Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (8):388-394
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Quantification of potential genotoxic impurity in Divalproex sodium drug substance by GC-MS method

K. V. S. N. Raju[*1], P. Madhava Reddy[1], N. Sreenivas[1], Hemant Kumar Sharma[1], K. S. R. Pavan Kumar[*1], V. Jagadeesh Kumar[1], G. Himabindu[2] and N. Annapurna [2]

[1]*Aurobindo Pharma Limited Research Centre-II, Survey No. 71&72, Indrakaran Village, Sangareddy Mandal, Medak -502329, Andhra Pradesh, India*
[2]*Department of Engineering Chemistry, AU College of Engineering, Andhra University, Visakhapatnam- 530003, Andhra Pradesh, India*

ABSTRACT

A sensitive rapid Gas Chromatography with mass spectrometry (GC-MS) method has been developed and validated for the determination of 1-Bromopropane content in Divalproex sodium drug substance. 1-Bromopropane has been considered as a potential genotoxic impurity. The lower level of detection was achieved on fused silica capillary column coated with 100% dimethylpolysiloxane stationary phase with Electron Impact ionization (EI) in Selective Ion Monitoring (SIM) mode. GC runtime was 25min employing programmed temperature with split mode using head-space injection technique. The developed method was validated for specificity, linearity, accuracy and precision. The detection and quantitation limits of 1-Bromopropane obtained were 0.05 µg/g and 0.10 µg/g respectively. The method was found to be linear in the range between 0.10 µg/g and 0.75 µg/g with correlation coefficient 0.9999. The average recovery obtained in Divalproex sodium drug substance was 97.3%. The developed method was found to be rugged for the determination of 1-Bromopropane in divalproex sodium drug substance. The detailed approach of experiments is discussed in the paper.

Key words: 1-Bromopropane, Divalproex sodium, Gas chromatograph with mass spectrometer

---

INTRODUCTION

Residual solvents in pharmaceuticals are used in the manufacture of drug substances which are to be evaluated and should be removed or controlled to the extent possible as they do not provide therapeutic benefit [1] and there are not completely removed by practical manufacturing techniques [2]. Divalproex Sodium (DS) is chemically known as Sodium hydrogen bis(2-propylvalerate) oligomer (Figure 1) having molecular weight as 310.41. It is a stable co-ordination compound combination of sodium valproate and valproic acid in a 1:1 molar ratio and formed during the partial neutralization of valproic acid with 0.5 equivalent of sodium hydroxide and it has been used successfully in the management of various painful neuropathies.

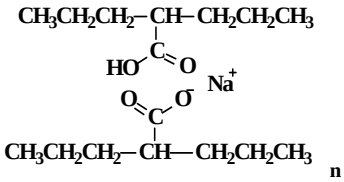


Figure. 1. Chemical structure of Divalproex Sodium

DS was discovered to decrease the likelihood of seizure in 1963.

DS is thought to work by increasing the levels of a brain neurotransmitter called gamma-aminobutyric acid (GABA). GABA is an inhibitory neurotransmitter, which means that its presence makes it harder for nerve cells (neurons) in the brain to become activated. It is believed that increasing GABA's inhibitory action on brain neurons accounts for the ability of divalproex sodium to decrease seizures and curb manic behaviors [3].

1-Bromopropane (BP) is one of the key raw materials used in the synthetic process of DS. Low levels of 1-Bromopropane may be present in the final drug substance as residual impurity. BP comes under potential carcinogenic impurity as per its structure as primary alkyl halides. The chemical structure of 1-Bromopropane is given in Figure 2.

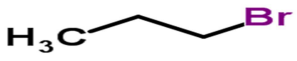


Figure 2. Chemical structure of 1-Bromopropane

Most of the countries are having their own specific guidelines for testing of pharmaceuticals for genotoxicity. As per regulatory requirements, the carcinogenicity and mutagenicity testing are requires, when the compound or its metabolite is structurally related to a known carcinogen or when the nature and action of the drug suggest a mutagenic/carcinogenic potential [4].

The report of carcinogens (thirteenth edition), monograph of BP, National Toxicology Program, U.S. Department of Health and Human Services reveals that BP is reasonably anticipated to be as human carcinogen based on sufficient evidences on its potential carcinogenicity [5]. Studies have been performed on carcinogenicity in rodents. These type of impurities pose an additional safety concern on clinical studies and as well as on patients. Henceforth, an extensive effort to be performed to control this type of impurity at safe concentrations.

Depakote tablets (divalproex sodium delayed release tablets) are for oral administration. Depakote tablets are supplied in three dosage strengths containing DS equivalent to 125 mg, 250 mg, or 500 mg of valproic acid. DS is generally considered safe and its maximum recommended dosage is 60 mg/kg/day; ie equal to 3000mg [6]**.** The impurity should not be present in drug substance or should be less than 0.5 µg/g as per TTC approach based on daily dosage of drug substance [7]. Therefore, keeping the view of the impurity level, we have chosen GCMS instead of GC as this technology takes full advantage.

Some of the analytical methods were already available in some of literatures for the determination of BP. China National Textile and Apparel Council has published the determination of the content of 1-Bromopropane in Textiles via Gas Chromatography-mass Spectrometric Method[8]. Vincent Bessonneau, Luc Mosqueron were reported more than 40 volatile organic compounds (VOCs) including aliphatic, aromatic and halogenated hydrocarbons, aldehydes, alcohols, ketones, ethers and terpenes measured in a teaching hospital in France by ATD/GC/MS [9]. Toxicological Sciences, Aug 2007 was published for Globin S-Propyl Cysteine and Urinary N-Acetyl-S-Propylcysteine as Internal Biomarkers of 1-Bromopropane Exposure by liquid chromatography–tandem mass spectrometry (LC/MS/MS) and gas chromatograph–mass spectrometry (GC/MS) [10].

To the best of our knowledge, determination of BP in the DS drug substance by GCMS has not been reported in literature till date. This paper describes the development, optimization and validation of GCMS method in accordance with ICH guideline Q2(R1) and United States Pharmacopeia.

MATERIALS AND METHODS

Chemicals, reagents and samples

The investigated samples DS drug substance was gifted from APL Research Centre laboratories (A division of Aurobindo Pharma Ltd., Hyderabad.). Analytical reagent (AR grade) 1-Bromopropane, ethanol, n-propanol, benzene, ethylchloride, N,N-dimethylformamide, 1-pentanoic acid, 2-Methyl pentanoic acid, 2-ethylpentanoic acid, Isopropyl pentanoic acid, 2-Butyl pentanoic acid, Valproic acid and Dimethylsulfoxide were procured from Fluka Germany, sodium chloride and HPLC water were procured from Merck, Mumbai, India.

Equipment

The gas chromatograph system with mass spectrometer was a Shimadzu GCMS-QP2010 equipped with Shimadzu AOC-5000 head space sampler (Make: Shimadzu Corporation, Kyoto, Japan) was used. The data handling system GCMS solution, version 2.53.00 SU1 was used to monitor the output signals and for processing. Capillary GC column (DB-1) (Make: J & W Scientific, Santa Clara, CA, USA) was used in this study.

Chromatographic conditions

The analysis was carried out on a fused silica capillary column of 30 m length, 0.32mm internal diameter and coated with 100% dimethylpolysiloxane stationary phase of 3 µm film thickness (DB-1). Helium gas was used as carrier gas, maintaining column pressure at 10kPa in a split mode with ratio of 1:4. The temperature of the capillary injector was set as 220°C and column oven temperature was programmed as initially 40°C maintained for 10min, then raised to 220°C at a rate of 15°C per minute held for 3min.

The mass parameters were set as follows.

Group & Events						
Start time	**End time**	**Avg. Mode**	**Event time (sec)**	**Channel (m/z)**	**Channel (m/z)**	**Channel (m/z)**
7.00	10.00	SIM	0.20	27	43	122

Ion source temperature & Interface temperature - 250°C, Threshold-200 and detector voltage is relative to the tuning result.

The head space conditions were maintained as follows. Cycle- HS-injection, syringe-2.5 ml-HS, sample volume-1 ml, incubation temperature-80°C, incubation time-20 min, agitation speed-500 rpm, agitation on/off time-10 seconds, syringe temperature-100°C, fill speed-1 ml/sec, pull up delay-500 msec, inject speed-1 ml/sec, pre/post injection delay-0 msec, syringe flushing-5 min, analysis time-35 min.

Preparation of solutions

Blank solution

Add 0.80 ml of water and 0.20 ml of Dimethylsulfoxide into the headspace vial containing about 0.5 g of sodium chloride and seal the vial immediately.

Standard stock solution

Accurately weigh and transfer about 25mg of 1-Bromopropane into a 25 ml clean, dry volumetric flask containing about 10 ml of Dimethylsulfoxide, mix and make up to volume with Dimethylsulfoxide. Dilute 1.0 ml of this solution to 50 ml with Dimethyl sulfoxide. Further dilute 0.5 ml of this solution to 50 ml with Dimethylsulfoxide.

Standard solution

Transfer accurately 0.2 ml of the standard stock solution into the headspace vial containing about 0.5 g of sodium chloride. Add 0.8 ml of water and seal the vial immediately.

Sample solution
Accurately weigh and transfer about 80mg of sample into the headspace vial containing about 0.5 g of sodium chloride. Add 0.8 ml of water and 0.2 ml of Dimethylsulfoxide and seal the vial immediately.

RESULTS AND DISCUSSION

Method development and optimization

The challenge is to achieve the detection and quantitation at low level using the Gas Chromatography Mass Spectrometry (GC-MS) to acquire the good separation with desired sensitivity. In this study, DB-1 column with 30 m length, 0.32mm internal diameter with 3 µm film thickness was employed for the GC-MS analysis. This column was chosen because of non-polar stationary phase, which is suitable for retaining the BP and resolving other analytes from BP and also DB-1 column shows an excellent robustness for several GC-MS methods as per previous hands on usage of column. Electron impact ionization (EI) mode was selected because EI is generally more robust and easy to transfer when compared to other GC-MS ionization techniques/mode.

Development trails were initiated on head space technique due to low volatile nature of BP. standard solution (0.5µg/g) of BP was prepared in dimethylsulfoxide with respective to sample concentration (100mg/ml) and same solution (0.25 ml of the standard solution and 0.75 ml of dimethylsulfoxide) was transferred in to head space vial and sealed the vial by screwing on the magnetic screw cap. The vial was incubated at 80°C for 20min and injected through AOC 5000 auto injector in to GC-MS (in scan mode). After completion of acquisition, the retention time of BP was extracted with the help of National Institute of Standards and Technology (NIST) library target analyte channels [mass-to-charge ratio(m/z)]. The spectrum of BP extracted from NIST library is given in Figure 3.

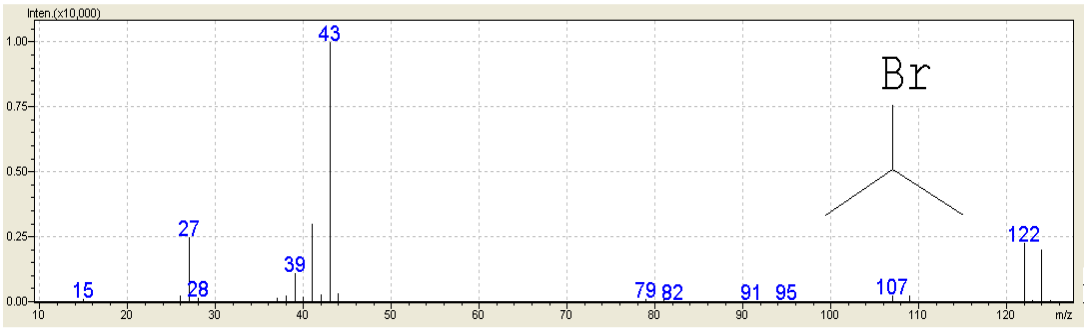


Figure 3. Spectrum of 1-Bromopropane (Extracted from NIST library)

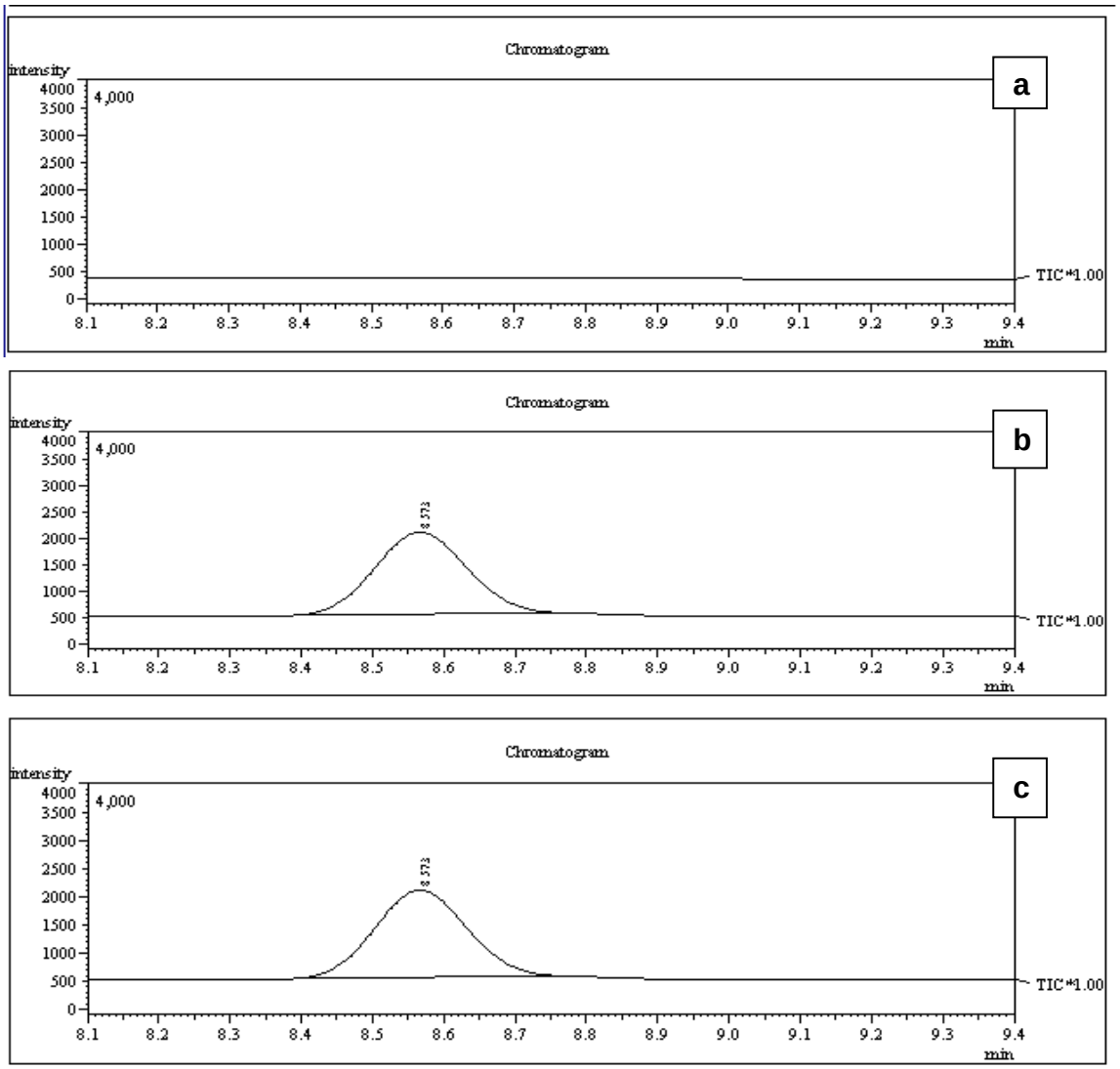
The effect of head space vial preparation and sample concentrations were verified for low level quantification. As a part of investigation, same solution (0.25 ml of the standard solution and 0.75 ml of dimethylsulfoxide) was transferred in to head space vial and sealed the vial by screwing on the magnetic screw cap. Then incubated at 80°C for 20min and injected through AOC 5000 auto injector in to GC-MS (in Selective Ion Mode [SIM]). In this vial preparation trail, result shows that the low detection level was difficult to achieve. Hence, the vial preparation has been modified by introducing water and sodium chloride. The scientific rationale in addition of water is, compounds that have low Partition Coefficient (K) values will tend to partition more readily into the gas phase and have relatively high responses for lower limits of detection and for addition of salt into the aqueous sample matrix is high salt concentrations in aqueous samples decrease the solubility of polar organic volatiles in the sample matrix and promote their transfer into the headspace, resulting in lower K values and sample concentration. After changing to the head space vial preparation and sample concentration, recovery has been attained at desired specification level. In this optimized head space vial preparation, there was no interference observed between sample matrix and analyte peak.

The effect of initial column temperature on the separation of BP with other analytes was investigated. The initial column temperature was varied from 40°C to 60°C in the split mode. The results show that the peak shape and separation of each analytes was satisfactory when the initial column temperature kept as 40°C comparatively other temperatures. Split mode was also modified based on response and for better peak shape purpose. Finally, the optimized method was validated as per ICH guideline Q2(R1)[11].

Validation

Specificity

As per ICH, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the developed method was verified in presence of residual solvents like ethanol, n-propanol, benzene, ethylchloride, N,N-dimethylformamide, 1-pentanoic acid, 2-methylpentanoic acid, 2-ethylpentanoic acid, isopropylpentanoic acid, 2-butylpentanoic acid, valproic acid, which were used in the synthesis process of DS. These solvents were injected individually to confirm the responses. Blank solution, as such sample solution, sample solution spiked with all solvents including BP and sample solution spiked with all solvents excluding BP were prepared and injected into GCMS and monitored the responses in SIM mode. The typical spectrograms are given in Figure 4.



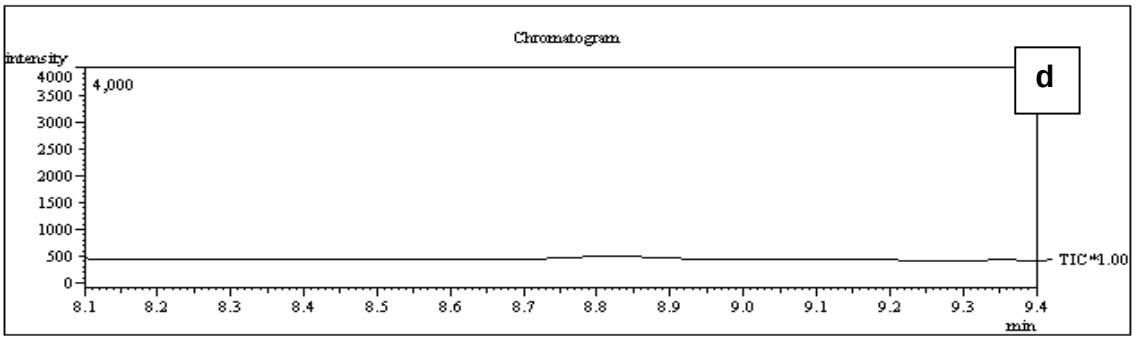


Figure 4. Typical GCMS total ion spectrograms of a) blank solution, b) standard solution, c) Divalproex sodium spiked with all known residual solvents including 1-Bromopropane and d) Divalproex sodium spiked with all known residual solvents excluding 1-Bromopropane

Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) values of BP was determined using visual evaluation. The predicted concentrations of LOD and LOQ of BP was verified for precision by preparing the solutions containing at about predicted concentrations and injected each six times into GCMS and calculating the %RSD of peak areas. The LOD/LOQ values are given in Table 1.

Linearity

A series of solutions were prepared using BP at concentration levels from LOQ to 150% of specification level (0.5ppm) and each solution was injected and calculating the statistical values like slope, intercept, STEYX and correlation coefficient from linearity plot drawn for concentration versus area. The statistical data is given in Table 1.

Table 1. Statistical data of linearity, LOD/LOQ for 1-Bromopropane

Statistical parameters	Results
Correlation coefficient	0.999
Concentration range (µg/g)	0.1-0.75
Intercept	-225
Slope(S)	20565
Limit of detection(µg/g)	0.05
Limit of quantification(µg/g)	0.1
Precision for Limit Of Detection (%R.S.D)	6.1
Precision for Limit Of Quantification (%R.S.D)	5.3
Calibration points	6

Table 2. Statistical data of precision experiments

Divalproex sodium		
ID	System precision[a]	Method precision[b] (µg/g)
1	11287	0.49
2	11306	0.46
3	11444	0.48
4	11459	0.46
5	11232	0.46
6	11559	0.48
Mean	11381	0.47
SD	125	0.01
% RSD	1.1	2.1
95% CI(±)	131	0.01

a : area of 1-Bromopropane
b: content of 1-Bromopropane

Precision

The precision (system precision) was evaluated by injecting six injections of standard solution of BP in to GCMS and calculating the % relative standard deviation (RSD). The method precision was checked by injecting six individual preparations of DS spiked with BP at specification level and calculated the % RSD of BP content. The achieved precision experiment results are reported in Table 2.

Accuracy

The accuracy study was carried out by preparing DS sample solutions in triplicate by spiking BP at LOQ level, 50%, 100% and 150% of specification level (0.5 µg/g) and calculated the percentage recovery. The accuracy experiment results are reported in Table 3.

Table 3. Accuracy data of 1-Bromopropane in Divalproex sodium

Accuracy (Average of 3 replicates)	1-Bromopropane			
	Level-I	Level-II	Level-III	Level-IV
Added (µg/g)	0.10	0.25	0.50	0.75
Recovered (µg/g)	0.09	0.26	0.48	0.70
Recovery (%)	87.6	102.7	96.0	93.3
R.S.D(%)	0.1	2.2	2.1	2.8
Overall recovery (%) (Average of 12 replicates)	97.3			

CONCLUSION

Method validation data demonstrated that the developed GC-MS method is sensitive, specific and as well as accurate for the estimation of 1-Bromopropane in Divalproex sodium drug substance. Hence, the validated GC-MS method can be employed in to the routine analysis.

Acknowledgements

The authors gratefully acknowledge the management of APL Research Centre-II (A Division of Aurobindo Pharma Ltd.), for allowing us to carry out the present work. The authors are also thankful to the colleagues of Analytical Research Department and Chemical Research Department for their co-operation.

REFERENCES

[1] United states pharmacopoeia, general chapter ⟨467⟩, Residual Solvents, USP39–NF34, **2016,** 339.
[2]International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Impurities: Guideline for Residual Solvents Q3C(R5), **2011**.
[3]Allan J Ellsworth, Mosby's Medical Drug Reference, **2007,** 1pck edition.
[4]L. Muller, R. J. Mauthe, C. M. Riley, M. M. Andino, D. D. Antonis, C. Beels, J. Degeorge etal, *Regul. Toxicol. Pharmacol*., **2006,** 44, 198-211.
[5] http://ntp.niehs.nih.gov/ntp/roc/content/profiles/bromopropane.pdf
[6] http://www.drugs.com/dosage/divalproex-sodium.html
[7] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf
[8] www.texleader.com.cn/en/summary/4069.html
[9] V. Bessonneau, L. Mosqueron, A. Berrubé, G. Mukensturm, S. B. Bataillon, J. P.Gangneux, O. Thomas, *Plos one.*, **2013,** 8(2), 1-14. http://dx.doi.org/10.1371/journal.pone.0055535
[10] H. Valentine, Kalyani Amarnath, Venkataraman Amarnath, W. Li, X. Ding, W. M. Valentine and G. Ichihara, *Toxicological Sciences*., **2007,** 98(2), 427-435.
doi:10.1093/toxsci/kfm126
[11]International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical procedures: Text and methodology Q2(R1), Step 4, **2005.**