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Preparation of Lacosamide Sustained-release Tablets and Their Pharmacokinetics in Beagles and Mini-pigs

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The aim of the present study was to improve dosing of lacosamide, a functionalized amino acid used as an antiepileptic agent, from twice daily to once daily for the convenience of patients. A sustained-release lacosamide tablet was developed and dissolution testing was employed to determine *in vitro* release behavior using water or buffer solutions at pH 1.2, 4.0, or 6.8. Lacosamide was released for 12 h from the sustained-release (SR) tablet, as compared to complete release within 1 h from an immediate-release Vimpat® tablet. Each formulation (100 mg) was orally administered to six beagle dogs and six mini-pigs under fasted conditions, and pharmacokinetic parameters such as the area under the concentration time curve (AUC_t), the maximum plasma concentration (C_{max}), and the time at which this occurred (T_{max}) were calculated. These results showed similar values for AUC_t, C_{max}, and T_{max} following oral administration of immediate-release (Vimpat®) and SR lacosamide tablets.

Key Words : Lacosamide, Sustained release tablet, Pharmacokinetics, Beagle dog, Mini-pig

Introduction

Lacosamide (*R*-2-actamido-*N*-benzyl-3-methoxypropionamide) is a functionalized amino acid with potent antiepileptic effects in rodent seizure models.[1-3] This compound is thought to work by selective enhancement of sodium channel slow inactivation, was effective and well-tolerated in clinical trials and is currently available for clinical use as immediate release tablets, oral solutions and intravenous injectable solutions.[4]

The pharmacokinetic profile of lacosamide exhibits low intra- and inter-patient variability. After single-dose oral or intravenous administration, the plasma concentration of lacosamide increases in a dose-dependent manner with oral doses up to 800 mg and intravenous doses up to 300 mg.[6,7] Lacosamide has an aqueous solubility of about 27 g/L, and is rapidly and completely absorbed by the body following first-order kinetics. T_{max} usually occurs between 1 and 4 h after administration and food does not affect the rate or extent of absorption. Lacosamide has an elimination half-life of about 13 h, making it an ideal candidate for twice daily dosing with an immediate release formulation.[1,5] However, This is not optimal because patient compliance decreases as the dosing frequency of a drug increases.[8] Moreover, the rapid and complete absorption of lacosamide after oral administration poses a problem as it can lead to a high C_{max} followed by a high steady-state concentration, resulting in a greater frequency of adverse pharmacological events. These include visual disturbances and prolongation of the P-R electrocardiogram interval between the start of atrial systole and the start of ventricular systole, leading to AV (atrioventricular) node blockage.[11,12] Large fluctuations in plasma lacosamide concentrations can also produce these unwanted side effects, which limit the usefulness of oral lacosamide in some patients.

For the reasons outlined above, there is a need for improved lacosamide formulations that are capable of providing stable therapeutic effects by maintaining a constant plasma drug level for an extended period. This would avoid the 'peak and trough'-related side effects, keeping plasma lacosamide below the minimum toxic concentration and within the therapeutic window. SR formulations may therefore contribute to improved outcomes for epilepsy patients by providing better seizure control, whilst reducing the potential for adverse events. A once-daily lacosamide SR tablet has been developed to provide more convenient dosing, potentially leading to improved compliance and a superior safety/efficacy ratio.[8-10]

In the present study, a sustained release ethylcellulose matrix containing lacosamide was prepared in an aqueous environment and evaluated *in vitro* and *in vivo*.

Experimental

Chemicals. Lacosamide was purchased from Aurobindo Pharma Limited (Andhra Pradesh, India) and dissolved in 70% methanol to form a 100 μg/mL stock solution. Vimpat® tablets (50 mg) were purchased from UCB Pharma S. A. (Brussels, Belgium). Ethylcellulose (Ethocel Std 7, Ethocel Std 7 FP) was purchased from Colorcon Korea (Suwon, Korea). Polyvinylpyrrolidone (Povidone K30) was purchased from BASF Korea (Seoul, Korea). Lactose (SuperTab® 11SD) was purchased from DMV (Veghel, The Netherlands). Colloidal silicon dioxide was purchased from Wacker Silicones (Singapore). Magnesium stearate was purchased from Nof

Table 1. Lacosamide sustained release tablet formulations

	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Tablet components (mg)				
Lacosamide	100	200	300	400
Spray-dried Lactose	40	80	120	160
Povidone K30	18	36	54	72
Ethylcellulose std 7	-	28	63	112
Ethylcellulose std 7 FP	35	42	42	28
Colloidal silicon dioxide	3	6	9	12
Magnesium stearate	2	4	6	8
Opadry 03B52165	6	12	18	24
Tablet weight (mg)	204	408	612	816

Corporation (Tokyo, Japan). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

Sustained Release Tablets. A range of tablet formulations was prepared by wet granulation (Formulations 1-4, Table 1). All the powders were passed through ASTM (American Society of Testing and Materials) 30 mesh. The required quantities of drug and spray-dried lactose were mixed thoroughly, and a sufficient volume of granulating agent (ethanolic solution of Povidone K-30) was added slowly. The granules were dried at 50 °C for 1 h before adding colloidal silicon dioxide and magnesium stearate as glidant and lubricant.

The practical weight of the tablets was calculated based on the drug content of the granulations, and the tablets were compressed (round punches) using a Carver press. Each tablet contained 100-400 mg of lacosamide and other pharmaceutical ingredients, as listed in Table 1.

Lacosamide Quantification. High performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were used to quantify sample lacosamide levels.[14] The optimal conditions of lacosamide detection by HPLC were achieved using an Agilent Technologies (USA) 1200 series HPLC with an Inertsil C8-3 column (150 mm × 4.6 mm, 5 μm) and UV detection using a photo-diode array UV/Vis detector at 210 nm (Agilent Technologies, 1100 series, USA). The column was maintained at 25 °C and the mobile phase consisted of phosphate buffer: acetonitrile (70:30, v/v) at pH 3.0. Chromatography was performed isocratically at a flow rate of 1 mL/min.

LC-MS/MS was performed using an AB-Sciex API 2000 triple quadruple mass spectrometer equipped with a turbo electrospray ion (ESI) source (Foster City, CA, USA). Samples (5 μL) were delivered into the ESI source via a micro-LC (Foster City, CA, USA) and autosampler (Agilent Technologies, 1200 series, USA) equipped with an Agilent zorbox XDB-C18 column (2.1 × 50 mm, 5 μm) at 30 °C. An isocratic mobile phase was used, consisting of degassed acetonitrile:10 mM ammonium acetate (25:75, v/v) with a flow rate of 250 μL/min for a total running time of 3 min. The retention times for ranitidine HCl (as an internal standard) and lacosamide were about 0.78 and 1.25 min, respectively. Lacosamide was detected by MS/MS using the multiple reaction monitoring (MRM) scan mode with positive ion detection. Quantitative analysis was carried out by MRM at *m/z* 251.2 → 108.2[15] for lacosamide and at *m/z* 315.1 → 176.1[16] for ranitidine HCl.

***In vitro* Tablet Dissolution Study.** Dissolution of the SR tablets was investigated using a modified version of the method described by Crowley *et al*. in 2004.[13] Dissolution profiles were determined at 37 °C in a dissolution tester using the paddle method at 50 rpm according to the Korea Pharmacopoeia (KP) dissolution procedure. The dissolution media were 0.01 N HCl buffer (pH 1.2), acetate buffer (pH 4.0), phosphate buffer (pH 6.8) and water. In addition, the drug release profile from a marketed lacosamide tablet (Vimpat®) was examined for comparison. In each test, a weighed quantity of each formulation was placed in 900 mL of the dissolution medium. At the time points indicated, 1 mL aliquots were withdrawn through a 10 mm filtering rod. Filtered samples were assayed for lacosamide by HPLC and data were expressed as the percentage of lacosamide released over time (n = 8 for each formulation).

***In vivo* Pharmacokinetic Studies.** Six male beagle dogs (12 ± 7 kg) and six mini-pigs (20 ± 5 kg) were used in the pharmacokinetic studies. All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals, from the National Institutes of Health (USA). All animals were kept in a temperature-controlled environment (22 ± 2 °C) with a 12 h light-dark cycle. They had free access to food and water, except for immediately prior to each experiment, when they were fasted overnight with free access to water. The study was carried out using a randomized crossover design, with the animals divided into two groups of three. Each dog or pig received either a SR tablet (formulation 1) or Vimpat® tablets, with a washout period of 2 weeks before switching to the other formulation. The SR tablet (100 mg lacosamide) was administered once daily, whereas Vimpat® (50 mg lacosamide) was administered twice daily. For evaluation of lacosamide levels following oral administration of Vimpat® to the dogs, 1 mL blood samples were collected from the jugular vein immediately before administration, and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 12.5, 13, 13.5, 14, 15, 18, 24, 30, 36, and 48 h after administration. Following administration of the SR formulation, blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 30, 36, and 48 h. For evaluation of lacosamide levels following oral administration of Vimpat® to mini-pigs, 1 mL blood samples were collected from the jugular vein immediately before administration, and at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 12.5, 13, 13.5, 14, 15, 18, 24, 30, 36, and 48 h after administration. Following administration of the SR formulation, blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 30, 36, and 48 h. All heparinized blood samples were immediately cooled on ice and then centrifuged at 3,000 rpm (4 °C) for 10 min. The plasma supernatant was stored at −70 °C prior to analysis.

Plasma Sample Preparation for Lacosamide Quantification. Internal standard solution (10 μL of 10 μg/mL raniti-

dine HCl in 70% methanol) was added to 50 μL of each dog or mini-pig plasma sample in microcentrifuge tubes and vortexed for 3 min. Methanol (700 μL) was added and the tubes were capped, vortexed for 3 min and centrifuged at 3,600 rpm, −4 °C for 10 min. The sample supernatant (100 μL) was transferred to a fresh tube before adding either 900 μL methanol (dog samples) or 700 μL methanol (mini-pig samples) and vortexing for 3 min. A 100 μL aliquot of the diluted sample was transferred to a HPLC vial and 5 μL samples were analyzed by LC-MS/MS, as described above. Calibration standards were analyzed, which had been prepared using dog plasma spiked with 30, 50, 100, 500, 1000, 5000, 10000, 16000 or 20000 ng/mL lacosamide, or mini-pig plasma spiked with 30, 50, 100, 500, 1000, 5000 or 10000 ng/mL lacosamide. These samples were analyzed in triplicate on each analytical run and the equations of the lines produced were y = 0.0004 x + 0.0656 (r = 0.9987) for dog plasma, and y = 0.0004 x + 0.0524 (r = 0.9970) for mini-pig plasma. The calibration curves were therefore linear over the relevant ranges of lacosamide concentrations.

Results and Discussion

***In vitro* Dissolution Studies.** The release of lacosamide from Formulation 1, as compared to Vimpat®, was investigated in dissolution media with a range of pH. The results, shown in Figure 1, indicated that lacosamide release was significantly slower from the ethylcellulose-containing Formulation 1. Release occurred slightly more slowly in dissolution media with acidic pH, suggesting that stomach acid may help slow release further. The release rates from formulations with different amounts of lacosamide (Formulations 1-4) are shown in Figure 2. These data indicated that release rate was not influenced by the lacosamide content of the formulation over the range studied (100-400 mg).

LC-MS/MS Assay Characterization. Plasma spiked with lacosamide was processed using the extraction method described above and the concentration of lacosamide determined using LC-MS/MS. The extraction recovery of lacosamide was found to be 90.3 ± 2.6%, 94.6 ± 1.3%, 92.9 ± 7.5%, 95.1 ± 2.7% at the spiked lacosamide concentrations of 30, 50, 1000 and 10000 ng/mL, respectively (data not shown), which was adequate for subsequent drug detection. In addition, the intra- and inter-day accuracy and precision of the LC-MS/MS quantification of lacosamide spiked into mini-pig plasma were examined at four concentrations, with five replicates for each. The results, summarized in Table 2, demonstrated that accuracy and precision values were within an acceptable range. Accuracy was assessed by calculating the percent deviation from the theoretical concentration of lacosamide spiked into mini-pig plasma. Precision was determined by calculating the coefficients of variation for intra- and inter-day replicates.

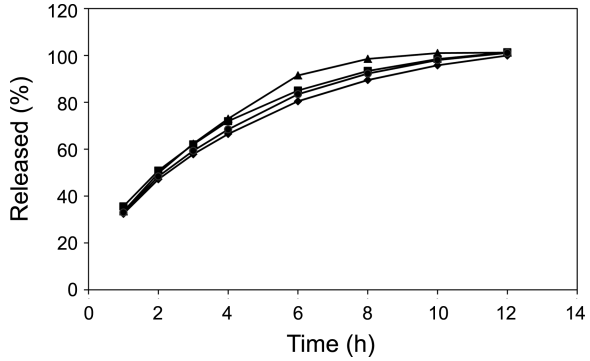

Figure 1. Dissolution profiles of the sustained release Formulation 1 when tablets were incubated with dissolution medium at pH 1.2 (◆), pH 4.0 (■), pH 6.8 (▲), or water (●). Data represent the mean ± S.D., *n* = 8.

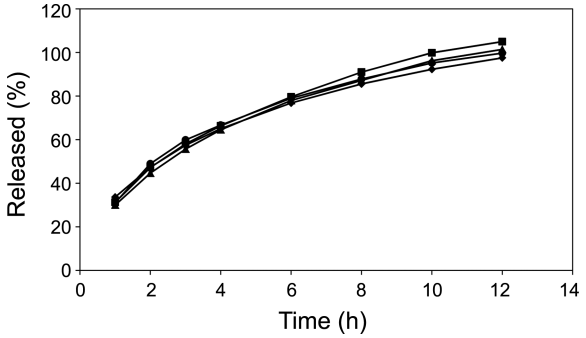

Figure 2. Dissolution profiles of all the sustained release tablets in water. Formulation 1 (◆), Formulation 2 (■), Formulation 3 (▲), Formulation 4 (●). Data represent the mean ± S.D., *n* = 8.

Table 2. Intra- and inter-day accuracy and precision of lacosamide quantification in spiked mini-pig plasma

	Low (30 ng/mL)	Low (50 ng/mL)	Middle (1000 ng/mL)	High (10000 ng/mL)
Intra-day accuracy and precision				
Mean	30.07	49.73	1031.89	10012.13
S.D.	0.25	0.46	8.56	57.02
%CV	7.21	5.74	4.98	7.78
%Deviation	1.29	2.79	2.40	1.71
n	5	5	5	5
Inter-day accuracy and precision				
Mean	31.04	51.61	1015.74	10087.32
S.D.	0.21	1.24	9.14	77.45
%CV	5.71	6.14	4.21	6.23
%Deviation	1.23	1.41	2.94	4.74
n	5	5	5	5

Pharmacokinetic Studies. The pharmacokinetics of oral SR Formulation 1 and Vimpat® (lacosamide 50 mg) were compared in beagle dogs and mini-pigs. The mean plasma concentrations of lacosamide in dogs and mini-pigs are presented in Figure 3 and Figure 4, respectively and the corresponding pharmacokinetic parameters (PK) are shown in Tables 3 and 4. Although Formulation 1 and Vimpat® produced similar maximum plasma concentrations in dogs (C_{max}), the time to reach this concentration (T_{max}) was longer for Formulation 1 and the area under the plasma concentration-time curve from 0 to infinity ($AUC_{(0-\infty)}$) was lower, as compared to Vimpat® (Table 3 and Fig. 3). The elimination

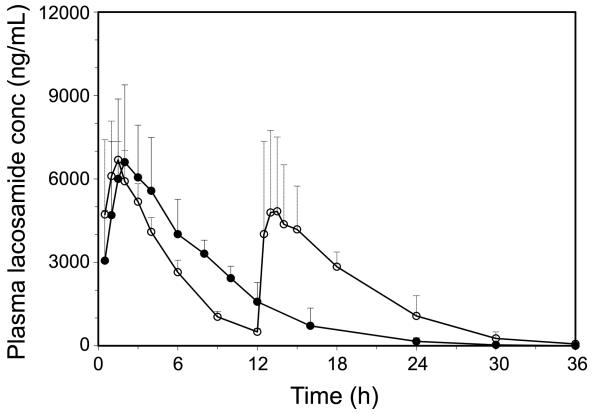

Figure 3. Plasma concentration of lacosamide in beagle dogs. Data represent the mean ± S.E., n = 6 for Vimpat® tablet (50 mg) (●), and sustained release Formulation 1 (○).

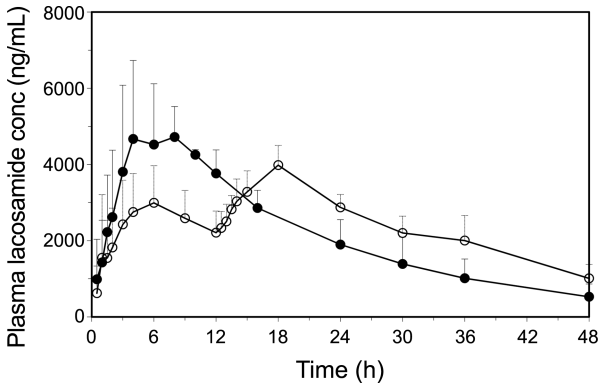

Figure 4. Plasma concentration of lacosamide in mini-pigs. Data represent the mean ± S.E., n = 6 for Vimpat® tablet (50 mg) (●), and sustained release Formulation 1 (○).

half-life of lacosamide was found to be 3-4 h in dogs, as compared to about 13 h in humans. This result indicated that the shorter gastrointestinal transit time in dogs may affect the absorption of lacosamide.[17,18] In mini-pigs, Formulation 1 and Vimpat® produced similar C_{max} and $AUC_{(0-\infty)}$ (Table 4 and Fig. 4). Kim *et al*. was showed that lacosamide has low variability in its PK parameters. C_{max} of lacosamide 50, 100 and 200 mg tablets were reached at 0.5-1.5 h and geometric means C_{max} (Coefficient of variation, CV%) were 1.63 (9.1), 2.85 (14.6) and 5.84 (24.3) μg/mL, respectively. The corresponding geometric means AUC(0-96) (CV%) were 27.15 (14.7), 51.95 (13.6) and 114.65 (15.8) μg/mL, respectively. In addition, there were no other ethnic differences in the PK profile of lacosamide between Koreans and Caucasians.[19]

In these studies performed in mini-pigs, a single 100 mg dose of lacosamide SR was bioequivalent to two 50 mg doses of lacosamide immediately release given 12 h apart. A once-daily SR tablet dosage form with the desired *in vivo* performance was successfully designed. However, further investigations in human are required to prove the clinical usability of the experimental SR formulations.

The antiepileptic drugs currently commercially available as SR, controlled-release or extended-release formulations are carbamazepine, valproate, phenytoin, oxcarbazepine, levetiracetam and topiramate.[20-22] New antiepileptic drugs are usually first launched and marked as conventional dosage forms or IR formulation. IR formulation of antiepileptic drugs with short half-lives, in fact, necessitate frequent administration to maintain drug concentrations over the dosing interval and produce wide fluctuations in drug concentrations throughout the day.[10] In some patients taking these formulations, optimal seizure control may require doses that produce peak drug concentrations (shortly after dosing) above the optimal therapeutic range for seizure control/drug tolerability, increasing the likelihood of toxicity.

The results indicated that lacosamide of SR tablet from ethylcellulose were slow released in mini-pig. This result suggested that ethylcellulose would be useful to deliver lacosamide in a pattern that allows improved dissolution rate, leading to be SR tablet. Also, the particle size of ethylcellulose was affected to same dissolution results regardless of the volume of lacosamide. We demonstrated the potential of ethylcellulose processed by SR tablet for improving oral delivery. Our results also indicate that the proposed method has been successfully applied to pharmacokinetic studies to determine the concentration of lacosamide in human plasma.

Table 3. Pharmacokinetics of lacosamide sustained release Formulation 1 and Vimpat® in dogs

Parameter	Formulation 1	Vimpat® (50 mg)
C_{max} (ng/mL)	6602 ± 2574	6685 ± 893
T_{max} (h)	2.0 ± 1.0	1.5 ± 4.7
$AUC_{(0-\infty)}$ (h ng/mL)	56043 ± 12928	74953 ± 5603

Data represent the mean ± S.E., n = 6. One tablet of Formulation 1 was administered (100 mg lacosamide) and two tablets of Vimpat® (50 mg lacosamide per tablet).

Table 4. Pharmacokinetics of lacosamide sustained release Formulation 1 and Vimpat® in mini-pigs

Parameter	Formulation 1	Vimpat® (50 mg)
C_{max} (ng/mL)	4720 ± 1190	3982 ± 619
T_{max} (h)	8.0 ± 3.7	18.0 ± 8.1
$AUC_{(0-\infty)}$ (h ng/mL)	104423 ± 19154	114003 ± 22499

Data represent the mean ± S.E., n = 6. One tablet of Formulation 1 was administered (100 mg lacosamide) and two tablets of Vimpat® (50 mg lacosamide per tablet).

Conclusion

From these observations of lacosamide SR tablet and pharmacokinetic behaviors could be efficacious in leading the SR profile of pharmacokinetic behaviors in beagle dogs and mini-pigs. According to the pharmacokinetic profiles, there was significantly equal to C_{max} and $AUC_{(0-\infty)}$. In addition, lacosamide SR tablet has simplified the dosage regimen and may help to improve patient compliance. These results will also be useful for further pharmacokinetic studies of lacosamide during clinical trial.

References

1. Horstmann, R.; Bonn, R.; Cawello, W.; Doty, P.; Rudd, G. D. *Epilepsia* **2002**, *43*, 188.
2. Kropeit, D.; Schiltmeyer, B.; Cawello, W.; Hammes, W.; Horstmann, R. *Epilepsia* **2004**, *45*, 123.
3. Cawello, W.; Boekens, H.; Bonn, R. *Eur. J. Drug Metab. Pharmacokinet.* **2012**, *37*, 241.
4. Beyreuther, B. K.; Freitag, J.; Heers, C.; Krebsfanger, N.; Scharfenecker, U. *CNS Drug Rev.* **2007**, *13*, 21.
5. Beydoun, A.; D'Souza, J.; Hebert, D.; Doty, P. *Expert Rev. Neurother.* **2009**, *9*, 33.
6. Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P.; Perucca, E. *Epilepsy Res.* **2002**, *51*, 31.
7. Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Perucca, E.; Tomson, T. *Epilepsy Res.* **2004**, *61*, 1.
8. Sommerville, K. W. *Epilepsy Res.* **2006**, *68*, 19.
9. Verrotti, A.; Salladini, C.; Di Marco, G.; Pisciella, F.; Chiarelli, F. *J. Child Neurol.* **2007**, *22*, 419.
10. Bialer, M. *CNS Drugs* **2007**, *21*, 765.
11. Ben-Menachem, E.; Biton, V.; Jatuzis, D.; Abou-Khalil, B.; Doty, P.; Rudd, G. D. *Epilepsia* **2007**, *48*, 1308.
12. Krause, L. U.; Brodowski, K. O.; Kellinghaus, C. *Epilepsy & Behavior* **2011**, *20*, 725.
13. Crowley, M. M.; Schroeder, B.; Fredersdorf, A.; Obara, S.; Talarico, M.; Kucera, S.; McGinity, J. W. *Int. J. Pharm.* **2004**, *269*, 509.
14. Valarmathi, R.; Farisha Banu, S.; Akilandeswari, S.; Senthamarai, R.; Dhharshini, C. S. *Int. J. Pharm. Chem. Sci.* **2013**, *2*, 181.
15. Kim, S. J.; Koo, T. S.; Ha, D. J.; Baek, M. K.; Lee, S. K.; Shin, D. S.; Moon, H. S. *Biomed. Chromatogr.* **2012**, *26*, 371.
16. Sun, X.; Tian, Y.; Zhang, Z.; Chen, Y. *J. Chromatogr. B* **2009**, *877*, 3953.
17. Kararli, T. T. *Biopharm. Drug Dispos.* **1995**, *16*, 351.
18. Klausner, E. A.; Lavy, E.; Friedman, M.; Hoffman, A. *J. Control Rel.* **2003**, *90*, 143.
19. Kim, S. E.; Gu, N.; Kim, B. H.; Fitchner, A.; Elshoff, J.; Cawello, W.; Jang, I. J.; Yu, K. S. *Pharmacology* **2012**, *89*, 172.
20. Rouitsa, E.; Burtonb, I.; Guénoléc, E.; Troenarua, M. M.; Stockis, A.; Sargentini-Maier, M. L. *Epilepsy Research* **2009**, *84*, 224.
21. Doughty, J.; Baker, G. A.; Jacoby, A.; Lavaud, V. *Epilepsy & Behav.* **2003**, *4*, 710.
22. Pellock, J. M.; Smith, M. C.; Cloyd, J. C.; Uthman, B.; Wilderd, B. J. *Epilepsy & Behav.* **2004**, *5*, 301.