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Review

Headspace GC-FID Based Estimation of Ethanol content in Indomethacin Oral Suspension

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Abstract

Indomethacin oral suspension is a widely used non-steroidal anti-inflammatory drug (NSAID) formulation intended for patients requiring flexible dosing, rapid therapeutic onset, or difficulty swallowing solid dosage forms. The suspension provides enhanced dosing accuracy, especially in pediatric and geriatric populations, and ensures uniform distribution of the drug within the gastrointestinal tract. Indomethacin exerts its pharmacological effect primarily by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, leading to a reduction in prostaglandin synthesis that mediates inflammation, pain, and fever. Due to its high lipophilicity, the drug quickly achieves therapeutic plasma concentrations, offering effective relief in conditions such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute gouty arthritis.The formulation contains ethanol as a co-solvent to improve solubility and maintain suspension stability. As ethanol is a volatile organic component, its accurate quantification is essential for product quality, patient safety, and regulatory compliance. Gas Chromatography (GC) with headspace analysis was employed for determining ethanol content in the formulation due to its sensitivity and selectivity. Method validation parameters, including system precision, linearity, method precision, intermediate precision, accuracy, specificity, and robustness, were thoroughly evaluated. The analytical method demonstrated excellent precision, linearity across the tested concentration range, accurate recovery of ethanol, and no interference from placebo or diluents at the retention times of ethanol and isopropyl alcohol. Robustness studies confirmed the reliability of the method under deliberate variations in analytical conditions. Overall, the validated GC method is suitable for routine quality control of ethanol in Indomethacin oral suspension.

Keywords: Indomethacin, Oral Suspension, Ethanol Determination, Gas Chromatography, Method Validation

INTRODUCTION

Indomethacin oral suspension is a widely used pharmaceutical formulation intended for patients who require flexible dosing, rapid onset of action, or have difficulty swallowing solid oral dosage forms.[1] Indomethacin, a potent non-steroidal anti-inflammatory drug (NSAID), is commonly prescribed for the management of inflammatory conditions such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gouty arthritis, and certain musculoskeletal disorders.[2] The oral suspension offers improved dosing accuracy in pediatric and geriatric populations and allows for more uniform drug distribution within the gastrointestinal tract. Owing to its high lipophilicity and ability to achieve therapeutic plasma levels rapidly, indomethacin oral suspension is preferred in conditions requiring prompt anti-inflammatory and analgesic effects.[3-5] The formulation typically contains the active drug dispersed in an aqueous vehicle with suspending agents, sweeteners, and stabilizers to maintain physical uniformity and ensure dose consistency throughout the treatment period. Pharmacologically, indomethacin exerts its action primarily through the inhibition of prostaglandin synthesis.[6-8] Its mechanism of action is centered on reversible blockade of the cyclooxygenase (COX) enzymes, COX-1 and COX-2. These enzymes catalyze the conversion of arachidonic acid into prostaglandins and thromboxanes, which are key mediators of inflammation, pain, fever, and vascular homeostasis. By inhibiting COX activity, indomethacin reduces the formation of prostaglandin E2 (PGE2), prostacyclin (PGI2), and other inflammatory mediators, thereby alleviating swelling, pain, and erythema associated with inflammatory disorders.[9] Additionally, the reduction in prostaglandin synthesis contributes to decreased sensitization of nociceptors, resulting in effective analgesia. Indomethacin also demonstrates antipyretic activity by lowering elevated body temperature.[10] It acts at the hypothalamic thermoregulatory center, where inhibition of PGE2 leads to normalization of the body's temperature set point. Beyond its classical COX inhibition, indomethacin may also reduce polymorphonuclear leukocyte migration and suppress immune cell activation, further contributing to its anti-inflammatory properties.[11] Although highly effective, indomethacin must be used with caution due to potential adverse effects, particularly gastrointestinal irritation, peptic ulceration, and renal function impairment.[12-13] The suspension form can minimize gastric irritation when taken with food and allows for titration to the lowest effective dose. Overall, indomethacin oral suspension remains a valuable therapeutic option for managing a wide range of painful and inflammatory conditions. Indomethacin oral suspension contains ethanol as a co-solvent to enhance solubility and maintain formulation stability.[14-16] Since ethanol is a volatile organic component, its quantification is essential for product safety and regulatory compliance. Gas Chromatography (GC) is the preferred method due to its high sensitivity, selectivity, and accuracy in measuring ethanol levels in liquid formulations.[17-20]

MATERIALS AND METHODS

Diluent Preparation

Diluent-1: Dimethylsulfoxide (DMSO)

Diluent-2: Transfer 600 mL of DMSO and 400 mL of water into a suitable container and mix well.

Diluent-3: Accurately weigh about 1000 mg of Isopropyl alcohol into 25 mL volumetric flask containing about 10 mL of diluent-2. Mix well and transfer the content into 2000 mL volumetric flask. Rinse the 25 mL volumetric flask with about 10 mL of diluent-2 for 3 to 4 times and transfer into 2000 mL volumetric flask. Dilute to volume with diluent-2 and mix well (Concentration of Isopropyl alcohol is about 500 µg/mL).

Blank Preparation

Transfer 2.0 mL of diluent-3 and add 1.0 g of accurately weighed Sodium chloride into a 20 mL headspace vial and immediately close with a crimp cap.

Standard Preparation

Preparation of Stock Standard Solution: Accurately weigh about 83mg of Ethanol in 25 mL volumetric flask containing about 10 mL of diluent-2. Dilute to volume with diluent-2 and mix well.

Preparation of Working Standard Solution: Pipette 10.0 mL of Stock standard solution into 50 mL volumetric flask and dilute to volume with diluent-3 and mix well. (Concentration is about 640 µg/mL of Ethanol)

Preparation of Working Standard in Headspace Vial: Pipette 2.0 mL of working standard solution and 1.0 g of accurately weighed Sodium Chloride into the same 20 mL headspace vial and immediately close with a crimp cap.

Sample Preparation

Take about 8.36 g of Indomethacin Oral Suspension (equivalent of 83 mg of ethanol) into 25 mL volumetric flask (For packaged product mix NLT 2 bottles of the Oral Solution and before mixing make sure the bottle cap for proper closing). Pipette 5.0 mL of water and dilute to volume with diluent-1 and mix well. Pipette 10.0 mL of sample preparation into 50 mL of volumetric flask and dilute to volume with diluent-3 and Mix well.

Sample in headspace vial Preparation: Pipette 2.0 mL of sample preparation and 1.0 g of sodium chloride into same headspace vial and crimp the vial.

Instrumental Parameters

Agilent Gas Chromatograph 6890N DB-624, 30 m x 0.32 mm, 1.80µm or equivalent

Oven

Initial Temperature : 40ºC
Initial Hold : 3 min
Ramp-1 – 30ºC /min
Final Temperature – 220ºC
Hold Time – 11 min
Run time – 20 min

Injector/Inlet

Injector Temperature - 170ºC
Split ratio – 1:5
Carrier gas - Nitrogen
Carrier gas flow - 3.00 mL/min (constant flow)

Detector

Detector – FID
Detector temperature - 260°C
Constant Makeup – 25.0 mL/min
Hydrogen flow- 30.0 mL/min
Air flow – 300 mL/min

Head Space 7897A

Vial Oven Temperature : 85°C
Loop Zone Temperature : 95°C
Transfer line Temperature : 110°C
GC Cycle Time : 35.00 min
Vial Equilibration Time : 15.0 min
Vial Pressurization time : 0.20 min
Loop Fill time : 0.30 min
Loop Equilibration Time : 0.10 min
Sample Inject : 1.0 min

METHOD VALIDATION

System Precision

A standard solution was prepared as per the method and injected. The % RSD for peak area ratio for Ethanol and Isopropyl alcohol from six (6)-replicate injections of the standard solution were calculated. The % RSD for the peak area ratio of ethanol and isopropyl alcohol from six (6) replicate injections of standard solution should be NMT 5.0.The USP tailing factor for ethanol and isopropyl alcohol peak should be NMT 2.0.

Linearity and Range

Standard solutions of varying concentrations ranging from 40% to 150% of the standard theoretical concentration were injected into GC system. The correlation coefficient square (r2) must be NLT 0.97.

Method Precision

Method precision was determined by injecting six (6)-individual samples of Indomethacin Oral Solution, 25mg/mL spiked with ethanol at the specification level. The samples were prepared as per the method. The Obtained results should be within the limits (90.0%-110.0%). The %RSD of Ethanol content from six (6)-sample preparations should be NMT 5.0.

Intermediate Precision

The method precision ruggedness (reproducibility) of the Ethanol content method was determined by preparing six (6)-individual samples of Indomethacin Oral Solution, 25mg/mL (without alcohol) spiked with ethanol by a second analyst on a different day using a different column on a different GC system. Samples were prepared as per the method. The Obtained results should be within the limits (90.0%-110.0%). The % RSD of Ethanol content from six (6) sample preparations should be NMT 5.0. The % difference in mean Ethanol content (%) between Method precision and Intermediate precision results should be NMT 10.0.

Method Accuracy

The recovery of Ethanol was performed by spiking varying amounts of Ethanol in Placebo of Indomethacin Oral Solution, 25 mg/5mL (without alcohol) at the levels of 50% to 120% of the standard theoretical concentration. The samples were prepared as per the method in triplicates for 50% to 120% levels and injected. The % recovery of Ethanol content should be between 95%-105%.

Specificity

Diluent-2, Diluent-3, standard, sample, control and spiked solutions were prepared and injected. No interference should be observed from diluent-2, diluent-3 and placebo (Control) at the retention time of Ethanol and Isopropyl alcohol.

Robustness

Vary important chromatographic parameters such as column oven temperature ± 5°C, carrier gas flow ±0.5 mL/min and head space oven temperature ± 10°C and inject the six (6)-replicates of standard preparation for each parameter and compare the system suitability. All the system suitability requirements must be met. b. Include the cautionary statement based on the results.

RESULTS AND DISCUSSION

Table 1. System Precision Results

S.No	Sample Name	Peak Area Ratio for Ethanol and IPA	USP Tailing
01	Standard – 1	0.945732	1.1
02	Standard – 2	0.940491	1.0
03	Standard – 3	0.942293	1.1
04	Standard – 4	0.932763	1.0
05	Standard – 5	0.933578	1.1
06	Standard – 6	0.928531	1.1
Mean		0.937232	
%RSD		0.7	

Table 2. Linearity Results

S.No	Sample Name	Peak Area Ratio for Ethanol and IPA
01	Linearity – 45%	0.364202
02	Linearity – 72%	0.604782
03	Linearity – 90%	0.856124
04	Linearity – 108%	1.004682

05	Linearity – 143%	1.423264
	Correlation Coefficient square	0.97

Table 3. Method Precision Results

S.No	Sample Name	Peak Area Ratio for Ethanol and IPA	Percent LC
01	Method Precision – 1	0.756026	103.0
02	Method Precision – 2	0.747985	102.0
03	Method Precision – 3	0.749562	102.1
04	Method Precision – 4	0.748195	102.0
05	Method Precision – 5	0.731458	99.7
06	Method Precision – 6	0.739682	100.8
Mean			101.6
%RSD			1.1

Table 4. Intermediate Precision Results

S.No	Sample Name	Peak Area Ratio for Ethanol and IPA	Percent LC
01	Intermediate Precision – 1	0.879346	119.1
02	Intermediate Precision – 2	0.784862	106.6
03	Intermediate Precision – 3	0.791946	107.0
04	Intermediate Precision – 4	0.770541	104.8
05	Intermediate Precision – 5	0.793542	107.8
06	Intermediate Precision – 6	0.789508	107.1
Mean			108.8
%RSD			4.8

Table 5. Method Accuracy Results

S.No	Sample Name	Response	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Average/%RSD
01	50% Rec -1	0.454601	328.21	321.91	98	99 / 1%
02	50% Rec -2	0.456868	326.61	323.51	99	
03	50% Rec -3	0.460708	326.93	326.23	100	
04	100% Rec -1	0.857543	625.47	607.24	97	98 / 1%
05	100% Rec -2	0.859121	617.02	608.36	99	
06	100% Rec -3	0.860722	614.63	609.49	99	
07	120% Rec -1	1.068091	774.31	756.33	98	98 / 1%
08	120% Rec -2	1.067431	781.40	755.87	97	
09	120% Rec -3	1.074882	773.59	761.14	98	

Table 6. Specificity Results (Retention times)

	Solvent Name		Diluent -3	Standard	Control	Spiked

S.No		Diluent -2				
01	Ethanol	2.062	2.062	2.060	2.062	2.060
02	IPA	2.541	2.540	2.540	2.540	2.540

Table 7. Robustness Results

S. No	Sample Name	%RSD	USP Tailing	
			Ethanol	IPA
01	Head space oven 90ºC	0.2	1.1	1.0
02	Head space oven 70 ºC	1.2	1.0	1.0
03	Column oven 35 ºC	0.5	1.1	1.0
04	Column oven 45 ºC	3.6	1.0	1.1
05	Carrier gas flow 3.5 mL/min	0.1	1.1	1.1
06	Carrier gas flow 4.5 mL/min	0.8	1.0	1.1

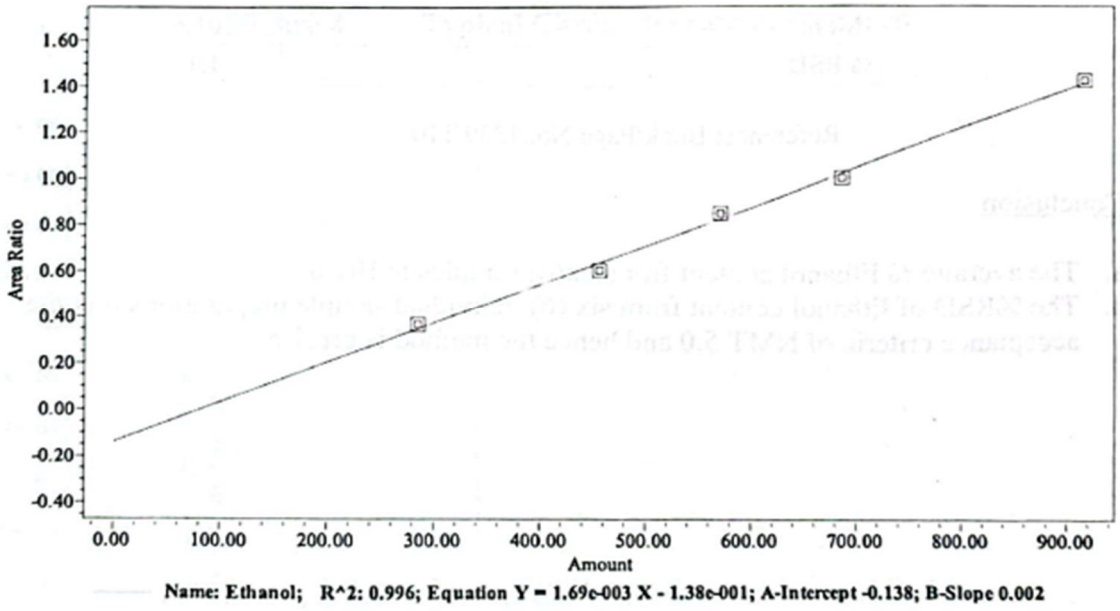

Figure 1. Linearity graph for Ethanol

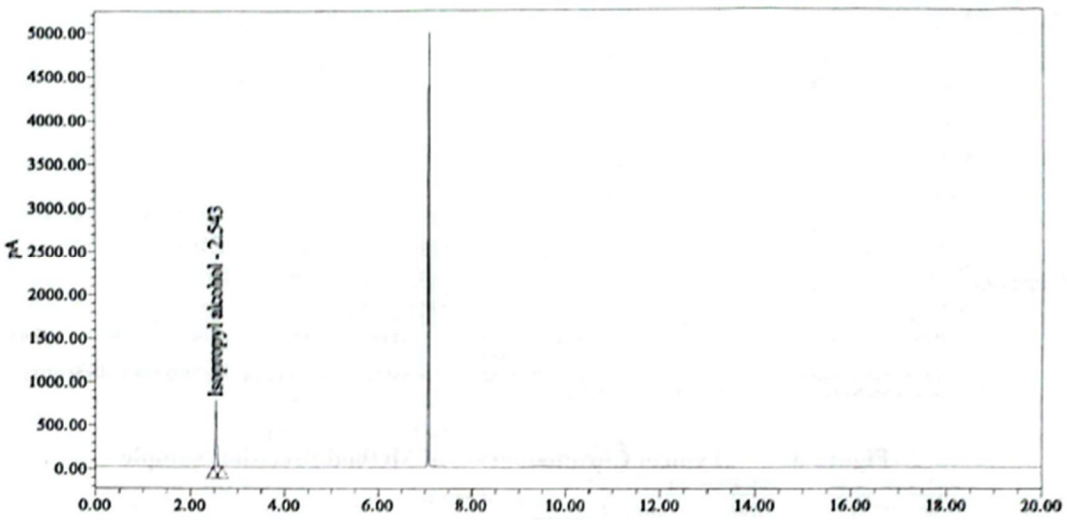

Figure 2. Typical Chromatogram for Diluent

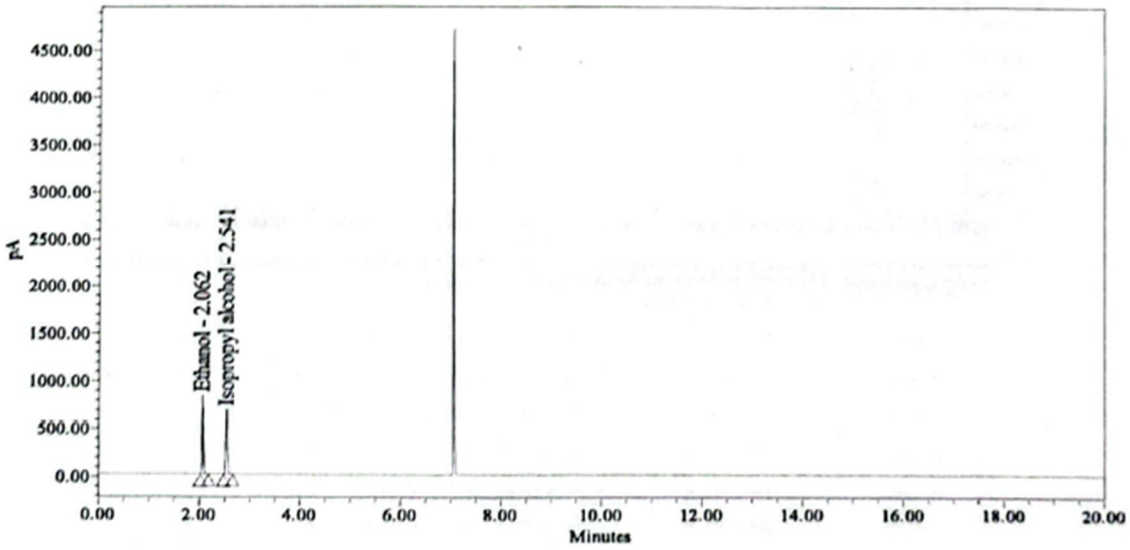

Figure 3. Typical Chromatogram for Standard

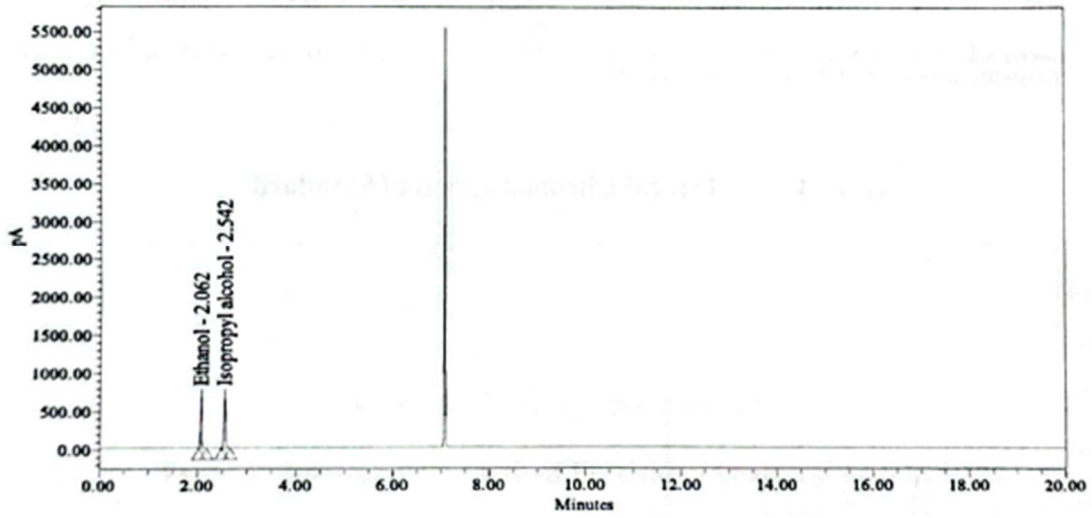

Figure 4. Typical Chromatogram for Sample

CONCLUSION

The % RSD for the peak area ratio of Ethanol and isopropyl alcohol from six (6) replicate injections is less than 5.0. The USP tailing factor for ethanol and isopropyl alcohol peaks were within the acceptance criteria of NMT 2.0, and hence the system is precise. The correlation coefficient square for Ethanol met the acceptance criteria of NLT 0.97 and the linear regression data shows that the method is linear over the entire concentration range of 45% to 145% of the standard theoretical concentration and is adequate for its intended purpose. The average % Ethanol content from six(6) samples is 101.6. The %RSD of Ethanol content from six (6) individual sample preparations met the acceptance criteria of NMT 5.0 and hence the method is precise. The Obtained results should be within the limits (90.0%-110.0%).The % RSD of Ethanol content from six (6) sample preparations should be NMT 5.0. The % difference in mean Ethanol content (%) between Method precision and Intermediate precision results should be NMT 10.0.The average % Ethanol content from six(6) samples is 108.8%.The %RSD of Ethanol content (%) from six (6) individual sample preparations is 4.8.The % difference in Mean Ethanol content (%) between method precision and intermediate precision results met the acceptance criteria of NMT 10.0, and hence the method is rugged. The % Recovery obtained for Ethanol content is within the range of 95%-105% and hence the method is accurate. No interference was observed at the retention time of Ethanol and Isopropyl alcohol from diluent-2, diluent-3 and placebo(control). Hence the method is specific. No significant change was observed in retention times, %RSD of peak area ratio of ethanol and isopropyl alcohol and USP tailing for the Standard solution for small variations in column oven temperature, Carrier gas flow rate and head space oven temperature. Hence the method is robust.Based on the above studies it is concluded that the method for Ethanol in Indometahcin Oral Suspension 25mg/5 mL is specific, precise, accurate, rugged, robust and linear over the concentration range.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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