Published in final edited form as:

Curr Protoc Chem Biol. 2012 December 1; 4(4): 299–309. doi:10.1002/9780470559277.ch120072.

In Vivo Rapid Assessment of Compound Exposure (RACE) for Profiling the Pharmacokinetics of Novel Chemical Probes

Danielle McAnally^{1,2}, Michael Vicchiarelli¹, Khandaker Siddiquee², and Layton H. Smith^{1,2}

¹Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute at Lake Nona 6400 Sanger Rd Orlando FL 32827.

²Cardiovascular Pathobiology Program, Orlando Florida.

Abstract

The RACE assay is an easy and efficient method for estimating the exposure of novel chemical probe compounds in mice. RACE is a truncated and compressed version of a traditional comprehensive in vivo pharmacokinetics study. The method uses a single standard formulation, dose, route of administration, and a small cohort of mice (n=4). Standardized protocols and an abbreviated sample collection scheme reduce the labor needed to perform both the in life and bioanalytical phases of the study. The procedure reduces the complexity of data analysis by eliminating all but one calculated pharmacokinetic parameter; estimated exposure (eAUC₂₀₋₁₂₀), a parameter that is sufficient to rank order compounds based on exposure, but is also easily determined by most software using the simple trapezoidal rule. The RACE assay protocol is readily applicable to early/exploratory studies of most compounds, and is intended to be employed by laboratories with limited expertise in pharmacology and pharmacokinetics.

Keywords

Pharmacokinetics; exposure; rapid; in vivo; high-throughput

INTRODUCTION

This unit describes a procedure for the efficient assessment of compound exposure when administered to a small cohort of mice, and an abbreviated sample collection scheme. The procedure can be readily adapted to determine dose linearity and tissue distribution studies.

The recent expansion in the accessibility of high-throughput screening and related technologies for chemical biology has yielded a wealth of new tools suitable to probe the function of individual proteins and pathways in a variety of contexts. As this trend continues, it is likely that many more probe compounds will be identified. Consequently there will be an explosion in both the availability and the subsequent application of novel small molecule tool compounds to address important questions of protein function in both normal and disease states. Some of these probes undoubtedly will be used in vivo, making an understanding of the pharmacokinetic (PK) profiles of these novel compounds critical, particularly in the light of applying them to different disease models such as transgenic or surgically modified mice, or other model organisms. It is important for investigators who wish to utilize these new tools to know the basic exposure levels that can be anticipated in

Correspondence to: Layton H. Smith.

dmcanally@sanfordburnham.orgmvicchiarelli@sanfordburnham.orgksiddiquee@sanfordburnham.orglhsmith@sanfordburnham.org.

their preferred animal disease model. Because the mouse is one of the most common animal models of disease, and is readily accessible to most research laboratories, here we present a procedure for the efficient assessment of compound exposure using a small cohort of mice and an abbreviated sample collection scheme.

Despite significant advances in the automation and throughput of *in vitro* assays of compound absorption, distribution, metabolism, excretion and toxicity (ADME/T), *in vivo* PK studies are still performed in a low-throughput manner. In addition, there are few labs outside of the pharmaceutical, biotech or contract research industries with the knowledge and capabilities to execute these studies. Therefore there is a need to establish and validate rapid high throughput *in vivo* PK study methods that can be operationally accessible to most research labs. The basic concept behind RACE was to produce a simple protocol that 1) did not require significant expertise in drug metabolism and pharmacokinetics studies; 2) used reagents and instruments common to most biology labs or that were easily purchased at a reasonable cost; and 3) utilized as little compound as possible. Key features of the RACE are the use of simplified and standardized protocols for the formulation and administration of the compound, the collection of fewer blood samples and the analysis of the samples, as well as a reduced number of animals. These features address several of the bottlenecks that decrease the throughput of traditional pharmacokinetic studies, and address existing barriers to their utility in academic labs environments. Ultimately the RACE approach simplifies and streamlines the process resulting in greater efficiency for the project experiments, and cost savings.

STRATEGIC PLANNING

Institutional animal care and use committee

Prior to the performance of the RACE (or any other experiment utilizing vertebrate animals) it is incumbent upon the Investigator to obtain the appropriate institutional approvals. For most Investigators, approval is obtained from the Institutional Animal Care and Use Committee (IACUC). The protocol described herein is approved by the Sanford Burnham Medical Research Institute at Lake Nona IACUC. It is designed to reduce the number of live animals used in PK studies, and makes use of techniques that minimize the potential for pain and discomfort to the mice in the study.

Vehicle/formulation selection

In a traditional *in vivo* PK protocol designed to support a drug discovery effort, the formulation of the compound for testing *in vivo* is based upon the outcome of several experiments each designed to select the best formulation for the compound based on its physiochemical properties, potential therapeutic application, and desired route and frequency of administration (Lee et al., 2003). In contrast, experiments using a chemical probe as a tool to test a hypothesis require only that the compound reach a level of exposure sufficient to exert the intended pharmacodynamic effect. More often than not, the probe compound is administered intraperitoneally, at the highest possible dose to elicit a specific effect while avoiding toxicities. With these experimental designs in mind, we have streamlined the formulation process for RACE by using a standard vehicle for all compounds tested. All compounds are formulated in a vehicle consisting of DMSO, Tween-80 and sterile water at a ratio of 10:10:80. In our experience, >90% of the compounds submitted are sufficiently soluble in this common vehicle. Compounds that require more extensive formulation should be considered unsuitable for this protocol.

Dose selection and route of administration

The dose selected for the proposed RACE assay will vary depending upon the goals of the experiment, the physicochemical properties of the compound itself, and the intended experimental outcome. Investigators who wish to study the pharmacokinetics of a compound for the first time should choose a dose that is suitably low to avoid unintended side effects, overt toxicity and nonspecific pharmacodynamic effects, while maintaining sufficient plasma levels to ensure accurate quantification of the compound in the plasma compartment by analytical methods such as LC/MS. It is suggested that initial studies utilize a dose range of 1 – 2 mg/kg. This dose range is based upon our own experience performing numerous assays in support of early discovery projects, and in consideration of the detection capabilities of most analytical instruments. Ultimately it is up to the PI to determine the appropriate dose to be tested. These suggestions are included here as a starting point.

Introduction

The protocol presented below details the methods and steps for the rapid, cost effective evaluation of compound exposure *in vivo*. Compound is administered to three mice, followed by blood collection at two time points (20 and 120mins). Whole blood samples are centrifuged and the plasma collected for analysis of compound concentration by LC/MS/MS. Depending upon the aim of the study and the number of compounds, doses, and routes of administration the entire *in vivo* portion of the procedure can be achieved within 3 hours (**Table 2**).

The relative ease and rapidity of the RACE assay will allow for the simultaneous study of several doses of compound. Such studies will be useful in determining the ratio of compound exposure to dose administered. Ideally there will be a linear relationship between the amount of compound administered, (i.e. the dose), and the exposure (i.e. the area under the curve (AUC)). A linear dose exposure relationship allows for the predictive outcome of pharmacodynamic effect based upon the amount of compound administered (**Table 3**).

Another alternative application of the RACE protocol is the determination of target tissue distribution. The protocol can be modified to include the collection of tissues (such as brain, liver, heart, skeletal muscle etc.) after the initial dose. By combining plasma and tissue drug level data, an Investigator can establish a pharmacokinetic/pharmacodynamic relationship. There are a few special considerations involved in utilizing the RACE assay for tissue distribution studies. The first is to determine the tissue distribution of interest and to consider the rate by which blood is delivered to that tissue. Tissues that comprise the circulatory system (including the heart and vasculature) are likely to be exposed to high levels of compound early in the study. Studies seeking to determine the tissue distribution from the plasma compartment to tissues of the cardiovascular system should collect tissues at earlier time points than the proposed 120 min. time point. Similar strategies can be applied to tissues with rapid and extensive rates of perfusion such as the brain. In contrast, studies seeking to determine the distribution of a compound from the plasma compartment to tissues of lesser rates of perfusion, such as adipose tissue, should consider collecting plasma and tissue at a later time point. Accordingly the RACE protocol timeframe should be extended to reflect the increased time necessary to permeate the tissue of interest.

Also of importance is the method's ability to provide a high throughput approach. Given that all compounds are prepared in the same vehicle formulation, as long as the route of administration remains the same across compounds there is only a need for one mouse to be dosed with the vehicle per day, further saving time and money. The protocol remains unchanged with the focus being shifted to the time at which each mouse receives an initial

dose of compound, followed by two blood collections at 20min and 120min post dose. The completion of seven compounds in one day is illustrated in **Table 4**.

Materials

- Sterile DMSO – Biotechnology Performance Certified Sigma Cat# D2438 Sigma-Aldrich (St. Louis, MO) Purity (USP) – 99.9% Storage – Room Temp Stability – 3 month after opening
- Sterile TWEEN80 – Sigma Cat# P4780-100mL Sigma-Aldrich (St. Louis, MO) Purity/Grade – Cell Culture Grade Storage – Room Temp Stability – 3 month after opening
- Sterile water – Double Processed Sigma Cat# W3500 Sigma-Aldrich (St. Louis, MO) Purity/Grade – Cell Culture Grade Storage – Room Temp Stability – 3 month after opening
- Sterile Goldenrod 5.0mm lancets – Medipoint Cat# Goldenrod5 Medipoint, Inc. (Mineola, NY) Storage – Room Temp
- Sterile EDTA blood collection tubes – Sarstedt Cat# 16.444.100 Sarstedt (Numbrecht, Germany) Storage – Room Temp
- Sterile 1mL syringe with 23 gauge – BD Cat# 305145 sterile needle (IP or SQ administration) Becton Dickinson (Franklin Lakes, NJ) Storage – Room Temp
- Acetonitrile - Sigma Cat #34967-4L Sigma-Aldrich (St. Louis, MO) Purity/Grade – LC-MS Chromasolv Storage – Room Temp Stability – 3 month after opening
- Indomethacin - Sigma Cat #I7378-10G Sigma-Aldrich (St. Louis, MO) Purity/Grade – 99.9% (TLC) Storage – Room Temp Stability – >12 month after opening
- Control Mouse Plasma - Bioreclamation Cat# MSEPLEDTA3-C57 Bioreclamation, LLC (Long Island, NY) Storage – -80°C Stability – 12 month after aliquot and storage in freezer
- Balance – Mettler Toledo XP205 Balance Mettler Toledo (Columbus, OH)
- Centrifuge - Eppendorf 5617R Centrifuge Operated at 14k rpm @ 4°C Eppendorf (Hamburg, Germany)
- API 4000 LC/MS/MS - AB Sciex (Framingham, MA)
- Acuity UPLC - Waters Corporation (Milford, MA)

Steps and Annotations

2-3 Weeks Pre Dosing

1. Determine and (order) request appropriate number mice/strain.
2. Coordinate/confirm with client dosing requirements.
3. Coordinate in vivo responsibilities with staff.
4. Confirm/order appropriate supplies.
5. Schedule/confirm vivarium laboratory room.

1-2 Days Pre Dosing

1. Verify mice number and weights within acceptable limits (23-26g).

2. Organize and assemble appropriate materials in vivarium.
3. Verify dosing route/conc., etc. with client and staff.
4. Calculate estimated dosing regimen based on mice weights.
5. If housed together give each mouse a unique identifier (ear punch, tag, etc.).

Day of Dosing

1. Prepare compound and vehicle formulation.
2. Number and weigh each mouse and determine dosage accordingly.
3. Dose each mouse via appropriate route of administration and time accordingly.
4. Blood collection from submandibular vein puncture in blood collection tubes at $t=20$ and $t=120$ minutes post dose (see schedule in **Tables 2-4** depending upon aim of study).
5. Stop bleeding by applying pressure with gauze, and store collected blood on ice.
6. Following the last blood collection, euthanize mice via an approved method that is consistent with local IACUC protocol approval and AVMA guidelines.
7. Freshly collected whole blood should be stored on wet ice, until plasma is separated from the cellular constituents by centrifugation. Centrifuge whole blood at 10,000 rpm for 10 minutes at 4°C to separate plasma. Following centrifugation, the plasma sample supernatant should appear clear, and golden in color. An obvious pellet of precipitated proteins should be observed at the bottom of the micro-tube. If foreign material(s) or other potential contaminants are observed in suspension, sample(s) should be centrifuged again for another 10 minutes.

Hemolysis (lysis of red blood cells) is a common issue when blood is collected via the mandibular vein. Some investigational compounds may cause hemolysis as well. Hemolysis is easily identified in plasma. Instead of being a clear golden color, the plasma will have a reddish brown tint as a result of the heme released from lysed red blood cells. Hemolysis will not affect the analysis of compound in the sample by LC/MS. Plasma samples that are hemolyzed should be analyzed as planned. When handling hemolyzed samples, special care should be taken to avoid contaminating the plasma sample with cellular debris.

8. Transfer plasma to appropriately labeled tubes and immediately store at -80°C until analysis.

1-4 Days Post Dosing

1. Bioanalytical sample analysis of samples via LC/MS/MS (or other suitable method).
2. Data analysis review and draft report generation.

Bioanalytical Sample Analysis

Sample extraction

Plasma samples should be extracted using a minimum precipitation reagent to plasma volume of 4:1 (v:v) in 1.7mL microcentrifuge tubes (conical bottom). A centrifugal speed is recommended to be between 10-14,000 rpm for minimum of 10 minutes to allow sufficient removal of precipitated proteins and other potential sample contaminants. Refrigeration during centrifugation is not required but recommended if the expected analyte(s) are labile. We typically use a setting of 4°C. It is not recommended to precipitate samples in 96-well

plates as this may lead to insufficient/contaminated sample supernatant due to the speed limitations for 96-well plates during centrifugation (3,700rpm).

Standard Curve

A minimum of 8 standard curve samples are prepared from control mouse plasma, spiked with defined concentrations of neat compound, and are analyzed in duplicate at the beginning of the analysis batch and the end. The upper limit of the standard curve dynamic range is typically estimated at 0.1% of the mg/kg dose and lower limit at 0.001%. For example, a compound dosed at 10mg/kg would have a standard curve dynamic range of 0.100-10.0 μ g/mL.

Control mouse plasma (20.0 μ L) is aliquotted into 1.7mL micro-centrifuge tubes followed by the additional of 200 μ L of cold internal standard (IS) spiking solution (Indomethacin, 1.00 μ g/mL in ACN). Double blank samples (control mouse plasma with no analyte or IS) receive 200 μ L of cold acetonitrile. Sample tubes are then capped and vortexed for 3 minutes at room temperature. After vortexing, samples are centrifuged at 14,000 rpm for 10 minutes at 4°C. The supernatant is then transferred from each tube into individual wells of a 96-well 2mL plate. The sample plate is then placed into the instrument autosampler, and stored at 15°C until analysis by LC/MS/MS. Control mouse plasma should be free of any interfering peaks associated with analyte(s) and IS.

LC/MS/MS tuning and method development

Due diligence regarding LC/MS/MS method development is recommended prior to sample analysis for each analyte. The following analyte dependent parameters are optimized during HPLC method development: HPLC column selection, mobile phase reagents, injection volume, autosampler wash reagents, gradient profile, column temperature, and sample diluent.

Mass spectrometry (MS) optimization, also known as tuning, is typically evaluated in both positive and negative modes using both Turbo Ion Spray (TIS) and Atmospheric Pressure Chemical Ionization (APCI) sources. Compound structure is routinely used to predict source choice (TIS vs. APCI), polarity, and fragmentation patterns. During TIS, analyte(s) in solution pass through a metal type capillary which transfers a charge to the analyte(s). During APCI, analyte(s) in solution flow through a heated nebulizer and are vaporized and a charge is applied to the analytes through a corona discharge needle. The transfer of charges causes the formation of charged ions commonly of the type [M+H], [M+2H], [M+NH₄], [M+Na] (positive mode) and/or [M-H] (negative mode). In Product Ion generation mode, the newly determined m/z undergoes Collision-induced dissociation (CID), or MS/MS fragmentation, in quadrupole 2 (Q2) and these daughter ions, or products, are detected in quadrupole 3 (Q3). Analyte specific fragments can then be identified, allowing for the application of multiple reaction monitoring (mrm) during data acquisition. MRM is essential for accurate quantification of analytes. Source parameter optimization based on flow rate includes: source temperature (typically 500°C), Curtain Gas (10), Nebulizer Gas (50), Auxillary Gas (50). Other arbitrary parameters that are also optimized include: Declustering Potential (DP), Collision Energy (CE), Collision Entrance Potential (CEP), Collision Exit Potential (CXP), Entrance Potential (EP).

Reagents and Solutions

All compound formulations are prepared the morning of the RACE in a DMSO:TWEEN80:H2O (10:10:80, v:v:v) solution.

Commentary

Background Information—Recent years have seen the development of hundreds of new chemical tool compounds suitable for probing the biology of the target in a variety of assays and contexts. Many of these probes have been developed by Investigators and laboratories at universities and research institutes. These efforts employed sophisticated high-throughput screening technologies and directed medicinal chemistry to produce probes that met specific potency and selectivity criteria, but were often not intended to produce molecules with drug-like properties. Thus, optimization of the compounds for use in a wide variety of animal models was outside of the scope of the effort.

A rudimentary knowledge of the pharmacokinetics of a given probe compound will be essential to their application in a well-considered in vivo study. Thus there is an immediate need for a rapid and accessible empirical approach to answering the question of what dose should be used in an in vivo study using these new tool compounds. The RACE assay is an easy and efficient method for answering this question. The method involves the use of a single standard formulation, a small cohort of mice, and a single dose level administered via a single route. Standardized formulations, dosing and sample collection protocols, along with an abbreviated sample collection scheme reduce the labor needed to perform both the in life and bioanalytical phases of the study. The procedure further reduces the complexity of data analysis by eliminating all but one calculated pharmacokinetic parameter. The protocol generates estimated exposure (eAUC₂₀₋₁₂₀), a parameter that is sufficient to rank order compounds based exposure, but is also easily determined by most graphing software using the simple trapezoidal rule.

In addition to the RACE protocol, there are few other experiments that seek to reduce the time and cost of full PK studies. For example, cassette dosing has been demonstrated to save time, money, and resources as well as providing full PK parameters (Berman et al., 1997). However, several limitations with the cassette approach have been identified. Of primary concern is the inability to predict and prevent drug-drug interactions when multiple compounds are co-administered. Further there are challenges to formulate multiple compounds in a single vehicle. All of these issues have led to a decline in frequency of using this approach (White and Manitpisitkul, 2001). Refinements in the cassette approach yielded the cassette accelerated rapid rat screen (CARRS), a procedure that addressed some of the limitations of cassette dosing, but others remain (Korfmacher et al., 2001). Another rapid PK study is the SnapshotPK. This approach is similar to the CARRS method, but abandoned cassette dosing in favor of pooled sample analysis, fewer time points and smaller blood volumes (Liu et al., 2008). All of these approaches seek to balance the need for detailed pharmacokinetic data on a large number of compounds, with the ultimate goal of selecting the compound that exhibits the most favorable pharmacokinetics. This is critical in a drug discovery effort, but has little applicability to the use of probe compounds in vivo. Most researchers are likely to use only one compound in a variety of assays to test a single hypothesis, so compound throughput is of less importance than the ease of conducting the study. RACE provides the non-industry-based investigator with an approach to answer the PK question that is directly applicable to the study in question. One important caveat of RACE is that it provides only a limited glimpse into the potential exposure of the compound, and is not an appropriate experimental paradigm for drawing conclusions on other PK parameters such as clearance, half-life, elimination etc.

Critical Parameters

Submandibular Bleeding of Mice: Special care and attention needs to be taken when performing all blood collections. The mouse should be held by the scruff of the neck and a 5.0mm lancet is recommended. One's ability to effectively and efficiently stick directly

behind the point at which the upper and lower jaws meet should be practiced prior to ensure confidence and correct technique. This is the site for the merging of the orbital veins, submandibular vein, and various other veins draining the facial area into the jugular vein (Golde et al., 2005). The lancets have been designed to prevent significant tissue damage and protection from an incision all the way through the mouth.

Blood Collection: Proper stick into the submandibular vein consistently yields 0.02-0.50mL of blood with little effect on the mouse post blood collection (Golde et al., 2005). If necessary adjust the volume of blood harvested at each time point, as each mouse will undergo two collections. To prevent hemolytic plasma samples, blood should fall freely into the collection tube.

Analysis of compounds in collected samples: This article assumes that labs engaged in even the most limited pharmacokinetic or pharmacodynamic studies using small molecules will have access to analytical instrumentation for the accurate quantification of compound in the samples collected. There are a number of compound detection methods, but liquid-chromatography coupled with tandem mass spectrometry is the most widely applied and available. For those investigators without local access to such resources there are numerous university labs and contract research organizations that provide this service for a reasonable fee. Of critical importance is the use of a reliable method to report accurate quantities of compound, sufficient compound for use in a standard curve, and plasma and tissue blanks for the assessment of matrix effects.

Troubleshooting

Compound is insoluble in the standard RACE vehicle: The use of standardized protocols streamlines the work-flow for RACE, and simplifies the data analysis. This however, is not meant to convey that the protocol is inflexible. In fact, its flexibility is an advantage to the RACE protocol. Thus, compounds that can be formulated in a vehicle other than the suggested standard formulation should be. The RACE assay is formulation agnostic, and could even be used to screen various excipients or formulations to determine which results in the greatest exposure. Similarly, a compound that formulates as a suspension is equally suitable for RACE.

Low compound exposure, no detectable compound: Perhaps the most common issue with RACE is the failure to detect significant levels of compound in the plasma. This is to be expected for probe compounds in the early exploratory phase, and for compounds in which metabolic stability has not been assessed. Unfortunately, there is little that can be done to improve bioavailability outside of modifying the compound at the chemical level. If the compound of interest has been shown to be metabolically stable or is predicted (via in silico analysis, or in vitro metabolic stability assay) to be so, then increasing the dose, administering via a different route (IV, or SQ), or using a different formulation may resolve the issue.

Compound is lethal at the administered dose: Often the compounds tested are toxic and cause death or significant adverse events such that the animals must be euthanized for humane reasons. During the RACE study, mice should be monitored for overt signs of toxic effects including labored breathing, ruffled fur, hunched posture, lethargy, low body temperature, ataxia (altered gate), and vocalization. Animals that exhibit these signs should be euthanized according to methods approved by the IACUC and the AVMA. In this case, if the dose administered is high (>20mg/kg), additional RACE assays using much lower doses may be successful and no cause toxic effects.

Anticipated Results—The procedures described above can be used to generate initial estimates of exposure for a novel chemical entity when administered at a specific dose via a specific route. Results from these studies can be used to profile novel compounds, rank order compounds from a series based on potential exposure, inform decisions on dose linearity, and predict tissue distribution.

Time Considerations—Based upon route of administration and familiarity ensure a feasible dosing and blood collection schedule has been prepared. A five minute gap between each dose is recommended initially, however with familiarity this can be adjusted. With practice, a dedicated technician can perform 7 RACE studies per day using the “high throughput” protocol outlined above and in Table 4. Thus the in vivo portion of the RACE study can be completed on as many as 35 compounds per week. If the samples from these 35 experiments are processed for LC/MS/MS analysis immediately following the end of each day's in vivo study, and the processing continues in parallel throughout the week, it is possible to have complete data sets for all 35 experiments in 10 days.

Acknowledgments

The authors would like to thank Dr. Greg Roth for his inspiration for the RACE assay, and acknowledge funding support from NIH Grant HG005033, and Florida Department of Health Grant 06-NIR-09.

Literature Cited

- Berman J, Halm K, Adkison K, Shaffer J. Simultaneous pharmacokinetic screening of a mixture of compounds in the dog using API LC/MS/MS analysis for increased throughput. *J Med Chem.* 1997; 40:827–829. [PubMed: 9083469]
- Golde WT, Gollobin P, Rodriguez LL. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. *Lab Anim (NY).* 2005; 34:39–43. [PubMed: 16195737]
- Korfmacher WA, Cox KA, Ng KJ, Veals J, Hsieh Y, Wainhaus S, Broske L, Prelusky D, Nomeir A, White RE. Cassette-accelerated rapid rat screen: a systematic procedure for the dosing and liquid chromatography/atmospheric pressure ionization tandem mass spectrometric analysis of new chemical entities as part of new drug discovery. *Rapid communications in mass spectrometry : RCM.* 2001; 15:335–340. [PubMed: 11241763]
- Lee YC, Zocharski PD, Samas B. An intravenous formulation decision tree for discovery compound formulation development. *Int J Pharm.* 2003; 253:111–119. [PubMed: 12593942]
- Liu B, Chang J, Gordon WP, Isbell J, Zhou Y, Tuntland T. Snapshot PK: a rapid rodent in vivo preclinical screening approach. *Drug discovery today.* 2008; 13:360–367. [PubMed: 18405850]
- White RE, Manitspitkul P. Pharmacokinetic theory of cassette dosing in drug discovery screening. *Drug Metab Dispos.* 2001; 29:957–966. [PubMed: 11408361]

A **B**

Compound	First X	Last X	Peak X	Peak Y	Total Area	Calculated eAUC**
A	20.0	120.0	120.0	158.00	10483	10.483 μg*hr/mL
B	20.0	120.0	120.0	98.87	7828	7.828 μg*hr/mL

Figure 1.

Example of data from a previously performed RACE study. The purpose of this study was to select which compound from a series exhibited the highest estimated exposure . **(A)**

Representative exposure data from two compounds in a series obtained by sequential RACE studies (n=3 mice/time point. Vehicle mice showed no compound in the plasma, data not shown). **(B)** Results from the GraphPad Prism5 AUC analysis. **eAUC (estimated exposure (AUC₂₀₋₁₂₀))

Table 1**List of non-standard abbreviations and non-standard acronyms**

ACN - Acetonitrile
ADME/T – Absorption, distribution, metabolism, elimination and toxicity
APCI - Atmospheric pressure chemical ionization
AUC – Area under curve
AVMA – American veterinary medical association
CARRS – Cassette accelerated rapid rat screen
CE - Collision energy
CEP - Collision entrance potential
CID - Collision-induced dissociation
CXP - Collision exit potential
DP- Declustering potential
DMSO – Dimethyl sulfoxide
eAUC – Estimated area under curve
EDTA – Ethylenediaminetetraacetic acid
EP – Entrance potential
IACUC – Institutional animal care and use committee
IP – Intraperitoneal
IV – Intravenous
MS - Mass spectrometry
m/z - Mass-to-charge ratio
MRM – multiple reaction monitoring
LC/MS/MS – Liquid chromatography/mass spectrometry/mass spectrometry
PK – Pharmacokinetic
RACE – Rapid assessment compound exposure
SQ – Subcutaneous injection
TWEEN80 – Polysorbate 80
TIS - Turbo ion spray
UPLC – Ultra performance liquid chromatography

Table 2

Sample dosing and blood collection schedule for one compound at a single concentration and route of administration.

Mouse	Compound	Concentration	Route of Administration	Dosing Time	Blood Collection 1 (20min)	Blood Collection 2 (120min)
1	A	10mg/kg	IP	8:00	8:20	10:00 *
2	A	10mg/kg	IP	8:05	8:25	10:05 *
3	A	10mg/kg	IP	8:10	8:30	10:10 *
4	Vehicle		IP	8:15	8:35	10:15 *

* Indicates euthanasia performed as part of the blood collection (i.e. terminal), or immediately following

Table 3

Sample dosing and blood collection schedule for one compound at three different concentrations using the same route of administration.

Mouse	Compound	Concentration	Route of Administration	Dosing Time	Blood Collection 1 (20min)	Blood Collection 2 (120min)
1	A	5mg/kg	PO	8:00	8:20	10:00 [*]
2	A	5mg/kg	PO	8:05	8:25	10:05 [*]
3	A	5mg/kg	PO	8:10	8:30	10:10 [*]
4	Vehicle 1		PO	8:15	8:35	10:15 [*]
5	A	10mg/kg	PO	8:40	9:00	10:40 [*]
6	A	10mg/kg	PO	8:45	9:05	10:45 [*]
7	A	10mg/kg	PO	8:50	9:10	10:50 [*]
8	A	20mg/kg	PO	8:55	9:15	10:55 [*]
9	A	20mg/kg	PO	9:20	9:40	11:20 [*]
10	A	20mg/kg	PO	9:25	9:45	11:25 [*]

^{*} Indicates euthanasia performed as part of the blood collection (i.e. terminal), or immediately following

Table 4

Sample dosing and blood collection schedule for the high-throughput RACE protocol

Mouse	Compound	Concentration	Route of Administration	Dosing Time	Blood Collection 1 (20min)	Blood Collection 2 (120min)
1	A	10mg/kg	IP	8:00	8:20	10:00*
2	A	10mg/kg	IP	8:05	8:25	10:05*
3	A	10mg/kg	IP	8:10	8:30	10:10*
4	B	10mg/kg	IP	8:15	8:35	10:15*
5	B	10mg/kg	IP	8:40	9:00	10:40*
6	B	10mg/kg	IP	8:45	9:05	10:45*
7	C	10mg/kg	IP	8:50	9:10	10:50*
8	C	10mg/kg	IP	8:55	9:15	10:55*
9	C	10mg/kg	IP	9:20	9:40	11:20*
10	D	10mg/kg	IP	9:25	9:45	11:25*
11	D	10mg/kg	IP	9:30	9:50	11:30*
12	D	10mg/kg	IP	9:35	9:55	11:35*
BREAK						
13	Vehicle		IP	12:30	12:50	2:30*
14	E	10mg/kg	IP	12:35	12:55	2:35*
15	E	10mg/kg	IP	12:40	1:00	2:40*
16	E	10mg/kg	IP	12:45	1:05	2:45*
17	F	10mg/kg	IP	1:10	1:30	3:10*
18	F	10mg/kg	IP	1:15	1:35	3:15*
19	F	10mg/kg	IP	1:20	1:40	3:20*
20	G	10mg/kg	IP	1:45	2:05	3:45*
21	G	10mg/kg	IP	1:50	2:10	3:50*
22	G	10mg/kg	IP	1:55	2:15	3:55*

Table 5

Compound requirements for the basic protocol at a range of doses

Dose (mg/kg)	Amount of Compound (mg, 100% purity) for 3 mice (6-8 weeks of age)
1	2
2	3
5	4
10	6
20	8
Bioanalysis Stds.	5