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Research Article

GAS CHROMATOGRAPHY METHOD DEVELOPMENT AND METHOD VALIDATION OF REDISUAL SOLVENT (ISOPROPYL ALCOHOL) IN MAGNESIUM VALPROATE

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ABSTRACT

Analysis is equally important in pharmacokinetics and in drug metabolism studies, both of which are fundamental to the assessment of bioavailabilty and the duration of clinical response. Quality assurance plays a central role in determining the safety and efficacy of medicine. An important aspect of development, productivity, distribution and use of drugs in their analysis. From the literature survey it was found that there was no suitable method available for the determination of residual solvent (organic volatile impurity)- Isopropul alcohol(IPA) in magnesium valproate. Hence it was deemed of interest to generate a method development and a supporting validation data for determination of residual solvent (organic volatile impurity) Isopropul alcohol (IPA) in magnesium valproate. The amount of IPA in magnesium valproate was determined by using gas chromatography using the flame ionisation detector. The peak of IPA in magnesium valproate was identified by comparing with the standard retention time and it is found to be around 9-10 minutes. The amount of isopropyl alcohol is magnesium valproate was carried out by gas chromatographic method using flame ionisation detector. The type of column is AT-1 capillary column. The flow rate was set at 1ml/min. The carrier gas used is nitrogen gas. The column temperature was maintained at 200°C and the detector temperature was maintained at 260°C. System suitability parameters such as resolution factor, tailing factor and number of theoretical plates were calculated Linearity was evaluated by visual inspection of plot of peak area as a function of analyte concentrate for IPA. The validation of the proposed gas chromatographic method was further verified by recovery studies. The percentage recovery range was found to be within 99.82-100.46% for IPA. This serves as a good index of the accuracy and reproducibility of the method.

The precision of the methodology was confirmed by repeating the procedure by preparing the three standard preparations of IPA and three samples preparations of magnesium valproate and injecting six replicate injections. All the parameters including the flow rate, temperature, split ratio and the sensitivity was maintained constant throughout the procedure.

Keywords: Magnesium valproate, Iso propyl alcohol, Residual solvent, Method development and method validation.

INTRODUCTION

Analysis is equally important in pharmacokinetics and in drug metabolism studies, both of which are fundamental to the assessment of bioavailabilty and the duration of clinical response. Quality assurance plays a central role in determining the safety and efficacy of medicine. An important aspect of development, productivity, distribution and use of drugs in their analysis. From the literature survey it was found that there was no suitable method available for the determination of residual solvent (organic volatile impurity)- Isopropul alcohol(IPA) in magnesium valproate. Hence it was deemed of interest to generate a method development and a supporting validation data for determination of residual solvent (organic volatile impurity) Isopropul alcohol(IPA) in magnesium valproate. The objective of the study is to demonstrate system suitability, method precision, linearity and range, accuracy\, specificity, limits of detection, limits of quantitation, ruggedness and robustness.

MATERIALS AND METHODS

Instrumentation: balance precise make Model 202A

Gas chromatogram netel 9100 with flame ionisation detector, 60Mx 0.25mm, ID fused silica capillary column coated with the 1.0µm film of stationary phase, Dimethyl polysiloxane (Altech -1), Hamilton syringe with 20µl loop.

DMF- Dimethyl formamide (Ranbaxy Chemicals)

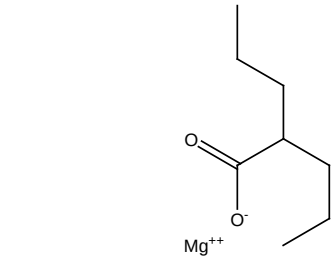
IPA- Isopropyl alcohol (Ranbaxy Chemicals)

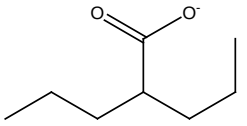
Magnesium Valproate- Anjan drugs Pvt limited.

Drug profile

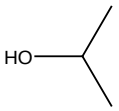
Magnesium valproate

It is a broad spectrum anti epileptic more potent in blocking Pentylene tetrazole seizures than in modifying maximal electroshock. It also inhibits the extablishment of chronic experimental seizure foci and prevents kindling. It produces little sedation or other central effects. It is an anti epileptic drug.[1]





magnesium valproate



isopropyl alcohol

A white crystalline powder, characteristic, soluble in methanol and DMF.

The oral absorption of magnesium valproate is good. It is 90% bound to plasma proteins completely metabolised in liver by oxidation (some metabolites are active) and glucoronide conjunction- excreted in urine. Plasma half life is 10-15hrs but anticonvulsant effects are longer lasting.

Isopropyl alcohol is 2-propanol. It is a pharmaceutical aid Solvent. It is a clear, colourless liquid, characteristic odour, spirituous and flammable. It is soluble in water and ether.

Operational Conditions

Mode of operation: Isothermal

Detector: FID

Column Temperature: 200°C

Injection Temperature: 220°C

Carrier gas: Nitrogen

Flowrate: 1ml/min

Injection volume: 1.0µl

Analytical Method Validation

The method used for the analytical method development as well as method validation is Gas Chromatographic method.

The diluents used for this method is DMF (dimethyl formamide). During the estimation of organic volatile impurity the drug has to be solubilised completely in the diluent such that the organic volatile impurity (solvent IPA) is completely released from the drug. Magnesium valproate is soluble in DMF and insoluble in water. Hence DMF is selected as diluent.

The type of column used in gas chromatography is AT-1 (dimethyl silicone) 60mx0.25mm ID fused silica capillary column coated with the 1.0µm film of stationary phase dimethyl polysiloxane. Since the column length is 60M the separation (resolution factor) between the IPA and DMF is found to be good. The presence of IPA in magnesium valproate is very low in ppm level. Hence the capillary column is selected.

Table 1: The ppm and percentage of final concentration

S. No	Standard Stock solution (ml)	Final dilution (ml)	Final Concentration (ppm)	Final Concentration in %
1	0.40	50	0.0628	20%
2	1.00	50	0.157	50%
3	2.00	50	0.314	100%
4	2.50	50	0.3925	125%
5	3.00	50	0.471	150%

Analytical method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical testing of a pharmaceutical product is necessary to ensure its purity, stability and efficacy. Analytical method validation is an integral part of the quality control system.

As per USFDA/ICH guideline definition –"Validation is establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product of service meeting its predetermined specifications and quality characteristics. Validation is thus the action of proving in accordance with the principle of GMP that any procedure, process, equipment method, material and activity actually leads to the expected results and produces a quality product.[2]

The purpose of validation of any equipment or process is achieved by means of validation protocol which details the test to be carried out frequency of testing and results expected ie the acceptance criteria.

Analytical validation refers to the evaluation and proving that any analytical method serves the intend purpose. It also ensures that the selected analytical method will give reproducible and reliable results adequate for intended purpose.[3]

The preparation and execution should follow a validation protocol preferably written in a step by step instruction format. The proposed procedure assumes that the instrument gas been selected and method developed.[4,5]

Parameters for Method Validation

The parameters as defined by the ICH and by other organisations and authors are as follows:

System suitability, Specificity, Precision, Accuracy, Linearity, Range, Limit of detection, Limit of Quantitation, Ruggedness, Robustness[6,7,8].

RESULTS & DISCUSSION

The amount of IPA in magnesium valproate was determined by using gas chromatography using the flame ionisation detector. The peak of IPA in magnesium valproate was identified by comparing with the standard retention time and it is found to be around 9-10 minutes.

Linearity was evaluated by visual inspection of plot of peak area as a function of analyte concentrate for IPA. the data regarding linearity for the IPA are given in Table 1. From the linearity studies the specified range was determined for the IPA is given as 0.0628ppm -0.471ppm.

Table 1: linearity data

Concentration	% of IPA	Peak area of IPA						RSD %	Correlation coefficient (r)
0.0628	20	2695	2669	2646	2701	2642	2684	0.93	
0.157	50	6686	6463	6692	6626	6627	6669	1.29	0.999
0.314	100	13286	13252	13226	13300	13259	13225	0.23	
0.3925	125	16480	16581	16647	16751	16596	16615	0.47	
0.471	150	20825	20886	20295	20146	20855	20912	1.64	

The amount of isopropyl alcohol is magnesium valproate was carried out by gas chromatographic method using flame ionisation detector. The type of column is AT-1 capillary column. The flow rate was set at 1ml/min. The carrier gas used is nitrogen gas. The column temperature was maintained at 200°C and the detector temperature was maintained at 260°C.

Table 2: System Suitability data

Parameter	IPA
Resolution factor	4.68
Tailing factor	1.18
Number of theoretical plates	23784

System suitability parameters such as resolution factor, tailing factor and number of theoretical plates were calculated and the results are presented in Table 2.

The symmetry factor or the tailing factor values for IPA was found to be 1.18 indicating the symmetrical nature of the peaks.

The number of theoretical plates was high and it was found to be 23784 indicating the efficient performance of the column.

The resolution factor between the IPA and dimethyl formamide was found to be 4.68.

The amount of IPA was carried out on magnesium valproate by calculating the sample solution with that of standard solution. The data regarding the amount present is depicted in table 3.

The amount of IPA was subjected to statistical analysis. The RSD values obtained are below 5 % indicating the precision of he applied methodology.

The validation of the proposed gas chromatographic method was further verified by recovery studies. The data regarding recovery studies was presented in table 4. The percentage recovery range was found to be within 99.82-100.46% for IPA. This serves as a good index of the accuracy and reproducibility of the method.

The precision of the methodology was confirmed by repeating the procedure by preparing the three standard preparations of IPA and three samples preparations of magnesium valproate and injecting six replicate injections. The table was represented for standard and sample are response in Table 5 and 6. The results obtained express the precision of the proposed method.

System suitability parameters such as resolution factor, tailing factor and number of theoretical plates were calculated and the results are presented in Table7 & 8.

Table 3: Amount of IPA Present

Sample	Peak Area response (Standard)	Peak Area response (Sample)	SD		RSD%		Amount of IPA present in ppm	Limit of IPA in ppm
Magnesium Valproate B.No BME 002	12044	1216	8.02	10.83	0.66	0.09	487.72	5000

Table 4: Accuracy of Isopropyl Alcohol

Injection No	Concentration in mg/ml IPA		
	0.2826 (90%)	0.3140(100%)	0.3454(110%)
	Observed area response for IPA		
I	12053	13280	14530
II	12000	13210	14480
III	12100	13200	14500
IV	12030	13280	14650
V	12005	13300	14490
VI	12040	13368	14512
% of RSD	0.30%	0.47%	0.43%
Recovery in mg/ml	0.2821	0.3143	0.3470
% of Recovery	99.82	100.09	100.46
Limit 90% to 110%			

Table 5: Precision (Standard)

Injection No	Observed area response for IPA		
	Standard I	Standard II	Standard III
I	12040	12041	12190
II	12518	12111	12801
III	13180	12139	12299
IV	13619	12539	12401
V	13296	11281	12883
VI	13093	12878	12604
% RSD	4.44%	4.42%	2.23%
RSD Limit NMT 5%			

Table 6: Precision (Sample)

Injection No	Observed area response for IPA		
	Sample I	Sample II	Sample III
I	1200	1195	1218
II	1225	1199	1225
III	1200	1200	1199
IV	1215	1211	1218
V	1216	1228	1220
VI	1218	1220	1218
% RSD	0.84%	1.09%	0.73%
RSD Limit NMT 5%			

Table 7: Linearity and range for IPA

Concentration	% of IPA	Peak area of IPA						RSD %	Correlation coefficient (r)
0.0628	20	2695	2669	2646	2701	2642	2684	0.93	
0.157	50	6686	6463	6692	6626	6627	6669	1.29	0.999
0.314	100	13286	13252	13226	13300	13259	13225	0.23	
0.3925	125	16480	16581	16647	16751	16596	16615	0.47	
0.471	150	20825	20886	20295	20146	20855	20912	1.64	

Table 8: System Suitability data

Parameter	IPA
Resolution factor	4.54 (NLT 3)
Tailing factor	1.18 (NMT 2)
Number of theoretical plates	23743 (NLT 15000)

This shows that the system suitability are within the limit. The RSD for the precision is not more than 5%. Linearity was evaluated by visual inspection of plot and peak area as a function of analyte concentration by isopropyl alcohol. From the linearity studies the specified range was determined for the isopropyl alcohol is given as 0.0628-0.471ppm. The limit of RSD is not more than 5% which shows that the method is capable of producing good response in FID detector.

The accuracy level is the percentage recovery which should be in the range of 100.19- 100.55%. The validation of developed method shows that the method is capable of showing good accuracy and reproducibility.

The percentage for the limit of detection is 0.000628%. this shows that then lowest concentration of IPA can be detected and the percentage for be limit of quantitation is 0.000628%.

By using different analyst at different day method ruggedness is satisfactorily by analysing the same sample at normal operating condition and IPA peak was detected. By changing the instrument, injection temperature and detector temperature method robustness is satisfactory by analysing the sample at a change in operating condition and the IPA code is detected.

The data related to the limit of detection, limit of quantification, accuracy of isopropyl alcohol, ruggedness and robustness are all given in table 9 to 12

Table 9: Limit of detection

Injection No	Detector response IPA (Area counts)
01	260
02	283
03	274
04	281
05	272
06	282
Mean	276
RSD 3.17%	

Table 10: Limit of Quantitation

Injection No	Detector response IPA (Area counts)
01	2649
02	2617
03	2700
04	2617
05	2723
06	2731
Mean	2673
RSD 1.94%	

Table 11: Accuracy of Isopropyl Alcohol

Injection No	Concentration in mg/ml IPA		
	0.2826	0.3140	0.3454
	Observed area response for IPA		
I	12001	13285	14540
II	12100	13290	14550
III	12015	13295	14545
IV	12090	13280	14560
V	12180	13260	14520
VI	12150	13300	14511
% of RSD	0.59%	0.11%	0.13%
Recovery in mg/ml	0.2834	0.3146	0.3473
% of Recovery	100.28	100.19	100.55
Limit 90% to 110%			

Table 11: Ruggedness

S. No.	Parameter	Normal condition	Ruggedness condition
1	Instrument	Netel GCIII	Netel GC III
2	Column	AT-1 60mx0.25mm	AT-1 60mx0.25mm
3	Column Temperature	180oC	180oC
4	Injector Temperature	200oC	200oC
5	Detector temperature	240oC	240oC
6	Carrier gas	Nitrogen	Nitrogen
7	Carrier gas flow	1.99	1.99
8	Injection Volume	1.0µl	V
9	Content of IPA	487.72ppm	485.49ppm

Table 12: Robustness

S. No.	Parameter	Normal condition	Ruggedness condition
1	Instrument	Netel GCIII	Netel GC III
2	Column	AT-1 60mx0.25mm	AT-1 60mx0.25mm
3	Column Temperature	180oC	180oC
4	Injector Temperature	200oC	200oC
5	Detector temperature	240oC	240oC
6	Carrier gas	Nitrogen	Nitrogen
7	Carrier gas flow	1.99	1.99
8	Injection Volume	1.0µl	V
9	Content of IPA	487.72ppm	484.10ppm

All the parameters including the flow rate, temperature, split ratio and the sensitivity was maintained constant throughout the procedure. The consolidated result is tabulated in Table 13

Table 13: Consolidated result tabulation

S. No.	Attributes	Observed Value			Acceptance limit
1	System Suitability	1.	Resolution	4.54	NLT 3.00
		2.	Tailing factor	1.18	NLT 2.00
		3.	Theoretical plates	23743/m	NLT 15000
2	Precision	1.	Standard	RSD	
		I		4.44%	NMT 5.00%
		II		4.42%	
		III		2.23%	
		2.	Sample		
		I		0.84%	
		II		1.09%	
		III		0.73%	
3	Linearity and Range			RSD	
		20% IPA		0.93%	NMT 5.00%
		50% IPA		1.29%	Correlation coefficient (r)
		100% IPA		0.23%	NMT 0.98
		125% IPA		0.47%	
		150% IPA		1.64%	
		Correlation coefficient (r)		0.999	
4	Accuracy	0.2826mg/ml (90%)		0.2834mg/ml (100.28%)	0.25434(90%)-0.31086 (110%)
		0.3149mg/ml (100%)		0.3146mg/ml (100.19%)	0.2826 (90%)-0.3454 (110%)
		0.3454mg/ml (110%)		0.3473mg/ml (100.55%)	0.31086 (90%)-0.37994 (110%)
5	Limit of detection			0.000628%	
6	Limit of quantitation			0.000628%	
7	Ruggedness			Satisfactory	Should be satisfactory
8	Robustness			satisfactory	Should be satisfactory

SUMMARY & CONCLUSION

Analytical methods play a vital role in new drug development, preformulation and formulation studies, stability studies, quality control testing and in quality assurance programs. So analysis are always in search of developing rapid and accurate new methods for analysis that are able to exist in routine analytical work. The present analytical work comprises of simple, precise, rapid, sensitive and accurate methods for the development and validation of residual impurity of isopropyl alcohol in magnesium valproate. This can be determined by gas chromatographic method.

The amount of isopropyl alcohol is magnesium valproate was carried out by gas chromatographic method using flame ionisation detector. The type of column is AT-1 capillary column. The flow rate was set at 1ml/min. The carrier gas used is nitrogen gas. The column temperature was maintained at 200^{o}C and the detector temperature was maintained at 260^{o}C.

The amount of isopropyl alcohol was found to be 487.72 ppm and it is subjected to statistican analysis. The RSD values obtained are below 5% indicating the precision of the applied methodology and the percentage recoveries vary from 99.82 – 100.55%.

The results obtained on the validation met the ICH and USP requirements. It inferred that the method was found to be simple, simple, precise and linear proportional ie accurate, reproducible and reliable. The method was found to have suitable application in routine laboratory analysis with a high degree of accuracy and precision.

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