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Short Communication:

Use of Subcutaneous and Intraperitoneal Administration Methods to Facilitate Cassette Dosing in Microdialysis Studies in Rats

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Running Title Page

Extravascular dosing in cassette brain microdialysis studies

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List of abbreviations

ANOVA, Analysis of variance; $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$, Brain interstitial fluid-to-plasma area under the curve ratio from 0 to 360 minutes; BBB, Blood-brain barrier; CNS, Central nervous system; (a)CSF, (Artificial) Cerebrospinal fluid; $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, Brain interstital fluid-to-plasma concentration ratio at steady-state; HPLC, High-performance liquid chromatography; ISF, Insterstitial fluid; Kp,uu, Brain-to-plasma unbound tissue partition coefficient; LC-MS, Liquid chromatography-mass spectrometry; IP, Intraperitoneal; IV, Intravenous; PK, Pharmacokinetic; q1h, dosed every hour; q2h, dosed every 2 hours; SAR, Structure-activity relationship; SC, Subcutaneous

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Abstract

Microdialysis is a powerful technique allowing for real-time measurement of unbound drug concentrations in brain interstitial fluid (ISF) in conscious animals. Use of microdialysis in drug discovery is limited by high resource requirement and low throughput, but this may be improved by cassette dosing. Administering multiple compounds intravenously (IV) of diverse physiochemical properties, it is often very challenging and time-consuming to identify a vehicle that can dissolve all the compounds. To overcome this limitation, the present study explores the possibility of administering a cassette dose of 9 diverse compounds (carbamazepine, citalopram, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine, and risperidone) in suspension, rather than in solution, by intraperitoneal and subcutaneous routes, and determining if this is a viable option for assessing BBB penetration in microdialysis studies. Repeated hourly subcutaneous dosing during the 6 hour microdialysis study allowed for the best attainment of distributional equilibrium between brain and plasma, resulting in less than two-fold of difference unbound brain to unbound plasma concentration ratio for the cassette dosing method versus the discrete dosing. Both subcutaneous and intraperitoneal repeated dosing can provide a more practical substitute for IV dosing in determining brain penetration of a cassette of diverse compounds in brain microdialysis studies. The results from the present study demonstrate that dosing compounds in suspension represents a practical approach to eliminate the technical challenge and labor-intensive step of preparation of solutions of a mixture of compounds and will enable the use of cassette brain microdialysis method in a CNS drug discovery setting.

Introduction

An important property of drugs is the rate and extent of blood-brain barrier (BBB) penetration. This is determined by physiochemical properties such as size, charge, polarity, lipophilicity as well as the drug's affinity for influx and efflux transporters (Abraham, 2004, Chikhale, et al., 1994, Lee, et al., 2001, Levin, 1980, Liu, et al., 2008). The property of brain penetration is critical for central nervous system (CNS) targets, in which efficacy can only be achieved if the compound reaches brain tissue and engages with the target. There is also a more general implication for all drugs: on- or off-target CNS activity in relation to free drug in brain tissue, according to the free drug hypothesis (Brodie, et al., 1960, Liu, et al., 2014, Tillement, et al., 1988). Therefore, it is advisable to have an understanding of brain penetration at an early stage of structure-activity relationship (SAR).

There are multiple methods currently employed for screening BBB penetration: some indirect and some direct. In silico methods utilize physiochemical properties, measured or predicted, to estimate permeability. In vitro screening methods such permeability measurement in cell monolayers or cells plated on transwell devices are commonly employed (Nicolazzo, et al., 2006). Screening usually stops short of in vivo methods for economical as well as ethical reasons. Furthermore, assessing BBB permeability in preclinical species requires destructive sampling. While some studies have used large numbers of animals to characterize kinetics of brain penetration (Chow, et al., 2011), in an industry setting, a single time point is often used with the assumption that steady-state conditions are reached.

One means of averting this assumption is the use of brain microdialysis to indirectly measure brain concentrations real-time in live, conscious animals (de Lange, et al., 1994, Liu, et al.,

2009). Unfortunately, this method requires surgical procedures that render it costly in the event that compounds are assessed discretely. Some efforts have been made to increase throughput of microdialysis studies by using cassette dosing (Deshmukh, et al., 2015), but these efforts have currently been confined to intravenous (IV) bolus and infusion dosing. In many cases in drug discovery research, compounds with very diverse physiochemical properties are being tested which makes finding a suitable vehicle to dissolve the compounds very difficult. This ultimately results in the use of doses which are very low, and for compounds with low probe recovery or low BBB penetration, dialysate concentrations are likely to fall below the limit of quantitation, which does not allow for a quantitative determination of brain penetration.

Identifying a suitable, non-toxic vehicle that can dissolve all the compounds at a sufficient concentration for a brain microdialysis study represents a main challenge for the application of cassette dosing for the brain microdialysis method. To overcome this challenge, we propose to dose the cassette compounds in suspension in a simple aqueous formulation in non-IV administration routes. In the present study, a cassette of diverse compounds (both in terms of structure and physiochemical properties, summarized in Supplemental Table 1) was dosed as a suspension to rats, either intraperitoneally (IP) or subcutaneously (SC), and a dosing regimen was explored that can be used to attain steady state conditions. This allowed for rigorous quantitative assessment of blood-brain partitioning via microdialysis, making microdialysis a more efficient and suitable tool for screening compounds of interest for BBB penetration in a drug discovery setting.

Materials and Methods

Animals and Surgery For the pharmacokinetic (PK) study, male Sprague-Dawley rats (n=3 rats per dosing arm) with cannulae implanted in the femoral artery were obtained from Charles River Laboratories, Inc. (Wilmington, MA). For the microdialysis studies, femoral artery-cannulated male Sprague-Dawley rats (250–350 g, 8-9 weeks old) with a surgically implanted microdialysis guide cannulae (CMA/12; CMA Microdialysis), were purchased from Charles River Laboratories, Inc. (Wilmington, MA). The guide cannulae had been implanted in the prefrontal cortex at 3.2 mm anteroposterior, 1.0 mm mediolateral, and 0.5 mm dorsoventral to the bregma and secured to the skull with screws and dental cement. Rats were acclimatized to the laboratory environment for 3 to 5 days before the study

PK Study A 9-compound cassette consisting of carbamazepine, citalopram, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine and risperidone was administered via subcutaneous (1 or 2 mg/kg) or intraperitoneal (2 mg/kg) injection in 1% methylcellulose, as a suspension. The dosing volume for subcutaneous administration was 2 ml/kg and the dosing volume for intraperitoneal administration was 5 ml/kg. Plasma samples were taken via the femoral artery cannula at 2, 3, 15, 30, 60, 120, 240, and 480 minutes after dosing. Blood was then centrifuged at 3,200 x g for 5 min at 4°C to obtain plasma.

Microdialysis Studies The principles of a microdialysis study are outlined by Durk (2018). In vitro recovery values of individual compounds for microdialysis probes were determined in a previous study (Deshmukh, et al., 2015) and are shown in Table 1. Approximately 16 hours before dosing, the rats were placed into individual BASi RATURN systems (Bioanalytical Systems, Inc., West Lafayette, IN) with access to food and water ad libitum. Dummy probes

were replaced with CMA 12/2-mm probes (CMA Microdialysis) and perfused with artificial cerebrospinal fluid (CMA Microdialysis) at a rate of 1 µl/min overnight using microdialysis pumps (CMA/102; CMA Microdialysis). On the day of the study, the outlets were connected to BASi Refrigerated HoneyComb Fraction Collectors (Bioanalytical Systems, Inc, West Lafayette, IN.) at 4°C and perfused at 1 µL/min. Rats (n = 4) received either a SC or an IP dose of a cassette of compounds, consisting of 1 or 2 mg/kg of citalopram, carbamazepine, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine and risperidone. The formulation vehicle was 1% methylcellulose in phosphate buffered saline, pH 7.4 and the compounds were administered as a suspension at a volume of 2 ml/kg. Three dosing regimens were tested, SC dosing every hour (q1h), SC dosing every 2 hours (q2h) and IP dosing q2h. IP dosing q1h was not used because q2h was the most frequent dosing regimen approved by the Genentech Institutional Animal Care and Use Committee. For all SC and IP repeat dose studies, site of injection was rotated between doses, as outlined in the Good Practice Guide to the Administration of Substances and Removal of Blood (Diehl, et al., 2001). For all three studies, blood samples were collected via femoral artery cannula 15, 30, 60, 120, 180, 240, 300, 330 and 360 after the first dose. Blood was then centrifuged at 3,200 x g for 5 min at 4°C to obtain plasma. The perfusate samples were serially collected from each animal for 30 minute intervals from -15 minutes predose to 6 hours post dose. The concentrations are reported at the midpoint of each interval. At 6 hours, the animals were euthanized by intravenous injection of Euthasol (solution of 750 mg/kg phenobarbital and 95 mg/kg phenytoin sodium). All the samples were stored at -20°C before analysis. All studies were approved by Genentech Institutional Animal Care and Use Committee.

Liquid Chromatography-Mass Spectrometry Standard curves and quality control samples were prepared by spiking a known amount of a mixture of the 9 compounds into a blank mixed matrix of rat plasma or artificial cerebrospinal fluid (CSF). A total of 25 µl of samples, 25 µl of calibration standards, or 25 µl of quality controls were mixed with 5 µl of internal standard (d3-naltrexone) and 200 µl acetonitrile. Following vortexing and centrifugation at 1500 x g for 10-15 minutes, 100 µl of supernatant was transferred to a 96-well plate and diluted with 50 µl water prior to analysis by HPLC-MS/MS.

Data Interpretation Samples were analyzed using two sets of standard curves and two sets of quality controls in each analytical run. The system consisted of a Shimadzu LC-30AM pump (Shimadzu, Columbia, MD), and an AB Sciex Qtrap 5000 (AB Sciex, Foster City, CA) mass spectrometer with a turbo ion spray interface. A 20 µl aliquot of each sample was injected into a Kinetex reverse-phase PFP (pentafluorophenyl) 2.6 µm 100A 50 x 2.1 mm column (Phenomenex, Torrance, CA). The lower limit of quantitation (LLOQ) ranged from 0.01 to 0.1 ng/ml. The assay accuracy was between 75% and 125%. Peak quantitation was performed using Analyst Software. For the PK study, PK parameters were determined using Pheonix WinNonlin®. For microdialysis studies, brain penetration was assessed by comparing the brain ISF:plasma concentration ratio of each compound at steady-state ($C_{ss,\ ISF}/C_{ss,\ u,\ p}$) and the brain ISF:plasma ratio of the area under the curve ($AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$) throughout the study. Differences between $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ and $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ for extravascular dosing regimens and intravenous infusion to steady-state (control dosing regimen) were assessed by one-way analysis of variance (ANOVA) and a *p* value of less than 0.05 was considered to be statistically significant. Differences in peak-to-trough ratio between subcutaneous and

intraperitoneal dosing regimens were compared using Student's 2-tailed t-test. A *p* value of less than 0.05 was considered to be statistically significant.

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Results

Single dose PK study: A single-dose cassette of all 9 compounds- citalopram, carbamazepine, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine and risperidone, was administered intraperitoneally or subcutaneously. All compounds were well-absorbed, reached maximum plasma concentration within one hour and were cleared from plasma as time progressed (Supplemental Fig. 1). Parameters for the PK data are summarized in Supplemental Table 2. The PK data was used to confirm that the dosing regimen used would result in estimated dialysate concentrations above the lower limit of quantitation, since $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ was known (using IV infusion to steady-state) and in vitro probe recovery was also known (Table 1). It was determined that 2 mg/kg would likely yield dialysate concentrations within the limit of quantification. This was confirmed in the microdialysis studies outlined in this paper. Plasma protein binding and *in vitro* probe recovery values are displayed in Table 1. It is suggested that prior to any microdialysis study with extravascular administration, a single dose PK study be used to ensure that adequate systemic exposures are attainable.

Repeated Subcutaneous Dose Microdialysis Study, every hour: The 9-compound cassette was administered subcutaneously, every hour, during the 6 hour dosing period. In general, brain: plasma ratio was greater than 1 for diphenhydramine and naltrexone, close to 1 for carbamazepine, metoclopramide and risperidone, and less than 1 for citalopram, desmethylclozapine, gabapentin and quinidine (Fig. 1). The $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ for each dosing regimen are summarized in Table 2. For both $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, only carbamazepine and gabapentin were significantly different than ratios determined by IV infusion to steady-state.

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Repeated Subcutaneous Dose Microdialysis Study, every 2 hours: The 9-compound cassette was administered subcutaneously, every 2 hours, during the 6 hour dosing period. In general, brain: plasma ratio was close to 1 for diphenhydramine and naltrexone, close to 1 for citalopram, carbamazepine, metoclopramide and risperidone, and less than 1 for, and gabapentin (Fig. 2). The $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ for each dosing regimen are summarized in Table 2. It should be noted that for desmethylclozapine and quinidine, the dialysate concentrations fell below the limit of quantitation, and thus, calculation of $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ was not possible. For $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ carbamazepine, metoclopramide and naltrexone were significantly different than values determined by IV infusion to steady-state. For $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, carbamazepine, diphenhydramine, gabapentin, metoclopramide and naltrexone were significantly different than values determined by IV infusion to steady-state.

Repeated Intraperitoneal Dose Microdialysis Study: The 9-compound cassette was administered intraperitoneally, every 2 hours, during the 6 hour dosing period. In general, brain: plasma ratio was greater than 1 for diphenhydramine and naltrexone, close to 1 for carbamazepine, metoclopramide and risperidone, and less than 1 for citalopram, desmethylclozapine, gabapentin and quinidine (Fig. 3). The $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ for each dosing regimen are summarized in Table 2. For $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ carbamazepine, gabapentin, metoclopramide and naltrexone were significantly different than values determined by IV infusion to steady-state. For $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, carbamazepine, diphenhydramine, metoclopramide and naltrexone were significantly different than values determined by IV infusion to steady-state.

Discussion

The results of this study demonstrate that repeated extravascular dosing of a cassette in suspension can be used in place of an intravenous cassette infused to steady-state when screening compounds in brain microdialysis studies. This is a significant improvement in avoiding extensive formulation work which may be required to deliver a diverse cassette of compounds as an intravenous dose in solution. In addition, further steps may be taken to re-use rats with microdialysis probes implanted; a number of studies have examined to what extent rats with implanted microdialysis probes may be re-used (de Lange, et al., 1994), but this appears to be dependent on the probe compound and also that study be performed under carefully controlled conditions. A different approach was taken by Durk et al, in which probes were implanted in each side of the brain and the same set of rats was used twice (2015), also saving considerable time and resources.

The set of compounds used in the present study was chosen because a cassette microdialysis dataset was already available following IV infusion (Deshmukh, et al., 2015), and these compounds are readily available to any researcher and can be used as internal standards for future studies, administered with unknown compounds to benchmark the extent of brain penetration. In addition to the compounds selected from Deshmukh et al, two additional compounds from Liu et al, quinidine and risperidone, were added in order to better represent P-gp substrates.

It should be noted that all three dosing regimens, in addition to IV infusion to steady state, consistently allow for the categorization of compounds as net efflux ($K_{p,uu} << 1$), passive distribution ($K_{p,uu} \approx 1$), or net uptake ($K_{p,uu} >> 1$). While more definitive studies may need to

be carried out to quantitatively assess brain concentrations for compounds of interest, the screening paradigm outlined in this study allows for early screening without extensive formulation work which may be required for IV dosing.

One further parameter which plays an important role in assessing brain: plasma ratio in such a short dosing duration is the rate at which tissue distribution occurs. If a compound is lipohilic and non-polar and the capillary membrane does not act as a physical barrier to the compound, then rate of distribution will be dependent on the vascular blood flow to that tissue. However, the presence of tight junctions at the BBB will limit the rate of absorption such that the rate-determining step will be permeation across the capillary membrane, which is related to molecular weight, charge and lipophilicity (Goresky, et al., 1970, Liu, et al., 2005). Because of this, large, charged and more polar compounds will take longer to distribute to tissues, and this was observed, as an example, for zwitterionic gabapentin versus lipophilic, weakly basic diphenhydramine.

Generally speaking, for all compounds in the 9-compound cassette, subcutaneous dosing resulted in $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ ratios that were closest to those obtained from IV infusion dosing, whereas intraperitoneal dosing resulted in ratios which were less accurate. The fewest significant differences were observed between IV infusion to steady state and the q1h subcutaneous dosing regimen compared to the other regimens, when $AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ values were compared between dosing regimens using one-way ANOVA. One possible explanation for this observation is that an intraperitoneal undergoes faster absorption to the systemic circulation versus a subcutaneous dose, and this is less similar to steady-state IV infusion than subcutaneous dosing. This is confirmed by comparing the peak-to-trough ratio for each dosing regimen. Hourly subcutaneous dosing yielded peak-to-trough ratios of 1 or less.

Statistical comparison of subcutaneous dosing and intraperitoneal dosing, administered every 2 hours, showed that for all compounds except citalopram, peak-to-trough ratio was significantly higher with intraperitoneal dosing vs. subcutaneous dosing (Supplemental Table 3). Since systemic clearance is the same no matter what route of administration is used, it is likely that apparent clearance is determined by rate of absorption, and this rate appears to be faster with intraperitoneal dosing vs subcutaneous dosing. Further studies may be needed to optimize the dosing regimens for individual compounds, but the q1h dosing regimen is a good starting point that works well for most compounds. This approach also minimized the chances of a sample being below the limit of quantification, especially in the dialysate, in which concentrations may be near or below the limit of detection due to low recovery, low brain penetration or a combination thereof.

Despite these differences in physiochemical properties and time to reach steady-state, repeated extravascular dosing was shown to be a suitable alternative to intravenous infusion to steady-state, in cassette microdialysis studies to determine brain penetration of a diverse set of compounds.

Authorship Contributions

Participated in research design: Durk, Liederer, Liu

Conducted experiments: Valle, Durk

Contributed new reagents or analytic tools: Durk, Ding

Performed Data Analysis: Durk, Deshmukh, Ding

Wrote or contributed to the writing of the manuscript: Durk, Liu

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Figure Legends

Figure 1 Unbound drug concentrations in plasma (dotted) and brain ISF (solid, calculated from recovery and dialysate concentration) following repeated, subcutaneous administration in rats, every hour. Data are mean ± standard deviation, n=4.

Figure 2 Unbound drug concentrations in plasma (dotted) and brain ISF (solid, calculated from recovery and dialysate concentration) following repeated, subcutaneous administration in rats, every 2 hours. Data are mean ± standard deviation, n=3. For quinidine and desmethylclozapine, dialysate concentrations were measurable in only 1 of the 3 rats and therefore standard deviation were not reported and brain: plasma ratios were not calculated.

Figure 3 Unbound drug concentrations in plasma (dotted) and brain ISF (solid, calculated from recovery and dialysate concentration) following repeated intraperitoneal administration in rats, every 2 hours. Data are mean ± standard deviation, n=4.

Table 1 Plasma protein binding and in vitro recovery of the cassette of compounds used in microdialysis studies

	$f_{u,\,p}$	In vitro probe recovery
Citalopram[a]	0.81 ± 0.20	0.264 ± 0.017
Carbamazepine[a]	0.27 ± 0.02	0.288 ± 0.013
Desmethylclozapine[a]	0.061 ± 0.004	0.320 ± 0.018
Diphenhydramine[a]	0.47 ± 0.04	0.272 ± 0.018
Gabapentin[a]	0.76 ± 0.05	0.225 ± 0.042
Metaclopramide[a]	0.50 ± 0.03	0.237 ± 0.019
Naltrexone[a]	0.53 ± 0.05	0.242 ± 0.015
Quinidine[b]	0.265 ± 0.02	0.294 ± 0.061
Risperidone[b]	0.080 ± 0.005	0.183 ± 0.041

a (Deshmukh, et al., 2015)

b (Liu, et al., 2009)

Table 2 Comparison of brain penetration between IV infusion to steady-state from Liu et al (2009) or Deshmukh et al (2015) and the three dosing regimens employed in the current study.

	$AUC_{0\text{-}360,\ ISF}/AUC_{0\text{-}360,\ u,\ p}$				$C_{ss,\ ISF}/C_{ss,\ u,\ p}$			
	IV Infusion (n=3 or 4)	SC q1h (n=4)	SC q2h (n=3)	IP q2h (n=4)	IV Infusion (n=3 or 4)	SC q1h (n=4)	SC q2h (n=3)	IP q2h (n=4)
Citalopram[a]	0.438 ± 0.131	0.293 ± 0.058	0.450 ± 0.284	0.467 ± 0.156	0.400 ± 0.145	0.310 ± 0.0912	0.569 ± 0.351	0.480 ± 0.139
Carbamazepine[a]	0.250 ± 0.0722	0.773 ± 0.0622†	0.779 ± 0.265†	1.31 ± 0.203†	0.249 ± 0.0532	0.860 ± 0.122†	0.913 ± 0.345†	1.37 ± 0.179†
Desmethylclozapine[a]	0.0902 ± 0.0673	0.0466 ± 0.0118	NC	0.0987 ± 0.0452	0.113 ± 0.040	0.0641 ± 0.026	NC	0.138 ± 0.0716
Diphenhydramine[a]	2.24 ± 0.437	2.06 ± 0.241	2.43 ± 0.957	3.38 ± 0.947	0.679 ± 0.191	2.16 ± 0.529	3.34 ± 1.33†	4.18 ± 1.17†
Gabapentin[a]	0.0153 ± 0.00514	0.0698 ± 0.0348†	0.0622 ± 0.0108	0.0747 ± 0.0230†	0.0155 ± 0.00481	0.0846 ± 0.0455	0.0799 ± 0.0185†	0.0841 ± 0.0307†
Metaclopramide[a]	0.0905 ± 0.0200	0.436 ± 0.0960	0.646 ± 0.317†	0.747 ± 0.130†	0.0875 ± 0.0201	0.411 ± 0.0981	0.704 ± 0.380†	0.803 ± 0.176†
Naltrexone[a]	0.441 ± 0.106	1.54 ± 0.267	1.55 ± 0.679†	2.78 ± 0.651†	0.407 ± 0.100	1.18 ± 0.309	1.88 ± 0.906†	3.55 ± 0.601†
Quinidine[b]	0.154§	0.105 ± 0.0378#	NC	0.143 ± 0.0528#	0.173 ± 0.109	0.0964 ± 0.0376	NC	0.146 ± 0.0685
Risperidone[b]	0.620§	0.549 ± 0.0850#	0.776 ± 0.645#	0.793 ± 0.204#	0.530 ± 0.117	0.520 ± 0.118	0.790 ± 0.653	0.696 ± 0.0938

a (Deshmukh, et al., 2015), n=4

b (Liu, et al., 2009)**,** n=3

NC denotes “not calculated”, because dialysate levels were below the limit of quantitation.

One-way ANOVA was not performed because individual animal data were not available for AUC ratio from Liu et al, 2009.

§ Standard deviation is not reported because individual animal data were not available for AUC ratio from Liu et al, 2009.

† p <0.05 between IV infusion to steady-state and the indicated extravascular dosing group.

Figure 1

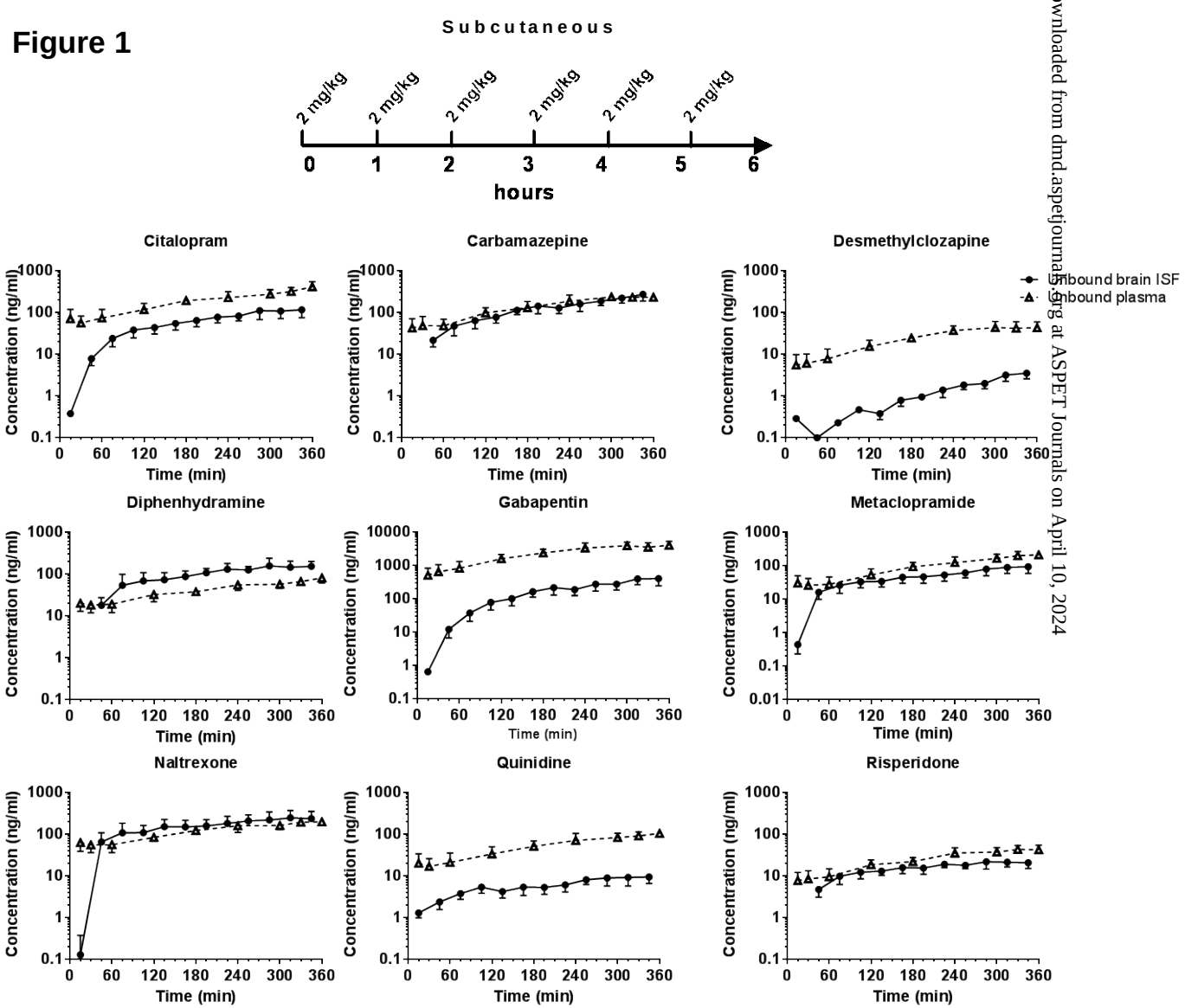


Figure 2

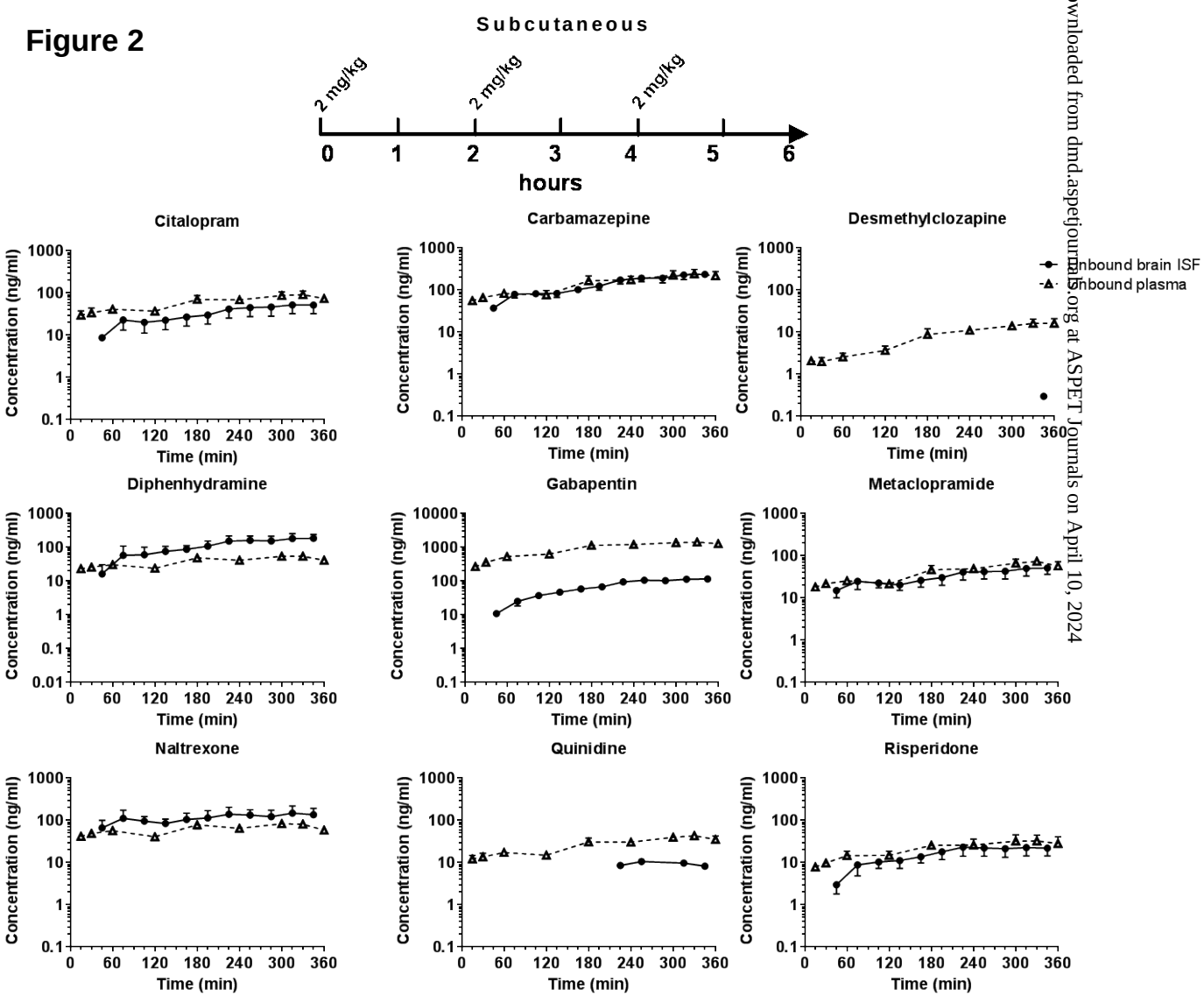
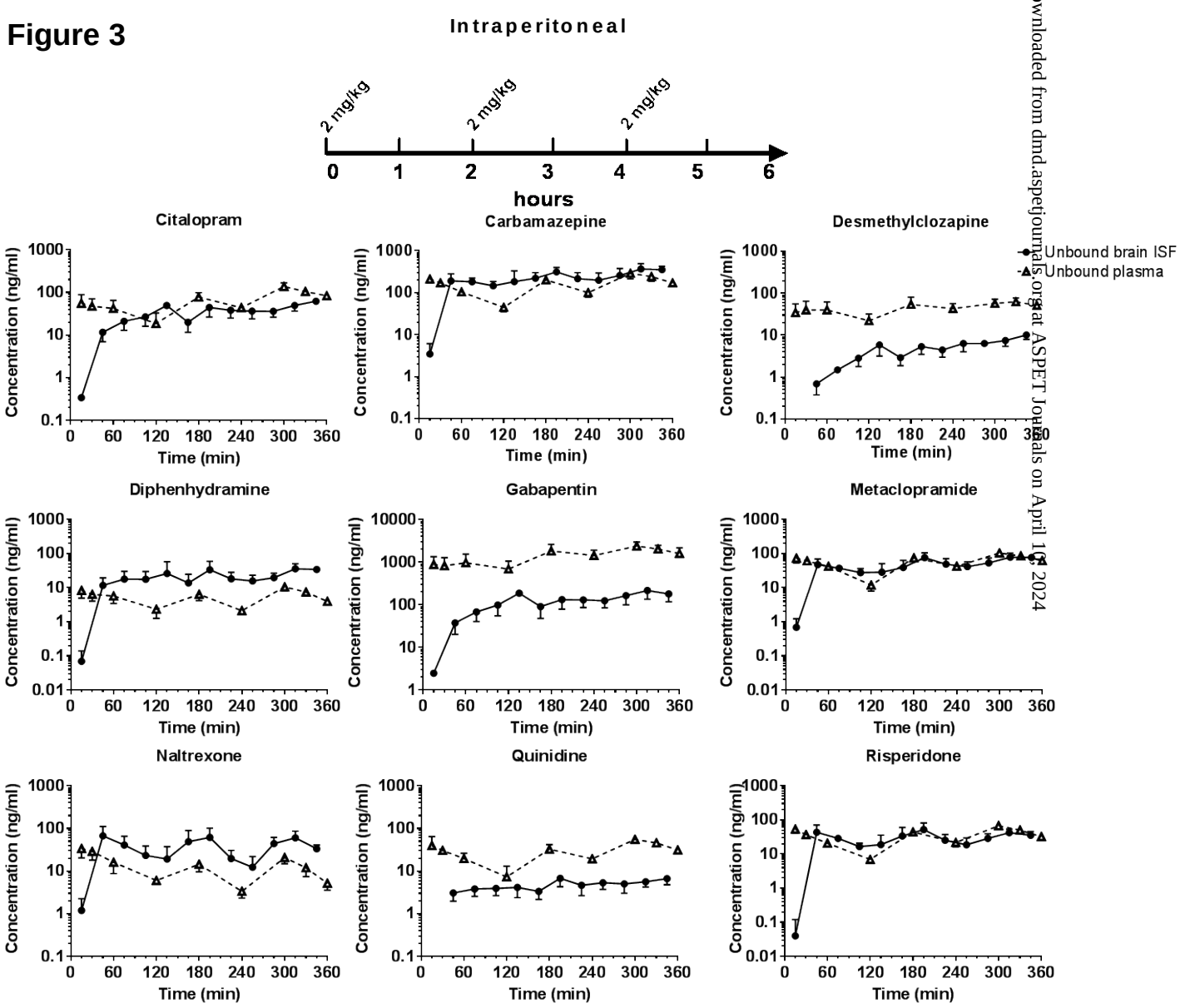


Figure 3



Supplemental Data

Use of Subcutaneous and Intraperitoneal Administration Methods to Facilitate Cassette Dosing in Microdialysis Studies in Rats

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Drug Metabolism and Disposition

Supplemental Table 1 Summary of physiochemical properties of the 9 compounds used in the cassette

Compound	MW (Da)	Charge	pKa	logP	PSA (Å²)	mdr1a/b KO/WT
Carbamazepine	236	Neutral	-	2.4	36	1.1
Citalopram	324	Basic	9.6	3.5	35	1.9
Desmethylclozapine	313	Basic	8.9	2.8	35	N.A.
Diphenhydramine	255	Basic	8.98	3.3	12	N.A.
Gabapentin	171	Zwitterionic	4.6 (acid), 9.9 (base)	-1.9	63	N.A.
Metaclopramide	300	Basic	9.6	2.2	58	6.6
Naltrexone	341	Basic	8.2, 9.6	1.9	70	N.A.
Quinidine	324	Basic	9.3	3.4	41	36
Risperidone	410	Basic	8.4	2.7	57	10

N.A. data not available

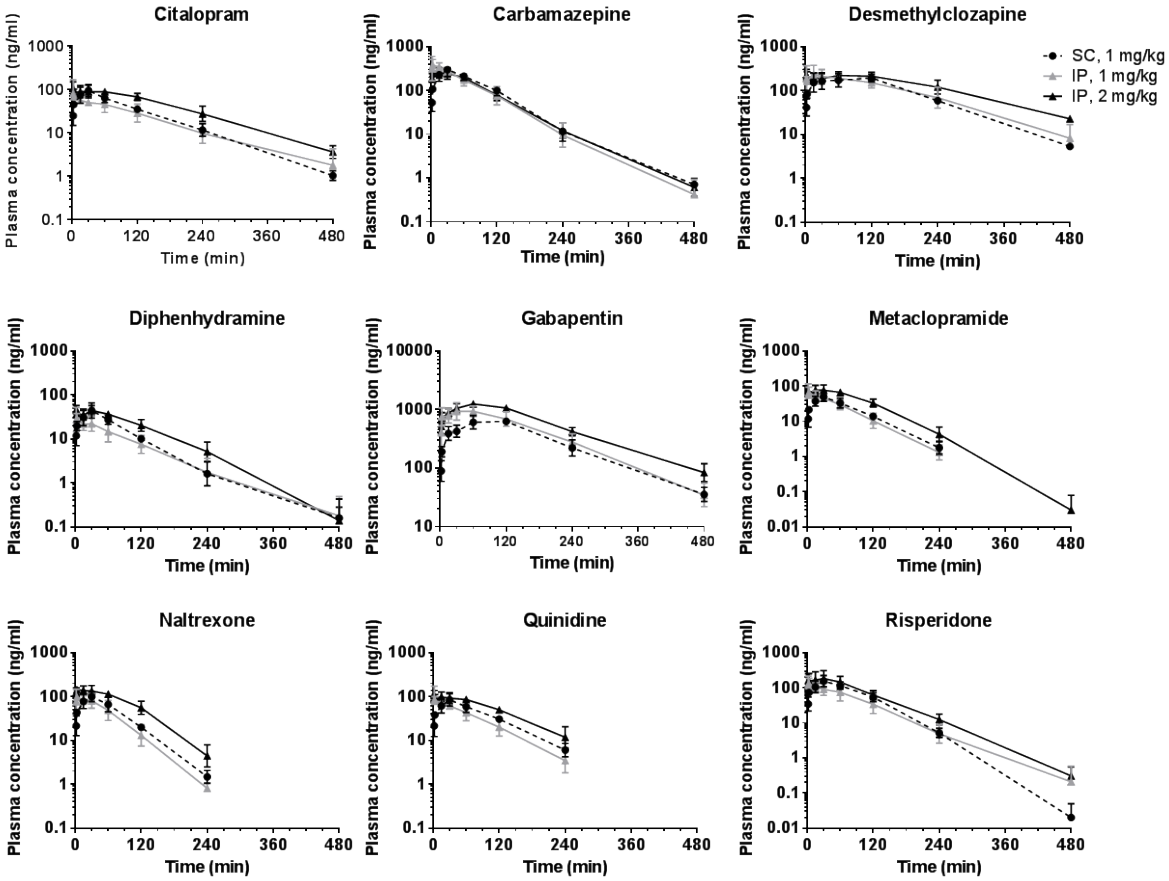
Supplemental Table 2 Summary of the pharmacokinetic parameters (Cmax, Tmax and terminal half-life) for each dosing regimen

Compound	Dosing regimen	C_{max} (ng/ml)	T_{max} (h)	$t_{1/2}$ (h)
Citalopram	SC, 1 mg/kg	95.6 ± 36.3	0.500 ± 0	1.17 ± 0.0465
	IP, 1 mg/kg	106 ± 67.1	0.361 ± 0.553	1.33 ± 0.614
	IP, 2 mg/kg	119 ± 37.6	0.511 ± 0.483	1.41 ± 0.0861
Carbamazepine	SC, 1 mg/kg	305 ± 132	0.417 ± 0.764	2.87 ± 2.09
	IP, 1 mg/kg	429 ± 191	0.111 ± 0.983	0.743 ± 0.185
	IP, 2 mg/kg	287 ± 64.0	0.583 ± 1.13	0.809 ± 0.127
Desmethylclozapine	SC, 1 mg/kg	212 ± 62.3	1.17 ± 0	3.87 ± 1.35
	IP, 1 mg/kg	268 ± 112	1.01 ± 0.00962	2.83 ± 0.461
	IP, 2 mg/kg	249 ± 71.3	0.694 ± 0.269	2.69 ± 0.354
Diphenhydramine	SC, 1 mg/kg	44.5 ± 21.9	0.500 ± 0.577	0.846 ± 0.334
	IP, 1 mg/kg	38.7 ± 15.5	0.0444 ± 0.289	0.900 ± 0.470
	IP, 2 mg/kg	52.5 ± 12.0	0.189 ± 0	0.837 ± 0.164
Gabapentin	SC, 1 mg/kg	657 ± 127	1.67 ± 0.577	5.88 ± 2.44
	IP, 1 mg/kg	1060 ± 305	0.833 ± 0.289	3.93 ± 1.15
	IP, 2 mg/kg	1250 ± 156	1.00 ± 0	3.38 ± 0.269
Metaclopramide	SC, 1 mg/kg	49.9 ± 17.2	0.500 ± 0	4.25 ± 6.10
	IP, 1 mg/kg	74.8 ± 43.6	0.0444 ± 0.00962	0.615 ± 0.121
	IP, 2 mg/kg	85.5 ± 25.7	0.417 ± 0.144	0.670 ± 0.0978
Naltrexone	SC, 1 mg/kg	105 ± 30.3	0.417 ± 0.144	3.68 ± 5.43
	IP, 1 mg/kg	128 ± 26.4	0.194 ± 0.265	1.16 ± 1.30
	IP, 2 mg/kg	155 ± 26.1	0.194 ± 0.265	0.598 ± 0.135
Quinidine	SC, 1 mg/kg	84.7 ± 32.8	0.667 ± 0.289	0.992 ± 0.326
	IP, 1 mg/kg	111 ± 65.5	0.111 ± 0.121	0.729 ± 0.248
	IP, 2 mg/kg	111 ± 32.7	0.511 ± 0.483	1.02 ± 0.457
Risperidone	SC, 1 mg/kg	156 ± 68.9	0.667 ± 0.289	3.19 ± 3.35
	IP, 1 mg/kg	169 ± 67.5	0.0444 ± 0.00962	0.726 ± 0.284
	IP, 2 mg/kg	206 ± 122	0.667 ± 0.289	0.761 ± 0.207

Supplemental Table 3 Peak-to-trough ratio, following the final dose, for each compound, for the subcutaneous and intraperitoneal dosing regimens, both q2h. The q1h dosing regimen was not included, since peak-to-trough ratio was 1 or less than 1 for all compounds.

Compound	SC q2h	IP q2h	$p < 0.05$*
Citalopram	1.29 ± 0.0776	1.64 ± 0.307	No
Carbamazepine	1.13 ± 0.0428	1.86 ± 0.193	Yes
Desmethylclozapine	1.02 ± 0.0277	1.37 ± 0.251	Yes
Diphenhydramine	1.40 ± 0.0710	2.99 ± 0.325	Yes
Gabapentin	1.14 ± 0.0698	1.81 ± 0.508	Yes
Metaclopramide	1.33 ± 0.146	1.88 ± 0.374	Yes
Naltrexone	1.55 ± 0.273	7.40 ± 2.37	Yes
Quinidine	1.25 ± 0.0766	2.06 ± 0.629	Yes
Risperidone	1.26 ± 0.136	2.38 ± 0.492	Yes

* Student's 2-talied t-test



Supplemental Figure 1 Total drug concentrations in plasma following a single dose of the 9-compound cassette in rats, by subcutaneous route, 1 mg/kg (dotted), intraperitoneal route, 1 mg/kg (solid grey) or intraperitoneal route, 2 mg/kg (solid black). Data are mean ± standard deviation, n=3. PK parameters are summarized in Supplemental Table 2. The PK data was used to confirm that the dosing regimen used would result in estimated dialysate concentrations above the lower limit of quantitation.