

## Analytical method development and method validation for the Assay of 5-chloro-2,3-dihydro-1H-inden-1-one by gas chromatography (GC)

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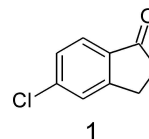
**Abstract :** Analytical procedure for method development and method validation of 5-chloro-2,3-dihydro-1H-inden-1-one to demonstrate its intended purpose characteristics applicable to identification, control of impurities and assay results.

**(Keywords:** GC, Method development, Validation, Assay)

### Introduction

GC method development and validation is a sensitive, accurate, reproducible, quantitative and versatile tool for the analysis of API Intermediate in pharmaceutical industry. This technique plays an important role in analysis of 5-chloro-2,3-dihydro-1H-inden-1-one (**1**) intermediate which being used in anticonvulsant anticholinergics and diary sulfonylureas, having potential activity against solid tumors drugs and pharmaceutical products<sup>1-3</sup>. However the use of GC is limited to volatile thermally stable compounds or the molecules that may undergo derivatization reactions to thermally stable products. 5-chloro-1-indanone GC assay method provided by the invention avoids environmental pollution problem caused by strong acid titration method and treatment of large amount of wastewater. Literature survey revealed that there are various methods available in the literature using HPLC, UV. Therefore the aim of the study was to develop simple, rapid, accurate, reproducible and economic assay by GC method for Simultaneous estimation of 5-chloro-2,3-dihydro-1H-inden-1-one. Assay method development and validation

play important role in the synthesis of 5-chloro-1-indanone during reaction monitoring and manufacture of pharmaceuticals. GC Method development<sup>4-5</sup> is the process of proving that an analytical method is acceptable for use to measure the concentration of a product in a specific compound<sup>6-7</sup>. Which allow simplified procedures to be employed to verify that an analysis is procedure, accurately and consistently will deliver reliable measurement of an active ingredient in a compound's preparation. The method validation is essential for analytical method development and tested extensively for specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, robustness and system suitability.



5-chloro-2,3-dihydro-1H-inden-1-one

### Results and Discussion:

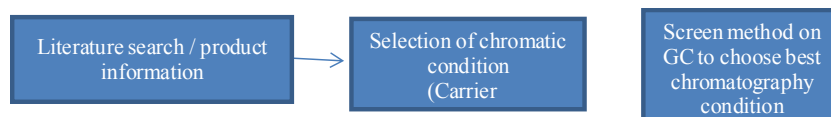
#### Aim of study:

Aimed to develop Analytical procedure for method development and Assay method validation by gas chromatography of 5-chloro-2,3-dihydro-1H-inden-1-one for an intended application. There are many more methods already known in literature which are suffering with various issues like use of critical reagents and tedious chemical titration method. It can be noticed that although more

and more researchers choose to use the chiral column, the costs of the columns can also be prohibitive and mobile phase composition can be rather limited 5c-5d for the various indone derivatives 7-10, 11. Many more method quoted in 12, 13, 14, 15-20 Our aim to develop the comparatively easy and economical reproducible method development and its validation as per

ICH. But no specific gas chromatographic methods has been reported till date, for the quantitative determination of 5-chloro-2,3-dihydro-1H-inden-1-one. This is the in the continuation of our recent research program published in recent article 2.

### 1.1 GC Method development flow chart



## 2. Chromatography condition:

**2.1 Selection of Column:** The selection of column is based on chemical and physical properties of material, changing the chemical feature of the stationary phase results its physical properties of material. 12. The various column attempted for the assay method development of 5-chloro-2,3-dihydro-1H-inden-1-one. 30m x 0.32mm ID x 1.8µm, DB-624 capillary column : Separation didn't found suitable with respect to raw material 3-(3-chlorophenyl)propionic acid and product 5-chloro-2,3-dihydro-1H-inden-1-one, 25 m x 0.32mm ID x 0.25 µm, BP-1 capillary column : Peak merged, Separation didn't found suitable, 30 m x 0.32mm ID x 1.0 µm, DB -1 capillary column : Separation is good between, peak shape is sharp between raw material 3-(3-chlorophenyl)propionic acid and product 5-chloro-2,3-dihydro-1H-inden-1-one

**2.2 Selection of Carrier Gas:** Carrier gases are used to move the solutes through the column for best suited 13. Hydrogen, Helium and nitrogen are common carrier gas. During method development nitrogen gas used as a carrier gas due to following reasons. a. Dryness b. Inertness c. cost Optimization of Column oven temperature program: To improve the resolution of raw material and product eluting peaks, decrease the initial temperature or increase the initial hold time. Optimization of Column oven temperature program play a very important role in development. This is due to the inlet pressure increase that accelerated the elocution of compounds because of a large pressure gradient between the column inlet and column outlet 12-13. Started from 100°C, 140°C and 160°C results are summarized in below table-1

| Table-1 |                    |                                              |
|---------|--------------------|----------------------------------------------|
| Sr.No.  | Column temperature | Remarks                                      |
| 1       | 100°C              | Response very low                            |
| 2       | 140°C              | Peaks obtained with low response             |
| 3       | 160°C              | Response good                                |
| 4.      | 290°C              | Peak shape with good response measured in mv |

On the basis of above result we concluded that 290°C Column oven temperature program is suitable for the development of 5-chloro-2, 3-dihydro-1H-inden-1-one.

## 3. Optimization of Injector type, temperature & injection volume:

Table-2:

| Sr. No | Injector type | Temp | Injection volume | Remarks           |
|--------|---------------|------|------------------|-------------------|
| 1      | Manual        | 280  | 1.0 µl           | Area % variation  |
| 2      | Auto sampler  | 250  | 1.0 µl           | Area % consistent |

On the basis of above result we concluded that Injector Auto sampler system type, & injection volume - 1.0 µl is suitable for the GC method development of 5-chloro-2,3-dihydro-1H-inden-1-one, which give Area % consistent and peak shape / size and response is good of GC chromatogram.

### Optimization of detector type & detector temperature:

**Table-3:**

| Sr. No | Detector type | Detector Temp(°C) | Remarks                         |
|--------|---------------|-------------------|---------------------------------|
| 1      | FPD           | 320               | Peak not observed               |
| 2      | NPD           | 320               | Peak not observed               |
| 3      | TCD           | 320               | Peak observed with low response |
| 4      | FID           | 300               | Very good response              |

On the basis of above result we concluded that detector type FID & detector temperature 300 is suitable to detect the peak for method development

### 3.2 Optimization of Sample preparation:

The basic goal of sample preparation is to ensure that these conditions are met, with additional goals that the preparation be

reproducible to meet quantitative analysis with respect to the (High response, sharp peak, consistent result and reproducible peak area) . The sample which was injected into the gas chromatograph is a liquid gas and it was volatile enough under the conditions of the inlet and column to traverse the instrument, and, ideally, the matrix interferences was not observed to avoid contaminate into the instrument or column

**Table-4:**

| Sr. No | Solvent                 | Dilution | Remarks                                             |
|--------|-------------------------|----------|-----------------------------------------------------|
| 1      | Acetone                 | 1000     | Turbid solution                                     |
| 2      | Ethyl acetate           | 1000 ppm | Turbidity and Layer observed                        |
| 3      | N,N Dimethyl form amide | 1000 ppm | Peak not separated                                  |
| 4      | Dichloromethane         | 1000 ppm | Clear solution and peak observed with good response |

On the basis of the above result we concluded that Dichloromethane is suitable solvent for sample preparation and 1000 ppm is suitable dilution.

### 2. Method Validation:

Proper selection of the methods depends upon the nature of the sample (volatile or nonvolatile molecule), its molecular weight, solubility and melting point. Factors that affect gas chromatographic analysis are column temperature, carrier gas flow-rate, injection temperature, split ratio, detector temperature and sample size<sup>14-15</sup>

#### 2.1 Material and method

The standard was purchased from Aldrich, GC grade solvent purchased from Spectrochem Pvt ltd, Mumbai (India). GC gas chromatographic system used to perform development and validation of this assay method was Shimadzu GC- 2030 equipped with flame ionization

detector (FID) and data processing system (Lab solution).

#### 2.2 Standard Preparation

A standard solution containing 1000 ppm of 5-chloro-2,3-dihydro-1H-inden-1-one was prepared in a 100 ml volumetric flask.

#### 2.3 Test Preparation

100mg sample weighed and transfer into a 100 ml volumetric flask. About 100ml MDC was added and sonicated for a minimum 20 min. with intermittent shaking. Then content was brought back to ambient temperature and diluted to volume with MDC. The sample was filtered through 0.45µm nylon syringe filter. The concentration 1000 ppm of 5-chloro-2,3-dihydro-1H-inden-1-one.

#### 2. Experimental details

**2.1 Chromatographic Conditions:** After optimization of Chromatographic condition below GC parameter used for validation sequence.

|                  |                  |                        |                   |           |
|------------------|------------------|------------------------|-------------------|-----------|
| Linear velocity  | 69.4 cm/sec      |                        |                   |           |
| Purge flow       | 0.0 ml/min       |                        |                   |           |
| Split ratio      | 40               |                        |                   |           |
| Injection volume | 1.0 $\mu$ l      |                        |                   |           |
| Detector         | FID              |                        |                   |           |
| Column           |                  | Rate $^{\circ}$ C/ Min | Temp $^{\circ}$ C | Hold time |
| Oven Programming | 0                | ---                    | 70                | 3         |
|                  | 1                | 10                     | 250               | 5         |
|                  | 2                | 10                     | 290               | 27        |
| FID temperature  | 300 $^{\circ}$ C |                        |                   |           |
| Sampling rate    | 40 msec          |                        |                   |           |
| H2/Air flow      | 40/400 ml/Minute |                        |                   |           |

| Column Details       | Column           | Length meter | ID     | Film thickness $\mu$ m |
|----------------------|------------------|--------------|--------|------------------------|
|                      |                  | 30           | 0.32mm |                        |
| Injector temperature | 250 $^{\circ}$ C |              |        |                        |
| Injection mode       | Split            |              |        |                        |
| Flow control mode    | Pressure         |              |        |                        |
| Pressure             | 135.4 kPa        |              |        |                        |
| Total flow           | 208.9 ml/min     |              |        |                        |
| Column Flow          | 5.09 ml / min    |              |        |                        |

### 5.3 Linearity

Method linearity is defined as the ability of the method to demonstrate that the test results are proportional to the concentration of (mg/ml). Method linearity was conducted by generating a calibration curve using different concentration levels of CN; C6 gas standards % each concentration level was analyzed using GC; FID in six replications.

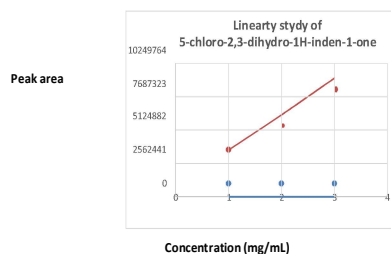


Fig-1

### 5.4 LOD and LOQ

The LOD and LOQ evaluated by serial dilutions of 5-chloro-2,3-dihydro-1H-inden-1-

one stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for 5-chloro-2,3-dihydro-1H-inden-1-one found to be 0.028  $\mu$ g/ml and the LOQ value 0.28  $\mu$ g/ml.

### 5.5 Precision

The developed method found precise with respect to the % RSD values and intermediate precision were 0.83 % and 0.42 %, respectively, which confirm that method was precise.

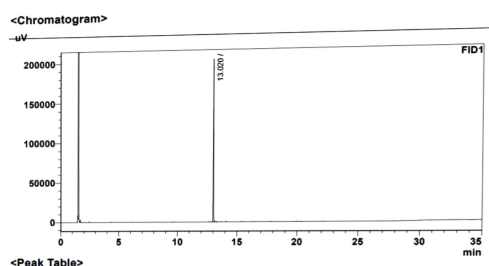
### 5.6 Accuracy

The GC area responses for accuracy determination are depicted in Table 6. Accuracy study was conducted by using standard addition method. A known concentration of standard substance (analyte) was added to blank preparation of sample matrix and recovery of analyte is calculated on the basis of area obtained in the chromatogram. The result shows that best recoveries (99.33-101.37 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table-6:

| Level % | Amount added Concentration (mg/ml) | Amount found Concentration (mg/ml) | % Recovery | % RSD |
|---------|------------------------------------|------------------------------------|------------|-------|
| 50      | 1.2532                             | 1.2500                             | 99.74      | 0.38  |
| 100     | 2.5064                             | 2.5122                             | 110.23     | 0.45  |
| 150     | 3.7515                             | 3.7510                             | 99.98      | 0.38  |

#### GC Chromatogram of 5-chloro-2,3-dihydro-1H-inden-1 as per Assay development method



was not decrease below the minimum percentage.

#### 5.7 System suitability

Six replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area determined for same. Acceptance criteria for system suitability found to be satisfactory, during all validation parameter.

#### 5.8 Robustness

The below experimental data shows that during all conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

#### 5.7 Solution stability study

The solution stability study at different intervals of time for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2 – 8 °C and ambient temperature, as during this time the Assay result

Table - 7

| Robust conditions              | % Assay |
|--------------------------------|---------|
| Column oven temp. at 260°      | 101.20  |
| Column oven temp. at 280°      | 101.51  |
| Carrier gas pressure at 210kPa | 99.57   |
| Carrier gas pressure at 190kPa | 102.18  |
| Injection volume 2.5µl         | 99.32   |
| Injection volume 3.5 µl        | 101.22  |
| Column change                  | 99.50   |

#### 2. Conclusion:

This procedure is to validate for assay method in 5-chloro-2, 3-dihydro-1H-inden-1-one by Gas chromatography (GC). This method is suitable for quantifying of assay of material. On the basis of above data, it was concluded that the developed method was validated as per the ICH guideline like specific, linear, precise, accurate, and robust. Because the method separates 5-chloro-2, 3-dihydro-1H-inden-1-one and all the degradation products formed under

variety of stress conditions it can be regarded as stability indicating.

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