Asian Journal of Chemistry; Vol. 30, No. 10 (2018), 2215-2219

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21405

Isolation and Spectral Characterization of Degradation Impurity in Perampanel Drug Substance Using UPLC-MS and NMR Spectroscopy: Validation of Assay Method by UPLC

SHAIK JOHN SAIDA[1,2,*], MUNIAPPAN MUTHUCHAMY[1], MURALIDHARAN KALIYAPERUMAL[2], CHIDANANDA SWAMY RUMALLA[2], RAMULU YANAKA[2] and S. VENKAT RAO[3]

[1]Bharath Institute of Higher Education and Research, Selaiyur, Chennai-600 073, India
[2]Department of Medicinal Chemistry, GVK Bioscience Pvt. Ltd., Hyderabad-500 076, India
[3]SMS Pharmaceuticals Ltd., Hyderabad-500 043, India

*Corresponding author: E-mail: saida_sk2005@yahoo.co.in

Received: 26 April 2018; *Accepted*: 17 July 2018; *Published online*: 31 August 2018; AJC-19046

A stability indicating reverse phase ultra performance liquid chromatography (UPLC) for perampanel and identification of degradation products of perampanel from forced degradation studies. Perampanel was subjected to stress conditions of acid, aqueous hydrolysis, oxidative, photolytic and thermal stress degradation. The degradation of perampanel was observed under acid hydrolysis, basic hydrolysis and peroxide and it was stable in thermal, photo degradation conditions. Good resolution was observed between drug and its degradation products with the proposed UPLC method was developed by using mobile phase formic acid in water and acetonitrile. The developed UPLC method was validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness. The linearity of method was performed concentration levels between 20 to 120 µg/mL with correlation coefficient 0.999. Assay recoveries were found to be 98.2 to 101.6 %. The developed UPLC method was found to be useful to determine the regular production of perampanel samples and its stability studies. Two new degradants were formed (**1-2**). Both acid and base hydrolysis resulted in the same compound **1**. However, oxidation by peroxide resulted in another new compound **2**. Both degradation products were completely characterized by HRMS and extensive NMR, spectrometry. Stability indicating validated method by UPLC and its degradation and cartelization studies were not reported elsewhere.

Keywords: Perampanel, Method validation, Degradation products, HRMS, NMR spectroscopy.

INTRODUCTION

Perampanel is an antiepileptic drug used in addition to other drugs to treat partial seizures and generalized seizures for people [1]. Perampanel chemically known as 2-(2-oxo-1-phenyl-5-pyridin-2-yl-1,2-dihydropyridin-3-yl)benzonitrile (Fig. 1). It was approved in 2012 and as of 2018 its optimal role in the treatment of epilepsy relative to other drugs was not clear [2]. Perampanel is the first antiepileptic drug in the class of selective non-competitive antagonist of AMPA receptors [3].

Perampanel drug was labeled in a black box warning because it may cause serious psychiatric and behavioural changes and homicidal or suicidal thoughts. Other side effects have included dizziness, somnolence, vertigo, aggression, anger, loss of coordination, blurred vision, irritability and slurred speech. Perampanel reduced the effectiveness of levonorgestrel oral contraceptives by about 40 % [1]. Women who may get pregnant should not take it as studies in animals show it may harm a fetus [4]. Perampanel is liable to be abused; high doses produced euphoria responses similar to ketamine. It is designated as a Schedule III controlled drug substance by the drug enforcement authority [1]. As of August 2016 perampanel had

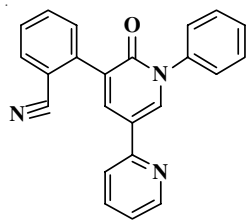


Fig. 1. Chemical structure of perampanel (m.f.: $C_{23}H_{15}N_3O$; m.w.: 349.38 g/mol)

been studied and development discontinued in migraine, multiple sclerosis, neuropathic pain and Parkinson's disease [5]. Few HPLC methods were reported in the literature for the analysis of perampanel in pharmaceutical formulation [6].

No UPLC methods were reported in major pharmacopoeias. Based on literature survey no methods were found on stability, degradation, isolation, identification and complete characterization of perampanel. The present drug stability test guide lines Q1A(R2) issued by international council on harmonization (ICH) suggested that stress study should be carried on a drug to establish its stability characteristics, leading to separation of degradation products and hence supporting the stability of the proposed analytical procedures. It also required that analytical test procedures for stability samples should be fully validated [7-9]. The method was developed and validated as per ICH guidelines and the proposed method was accurate, rapid, specific and reproducible for determination of perampanel in bulk drugs.

EXPERIMENTAL

The drug perampanel (API) was a gift sample received from SMS pharmaceutical Ltd, India. Solvents and buffers chromatographic grade acetonitrile and formic acid form Merk brand and DMSO-d_6 with TMS 0.03 % (v/v), D_2O solvents from Cambridge isotope limited, water used from Milli-Q grade.

Waters make ultra high performance liquid chromatography (UHPLC) connected with PDA detector is used for method development and validation and processed with Empower software. and Gilson make Preparative HPLC with liquid handler GX-271, pump module 331 & 332 connected with PDA detector, The Waters make HRMS Q-TOF Micro Mass with ion source ESI mode, Capillary voltage: 2800 V; Sample voltage: 30 V; Source temperature: 140 °C and Leucine encephalin (*m/z* 555.6228) is used internal standard for HRMS analysis and the data was processed with Mass Lynx software. To determine the functional groups by IR spectrum, FT-IR is used from Shimadzu and NMR (400 MHz) instrument is used from Bruker.

Chromatographic and spectroscopic conditions: Degradation impurities were purified by using Gilson prep-HPLC with column Kromasil C18 (250 × 20 mm) 5 µ with mobile phase A: 0.1 % formic acid in Aquas and B: acetonitrile with gradient elution % B: 0/5, 10/60, 15/90, 15.5/5, 20/5 with a flow rate of 20 mL/min at room temperature. The crude degradation reaction material was dissolved in mobile phase, filtered and injected 300 µL/inj and purification was monitored at 215 and 290 nm.

^1H, ^{13}C and 2D NMR was recorded with 400 MHz NMR instrument and the degradation impurities were recorded in DMSO-d_6 solvent. Carbon-proton connectivity was confirmed by 2D NMR like COSY, HSQC, HMBC,^{15}NHSQC and ^{15}NHMBC and the hydrogen's attached with hetero atoms were further confirmed by D_2O exchange experiment.

The waters make Q-TOF micro mass high resolution mass spectrometry (HRMS) is used to find out the accurate mass of the degradation impurities and the data was monitored in positive mode.

For method development water UPLC is used with column Aquity BEH (100 × 2.1) mm 1.7 µ with a buffer combination of buffer A: 0.1 % formic acid in Aquas and % B: acetonitrile with isocratic elution mode in a ration of 60:40 (A:B), flow: 0.4 mL/min at 40 °C. Chromatograms were monitored at 290 nm. The functional group information was done by infrared spectrum in solid state as KBr medium.

Preparation of solutions

Stress conditions: The drug was dissolved in 0.5 N HCl solution and stirred for 12 h at 60 °C and the reaction was monitored by UPLC-MS. For oxidative degradation drug was dissolved in 30 % peroxide solution and refluxed for 2 days at 60 °C and for base degradation drug was dissolved in 0.5 N NaOH and stirred for 16 h and the degradation was not observed in other stress conditions photo and thermal study.

Purification of degradation compounds: Acid and oxidative degradation crude mixtures were slightly diluted with mobile phase and filtered.

The filtered solution was taken up for the purification by reverse prep-HPLC. Isolated fractions were collected and lyophilized to get fine solid compounds.

Preparation of standard solution: The drug substance 200 µg/mL was transferred in to 100 mL of volumetric flask and dissolved in 50 mL of acetonitrile and buffer initially and further make-up to the mark. Stock solution was sonicated for 0.5 h and filtered. Further dilutions (20-120 µg/mL) were prepared from stock solution to check linearity and system suitability tests.

RESULTS AND DISCUSSION

Method development and validation of drug substance by UPLC conditions and isolation of its degradation impurities: A new stability indicating method for perampanel and its degradation products was developed. Structures of the degradants were also established. The chromatogram is shown in Fig. 2.

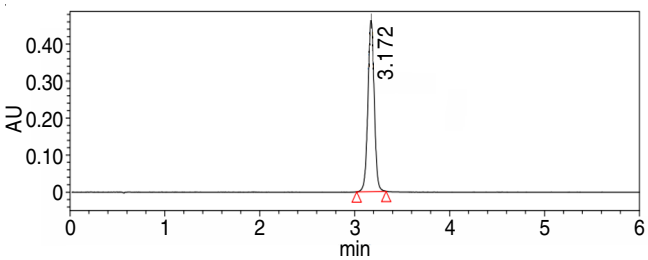


Fig. 2. Chromatogram of perampanel drug

Degradation behaviour of perampanel: Table-1 shows the degradation details of perampanel. Two major impurities were found in acid and oxidative degradation.

In acid degradation 17.02 % of new impurity was found (Fig. 3) and in base degradation almost negligible impurity was observed which is matching with acid impurity. In oxidative degradation 11.68 % of new impurity was found (Fig. 4).

Characterization of degradation products

Acid degradation product-A: Compared with drug substance ^1H NMR of acid degradation product was shown two extra peaks at 7.13 and 7.60 ppm with proton each and it got exchanged in D_2O exchange experiment. These two protons

TABLE-1
DEGRADATION DETAILS OF PERAMPANEL

Degradation product	RT (min)	Area (%)	USP resolution	USP tailing	Purity
		Acid degradation			
Degradation impurity-A (in acid)	0.951	17.02		1.14	Pass
Perampanel	3.192	82.98	25.35	1.01	Pass
		Base degradation			
Degradation impurity (in base)	0.948	2.96		1.18	Pass
Perampanel	3.189	97.04	25.86	1.02	Pass
		Oxidative degradation			
Degradation impurity-B (in peroxide)	0.987	11.68		1.18	Pass
Perampanel	3.186	88.32	23.96	1.02	Pass

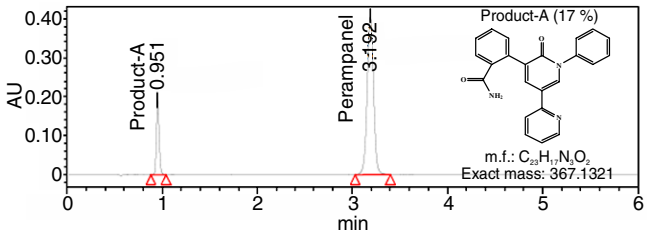


Fig. 3. Acid degradation product-A of perampanel

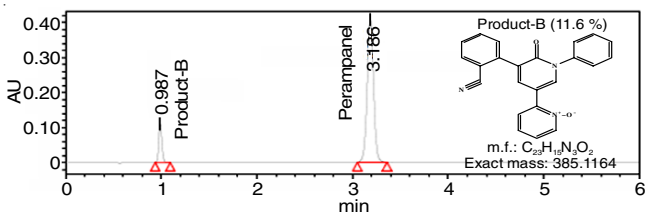
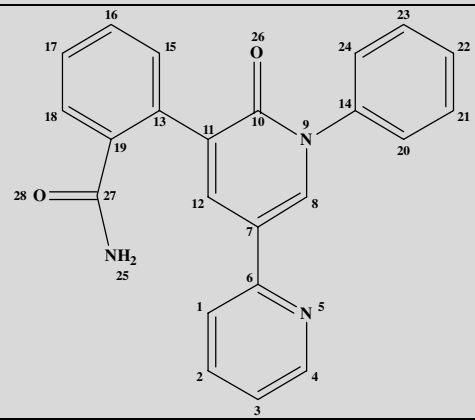
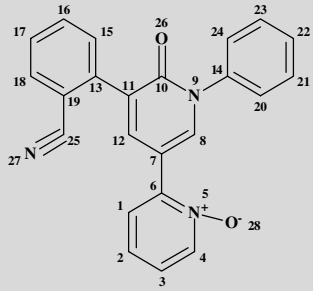


Fig. 4. Oxidative degradation product-B of perampanel

TABLE-2
^{1}H AND ^{13}C NMR ASSIGNMENTS OF ACID AND OXIDATIVE DEGRADATION PRODUCTS A & B





Assignment	Degradation product-A				Degradation product-B			
	Type of carbon	^{13}C (ppm)	^{1}H (ppm)	^{15}N (ppm)	Type of carbon	^{13}C (ppm)	^{1}H (ppm)	^{15}N (ppm)
1	CH	118.8	7.95		CH	125.5	7.61	
2	CH	137.1	7.82		CH	126.3	7.35	
3	CH	121.8	7.28		CH	124.3	7.22	
4	CH	149.2	8.58		CH	140.5	8.35	
5	N			301.2	N			285.7
6	C	152.8			C	144.6		
7	C	117.2			C	110.7		
8	CH	136.2	8.36		CH	141.3	8.83	
9	N			186.1	N			186.1
10	C=O	160			C=O	160.1		
11	C	132.6			C	128.4		
12	CH	135.6	8.21		CH	140.6	8.09	
13	C	135.7			C	139.8		
14	C	141.2			C	140.4		
15	CH	130.6	7.46		CH	131.2	7.82	
16	CH	129.5	7.56		CH	132.4	7.63	
17	CH	127.5	7.42		CH	128.4	7.45	
18	CH	127.3	7.57		CH	133.2	7.77	
19	C	136.8			C	112.1		
20,24	CH	126.8	7.51		CH	129.6	7.51	
21,23	CH	129	7.55		CH	126.6	7.51	
22	CH	128.2	7.48		CH	128.3	7.45	
25	$-NH_2$		7.58 & 7.13	108	N			

connected with one nitrogen which is confirmed by ^{15}NHSQC, its clearly indicated that one extra –NH_2 group is present in acid degradation product. Structure was further confirmed by ^{13}C NMR, HSQC, HMBC and ^{15}NHMBC.

Carbon-hydrogen connectivity was exactly matching to the acid degradation product-A. For more accuracy results HRMS was performed to know the accurate protonated ion mass *m/z* 368.1391 with chemical formula $C_{23}H_{18}N_3O_2$ with less error 2 ppm.

Infrared spectrum was compared with perampanel, observed absence of –CN at 2217 cm^{-1} and presence of –NH_2 at 3179 cm^{-1} & 3339 cm^{-1} and other major frequencies are conforms to degradation product-A. Table-2 shows the assignments of ^{1}H and ^{13}C NMR assignments of the degradation product-A.

Oxidative degradation product-B: Number of protons was same for both perampanel and degradation product-B but they differ in their mass. To know accurate mass of this compound was analyzed by HRMS found its molecular protonated ion mass *m/z* 366.1248, with chemical formula $C_{23}H_{16}N_3O_2$ with less error 1.4 ppm data was confirms to the degradation product-B. Infrared spectrum N-O stretching is observed at 936 cm^{-1} and –CN observed at 2217 cm^{-1} and other major frequencies were conforms to degradation product-B. Proton-carbon connectivity was confirmed by ^{1}H NMR, ^{13}C NMR, HSQC, HMBC and ^{15}NHMBC. NMR data was compared with drug substance the pyridine nitrogen value of degradation product was compared with perampanel, pyridine nitrogen in perampanel at 301.2 ppm was missing and appeared at 285.7 ppm, which clearly confirms to N-oxide product and other correlation were confirmed by 2D NMR. Table-2 shows the ^{1}H and ^{13}C NMR assignments of degradation product-B. The ^{15}NHMBC data of the drug along with the two degradation products is given in Table-3.

TABLE-3
NITROGEN (ppm) VALUES

	Nitrogen ppm values by ^{15}N HMBC		
	N (5)	N (9)	NH_2
Perampanel (pure drug)	301.2	186.1	–
Acid degradation product-A	303.4	182.1	107.4
Oxidative degradation product-B	285.7	186.1	–

Method validation: Proposed method was developed as per ICH guidelines, total six duplicate injections were performed to check the precision of proposed method.

Recovery 100.1 % was observed with proposed method which clearly indicates the accuracy of the method. Linearity was performed for concentration levels 20-120 µg/mL and calculated R^2 is within the acceptable limits. LOD & LOQ were found 0.03 and 0.06 µg/mL, respectively.

Six duplicate injections was performed to check the precision of proposed method and % RSD is within the limit. Accuracy and recovery of the method was proved by spiking 10 % of known concentration to the standard concentrations like 80, 100 and 120 % and the assay % is observed 98.2, 100.6 and 101.6, respectively. Solution stability was checked for the drug storage at specific temperature (2-8 °C) for one month, material was stable in solution.

Method robustness was checked by changing few parameters like temp ± 5 °C, pH ± 0.2 and flow ± 0.2 mL, based on the above trials there is no major changes were observed that the method was robust. Method validation parameters were shown in Tables 4-7.

TABLE-4
LINEARITY OF PERAMPANEL

Concentration of drug (µg/mL)	Retention time (min)	Peak area
20	3.110	437665
40	3.128	869179
60	3.135	1304423
80	3.127	1742361
100	3.135	2152675
120	3.124	2592093

TABLE-5
METHOD PRECISION OF PERAMPANEL

Precision results	
Inter-day	Intra-day
Area (Mean ± standard deviation), no. of inj. = six	Area (Mean ± standard deviation), no. of inj = six
2214788 ± 14497	21498931 ± 2883

Conclusion

The new advanced UPLC method is suitable to determine perampanel in bulk drugs. Because of short run time this method is very useful in bulk drugs to run many injections during reaction monitoring of perampanel. Proposed method is suitable to calculate low concentration level of LOD (0.03 µg/mL) and LOQ (0.06 µg/mL).

TABLE-6
RECOVERY STUDIES OF PERAMPANEL WITH KNOWN CONCENTRATION

S. No.	Accuracy		Accuracy		Accuracy	
	80 %	80 % + spike 10 % of known con.	100 %	100 % + spike 10 % of known con.	120 %	120 % + spike 10 % of known con.
1	1762590	1946400	2152670	2397990	2598616	2881563
2	1749682	1931057	2156033	2408838	2608335	2880873
3	1758712	1941517	2151521	2347355	2608052	2843761
Mean	1756994.667	1939658	2153408	2384727.667	2605001	2868732.333
SD	6623.144369	7838.611038	2344.78549	32817.02205	5531.382377	21628.56078
% RSD	0.376958707	0.404123358	0.10888719	1.376132902	0.212337054	0.753941402
Recovery (%)	98.2		100.6		101.6	
Average % of recovery	100.1					

TABLE-7
REGRESSION OF UPLC PROPOSED METHOD FOR PERAMPANEL

Parameters	Proposed UPLC method
RT (min)	3.17
Theoretical plates (n)	14900
Symmetry (tailing)	1.1
Linearity concentrations (ppm)	20-120
Regression coefficient (R^2)	0.999
Limit of detection (LOD) (µg/mL)	0.03
Limit of quantification (LOQ) (µg/mL)	0.063

Hence the precision, linearity, repeatability, rapidness, simplicity and economical of UPLC method is having more advantage of other existed HPLC methods.

In acid degradation study the product-A is formed with mass *m/z* 367.13 and having chemical formula $C_{23}H_{17}N_3O_2$ and in oxidative degradation product-B was formed with mass *m/z* 365.11 having chemical formula $C_{23}H_{15}N_3O_2$, both acid and oxidative degradation products (A & B) were new and fully characterized and confirms to the structure.

The method is confirmed its suitability to determine stability indicating method for perampanel in bulk drug industries.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J.E. Frampton, *Drugs*, **75**, 1657 (2015); https://doi.org/10.1007/s40265-015-0465-z.
2. P. Patsalos and F. Besag, *Neuropsychiatr. Dis. Treat.*, **12**, 1215 (2016); https://doi.org/10.2147/NDT.S83842.
3. D.J. Chong and A.M. Lerman, *Curr. Neurol. Neurosci. Rep.*, **16**, 39 (2016); https://doi.org/10.1007/s11910-016-0640-y.
4. J. Greenwood and J. Valdes, *Pharm. Therap*., **41**, 683 (2014);
5. A.B. Ettinger, A. LoPresti, H. Yang, B. Williams, S. Zhou, R. Fain and A. Laurenza, *Epilepsia*, **56**, 1252 (2015); https://doi.org/10.1111/epi.13054.
6. C.J. Patel, S.S. Patel and M.M. Patel, *Int J. Pharm. Drug. Anal*., **5**, 229 (2017).
7. Stability Testing of New Drug Substances and Drug Products Q1 A (R2), International Conference on Harmonization, IFPMA, Geneva (2003).
8. USP 2011 United States of Pharmacopoeia, United States Pharmacopeia Drug Stability Principles and Practices, edn 34 (2000),
9. M. Bakshi and S. Singh, *J. Pharm. Biomed. Anal.*, **28**, 1011 (2002); https://doi.org/10.1016/S0731-7085(02)00047-X.