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Bioanalytical method for evaluating the pharmacokinetics of the GCP-II inhibitor 2-phosphonomethyl pentanedioic acid (2-PMPA)

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Abstract

2-Phosphonomethyl pentanedioic acid (2-PMPA) is a potent and selective inhibitor of glutamate carboxypeptidase-II, an enzyme which catabolizes the abundant neuropeptide N-acetyl-aspartyl-glutamate (NAAG) to N-acetylaspartate (NAA) and glutamate. 2-PMPA demonstrates robust efficacy in numerous animal models of neurological disease, however its pharmacokinetics has not yet been fully described. 2-PMPA is a highly polar compound with multiple negative charges causing significant challenges for analysis in biological matrices. Here we report a derivatization method for the acidic groups that involved protein precipitation with acetonitrile followed by reaction with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA). The silylated analyte with transitions (683→551.4) and the internal standard (669→537.2) were monitored by tandem mass spectrometry with electrospray positive ionization mode. The method was subsequently used to evaluate 2-PMPA pharmacokinetics in rats. Intraperitoneal administration of 100 mg/kg 2-PMPA resulted in maximum concentration in plasma of 275 µg/mL at 0.25 h. The half-life, area under the curve, apparent clearance, and volume of distribution were 0.64 h, 210 µg×h/mL, 7.93 mL/min/kg, and 0.44 L/kg, respectively. The tissue/plasma ratios in brain, sciatic nerve and dorsal root ganglion were **0.018, 0.120 and 0.142** respectively. In summary, a sensitive analytical method for 2-PMPA is reported that can be employed for similarly charged molecules.

Keywords

2-PMPA; glutamate carboxypeptidase-II; derivatization; pharmacokinetics; brain/plasma ratio

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1. Introduction

Glutamate carboxypeptidase II (GCP-II) (EC3.4.17.21) is a membrane-bound zinc metallopeptidase involved in the hydrolysis of N-acetylaspartylglutamate (NAAG) into N-acetylaspartate (NAA) and glutamate. Glutamate an essential excitatory neurotransmitter in brain, but at excessive extracellular levels is linked to both acute and chronic neurological disorders, including stroke, traumatic brain injury, amyotrophic lateral sclerosis, Alzheimer's dementia and Parkinson's disease [1-3]. Small molecule inhibitors of GCP-II have been shown to selectively decrease excess extracellular glutamate following injury, while having no effect on basal levels [4]. This effect is thought to be mediated both via increased NAAG which results in mGluR3 agonism as well as decreased glutamate. Thus GCP-II inhibition has been proposed as a possible therapeutic strategy for treatment of neurological disorders associated with glutamate excitotoxicity. 2-Phosphonomethyl pentanedioic acid (2-PMPA) is a highly potent and selective inhibitor of GCP-II, with an IC₅₀ value of 300 pM [5]. 2-PMPA has shown remarkable specificity for GCP-II with no apparent affinity for over 100 different receptors, ion channels, transporters, and enzymes including several glutamatergic sites such as NMDA, AMPA, and metabotropic glutamate receptors [4].

2-PMPA has been evaluated in several *in vitro* and *in vivo* models of neurological and psychiatric disorders, where it has been demonstrated to decrease extracellular glutamate and/or provide therapeutic benefit in models of ischemic brain injury [4, 6], neuropathic pain [7-9], peripheral neuropathies [7, 9], formalin-induced agitation [10], seizures [11, 12], morphine tolerance [13], cocaine-associated sensitization [14], amyotrophic lateral sclerosis [15], and most recently in cognition dysfunction associated with multiple sclerosis [16].

Although the utility 2-PMPA has been demonstrated in several animal models of disease the compound's extreme hydrophilic nature has impeded its development as a therapeutic drug. Most of the preclinical studies mentioned above have administered 2-PMPA at doses of 50-100 mg/kg i.p. or i.v. to produce the therapeutic effects despite its picomolar potency *in vitro*. The discrepancy between its excellent potency and use of large doses to produce efficacy warrants evaluation of pharmacokinetics of 2-PMPA for better understanding of its pharmacological effects as well as to facilitate its development. To date, no report has been presented evaluating the full pharmacokinetics of 2-PMPA. More importantly, it has been reported that 2-PMPA achieves micromolar concentrations in the brain [4, 17], however, its brain exposures relative to plasma (brain/plasma ratio) have never been evaluated.

Given the abundance of *in vivo* efficacy data, it would be beneficial to systematically quantitate 2-PMPA plasma pharmacokinetics as well as its penetrability into tissue and brain. However, the multiple negatively charged groups of the compound causes major challenges in its analysis particularly from plasma matrix by simple reverse phase chromatography. Here we report a relatively simple, selective and specific method employing N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) as a derivatizing agent [18]. This method was advantageous in allowing silylation of both the phosphonic acid and carboxylic acids on 2-PMPA, and enabling its detection by liquid chromatography with tandem mass spectrometry (LC/MS/MS). The method was validated for accuracy, precision, sensitivity, specificity, calibration curve range, and reproducibility according to the FDA guideline for validation of bioanalytical methods [19]. The method was then applied for the determination of 2-PMPA in plasma and tissues to characterize its pharmacokinetic parameters after i.p. administration in rats.

2. Materials and Methods

2-PMPA and 2-PMSA (internal standard) were synthesized as reported previously [5]. Formic acid (98%, v/v in water), and acetonitrile (HPLC grade) were obtained from EMD Chemicals Inc. (Gibbstown, NJ, USA). Deionized water was obtained from a Milli-Q-UF system (Millipore, Milford, MA, USA) and used for all aqueous solutions. Drug-free (blank) heparinized rat plasma was obtained from Innovative Research Inc. (Plymouth, MN, USA). N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). All other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO).

2.1 Calibration standards and quality control samples in plasma

All calibration standards and quality control (QC) samples of 2-PMPA were prepared fresh each day, prior to analysis. A stock solution of 1 mg/mL was prepared by dissolving accurately weighed 2-PMPA in methanol. The working solutions were prepared by stepwise dilution of stock in acetonitrile/water (1:1, v/v). Calibration standards were prepared at seven different levels by spiking heparinized rat plasma with appropriate working stock to obtain a final concentration of 0.1, 0.2, 0.5, 1, 2, 5 and 10 µg/mL, in duplicate. QC samples were prepared independently in blank plasma samples at five 2-PMPA concentrations including the lower limit of quantitation (LLOQ; 0.1 µg/mL), the low QC (LQC; 0.3 µg/mL), the medium QC (MQC; 4 µg/mL), the high QC (HQC; 8 µg/mL), and the above upper limits of quantitation (AULQ; 80) QC (after 1:10 dilution).

Linearity of calibration curves was computed based on peak area ratio (area of analyte/area of IS) using a least-squares linear regression analysis with 1/[nominal concentration] weight. The concentration of each standard was back calculated from calibration curve parameters which were then used to obtain values for the QC and unknown samples by interpolation.

2.2 Sample Preparation

The structures of underivatized 2-PMPA, and 2-PMSA are shown in Fig. 1. The preparation of the silyl ester derivatives of 2-PMPA (Fig. 2) from plasma samples consisted of protein precipitation, sample evaporation (300 µL supernatant), followed by the derivatization procedure as detailed below.

2-PMPA concentrations in rat plasma were determined using the validated method described above. The same method was employed for determining tissue concentrations of 2-PMPA. Prior to extraction, frozen samples were thawed at ambient temperature. For plasma samples, 100 µL of the calibration standard were transferred into glass test tubes. For brain tissues, the samples were weighed in a 1.7 mL silanized vials to which twice the volume of methanol (dilution 1:2) was added. For sciatic nerve and DRGs, 10 times the volume was added (1:10 dilution). The calibration curve for the tissues was developed in methanol. Sample preparation involved a single liquid extraction by addition of 300 µL of acetonitrile with internal standard (i.e., 1 µg/mL of 2-PMSA in acetonitrile for plasma and 0.1 µg/mL for tissues), followed by vortexing for 30 sec and then centrifugation at 2000 g for 10 min. Supernatant was transferred (~ 300 µL) and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was reconstituted with 100 µL of acetonitrile, samples were vortexed and 100 µL of derivatizing agent (MTBSTFA) was added. The samples were covered in parafilm, and heated at ~ 80 °C for 1 h [20]. At the end of 1 h, the derivatized samples were allowed to cool at room temperature and 100 µL of acetonitrile was added to each sample. The sample was transferred to a 250 µL polypropylene autosampler vial sealed with a teflon crimp cap and a volume of 10 µL was injected onto the ultra performance

liquid chromatography (UPLC) instrument for quantitative analysis using a temperature-controlled **autosampler** operating at 10°C.

2.3 Chromatographic and mass-spectroscopic conditions

Chromatographic analysis was performed using Waters Acquity UPLC (Milford, MA, USA). The mobile phase used for the chromatographic separation composed of 0.1% formic acid in acetonitrile and 2 mM ammonium acetate in water (80:20) delivered isocratically at a flow rate of 0.3 mL/minute. Separation of the analyte was achieved at ambient temperature using Waters **X-terra**®, RP18, 3.5 µm, (2.1 × 10 mm; Milford, MA, USA) column. The analyte was monitored using an AB SCIEX 5500 triple-quadrupole mass-spectrometric detector (Applied Biosystems, Foster City, CA, USA) that was equipped with an electrospray interface, and operated in a positive ion mode. The instrument was controlled by the Analyst version 1.5 software (Applied Biosystems). Samples were introduced to the interface through Turbo Ion Spray with the temperature setting at 450°C. A high positive voltage of 5.5 kV was applied to the ion spray. Nitrogen was used as the nebulizer gas and curtain gas, and argon as a collision gas with the settings of 30, 40 and 7, respectively. Other parameters were employed to optimize conditions for silylated 2-PMPA including declustering potential (DP) of 236, entrance potential (EP) of 10, collision energy (CE) of 40, and collision cell exit potential (CXP) of 54. The spectrometer was programmed in multiple reaction monitoring mode to allow the transition of the parent ion of derivatized 2-PMPA at m/z 683.0 and that of the internal standard 2-PMSA at m/z 669.0 pass through the first quadrupole (Q1) and into the collision cell (Q2). The daughter ions for derivatized 2-PMPA (m/z 551.4) and the internal standard (m/z 537.2) were monitored through the third quadrupole (Q3).

2.4 Specificity and Validation

The method specificity was evaluated by visual inspection of extracted rat plasmas from six different sources for the potential interferences from endogenous or exogenous substances.

The validation runs for the method were performed on three consecutive days. This included a calibration curve processed in duplicate, QC samples at five different concentrations in quintuplicate, and a single blank plasma and zero-level standard (blank with internal standard). Estimates of the between-run precision were obtained by one-way analysis of variance (ANOVA) as described previously [21]. The carry-over effect was evaluated following previously described methods [22]. In brief, blank mobile phase was injected following plasma samples containing the highest analyte concentration (10 µg/mL).

2.5 Stability

Bench-top stability of 2-PMPA in rat plasma was assessed for QC samples at 0.3, 4, 8, and 80 µg/mL (after 1:10 dilution) at room temperature for 4 h. Freeze/thaw stability was assessed for the QC samples thawed at room temperature and refrozen at –80°C over three cycles. The long-term stability of 2-PMPA in rat plasma at –80°C was evaluated by assaying QC samples 4 weeks following –80°C storage. The autosampler stability of derivatized 2-PMPA in rat plasma at 10°C was evaluated by assaying QC samples immediately and then reanalyzing after 18 h storage in the autosampler. Freshly processed standard samples were used to quantitate all the QC samples. All stability QC samples were analyzed in triplicate.

2.6 Matrix effect

The matrix effect in plasma was evaluated in QC samples at 0.3, 4, 8, and 80 µg/mL (after 1:10 dilution). Peak area ratios of 2-PMPA and internal standard directly spiked in

acetonitrile were compared to peak area ratio of 2-PMPA and internal standard in rat plasma post extraction.

2.7 Pharmacokinetics of 2-PMPA in rats

All the animal studies were performed as per protocols approved by the Institutional Animal Care and Use Committee, at Johns Hopkins University.

Male Wistar rats (6-8 weeks; weighing 200-250 g) obtained from Harlan® Laboratories (Indianapolis, IN) were maintained in a controlled environment and allowed food and water available ad libitum. 2-PMPA was administered to male Wistar rats as a single intraperitoneal (i.p.) dose. Dosing solutions were prepared on the day of the experiment in 50 mM HEPES buffered saline, (and pH adjusted to 7.4). At 0.08, 0.25, **0.5**, 1, 2, and 4 h post dose, blood samples were collected in heparinized microtubes by cardiac puncture immediately before sacrifice. Tissues (brains, sciatic nerves and DRG's) were dissected after exsanguination and immediately flash frozen (-80°C). Plasma was prepared by centrifugation immediately after collection of blood samples. 2-PMPA was assayed in plasma and tissues by the developed LC/MS/MS method described above. Samples with concentrations above the upper limit of quantitation were serially diluted to be within the analytical range. Tissue samples were then quantitated in $\mu\text{g/g}$ as: nominal concentration ($\mu\text{g/mL}$) $\times$ standardized dilution $\times$ additional dilution (where applicable).

2.8 PK analysis

Mean plasma and tissue concentrations of 2-PMPA were analyzed using non-compartmental method [23] as implemented in the computer software program WinNonlin Professional version 5.3 (Pharsight Corp., Mountain View, CA). The maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were the observed values. The area under the plasma concentration time curve (AUC) value was calculated to the last quantifiable sample (AUC_{last}) by use of the log-linear trapezoidal rule. The AUC values were extrapolated to infinity (AUC_{inf}) by dividing the last quantifiable concentration by the terminal disposition rate constant (λ_z), which was determined from the slope of the terminal phase of the concentration-time profile using a uniform weighting factor. The **elimination** half-life ($t_{1/2}$) was determined by dividing 0.693 by λ_z . The apparent volume of distribution (V_d/F) were calculated as dose divided by the product of (AUC_{inf}) and λ_z and the apparent clearance (Cl/F) was calculated by dividing the dose administered by AUC_{inf} . If the percent AUC extrapolated was $>20\%$ or the r^2 on the λ_z was <0.9 , the AUC_{inf} , Cl/F , V_d/F and $t_{1/2}$ were not reported.

3. Results

3.1 Mass Spectrometric detection

The structures of 2-PMPA and 2-PMSA are presented in Fig. 1. The structure of the silylated 2-PMPA is presented in Fig. 2. The mass spectra of derivatized 2-PMPA and 2-PMSA are shown as molecular ions $[\text{MH}]^+$ at m/z 683 and m/z 669 respectively (Fig. 3). The major fragments selected for subsequent monitoring in the third quadrupole for the derivatized 2-PMPA and 2-PMSA were at m/z 551.4 and 537.2 (Fig. 3) respectively. The precursor ions represent t-butyldimethyl-silylation (TBDMS) at all of the four acidic groups. The daughter ions represent desilylation at one of the functional groups.

3.2 Linearity of detector responses

Linearity of calibration curves calculated from peak area ratio (area of analyte/area of IS) as function of the nominal concentration was assessed by weighted ($1/\text{concentration}$) least

square regression over the range 0.1-10 µg/mL in plasma. Linear regression provided a unit slope and an intercept not significantly different from zero. For 2-PMPA plasma samples, the mean $\pm$ standard deviation of the equation was 0.0008 ± 0.0003 for the intercept and 0.00003 ± 0.00001 for the slope with a correlation coefficient 0.9984 ± 0.0009 .

For each point on the calibration curve in plasma, the concentrations back-calculated from the equation of the regression analysis were always within 96.4-103% for 2-PMPA (Table 1). The LLOQ for the derivatized 2-PMPA in plasma was established at 0.1 µg/mL with an accuracy within 8.4% and a precision 6.8%, and was associated with a mean ($\pm$ standard deviation) signal-to-noise ratio for 15 observations of 25.5 ± 4.9 . The LLOQ was based on preliminary experiments and was sufficient for pharmacokinetic studies in plasma following i.p. administration, which is the most common route of administration employed in published preclinical studies for 2-PMPA.

MS/MS, being a highly sensitive method, can be affected by carry-over. No detectable carry-over occurred in mobile phase runs after the highest concentration plasma sample determinations. The peak area in blank sample injected after highest calibrator (i.e. 10 µg/mL) was below 20% of peak area of LLOQ (0.1 µg/mL) indicating no impact on results presented.

3.3 Selectivity and specificity

No interfering peaks were observed in the chromatograms of blank plasma from 6 different rats. The LC/MS/MS method exhibits high specificity because only ions derived from the analytes of interest are monitored. The mean ($\pm$ standard deviation) retention times for the silyl ester derivatives of 2-PMPA and IS 2-PMSA were 1.35 ± 0.006 and 1.24 ± 0.006 min respectively. The total chromatographic run time was 5 min. The selectivity for the analysis is shown by symmetrical resolution of the peaks, with no significant chromatographic interference around the retention times of the analyte and internal standard in analyte-free specimens. Representative chromatograms of blank rat plasma, plasma spiked with internal standard and 2-PMPA, as well as rat plasma sample collected at 60 min after i.p. administration are shown in Fig. 4.

3.4 Accuracy and precision

The between and within day precision and accuracy of the assay for derivatized 2-PMPA were determined in plasma by analyses of 5 of QC samples in quintuplicate at LLOQ, the low QC, medium QC, high QC, and AULQ QC (after 1:10 dilution). For between day precision and accuracy, the complete analytical procedure was performed on three consecutive days and the data from the QC samples were examined by a one-way analysis of variance (ANOVA). The within run and the between-run variability (precision) for QC samples were within 12.1% for the derivatized 2-PMPA. Similarly, the mean predicted concentration accuracy ranged from 92.9% to 108.4% (Table 2). These results indicate that the method was reliable and reproducible for pharmacokinetic assessment of 2-PMPA.

3.5 Stability

The results of stability experiments using QC samples are detailed in Table 3. 2-PMPA was found to be stable at room temperature for 4h, repeated freeze/thaw cycles at -80°C , frozen condition at -80°C for 28 days, and in the autosampler at 10°C for at least 18 h, supporting the routine pharmacokinetic analyses of 2-PMPA.

3.6 Matrix effect

Matrix effects were evaluated with QC samples. The mean area responses of post-extracted samples compared with the response of analytes from neat standard samples dissolved in

mobile phase at equivalent concentrations was 112%, 111%, 109% and 100% from 0.3, 4., 8, and 80 (after 1:10 dilution) $\mu\text{g/mL}$, respectively. These data suggest no ion suppression occurred.

3.7 Pharmacokinetic Results for 2-PMPA

Plasma and tissue concentration-time profiles of 2-PMPA in rats are shown in Fig. 5. The pharmacokinetic parameters are presented in Table 4. After a single intraperitoneal dose of 100 mg/kg, 2-PMPA was readily absorbed and was detectable within the first 5 min in plasma. The peak plasma concentration (C_{max}) was found to be 275 $\mu\text{g/mL}$ and was observed at 0.25 h. The elimination $t_{1/2}$ value in plasma was calculated to be 0.64 h. The apparent volume of distribution was low and calculated to be 0.44 L/kg; and apparent clearance of 2-PMPA from plasma was 7.93 mL/min/kg (Table 4).

In addition to plasma, 2-PMPA was detected in all tissues (Fig. 5; Table 4) particularly in sciatic nerve and dorsal root ganglion (DRG's). The distribution to the brain, sciatic nerve and DRG was rapid and all of the tissues had measurable concentrations of 2-PMPA within the first 5 min. The C_{max} in sciatic nerve and DRG like plasma were also achieved at 0.25 h and were observed to be 18.5 $\mu\text{g/g}$ and 30.7 $\mu\text{g/g}$ respectively while in brain the maximum concentrations were almost 10 fold lower and more variable achieving only 3.28 $\mu\text{g/g}$ at 0.08 h. The (AUC_{inf}) was highest in plasma at 210 $\text{h}\times\mu\text{g/mL}$. The sciatic nerve and DRGS showed almost similar exposures of 24.4 $\text{h}\times\mu\text{g/g}$ and 30.6 $\text{h}\times\mu\text{g/g}$, based on AUC_{last} and AUC_{inf} , respectively, followed by brain which was 4.41 $\text{h}\times\mu\text{g/g}$ (Table 4). The relative exposures of tissues to plasma based on (AUC_{last}) were calculated to be 0.018 for brain and 0.120 and 0.142 for sciatic nerve and DRG, respectively. On average, the relative exposures in brain were lowest whether based on C_{max} or AUC_{last} .

4. Discussion

2-PMPA was the first potent and selective GCP-II inhibitor reported in 1996 by Jackson et al. Although hundreds of GCP-II inhibitors have been synthesized since, none have shown the potency, selectivity, and wide-spread efficacy in *in vitro* and *in vivo* models as 2-PMPA. In fact, 2-PMPA has shown therapeutic utility in over twenty preclinical models of neurological disorders wherein altered glutamatergic transmission is presumed pathogenic [24]. Although 2-PMPA is the most extensively studied GCP-II inhibitor, to-date there has been no report on its pharmacokinetics analysis perhaps due to a lack of a standard analytical assay which is particularly challenging owing to its extreme hydrophilic nature.

The present study describes a validated assay for analysis of 2-PMPA in rat plasma. MTBSTFA, was considered an appropriate reagent for derivatization, since it readily allowed esterification of all four acidic groups on 2-PMPA. The derivatization was essential for the transformation of this highly polar and poorly ionizable molecule, making it suitable for detection by LC/MS/MS. The transformation of 2-PMPA to a large non-polar molecule, however, led to peak fronting. The fronting may be improved with better chromatography which is a current focus of our laboratory. Overall, however, the method was reliable and reproducible with a relatively simple sample preparation and allowed systematic 2-PMPA pharmacokinetics assessment in both plasma and tissues including its presumed target site of action in brain and peripheral nerve.

Using this procedure, we report that following i.p. administration of 100 mg/kg, 2-PMPA was absorbed rapidly, reaching a maximum concentration of 275 $\mu\text{g/mL}$ ($\sim 1200 \mu\text{M}$) in plasma and 3.27 $\mu\text{g/g}$ ($\sim 15 \mu\text{M}$) in brain. The brain concentrations are consistent with a previous report of 50 μM concentration in brain at 1 h following a 100 mg/kg i.p. dose of radiolabeled 2-PMPA [4]. However these concentrations in brain may be misleading. When

assessing the brain exposures relative to plasma based on AUC_{inf} , the brain to plasma ratio was calculated to be 1.8%. Given this low ratio, it is conceivable that some of measured 2-PMPA in the brain may be due to residual blood contamination [24]. However, in another study conducted by Nagel et al. using in vivo microdialysis [17] it was reported that i.p. administration of 2-PMPA (50 mg/kg) produced a mean peak concentration of 29.6 μ M in dialysate. Although plasma 2-PMPA levels were not measured in this study for comparison, these data suggest that significant blood contamination may not be relevant since measured ECF concentrations are not typically thought to be indicative of blood contamination. The disparity between the two studies is perhaps due to differences in assays and methodologies between labs and further emphasizes the need for a robust validated assay for 2-PMPA.

In contrast to the central nervous system, in the peripheral nervous system the tissue to plasma ratio was ~14%, suggesting better penetrability at these sites. These data are consistent with the generally more potent efficacy of GCP-II inhibitors in the peripheral nervous system models including model of chemotherapy-induced peripheral neuropathy [25], diabetic neuropathy and neuropathic pain [25].

In summary, we present, for the first time, a robust, sensitive, and reliable bioanalytical method to quantitate 2-PMPA from plasma and tissues. Using this method we report the first systematic pharmacokinetic analysis of 2-PMPA, one of the most potent, selective, and well-studied GCP-II inhibitors. Having access to a validated assay and its pharmacokinetic data may further aid in conducting PK/PD analysis, employing specific strategies such as prodrug development, formulation enhancements or alternative routes of delivery and ultimately enable its clinical development

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Abbreviations

GCP-II	glutamate carboxypeptidase-II
PSMA	prostate specific membrane antigen
NAALADase	N-acetylated-alpha-linked acidic dipeptidase
NAAG	N-acetyl-aspartyl glutamate
2-PMPA	2-phosphonomethyl pentanedioic acid
2-PMSA	2-phosphonomethyl succinic acid
DRG	dorsal root ganglion
MTBSTFA	N-tert-butyldimethylsilyl-N-methyl trifluoroacetamide
LLOQ	lower limit of quantitation
LQC	low quality control
MQC	medium quality control
HQC	high quality control
AULQ	above upper limits of quantitation
DP	declustering potential

EP	entrance potential
CE	collision energy
CXP	collision cell exit potential
ANOVA	one-way analysis of variance
AUC	area under the concentration-time curve
C_{max}	maximum plasma concentration
T_{max}	time to C _{max}

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Highlights

- 2-PMPA is a potent and selective glutamate carboxypeptidase II inhibitor
- 2-PMPA has shown efficacy in >20 animal models of neurological/psychiatric diseases
- Bioanalysis of 2-PMPA has been challenging due to its multiple negative charges
- We report derivatization method enabling 2-PMPA detection by LC/MS/MS
- We report, for first time, 2-PMPA plasma pharmacokinetics and tissue distribution

A

2-phosphonomethyl pentanedioic acid

B

2-phosphonomethyl succinic acid (internal standard)

Fig. 1. Chemical structure of (A) 2-phosphonomethyl pentanedioic acid (2-PMPA) and (B) 2-phosphonomethyl succinic acid (2-PMSA; internal standard).

Fig.2.

Derivatization reaction for 2-PMPA with *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA). Four TBDMS groups were incorporated on each of the acidic groups (i.e. two on the carboxylates and two on phosphonic acid) forming a nonpolar compound that could be retained on a reverse phase column.

Fig.3.
The mass spectra of daughter scan for (A) silylated 2-PMPA at m/z 683 $\rightarrow$ 551.4 and (B) silylated 2-PMSA at m/z 669 $\rightarrow$ 537.2

Fig. 4. Chromatograms of blank rat plasma (A, B), plasma spiked with 0.1 $\mu\text{g/mL}$ 2-PMPA (C), 1 $\mu\text{g/mL}$ 2-PMSA (D), and rat plasma 1 h after receiving a 100 mg/kg i.p. dose of 2-PMPA. (E, F).

Fig. 5.
Mean concentration profiles for 2-PMPA in rat plasma (●), brain (◆), sciatic nerve (■) and DRG (▼) following i.p. administration of 100 mg/kg. (* represents n=1 rats at 30 min time point)

Table 1Back-calculated concentrations from calibration curves for 2-PMPA in plasma^a

Condition	Calculated Concentration ($\mu\text{g/mL}$) ^b	Accuracy (%)	Precision(%)	
			Within-Run	Between-Run
0.1	0.102 ± 0.007	102	5.85	3.49
0.2	0.206 ± 0.017	103	10.3	_c
0.5	0.504 ± 0.038	101	7.84	_c
1	0.964 ± 0.098	96.4	11.5	_c
2	1.93 ± 0.13	96.6	8.52	_c
5	5.06 ± 0.25	101	4.51	2.47
10	9.88 ± 0.61	98.8	7.23	_c

^aPerformed in duplicate on 3 separate days.^bValues are mean $\pm$ standard deviation.^cNo significant variation was observed as a result of performing the assay in different runs.

Table 2Assessment of accuracy and precision of 2-PMPA in rat plasma^a

Nominal Concentration (µg/mL)	Calculated Concentration (µg/mL)	Accuracy (%)	Precision (%)	
			Within-Run	Between-Run
0.1	0.108 ± 0.0	108.4	6.84	6.76
0.3	0.304 ± 0.0	101.2	4.89	9.34
4	4.04 ± 0.0	100.9	6.87	8.97
8	7.45 ± 0.004	92.9	4.29	_c
8 (1:10) ^d	7.69 ± 0.003	96.1	3.54	12.10

^a Performed in quintuplicate on three separate days^b Not determined^c No significant variation was observed as a result of performing the assay in different runs.^d Sample diluted 1:10 (v/v) prior to analysis

Table.3

Assessment of stability for 2-PMPA in rat plasma

Conditions	Nominal Concentration (µg/mL)	Calculated Concentration. (µg/mL)	%RE
Bench Top (Room temperature)			
	0.3	0.326 ± 0.009	8.30%
	4	4.22 ± 0.28	5.48%
	8	8.84 ± 0.18	10.5%
	8 ^a	7.89 ± 0.17	-1.35%
Freeze Thaw (-80°C)			
Cycle 1	0.3	0.349 ± 0.005	16.0%
	4	4.26 ± 0.11	6.46%
	8	7.86 ± 0.10	-1.80%
Cycle 2	0.3	0.318 ± 0.017	5.86%
	4	3.99 ± 0.05	0.01%
	8	7.59 ± 0.14	-5.10%
Cycle 3	0.3	0.355 ± 0.009	18.6%
	4	3.89 ± 0.19	-2.71%
	8	7.66 ± 0.33	-4.30%
Autosampler (10°C)			
	0.3	0.338 ± 0.014	12.7%
	4	4.27 ± 0.048	6.70%
	8	8.06 ± 0.45	0.80%
	8 ^a	8.14 ± 0.47	1.73%
Long Term (-80°C)			
28 days	0.3	0.283 ± 0.85	-15.2%
	4	3.85 ± 0.13	-7.14%
	8	7.73 ± 0.27	-6.80%
	8 ^a	8.11 0± 0.28	5.33%

^aSample diluted 1:10 (v/v) prior to analysis

Table 4

Pharmacokinetics parameters of 2-PMPA following i.p. administration (100 mg/kg) in rats

	$C_{\max}^a$ ($\mu\text{g/mL}$, $\mu\text{g/g}$)	$T_{\max}^a$ (h)	AUC_{last}^a ($\text{h}\cdot\mu\text{g/mL}$ or $\text{h}\cdot\mu\text{g/g}$)	$(AUC_{\text{inf}})^a$ ($\text{h}\cdot\mu\text{g/mL}$ or $\text{h}\cdot\mu\text{g/g}$)	Cl/F^b (mL/min/kg)	V_d/F^b (L/kg)	$t_{1/2}^a$ (h)	Tissue/Plasma Ratio ^c
Plasma	275	0.25	207	210	7.93	0.44	0.64	
Brain	3.28	0.08	4.27	N.R. ^e	N.C. ^d	N.C. ^d	N.C. ^d	0.020
Sciatic Nerve	18.6	0.25	24.5	N.R. ^e	N.C. ^d	N.C. ^d	N.C. ^d	0.120
Dorsal Root Ganglion (DRG)	30.7	0.25	29.4	30.6	N.C. ^d	N.C. ^d	N.C. ^d	0.142

^aData are presented as mean.; n=3 rats per time point (except for 0.5 h, where n=1), Data are presented as $\mu\text{g/mL}$ for plasma or $\mu\text{g/g}$ for tissue.^bFor i.p. data, apparent clearance (Cl/F) and apparent volume (V_d/F) are presented^cBased on AUC_{last} for plasma^dNot Calculated^eNot reported; AUC%extrapolated >20%