mix, the sample is extracted with 5 ml of 20% (v/v) ethyl acetate in pentane for 1 h using a horizontal shaker. The samples were centrifuged for 10 min at ca. 3000 *g*. The upper organic layer was transferred to a clean tube and evaporated to dryness in a nitrogen evaporator (Zymark TurboVap). The residue is dissolved in 0.25 ml of methanol and transferred to a GC injection vial and evaporated to dryness in a vacuum centrifuge (Savant Speed-Vac). The residue was reconstituted in 10 to 20 μl of ethanol.

#### 2.2.2. HPLC–APCI-MS–MS

A 40-μl aliquot of serum was added to a polypropylene microcentrifuge tube and subjected to protein precipitation with 100 μl of acetonitrile–methanol (90:10) containing 1 ng/μl of the internal standard (SCH 56580). After vortexing for 30 s and centrifugation at 12 000 *g* for 8 min, the supernatant was transferred into HPLC injection vials containing low-volume inserts.

### 2.3. Preparation of calibration samples

#### 2.3.1. GC–TSD

Standard curves were prepared by spiking SCH 44342 into drug-free mouse serum at the following concentrations: 25, 50, 100, 250, 1000, 2500 and 5000 ng/ml. Aliquots of each standard (0.1 ml) were processed in the same manner as the study samples.

#### 2.3.2. HPLC–API-MS–MS

Standard curves were likewise prepared by spiking SCH 44342 into drug-free mouse serum at the following concentrations: 10, 25, 50, 100, 250, 1000, 2500, 5000, 10 000, 20 000, 30 000 and 50 000 ng/ml. Duplicate 40-μl aliquots of each standard were processed in the same manner as the study samples.

### 2.4. Instrumentation and operating conditions

#### 2.4.1. GC–TSD

A 1–2 μl portion of the sample extract was injected onto a Varian 3500 capillary gas chromatograph equipped with a Model 8100 autosampler, septum programmable injector (SPI) and a capillary TSD system operated in the most sensitive setting (bead power 3.5 amps). Gas flow-rates for TSD were 4.4 and 100 ml/min for hydrogen and air, respectively. Helium (1.2 ml/min) was used as the carrier gas, with nitrogen (20 ml/min) plumbed as a capillary make-up gas. Separation of the analyte and internal standard was achieved with a J&W DB-5 capillary column (15 m×0.32 mm, 0.25 μm film thickness) using a temperature program of 230°C (3 min) to 280 at 10°C/min (hold for 7 min), followed by a post-run to 320°C. The SPI and TSD system were held at 285 and 340°C, respectively.

#### 2.4.2. HPLC–APCI-MS–MS

The HPLC system consisted of a Waters 600S Controller, 616 pump and WISP 717 Plus autosampler. Chromatographic separation of the analyte and the internal standard was achieved with a Waters Symmetry $C_{18}$ column (5 μm packing material, 50× 3.9 mm) using a two-solvent gradient system: A (0.01 *M* ammonium acetate in methanol–water (20:80) and B (0.01 *M* ammonium acetate in methanol). At a constant flow-rate of 0.8 ml/min, a linear gradient from 55 to 85% B was run over 2.5 min, held for 1.5 min and then re-equilibrated to 55% B over 3.5 min. The effluent from the HPLC system was connected directly to a Finnigan TSQ 7000 MS system equipped with a standard electrospray (ESI)/atmospheric pressure chemical ionization (APCI) source in the positive ion APCI mode. A switching valve (Valco Instruments, Model EC6W) was used to divert the HPLC effluent into a waste container for the first 1 min of the HPLC run time to minimize contamination of the APCI interface. The MS–MS reaction selected to monitor SCH 44342 was the transition from *m/z* 430 ($MH^+$) to a fragment resulting from cleavage of the molecule in the collision cell (argon gas pressure at approx. 2 mTorr and collision energy of 17 eV; 1 Torr=133.322 Pa) as indicated in Fig. 1 (*m/z* 311). The internal standard (a similar analog) was monitored using the transition from *m/z* 444 ($MH^+$) to its most abundant fragment ion (*m/z* 325) at a collision energy of 18 eV. Each transition was alternately monitored with a dwell time of 0.5 s. An icl procedure was written to control the operation of the switching valve and the MS–MS transitions.

### 2.5. Calculation of pharmacokinetic parameters

Mean concentrations were used for the calculation of all pharmacokinetic parameters, including half-life ($t_{1/2}$), concentration ($C_{max}$) and time of maximum concentration ($T_{max}$). Area under the concentration–time curve (AUC) utilized the linear trapezoidal rule.

## 3. Results

### 3.1. GC–TSD assay

A GC chromatogram from a sample of mouse serum spiked with the internal standard (SCH 29851) and SCH 44342 at 0 and 1000 ng/ml is shown in Fig. 2. The background noise in the chromatogram was sufficiently low to allow a lower limit of quantitation of about 25 ng/ml. During the course of the method development, several liquid–liquid extraction solvent systems were evaluated. The final solvent of 20% ethyl acetate in pentane showed greater than 80% extraction efficiency for SCH 44342 in mouse serum. Nonetheless, several

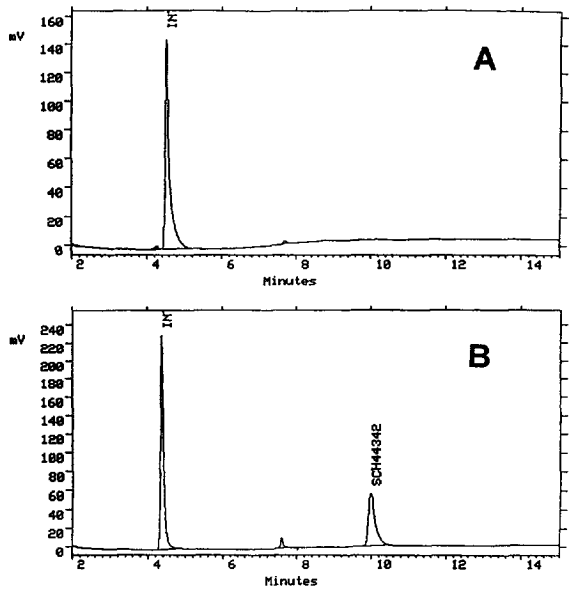


Fig. 2. GC chromatograms of mouse serum spiked with the internal standard (SCH 29851) and SCH 44342 at (A) 0 ng/ml and (B) 1000 ng/ml.

other analogs in this series of FPT inhibitors appeared to be much more difficult to extract from serum as effectively and showed greater levels of chromatographic noise (unpublished data). This was perhaps the most compelling reason for switching to the HPLC–API-MS–MS methodology, which did not require extensive sample preparation method development.

The calibration curve for the GC assay consisted of eight standard concentrations from 25 to 5000 ng/ml and was constructed using least squares linear regression with $1/y$ weighting. A typical calibration curve is defined by the following equation: $y=0.0007705x-0.0069$ where $y$ is the ratio of peak heights for the analyte and internal standard and $x$ is the concentration of analyte in ng/ml. The response was linear as indicated by an $r^2$ value of 0.9963. Accuracy, expressed as the deviation from the nominal value, was between −19 and +12%.

### 3.2. HPLC–API-MS–MS assay

Mass chromatograms of mouse serum spiked with the internal standard and SCH 44342 at 0 and 10 ng/ml are shown in Fig. 3. The signal-to-noise ratio is similar to that seen in the GC chromatogram (Fig. 2) containing 100 times the amount of SCH 44342. Thus, the increased sensitivity of the HPLC–API-MS–MS assay allowed the lower limit of quantitation to be set at a level of 10 ng/ml, using less than half the amount of serum (40 μl compared to 0.1 ml). In addition, the dynamic range of the assay was found to be much greater, and allowed the upper limit of quantitation to be set at 50 000 ng/ml. The calibration curve for the HPLC–MS–MS assay consisted of thirteen standard concentrations in duplicate from 10 to 50 000 ng/ml and was constructed using a power function of the form $y=a+bx^c$, where $y$ is the peak area ration of analyte and internal standard, $x$ is the concentration in ng/ml and $a$, $b$ and $c$ are the intercept, slope and exponent, respectively. A typical calibration curve is defined by the following equation: $y=-0.000245+0.000215x^{0.9784}$. The correlation coefficient ($r^2$) values was 0.999999 for the 4–5 orders of magnitude of the calibration curve. Accuracy (% bias) was less than 12% at all concentrations.

## 4. Discussion

Concentrations of SCH 44342 in mouse serum following oral and intravenous administration at 25 mg SCH 44342/kg are shown in Fig. 4 (HPLC–API-MS–MS data). SCH 44342 was rapidly absorbed following oral administration, achieving a maximum concentration ($C_{max}$) of 1.02 μg/ml at 5 min post-dose. The area under the serum concentration–time curve ($AUC_{0-1\,h}$) was 0.37 and 1.75 μg h/ml, respectively, following oral and intravenous administration. The oral bioavailability was thus 21%. The elimination half-life of SCH 44342 after intravenous administration was determined to be less than 10 min [5].

The serum concentration–time profile of SCH 44342 after a single oral dose was compared with the various indicators of FPT potency for this compound (Fig. 4). The amount of time when the serum concentration was greater than each of these measures of FPT potency was determined from this curve, and found to be less than 0.5 h for the FPT $IC_{50}$, and no time above the cos $IC_{50}$. Thus, the pharmacokinetics of this compound would suggest

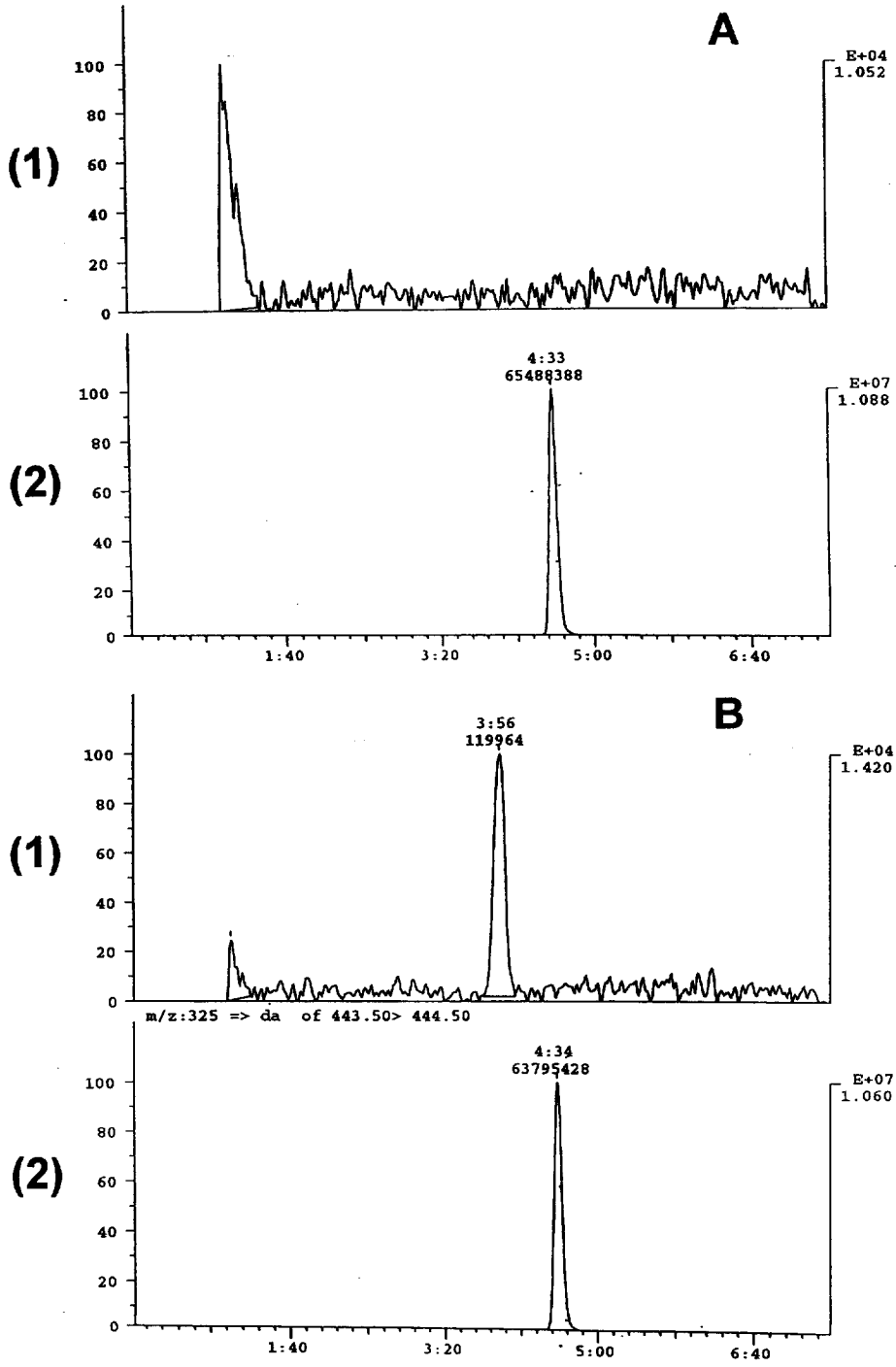


Fig. 3. Mass chromatograms of mouse serum spiked with the internal standard (a similar analog) and SCH 44342 at (A) 0 ng/ml and (B) 10 ng/ml. Windows 1 and 2 monitor the transitions from *m/z* 430 to *m/z* 311 (SCH 44342) and from *m/z* 444 to *m/z* 325 (internal standard), respectively.

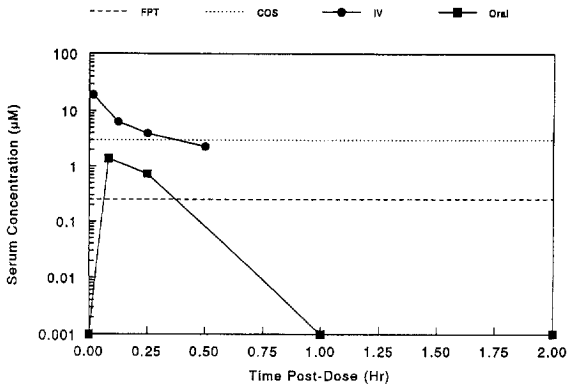


Fig. 4. Serum concentration–time profile of SCH 44342 following oral and intravenous administration at 25 mg/kg in the nude mouse.

that its duration may not be sufficient for optimum activity in vivo. Consistent with this finding is a recent study where SCH 44342 did not show inhibition of tumor growth in a nude mouse model [5]. It was speculated that improving the pharmacokinetic profile of compounds in this series of FPT inhibitors would improve their potential as antitumor agents.

An ambitious program was thus undertaken to continue to screen for FPT inhibitors with improved intrinsic potency, but also to rapidly evaluate the pharmacokinetics of these compounds in animal models. This latter screening project required the use of LC–API-MS–MS methods similar to the one presented here, primarily due to the much quicker analytical method development.

## 5. Conclusions

Switching from GC to LC–API-MS–MS for analysis of serum/plasma samples from these pharmacokinetic studies reduced the time required from 2–4 weeks for each compound to an average of two compounds per week. In addition, the use of various tandem mass spectrometric scanning methods [6] allowed us to search for and identify metabolites of the candidate compounds. Knowledge about the sites of metabolism allowed chemical research to synthesize new molecules with protected metabolic sites, thereby improving the pharmaockinetic stability of the later compounds in this series. Thus, LC–API-MS–MS not only increased the rate at which candidate compounds could be evaluated in a pharmacokinetic screening program, but its additional qualitative capabilities significantly improved the type of pharmacokinetic data which could be provided to our collaborators in tumor biology and chemical research.

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