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Research Article

Development and Validation of Stability-Indicating RP-HPLC Method for the Estimation of Cuminaldehyde

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ABSTRACT
Cumin aldehyde is an herbaceous plant *(Cuminum cyminum L.)* volatile oil that used as a regular spice in kitchen foods. It also has some pharmacological properties such as analgesic, hepato-protective, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antioxidant and anticancer. The key objective of the present study was to develop and validate the RP-HPLC method for the estimation of cumin aldehyde. According to the ICH guidelines, RP-HPLC stability indicating method was used in which reverse phase enable Cosmosil C_{18} column (250 × 4.6 mm, 5µm) used in isocratic mode. For the estimation of cumin aldehyde, sodium sulphate: acetonitrile: methanol (20:73:7 v/v) was used as a mobile phase and it was delivered at flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL and elute was analyzed by a UV detector at 326 nm. Linearity was observed between the concentration range of 20 µg mL^{-1} -140 µg mL^{-1} and the correlation coefficient R^2 value was found to be 0.997 ± 0.5. The method was accurate and recovery was found to be in the range of 98.06 -100.40 %. The limit of detection of cumin aldehyde was found to be 1.04 µg mL^{-1} and limit of quantitation was found to be 3.16 µg mL^{-1}. Cumin aldehyde was subjected to stress conditions including acidic, alkaline, neutral, oxidation, and dry heat degradation. Cumin aldehyde was more sensitive to acidic, dry heat and oxidative degradation and it is stable at alkaline conditions. The method was validated according to ICH guidelines.

Keywords*:* Cumin aldehyde, HPLC method, Validation, Stability-indicating.

INTRODUCTION

Cumin *(Cuminum cyminum L.)* is a small annual and herbaceous plant belonging to the Apiaceae family. It is one of the popular spices regularly used as a flavouring agent. *C. cyminum* seeds have been used for treatment of toothache, dyspepsia, diarrhea, epilepsy and jaundice[1-3]. The proximate composition of the seeds indicates that they contain fixed oil (approximately 10%), protein, cellulose, sugar, mineral elements and volatile oil. Cumin seeds contain volatile oil (1–5%) that imparts the characteristic aroma to the seeds. The applicable part of cumin is the fruit/seed[4]. Cumin is a rich source of iron. Cumin oil and its constituent have been reported to exhibit strong larvicidal and antibacterial activity. Cumin aldehyde (4-isopropylbenzaldehyde) is a yellow-brown pleasant volatile oil. It has molecular formula $C_{10}H1_2O$ and molecular weight 148.21 g/mol. The chemical structure of cumin aldehyde is shown in Fig. 1. Cumin aldehyde is insoluble in water, soluble in ethanol, methanol and other organic solvents. Cumin aldehyde has been reported to lower blood pressure in human sand in different animal models of hypertension and also decrease heart rate. It also has analgesic, hepato-protective, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antioxidant and anticancer properties[5-7].

MATERIALS AND METHODS

Instrumentation

The current research work was performed on a Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with reverse phase C_{18} column (250 mm x internal diameter 4.6 mm x particle size 5µm). Sample injection was done via a 20 µL loop. UV- Visible detector (Shimadzu SPD-20A) was used for detection purpose and

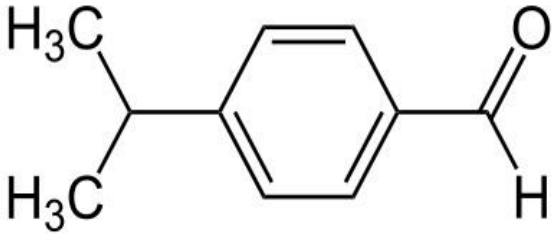

Figure 1: Chemical structure of Cumin aldehyde.

output signal was monitored and integrated by LC-solution

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Table 1: Optimization of RP-HPLC method.

Method parameter	Optimized value
Column	C_{18} (250 mm x 4.6 mm x 5µm) column
Wavelength of detection	326 nm
Mobile phase	Sodium sulphate: acetonitrile: methanol (20: 73: 7 v/v)
Pump mode	Isocratic
Flow rate	1.0 mL min^{-1}
Run time	10 minutes
Volume of injection	20 µL
Temperature	25 ± 2ºC

Table 2: Linear regression data for calibration curves.

Parameters	Cumin aldehyde
Linearity range µg/ml	20-140
r^2 ± SD*	0.997 ± 0.5
Slope ± SD*	5123.4 ± 1037.06
Intercept ± SD*	85010 ± 1302.02
Y= mx + c	Y = 5123.4x + 85010

software.

Reagents and Materials

Cumin aldehyde was purchased from Hi-media laboratories Mumbai, India. HPLC grade acetonitrile and other solvents (Mfg by: Merck Ltd., Mumbai) were used.

Preparation of Mobile Phase

Different ratios of mobile phase compositions were tried to optimize the RP-HPLC parameters but to get a satisfactory separation and good peak symmetry for cumin aldehyde, a mobile phase composition of sodium sulphate, acetonitrile and methanol were used in the ratio of 20: 73: 7 (v/v). The mobile phase was filtered under vacuum through 0.22 µm nylon membrane filter and degassed by using sonicator.

Preparation of standard stock solutions

Standard stock solutions of cumin aldehyde was prepared by dissolving 10 mg in 10 mL of methanol in 10 mL volumetric flask with shaking and then volume was made up to the mark of 10 mL with the methanol to get standard stock solution of 1000 µg mL^{-1} respectively. The stock solution were degassed by using sonicator and filtered through a 0.22 µm nylon membrane filter. From this stock solution different aliquots were prepared.

Preparation of Standard Calibration curves of Cumin aldehyde

A reverse phase 4.6 × 250 mm Cosmosil C_{18} HPLC column with 5 µm (particles) packing was used as a chromatographic column for the estimation of cumin aldehyde. The column oven temperature and the HPLC system were maintained at 25 ± 2ºC. The mobile phase sodium sulphate: acetonitrile: methanol (20: 73: 7 v/v) was delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL. Elute was analyzed by a UV detector. Optimization of RP-HPLC method is shown in Table 1. The standard calibration curve was prepared from the stock solutions (1000 µg mL^{-1}). The different aliquots were pipetted into a series of 10 mL volumetric flasks and the volume was made up to the mark with methanol to obtain a set of solutions of cumin aldehyde having concentration range 20-140 µg mL^{-1} each. 20 µL solutions was injected into HPLC system and peak areas were measured by UV detector set at 326 nm to obtain the calibration curve.

Validation of the Proposed Method

The proposed method was validated According to the International Conference on Harmonization (ICH) guidelines; the proposed method was validated for different parameters like linearity, precision, repeatability and reproducibility, accuracy, limit of detection (LOD) and limit of quantification (LOQ)[8-11].

Range

Range is the interval between upper and lower concentration (amount) of analyze in sample for which it has been demonstrated that the analytical method has suitable level of precision, accuracy and linearity. The linear response was observed at 326 nm over a range of 20-140 µg mL^{-1} for cumin aldehyde and the calibration curve of cumin aldehyde are shown in Fig. 2.

Precision

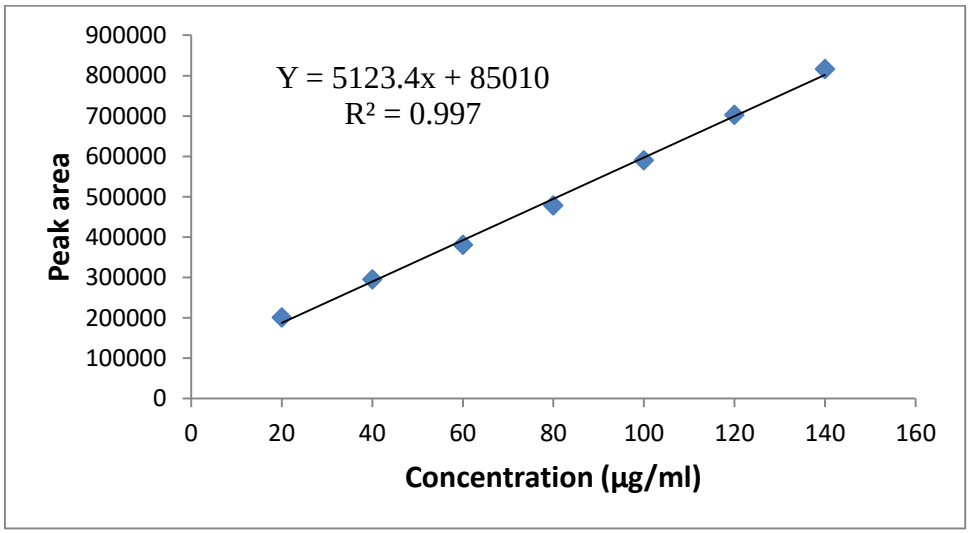

Figure 2: Standard calibration curve of Cumin aldehyde.

Table 3: Repeatability studies (Accuracy).

Precision		Amount (µg mL^{-1})	Area	Mean Area ± SD*	% RSD*
		80	478296		
		80	478012		
Repeatability	(n=6)	80	478176	478181.05 ± 405.07	1.01
		80	478093		
		80	478292		
		80	478219		

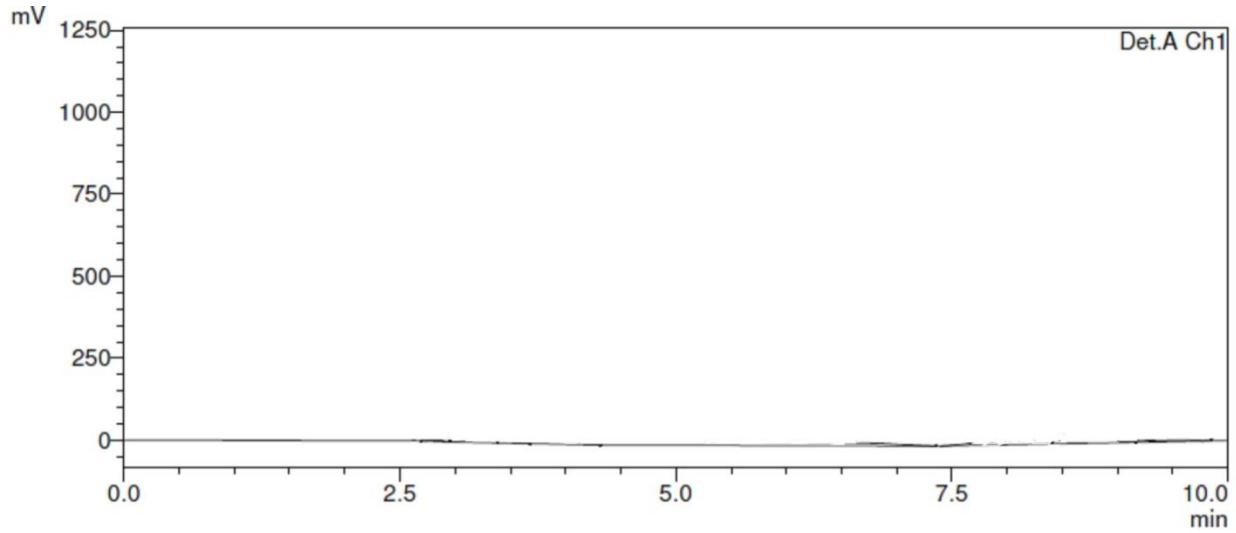

Figure 3: Chromatogram of blank sample.

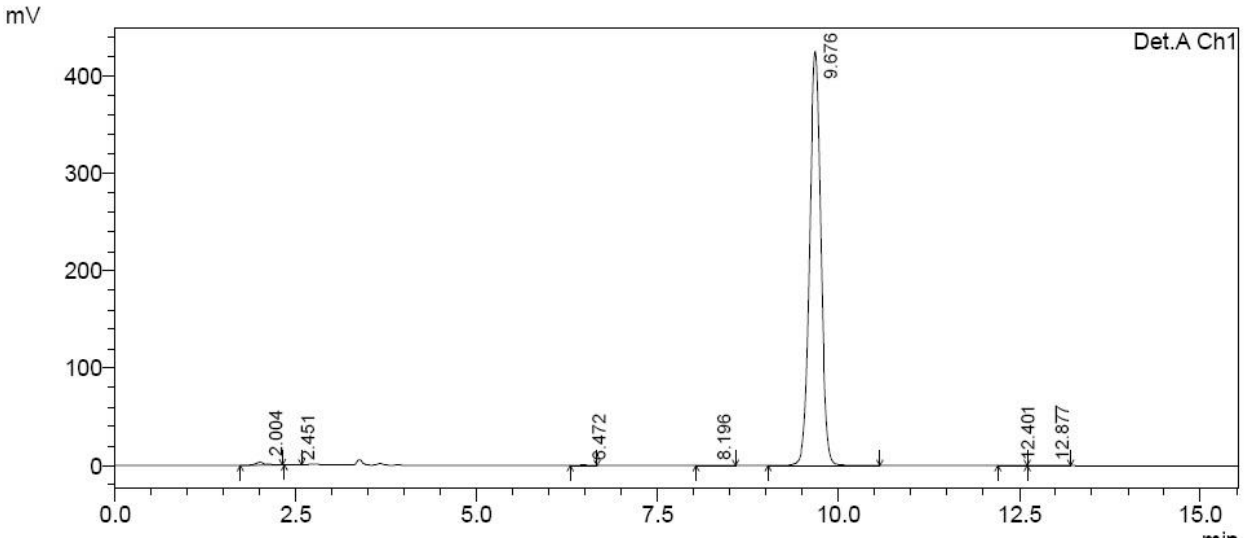

Figure 4: Chromatogram of Cumin aldehyde.

The intra-day and inter-day precision of the method was evaluated by repeatability and intermediate precision studies at three concentration levels (20 µg, 80 µg, and 140 µg). The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of % RSD (relative standard deviation).

Repeatability

Method precision of experiment was performed by preparing the standard solution of cumin aldehyde (80 µg mL^{-1}) for six times without changing the parameters of the proposed method. The results were reported in terms of percent relative standard deviation.

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was determined and analyzed at three different concentrations (20 µg, 80 µg, and 140 µg) on 3 times on the same day and on 3 different days over a period of 1 week.

Accuracy (Recovery study)

The accuracy of the proposed method was determined by calculating the recovery of cumin aldehyde by the standard addition method. Known amounts of standard solutions of cumin aldehyde was added at 80 %, 100 % and 120 % w/w level to pre analyzed sample solutions of cumin aldehyde.

Table 4: Intraday precision studies.

Precision	Amount (µg mL^{-1})	Area	Mean Area ± SD	% RSD*
Intra-day (n=3)	20	201063		
	20	200977	201019.64 ± 129.05	0.281
	20	201019		
	80	478296		
	80	478162	478227.29 ± 881.04	0.590
	80	478224		
	140	816055		
	140	815993	816040.02 ± 307.06	1.025
	140	816071		

Table 5: Inter-day precision studies.

Precision	Amount (µg mL^{-1})	Area	Mean Area ± SD	% RSD*
Inter-day (n=3)	20	201063		
	20	200977	201019.64 ± 129.05	0.281
	20	201019		
	80	478296		
	80	478162	478227.29 ± 881.04	0.590
	80	478224		
	140	816055		
	140	815993	816040.02 ± 307.06	1.025
	140	816071		

Table 6: Results for accuracy studies.

Level (n=3)	Amount Added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% recovery
80%	50	49.026	98.04
		49.071	98.14
		49.010	98.02
100%	75	74.082	98.77
		75.009	100.012
		75.002	100.002
120%	100	100.603	100.603
		100.217	100.217
		100.409	100.409

Table 7: Summary of the Validation Parameters.

Parameters (Unit)	Cumin aldehyde
Linearity range (µg mL^{-1})	20 -140
Correlation Coefficient ± SD, n = 6	0.997 ± 0.5
Precision (%RSD)	
Inter day % RSD	1.025
Intraday % RSD	0.281
Recovery (%), n=3	100.40
Limit of detection (µg mL^{-1})	1.04
Limit of quantitation (µg mL^{-1})	3.16

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of cumin aldehyde was derived using following equation as per ICH guidelines.

LOD = 3.3 x σ/S

LOQ = 10 x σ/S

Where, σ = Standard deviation of the y-intercept

S = Mean slope of the calibration curve.

Force Degradation Studies

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and/ or validate the stability indicating power of the analytical procedures used.

Forced degradation is a powerful tool used routinely in pharmaceutical development in order to develop the stability indicating method that leads to quality stability data and to understand the degradation pathways of the drug substances and drug products. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays. Cumin aldehyde was stressed under various conditions (acid, base, neutral, oxidation and dry heat) to perform forced degradation studies. The peaks of degraded products were well separated from the analyze peak with good resolution which indicates that the developed method is stability indicating.

Acid Induced Degradation

Stock solution (10 mL) of cumin aldehyde (1000 µg mL^{-1}) was treated with 1 mL of 0.1M HCl and this reaction mixture was refluxed at 70ºC for about 1 h. After 1 h, the solution was neutralized using 1 mL of 0.1M NaOH solution and then injected into HPLC system.

Base Induced Degradation

Stock solution (10 mL) of cumin aldehyde (1000 µg mL^{-1}) was treated with 1 mL of 0.1M NaOH and this reaction mixture was refluxed at 70ºC for about 1 h. After 1 h, the solution was neutralized using 1 mL of 0.1M HCl solution and then injected into HPLC system.

Oxidative Degradation

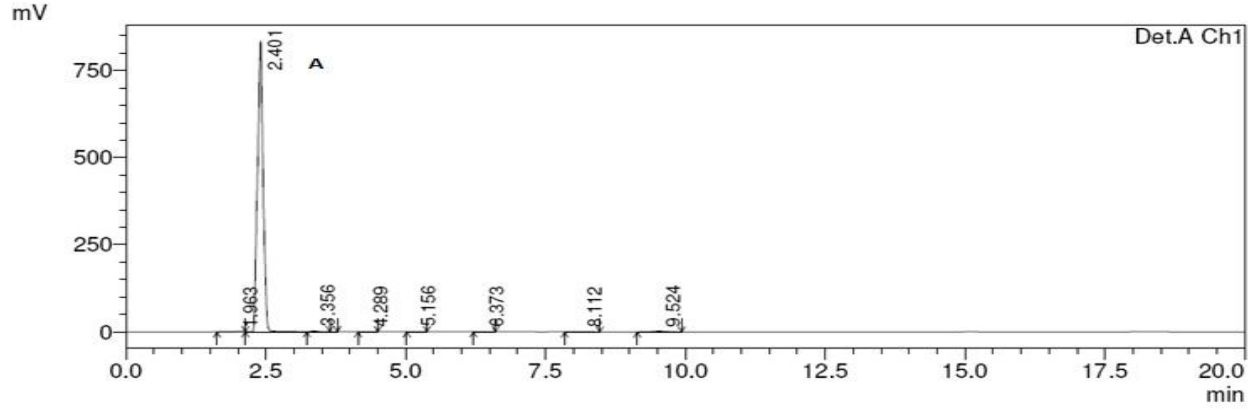

Figure 5: Chromatogram of Acid degradation of Cumin aldehyde.

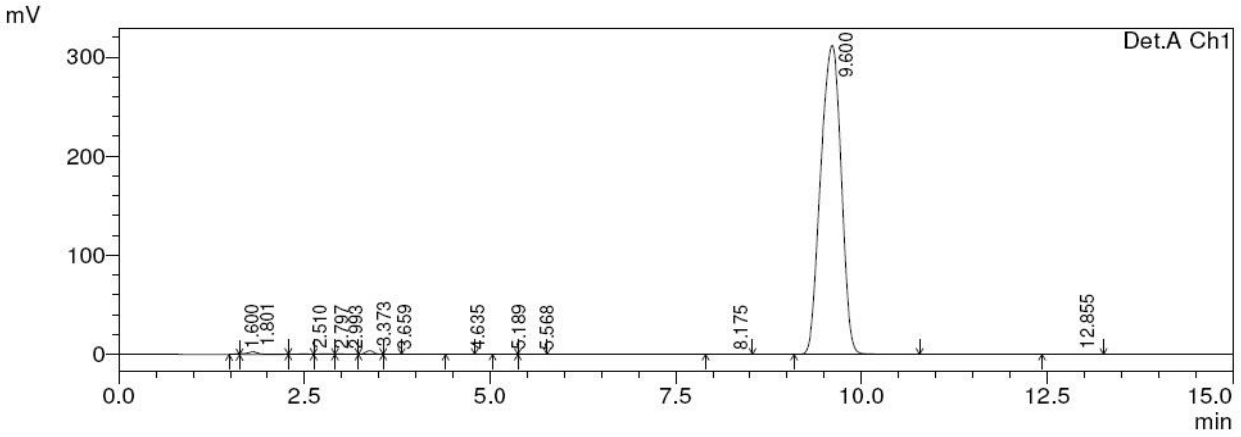

Figure 6: Chromatogram of Alkaline degradation of Cumin aldehyde.

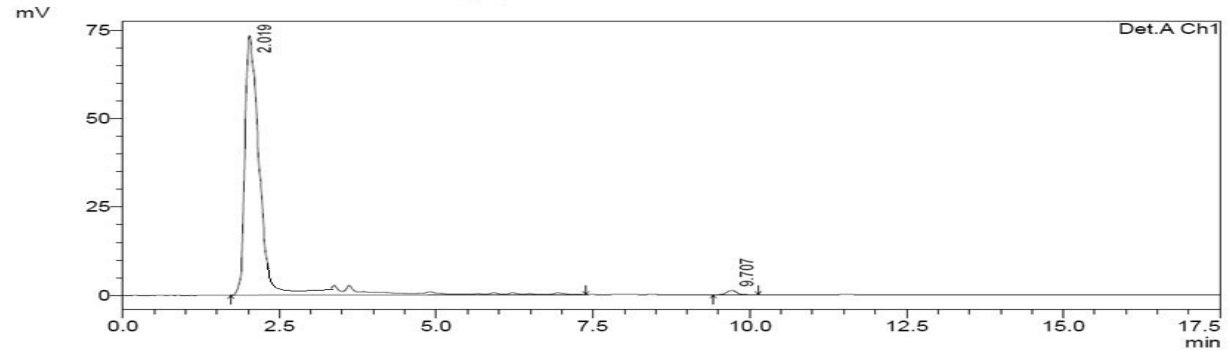

Figure 7: Chromatogram of Oxidative Degradation of Cumin aldehyde.

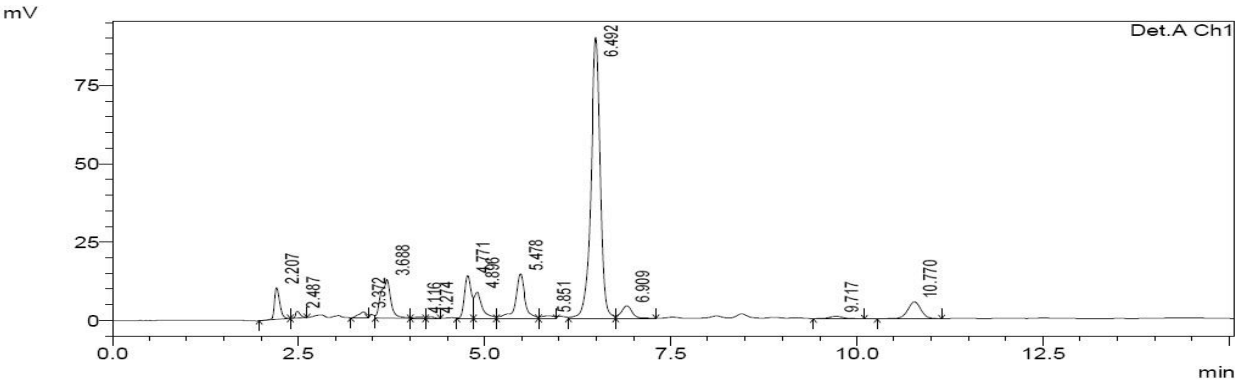

Figure 8: Chromatogram of Dry heat Degradation of Cumin aldehyde.

Stock solution (10 mL) of cumin aldehyde (1000 µg mL^{-1}) was transferred to separate round bottom flask, to this 10 ml of 3% hydrogen peroxide was added and this reaction mixture was kept for 1 h at 70°C. Sample was diluted and mixed well and injected into HPLC system.

Dry Heat Degradation

Accurately weighed 10 mg of cumin aldehyde spread in a petri dish and kept in oven at 105°C for about 8 h and then cumin aldehyde was diluted with mobile and filter through 0.45µm filter and injected into HPLC system.

RESULTS AND DISCUSSION

Several mobile phase compositions were tried to validate RP-HPLC method and to estimate cumin aldehyde. A satisfactory separation, good peak symmetry, better reproducibility and repeatability of cumin aldehyde were obtained with a mobile phase comprising of Sodium sulphate: acetonitrile: methanol (20: 73: 7 v/v) at a flow rate of 1.0 mL min^{-1}. Quantification was achieved with UV Visible detector at 326 nm.

In proposed validation methods, retention time of blank (Fig. 3) and cumin aldehyde was recorded and it was found at 9.67 min as shown in Fig. 4.

The calibration graphs for cumin aldehyde was constructed by plotting the area versus their corresponding concentrations, good linearity was found over the range of 20-140 µg mL^{-1} for cumin aldehyde with co-efficient of correlation (R^2) = 0.997 ± 0.5. The regression characteristics and validation parameters are reported in Table 2. The repeatability, intra-day and inter-day precision of cumin aldehyde are summarized in Table 3, Table 4 and Table 5, respectively. The mean recoveries were found 100.40 ± 0.05 % respectively for cumin aldehyde by the standard addition method shown in Table 6. The limit of detection of cumin aldehyde was found to be 1.04 µg mL^{-1} and limit of quantitation was found to be 3.16 µg mL^{-1}. The summary of proposed validation parameters of cumin aldehyde was shown in Table 7. The stress or forced degradation study of cumin aldehyde was evaluated by HPLC and shown in Fig. 5, 6, 7, and Fig. 8 respectively. Cumin aldehyde was more sensitive to acidic, dry heat and oxidative degradation. It was found to be stable under the alkaline condition and results are summarized in Table 8.

Table 8: Summary of forced degradation studies of Cumin aldehyde.

Stress conditions	Time (h) and Temperature (°C)	Amount of cumin aldehyde degraded (%)	Amount of cumin aldehyde recovered (%)
Acid (0.1 M HCL)	1 h at 70°C	61.53	38.47
Alkali (0.1M NaOH)	1 h at 70°C	9.16	90.84
Oxidative H_2O_2 (3 %)	1 h at 70°C	69.03	30.97
Dry heat	8 h at 105°C	32.82	67.18

CONCLUSION

The present research work describes a simple and sensitive RP-HPLC method for the estimation of cumin aldehyde. Various ratio of Sodium sulphate: acetonitrile: methanol mobile phase were tried and found that (20: 73: 7 v/v) proportion of the mobile phase favored the separation and elution of cumin aldehyde. The results of accuracy and precision were in good agreement with the threshold limits of validation parameters as per ICH guidelines.

The intra-day and inter-day variability and accuracy results of cumin aldehyde were found in acceptable limit. Simplicity of the method, economical nature and low limit of detection and quantification makes the method superior to the other reported HPLC methods. The developed method was applied for the stability studies of cumin aldehyde.

LIST OF ABBREVIATIONS

%- Percent, λ_{max} - Wavelength of maximum absorbance, °C- Degree Celsius, Cm- centimeter, µg- Micro gram, Hour-h, LOD - limit of detection , LOQ - limit of quantification, mg- Milligram, Min – Minute, mL – Milliliter, µL – Microliter, r^2 - Regression coefficient, RP-HPLC- Reverse phase-high performance liquid Chromatography, rpm - Revolutions per minute, SD- Standard deviation, t_R - Retention time, UV- Ultra Violet

CONFLICT OF INTEREST

The authors declared that there are no conflicts of interest.

REFERENCES

1. AlDisi SS, Anwar MA, Eid AH. Anti-hypertensive Herbs and their Mechanisms of Action: Part-I. Frontiers in Pharmacology 2016; 6:323.
2. Rebey IB, Karoui IJ, SellamiIH, et al. Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum L.*) seeds. Industrial Crops and Products 2012; 36: 238–245.
3. Johri RK. Cuminum cyminum and Carum carvi: An update. Pharmacognosy Reviews 2011; 5 (9):63-72.
4. Singh RP, Gangadharappa HV, Mruthunjaya K. *Cuminum cyminum* – A Popular Spice: An Updated Review. Pharmacog J 2017; 9(1): 73-82.
5. Hassan El-Ghorab A, Nauman M, Anjum FM, Hussain S, Nadeem M. A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). J. Agric. Food Chem 2010; 58: 8231–8237.
6. Rebey IB, Jabri-Karoui I, Hamrouni-Sellami I, Bourgou S, Limam F, Marzouk B. Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Industrial Crops and Products 2012; 36: 238–245.
7. Bisceglie F, Pinelli S, Alinovi R, Goldoni M, Mutti A, Camerini A, Piola L, Tarasconi P, Pelosi G. Cinnamaldehyde and cuminaldehyde

thiosemicarbazones and their copper (II) and nickel (II) complexes: A study to understand their biological activity. Journal of Inorganic Biochemistry 2014; 140: 111–125.
8. Yousif Al-Hashemi FH. Chromatographic separation and identification of some volatile oils, organic acids and phenols from the seeds of *cuminum cyminum* growing in Iraq. IJRRAS 2014; 19 (1): 80-90.
9. Morshedi D, Aliakbari F, Tayaranian-Marvian A, Fassihi A, Pan-Montojo F, P´erez-S´anchez H. Cumin aldehyde as the major component of *Cuminum cyminum*, a natural aldehyde with inhibitory effect on alpha-syncline fibrillation and cytotoxicity. Journal of Food Science 2015. DOI: 10.1111/1750-3841.13016.
10. Kulkarni S, Sane A, Bhise K, Patil A, Dhamole P, Desai S. Development of extraction methods and quantification of safranal by high performance liquid chromatography from *cuminum cyminum* L. and studying its antimicrobial properties. IPCBEE 2014; 64. DOI: 10.7763.
11. Chen Q, Gan Z, Zhao J, Wang Y, Zhang S, Li J, Ni Y. *In vitro* comparison of antioxidant capacity of cumin (*Cuminum cyminum* L.) oils and their main components. LWT - Food Science and Technology 2014; 55; 632-637.