IJPSR (2011), Vol. 2, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 February, 2011; received in revised form 14 March, 2011; accepted 06 April, 2011

DEVELOPMENT AND VALIDATION OF A HEADSPACE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF RESIDUAL SOLVENTS IN LEVETIRACETAM (API)

Ankur Shukla*, R. K. Jat, Pankaj Sharma and Yogendra Patel

Department of Medicinal Chemistry & Drug Discovery, Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India

Keywords:

Levetiracetam,
Keppra,
AEDs,
SV2A,
GC HS,
Validation

Correspondence to Author:

Ankur Shukla

Department of Medicinal Chemistry & Drug Discovery, Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India

Abbreviations:

LEV: Levetiracetam,
gm: Gram,
ml: Mili liter,
m: Meter,
mm: Mili Meter
AEDs: Antiepileptic drugs,
GC HS: Head space gas chromatographic,
SV2A: Synaptic vesicle protein 2A

ABSTRACT

Levetiracetam is a new antiepileptic medication that is effective as adjunctive therapy in the treatment of partial onset seizures in adults and children 4 years of age and older with epilepsy. Levetiracetam is an antiepileptic drug available as a clear, colorless, grape-flavored liquid (100 mg/mL) for oral administration. It is the S-enantiomer of etiracetam, structurally similar to the prototypical nootropic drug piracetam. Levetiracetam shows great potential as a safe and effective agent for the treatment of neonatal seizures based on its efficacy and safety profile in other age groups, its mechanism of action is still not much known.

Materials and Methods: The method development and its validation were performed on Agilent's gas chromatographic system equipped with Flame Ionization detector and head space analyzer. The method involved in SPB^{TM}- 624, Supelco, 60 m length, 0.32 mm internal diameter, and 1.8 µm film thickness column using nitrogen gas as a carrier. The flow rate was 1.5 mL per minute and flame ionization detector (FID) was used.

Results: During method validation, parameters such as precision, linearity, accuracy, limit of quantification and detection and specificity were evaluated, which remained within acceptable limits.

Conclusions: The method has been successfully applied for the quantification of the amount of residual solvents present in Levetiracetam bulk drug. The method presents a simple and reliable solution for the routine quantitative analysis of residual solvents in Levetiracetam bulk drug.

INTRODUCTION: The need for a rapid and reliable method for the determination of residual solvents has become significant due to the toxicity of residual solvents in drug substances and drug products [1]. The determination of residual solvents in drug substances, excipients or drug products is known to be one of the most difficult and demanding analytical tasks in the pharmaceutical industry. Furthermore, determination of the polar residual solvents in pharmaceutical preparations is still an analytical challenge mainly because these compounds are quite difficult to remove from water or polar solvents [2].

Many pharmaceutical products have to be analyzed for residual solvents at different stages of their development (raw materials, intermediate products and final product). Organic solvents such as Methanol, ethanol, Acetone**,** dichloromethane, Isopropyl alcohol, Acetonitrile, Methyl ethyl ketone, Ethyl acetate, Tetrahydrofuran, Cyclohexane, are frequently used in the pharmaceutical industry. The manufacturing of new active pharmaceutical ingredients (APIs) under Good manufacturing practices (GMP) conditions demands adequate control of quality of the different ingredients used in the synthesis. Organic residual solvents have therefore to be controlled during any GMP synthesis. Headspace gas chromatography (HSGC) is the most favored technique for the analysis of volatiles and semi volatile organics in solid, liquid, gas samples [3, 4, 5, 6, 7].

MATERIALS & METHODS:

Chemicals and reagents: Levetiracetam was obtained from Analytical research department of Jubilant Life Scinces, Noida, India. Milli Q water was obtained from inhouse Milli Q water plant. Dimethyl sulfoxide (Spectrochem), Methanol, Acetone, Dichloromethane, Isopropyl Alcohal, Acetonitrile, Methyl Ethyl Ketone, Ethyl Acetate, Tetrahydrofuran, Cyclohexane were purchased from Sigma-Aldrich, ethanol (Changshu Yangyuan), Toulene (Merk).

Apparatus and Chromatographic Condition: The analysis for the determination of residual solvents and its validation was performed on Agilent's gas chromatograph equipped with headspace sampler and a flame ionization detector (FID). SPB^{TM}- 624, Supelco, 60 m length, 0.32 mm internal diameter, and 1.8µm film thickness column was used for the validation. The final validated HSGC method for the separation of residual solvents used a flow rate of 1.5ml/min. Oven temperature was maintained at 40°C for 22 min, and then a linear thermal gradient of 30°C/min to 220°C was used with a final hold of 8 min. Total run time was 36.0 min. Nitrogen was used as a carrier gas at a constant flow rate of 1.5ml/min.

Preparation of Standard and Sample vials: A common standard stock solution in N,N-dimethylsulfoxide containing all the known residual solvents of Levetiracetam API was prepared in such a way that it has a final concentration of methanol, ethanol, acetone, isopropyl alcohol, ethyl acetate, cyclohexane, methylethyl ketone, dichloromethane, acetonitrile, tetrahydrofuran and toluene are 3000 ppm, 5000 ppm, 5000 ppm, 5000 ppm, 5000 ppm, 3880 ppm, 5000 ppm, 600 ppm, 410 ppm, 720 ppm and 890 ppm respectively, for a sample concentration of 50 mg per mL.

The standard vial was prepared with 2 ml of the standard solution and the sample vials were prepared with approximately 0.1 gram of sample with 2 ml N, N-dimethylformamide as diluents.

Method Validation: The method validation was done by evaluating specificity, limit of detection (LOD) and limit of quantitation (LOQ), linearity, accuracy, repeatability, and method precision of residual solvents as indicated in the ICH guideline Q2B 'Validation of Analytical Procedures: Methodology [15].

Specificity: Levetiracetam API sample was spiked with all solvents individually and each sample was chromatographed to examine interference, if any, of the residual solvents peak with each other.

Linearity: The linearity of the method was determined by making injection of each residual solvent over the range of limit of quantifications (LOQ) to 200% of the standard concentration as mentioned earlier.

System precision: Six vials of standard solution were prepared and chromatographed in the GC system.

Method precision: A single batch of Levetiracetam API was prepared six times and analyzed by the proposed method.

Detection and Quantification limits: The LODs of residual solvents in Levetiracetam were determined based on signal-to-noise ratio of 3:1. The LOQs of residual solvents were determined based on signal-to-noise ratio 10:1. Six replicates were performed at each level.

Accuracy: Known amount of sample (about 100 mg) was taken separately in nine different vials and spiked with known quantities of methanol, ethanol, acetone, isopropyl alcohol, ethyl acetate, cyclohexane, methylethyl ketone, dichloromethane, acetonitrile, tetrahydrofuran and toluene at three different levels in triplicate

System Suitability: A system suitability criterion was taken to be the resolution between the critical pairs, Methyl Ethyl Ketone & Ethyl acetate. The system suitability was evaluated by injecting the standard solution on various days before starting the any exercise during validation study.

RESULT & DISCUSSION:

Method development: An understanding of the nature of the various residual solvents present in API is the foremost prerequisite for successful method development in HSGC. In addition, successful method development should result in a fast, simple and time efficient method that is capable of being utilized in a manufacturing setting. Following were the stepwise strategies for the method development in our case.

Column selection: The primary goal of column selection was to resolve a total of 10 residual solvents (i.e. methanol, ethanol, acetone, isopropyl alcohol, ethyl acetate, cyclohexane, methylethyl ketone, dichloromethane, acetonitrile, tetrahydrofuran and toluene,) which were used during the synthesis and manufacturing of Levetiracetam. Several columns were initially investigated to finalize a single method for the separation and quantitation of solvents. Wall-coated capillary columns of various brands with a variety of phases and dimensions have been investigated, e.g., column A (30 m length, 0.32 mm i.d. with a stationary phase of 6% cynopropyl phenyl and 94% dimethyl polysiloxane film of 3.0 µ), RTx-624 (30 m length, 0.32 mm i.d. with a stationary phase of 6% cynopropyl phenyl and 94% dimethyl polysiloxane film of 1.8 µ).

In the above column, the response was found to be comparatively lower and peak shapes were found to be unsatisfactory. Also, there was a problem in resolving the closely related peaks specially 2-methylpentane and dichloromethane. Therefore, SPB^{TM}- 624, Supelco, 60 m length, 0.32 mm internal diameter, and 1.8µm film thickness proved to be the best column that could fulfill all the needs of the method, i.e., higher sensitivity, shorter runtime and higher resolution between the critical pairs.

Thermal program and thermal gradient: A linear thermal gradient was chosen to provide elution of the solvents' peak during the isothermal segment of the chromatographic run for better quantification. An initial hold of 22 min at 40°C and a linear thermal gradient to 220°C at 30°C/min was found to give the best peak shape and retention without affecting the resolution.

The headspace method was optimized in such a way that maximum amount of the solvents present in the sample get evaporated for the detection. For this the standard and sample vials were heated at Oven equilibration temperature 100°C, Loop temperature 105°C, Transfer line temperature 110°C, GC cycle time

45 minutes, Oven/vial, equilibration time 15 minutes, Pressurization time 0.5 minutes, Loop fill time 0.2 minutes, Injection time 0.5 minutes, Loop equilibration time 0.5 minutes Vial pressure 14 psi, Vial shake Off was found to be suitable for getting a good response. (**Table 1**) shows the complete headspace condition.

TABLE 1: HEADSPACE CONDITION

Oven equilibration temperature	100°C
Loop temperature	105°C
Transfer line temperature	110°C
GC cycle time	45 minutes
Oven/vial, equilibration time	15 minutes
Pressurization time	0.5 minutes
Loop fill time	0.2 minutes
Injection time	0.5 minutes
Loop equilibration time	0.5 minutes
Vial pressure	14 psi
Vial shake	Off

Method validation:

Specificity: The relative retention time of the all-residual solvents indicated that they were well separated from each other.

Linearity: The FID detector response was shown to be linear over this range with correlation coefficients (R^2) higher than 0.99 (**Table 2**, **Figure 1**).

TABLE 2: LINEARITY OF DICHLOROMETHANE

Linearity level	Conc. (ppm)	Mean area
QL	45.33	2.66
15 % level	-	-
30 % level	-	-
50 % level	50.37	3.06
75 % level	75.55	4.37
100 % level	100.73	5.66
125 % level	125.92	7.29
150 % level	151.10	8.52
Slope	**0.06**	**5.04**

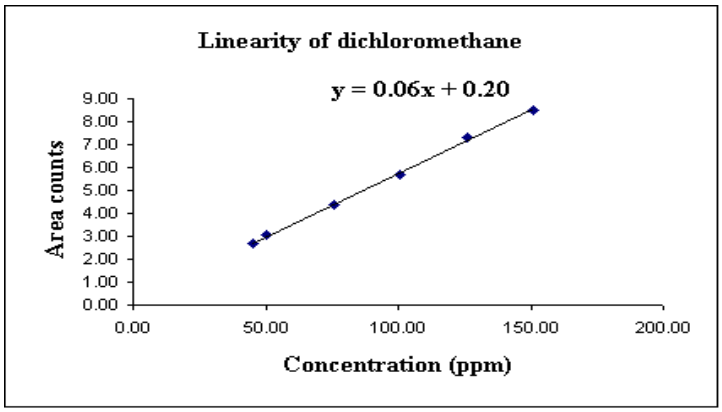


FIG. 1: LINEARITY OF DICHLOROMETHANE

System precision: Results indicate an acceptable level of precision for the analytical system (**Table 5**). The % relative standard deviation (%RSD) of area obtained from six standard vials was calculated to show the system precision. An acceptance criterion for system precision was taken to be not more than (NMT) 8% for six vials.

TABLE 3: SYSTEM PRECISION

Solvent name	Methanol	Acetone	Dichloro-methane ketone	Cyclohexane	Toluene
Injection	Area	Area	Area	Area	Area
1	234.11	1346.60	6.05	3768.36	185.08
2	206.11	1309.47	5.83	3830.82	163.28
3	207.02	1314.40	5.73	3855.49	163.91
4	202.72	1297.92	5.74	3812.59	159.52
5	202.16	1295.82	5.64	3800.98	158.84
6	207.53	1304.04	5.78	3794.25	163.17
Mean	209.94	1311.38	5.80	3810.42	165.63
SD	12.049	18.603	0.140	30.238	9.760
%RSD	5.74	1.42	2.41	0.79	5.89

Method precision: The %RSD values indicate that the method has an acceptable level of precision. The %RSD values of area obtained from six sample vials were calculated to show the method precision. An acceptance criterion for method precision was taken to be NMT 10% for six preparations.

Detection and quantification limits: (Table 4) show the quantification and detection limits, respectively, for the samples.

TABLE 4: QUANTIFICATION AND DETECTION LIMITS

Name	Detection limit (ppm)	Quantitation limit (ppm)
Methanol	14.92	44.75
Acetone	4.98	14.95
Dichloromethane	15.43	46.30
Cyclohexane	1.07	3.22
Toluene	4.99	14.96

Accuracy: The accuracy was evaluated by the % recoveries of residual solvents spiked in the sample. An acceptance criterion for accuracy was that the recovery should be in the range of 80-120%. The recoveries of residual solvents ranged between 93.06 and 106.6%. Results indicate that the method has an acceptable level of accuracy (**Table 5**).

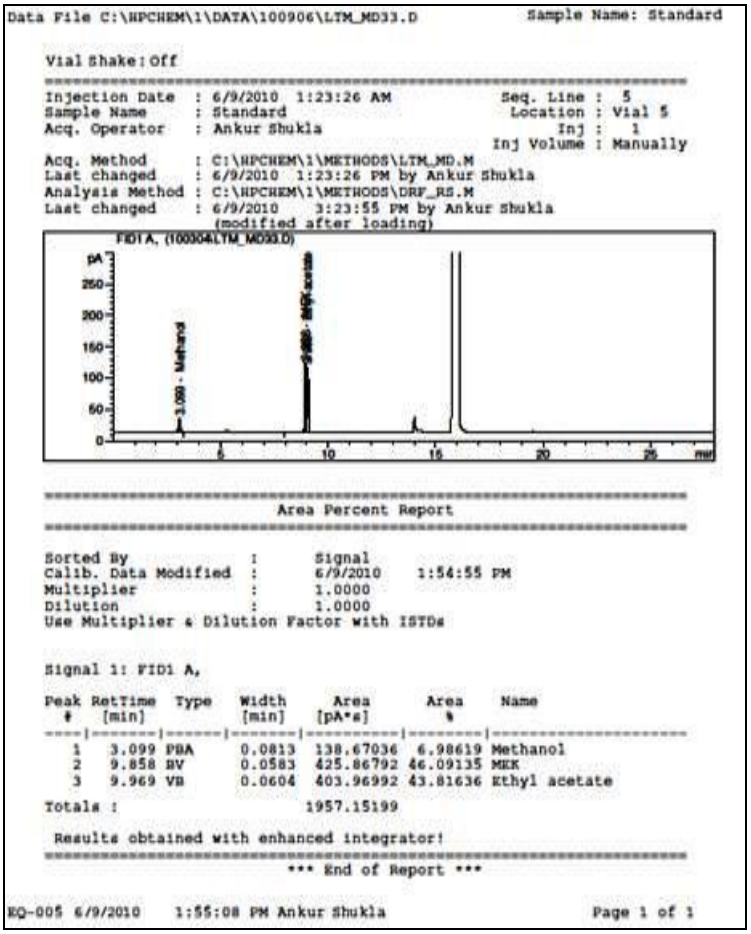
TABLE 5: SYSTEM SUITABILITY DATA- ACCURACY

System suitability data – accuracy		
System suitability parameter	**Results**	**Acceptance criteria**
Resolution (Acetone and dichloromethane peaks)	9.15	Should be more than 2.0
%RSD (Methanol)	1.93	Should be less than 10.0
%RSD (Acetone)	1.03	
%RSD (Dichloromethane)	1.19	
%RSD (Cyclohexane)	0.99	
%RSD (Toluene)	2.64	

System suitability: Using the system suitability software, resolution between Methyl Ethyl Ketone & Ethyl acetate was calculated. The criterion for system suitability was that the resolution between critical pair mentioned above should not be less than 1.5 and it was found well above the minimum passing limit (**Table 6**).

TABLE 6: SYSTEM SUITABILITY DATA- REPEATABILITY

System suitability data – repeatability		
System suitability parameter	**Results**	**Acceptance criteria**
Resolution (Acetone and dichloromethane peaks)	8.98	Should be more than 2.0
%RSD (Methanol)	5.74	Should be less than 10.0
%RSD (Acetone)	1.42	
%RSD (Dichloromethane)	2.41	
%RSD (Cyclohexane)	0.79	
%RSD (Toluene)	5.89	



CHROMATOGRAM: VIAL SHAKE PARAMETER

CONCLUSION: A single, rapid and highly selective HSGC method was developed and validated for the quantification of residual solvents present in Levetiracetam bulk drug through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The residual solvents methanol, ethanol, acetone, isopropyl alcohol, ethyl acetate, cyclohexane, methylethyl ketone, dichloromethane, acetonitrile, tetrahydrofuran and toluene were determined. The method was shown to be specific for Levetiracetam and was applied successfully to monitor and control these solvents on a manufacturing level. The method was found to be applicable for the routine analysis of the Levetiracetam in pharmaceutical firms.

ACKNOWLEDGEMENT: The author would like to thank Mr. R K Jaat (Gyan Vihar University, Jaipur, India) for his contribution in restructuring the article.

REFERENCE:

1. Sugimoto M, Suzuki H, Akimoto K, Kuchiki A, Nakagawa H. Determination of residual solvents in drug substances by gas chromatography with thermal desorption cold trap injection. Chem Pharm Bull 1995; 43:2010-3.
2. B'Hymer C. Residual solvent testing: A review of gas chromatographic and alternative techniques. Pharm Res 2003; 23:337-44.
3. Camarasu CC. Unknown residual solvents identification in drug products by headspace solid phase microextraction gas chromatography-mass spectrometry. J Chromatographia 2002; 56:S131-5.
4. Raghani AR. High speed gas chromatographic analysis of solvents in pharmaceutical using solid phase microextraction. J Pharm Biomed Anal 2002; 29:507-18.
5. Coran SA, Giannellini V, Furlanetto S, Bambagiotti-Alberti M, Pinzauti S. Improving Gas chromatographic determination of residual solvents in pharmaceuticals by the combined use of head space solid-phase microextraction and isotopic solution. J Chromatogr A 2001; 915:209-16.
6. Rocheleau MJ, Titley M, Bolduc J. Measuring residual solvents in pharmaceutical samples using fast gas chromatography techniques. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 805:77-86.
7. Legrand S, Dugay J, Vial J. Use of solid-phase microextraction coupled with gas chromatography for the determination of residual solvents in pharmaceuticals in pharmaceutical products. J Chromatogr A 2003; 999:195-201.
8. Walawalkar MG. A new antimalarial candidate. Curr Sci 2004; 87:1035-7.
9. Kaiser M, Wittlin S, Nehrbass-Stuedli A, Dong Y, Wang X, Hemphill A, et al. Peroxide bond-dependent antiplasmodial specificity of artemisinin and OZ277 (RBx11160). Antimicrob Agents Chemother 2007; 51:2991-3.
10. Kreidenweiss A, Mordmóller B, Krishna S, Kremsner PG. Antimalarial activity of a synthetic endoperoxide (RBx-11160/OZ277) against Plasmodium falciparum isolates from Gabon. Antimicrob Agents Chemother 2006; 50:1535-7.
11. Uhlemann AC, Wittlin S, Matile H, Bustamante LY, Krishna S. Mechanism of antimalarial action of the synthetic trioxolane RBX11160 (OZ277). Antimicrob Agents Chemother 2007; 51:667-72.
12. Maerki S, Brun R, Charman SA, Dorn A, Matile H, Wittlin S. In vitro assessment of the pharmacodynamic properties and the partitioning of OZ277/RBx-11160 in cultures of Plasmodium falciparum. J Antimicrob Chemother 2006; 58:52-8.
13. International Conference on Harmonization, Impurities in new drug substances, Q3A (R2), 2006.
14. Reddy P, Reddy MS. Residual Solvents determination by HS-GC with flame ionization detector in omeprazole pharmaceutical formulations. Int J Pharm Tech Res 2009; 1:230-4.
15. International Conference on Harmonization, Validation of Analytical Procedures: Methodology, Q2B, 1996.

****