IJPSR (2026), Volume 17, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 February 2026; received in revised form, 26 March 2026; accepted, 23 April 2026; published 01 July 2026

DEVELOPMENT AND VALIDATION OF RESIDUAL SOLVENT DETERMINATION BY GAS CHROMATOGRAPHY- FID IN EDARAVONE FORMULATION

K. Sobiya *, K. Nitheesh Kumar, M. Gandhimathi and S. Santhosh Kumar

Department of Pharmaceutical Analysis, Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Coimbatore - 641044, Tamil Nadu, India.

Keywords:

GC-FID, Edaravone, Residual Solvents, Methanol, Validation

Correspondence to Author:
Mrs. K. Sobiya

Assistant Professor,
Department of Pharmaceutical Analysis, Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Coimbatore - 641044, Tamil Nadu, India.

E-mail: rakshadeva28@gmail.com

ABSTRACT: An accurate, precise, robust, and sensitive gas chromatographic method with flame ionization detection (GC-FID) was developed for the determination of residual solvents in edaravone injection formulation. Impurities may be present in final pharmaceutical products. However, their presence and control in drug substances and drug products are regulated according to pharmacopoeial standards and ICH guidelines. Pharmaceutical products generally contain three types of impurities: organic, inorganic, and residual solvents. Residual solvents are volatile organic chemicals used during the manufacture of drug substances or products. In this study, a simple and sensitive GC method was developed and validated for the determination of methanol as a residual solvent in edaravone formulation. Chromatographic separation was performed on an AB-Innowax polar capillary column (30 m × 0.25 mm × 0.25 µm) using nitrogen as carrier gas at a flow rate of 1.7 mL/min. The temperature program was set at 50 ºC (1 min), increased to 70 ºC (2 min), then raised to 200 ºC at 10 ºC/min then raised to 250 ºC and held for 5 min. Injection was performed in split mode (7:1) with 1 µL volume. Methanol retention time was 2.482 min. Injector and detector temperatures were 250 ºC. DMSO was used as solvent. The method showed linearity (0.6–3.0 µg/mL; $r^2 = 0.9994$) and was validated as per ICH Q2(R2) guidelines. Successful application confirmed suitability for methanol determination in edaravone injection because the only one solvent methanol was used during the synthesis of edaravone (HY-B0099).

INTRODUCTION: The residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacturing of drug substances or excipients, or in the preparation of drug products, but are not completely removed by practical manufacturing techniques.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.17(7).2184-90

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(7).2184-90

According to ICH guidelines, residual solvents are divided into three classes from most toxic solvents to solvents with insignificant toxicological effect on human health [1, 2]. Gas chromatography is the primary method for analyzing residual solvents in pharmaceutical substances and products, which involves various sample introduction techniques, such as headspace, solid phase microextraction and direct injection of the analyte.

High selectivity and excellent sensitivity for volatile compounds makes gas chromatography as one of the practical and most popular method for determining the residual solvents in pharmaceutical substances and products [3-6].

Edaravone is an amphiphilic, 2-pyrazolin-5-one derivative that was initially approved in 2001 as a treatment for acute ischemic stroke due to its ability to mitigate the oxidative damage following a stroke event. In 2015 edaravone received approval for the treatment of motor neuron disease (MND), as oxidative damage causes the progressive degeneration of motor neurons in the brain and spinal cord of MND patients. Edaravone exerts its antioxidant effects by free radical scavenging, inhibiting superoxide and hydroxyl radical-dependent lipid peroxidation and thereby preventing damage to brain cells. Edaravone is slightly soluble in water, freely soluble in organic solvents like methanol and acetonitrile. The boiling point of edaravone is 191 ºC [7]. Bhumi Patel *et al* have reported First derivative Spectroscopic Method for Simultaneous Estimation of Edaravone and Argatroban in synthetic mixture [8]. Li jin-lin *et al* have described a HPLC method to determine phenyl hydrazine residues in Edaravone [9]. Gandhimathi. M *et al* have reported a HPTLC method for estimation of Edaravone in human plasma [10]. However, to best of our knowledge, residual solvent analysis in Edaravone and its marketed formulation have not been reported. The present work aims to develop a rapid, accurate and precise gas chromatographic method for quantification of residual solvents in Edaravone formulation using flame ionization detector.

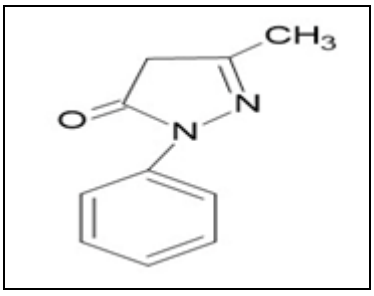

FIG. 1: STRUCTURE OF EDARAVONE [11]

MATERIALS AND METHODS:

Chemical and Reagents: Methanol was purchased from Lobachemie Pvt. Ltd., Mumbai that meets HPLC, DMSO was procured from Isochem Laboratories, Kochi, India. Edaravone injection IP (1.5 mg/ml) was purchased from Sun Pharma Laboratories Ltd., Assam, India.

Instrumentation: Nitrogen was used as the carrier gas for the analysis, which was carried out on a Mayura Analytical LLP gas chromatography model 1100 with an AB-Innowax capillary column and FID detector.

Chromatographic Conditions: The analysis for the determination of residual solvents and its validation was performed on Mayura Analytical LLP gas chromatography equipped with a flame ionization detector (FID). AB-Innowax capillary 30 m length, 0.25mm internal diameter, and 0.25µm film thickness column was used for the validation. The final validated GC-FID method for the separation of residual solvents used a flow rate of 1.7ml/min. Oven temperature was maintained at 50 ºC – hold 1min to 70 ºC at the rate of 10 ºC/min, then increased to 200 ºC at a rate of 10 ºC/min and then raised to 250 ºC finally held for 3 minutes. Injector and detector temperature was maintained at 250 ºC. Nitrogen was used as a carrier gas at a constant flow rate of 1.7ml/min. The injection was carried out in split mode with a split ratio of 7:1 with injection volume of 1µL.

Preparation of Standard Solution: The standard stock solution was prepared by taking 100µl of methanol (0.8g/mL) and making up the volume with DMSO in a 10ml volumetric flask. From the above solution 3.8ml was taken and made up to 10ml with DMSO. The final concentration of standard solution was 3mg/ml of methanol (3000 ppm). (i.e., 3mg/mL equals 3000 ppm which is the daily limit of methanol as residual solvent).

Preparation of Sample Solution: Injection containing 1.5mg/mL of edaravone was selected for the study. The sample solution was prepared by taking 2.5mL of the 1.5mg/mL injection and making it to 10mL with DMSO. The concentration of the sample solution was 375µg/mL.

Spiked Sample Solution: The spiked sample solution was prepared by adding 38µl of methanol to the formulation sample solution. The concentration of methanol was 3mg/ml (3000 ppm). (i.e., 3mg/mL equals 3000 ppm which is the daily limit of methanol as residual solvent), (here 38µl of methanol was added to achieve 3000 ppm).

Method Validation: The validation of the developed method was carried out in terms of specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness as per ICH Q2(R2) guidelines [12, 13].

Specificity: The specificity of the method was investigated by the diluent (DMSO) with the residual solvent (methanol) separation using retention time and assessing the absence of interference from impurities.

Linearity: The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to concentration of the analyte in the sample. The calibration graph was plotted with measured peak area against concentration. From the calibration graph, good linearity was observed for standard (methanol) and spiked sample solution. The slope, intercept and correlation coefficient (r^2) values were calculated.

Detection Limit (DL) and Quantification Limit (QL): The Detection Limit and quantification were calculated from the standard deviation of the y-intercepts and slope of the calibration curves of the standard and spiked sample.

Precision: Precision was studied by analysis of the standard methanol at three different concentration levels injected three times and their %RSD values were calculated.

Accuracy: The accuracy study was carried out by standard addition method to ensure the accuracy and reliability of the proposed method. It was done by addition of known quantities of the standard methanol with the analyzed sample and the content was reanalyzed by the developed method.

System Suitability: System suitability test parameters to be established and validated, parameters like tailing factor, number of theoretical plates (N), capacity factor (k'), resolution (Rs) and retention time (Rt) *etc.* were obtained from the standard.

Robustness: Robustness testing was performed by varying the operational parameters, one at a time, such as flow rate and ramp temperature and results were observed.

RESULT & DISCUSSION:

Method Development: Capillary column contains poly ethylene glycol (PEG) as a stationary phase, which is very important factor that will greatly affect the effectiveness of the chromatographic separation. Hence, the operating temperature of oven and pressure control were optimized. Linear temperature programme was chosen for better quantification. The formulation was initially solubilized in dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). Based on good resolution, solubilizing and volatilizing character, DMSO was selected as the solvent for the formulation.

Chromatographic Condition: It is optimized by adjusting the pressure based on column length and dimensions, using a linear temperature program for residual solvent analysis with DMSO as diluent, and employing a split injector so that only a controlled fraction of the vaporized high-concentration sample entered the column while the remainder was vented out. The complete Chromatographic condition is shown in **Table 1**.

TABLE 1: GC OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Column	AB-Innowax capillary column (30m × 0.25mm × 0.25 µm)
FID detector gas	Hydrogen – 15psi and Air/Oxygen – 5psi
Make up gas	17psi
Carrier gas	Nitrogen
Flow rate	1.7 mL/min
Split ratio	7:1
Detector	FID
Injector and detector temperature	250 ºC
Injection volume	1µl
Oven temperature program	50ºC (1 min), increased to 70ºC (2 min), then raised to 200 ºC at 10ºC/min then raised to 250 ºC and held for 5 min

Quantification of Residual Solvent in Formulation: GC-FID method also answerable for residual solvent analysis for direct injection by LOD, for quantification we have to use spiking method. Injection containing 1.5mg/mL of edaravone was selected for this study. The presence of trace amount of methanol in the formulation was confirmed by injecting the sample (formulation) at the concentration of 375µg/mL. Furtherly the quantification of methanol present in the formulation was carried out by standard addition method i.e., spiking known concentration of standard in the formulation. The calibration data of both the standard and spiked sample were plotted on the same calibration curve as shown in the **Fig. 2** and the unknown concentration of methanol was

determined. The amount of residual solvent quantified in the formulation was 1µg/mL. The edaravone injection used in the study contains total volume of 20 mL, therefore the total amount of residual solvent present in the injection was 20µg, which was found to be within the acceptable limit specified by the ICH guidelines.

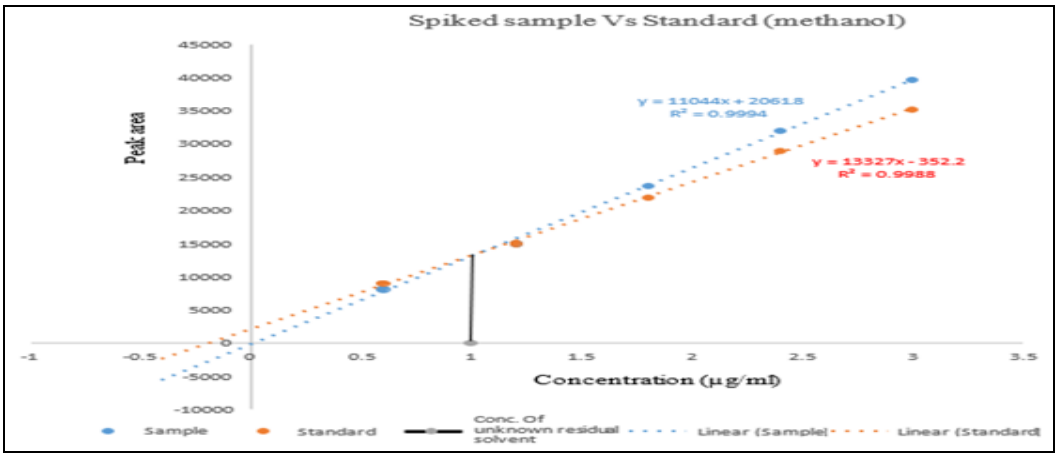

FIG. 2: CALIBRATION GRAPH FOR SPIKED SAMPLE AND STANDARD

Method Validation: The developed method was validated as per ICH guidelines Q2(R2).

Specificity: The methods specificity was evaluated by separating the residual solvent (methanol) from the diluent (DMSO) based on retention time and confirming no interference from impurities as shown in **Fig. 3.** And the chromatogram of blank (DMSO) is shown in **Fig. 4.**

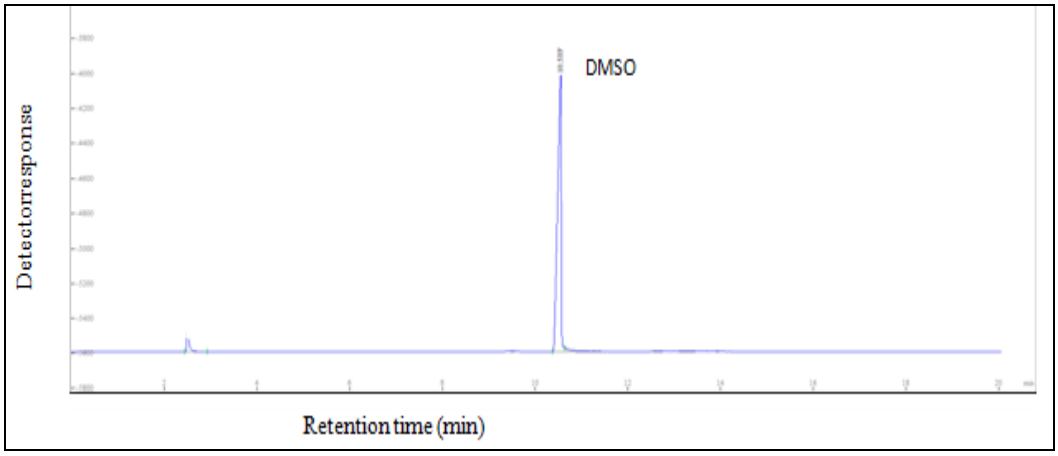

FIG. 3: CHROMATOGRAM FOR METHANOL IN DMSO

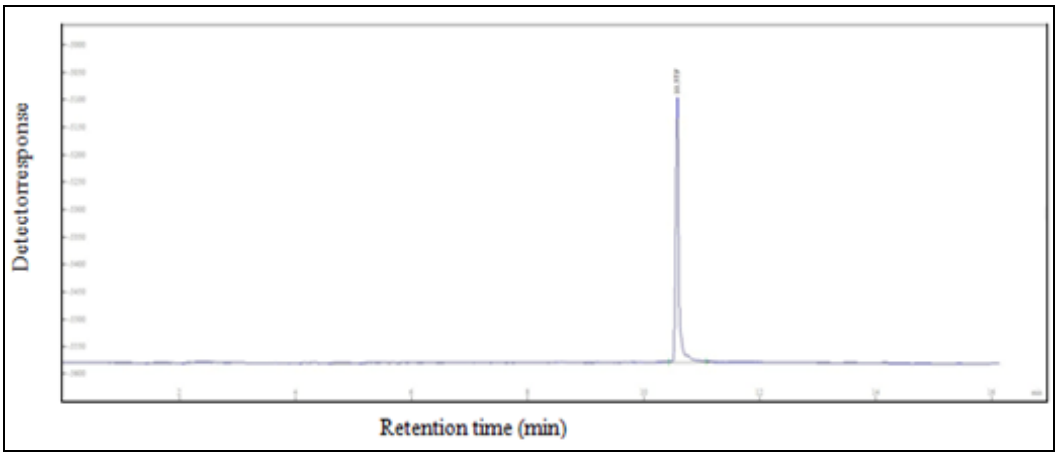

FIG. 4: CHROMATOGRAM OF BLANK (DMSO)

Linearity: The linearity of the method was determined by injecting standard (methanol) and spiked sample solution over the calibration levels of five ranges between 0.6- 3.0 µg/ml. The linear graphs obtained are shown in the **Fig. 2**. The regression data are shown in **Table 2**.

TABLE 2: REGRESSION DATA

	Linearity range (µg/ml)	Linear equation	Correlation coefficient (r^2)
Standard (methanol)	0.6-3	y=11044x+2061.8	0.9994
Spiked sample	0.6-3	y=13327x-352.2	0.9988

Detection Limit (DL) and Quantification Limit (QL): The limit of detection and quantification were calculated from the standard deviation of the y-intercepts and slope of the calibration curves of the standard and spiked sample using the formulae given below,

$$\text{Limit of detection} = 3.3 \times \sigma/S$$

$$\text{Limit of quantification} = 10 \times \sigma/S$$

Where, σ is the standard deviation of the y-intercepts of regression lines S is the slope of the calibration curve.

The detection limit and quantification limit of methanol was found to be 0.295 µg/ml and 0.895 µg/ml.

Precision: Intraday and interday precision was assessed by analyzing standard methanol at three concentration levels, with each injected three times on the same day and the %RSD were shown in **Table 3** and **Table 4.**

TABLE 3: INTRADAY PRECISION DATA

Concentration (µg/ml)	Peak area	%RSD
	13560	
1.2	13138	1.646
	13459	
	20775	
1.8	20120	1.697
	20653	
	27715	
2.4	28435	1.674
	27559	

TABLE 4: INTERDAY PRECISION DATA

Concentration (µg/ml)	Peak area	%RSD
	13450	
1.2	12902	2.904
	13650	
	20665	
1.8	19859	2.097
	20504	
	28702	
2.4	28384	1.377
	27926	

Accuracy: Accuracy was assessed by standard addition method in which known quantities of standard methanol were added to the sample and reanalyzed by the developed method. The %RSD are shown in **Table 5**.

TABLE 5: ACCURACY DATA

Level	Spiked concentration	% Recovery	%RSD
80%	1.44 µg/ml	99.3	1.45
100%	1.8 µg/ml	100.2	1.23
120%	2.16 µg/ml	99.8	0.86

Robustness: Robustness was performed by varying the optimized conditions (±1 °C ratio of column temperature, ±0.05 mL/min flow rate), with chromatograms showing results nearly identical so the developed method was found to be robustness. As shown in the **Table 6.**

TABLE 6: ROBUSTNESS

Conditions	Retention time	Area	Tailing factor
1.65 ml/min	2.484	36832	1.58
1.75 ml/min	2.480	36681	1.57
50 ºC (1 min), increased to 70 ºC (2 min), then raised to 200 ºC at 11 ºC/min then raised to 250 ºC and held for 5 min	2.482	36758	1.58
50 ºC (1 min), increased to 70 ºC (2 min), then raised to 200ºC at 9ºC/min then raised to 250 ºC and held for 5 min	2.482	36752	1.58

Stability: The result of the sample and standard solution stability showed that the solution stored under room temperature conditions at a concentration 3 µg/ml was stable for up to 4 hours as shown in **Table 7.**

TABLE 7: STABILITY

Time (hours)	Standard solution (peak area)	Sample solution (peak area)
1	35820	40027
2	35532	39163
3	35317	39341
4	35129	39592
5	34986	39247

System Suitability: System suitability parameters like tailing factor, number of theoretical plates (N), capacity factor (k') and resolution (Rs), retention time (Rt) etc. were obtained from the standard chromatograms are shown in **Table 8.**

TABLE 8: SYSTEM SUITABILITY RESULTS

Parameters	Methanol	Limits
Retention time	2.482±0.1 min	-
Theoretical plates	14291	N > 2000
Tailing factor	1.58	<2
Resolution	3.8	>2

CONCLUSION: A simple, cost-effective gas chromatographic method for identification and quantification of methanol present in the edaravone formulation. The developed method is precise, specific, accurate and validated according to ICH guidelines. This developed GC-FID method demonstrated precise, economical and commercially viable quantitative technique for residual solvents determination in edaravone drug and formulation which will be advantageous for industrial scale manufacturing. This method can also be applied for analysis of edaravone formulation for the presence of methanol by research laboratories for its simplicity and low cost.

ACKNOWLEDGEMENTS: The authors are thankful to SNR Sons Charitable Trust, Coimbatore-641044, Tamil Nadu, India for providing adequate facilities in our institution for carrying out this work.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). Impurities: guideline for residual solvents Q3C(R8). Step 4 version. Geneva: ICH; 2021.
2. International Conference on Harmonization (ICH). Guidance for industry: Q3C impurities-residual solvents. Geneva: ICH; 1997.
3. Fowlis IA: Gas chromatography. 2nd Ed Chichester: John Willey and Sons 2008; 21-57.
4. Grant DW: Capillary gaschromatography. New Delhi: John Willey and Sons 2009; 10-20: 40-50.
5. Indian Pharmacopoeia Commission, Indian Pharmacopoeia. Ghaziabad: Indian Pharmacopoeia Commission. Gas chromatography 2022; 1: 23.
6. Kaur G and Sharma S: Gas chromatography: a brief review 2017.
7. Ministry of health, labor and welfare. The Japanese Pharmacopoeia, 18th edition, Tokyo: Ministry of health, labor and welfare 2012; 915-917.
8. Patel B: First derivative Spectroscopic Method for Simultaneous Estimation of Edaravone and Argatroban in

synthetic mixture. Asian Journal of Research in Pharmaceutical Sciences 2014; 5(1): 18.
9. Li Jin-lin: Determination of Phenyl hydrazine Residues in Edaravone by HPLC. Journal of China Pharmacy 2008; 31: 2454-5.
10. Gandhimathi M, Kumar MS, Baghla R and Ravi TK: RP-HPTLC Method for the *in-vitro*. Estimation of Edaravone in Human. Plasma. Indo-Pacific Journal of Soft-Power 2010; 72(2).
11. PubChem.Edaravone[Internet].Pubchem.ncbi.nlm.nih.gov. Availablefrom: http://pubchem.ncbi.nlm.nih.gov/compound/Edaravone
12. International Council for Harmonization Validation of analytical procedures: text and methodology Q2(R1). Geneva: ICH 2005; 1–13.
13. Napte B: Analytical method validation for pharmaceuticals. Pharma Growth HUB Vol. 1. Chapter – 10: 170-203.

How to cite this article:
Sobiya K, Kumar KN, Gandhimathi M and Kumar SS: Development and validation of residual solvent determination by gas chromatography- fid in edaravone formulation. Int J Pharm Sci & Res 2026; 17(7): 2184-90. doi: 10.13040/IJPSR.0975-8232.17(7).2184-90.