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ORIGINAL RESEARCH PAPER

A rapid and sensitive High-Performance Liquid Chromatography-tandem Mass Spectrometry method for determining apremilast in beagle dog plasma and urine: Application in a pharmacokinetic study

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ABSTRACT

A rapid and sensitive High-Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC/MS/MS) method for determining apremilast in beagle dog plasma and urine samples was developed and validated using clopidogrel as the internal standard (IS). Apremilast was extracted from the plasma and urine samples by liquid-liquid extraction using methyl tert-butyl ether. Chromatographic separation was performed using a C₈ column with gradient elution and a mobile phase containing methanol and 0.1% formic acid. Quantification was achieved in multiple reaction monitoring (MRM) mode with a transition of m/z 461.3→178.2 for apremilast and m/z 322.2→184.1 for clopidogrel (IS). This method was validated regarding its specificity, linearity, precision, accuracy, and stability. The lower limit of quantification (LLOQ) for this method was 5 ng/mL, and the calibration curve was linear over 5–1,000 ng/mL. The intra- and inter-run coefficients of variance (CV) of apremilast in plasma samples were less than 12.92% and 10.64%, respectively, while in urine samples, the CV were less than 11.84% and 10.20%, respectively. The samples were stable under the tested conditions. This method was successfully applied to a pharmacokinetic study in beagle dogs following oral administration of 10 mg of apremilast.

KEYWORDS

apremilast, HPLC-MS/MS, pharmacokinetics, urine excretion

INTRODUCTION

Apremilast is a novel oral agent that inhibits phosphodiesterase 4 (PDE 4) [1, 2]. Psoriasis and psoriatic arthritis are diseases driven by the overproduction of inflammatory mediators released by innate and adaptive immune cells [3, 4]. PDE inhibitors increase anti-inflammatory agent secretion and also inhibit the release of inflammatory mediators [5, 6]. Because PDE 4 is a PDE expressed in many types of innate and adaptive immune cells, it has become a potential target for treating psoriasis and psoriatic arthritis [7–9]. Recent trials demonstrated the efficacy of apremilast in patients with moderate-to-severe psoriasis. In addition, apremilast also showed a regulatory effect on innate immunity, and this activity is more potent than that on adaptive immunity [10–12]. Apremilast was approved by the FDA in

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March of 2014 for the treatment of psoriasis and psoriatic arthritis in adult patients. It is the first oral formulation approved by the FDA in over two decades for these diseases [13]. The trade name for apremilast is Otezla, and it is manufactured by Celgene Corporation (Summit, NJ, USA) as 10-mg, 20-mg, and 30-mg tablets [14].

As a new oral drug, a pharmacokinetic study is required to describe its disposition *in vivo*. To facilitate the pharmacokinetic study, an assay for quantifying apremilast in both plasma and urine samples is necessary. Although Hoffmann and Wu had developed assay methods to evaluate the disposition and metabolism of apremilast in humans, they both used [^{14}C]-labeled apremilast rather than normal tablets [15]. Liu's method has been used to study the drug interactions with apremilast by liquid chromatography-tandem mass spectrometry [16, 17]. However, no study has reported detailed information of their assay in accordance with the specific guidelines issued by EMEA or FDA. Thus, in this study, a sensitive, rapid, and specific LC-MS/MS method was developed and applied to a pharmacokinetic study involving beagle dogs following a single oral dose of 10 mg of apremilast.

EXPERIMENTAL

Reagents and chemicals

The apremilast standard was provided by Shenzhen Yuanyang BioTechnology Co. Ltd. (Guangdong, China), and apremilast tablets were provided by Chengdu Xian Yi Medical Technology Co. Ltd. (Sichuan, China). Clopidogrel (internal standard, IS) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was purchased from Sigma (St Louis, MO, USA). Formic acid with a purity >99% was obtained from Dikma Technology (Lake Forest, CA, USA). The deionized water was distilled in our laboratory using a Millipore AFS-10 water-purification system (Millipore, Billerica, MA, USA).

Beagle dogs (6.5–7.5 kg) were provided by Chengdu Darso Experimental Animal Co. Ltd. (Sichuan, China). Before administration, they fasted overnight and were allowed free access to water. All animal handling procedures were in accordance with the Regulations of Experiment Animal Administration issued by the State Committee of Science and Technology of China.

Preparation of standard calibration and quality control samples

The apremilast stock solution (100 $\mu\text{g/mL}$) was prepared by dissolving an appropriate amount of apremilast standard in methanol and further diluting the standard solution in methanol to concentrations of 0.1, 0.2, 0.8, 2, 10, 20, 32 and 40 $\mu\text{g/mL}$, which served as the working solutions. The stock solution of clopidogrel (IS) was prepared in methanol at a concentration of 200 $\mu\text{g/mL}$ and was further diluted to

generate working solutions of 1 $\mu\text{g/mL}$. All the solutions were stored at 4 °C and tested for stability over, at least, 50 days. These working solutions were used to prepare the standard curve and quality control (QC) samples.

The calibration standards and QC samples with concentrations of 5, 10, 40, 100, 500, 1,000, 1,600 and 2,000 ng/mL were prepared by spiking 10 μL of the corresponding working solution into 200 μL of blank plasma or urine (5, 100, 500 and 1,600 ng/mL were selected as LLOQ, low, medium, and high QC samples, respectively). All the samples were stored at –20 °C with the real plasma and urine samples until analysis.

Sample processing

To analyze the beagle dog plasma or urine samples, 50 μL of 5% aqueous formic acid and 20 μL of the IS working solution were added to 200 μL of plasma or urine and then mixed well by vortexing briefly. Liquid–liquid extraction was then performed by adding 1 mL of methyl tert-butyl ether followed by vortex extraction for 3 min (IKA Vortex Genius 3 Vortex, Wilmington, NC, USA). After centrifugation at 12,000 rpm for 5 min, the upper organic layer was transferred into another neat tube and fully evaporated to dryness at 35 °C under a stream of nitrogen (Turbovap Zymark, Hopkinton, MA, USA). The dry residue was reconstituted with 100 μL of methanol, and a 5 μL aliquot was injected into the high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) system for analysis.

HPLC-MS/MS analysis

The LC-MS/MS analysis was performed using a Shimadzu SCL-10A HPLC system (Shimadzu, Japan) coupled to an API 3000 mass spectrometer (Sciex, Framingham, MA, USA). Data acquisition and analyses were performed using the Analyst software version 1.4.2 (Sciex, Framingham, MA).

The mass spectrometer was operated in a positive ion electrospray mode to measure the apremilast and IS. Quantification was achieved in multiple reaction monitoring (MRM) mode by detecting m/z transitions of 461.3–178.2 for apremilast and m/z 322.2–184.1 for IS. The API 3000 instrument parameters were set as follows: nebulizer, 12 psi; curtain gas, 10 psi; collision gas, 6 psi; electrospray voltage, 4,000 eV; and ion source temperature, 450 °C.

Chromatographic separation was achieved using an XTerra[®] MS C₈ column (5 μm , 2.1 $\times$ 150 mm, Waters). The column temperature was set at 40 °C. Analytes were eluted with a mobile phase consisting of methanol (containing 0.1% formic acid) (B) and 0.1% aqueous formic acid (A) using the gradient described below. The flow rate was set at 0.4 mL/min. The elution began with 30% B and changed linearly to 75% over 0.5 min. The mobile phase composition remained constant from 0.5 to 1.8 min. Starting at 1.8 min, the mobile phase composition was restored to 30% B over 0.2 min and maintained for 1 min.

Validation of the method

This method was validated in terms of specificity, selectivity, sensitivity, linearity, precision, accuracy, recovery, matrix effect and stability according to the guidelines for industry, Bioanalytical Method Validation published by the US Food and Drug Administration (2018) [18].

Specificity and selectivity. The specificity and selectivity were evaluated by monitoring the endogenous interference from blank beagle dog plasma or urine collected from six different individual subjects.

Sensitivity and linearity. The calibration standards were prepared by spiking the working solution into the blank beagle dog plasma or urine samples. The calibration curve was constructed by plotting the peak area ratios (R) of apremilast to IS versus the nominal concentrations (C) of apremilast and fitted using weighted least squares linear regression (the weighing factor was $1/C^2$). The lower limit of quantification (LLOQ) was defined as the lowest analytical concentration at which the analyte peak was identifiable (signal-to-noise ratio of at least 10:1), discrete, and reproducible with the precision within 20% and accuracy between 80% and 120%.

Precision and accuracy. The intra-day and inter-day precision and accuracy were determined by analyzing QC samples at four different concentration levels on three separate days. The precision was defined as the relative standard deviation (RSD) of the QC samples of six replicates, and the accuracy was assessed as the percentage of the nominal concentration (%). RSD should be within 15% for each QC samples except the sample at LLOQ, which should not deviate by more than 20%. The accuracy should be between 85 and 115% for QC samples except LLOQ, which should not exceed the range of 80–120%.

Extraction recovery and matrix effects. The extraction recovery was assessed by comparing the peak areas of the extracted QC samples (low, medium and high concentrations) with the peak areas measured for the sample spiked with the corresponding working solution after extraction. Unextracted standard solutions contained an equivalent amount of apremilast. The results correspond to the absolute extraction recoveries by eliminating the influence of matrix effect. The relative matrix effect for apremilast was evaluated by comparing the peak areas of the analyte in the extracted blank plasma or urine samples from six different drug-free beagle dogs.

Stability. The stability of apremilast was tested using three different QC sample concentrations under various experimental conditions, including three freeze-thaw cycles, storage for 80 days at $-20\text{ }^{\circ}\text{C}$, maintained in autosampler vials at $4\text{ }^{\circ}\text{C}$ for 12 h, and at room temperature for 8 h. A deviation of less than 20% of the original sample was considered stable.

Application of the method to a pharmacokinetic study

The assay was used to determine the apremilast plasma concentration in twelve beagle dogs (six male and six female) following oral doses of 10 mg of apremilast. Blood samples were collected before administration and at 0.17, 0.33, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 9, 12, 24, 36 and 48 h after dosing. Approximately 4 mL of blood were drawn and placed in heparinized tubes. The plasma was separated by centrifugation at 4,000 rpm for 5 min and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Urine was collected before administration and at 1, 2, 4, 6, 9, 12, 24 and 48 h after dosing and the volume was recorded. The partial urine samples (approximately 2 mL) were stored at $-20\text{ }^{\circ}\text{C}$ until analysis. The urine obtained was stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

The plasma and urine concentrations of apremilast were determined using the LC-MS/MS method described above. Pharmacokinetic parameters were calculated for apremilast using a model-independent method. The maximum plasma concentration ($C_{\max}$) and peak time ($t_{\max}$) were determined by directly inspecting the concentration–time curve. The elimination rate constant (k_{el}) was calculated through the semi-log linear regression of the terminal phase of the plasma concentration–time curve. The elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_{\text{el}}$. The area under the plasma concentration–time curve ($\text{AUC}_{0-48\text{h}}$) to the last measurable plasma concentration was calculated using the semi-log linear trapezoidal rule. The renal clearance was determined by the equation of ($\text{CL}_{\text{R}} = \text{Xu}/\text{AUC}$, where Xu is the total amount of drug recovered in urine, which was determined by multiplying the volume of the samples by their corresponding concentrations). For the data points below the LLOQ, a value of zero was assigned for the pharmacokinetic analysis.

RESULTS AND DISCUSSION

Mass spectrometry

The analyte and IS responded best to the positive ionization mode with the protonated ions $[\text{M}+\text{H}]^{+}$ being the major peaks for parent ions of both compounds. Their product ion mass spectra are shown in Fig. 1. MRM mode was used to identify the molecules by monitoring the transition of m/z from 461.3 to 78.2 for apremilast and from 322.2 to 184.1 for IS.

Representative chromatograms were obtained from blank plasma and urine (Fig. 2), blank plasma or urine spiked with the analyte and IS (Fig. 3), and plasma and urine samples from a representative subject at 2 h after dosing (Fig. 4). The chromatographic conditions were optimized for high sensitivity, speed, peak shape, and resolution. Under the optimum conditions, the retention times were 1.07 min for apremilast and 1.37 min for IS in plasma samples and 1.09 min for apremilast and 1.36 min for IS in urine samples.

Fig. 1. Chemical structures of apremilast (a) and clopidogrel (IS) (b) with fragmentation and the product ion spectrum of $[M+H]^+$

An HPLC-MS/MS in negative mode for determination of apremilast in rat plasma has been reported earlier [19]. In our study, apremilast was able to be detected using positive mode with equivalent sensitivity. The previous study showed that the sodium $[M+Na]^+$ (m/z 478.09) and ammonium $[M+NH_4]^+$ adducts (m/z of 483.02) had stronger signals than the protonated ions, which may cause the interference leading to lower signal intensity. Whereas, the protonated molecule of apremilast showed the highest intensity in our study, although the other two adducts were presented as

well. The results suggested that the mass reactions vary in different systems, so the optimization of mass conditions for mass detection is always necessary in order to achieve the best specificity and sensitivity.

Assay validation

Specificity. The analysis of the analyte and IS using the MRM function was highly selective. Endogenous substances in both plasma and urine samples did not exhibit

Fig. 2. MRM chromatograms for (a) blank plasma for apremilast, (b) blank plasma for clopidogrel, (c) blank urine for apremilast, and (d) blank urine for clopidogrel

Fig. 3. MRM chromatograms for (a) 5 ng/mL of apremilast and (b) IS (1 μ g/mL) in plasma, and (c) 5 ng/mL of apremilast and (d) IS (1 μ g/mL) in urine

Fig. 4. MRM chromatograms for (a) apremilast and (b) IS (1 μ g/mL) in a plasma sample obtained from a subject 2 h after dosing, and (c) apremilast and (d) IS (1 μ g/mL) in a urine sample obtained from a subject 2 h after dosing

interference or significant ion suppression. Representative chromatograms were obtained from blank plasma and urine, blank plasma or urine spiked with the analyte and IS, and plasma and urine samples from a representative subject at 2 h after dosing and are shown in Figs. 2–4.

Calibration curves and LLOQ. For the plasma sample, the calibration curves were linear in the concentration range 5–2,000 ng/mL: $Y = 0.0038C + 0.05$ (where Y is the peak area ratio of apremilast to IS and C is the nominal concentration), $R^2 = 0.9971$. The LLOQ was 5 ng/mL.

For urine samples, the calibration curves were linear in the concentration range 5–2,000 ng/mL: $Y = 0.0013C - 0.0003$ (where Y is the peak area ratio of apremilast to IS and C is the nominal concentration), $R^2 = 0.9996$. The LLOQ was 5 ng/mL.

Because of the high sensitivity of our method, the apremilast concentrations in beagle dog plasma and urine could be determined up to 48 h after dosing.

Precision and accuracy. The intra- and inter-day precision and accuracy of the assay are summarized in Table 1. The intra- and inter-day precisions were 4.99–12.92% and 3.82–10.64%, respectively, with accuracies ranging from 98.43 to 101.39% in plasma samples. In urine samples, the intra-day and inter-day precisions were 3.53–11.84% and 4.10–10.20%, respectively, with accuracies ranging from 97.23 to 104.93%.

Matrix and recovery. For the plasma samples, the mean apremilast extraction recoveries were $77.19 \pm 4.82\%$, $79.23 \pm 3.91\%$ and $79.46 \pm 3.26\%$ at concentrations of 100, 500 and 1,600 ng/mL, respectively. The mean extraction recovery was $69.57 \pm 3.37\%$ for IS. The absolute matrix effects were within the range of 91.73–105.18% for apremilast and 91.89–102.04% for IS.

For the urine samples, the mean apremilast extraction recoveries were $73.86 \pm 5.58\%$, $73.50 \pm 5.55\%$ and $75.21 \pm 4.96\%$ at concentrations of 100, 500 and 1,600 ng/mL, respectively. The mean extraction recovery was $64.92 \pm 4.65\%$ for IS. The absolute matrix effects were within the range of 93.69–107.58% for apremilast and 92.89–104.40% for IS. These results indicated no obvious matrix effects.

Stability. The analyte was stable in dog plasma and urine after storage for 80 days at -20°C or for 8 h at room temperature with deviations of $<8.59\%$ and $<5.76\%$, respectively, from the baseline level. No significant apremilast degradation was observed when the samples were maintained in the auto-sampler for up to 12 h at 4°C (with deviations from the baseline level of less than 7.91% for plasma and 8.13% for urine). The analyte was also stable over three freeze-thaw cycles.

Application of the method in beagle dogs

The validated LC-MS/MS method was successfully applied to a pharmacokinetic of apremilast in the beagle dogs following oral doses of 10 mg, which is demonstrated from the mean plasma concentration–time curve (Fig. 5). This method provides sufficient sensitivity and reliability for determining apremilast concentrations for the pharmacokinetic study.

Only 9.18–13.33% (mean $11.23 \pm 1.9\%$) of the apremilast was recovered, in urine samples over the collection period as the parent compound (Fig. 6), indicating extensive metabolism *in vivo*. The elimination half-life ($t_{1/2}$) of 5.92 ± 0.96 h was observed for apremilast. The main pharmacokinetic parameters are shown in Table 2.

Fig. 5. Mean plasma concentration–time course for apremilast in 12 beagle dogs following a single oral dose of 10 mg of the drug

Table 1. Precision and accuracy of the developed method for determining apremilast in beagle dog plasma and urine samples (the data are based on an assay with six replicates per day on three different days)

Conc. (ng/mL)	Intra-day determination (n = 6)				Inter-day determination (n = 6)			
	5	100	500	1,600	5	100	500	1,600
Plasma								
Measured concentration	4.92 ± 0.64	100.50 ± 9.97	503.67 ± 41.69	$1,589.83 \pm 79.33$	4.93 ± 0.52	101.39 ± 6.95	497.72 ± 26.63	$1,596.50 \pm 61.04$
Accuracy (%)	98.43	100.50	100.73	99.36	98.68	101.39	99.30	99.78
Precision (%)	12.92	9.93	8.28	4.99	10.64	6.85	5.35	3.82
Urine								
Measured concentration	5.25 ± 0.62	97.67 ± 7.74	486.17 ± 17.15	$1,583.35 \pm 89.22$	5.03 ± 0.51	99.38 ± 5.78	497.22 ± 24.07	$1,594.49 \pm 65.34$
Accuracy (%)	104.93	97.67	97.23	98.96	100.58	99.38	99.44	99.66
Precision (%)	11.84	7.92	3.53	5.63	10.20	5.82	4.84	4.10

Fig. 6. The mean quantity of apremilast (expressed as a percentage of the dose) excreted in urine in 12 beagle dogs following a single oral dose of 10 mg of the drug

Table 2. Pharmacokinetic parameters of apremilast after oral administration of 10 mg of the drug (mean value $\pm$ S.D., $n = 12$)

Parameters	Mean $\pm$ SD
T_{max} (h)	1.48 $\pm$ 0.70
C_{max} (ng/mL)	1,097.6 $\pm$ 272.3
$t_{1/2}$ (h)	5.92 $\pm$ 0.96
k_{el} (h^{-1})	0.12 $\pm$ 0.02
AUC_{0-t} (ng $\cdot$ h/mL)	10,825.8 $\pm$ 4,385.6
$AUC_{0-\infty}$ (ng $\cdot$ h/mL)	10,933.9 $\pm$ 4,424.4
X_u (mg)	1.123 $\pm$ 0.190
CL_R (mL/h)	102.6 $\pm$ 17.4

The pharmacokinetics of apremilast in beagle dogs was for the first time reported, especially the data of renal clearance of apremilast. A much shorter elimination half-life in dogs (5.92 h) was overserved compared to rats (11.4 h). Since only the blood samples were collected up to 20 (less than 2 half-lives) in the previous study [19], the result may not be reliable. In our study, the duration of sampling was extended to 48 h, which covered more than 5 half-lives to include the entire elimination phase.

The urine excretion data were also collected which showed that only 11% of the dose was recovered in renal clearance. The result indicated that hepatic clearance is the major pathway for apremilast elimination *in vivo*, which may explain why the impact of renal impairment on the pharmacokinetics of apremilast was shown by Liu et al. was not significant [16].

The data in our study is also useful to understand the pharmacokinetic difference in different species, which could provide important information for physiologically based pharmacokinetic modeling.

CONCLUSION

A specific, sensitive, accurate, and rapid LC-MS/MS method for determining apremilast in beagle dog plasma and urine was developed and validated. The method was successfully applied to a pharmacokinetic study involving beagle dogs following the oral administration of apremilast.

REFERENCES

- Mahmood, T.; Zaghi, D.; Menter, A. Emerging oral drugs for psoriasis. *Expert Opin. Emerg. Drugs* **2015**, 20(2), 209–20.
- Man, H. W.; Schafer, P.; Wong, L. M.; Patterson, R. T.; Corral, L. G.; Raymon, H.; Blease, K.; Leisten, J.; Shirley, M. A.; Tang, Y.; Babusis, D. M.; Chen, R.; Stirling, D.; Muller, G. W. Discovery of (S)-N-[2-[1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonyl-ethyl]-1,3-dioxo-2,3-dihydro-1H-indol-4-yl] acetamide (apremilast), a potent and orally active phosphodiesterase 4 and tumor necrosis factor- α inhibitor. *J. Med. Chem.* **2009**, 52(6), 1522–4.
- Lowes, M. A.; Bowcock, A. M.; Krueger, J. G. Pathogenesis and therapy of psoriasis. *Nature* **2007**, 445(7130), 866–73.
- Schafer, P. Apremilast mechanism of action and application to psoriasis and psoriatic arthritis. *Biochem. Pharmacol.* **2012**, 83(12), 1583–90.
- Claveau, D.; Chen, S. L.; O'Keefe, S.; Zaller, D. M.; Styhler, A.; Liu, S.; Huang, Z.; Nicholson, D. W.; Mancini, J. A. Preferential inhibition of T Helper 1, but Not T Helper 2, cytokines in vitro by L-826,141 [4-{2-(3,4-Bisdifluoromethoxyphenyl)-2-{4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-phenyl}-ethyl}-3-methylpyridine-1-oxide], a potent and selective phosphodiesterase 4 inhibitor. *J. Pharmacol. Exp. Ther.* **2004**, 310(2), 752–60.
- Eigler, A.; Siegmund, B.; Emmerich, U.; Baumann, K. H.; Hartmann, G.; Endres, S. Anti-inflammatory activities of cAMP-elevating agents: enhancement of IL-10 synthesis and concurrent suppression of TNF production. *J. Leukoc. Biol.* **1998**, 63(1), 101–7.
- Raychaudhuri, S. P.; Raychaudhuri, S. K.; Genovese, M. C. IL-17 receptor and its functional significance in psoriatic arthritis. *Mol. Cell. Biochem.* **2012**, 359(1–2), 419–29.
- Serezani, C. H.; Ballinger, M. N.; Aronoff, D. M.; Peters-Golden, M. Cyclic AMP: master regulator of innate immune cell function. *Am. J. Respir. Cell. Mol. Biol.* **2008**, 39(2), 127–32.
- Uyemura, K.; Yamamura, M.; Fivenson, D. F.; Modlin, R. L.; Nickoloff, B. J. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. *J. Invest. Dermatol.* **1993**, 101(5), 701–5.
- Kavanaugh, A.; Mease, P. J.; Gomez-Reino, J. J.; Adebajo, A. O.; Wollenhaupt, J.; Gladman, D. D.; Hochfeld, M.; Teng, L. L.; Schett, G.; Lespessailles, E.; Hall, S. Longterm (52-week) results of a phase III randomized, controlled trial of apremilast in patients with psoriatic arthritis. *J. Rheumatol.* **2015**, 42(3), 479–88.
- Kavanaugh, A.; Mease, P. J.; Gomez-Reino, J. J.; Adebajo, A. O.; Wollenhaupt, J.; Gladman, D. D.; Lespessailles, E.; Hall, S.; Hochfeld, M.; Hu, C.; Hough, D.; Stevens, R. M.; Schett, G. Treatment of psoriatic arthritis in a phase 3 randomised, placebo-controlled trial with apremilast, an oral phosphodiesterase 4 inhibitor. *Ann. Rheum. Dis.* **2014**, 73(6), 1020–6.
- Schett, G.; Wollenhaupt, J.; Papp, K.; Joos, R.; Rodrigues, J. F.; Vessey, A. R.; Hu, C.; Stevens, R.; de Vlam, K. L. Oral apremilast in the treatment of active psoriatic arthritis: results of a multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum.* **2012**, 64(10), 3156–67.
- Papp, K.; Reich, K.; Leonardi, C. L.; Kircik, L.; Chimenti, S.; Langley, R. G.; Hu, C.; Stevens, R. M.; Day, R. M.; Gordon, K. B.; Korman, N. J.; Griffiths, C. E. Apremilast, an oral phosphodiesterase 4 (PDE4) inhibitor, in patients with moderate to severe

- plaque psoriasis: Results of a phase III, randomized, controlled trial (Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis [ESTEEM] 1). *J. Am. Acad. Dermatol.* **2015**, 73(1), 37–49.
14. Sigler, J. Drug updates and approvals: 2014 in review. *J. Nurse Pract.* **2014**, 39(12), 14–23; quiz 23–4.
15. Hoffmann, M.; Kumar, G.; Schafer, P.; Cedzik, D.; Capone, L.; Fong, K. L.; Gu, Z.; Heller, D.; Feng, H.; Surapaneni, S.; Laskin, O.; Wu, A. Disposition, metabolism and mass balance of [(14)C]apremilast following oral administration. *Xenobiotica* **2011**, 41(12), 1063–75.
16. Liu, Y.; Zhou, S.; Assaf, M.; Nissel, J.; Palmisano, M. Impact of renal impairment on the pharmacokinetics of apremilast and metabolite M12. *Clin. Pharmacol. Drug Dev.* **2016**, 5(6), 469–79.
17. Liu, Y.; Zhou, S.; Nissel, J.; Wu, A.; Lau, H.; Palmisano, M. The pharmacokinetic effect of coadministration of apremilast and methotrexate in individuals with rheumatoid arthritis and psoriatic arthritis. *Clin. Pharmacol. Drug Dev.* **2014**, 3(6), 456–65.
18. U.S. Department of Health and Human Services. *Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine (CVM) Bioanalytical Method Validation Guidance for Industry.* **2018**.
19. Iqbal, M.; Ezzeldin, E.; Al-Rashood, S. T.; Imam, F.; Al-Rashood, K. A. Determination of apremilast in rat plasma by UPLC-MS/MS in ESI-negative mode to avoid adduct ions formation. *Bioanalysis* **2016**, 8(14), 1499–508.