International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.3, No.1, pp 342-347, Jan-Mar 2011

Validation of an Analytical Method for assay of Magnesium Valproate by Gas Chromatography

M.A.Ambasana[1]*,H.O.Kaila[1], R.S.Thakkar[1], H.T.Saravaia[1] and A.K.Shah[1]

[1]Department of Chemistry, Saurashtra University,Rajkot - 360 005,Gujarat, India

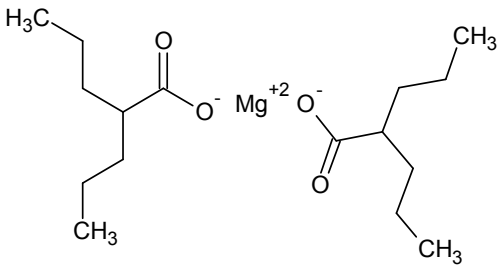
*Corres.author: ambasanamrunal@gmail.com
Contact Number: +91-9904949247*

Abstract: A simple, precise, and accurate stability indicating gas chromatographic method has been developed and validated for assay of Magnesium Valproate. The Chromatographic system used to perform development and validation of this assay method consisted of Shimadzu 2014 G.C. with Flame Ionization Detector and a manual injector. The method was validated for specificity, linearity, precision, accuracy, robustness, and solution stability. The specificity of the method was determined by stress testing of the drug (forced degradation). Response was a linear function of drug concentration in the range 2000-8000 µg/mL (r= 0.999). The LOD value for Magnesium Valproate was found to be 0.1 ppm and the LOQ value 1.0 ppm respectively. Accuracy (recovery) was between 99.54 and 100.28%. Same method is also well applicable for estimation of Sodium, Disodium, Calcium and Lithium salt of Valproic acid.
Keywords: Magnesium Valproate, Gas Chromatography, Method Validation.

INTRODUCTION:

Various Valproic acid salts have diverse biological activity specifically it is used as anticonvulsant medicine from last few decades. Presently this work describes the analysis of magnesium salt of Valproic acid through gas Chromatography and the same method of analysis can also applicable to Sodium, Calcium and Lithium Valproate as well as its process intermediates, all the Valproate has explicit biologically activity. Few of them are included in pharmacopoeia while others are under advance clinical trials.

Magnesium Valproate is white or almost white hygroscopic crystalline powder, slightly soluble in water, freely soluble in heptane and methanol. The molecular weight of Magnesium Valproate is 310.71 g/mole with molecular formula of $C_{16}H_{30}O_4Mg$. Magnesium Valproate is chemically known as Magnesium 2-propylpentanoate. Its dissociation constant (pKa) 4.6 reported, the melting point of Magnesium Valproate is 220°C and Flash point of the same 166.6°C [1][2][3][4][5][6].



Chemical Structure of Magnesium Valproate

EXPERIMENTAL:

CHEMICALS AND REAGENTS:

Magnesium Valproate reference standard (label claim 99.92% pure) and working standards were provided by the PARTH LABORATORIES PVT. LTD., Rajkot, Gujarat, India. HPLC-grade Heptane was from Spectrochem Pvt. Ltd. (Mumbai, India). Hydrochloric acid (35%) and

Sodium hydroxide pellets and 30% (v/v) were from Ranbaxy Fine Chemicals (New Delhi, India).

INSTRUMENTAL:

The Chromatographic system used to perform development and validation of this assay method consisted of Shimadzu 2014 G.C. with Flame Ionization Detector and a manual injector (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system, GC Solutions.

CHROMATOGRAPHIC CONDITION:

Instrument	: SHIMADZU 2014
Column	: BP-20, fused-silica capillary column 30m × 0.53mm, coated with wide-bore macrogol 20000, 2-nitro terephthalat (0.5µm)
Detector	: FID, 220º C
Injector	: Manual Injector, 220º C
Column Flow	: 8.25 mL/minute
Total Flow	: 36 mL/minute
Purge Flow	: 3 mL/minute

COLUMN TEMPERATURE PROGRAMMING

Rate (˚C/min)	Temperature (˚C)	Hold Time (min)
-	80˚c	2 min.
10	130˚c	10 min.

PREPARATION OF STANDARD SOLUTIONS:

Dissolve 1.000 g of the Magnesium Valproate (RS) in 20 ml of water. Add 10 ml of dilute sulphuric acid and shake with 20 ml of heptane and allow to stand for 10 minutes. Separate out the upper layers containing Valproic Acid and shake with pinch of anhydrous sodium sulphate. Filter and evaporate the filtrate, at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue, accurately weigh 0.250g of residue in 5 mL vol. flask and dilute up to the mark with diluent, shake well and pipette out 1mL of solution in to the 10 ml vol. flask and dilute up to the mark with diluent.

PREPARATION OF STANDARD SOLUTIONS:

Dissolve 1.000 g of the Magnesium Valproate working standard in 20 ml of water. Add 10 ml of dilute sulphuric acid and shake with 20 ml of heptane and allow to stand for 10 minutes. Separate out the upper layers containing Valproic Acid and shake with pinch of anhydrous sodium sulphate. Filter and evaporate the filtrate, at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue, accurately weigh 0.250g of residue in 5 mL vol. flask and dilute up to the mark with diluent, shake well and pipette out 1mL of solution in to the 10 ml vol. flask and dilute up to the mark with diluent.

METHOD VALIDATION:

APPLICATION OF TRESS (FORCED DEGRADATION STUDY):

To perform the forced degradation study exactly 1.00gm of active ingredient was subjected to acidic, alkaline, thermal and photolytic conditions. For acidic degradation the drug was heated under reflux with 0.1 M HCl at 80°C for 2 hrs and the mixture was neutralized. For alkaline degradation the drug was treated with 0.1 M NaOH at 80°C for 2 hrs and the mixture was neutralized. For thermal degradation the powdered drug was exposed at 110°C for 48 hrs. For photolytic degradation the powdered drug was exposed to sunlight for 24 hrs.

LINEARITY:

Seven solutions were prepared containing 2000 to 8000 µg/mL Magnesium Valproate, concentrations which corresponded to 40, 60, 80, 100, 120, 140, and 160%, respectively, of the test solution concentration. Each solution was injected in duplicate. Linearity was evaluated by linear-regression analysis.

PRECISION:

System precision was evaluated by analyzing the standard solution five times and method precision (repeatability) was evaluated by assaying six sets of test samples prepared for assay determination. Injected in duplicate on the same day (intraday precision).Method precision was determined by performing the same procedures on a different day (interday precision), and by another person under the same experimental conditions (intermediate precision).

ACCURACY:

Accuracy was assessed by determination of the recovery of the method at three different concentrations (corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard preparation. For each concentration, three sets were prepared and injected in duplicate.

ROBUSTNESS:

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factor chosen for this study were the flow rate (±1.0 mL/min), Initial temperature of the column (±10˚C) and using different lots of GC column.

SOLUTION STABILITY:

The stability of the test solution was evaluated by two ways as short term stock solution stability (STSSS) for 12 hours & long term stock solution stability (LTSSS) for 12 days the solution was stored at room temperature and 2-5˚C and tested at intervals of 12 hours & 12 days. The responses for the aged solution were evaluated using a freshly prepared standard solution.

SYSTEM SUITABILITY:

A system suitability test for the chromatographic system was performed before each validation parameter. Five replicate injections of standard preparation were injected and Average, Standard Deviation and % RSD of peak area were determined for same.

TABLE 1: SUMMERY OF ACCURACY STUDY

Accuracy Level	Amount added	Amount found	% Recovery	Mean	Standard dev.	% RSD
50%	2504	2504.62	100.02	100.28	0.45	0.45
	2496	2496.31	100.01			
	2492	2512.28	100.81			
100%	5002	5003.45	100.02	99.48	1.01	1.02
	5000	4915.65	98.31			
	4992	4998.12	100.12			
150%	7500	7459.79	99.46	99.54	0.16	0.16
	7496	7454.46	99.44			
	7494	7474.83	99.74			

RSD Relative Standard Deviation

TABLE 2: SUMMERY OF ROBUSTNESS STUDY

Conditions	Assay (%)	R T(min)	%RSD of System Suitability
9.25 ml/min Flow	99.46	6.005	1.23
7.25 ml/min Flow	99.20	6.496	0.97
Initial temp 90˚C	98.44	5.216	1.67
Initial temp 70˚C	99.70	7.252	0.88
Column change	98.98	6.238	0.75

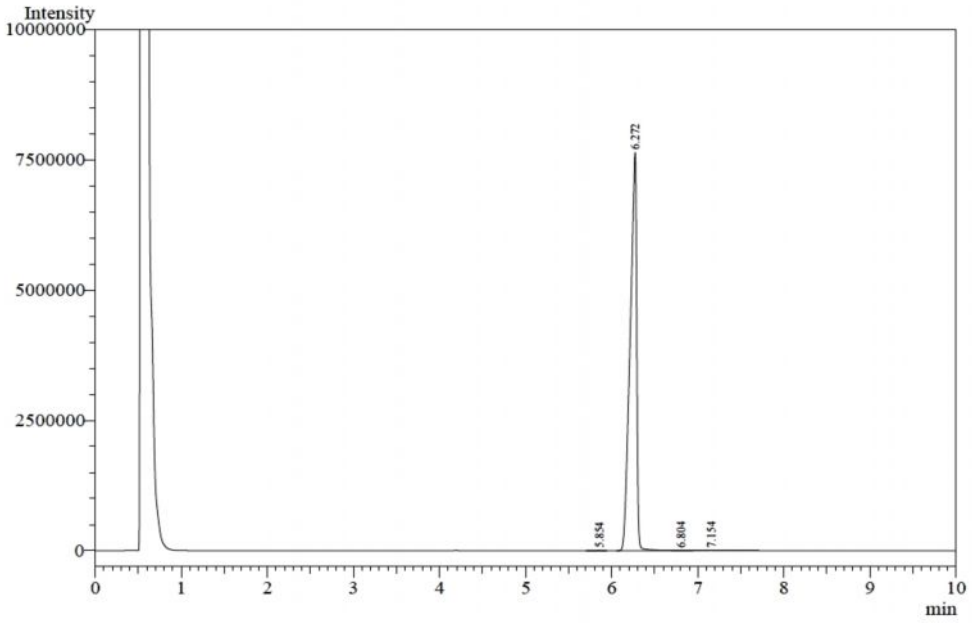
TABLE 3: SUMMERY OF SYSTEM SUITABILITY PARAMETERS

Parameter	Average	S.D.	%RSD
Specificity	41061114	483578.18	1.34
Linearity	40948951	187631.61	0.45
LOQ & LOQ	41113931	190180.75	0.46
Meth. Precision	40676288	273407.77	0.68
Int. Precision	40779575	287533.08	0.71
Accuracy	40531457	477979.64	1.18
Robustness	40462557	451058.36	1.02

TABLE – 4: RESULT OF FORCED DEGRADATION STUDY

Conditions used	Reflux time	Results
0.1N HCl	2 hrs.	No degradation
0.1N NaOH	2 hrs.	No degradation
Light	24 hrs.	No degradation
Heat	110˚C, 2 days	No degradation

FIGURE 1: CHROMATOGRAM OF STANDARD PREPARATION



RESULTS AND DISCUSSION:

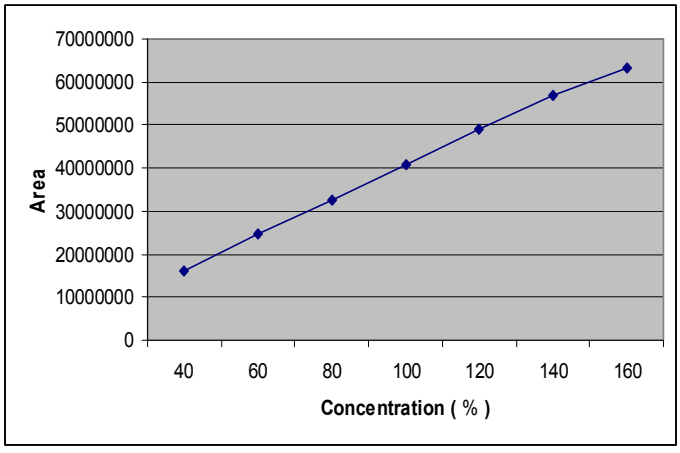
Presently this work describes the analysis of Magnesium salt of Valproic acid through gas Chromatography and the same method of analysis can also applicable to Sodium, Calcium and Lithium Valproate. The basic chromatographic conditions were designed to be simple and easy to use and reproduce and were selected after testing the different conditions that affect GC analysis, for example column, Initial temperature and flow rate of column, detection temperature, concentration of analyte, etc. Solutions of standard and test preparations were found to be stable in solvent.

After development of the analytical method, it was validated in accordance with ICH Guidelines. This furnished evidence the method was suitable for its intended purpose. The specificity of the method was also evaluated by checking chemical and physical forced degradation study. Observations and data of degradation study suggests that Magnesium Valproate is quite stable compound when refluxed with acid or alkali at 80˚ C for 2 hrs or even when heated in oven for 2 days at 110˚c or expose to UV light for 24 hrs in closed condition.

To determine linearity a calibration graph was obtained by plotting Magnesium Valproate concentration against peak area. Linearity was good in the concentration range 2000-8000 µg/mL. The regression equation was y = 46,956x+85,486 where x is the concentration in µg/mL and y is the peak area in absorbance units; the correlation coefficient was 0.999.

The developed method was found precise as the %RSD for the system precision was 0.67% for method precision, on the same day (intraday) and 0.70% for intermediate precision on next day (interday).

FIGURE 2: EVALUATION OF LINEARITY STUDY



Accuracy was assessed by determination of the recovery of the method at three different concentrations (corresponding to 50, 100 and 150% of test solution concentration). Known amounts of Magnesium Valproate (2500, 50000, and 7500 µg/ml) were taken and the %Recovery of Magnesium Valproate was calculated for each concentration, three sets were prepared and injected in

duplicate. % Recovery was calculated at each level and recorded as shown in Table-1. The mean recovery of Magnesium Valproate was between 99.48 and 100.28%, which is satisfactory.

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value (Table 2). System suitability data were also found to be satisfactory during variation of the analytical conditions (Table 3). The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions.
The stability of the test solution was evaluated by two ways as short term stock solution stability (STSSS) for 12 hours & long term stock solution stability (LTSSS) for 12 days the solution was stored at room temperature and 2-5[degrees] C and tested at intervals of 12 hours & 12 days. The responses for the aged solution were evaluated using a freshly prepared standard solution.

Table shows the results obtained in the solution stability study at different time intervals for the test preparations. It was concluded that the test preparation solution was stable up to 12 hrs at room temperature and 12 days at 2˚-5˚C because during this time, the result did not decrease below the minimum percentage.

Before each measurement of validation data a system suitability test was performed by measurement of general characteristics such as peak asymmetry, number of theoretical plates, and RSD (%) of peak area observed for a standard solution. The values obtained were satisfactory and in accordance within limits.

The intensive approach described in this manuscript was used to develop and validate a Gas chromatographic method that can be used for analysis of diverse salts of Valproic Acid, its process intermediates and pharmaceutical dosage form.

Method can be regarded as stability indicating as active substance was subjected to chemical and physical force degradation. Observations and data and suggests that Magnesium Valproate is quite stable compound, no degradation products produced during stress test.

CONCLUSION:

This method for assay and determination Magnesium Valproate was successfully developed and validated for its intended purpose. The method was found specific, linear, precise, accurate, and robust. This method could be recommended to the industry for impurity profiling, related substance and quality control of drug content in pharmaceutical preparations as the study of assay determination of various brands found to be satisfactory and within the limits.

ACKNOWLEDGEMENTS:

The authors are grateful to the PARTH Laboratories, Rajkot, Gujarat (India) for providing working standards and reference standards and Saurashtra University, Rajkot, Gujarat (India), for providing instrumental facilities.

REFERENCES:

1. Borowicz K.K., Kimber-Trojnar Z., Ratnaraj N., Patsalos P.N., Luszczki J.J. and Czuczwar S.J., Isobolographic analysis of interactions between losigamone and conventional antiepileptic drugs in the mouse maximal electroshock model, European Neuropsychopharmacology, 2007, 17(2), 94-101.
2. Gobbi G. and Janiri L., Sodium- and magnesium- valproate in vivo modulate glutamatergic and GABAergic synapses in the medial prefrontal cortex, Can. Psychopharmacology, (Berlin, Germany), 2006, 185(2), 255-262.
3. Borowicz K.K., Piskorska B., Luszczki J. and Czuczwar S.J., Influence of SIB 1893 - a selective mGluR5 receptor antagonist – on the anticonvulsant activity of conventional antiepileptic drugs in two models of experimental epilepsy, Polish Journal of Pharmacology, 2003, 55(5), 735-740.
4. Canger R. and Guidolin L., Clinical efficacy and tolerability of magnesium valproate as immunotherapy in patients with generalized or partial epilepsy, A non-blinded study of 320 patients, Clinical Drug Investigation, 2000, 20(4), 215-221.
5. Onofrj M., Colangelo U., Malatesta G., Di Giovanni M., Comparative study of preparations of sodium and magnesium valproate (VPA) in treatment of patients with epileptic seizures, Rivista di neurologia, 1988, 58(6), 223-230.
6. Lalic M., Cvejic J., Popovic J., Bozic K., Golo-Corbin-Kon S., Al-Salami H., Mikov M., Lamotrigine and valproate pharmacokinetics interactions in epileptic patients, European

Journal of Drug Metabolism and pharmacokinetics, 2009, 34(2), 93-99.

7. Subasranjan A., Suresh P., Srinivasulu C., Hemant R., Stability-indicating gas chromatography method for determination of divalproex sodium impurities in pharmaceutical preparation. Journal of Drug Testing and Analysis, 2010, 2(4), 182-187.
8. Lau-Cam CA and Roos RW: HPLC method with precolumn phenacylation for the assay of valproic acid and its salts in pharmaceutical dosage forms. Journal of Liquid Chromatography & Related Technologies, 1997; 20(13): 2075-2087.
9. Hao R, Yan C and Liu Y: Determination of magnesium and sodium valproate in serum by high performance liquid chromatography after precolumn derivatization. Yaowu Fenxi Zazhi, 1997; 17(3): 172-174.
10. Balbi A, Sottofattori E, Mazzei M and Sannita WG: Study of bioequivalence of magnesium and sodium valproates. Journal of Pharmaceutical and Biomedical Analysis, 1991; 9(4): 317-321.
11. Hu M, Huang S and Xiao B: Determination of magnesium valproate in serum by gas chromatography. Chinese Journal of Chromatography, 1987; 5(6): 362-364.

*****