1 **Simple and Rapid LC-MS/MS Method for Determination of Perampanel in**

2 **Human Plasma and Application to Bioequivalence Study**

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13**Abstract**

14Background: Perampanel (PER) is a third-generation anti-epileptic drugs (AEDs).
15Several methods have been developed for the quantification of perampanel in plasma.
16The pharmacokinetic characteristics of perampanel in healthy Chinese Subjects have
17not been comprehensively reported. Objective: A simple, fast and sensitive LC-
18MS/MS method was established and validated for the quantification of perampanel in
19human plasma and its application to a bioequivalence study. Methods:
20Chromatographic separation was accomplished on a ZORBAX Eclipse XDB-Phenyl
21column (4.6 mm × 75 mm, 3.5 μm) using a binary gradient with mobile phase A (5
22mmol/L ammonium acetate containing 0.1% formic acid) and B (acetonitrile-water
23(95:5, v/v)) at a flow rate of 0.9 mL/min and sample preparation was by one-step
24protein precipitation via acetonitrile. Results: The total run time in this study was 4.5
25min and the retention time of perampanel and perampanel-d5 (internal standard) were
262.30 min and 2.32 min, respectively. The method was developed and validated over
27the concentration range of 2.00-500 ng/mL for perampanel, with correlation
28coefficient greater than 0.9992. The inter-day precision was 3.1%-3.8% and accuracy
2998.9%-103.5%. The intra-day precision was 2.4%-6.8% and accuracy 97.6%-104.9%.
30The extraction recovery ranged from 99.23%-103.84% and the matrix effect was not
31significant. Perampanel was proved to be stable in solution and human plasma under
32the different tested conditions. The validated method was successfully applied to a
33randomized, open-label, 2-period, crossover bioequivalence study in healthy Chinese
34subjects, and the results indicated that bioequivalence was achieved for 2 formulations

35of the 4-mg perampanel tablet under both fasting and fed conditions, and both
36treatments were safe and well tolerated by all study subjects. Conclusion: The
37validated method was successfully applied to a bioequivalence study of perampanel in
38human plasma and has achieved satisfactory results.
39
40***Keywords:*** Perampanel, LC-MS/MS, human plasma, bioequivalence study, protein
41precipitation, pharmacokinetic

42 **1. Introduction**

43 Epilepsy is one of the most common chronic nervous system diseases and it is the
44 second most common neurological disease after headache [1]. It affects about 50
45 million people of different ages in the world, of which nearly 85% of the cases occur
46 in developing and underdeveloped countries [2, 3]. Epilepsy is primarily caused by
47 infection, head injury, birth trauma, hypoxic-ischemic insult, or any of a number of
48 other perturbations of nervous system function [4]. Epilepsy is a treatable disease,
49 most patients are treated with anti-epileptic drugs (AEDs), and up to 70% of them
50 have disappeared seizures [5].

51

52 Perampanel (2-[2-oxo-1-phenyl-5-pyridin-2-y1-1,2 dihydropyridin-3-y1] benzonitrile
53 hydrate) is a novel non-competitive selective antagonist at the postsynaptic ionotropic
54 alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) glutamate
55 receptor. Studies suggest that AMPA receptor antagonism could lead to inhibition of
56 seizure generation and spread reducing neuronal excitability [6-8]. Perampanel has
57 been approved by the US Food and Drug Administration and by the European
58 Medicines Agency for the adjunctive therapy of focal seizures and primary
59 generalized tonic–clonic seizures associated with idiopathic generalized epilepsy in
60 patients aged 12 years and older [9]. Perampanel is a third-generation AEDs, which
61 shows advantages in terms of efficacy, safety and tolerability [10]. Perampanel has a
62 high protein-binding ratio (> 95 %) and long half-life (~105 h) and is mainly
63 metabolized by cytochrome P450 (CYP) 3A4 [11, 12]. The median time to reach peak

64concentration varies between 0.5 and 2.5 hours in fasting patients and increases up to

65three hours after food intake [9].

66

67Upon surveying the literature, several analytical methods have been proposed for the

68evaluation of perampanel plasma concentration, using different detection techniques.

69A number of methods including high performance liquid chromatography (HPLC)-

70ultraviolet (UV) detector [13], HPLC-fluorescence (FL) detector [14, 15], HPLC-

71UV/FL [16] have been established to measure perampanel concentration in human

72plasma. Most of these methods have low sensitivity, require a relatively large volume

73of plasma, and involve a time-consuming extraction process. David Paul developed an

74ultra high-performance liquid chromatography-quadrupole time-of-fight-mass

75spectrometry (UHPLC-QTOF-MS) method, which has been developed to measure

76perampanel and its metabolites in both rat plasma and rat brain homogenate [17].

77Currently, LC-MS/MS methods are considered the first choice as a platform for the

78bioanalytical assay. But the reported LC-MS [18] and LC-MS/MS [19] methods have

79relatively long sample run time and complicated sample extraction, such as a liquid-

80liquid extraction procedure or a time-consuming evaporation process. These methods

81may not be feasible for high throughput sample analysis. Our lab has previously

82developed a method of impurities in perampanel bulk drugs using HPLC and gas

83chromatography (GC) [20].

84

85

86Bioequivalence studies provide compelling evidence for generic drugs that are 87comparable to innovators in pharmacokinetics [21, 22]. Shiba S reported 88bioequivalence experiments in fasting condition for the original tablet and granule 89formulations [23]. Yerino GA reported bioequivalence experiments in fasting 90condition for European populations [24]. There has been no evaluation of the 91bioequivalence of tablets in Chinese subjects under fasting and fed conditions. 92However, the above literature only evaluated the pharmacokinetics in fasting 93condition and within 168 h after administration, so as to evaluate the bioequivalence. 94This may not be sufficient to evaluate the bioequivalence of perampanel tablets. In 95this study, we performed a bioequivalence study under both fasted and fed conditions. 96The half-life of perampanel is 105 hours, the time point of blood collection reached 3-975 half-lives in this study.

98

99The objective of the present study is to develop a simple, rapid, specific, sensitive and 100reproducible LC-MS/MS method for the determination of perampanel in human 101plasma using protein precipitation method. This method showed low plasma 102consumption and simple pretreatment with a short analysis time. The proposed 103method was successfully applied to a bioequivalence study of 4 mg perampanel tablet 104formulation in 60 healthy subjects under fasting and fed conditions.

105

106**2. Materials and methods**

107*2.1. Chemicals and reagents*

108 Perampanel (PER, 96.1% purity) and Perampanel-d5 (internal standard, IS, ≥95.9%
109 purity) were purchased from TLC Pharmaceutical Standards Ltd. (Ontario, Canada).
110 Formic acid, acetonitrile and methanol of HPLC grade were purchased from Merck
111 (Darmstadt, Germany). LC-MS grade formic acid and ammonium formate were
112 products of Aladdin (Shanghai, China). Ultrapure water was obtained from Direct-Q
113 3UV Water Purification System (Millipore, Massachusetts, USA).
114
115 *2.2. Instrumentation and analytical conditions*
116 *2.2.1. Chromatography*
117 A Shimadzu 30AD HPLC system (Shimadzu, Kyoto, Japan) was used for
118 chromatographic separation with a ZORBAX Eclipse XDB-Phenyl column (4.6
119 mm×75 mm, 3.5 μm) maintained at 40°C. The gradient mobile phases consisted of
120 (A) 5 mmol/L ammonium acetate containing 0.1% formic acid and (B) acetonitrile-
121 water (95:5, v/v) , which were eluted at a flow rate of 0.9 mL/min. Gradient elution
122 program was as follows: 0.00-0.50 min, B: 55%; 0.50-2.30 min, B: 55%~75%; 2.30-
123 3.50 min,
124 B: 75%; 3.50-4.00 min, B: 75%~55%; 4.00-4.50 min, B: 55%. The total run time was
125 4.5 min per assay. Under these conditions, PER and IS typically eluted after 2.30 min
126 and 2.32 min, respectively.
127
128 *2.2.2. Mass spectrometry*
129 Perampanel and IS detection and quantification was performed using API 5500 triple

130quadrupole mass spectrometer (AB Sciex LLC, CA, USA) with a Turbo ionspray™ 131probe in positive mode operating at 5500V. To enhance sensitivity and specificity, 132multiple reaction monitoring (MRM) was applied. The source-dependent parameters 133were set as follows: curtain gas, 25 psi; collision gas, 7 psi; medium temperature, 134550°C; nebulizer gas (GS1) at 55 psi and heater gas (GS2) at 55 psi. The compound 135parameters including the declustering potential (DP), entrance potential (EP), 136collision energy (CE), and collision cell exit potential (CXP) were 100 V, 10 V, 36 V, 137and 18 V for PER, respectively, and 135 V, 10 V, 31 V, and 18 V for IS, respectively. 138Data acquisition and processing was completed using Analyst software (version 1391.6.3). The *m/z* transition used to monitor PER was 350.2 for the protonated molecule 140to 247.2 for the fragmention. The *m/z* ratio of the parent ion and product ion for IS 141were 355.2 and 248.2, respectively.

142

143*2.3. Preparation of calibration standards and quality control samples*

144Stock solutions of PER and IS at 1.00 mg/mL were prepared in acetonitrile. The stock 145solutions were stored at the condition of -20°C. Perampanel working standard 146solutions were prepared by serial dilution and IS working standard solution of 100 147ng/mL was prepared by dilution with methanol-water (1:1, v/v). Perampanel 148calibration standards and quality control (QC) samples were prepared by spiking 10 149μL of the appropriate perampanel working solution in 190 μL of blank human plasma 150with sodium heparin, resulting in calibration standard concentrations of 2.00, 4.00, 15110.0, 50.0, 150, 250, 450, and 500 ng/mL. . The QC samples were prepared at three

152different concentration levels, 6.00, 30.0 375375, 30.0, 6.00 ng/mL in addition to the
153lower limit of quantitation (LLOQ, 2.00 ng/mL).

154

155*2.4. Sample extraction*

15650 μL of internal standard was added to 500 μL plasma samples, and then protein
157precipitation was initiated by adding 400 μL of acetonitrile to each sample. The
158mixture was vortexed for 5 min followed by centrifuging at 4000 rpm, 4°C for 5 min.
159Then 500 μL of supernatant was removed and diluted with 400 μL acetonitrile-water
160(1:1, v/v). Subsequently, 2 μL of the solution was injected into LC–MS/MS system.

161

162*2.5. Method validation*

163An assay method validation study was performed in accordance with bioanalytical
164guidelines [25].

165*2.5.1. Selectivity and carryover*

166The selectivity of the method was examined by analysing 6 sources of blank human
167plasma which were individually analyzed and evaluated for interference at the
168retention times of the analyte and IS. Furthermore, the interference of IS to analytes
169was assessed by three different lots of blank human plasma spiked with IS, the
170interference of analytes to IS was assessed by three duplicates of upper limit of
171quantitation (ULOQ) without IS. Responses detected and attributable to interfering
172components should not be more than 20% of the analyte response at LLOQ and not
173more than 5% of the IS response in the LLOQ sample for each matrix.

174

175Carryover was detected by injecting blank samples subsequently after injecting 176ULOQ sample in calibration standard. The carryover should be within 20% of the 177analyte response at the LLOQ and 5% of the response for the IS.

178

179*2.5.2. Linearity*

180Linearity within the standard concentration range, 2.00–500 ng/mL, in human plasma 181was evaluated. The calibration curves were constructed by plotting the ratio of 182perampanel signal to IS signal as a function of perampanel concentrations. The 183equation model was obtained by weighted least squares linear regression analysis with 184a weighting factor of $1/x^2$. To assess linearity, the accuracy of the back-calculated 185concentrations of each calibration standard should be within ±15% (±20.0% at 186LLOQ) of the nominal concentration and the correlation coefficient (r^2) should be 187higher than 0.99. At least 75% of calibration standards and at least 6 calibration 188standard levels should meet the above criteria.

189

190*2.5.3. Precision and accuracy*

191The intra-day and inter-day precision and accuracy were determined by analysis at 4 192concentration levels, 2.00 ng/mL (LLOQ), 6.00 ng/mL (low QC), 30.0 ng/mL 193(medium QC) and 375 ng/mL (high QC), covering the calibration curve range. For 194evaluation of intra-day and inter-day precision and accuracy, 6 samples of each QC 195concentration were analyzed at 3 different days. The acceptance criterion is that the 196overall accuracy at each concentration level should be within ±15% (±20.0% at

197LLOQ) of the nominal concentration. The precision (coefficient of variation, CV%,)
198of the concentrations determined at each level should not exceed 15% (±20.0% at
199LLOQ). At least 50% of the samples at each QC level and at least 2/3 of all QC
200samples must meet the above criteria.

201

202*2.5.4. Matrix effects and extraction recovery*

203The matrix effect evaluated by analysing 6 replicates of low, medium and high QCs,
204each prepared using matrix from at least 6 different sources. It was determined by
205calculating peak area ratios of perampanel and IS with matrix and neat
206solution.Matrix effect was assessed using the CV% of the IS-normalized matrix factor
207from 6 batches of human plasma. When the CV% of the IS normalized matrix factor
208of each QC level does not exceed 15.0, it is considered acceptable.

209

210Extraction recovery was evaluated by comparing the peak areas of the analytes or IS
211of the un-extracted sample to that of the extracted sample. For extracted samples,
212proceed as described under Section 2.4. Meanwhile, for un-extracted samples,
213perampanel and IS working solution was added to supernatant so that the final
214concentration is consistent with the extracted sample concentration. Three QC levels
215(375, 30.0 and 6.00 ng/mL) were evaluated in six replicates. The CV of the recovery
216for each QC level should be no more than 15.0%.

217

218*2.5.5. Dilution integrity*

219Dilution integrity was evaluated to ensure that sample dilution with the same matrix
220did not have any effects on the reliability of the method. Perampanel spiked human
221plasma samples were prepared at concentrations of 1500 ng/mL. These solutions were
222further diluted with pooled human plasma four folds in six replicates and analyzed.
223
224*2.5.6. Stability*
225The stability of perampanel and IS in human plasma and stock solution was tested
226under different storage conditions. Stock solution and work solution stabilities were
227evaluated after 46 h at room temperature and 51 days in -20°C refrigerator,
228respectively. Matrix stability was assessed with LQC and HQC samples under
229different conditions. The experiments were performed in three replicates at each QC
230level to determine freeze-thaw stability (5 cycles at −80°C temperature), short-term
231stability (at room temperature for 28 h), post-preparative stability (in auto-sampler),
232and long-term stability (at −80°C for 100 days).
233
234*2.5.7. Application to bioequivalence study*
235The validated method was used to the quantitation of perampanel in human plasma
236samples obtained from a bioequivalence study comparing reference perampanel tablet
237(Eisai Co.,Ltd., Japan) and test perampanel tablet (Nanjing Healthnice Pharmaceutical
238Co., Ltd., China). Sixty healthy Chinese volunteers enrolled in randomized, single-
239dose, open-label, two-period, two-treatment, two-sequence cross over bioequivalence
240study under fasting condition and fed condition. The study was conducted in

241compliance with the ethical principles of the Helsinki Declaration 's. Signed and
242dated informed consent was obtained from all subjects. Of these, 30 volunteers were
243in the fasting group and an additional 30 volunteers were in the fed group.
244
245Under fasting condition, the subjects were administered 4 mg perampanel tablet with
246240 mL of water with a wash-out period of 42 days. Venous blood samples were
247collected before dosing and at 0.083, 0.17, 0.33, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3,
2484, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336, 360 and
249384 h. Under fed condition, the subjects were administered test or reference products
250with 240 mL of water after a high-fat, high-calorie breakfast based. An open-label,
251randomized, single-dose study has shown that food affects the rate of absorption, so
252venous blood samples were collected at administration 0.17, 0.33, 0.67, 1, 1.33, 1.67,
2532, 2.33, 2.67, 3, 3.33, 3.67, 4, 4.5, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216,
254240, 264, 288, 312, 336, 360 and 384h. At predetermined time points, Approximately
2554 mL of blood samples were collected into heparin sodium anticoagulation negative
256pressure blood collection tube, stored on ice for no more than 1 h, and centrifuged at
2572000 × g and 4°C for 10 min. Plasma was separated and stored at −80°C until
258analysis.
259

260**3. Results and discussion**

261*3.1. Method development*

262The objective of the present work was to develop and validate a simple, selective and

263sensitive method for perampanel in human plasma by LC-MS/MS for routine sample

264analysis. In this study, stable isotope-labeled perampanel-d5, which had

265chromatographic and mass spectrometric behavior similar to perampanel, was

266considered as IS. Perampanel and IS demonstrated improved sensitivity using ESI

267source than APCI, with a relatively stronger response in the positive-ion mode. The

268mass parameters were optimized for the quantification of perampanel and IS in human

269plasma. MRM mode of *m/z* 350.2→247.4 (perampanel) and *m/z* 355.0→248.2 (IS)

270was employed in this study. Collision energy, nebulizing gas flow, heater gas flow,

271declustering potential, entrance potential, collision cell exit potential, temperature

272were optimized to achieve the maximum MS responses. The optimum for parameters

273is present in Section 2.2. The fragmentation mass spectra of perampanel and

274perampanel-d5 are shown in Figure 1 (a, b).

275

276Chromatography conditions, including mobile phase, flow rate, column type and

277injection volume, were evaluated in order to achieve the best peak shape, and

278retention for perampanel and perampanel-d5 while minimizing the total sample

279running time. Different combinations of water-acetonitrile or methanol-acetonitrile

280and acidic buffers (ammonium formate/formic acid, ammonium acetate/acetic acid) of

281different strengths (2.0-10 mM) were tested as mobile phase. After the optimization of

282various mobile phase compositions and chromatographic columns, the selected

283mobile phase was consisted of acetonitrile-water (95:5, v/v) (A) and water (B)

284containing 5 mmol/L ammonium acetate and 0.1% formic acid. Further, several

285stationary phases were tested to optimize the proposed chromatographic method such
286as Zorbax Eclipse XDB-Phenyl column (150 mm × 4.6 mm, 5 μm), Ultimate XB-C18
287column (100 mm × 4.6 mm, 5 μm) and Zorbax Eclipse XDB-Phenyl column (75 mm
288× 4.6 mm, 5 μm). The use of C18 columns resulted in poor peak shape. Trials by
289Zorbax Eclipse XDB-Phenyl column exhibited long analysis time. Optimum
290performance was displayed using Zorbax Eclipse XDB-Phenyl column in terms of
291high resolution, short run time and symmetrical peaks. In addition, the effect of total
292flow rate was also studied from 0.5 to 1.0 mL/min, which was responsible for
293acceptable chromatographic peak shapes and to separate endogenous peaks. Flow rate
294set at 0.9 mL/min produced a good peak shape and brought the running time to 4.5
295min. Final chromatographic separation was performed at 40°C with a gradient mobile
296phase (0.9 mL/min) and a Zorbax Eclipse XDB-Phenyl column (75 mm × 4.6 mm, 5
297μm). The total running time was 4.5 min under the above chromatographic conditions.
298The injection volume was 2 μL.

299

300Protein precipitation was used in the sample preparation. Acetonitrile and methanol
301were tried as deproteinizing agents in the study. Acetonitrile produced cleaner plasma
302sample, and no matrix interference was observed for analyte and IS.

303

304*3.2. Method validation*

305*3.2.1 Selectivity and carryover*

306In the selectivity assessment, no interfering peaks that eluted at the retention times of

307perampanel and the IS were detected in the blank plasma samples obtained from six
308individuals Figure 2 (a-d). Validation results showed that the IS did not interfere with
309the analyte, and the analyte did not interfere with the internal standard. The carryover
310assessment confirmed that no carryover occurred, and minimal peaks were observed
311at the retention times of perampanel and the IS in the blank samples injected just after
312the ULOQ sample.

313

314*3.2.2. Linearity*

315Linearity was evaluated by preparing eight calibration curves in human plasma, and
316calibration standards covering the range of 2.00–500ng/mL for analyte. The best
317linear fit and least-squares residuals for the calibration curve were achieved with a
3181/x^2 weighting factor, giving a correlation coefficient $r^2 \geq 0.99$. The typical regression
319equation of the calibration curve was y=0.0229x+0.00776, where y is the peak area
320ratio of the analyte to the IS, and x is the concentration of the analyte.

321

322*3.2.3. Precision and accuracy*

323The inter- and intra-day precision and accuracy for perampanel in human plasma were
324presented in Table 1. For LLQC, low, medium, and high QC, the mean accuracies of
325the inter-day QC samples ranged from 98.9% to 103.5%, with CVs ranging from
3263.1% to 3.8%. The mean intra-day accuracies of QC samples ranged from 98.7% to
327104.9%. The CVs for all intra-day QC samples ranged from 2.4% to 6.8%. The results
328indicated that this method was precise and accurate.

329

330 *3.2.4. Matrix effects and extraction recovery*

331 The matrix effect and extraction recovery values for analyte at LQC, MQC and HQC
332 are presented in Table 2. The mean IS normalised matrix factors of the 6 different
333 sources of blank plasma on perampanel at three QC levels (6.00, 30.0 and 400 ng/mL)
334 were 102.73% ± 3.54%, 102.23% ± 1.05% and 103.83% ± 1.10%, respectively. The
335 results were within the acceptable limits (100 ± 15.00%), and there was no ion
336 suppression or ion enhancement in the plasma matrix on detection of the perampanel
337 or IS. The mean extraction recoveries for perampanel at three QC concentrations were
338 99.50%, 103.10% and 99.23%, respectively. The CV of extraction recovery was
339 within 1.7%. The results indicated that the chosen sample extraction procedure
340 resulted in satisfactory extraction recoveries with negligible matrix effect, not
341 significant concentration dependent, and the extraction method was reproducible.

342

343 *3.2.5. Dilution integrity*

344 Six samples at 1500 ng/mL were prepared, diluted with the same biological matrix to
345 5.0 times (dilution factor) analyzed. The precision for dilution integrity was 2.0%, and
346 the accuracy result was 101.9%. The results were within the acceptance limit of 15%
347 (CV) for precision and 85.0–115.0% for accuracy.

348

349 *3.2.6. Stability*

350 The stability of perampanel in human plasma and stock solution was tested under

351different storage conditions. The results are presented in Table 3. The stock standard

352solutions of perampanel was stable in a refrigerator at -20°C for 51 days and was

353stable at controlled room temperature (25 ± 5°C) for 46 h. Perampanel in human

354plasma was stable for three freeze–thaw cycles, for at least 28 h in human plasma at

355room temperature (25°C), for 100 days at −80°C. The post-preparative stability was

356also studied. These results suggest that perampanel was stable for application in the

357routine analysis.

358

359*3.2.7. Application to bioequivalence study*

360A validated method was used for bioequivalence studies of perampanel. All subjects

361received a single oral dose of 1 tablet, each containing 4 mg of perampanel in the

362morning of each trial period, on either the test or reference drug. The specific test

363scheme is present in Section 2.5.7. All 30 randomized subjects from fed conditions

364were included in the pharmacokinetic analyses. In the fasted study, two cases were

365withdrawn because of sudden situation. Consequently, only 28 subjects were included

366in the PK analysis of the reference PER tablet.

367The mean plasma concentration–time curves of profiles of the two formulations of

368perampanel after oral administration of 4 mg to Chinese healthy volunteers are

369presented in Figure 3.(a, b). The pharmacokinetic parameters were calculated through

370non-compartmental analysis using DAS 3.2.8 software (Table 4). Pharmacokinetic

371parameters (T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{½}$) were similar between the reference and

372test products. The 90% confidence interval of the geometric mean ratios of

373test/reference were within 80%-125% for C_{max}, $AUC_{0\text{–}t}$ and $AUC_{0\text{–}\infty}$ as shown in Table

3745. The difference between means of T_{max} is not significant (P > 0.05) with respect to

375DAS Signed-Rank Test.

376The pharmacokinetic parameters of reference product were analyzed. In fasting

377condition, the $AUC_{0\rightarrow\infty}$, T_{max} and $t_{½}$ of chinese subjects were similar to literature

378reported [26]. Compared with the fasting condition, the absorption of PER in chinese

379subjects was delayed, without affecting the absorption degree under fed

380conditions，and it was in accordance with the drug instructions of reference product

381[9].

383

384 **4. Conclusions**

385A simple, rapid, sensitive, selective and reproducible LC-MS/MS method was

386successfully developed and validated for the quantitation of perampanel in human

387plasma, and it was applied to a bioequivalence study. Sample preparation by protein

388precipitation was fast and simple, in one step and demonstrated good recovery results.

389The method was fully validated according to US-FDA guidelines [25]. The low limit

390of quantification allows utilization of the method for clinical and pharmacokinetic

391studies. The high throughput of the method permits the analysis of large number of

392human plasma samples per day. The method was successfully applied to a

393bioequivalence study in healthy Chinese human volunteers.

394

395**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

396The study was approved by ethics committee of Nanjing Technology University.

397

398**HUMAN AND ANIMAL RIGHTS**

399No animals were used in this research. All human research procedures followed were 400in accordance with the ethical standards of the committee responsible for human 401experimentation (institutional and national), and with the Helsinki Declaration of 4021975, as revised in 2013.

403

404**CONSENT FOR PUBLICATION**

405Signed and dated informed consent was obtained from all subjects.

406

407**AVAILABILITY OF DATA AND MATERIALS**

408Not applicable.

409

410**FUNDING**

411None.

412

413**CONFLICT OF INTEREST**

414The authors declare no conflict of interest, financial or otherwise.

415

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418

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514 **LEGENTS TO FIGURES**

515 Figure.1 MS/MS spectra of (a) perampanel and (b) perampanel-d5 (IS)

516 Figure.2 Representative chromatograms of perampane and perampanel-d5 in (a)

517double

518blank plasma; (b) blank plasma; (c) blank plasma spiked with perampanel at the

519LLOQ (2.00 ng/mL); (d) plasma sample obtained at 2.5 h after oral administration.

520Figure.3 Mean plasma concentration-times profile of perampanel after oral

521administration of test product and reference product to 28 and 30 healthy Chinese

522subjects under fasting (a) and fed (b) condition.