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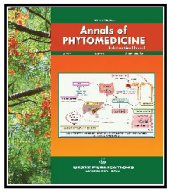

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Analytical method development and validation of residual solvents in ethosuximide by GC-headspace technique

Afroz Patan◆ and A. Poojah

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram-600117, Chennai, Tamil Nadu, India

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Abstract

A precise, reliable GC approach has been created for estimating the amount of isopropyl alcohol (class-III residual solvent) content in ethosuximide by GC-headspace technique. The chromatographic separation was carried out using dimethyl acetamide as the mobile phase. A gas chromatographic system with the capillary column, DB-624 with dimensions of 30 m × 0.53 mm × 3 µm has been utilized for the separation of analytes. The flow rate was maintained at a mode of consistent flow with 2.0 ml/min. The temperature of the column was been maintained at 40°C for 11 min, elevated to 240°C with a speed of 30°C per min, and held at 240°C for 12.33 min. Nitrogen has been utilized as the carrier gas. The temperature of the injection has been kept at 180°C. The detector temperature has been maintained at 250°C. The gas split ratio was 20:1. The data sensitivity has been acquired at 20 Hz/ 0.1. The make-up gas flow has been kept constant at 30 ml/min. The fuel flow has been upheld at 40 ml/min. The oxidizer flow was found to be 400 ml/min. The run time was 30 min. By the ICH guidelines, the analytical procedure was validated for ethosuximide. The approach has been discovered to be linear, exhibiting a coefficient of correlation of 0.99 and a y-intercept value of 0.74.

1. Introduction

Chromatography is an essential physicochemical technique that permits the detection, segregation, and refinement of the constituent elements of a mixture employed in both quantitative as well as qualitative studies (Coskun and Ozlem, 2016). The concept that fluid stationary phases, also known as stable phases, and mixtures of molecules placed on interfaces as well as solid materials, move with the aid of phase with mobility and split themselves from others is the basis of chromatography. The molecular characteristics associated with adsorption (liquid and solid), partition (liquid and solid), affinity, or variations in their molecular masses are among the elements that significantly impact this procedure of separation (Cuatrecasas *et al.,* 1968; Porath, 1997). Gas chromatography is the collective term for a set of analytical procedures for separation which is used for examining compounds that are volatile in the gaseous state. To isolate analytic compounds from the sample, gas chromatography (GC) requires immersing the individual components of the sample in a solvent and evaporating them. This creates a phase that remains stationary along with a mobile phase (Colin F. Poole, 2003). In addition to observing enzyme connections, gas-liquid chromatography plays a role in separating the amino groups as well as esters, alcohol and along lipids (Gerberding and Byers, 1998).

Corresponding author: Dr. Afroz Patan
Assistant Professor, Department of Pharmaceutical Chemistry and Analysis School of Pharmaceutical Sciences Vels Institute of Science, Technology and Advanced Studies (VISTAS) Pallavaram- 600117, Chennai, Tamil Nadu, India
E-mail: afroz.sps@velsuniv.ac.in
Tel.: +91-9176080692

In pharmaceutical companies, volatile organic chemical substances are generated or utilized during the manufacturing of medication components, excipients, or drug products and are referred to as residual solvents. Not all of the solvents are eliminated using a realistic production method (Akella Anuradha *et al.,* 2023). Isopropyl alcohol belongs to class III residual solvents (solvents with low toxic potential). One isomer of 1-propanol is isopropyl alcohol (2-propanol). It is a transparent fluid with antiseptic qualities. It serves as a solvent and is employed in the production of acetone and its derivatives. It is applied topically as an antiseptic (Indumathy *et al.,* 2023). IPA is a colorless and volatile liquid with a strong musty smell similar to rubbing alcohol. It possesses a 53°F flash point. The vapours cause slight irritation to one's nose, eyes, as well as throat and are heavier than air. It has a density of around 6.5 pounds per gallon. 2-propanol is also used to create coloring agents, window cleaning products, anti-freeze, medications, soaps, beauty products, skincare products, hair therapies, and perfumes. It is sold as rubbing alcohol in a 70% aqueous solution (Subhamalar *et al.,* 2023).

One of the most common neurological disorders is epilepsy (McCrea and Sarah, 2002). Ethosuximide, sometimes referred to as 2-ethyl-2-methylsuccinimide, is a type of succinimide that is frequently used in the treatment of seizures that are referred to as "petit mal seizures" (absence seizures) (Mares *et al.,* 1994; Zimmerman and Burgmeister, 1958). Whether taken alone or in combination, this drug is the best choice for managing pediatric patients with petit mal (generalized absence), epilepsy (Gören and Onat, 2007). Typical absence seizures are caused by complex interactions between the thalamus, a part of the brain, and the cerebral cortex. Ethosuximide decreases the frequency of seizures in approximately half of the patients and

controls almost all of the patients' absence seizures (Browne *et al.,* 1975; Berkovic *et al.,* 1987). Ethosuximide is known by its IUPAC name, 3-ethyl-3-methylpyrrolodin-2,5-dione. The chemical formula of ethosuximide is $C_7H_{11}NO_2$. The molecular weight in grams per mole is 141.1677. Ethosuximide is a white, crystalline medication that belongs to the BCS class-I chemical class. Ethosuximide is less soluble in organic solvents and dissolves well in water (Afroz Patan *et al.,* 2023).

The literature review (Mitesh Bhatt *et al.,* 2010; El-Shabrawy *et al.,* 2018; Nabizadeh *et al.,* 2023; Sghendo *et al.,* 2002; Heipertz *et al.,* 1977; Ou and Rognerud, 1984; Galan Valiente *et al.,* 1989) states that no analytical methodologies have been created for the estimation of residual solvents in ethosuximide capsules.

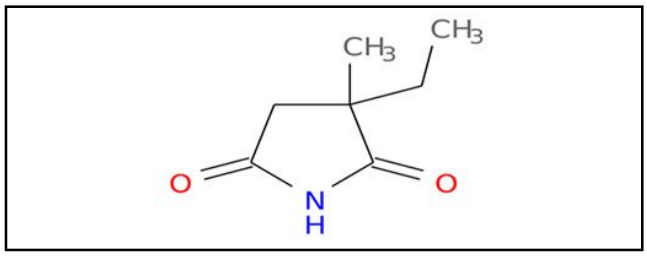

Figure 1: Structure of ethosuximide.

2. Materials and Methods

2.1 Chemicals and reagents

Dimethyl acetamide of GC grade was used.

2.2 Instruments and chromatographic conditions

A gas chromatography system (Shimadzu) and analytical balance were used for the analysis. A gas chromatographic system with the capillary column, DB-624 with the dimension of 30 m × 0.53 mm × 3 µm has been utilized for the separation of analytes. The flow rate was maintained at a mode of consistent flow with 2.0 ml/min. The temperature of the column has been maintained at 40°C for 11 min, elevated to 240°C with a speed of 30°C per min, and held at 240°C for 12.33 min. Nitrogen has been utilized as the carrier gas. The temperature of the injection has been kept at 180°C. The detector temperature has been maintained at 250°C. The gas split ratio was 20:1. The data sensitivity has been acquired at 20 Hz/ 0.1. The make-up gas flow has been kept constant at 30 ml/min. The fuel flow has been upheld at 40 ml/min. The oxidizer flow was found to be 400 ml/min. The run time was 30 min. By the ICH guidelines, the analytical procedure was validated for ethosuximide. The approach has been discovered to be linear, exhibiting a coefficient of correlation of 0.99 and a y-intercept value of 0.74.

2.3 Headspace parameters

The GC-headspace instrument was used. The vial temperature was maintained at 50ºC. The loop temperature was maintained at 130ºC. The transfer line temperature was maintained at 140ºC. The vial equilibrium time was found to be 15 min. The GC cycle time was found to be 40 min. The shaking speed was maintained at medium. The loop volume was found to be 1.0 ml. The injection volume was maintained at 1.0 µl. The injection time was found to be 1.0 min. The pressurization time was maintained at 3.0 min. The loop fill time was found to be 0.2 min. The loop equilibrium time was maintained at 0.2 min. The mixer was kept on. The mixing time was found to be 2.0 min. The mix stabilizer time was maintained at 0.5 min. The pressure was maintained at 15 psi.

2.4 Preparation of solutions for analysis

2.4.1 Diluent

Used dimethyl acetamide (DMA) as diluent.

2.4.2 Preparation of blank solution

Pipetted out 5 ml of DMA in to 20 ml head space vial. Placed the cap along with the septum and crimped the cap to seal it.

2.4.3 Preparation of standard solution

About 256.0 mg of isopropyl alcohol WS/RS was weighed and mixed well in a 100 ml volumetric flask with 30 ml of diluent. Mixed thoroughly and diluted to volume using the diluent. 10 ml was pipetted into a 100 ml volumetric flask, diluted with diluent to volume, and thoroughly mixed. 5 ml was transferred to 6 distinct headspace vials. Placed the septum and cap, then crimped the cap to seal it.

2.4.4 Preparation of check standard solution

About 256.0 mg of isopropyl alcohol WS/RS was weighed and mixed well in a 100 ml volumetric flask with 30 ml of diluent. Mixed thoroughly and diluted to volume using the diluent. 10 ml was pipetted into a 100 ml volumetric flask, diluted with diluent to volume, and thoroughly mixed. 5 ml was transferred to 6 distinct headspace vials. Placed the septum and cap, then crimped the cap to seal it.

2.4.5 Preparation of placebo

Weighed 2 whole capsules (about 1280.0 mg) and transferred them into a headspace vial. Pipetted out about 5 ml of diluent into the headspace vial. Mixed well by handshake for 5 min. Placed the cap along with the septum and crimped the cap to seal it.

2.4.6 Preparation of unspiked sample

Weighed 2 whole capsules (about 1280.0 mg) and transferred them into a headspace vial. Pipetted out about 5 ml of diluent into the headspace vial. Mixed well by handshake for 5 min. Placed the cap along with the septum and crimped the cap to seal it.

2.4.7 Preparation of spiked sample

Weighed about 2 whole capsules (about 1280.0 mg) and transferred in into a headspace vial. Pipetted out about 5 ml of standard solution into to headspace vial. Mixed well by handshake for 5 min. Placed the cap along with the septum and crimped the cap to seal it.

2.4.8 Preparation of bracketing standard solution

Used standard preparation as the bracketing standard solution.

3. Procedure for standard preparation

- Equal volumes (1 µl) of diluent were injected as a blank separately, followed by 6 replicate administrations of the standard preparation, a pair of replicate injections of the check standard preparation, a single injection of every test preparation, and a single injection of the standard injection.

- The chromatograms were recorded, and the responses for the major peak were measured.
- The chromatograms were recorded, documented in the instrument and column usage log book, and evaluated using the results.

4. Results

The safety of a drug product is dependent not only on the toxicological properties of the active drug substance itself, but also on the impurities that it contains. Therefore, identification, quantification, and control of impurities in the drug substance and drug product, are an important part of drug development and regulatory assessment. ICH Q3C R9 address issues relevant to the regulation of residual solvents drug substance and drug product.

The ICH guidelines were followed in the development, optimization, and validation of the analytical technique to ensure that the analytical solutions met the requirements for system suitability, specificity, linearity, accuracy, precision, robustness, and stability of analytical solutions. According to ICH guidelines (ICH Q3C R9), the values obtained from all the validation parameters for this method are present well within the acceptance criteria.

4.1 Method validation

4.1.1 System suitability

A system's compatibility assessment is a crucial component of a variety of analytical techniques, such as GC and HPLC. These examinations are predicated on the idea that the test, electrical apparatus, analytical procedures, and the samples that need to be examined all form an essential component of the operational system and can be assessed as such. In accordance with protocol, the GC system is injected six times with a standard solution of isopropyl alcohol to determine the system's suitability**.** The tailing factor of isopropyl alcohol should be NMT 2.0 at its peak. The theoretical count of plates for isopropyl alcohol should be NLT 2000 at its peak. The retention time of isopropyl alcohol was found to be 8.71. The % relative standard deviation of retention time of isopropyl alcohol (NMT 1.0 %) was found to be 0.01. The % relative standard deviation of the area of isopropyl alcohol (NMT 15.0 %) was found to be 2.1%. Table 1 shows the results of the system suitability of isopropyl alcohol.

Table 1: Results of system suitability

S. No.	Name of the solvent	Area (µVs)	Retention time (min)	Theoretical plate	Tailing factor
1.	Isopropyl alcohol	27891	8.71	28909	1.0

4.1.2 Specificity

The ability of the procedure to quantify analyte levels when the matrix constituents are present is referred to as specificity. Analyte identification, blank, and placebo interference with the analyte, as well as their combination, illustrate the analysis's specificity. The following solutions are produced and injected into the GC system by protocol to establish specificity. The outcome demonstrates that blank and placebo peaks do not interfere with the retention time of isopropyl alcohol in ethosuximide (finished product). The percentage recovery between the check standard preparation and standard preparation (85.0%-115.0%) was found to be 103.6%. The percentage of system drift between bracketing standard and standard (85.0%-115.0%) was found to be 95.8%. Table 2 shows the specificity results of isopropyl alcohol.

Table 2: Specificity results

S.No.	Preparations	Observation		
		Interference	Retention time (min)	Peak response
1.	Standard solution	No interference	8.7	28667
2.	Spiked sample solution	No interference	8.8	28097

4.1.3 Linearity and range

4.1.3.1 Linearity

An analytical procedure is said to be linear if it can yield outcomes which are directly proportionate to the analyte's concentration (amount) in the sample over a certain range of values.

4.1.3.2 Range

It refers to the range of analyte concentrations between the highest and lowest wherein the analytical technique's precision, linearity, and accuracy are appropriate. By carrying out five test concentrations of substances, ranging from 50% to 150% of the working concentrations of substances, as directed by protocol, the linearity of the approach is determined. About the 100% active concentration of isopropyl alcohol, the standard solutions are created at concentrations of 50%, 75%, 100%, 125%, and 150%. A graph is produced between area (on the Y-axis) and concentration (on the X-axis) depending on the area achieved with each level concentration of linearity and LOQ. The information is provided below. Figure 2 describes the linearity graph of isopropyl alcohol. Table 3 shows the linearity results of isopropyl alcohol.

Table 3: Linearity results

Std. wt. in mg	Potency in %	Conc. in %	Conc. in ppm	Average of IPA peak area
LOQ				
247.7 mg	100.0	9	22.29	2858
Linearity				
251.0 mg	100.0	50	125.5000	14395
		75	188.2500	21452
		100	251.0000	28608
		125	313.7500	35166
		150	376.5000	44918

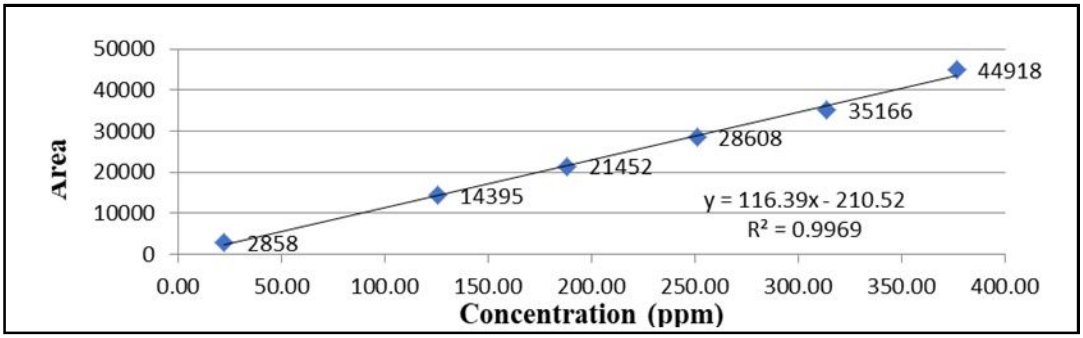

Figure 2: Linearity graph of isopropyl alcohol.

Table 4: Linearity observations

Linearity and range parameter	Obtained value		Acceptance standards
Correlation coefficient(r2)	0.9969		NLT 0.99
Percentage of y-intercept	0.74		±10.0 %
The percentage RSD of the peak area	LOQ	150 %	
from six standard preparations of lower	8.4	2.0	NMT 15.0 %
and higher replicates (LOQ and 150 %)			

4.1.4 Accuracy

The measure of accuracy is how closely the test findings produced by the procedure match the actual value. The percentage recovery using known, additional quantities of analytes is a popular method to express accuracy. A measure of an analytical method's exactness is its accuracy.The four concentrations will be used to evaluate accuracy (LOQ, 50%, 100%, and 150%).Spike Isopropyl alcohol into placebo capsules at LOQ, 50%, 100%, and 150% concentration and perform analysis as per the method. The average outcome at each concentration will subsequently be displayed as a percentage of the total amount recovered at each level or concentration. By the instructions, standard, check standard, and unspiked sampling solutions were created. Concentrations of LOQ, 50%, 100%, and 150% were used for preparing spiked sample solutions. Triplicate injections are given for each solution. The average % recovery of isopropyl alcohol at LOQ levels, 50% level, 100% level, and 150% levels (90.0 % to 110.0%) were found to be 96.4%, 98.7%, 103.1%, and 104.1%, respectively. The % RSD of isopropyl alcohol at LOQ level, 50% level, 100% level, and 150% levels (NMT 15.0 %) were found to be 3.6%, 7.2%, 7.9%, and 7.3%, respectively. Table 5 describes the accuracy results of isopropyl alcohol. The results obtained are described below as follows.

Table 5: Accuracy results

Accuracy level	Added amount (in ppm)	Recovered amount (in ppm)	Percentage of recovery (in %)	Average of %recovery	SD	RSD (in %)
LOQ	22.9695	23.0113				
	22.0216	95.8732	100.1819	96.4	3.6	3.7
	21.3871	93.1108				
50%	127.4097	122.1505				
	136.1341	106.8475	95.8722	98.7	7.1	7.2
	119.0833	93.4648				
100%	254.8195	276.7632				
	238.7786	93.7049	108.6114	103.1	8.2	7.9
	272.7064	107.0194				
150%	382.2292	363.9510				
	413.8190	108.2646	95.2180	104.1	7.7	7.4
	415.8814	108.8041				
			Overall	100.6	6.7	6.6

4.1.5 Precision

The extent of consistency between individual test findings obtained from applying an analytical technique repeatedly to many samples of a homogenous mixture is known as the procedure's precision. By using a homogeneous sample, the analytical method's precision is assessed.

4.1.5.1 System precision

Six replicate injections of the isopropyl alcohol standard solution will be used to test the precision of the system. The retention time of isopropyl alcohol was found to be 8.91. The % RSD of retention time of isopropyl alcohol (NMT 1.0%) was found to be 0.01% and the % RSD of the area of isopropyl alcohol (NMT 15.0 %) was found to be 5.4%. Table 6 shows the results of the system precision of isopropyl alcohol.

4.1.5.2 Method precision

The isopropyl alcohol amount in ethosuximide will be analyzed in two replicates of unspiked and six replicates of spiked samples to show the method's precision (reproducibility). The isopropyl alcohol content was found to be not detected in the two replicate injections of unspiked sample preparations. The average ppm of isopropyl alcohol content from 6 replicate spiked sample preparations (900 to 1100 ppm) was found to be 1006.4 ppm. The % RSD of isopropyl alcohol content from 6 replicate spiked sample preparations (NMT 15.0%) was found to be 0.1%. The confidence limit of IPA in 6 replicate spiked sample preparations (± 5.0%) was 1.5%.

4.1.5.3 Intermediate precision

Analyzing the amount of isopropyl alcohol in ethosuximide in two replicates of unspiked samples and six replicates of spiked samples, applying an alternative device, an alternative column, on a separate day, as well as a different lab and different analyst, revealed intermediate precision. Table 7 shows the results of IPA in the unspiked sample. Table 8 shows the results of IPA in spiked samples.

Table 6: Results of system precision

S.No.	Name of the solvent	Area (µVs)	Retention time (min)	Theoretical plate	Tailing factor
1.	Isopropyl alcohol	27891	8.91	28909	1.0

Table 7: Results of IPA in the unspiked sample

GC I.D.	AD / EQP / 017	QC / EQP / 207
Column I.D. No.	SGCA0002	SGC0051
	PPM of isopropyl alcohol	
Preparation	Method precision	Intermediate precision
1	ND	ND
2	ND	ND
Average	ND	ND
Average of 4 samples (MP+IP)	NA	
SD	NA	NA
SD of 4 samples (MP+IP)	NA	
%RSD	NA	NA
%RSD of 4 samples (MP+IP)	NA	
Confidence limits	NA	NA
Confidence limits of 4 samples (MP+IP)	NA	

*NA= not applicable, ND= not detected.

4.1.6 Robustness

Robustness is a measure of an analytical procedure's resistance to extremely tiny, purposeful modifications to method parameters. Robustness also reflects the procedure's level of reliability under normal circumstances of operation. The robustness of the analytical method for the content of isopropyl alcohol in ethosuximide is demonstrated by performing the method with purposeful, tiny changes in column temperature (± 2°C), flow rate (± 0.2 ml), and vial equilibrium time (± 5 min).

4.1.6.1 Effect of variation in flow rate (± 0.2 ml)

The flow rate fluctuations of 1.8, 2.0, and 2.2 milliliters per min (ml/min) show the analytical method's robustness by the protocol. By the procedure, the standard, check standard, unspiked sample, and spiked sample solutions have been created and injected into the GC. Table 10 shows the results of isopropyl alcohol with variations in flow rates. The outcomes are enumerated below.

4.1.6.2 Effect of variation in column temperature (°C)

The tiny temperature changes (38, 40, and 42°C) in the column as per protocol show how resilient the analytical approach is. By the procedure, the standard, check standard, unspiked sample, and spiked sample solutions have been created and injected into the GC. Tables 11 and 12 show the results of isopropyl alcohol with variations in column temperatures. The outcomes are enumerated below.

Table 8: Results of IPA in the spiked sample

GC I.D.	AD / EQP / 017	QC / EQP / 207
Column I.D. No.	SGCA0002	SGC0051
PPM of isopropyl alcohol		
Preparation	**Method precision**	**Intermediate precision**
1	1003.9943	990.3450
2	1004.1869	1013.5916
3	1008.4103	995.6776
4	1007.8830	992.2965
5	1006.3799	1007.1410
6	1007.5823	1006.0628
Average	1006.4060	1000.852
Average of 12 samples (MP+IP)	1003.6290	
SD	1.9	9.3
SD of 12 samples (MP+IP)	7.0	
%RSD	0.1	0.9
%RSD of 12 samples (MP+IP)	0.7	
Confidence limits	1.5	2.5
Confidence limits of 12 samples (MP+IP)	4.0	

% RSD value of isopropyl alcohol for 4 replicate unspiked samples (MP+IP), and 12 spiked sample preparations (MP+IP) ensures that the procedure is accurate and repeatable.

Table 9: Results of SST of flow rate

S.No.	Name of the solvent	Area (µVs)	Retention time (min)	Theoretical plate	Tailing factor
1.	Isopropyl alcohol - low flow rate (1.8 ml/min)	29630	8.70	29366	1.1
2.	Isopropyl alcohol - actual flow rate (2.0 ml/min)	29422	8.71	28761	1.1
3.	Isopropyl alcohol - high flow rate (2.2 ml/min)	29832	8.73	25810	1.1

Table 10: Results of IPA in variations in flow rate

System suitability parameters	Observation			Acceptance
Flow rate (ml/min)	**1.8 ml/min**	**2.0 ml/min**	**2.2 ml/min**	**criteria**
The tailing factor of isopropyl alcohol peak	1.1	1.1	1.1	**NMT 2.0**
The theoretical plate count of isopropyl alcohol peak	29366	28761	25810	**NLT 2000**
The % RSD of the retention time of isopropyl alcohol peak	0.0	0.0	0.0	**NMT 1.0 %**
The average of isopropyl alcohol content determined through two replicate spiked sample preparations at three distinct flow rates	1020	1019	953	**900 to 1100 ppm**
The % RSD of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct flow rates	9.3	8.1	0.6	
The average total amount of % RSD of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct flow rates		6.6		
The percentage of recovery from standard preparations and check standard preparations	98.0	91.5	98.1	**85.0 % to 115.0 %**
The percentage of system drift between bracketing standard and standard	90.6	87.0	93.8	

Table 11: Results of SST of column temperature

S.No	Name of the solvent	Area (µVs)	Retention time (min)	Theoretical plate	Tailing factor
1.	Isopropyl alcohol - low column temperature (38°C)	31630	8.70	27940	1.0
2.	Isopropyl alcohol - actual column temperature (40°C)	30923	8.71	27756	1.1
3.	Isopropyl alcohol - high column temperature (42°C)	33584	8.73	29418	1.1

Table 12: Results of IPA in variations in column temperature

System suitability parameters	Observation			Acceptance
Column temperature (°C)	**38°C**	**40°C**	**42°C**	**criteria**
The tailing factor of isopropyl alcohol peak	1.0	1.1	1.1	**NMT 2.0**
The theoretical plate count of isopropyl alcohol peak	27940	27756	29418	**NLT 2000**
The % RSD of the retention time of isopropyl alcohol peak	0.0	0.0	0.0	**NMT 1.0 %**
% RSD of the isopropyl alcohol peak area	4.9	3.3	2.1	**NMT 15.0 %**
the average of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct column temperatures	963.9	971.2	1005.2	**900 to 1100 ppm**
The average total amount of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct column temperatures		980.1		**900 to 1100 ppm**
The % RSD of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct column temperatures	0.2	0.8	1.8	**NMT 15.0 %**
The average total amount of % RSD of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct column temperatures		2.2		**NMT 15.0 %**
The percentage of recovery from standard preparations and check standard preparations	94.3	93.5	91.6	**85.0 % to 115.0 %**
The percentage of system drift between bracketing standard and standard	102.4	100.4	104.4	

Table 13: Results of SST of vial equilibrium time

S.No	Name of the solvent	Area (µVs)	Retention time (min)	Theoretical plate	Tailing factor
1.	Isopropyl alcohol - low vial equilibrium time (10 min)	29751	8.90	28081	1.1
2.	Isopropyl alcohol - actual vial equilibrium time (15 min)	30407	8.91	29219	1.1
3.	Isopropyl alcohol - high vial equilibrium time (20 min)	30898	8.93	28405	1.1

Table 14: Results of IPA in variations in vial equilibrium time

System suitability parameters	Observation			Acceptance
Vial equilibrium time	**10 min**	**15 min**	**20 min**	**criteria**
The tailing factor of isopropyl alcohol peak	1.0	1.1	1.1	**NMT 2.0**
The theoretical plate count of isopropyl alcohol peak	28081	29219	28405	**NLT 2000**
The % RSD of the retention time of isopropyl alcohol peak	0.0	0.0	0.0	**NMT 1.0 %**
% RSD of the isopropyl alcohol peak area	6.1	7.9	1.9	**NMT 15.0 %**
The average of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct vial equilibrium time	970	960	997	**900 to 1100 ppm**
The % RSD of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct vial equilibrium time	2.7	0.0	0.1	
The average total amount of % RSD of isopropyl alcohol content was determined through two replicate spiked sample preparations at three distinct vial equilibrium time		2.1		
The percentage of recovery from standard preparations and check standard preparations	103.5	95.5	104.9	**85.0 % to 115.0 %**
The percentage of system drift between bracketing standard and standard preparations	108.1	103.3	98.2	

Table 15: Results of IPA's solution stability

Time intervals	Isopropyl alcohol		
	Standard	Unspiked sample	Spiked sample
Initial	30851	ND	30030
After 12th h	28905	ND	30729
After 24th h	31776	ND	28348
After 36th h	28787	ND	28548
After 48th h	28494	ND	27680
Average	29762	ND	29067
Std. dev	1460.7372	NA	1264.8818
% RSD	4.9	NA	4.3

4.1.6.3 Effect of variation in vial equilibrium time

Small modifications in the vial equilibrium time (10 min, 15 min, and 20 min) as per procedure show how resilient the analytical process is. By the procedure, the standard, check standard, unspiked sample, and spiked sample solutions have been created and injected into the GC. Tables 13 and 14 show the results of isopropyl alcohol with variations in vial equilibrium time. The outcomes are enumerated below.

4.1.7 Stability of analytical solution

By injecting the solutions at various times after preparation, the stability of the standard and sample solutions will be illustrated. Up to 48 h, solutions must be injected once every 12 h. The area obtained at various time intervals will be used to determine the stability of the analytical solution.

By injecting standard, unspiked, and spiked sample preparations every 12 h upto 48 h, the stability of the solution is established. The following calculations indicate the percentage RSD of the area of the standard, unspiked, and spiked sample solutions. Table 15 shows the results of solution stability of isopropyl alcohol.

5. Discussion

The present effort is centered on developing an analytical approach for the estimation and validation of isopropyl alcohol (class-III residual solvent) content in ethosuximide by GC-head space technique. When developing a method, several key parameters play a crucial role in achieving optimal results. Selectivity and Resolution depends on column selection and mobile phase selection. Optimum mobile phase is selected based upon getting desired retention and resolution. Method development software is used for predictive runs. Method development is an iterative process, and understanding these parameters helps enhance the experimental process, multiple columns and mobile phases are evaluated to determine the best results.

The chromatographic separation was carried out using dimethyl acetamide as the mobile phase. A gas chromatographic system with the capillary column, DB-624 with the dimension of 30 m × 0.53 mm × 3 µm has been utilized for the separation of analytes. The flow rate was maintained at a mode of consistent flow with 2.0 ml/min. The temperature of the column has been maintained at 40°C for 11 min, elevated to 240°C with a speed of 30°C per min, and held at 240°C for 12.33 min. Nitrogen has been utilized as the carrier gas. The temperature of the injection has been kept at 180°C. The detector temperature has been maintained at 250°C. The gas split ratio was 20:1. The data sensitivity has been acquired at 20 Hz/ 0.1. The make-up gas flow has been kept constant at 30 ml/ min. The fuel flow has been upheld at 40 ml/ min. The oxidizer flow was found to be 400 ml/ min. The run time was 30 min. The IPA column temperature is taken in range 38, 40 and 42°C in robustness to check the stability of the column as for every run the column has to come back to intial temperature 40°C from 240°C. By the ICH guidelines, the analytical procedure was validated for ethosuximide. The approach has been discovered to be linear, exhibiting a coefficient of correlation of 0.99 and a y-intercept value of 0.74.

6. Conclusion

The outcomes of the validation of the analytical method demonstrate that the estimation of residual solvents (isopropyl alcohol) content in ethosuximide capsules by GC-headspace technique is suitable, specific, linear, accurate, precise, robust as well and stable. As a result, this approach is deemed validated and suitable for subsequent analysis.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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