

# An LC/MS Assay for Analysis of Vorinostat in Rat Serum and Urine: Application to a Pre-Clinical Pharmacokinetic Study

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## Abstract

The utility of currently reported liquid chromatography mass spectrometry methods for quantitation of vorinostat in plasma or serum may be limited. These methods employ time consuming and expensive procedures such as solid phase extraction or limited-access techniques such as high turbulence liquid chromatography coupled with column switching. This study offers a simple isocratic LC/MS method validated for determination of vorinostat in both rat serum and urine. Proteins were precipitated from serum using ice-cold acetonitrile and daidzein was used as internal standard. Separation was achieved using a Phenomenex® Luna® C<sub>18</sub> (2) (5 µm, 250 x 4.60 mm) column and isocratic elution with a mobile phase consisting of acetonitrile, water, and formic acid (30:70:0.1, v/v/v). Mass spectrometry detection using electrospray positive-mode ionization with selected ion monitoring detection was employed. The calibration curves were linear ranging from 0.05 to 50 µg/ml in serum and from 0.5 to 25 µg/ml in urine. In both biological matrices, assay precision was <15% (RSD) and the intra- and inter-run bias were within acceptable range (-15% to 15%). No degradation of vorinostat in either matrix was found following three freeze-thaw cycles and 24 h storage in the auto-injector. The lower limit of quantitation in rat serum and urine were 0.05 and 0.5 µg/ml, respectively. The upper limit of quantitation in rat serum and urine were 50 and 25 µg/ml, respectively. This assay was applied successfully to a pre-clinical study of vorinostat pharmacokinetics in rats.

**Keywords:** LC/MS; Vorinostat; Pharmacokinetics; Rats; Histone deacetylase

**Abbreviations:** LC/MS: liquid chromatography/mass spectrometry; SIM: single ion monitoring; LOQ: limit of quantitation; HDAC: histone deacetylase; m/z: mass to charge ratio; RSD: relative standard deviation; SD: standard deviation; SEM: standard error of mean; QC: quality control; PEG400: polyethylene glycol 400; MC: methylcellulose; PAR: peak area ratio; CDL: curved desolvation line; MRM: multiple reaction monitoring; IS: internal standard

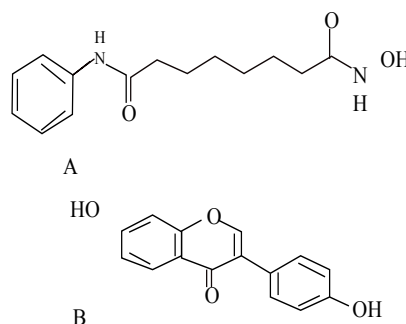
## Introduction

Histone deacetylase (HDAC) is an enzyme that removes acetyl groups from lysine residues of proteins, including histones and transcription factors [1]. Certain cancers overexpress HDACs resulting in over-compaction of the histone-DNA complex and repression of gene transcription for an array of genes including those for cell-cycle control, apoptosis, and tumor suppression [2]. HDAC inhibitors are currently under clinical evaluation for chemotherapeutic monotherapy [3,4] and as an adjunct treatment with existing agents [5,6].

The HDAC inhibitor, suberoylanilide hydroxamic acid, ((N-hydroxy-N'-phenyl-octanediamide), SAHA, vorinostat, Figure 1A), was originally developed for its anti-neoplastic effects and was approved by US FDA for the treatment of cutaneous T cell lymphoma (CTCL) in October 2006 [7,8]. Vorinostat is marketed by Merck & Co., Inc. as an oral capsule under the brand name Zolinza® [2].

In the biomedical literature, a small number of liquid chromatography mass spectrometry (LC/MS) methods for quantitation of vorinostat in plasma or serum are reported. To our knowledge, only one validated LC/MS/MS method of vorinostat in both human serum and urine is reported [9], however, it utilizes high turbulence liquid chromatography coupled with limited-access column switching technol-

ogy. Other methods including that by Kelly et al. [10] have reported an LC/MS method for determination of vorinostat in human plasma [10]. In this method, solid phase extraction (SPE) of human plasma was performed which is time consuming and expensive. In addition, use of human plasma has been shown to cause stability problems of vorinostat [9]. Many of the other reported methods of vorinostat analysis



**Figure 1:** Structures of (A) vorinostat and (B) the internal standard, daidzein.

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utilized tandem mass spectrometry detection, which may limit their utility as access to a MS/MS system is often limited [11,12]. Additionally, to our knowledge, few methods for analysis of vorinostat in rat serum or plasma are reported in the literature. In one method, analysis of vorinostat in rat plasma was conducted using LC-MS/MS with electrospray ionization with lower and upper limits of quantitation (LOQ) of 1 ng/ml and 2000 ng/ml, respectively [13]. Another LC/MS analysis of vorinostat in rat serum using multiple reaction monitoring (MRM) detection mode was developed with lower and upper LOQ of 5 ng/ml and 5000 ng/ml, respectively [14]. Both methods employed gradient elution and were validated for determination of vorinostat in rat serum only with relatively low upper limit LOQ.

Development of parenteral formulations of vorinostat has been hindered by its low water solubility, approximately 0.2 mg/ml, [15]. Also, pharmacokinetics of vorinostat are not optimal [2,13,14,16]. Because of this, new formulations of vorinostat for oral and intravenous administration are in development in our laboratory to improve the solubility and pharmacokinetics of vorinostat. These formulations can drastically improve solubility of hydrophobic compounds thereby greatly altering their pharmacokinetics. Appropriately, preliminary pharmacokinetic studies of these formulations indicated serum levels of vorinostat were much higher than the upper LOQ of the reported assays. Therefore, a simple isocratic LC/MS assay was developed and validated over a concentration range with lower and upper LOQ facilitating quantitation of vorinostat in both rat serum and urine following oral and intravenous administrations. In addition, the application of this LC/MS to a pre-clinical study of vorinostat pharmacokinetics in rats was investigated and findings were compared to previously reported data.

## Experimental

### Chemicals and reagents

Vorinostat and daidzein were purchased from LC laboratories (Woburn, MA, USA). HPLC-grade acetonitrile, water, and analytical-grade formic acid and polyethylene glycol 400 were purchased from J.T. Baker (Philipsburg, NJ, USA). Methylcellulose was purchased from Sigma-Aldrich (St. Louis, MO, USA). Male Sprague-Dawley rats ( $\approx 0.29$  kg) were obtained from Simonsen Labs (Gilroy, CA, USA). Ethics approval for animal experiments was obtained from the Institutional Animal Care and Use Committee of Washington State University.

### Chromatographic system and conditions

A liquid chromatographic-electrospray ionization-mass spectrometry (LC-ESI-MS) system was employed. A Shimadzu LCMS-2010 EV liquid chromatograph mass spectrometer system (Kyoto, Japan) connected to the LC portion consisting of two LC-10AD pumps, a SIL-10AD VP auto injector, a SPD-10A VP UV detector, and a SCL-10A VP system controller was used. Data analysis was accomplished using Shimadzu LCMS Solutions Version 3 software. The mass spectrometer conditions consisted of a curved desolvation line (CDL) temperature of 200°C and a block temperature of 200°C. The detector voltage was 1.5 kV. Vacuum was maintained by an Edwards® E2M30 rotary vacuum pump (Edwards, UK). Liquid nitrogen (Washington State University Central Stores) was used as a source of nebulizer gas (1.5 L/min). A Phenomenex® Luna® C<sub>18</sub> (2) column (250 x 4.60 mm, 5 µm) was utilized. The mobile phase consisted of acetonitrile,

water, and formic acid (30:70:0.1, v/v/v) that was filtered (0.2 µm) and degassed under reduced pressure prior to use. Separation was carried out isocratically at a flow rate of 0.6 ml/min. Vorinostat and the internal standard, daidzein (Figure 1B) were monitored in selected ion monitoring (SIM) positive mode at *m/z* 265 and 255, respectively.

### Stock and working standard solutions

Methanolic stock solutions of vorinostat (100 µg/ml) and the internal standard, daidzein (100 µg/ml) were prepared, protected from light, and stored at -20°C between uses for no longer than 3 months. Calibration standards were prepared daily from the stock solution of vorinostat by sequential dilution with either rat serum or urine as the matrix, forming a series of concentrations. For serum, the concentrations used were 0.05, 0.5, 1, 5, 10 and 50 µg/ml with three quality control concentrations namely, 0.1, 12.5 and 25 µg/ml. For urine, the concentrations used were 0.5, 1, 5, 10, 12.5 and 25 µg/ml with quality control concentrations at 0.75, 7.5 and 18.75 µg/ml.

### Sample preparation

To the working standards or samples (100 µL), 50 µL of internal standard solution (100 µg/ml) was added. One ml ice-cold acetonitrile was added to serum samples for efficient precipitation of plasma proteins. Urine samples were extracted with 0.5 ml of methanol. Samples were then vortexed for 30 s (Vortex Genie-2, VWR Scientific, West Chester, PA, USA) and centrifuged at 10,000 rpm for 5 min at room temperature (25 ± 1°C) (Beckman Microfuge centrifuge, Beckman Coulter Inc., Fullerton, CA, USA). The supernatant was transferred to new eppendorf tubes and evaporated to dryness by a stream of nitrogen gas. The residue was reconstituted with 100 µL of mobile phase, vortexed for 30 s, and centrifuged at 10,000 rpm for 5 min at room temperature (25 ± 1°C). The supernatant was transferred to HPLC vials and 10 µL was injected into the LC/MS system.

### Precision, accuracy, and recovery

The within-run precision and accuracy of six replicate assays were examined using six different concentrations of vorinostat over the concentration range 0.05-50 µg/ml in serum and 0.5-25 µg/ml in urine used to construct calibration curves in both matrices. As well, the within-run precision and accuracy were investigated for the three quality control (QC) samples, namely 0.1, 5, and 25 µg/ml in rat serum and 0.75, 7.5 and 18.75 µg/ml in rat urine. The between-run precision and accuracy of the assays were determined from the results of six replicate assays on six different days during a one-week period for both calibration and QC samples. The precision was evaluated by the relative standard deviation (RSD). The accuracy was estimated based on the mean percentage error of measured concentration to the actual concentration. Recovery (extraction efficiency) of vorinostat from rat serum and urine was assessed using three concentrations namely 0.05, 5.0, and 50.0 µg/ml in rat serum and 0.5, 5.0 and 25 in rat urine (*n* = 3) by comparing the peak area ratio (PAR) of sample normally prepared in the respective matrix as described above to the PAR of the corresponding reference sample prepared by adding a working standard solution to a processed blank sample.

### Stability of vorinostat samples

The freeze-thaw stability of vorinostat was tested using three concentrations representing low, middle and high concentrations over

the used range including the three QC samples, namely 0.05, 5.0, and 50 .0 µg/ml in rat serum and 0.5, 5.0 and 25 in rat urine. A freshly prepared stock solution of vorinostat (100 µg/ml) in methanol was used for the samples. These samples were analyzed without being initially frozen and then stored at -20 °C and thawed at room temperature (25 ± 1 °C) for three cycles.

The stability of vorinostat in normally prepared serum and urine samples during run-time in the LC/MS auto-injector was investigated ( $n = 3$ ) using samples of three concentration levels, above-mentioned for both matrices under freeze-thaw stability. Samples were prepared and injected directly after preparation, then kept in the sample rack of the auto-injector and re-injected into the LC/MS system 24 h after preparation. Finally, the PAR values of the latter were compared to the former.

### Pharmacokinetic disposition of vorinostat in rats

Male Sprague-Dawley rats (234-275 g) were obtained from Simonsen Labs (Gilroy, CA, USA) and given food (Purina Rat Chow 5001) and water ad libitum in our animal facility for at least 3 days before use. Rats were housed in temperature-controlled rooms with a 12 h light/dark cycle. The day before the pharmacokinetic experiment, the right jugular veins of the rats were catheterized with sterile silastic cannula (Dow Corning, Midland, MI, USA) under isoflurane anesthesia (Isothesia®, Butler Animal Health Supply, Dublin, OH, USA). This involved exposure of the vessel prior to cannula insertion. After cannulation, the Intramedic PE-50 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) connected to the cannula was exteriorized through the dorsal skin. The cannula was flushed with 0.9% saline. The animals were transferred to metabolic cages and fasted overnight. Animal use protocols were approved by The Institutional Animal Care and Use Committee at Washington State University, in accordance with "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985).

On the day of pharmacokinetic experiment, the previously catheterized rats as described above were dosed with vorinostat either intravenously ( $n = 3$ , 10 mg/kg) as polyethylene glycol 400 (PEG400) solution or orally ( $n=3$ , 50 mg/kg) as a suspension in 2% methylcellulose. The animals were transferred to metabolic cages and fasted overnight. Serial blood samples (≈ 0.30 ml) were collected from the cannula at 0, 1, 15 and 30 min, then 1, 2, 4, 6, 12, 24, 48, 72, 96, and 120 h after iv administration, or at 0, 15 and 30 min, then 1, 2, 4, 6, 12, 24, 48, 72, 96, and 120 h after oral administration. Each blood sample was collected into regular polypropylene microcentrifuge tubes and following centrifugation; the serum was collected and stored at -20 °C until analyzed. Urine samples were collected at 0, 2, 6, 12, 24, 48, 72, 96 and 120 h following both intravenous and oral administration and were stored at -20 °C until analyzed.

### Data analysis of rat serum and urine

Quantification was based on calibration curves obtained using the PAR of vorinostat to the internal standard, against vorinostat concentrations using unweighted least squares linear regression. Pharmacokinetic analysis was completed using data from individual rats for which the mean and standard error of the mean (SEM) were calculated for each group except for  $T_{max}$  which was represented as median and range. Pharmacokinetic parameters were estimated using

WinNonlin® using noncompartmental analysis. The apparent terminal elimination rate constant ( $K_E$ ) was estimated from the slope of the log-linear phase of declining serum concentration vs. time plot. The half-life ( $t_{1/2}$ ) was calculated using the following equation:  $t_{1/2} = 0.693/K_E$ . The area under the concentration time curve ( $AUC_{0-Clast}$ ) was calculated using the linear/logarithmic trapezoidal method. Summation of ( $AUC_{0-Clast}$ ) and the concentration at the last measured point divided by  $K_E$  yielded  $AUC_{0-\infty}$ . Mean residence time (MRT) was calculated by dividing  $AUMC_{0-\infty}$  by  $AUC_{0-\infty}$ , and clearance (CL) by dividing dose by  $AUC_{0-\infty}$ . The fraction excreted in urine unchanged ( $F_e$ ) was calculated by dividing the total cumulative amount of vorinostat excreted in urine ( $\Sigma Xu$ ) by the dose, renal clearance ( $CL_{renal}$ ) by multiplying  $F_e$  by CL, and non-renal clearance ( $CL_{NR}$ ) by subtracting  $CL_{renal}$  from CL. The  $C_{max}$  and corresponding  $T_{max}$  were obtained directly from the observed data. Absolute bioavailability (F) was determined by dividing  $AUC_{0-\infty, oral}$  by  $AUC_{0-\infty, IV}$  with dose adjustment.

## Results and Discussion

### Chromatography

Separation of vorinostat and daidzein (IS) in rat serum and urine was successfully achieved. No interfering peaks were co-eluted with the investigated compounds (Figure 2). The retention times of vorinostat and daidzein were approximately 10.5 and 17 min, respectively.

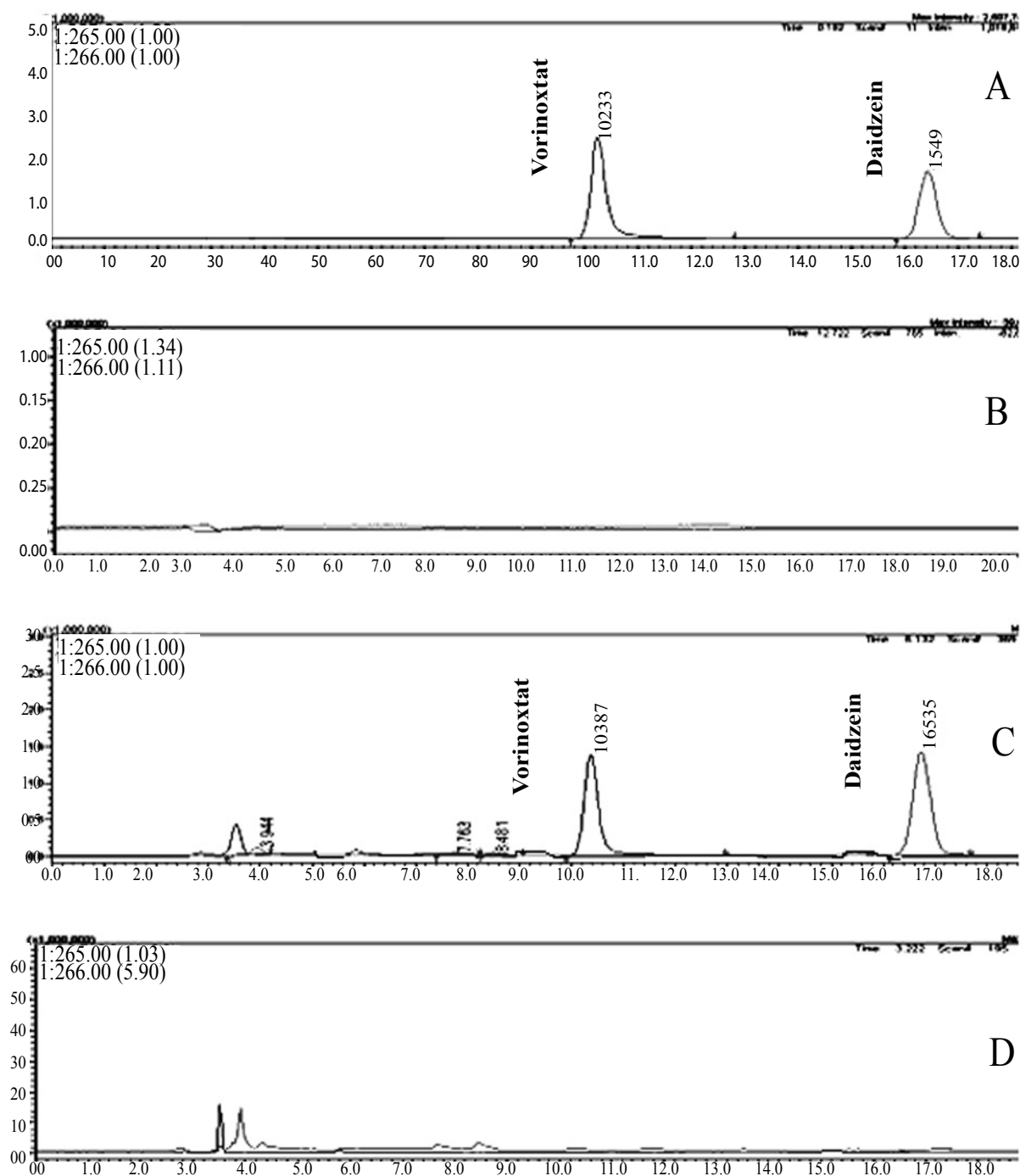
The performance of the LC/MS assay was evaluated according to the following parameters: peak shape and purity, interference from endogenous substances in serum or urine, linearity, limit of quantitation (LOQ), freeze-thaw stability, stability of reconstituted extracts, precision and accuracy. Various compositions of the mobile phase were tried for the best resolution of the drug and internal standard. The optimal separation was obtained with the use of a mobile phase consisting of acetonitrile, water and formic acid (30:70:0.1v/v/v) and a flow rate 0.6 ml/min. Acceptance criteria were assessed in accordance with US FDA bioanalytical method validation guidelines [17].

### Linearity and limit of quantitation (LOQ)

Excellent linear relationships were found between the PAR of vorinostat to the internal standard and the corresponding concentrations of vorinostat in both serum ( $r^2 \geq 0.999$ ) over a range of 0.05–50 µg/ml and urine ( $r^2 \geq 0.998$ ) over a range of 0.5-25 µg/ml. The mean regression lines from the validation runs were described by vorinostat (µg/ml) =  $0.0280 \pm 0.00015x + 0.0032 \pm 0.00020$  for serum and =  $0.0310 \pm 0.000067x + 0.0036 \pm 0.000046$  for urine. The lower LOQ of this assay was 0.05 µg/ml in rat serum and 0.5 µg/ml in rat urine with the respective between-day relative standard deviation of 12.91% and 5.41%; and respective between-day bias of -8.31% and -14.35% for vorinostat. The upper LOQ of this assay was 50 µg/ml in rat serum and 25 µg/ml in rat urine with the respective between-day relative standard deviation of 0.13% and 0.78%; and respective between-day bias of 0.09% and - 0.47% for vorinostat. The back-calculated concentrations were within the acceptance criteria.

### Precision, accuracy and recovery

The within- and between-run precision (RSD) calculated during replicate assays ( $n = 6$ ) of vorinostat in rat serum and urine was <15% over a wide range of concentrations (Table 1). The intra- and inter-run bias determined during the replicate assays for vorinostat was within



**Figure 2:** Representative chromatograms of: (A) rat serum containing the internal standard, daidzein, and vorinostat at a concentration of 50 µg/ml.; (B) blank rat serum showing no interfering peaks co-eluted with the compounds of interest; (C) rat urine containing the internal standard, daidzein, and vorinostat at a concentration of 25 µg/ml; and (D) blank rat urine showing no interfering peaks co-eluted with the compounds of interest.

the acceptable range: -15% to 15% (Table 1). The recovery (extraction efficiency) of vorinostat was assessed over the three selected samples representing the low, middle and high concentrations of the working range in both serum (0.05, 5, 50 µg/ml,  $n = 3$ ) and urine (0.5, 5, 25 µg/ml,  $n = 3$ ). The recovery values were  $\geq 99.83\%$  in serum and  $\geq 97.45\%$  in urine, (Table 2). These data indicate that the developed LC/MS method is reproducible and accurate.

### Stability of vorinostat samples

The stability of vorinostat following three freeze-thaw cycles and storage in the auto-injector at room temperature ( $25 \pm 1^\circ\text{C}$ ) for 24 h are reported in (Table 2). The percentage recovery of vorinostat was  $\geq 97.56\%$  in rat serum and  $\geq 97.48\%$  in rat urine following three freeze-thaw cycles indicating no degradation of this compound in either

| Biological Matrix | Added (µg/ml) | Vorinostat concentration (µg/ml) |             |            |             |            |             |
|-------------------|---------------|----------------------------------|-------------|------------|-------------|------------|-------------|
|                   |               | Observed (mean)                  |             | RSD(%)     |             | Bias (%)   |             |
|                   |               | Within-day                       | Between-day | Within-day | Between-day | Within-day | Between-day |
| Serum             | 0.05          | 0.051                            | 0.046       | 14.28      | 12.91       | -3.31      | -8.31       |
|                   | 0.5           | 0.43                             | 0.46        | 6.17       | 14.81       | 14.40      | -8.66       |
|                   | 1             | 0.93                             | 0.99        | 1.86       | 6.22        | -7.30      | -0.84       |
|                   | 5             | 5.37                             | 5.13        | 2.73       | 3.50        | 7.3        | 2.63        |
|                   | 10            | 9.71                             | 9.94        | 1.55       | 2.69        | -2.90      | -0.65       |
|                   | 50            | 50.22                            | 50.05       | 0.290      | 0.13        | 0.44       | 0.09        |
| Urine             | 0.5           | 0.46                             | 0.43        | 10.88      | 5.41        | -8.28      | -14.35      |
|                   | 1             | 0.96                             | 0.91        | 5.32       | 9.70        | -4.49      | -9.13       |
|                   | 5             | 5.64                             | 5.08        | 13.65      | 6.08        | 12.72      | 1.63        |
|                   | 10            | 10.47                            | 9.98        | 2.14       | 4.72        | 4.72       | -0.18       |
|                   | 12.5          | 12.94                            | 12.69       | 1.01       | 3.39        | 3.56       | 1.52        |
|                   | 25            | 25.83                            | 24.88       | 0.84       | 0.78        | 3.32       | -0.47       |

**Table 1:** Within- and between-day precision and accuracy of the assay for vorinostat in rat serum and urine ( $n = 6$ , mean, RSD and bias).

| Biological Matrix | Vorinostat concentration (µg/ml) | %Recovery (Extraction efficiency) | %Recovery (Freeze-Thaw stability) | %Recovery (Auto-injector stability) |
|-------------------|----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|
| Serum             | 0.05                             | 99.83 ± 2.74                      | 99.10 ± 4.55                      | 98.74 ± 3.58                        |
|                   | 5                                | 100.13 ± 1.37                     | 97.56 ± 2.08                      | 99.83 ± 7.29                        |
|                   | 50                               | 101.37 ± 3.32                     | 99.70 ± 3.89                      | 100.50 ± 7.47                       |
| Urine             | 0.5                              | 99.92 ± 3.61                      | 97.48 ± 3.17                      | 99.43 ± 5.19                        |
|                   | 5                                | 97.45 ± 0.10                      | 100.21 ± 1.80                     | 99.87 ± 4.43                        |
|                   | 25                               | 100.22 ± 3.28                     | 98.02 ± 1.23                      | 99.05 ± 4.89                        |

**Table 2:** Recovery and stability of vorinostat in rat serum and urine ( $n = 3$ , mean, and SD).

matrix. The percentage recovery of vorinostat was  $\geq 98.74\%$  in serum and  $\geq 99.05\%$  in urine after 24 h storage in the auto-injector at room temperature ( $25 \pm 1^\circ\text{C}$ ) confirming the stability of this compound in both matrices under these conditions.

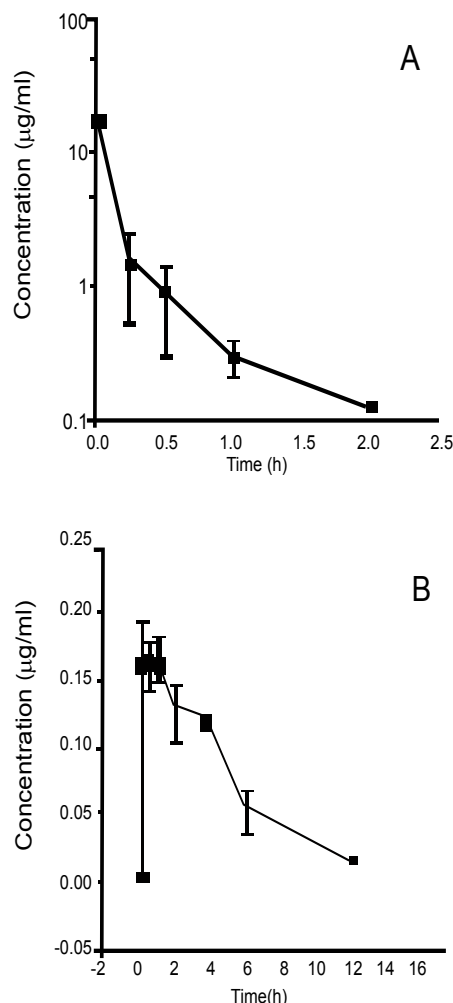
### Pharmacokinetics of vorinostat in rats

The LC/MS method was successfully applied to the determination of vorinostat in a pre-clinical pharmacokinetic study in male Sprague-Dawley rats ( $n = 3$  for each treatment group). Following administration of vorinostat either intravenously (10 mg/kg) or orally (50 mg/kg), the disposition in serum was investigated (Figure 3). The serum concentration versus time profiles observed for vorinostat in PEG400 shows that vorinostat was rapidly cleared from the body and was detected only up to 2 h post-dosing, (Figure 3A). Vorinostat in PEG400 after intravenous administration demonstrated a short half-life ( $t_{1/2} = 0.57 \pm 0.05$  h), low area under curve ( $AUC_{0-\infty} = 2.46 \pm 1.61$  µg.h/ml), short mean residence time ( $MRT = 0.54 \pm 0.13$  h) and a rapid serum clearance ( $CL = 8.93 \pm 4.02$  L/h/kg). Similar results of vorinostat in rat serum have been reported following intravenous administration at a dose of 2 mg/kg [14].

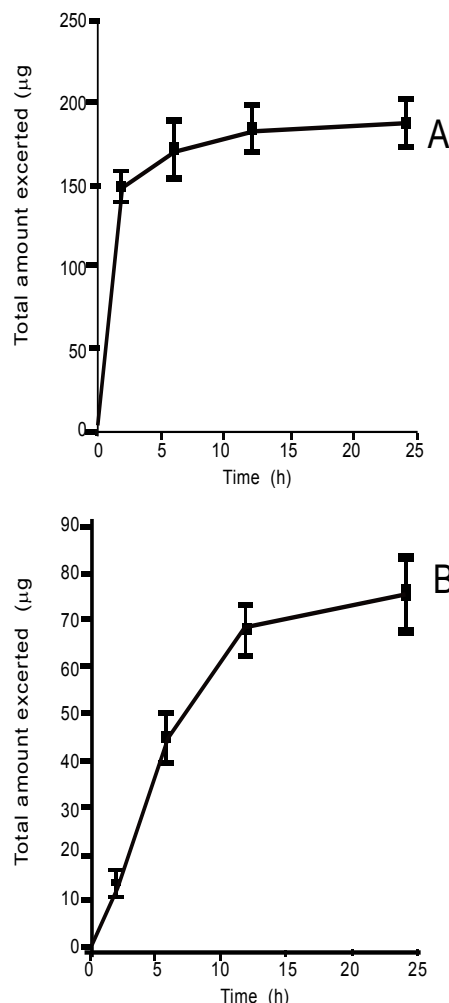
Following oral administration at 50 mg/kg, vorinostat in 2% methylcellulose (MC) suspension was detected in serum up to 12 h, (Figure 3B). The serum concentrations of vorinostat given orally appeared to decline with a mean serum elimination half-life ( $t_{1/2}$ ) of  $3.22 \pm 0.55$  h. The area under the curve, representing the total amount of drug

exposure in the serum over time, was  $0.99 \pm 0.12$  h.µg/ml. The absorption was fast as indicated by a short time to maximum concentration ( $T_{\max} = 0.25$  as median with a range of 0.75) and a concentration maximum ( $C_{\max}$ ) of  $0.21 \pm 0.03$  µg/ml. However, the absolute bioavailability was low ( $7.03 \pm 0.63\%$ ). Previous pharmacokinetic studies also report a short  $T_{\max}$  ( $0.17 \pm 0.0$  h) and low oral bioavailability (6.9%) of vorinostat following oral administration of (5 mg/kg) to Wistar rats [13]. In another study, such short  $T_{\max}$  ( $0.3 \pm 0.1$  h) and low oral bioavailability (11%) of vorinostat following oral (10 mg/kg) dosing of rats were also documented [14]. The authors suggested this was related to a high first-pass effect and predominant hepatic biotransformation. Herein, the total oral clearance of vorinostat ( $CL/F$ ) was determined to be  $52.09 \pm 6.30$  L/h/kg, which was predominated by non-renal clearance ( $CL_{\text{NR}}/F = 51.80 \pm 6.28$  L/h/kg) with only minor renal clearance ( $CL_{\text{renal}}/F = 0.29 \pm 0.03$  L/h/kg).

Following intravenous administration, the total amount excreted in urine plot (Figure 4) illustrates that only a fraction of the vorinostat dose was excreted in the urine following both intravenous and oral administration. This small amount of vorinostat was excreted in urine by 72 h following intravenous administration and 24 h following oral administration (Figure 4A, 4B, respectively). The fraction excreted unchanged ( $F_e$ ) of vorinostat was  $7.54 \pm 1.68\%$  from PEG400 and  $0.56 \pm 0.02\%$  from 2% MC suspension. Both the amounts of vorinostat excreted unchanged in urine and fraction excreted unchanged ( $F_e$ ) following oral administration were lower than those experienced after



**Figure 3:** Concentration-time profile of vorinostat in rat serum ( $n = 3$ ) following (A) intravenous (10 mg/kg) and (B) oral (50 mg/kg) administrations (means  $\pm$  SEM).



**Figure 4:** Total amount excreted plot of vorinostat in rat urine ( $n = 3$ ) following (A) intravenous (10 mg/kg) and (B) oral (50 mg/kg) administrations (means  $\pm$  SEM).

intravenous dosing indicating that the hepatic metabolism is a major contributor of vorinostat elimination. This agrees with the previously reported findings of predominant hepatic biotransformation of vorinostat [14].

## Conclusion

The developed LC/MS method for vorinostat is sensitive, reproducible, specific, linear over a suitable concentration range, precise and accurate. No degradation of vorinostat in rat serum and urine was detected following three freeze-thaw cycles and storage in the auto-injector at room temperature for 24 h. In addition, this assay method has been applied successfully in a pre-clinical study of the pharmacokinetics of vorinostat in rat serum and urine after intravenous and oral administration and the results were in agreement with previously reported data. Using this method, it is possible to characterize and evaluate newly developed drug delivery systems of vorinostat intended for oral and intravenous administration with improved serum and urine disposition.

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