

## Overview

- A PK Calculator module was developed in Gubbs Mass Spec Utilities (GMSU). The purpose of the software is to allow the user to review LC/MS/MS raw data and calculate final Pharmacokinetic parameters in one easy to use package.
- Since the raw data review and PK calculations are done in the same software in GMSU PK Calculator, the need for the user to export/import data into a secondary PK calculation software is eliminated. In GMSU PK Calculator, the PK calculations are up-dated in "real time" as chromatographic peak integrations or calibration settings are changed.
- 10 Novartis NCE's were dosed orally and intravenous to rats and plasma concentrations were determined.
- The LC/MS/MS raw data was acquired on a TSQ Quantum Ultra with Xcalibur 1.4.2 and Quick Quan 2.0.
- The GMSU PK results were compared with WinNonlin 4.0.1 and Watson 7.0.
- The GMSU calculated PK values of AUC,  $t_{1/2}$  and Clearance shows good correlations with the results from WinNonlin and Watson.

## Introduction

- The advances in HT-bioanalytics have greatly reduced the sample turn around times in bioanalysis. The effort in reducing the sample analysis time has now shifted the bottleneck from sample analysis to data reviewing and data interpretation.
- We have developed and evaluated a new module of GMSU called PK Calculator designed to simplify the general LC/MS/MS raw data and PK result calculation and reviewing process in a non-GLP Discovery environment in support of early preclinical *in-vivo* PK screening.
- The purpose of the software is to allow the user to review LC/MS/MS raw data and final PK parameters in one easy to use package, without having to export LC/MS/MS results into a secondary processing software for final PK calculations.
- To verify the performance of the GMSU PK Calculator, it was evaluated against WinNonlin and Watson.

## Method

- 10 Novartis NCE's were dosed orally and intravenous to rats and plasma samples were collected.
- The samples were analyzed on a TSQ Ultra from Thermo Fisher Scientific. Xcalibur 1.4.2 and Quick Quan 2.0 was used for method development and to acquire the raw data.
- GMSU PK Calculator was used to open LC/MS/MS raw data and to review the chromatographic peak integrations and calibration settings.
- After the PK study designs were assigned, non-compartmental PK calculations were performed "on the fly". The results is graphically displayed in a plasma concentration vs time plot and in a semi logarithmic plot.
  - CL,  $t_{1/2}$  and AUC were calculated.
  - In the AUC calculations for the IV dose, the concentration value at  $t = 0$ hr was set to being equal to the concentration at  $t = 0.083$ hr.
- The PK parameters in GMSU are defined as:

$$t_{1/2} = 0.693/k$$

$$AUC = \text{trapezoidal method } [b(c+a)/2]$$

$$C_{max} = \text{set to highest conc. value}$$

$$\text{Clearance} = \text{Dose}/AUC$$

- The sample concentrations from the data set were imported into WinNonlin 4.0.1 as Excel files and non-compartmental PK analysis was performed.
  - CL,  $t_{1/2}$  and AUC were calculated.
  - In the AUC calculations for the IV dose, the concentration value at  $t = 0$ hr was set to being equal to the concentration at  $t = 0.083$ hr.
- The peak area values from the data set were imported into Watson 7.0 as Xcalibur Excel results files. The analytical regressions and PK calculations were performed in Watson.
  - CL,  $t_{1/2}$  and AUC were calculated.
  - In the AUC calculations for the IV dose, the concentration value at  $t = 0$ hr was extrapolated from the plasma profile.

## Results

- User friendly LC/MS/MS raw data reviewing in GMSU allows the user to quickly get an overview of the results.



Fig 1. Chromatographic view where the user can adjust peak integration, smoothing and exclude calibration points.



Fig 2. Calibration view where the user can adjust curve fit, weighting, standard concentration value and toggle the use of internal standard.

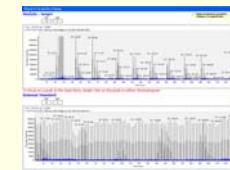


Fig 3. HT Acquisition Display gives the user and overview of the results.

- PK parameters are calculated in GMSU eliminating the need to export/import raw data into a secondary software package such as Watson or WinNonlin.



Fig 4. Assign study design. The user can define dose form, time points, species and animal number.



Fig 5. PK calculator. Mean concentration values are used for all calculations and the user defines what time points to use for AUC, CL and  $t_{1/2}$ . Any change in chromatographic peak integration or calibration settings are up-dated in real time in the PK results view.

- The values of AUC,  $t_{1/2}$  and Clearance from the GMSU PK Calculator were compared to WinNonlin and Watson. The GMSU results showed good correlation with the results from WinNonlin. The results did not correlate equally well with the results from Watson, but this can be explained by a difference in how the concentration data was treated.

	Statistics IV dose		Statistics PO dose	
	Mean AUC	% Stdev AUC	Mean AUC	% Stdev AUC
Cpd 1	1394	2.5	1313	1.3
Cpd 2	1089	0.5	1492	0.1
Cpd 3	321	0.1	31	7.3
Cpd 4	473	0.7	433	1.4
Cpd 5	125	2.3	BLOOQ	BLOOQ
Cpd 6	1359	1.2	1554	0.8
Cpd 7	350	0.0	342	4.0
Cpd 8	1604	0.3	87	1.2
Cpd 9	294	0.0	530	0.2
Cpd 10	366	0.1	633	0.2

Fig 6. Comparison of AUC values from GMSU PK Calculator and WinNonlin. The mean percent standard deviation between the two software's were 1.3%, showing good correlation. The variability increased when the results from GMSU and WinNonlin were compared to the results from Watson. The reason for the difference in AUC values can be explained by how the plasma concentration at  $t_0$  hr was treated. In the GMSU and WinNonlin calculations the concentration at  $t_0$  hr was set to being equal to the concentration at the first collected time point ( $t_{0.083}$  hr) for IV dose and zero for PO dose. Whereas, for the Watson calculations the extrapolated concentration value for  $t_0$  hr was used for the IV dose. Also, mean concentration values were used in the GMSU and WinNonlin calculations. Individual concentration values for each animal was used for the calculations in Watson.

The half life  $t_{1/2}$  calculations were also compared between the three different systems and the results showed good correlation between GMSU, WinNonlin and Watson.

## Conclusions

- GMSU PK Calculator is an easy to use tool that drastically decreases the time needed to evaluate LC/MS/MS raw data and calculate PK results.
- The raw data review and PK calculations are done in the same software eliminating the need to export/import data into a secondary PK calculation software. In GMSU the PK calculations are up-dated in "real time" as chromatographic peak integrations or calibration settings are changed.
- We have demonstrated that the GMSU PK calculator performs as well as WinNonlin and Watson in an early drug discovery setting in support of early preclinical *in-vivo* PK screening.
- Future versions of the GMSU PK Calculator will include the capability to extrapolate the concentration value at  $t_0$  hr for the IV AUC determination.