ORIGINAL RESEARCH

A SENSITIVE ISOCRATIC REVERSED PHASE HPLC METHOD FOR ASSAY DETERMINATION OF 4-IMIDAZOLECARBOXALDEHYDE

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ABSTRACT:

A reversed-phase high performance liquid chromatography method was developed for the assay determination of 4-imidazolecarboxaldehyde.An isocratic reversed phase high performance liquid chromatography method with good separation was achieved on Chemcobond 5-ODS-H, (250mm× 4.6 mm,5µ) as stationary phase and mobile phase as buffer solution and acetonitrile (70:30). The flow rate of the mobile phase was 0.8 ml/min and the total elution time including the column re-equilibration was approximately 10 min. The UV detection wavelength was 260 nm, injection volume was 5µl and experiments were conducted at 30 °C temperature. The calibration curve was linear r^2 = 0.999 over 4-imidazolecarboxaldehyde concentrations ranging from 100 µg to 300 µg/ml (n=6). The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the linearity range respectively. The method has recovery of above 98% and method precision RSD of below 1.5%. The validated method was successfully applied for the assay determination of 4-imidazolecarboxaldehyde in bulk manufacturing for pharmaceutical industry.

KEYWORDS: 4-imidazolecarboxaldehyde, Isocratic, Assay, intermediate, UV-detection.

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1. INTRODUCTION:

4-ICA is a 4-formyl derivative of imidazole used in the preparation of C17, 20-lyase inhibitor for the treatment of androgen-dependent prostate cancer. 4-ICA is the intermediate material for some biologically active compounds such as antimalarial drug. 4-ICA is also used as pesticide raw material for synergist, insecticides and fungicides of boric acid agent; In addition, it is also used as raw material of urea-formaldehyde resin curing agent, photographic medicine, adhesive, coating, vulcanizer, antistatic agent, etc. 4-ICA is used for preparation of organic synthetic intermediates, drugs and pesticides[1-10]. It also used as analytical reagent. Oxidation of 1-histidine by tert-butylhydroperoxide[2], pharmaceutical and cosmetic compositions, comprising urocainic acid derivatives as radical scavengers or antioxidants, resign and synthesis of potent non-peptidic farnesyl transferase inhibitors based on a terphenyl scaffoid [4-5]. An assay method development is important to identify, determine the purity and control the quality of a compound. Assay determination of 4-ICA was performed by HPLC with UV detection. The accurate calculate assay by the method is used in synthesis drug. The method was intended to be simple and also posses the essential, LOQ and LOD, linearity, precision, recovery and accuracy, robustness, solution stability for all studied compounds. Thus, it would be of particular interest to develop and validate an isocratic, reversed-phase high performance liquid chromatography method with UV-detector according to ICH guidelines[11-12] for the assay determination and routine analysis to check purity and presence of related substance in 4-ICA in pure product.

2. MATERIALS AND METHODS

2.1: Instrumentation and software: The HPLC system from Agilent HPLC 1100 Series with Variable Wavelength Detector (VWD), Diol Array Detector (DAD) Microprocessor, quaternary pump, Agilent Technologies international sarl, 1100 series, auto sample, micro auto sample, preparative auto sample and thermostatic column compartment were used for this entire study. Chromatographic separation was achieved on Chemcobond 5-ODS-H, (250mm× 4.6 mm,5µ) as stationary phase .

***2.2 :Chemicals and reagents*:** Acetonitrile (HPLC Grade), Potassium dihydrogen phosphate (KH_2PO_4), were purchase from SD-Fine Chemical Ltd., India. Milli-Q water was used for the experiments.

2.3: Standards and Sample Materials: The Pharmaceutical grade 4-ICA (99.9%), were gifted by Shri Hari Ohm Chemicals & Pharmaceutical Company, India. Other chemicals used for the analysis (AR grade) were procured from Spectrochem & SD Fine Ltd., Ahmadabad, India.

2.4: Details of Method: The chromatographic Quantitative (Assay) analysis was performed on Chemcobond 5-ODS-H, (250mm× 4.6 mm, 5µ) as stationary phase. Mobile phase as (preparation of buffer solution dissolved 6.5 gm of KH_2PO_4 in 1000 ml milli-Q water and filter it through 0.45µ membrane filter) and acetonitrile was mixed in the ratio of (70:30 ml). The isocratic flow rate was 0.8 ml/min, injection volume was 5µl and system detection was performed at 260 nm .

2.5: Preparation of solutions:

Standard preparation

Standard stock solutions of 4-ICA (1.0 mg/ml) was prepared by direct weighing of standard substance with dilute dissolution in Milli-Q water. The standards for the calibration curve were prepared in volumetric flasks (10 ml) using standard stock solutions by serial dilution to yield concentration of 100 µg to 300 µg/mL, (n=6).

Assay preparation

Vials of 4-ICA for Injection (0.30 mg) were separately dissolved in 1 ml Milli-Q water for injection to obtain a concentration of 300 µg/ml..All determinations were conducted in triplicate.

2.6: Method Validation

Validation of the developed method for the determination of 4-ICA was performed according to the ICH guidelines "Validation of analytical procedures: text and Methodology Q2B(R), Q2B(R)"[11-12] with standards and bulk drug , The system suitability along with method selectivity, specificity, linearity, range, precision (repeatability) and intermediate precision, accuracy, limits of detection and quantification, is short term and long term stability of the analytes in the prepared in the prepared solutions were demonstrated.

2.7 : Validation Tests Study:

2.7.1: Linearity and Calibration curve, Limit of Detection and Limit of Quantification:

Standards for linearity at five calibration curves consisting of the validation was performed on three separate days, with seven standard calibration level lines on each day.

Study design: Determine the LOD and LOQ was determined as per the following sequence. The data was evaluated and a linearity plot from the level was drawn to detect 150.0 % of specification limit. Procedure of injected blank (diluent), System suitability solution for retention time conformation and concentration solution level of 150% (300 µg/ ml) to 50% (100µg/ml) for Linearity. Assay results for 4-ICA are included in Table 1.

The limit of quantification (LOQ) and limit of detection (LOD) were estimated using the following equations.

LOQ=10 б /s and LOD=3.3 б /s

Where 'б' is the standard deviation of intercept and 's' is the slope of the calibration curve

The Relative Standard Deviation (RSD) was estimated using the following equation.

RSD = (100×SD)/AVG

Where 'AVG' is the number of results in AVERAGE result, 'SD' is the Standard Deviation.

2.7.2: Precision and Recovery (Accuracy)

The intra-day and inter-day precision were determined by analyzing three replicates of quality control samples at concentrations of 50,100,150 µg/ml (4-ICA) on the same day and three times on three days, respectively. The intermediate precision and method precision was evaluated by the relative standard deviation (RSD) and acceptable range of RSD was not more than 1.5 %. The accuracy was assessed by the method that was calculated by comparing the determined concentrations.

Calculation :

The % Assay w/w (on an anhydrous basis) of 4-ICA was calculated by following formula:

$$\%\ \text{Assay w/w (on an anhydrous basis)} = \frac{At}{As} X \frac{Ws}{Wt} X \frac{100}{100\text{-}\%\text{Water}} X\ P$$

Where,

At= Mean Peak area of 4-ICA in Test sample solution.

As= Mean Peak area of 4-ICA in Standard sample solution.

Ws= Weight of 4-ICA Standard in Standard sample in mg

Wt= Weight of 4-ICA Test sample in mg.

P= % Potency of 4-ICA Standard.

2.7.3: Stability

Stability of the lab samples were evaluated after short-term storage (at RT for Initial, 12,24,36,48 hrs), after long-term storage (at -5^{0}C for Initial, 12,24,36,48 hrs). The stability of standard stock solution was also evaluated.

Statistical analysis

The data were submitted to statistical analysis using Excel software and SPSS.

3. RESULTS AND DISCUSSION

3.1 : Optimization of chromatographic conditions for HPLC-UV assay of 4-ICA intermediates.

4-ICA peak was monitored at different wavelength (215,225,235,240 and 260nm)[1] and a compromise of 260 nm was selected as the optimum wavelength for HPLC analysis because it maximizes the peak symmetry of 4-ICA at that wavelength While giving a flat baseline Fig.1. Furthermore the UV-spectra of aqueous solution of 4-ICA showed the maximum absorption at wavelength 260 nm.

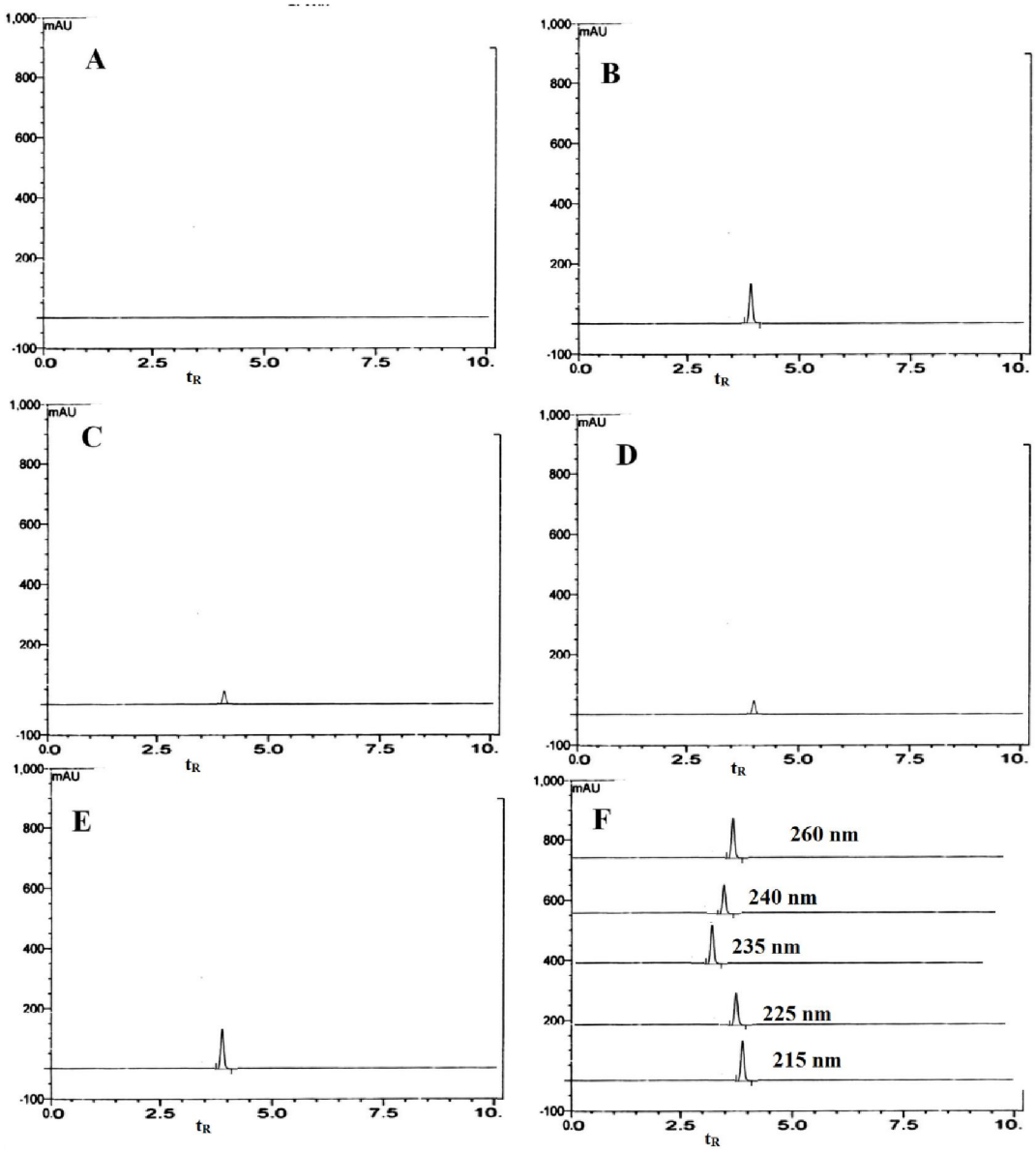

Figure 1: Chromatograph for method development (A) Blank, (B) reference of 4-ICA solution,(C) (LOD solution,(D)LOQ solution, (E) test solution of 4-ICA (F) Test solution deferent UV nm value.

3.2: Method Validation.

Method validation was performed as per ICH guideline Q2A and Q2B includes several parameters like precision, linearity, accuracy, robustness, LOD and LOQ. These parameters are studied systematically for both Assay and purity determination on HPLC [17-18] A typical chromatogram of the separation of the results in tablets are included in Table 1. To control the qulity of the compound quantitative analysis is required so for quantitative analysis method development for assay determination is important. We than developed the HPLC method with UV detection accurate Assay determination. The developed method was validated as per ICH guideline. The method was intended to be sample and also possessed the essential parameter such as LOD , LOQ and linearity.

3.2.1: Linearity and range on LC-UV

All calibration curves for 4-ICA presented a good coefficient of determination (R^2) 0.999 A lack-of-fit test was performed for all calibration curves and the calculated R^2-values of the

representative curves, system suitability parameter was complied. The correlation coefficient would not be less than 0.98. Y- Intercept ≤25% referred to the calculated response of the x-value corresponding to the concentration of the specification limit. Representative linearity and range results in tablets are included in Table 1, Table 2 and linearity chart is shown in fig.2.

Table 1. Concentration solution level of 150% (300 µg/ ml) to 50% (100µg/ml) for Linearity and Assay results for 4-ICA.

Concentration level (%)	Final concentration (µg/ml)	At	As	Ws	Wt	P	% of water	Assay
50	100	221380	4435275	2	0.1	99.9	0.2	99.92
80	160	353844	4435275	2	0.16	99.9	0.2	99.82
100	200	442998	4435275	2	0.2	99.9	0.2	99.98
120	240	531982	4435275	2	0.24	99.9	0.2	100.05
150	300	663954	4435275	2	0.3	99.9	0.2	99.89

At= Mean Peak area of 4-ICA in Test sample solution. As= Mean Peak area of 4-ICA in Standard sample solution. Ws= Weight of 4-ICA Standard in Standard sample in mg. Wt= Weight of 4-ICA Test sample in mg. P= % Potency of 4-ICA Standard.

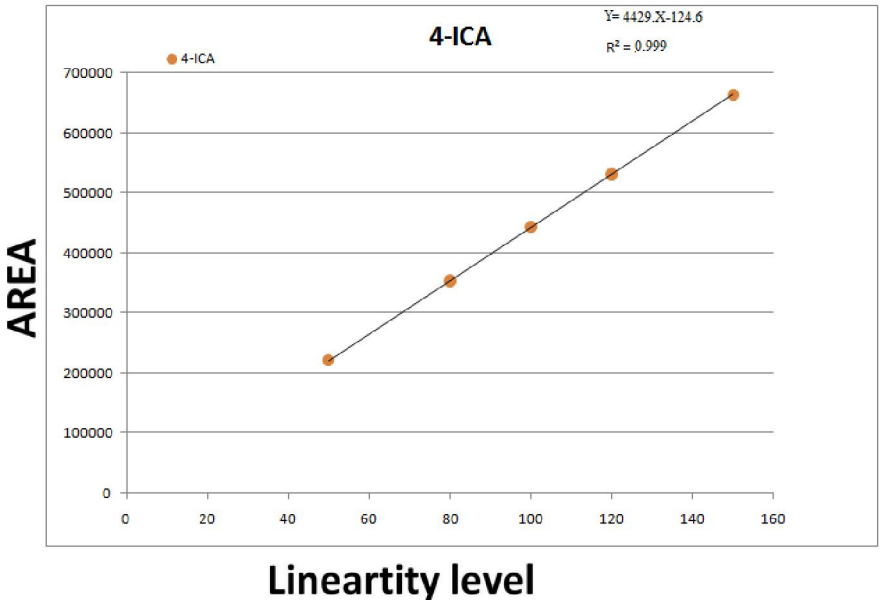

Figure 2: Plot of Area verses linearity level (50% to 150%) and confidence level for 4-ICA correlation (R^2) 0.999, Y- Intercept 4429.×-124.6 and .The correlation coefficient would not be less than 0.995. Y- Intercept ≤25% referred to the calculated response of the x-value corresponding to the concentration(linearity level) of the specification limit.

Table 2. Linearity, t_R(retention time), Coefficient of determination

(R^2), Y-intercept, LOD and LOQ data for proposed Method of 4-ICA

Substance	Range (µg/ml)	t_R(retention time)	Rt_R(relative time)	Coefficient of determination (R^2)	Y-intercept	LOQ	LOD
4-ICA	100-300	3.9	1	0.999	4429.×-124.6	0.26	0.08

3.2.2: Precision and Stability on LC -UV

The results obtained for intermediate precision and intraday precisions are presented in table 3. Method precision has a RSD below 1.5 % for Intraday and 2.0 % for interday precision, which comply with the acceptance criteria proposed (RSD below 2 %). Results for 4-ICA are included in Table 3.

Table 3. Summary of intermediate Precision and Method Precision

Set No.	Method Precision %	Set No.	Method Precision %	Set No.	Intermediate Precision %	Set No.	Intermediate Precision %
1	99.95	4	100.06	1	100.35	5	101.11
2	100.46	5	99.55	2	100.89	6	100.04
3	99.93	6	99.72	3	100.88	7	100.73
Mean	100.11		99.77		100.70		100.62
Stdv %	0.24		0.21		0.25		0.44
RSD %	0.24		0.21		0.25		0.44

Stability

Short-term stability: Lab samples were kept at room temperature for initial, 12,24,36,48 hrs and analyzed. The accuracy for samples RSD % ranged from 0.38 to 94% after short term stability testing.

Long-term stability: Lab samples were kept at-5^0C for Initial, 12,24,36,48 hrs. The samples were thawed at room temperature and analyzed. The accuracy for samples RSD % ranged from 0.41 to 0.80 % after long term stability testing. Results for 4-ICA are included in Table 4.

3.2.3: Accuracy

Accuracy was determined by evaluating the recovery of analyte. The percent recovery between theoretical (C_{theo}) and calculated (C_{calc}) concentration was derived by the following equation.

Recovery (%) = (C_{theo}/C_{calc}) ×100

The accuracy of the lab samples ranged from 98 to 100 %, indicating excellent accuracy of the proposed HPLC method Table 4.

Table 4. Summary of Stability Data for Proposed Method of 4-ICA

Solution stability for standard and test preparation at ambient temperature					
	Initial	After 12 hours	After 24 hours	After 36 hours	After 48 hours
Replicte	Standard Area	Standard Area	Standard Area	Standard Area	Standard Area
1	4425158	4435284	4407487	4497258	4478665
2	4402569	4412557	4445442	4425654	4402566
3	4412947	4415672	4402551	4414524	4412627
4	4426255	4467287	4487468	4488527	4473452
5	4447492	4415675	4415251	4425656	4494583
Mean	4422884	4429295	4431640	4450324	4452379
Stdv	16833.98	23078.73	35374.42	39246.11	41766.49
% RSD	0.38	0.52	0.80	0.88	0.94
Solution stability for standard and test preparation at -5^{0}C temperature					
	Initial	After 12 hours	After 24 hours	After 36 hours	After 48 hours
Replicte	Standard Area	Standard Area	Standard Area	Standard Area	Standard Area
1	4425256	4476269	4473651	4489567	4486867
2	4435429	4461698	4493653	4433856	4413554
3	4459455	4425947	4411527	4426849	4425269
4	4452898	4462584	4453846	4421591	4410921
5	4415751	4436546	4434488	4401359	4473859
Mean	4437758	4452609	4453433	4434644	4442094
Stdv	18344.02	20680.84	32177.68	33000.72	35647.60
% RSD	0.41	0.46	0.72	0.74	0.80

4. CONCLUSION:

The reversed phase HPLC-UV method was developed and validated as per ICH guidelines. This method is simple, precise, rapid, accurate, linear and selective for the assay determination of 4-ICA in the synthesis drug intermediate. The measured signal was shown to be precise, accurate and linear over the concentration range tested (100-300µg/ml) with a correlation coefficient better than 0.999. This method can be useful for routine analysis and quality control with limit of specification of 4-ICA in pharmaceutical industry.

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7. ABBREVIATIONS:

4-ICA : 4-imidazolecarboxaldehyde
API: Active pharmaceutical ingredient;
ICH: International conference of Harmonization;
LOQ: limit of Quantification;
RT: Room temperature;
t_R: Retention time;
Rt_R: Relative time;
R_S: Resolution;
R^2: Coefficient of determination

CONFLICT OF INTEREST REPORTED: NIL; SOURCE OF FUNDING: NONE REPORTED