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Gas Chromatographic method development approaches and validation for pharmaceutical products

Parag Das[1*], Ankit Gangani[2], Animesh Maity[1]

[1]Oman Pharmaceutical Products Co. LLC, Muscat, Sultanate of Oman, Oman.
[2]Hamlai Industries Pvt. Ltd., Sanand, Ahmedabad, India.

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ABSTRACT: Gas Chromatography (GC) is a very sensitive and versatile technique for the analysis of volatile mixtures in Active pharmaceutical ingredients - API, excipients and Pharmaceutical dosage forms. Volatile mixtures can be residual solvents or other volatile impurities which present within the permissible limits laid down by International Conference on Harmonization (ICH) guidelines ICH Q3C. Method development is the process of proving that an analytical method is acceptable for use to measure the concentration of an API in a specific compounded dosage form which allow simplified procedures to be employed to verify that an analysis procedure, accurately and consistently will deliver a reliable measurement of an active ingredient in a compounded preparation. Method validation is a procedure of performing numerous assessments designed to verify that an analytical test system is suitable for its intended reason and is capable of providing beneficial and legitimate analytical data.

Corresponding author*
Dr. Parag Das
Vice President – Technical
Oman Pharmaceutical Products Co. LLC
Muscat, Sultanate of Oman
Tel: +968-97044187
Mail ID: paragdas@omanpharma.com

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INTRODUCTION:

Gas Chromatography is a common type of chromatography technique utilized in analytical chemistry for separating and analyzing compounds which will be vaporized without decomposition. Typical use of gas chromatography is to separate different volatile components of complex mixture and to ascertain the purity of the selected compound. Separations are achieved by GC through a series of partitions between a moving gas phase and a stationary phase (Solid or

liquid) held in a small diameter tube (the column) after a sample mixture is injected through an injector port. A detector then monitors the composition of the gas stream coming from the column carrying separated components and detects, and therefore the resulting signals provide the input for data acquisition [1,2].

Methods are being developed for the products when there's no pharmacopoeial method available or when modification in existing method is required for better precision and ruggedness.

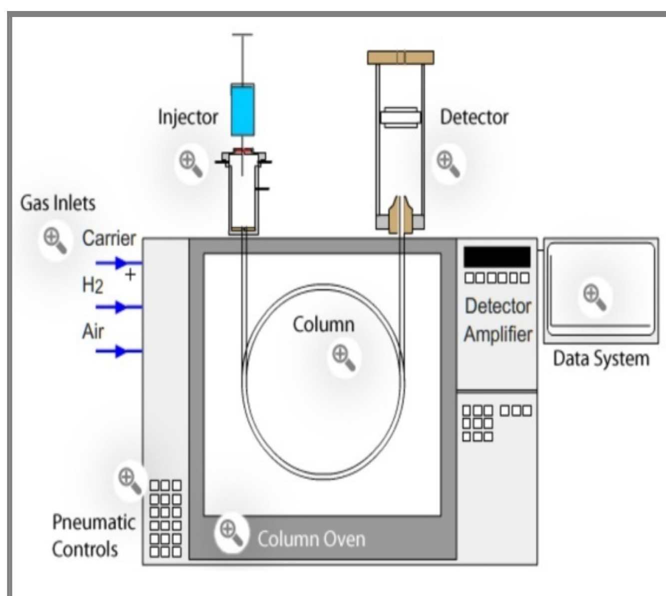

Fig 1. Modern Gas chromatograph.

There are several steps involved in the GC method development as following - Prior knowledge of sample / define separation goals, selection of chromatographic conditions (selection of carrier gas, column, injector and detector), selection of GC method (preliminary run), estimate best separation (alteration in chromatographic conditions), check for problems and troubleshoot, check for requirement of special procedure (recovery of purified material, qualitative analysis and quantitative analysis) and validation of method for routine laboratory use.

Apart from the above steps, other factors considered for GC method development are column selection (stationary phase and dimensions: column Id, length and film thickness), carrier gas selection (Nitrogen, Helium and Hydrogen: Flow rate), temperature of oven (initial temperature, initial hold, ramp rate, final temperature and final hold temperature), injector temperature and detector temperature [3,4].

For pharmaceutical products, split/split-less liner (injector part) and Flame Ionization Detector are mainly used.

Method development steps:

- Knowledge of properties of sample.
- Selection of chromatographic conditions.
- Developing the approach of analysis.
- Sample Preparation.
- Method Optimization.
- Method Validation.

Method Validation steps:

Analytical methods are being validated in accordance to ICH guidelines of Q2 (R1). The validation parameters are - System Suitability, Specificity, Linearity, Precision, Limit of Detection, Limit of Quantification and Robustness [5].

Basic criteria for new method development of drug analysis are as following [6]:

- The drug might not be official in any pharmacopoeias.
- A proper analytical procedure for the drug might not be available within the literature due to patent regulations.
- Analytical methods might not be available for the drug in the formulation under consideration due to the interference caused by the formulation excipients.
- Analytical methods for the quantification of the drug in biological fluids might not be available.
- Analytical methods for a drug in combination with other drugs may not be available.

DEVELOPING THE APPROACHES FOR ANALYSIS:

Two general approaches to GC method development are followed – First one is to start from a current method, evaluate the same for system suitability compliance. If all are meeting the pre-determined acceptance criteria, then proceed for validation. If any minor modifications are required to achieve the system suitability criteria and resolution between the peaks of interest, the changes can be done and then validation can be initiated. The second one is to develop a complete new method on the basis of the physical properties of the solvents being estimated, their tolerance limits, sample matrix, extraction procedure etc. Proper selection of the methods depends upon the character of the sample (volatile or non-volatile molecule), its molecular mass, solubility and boiling point. Factors that affect gas chromatographic analysis are - column temperature, carrier gas flow-rate, injection temperature, split ratio, detector temperature and sample size. All of these parameters are selected on the basis of trials and considering the system suitability parameters results. Typical parameters of system suitability are e.g.

retention time should be more than 5 min, the theoretical plates should be more than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be more than 2, % relative standard deviation (RSD) of the area of analyte peaks in standard chromatograms should not be more than 5.0 to 15.0 % looking for pharmaceutical industries [7,8].

Understanding the Physicochemical properties of sample:

In its early stages of development, it had been applied to the analysis of gases and vapours from very volatile components. The mixture, which may be a gas, a liquid or a vapour in some cases, has to be separated and analyzed by GC. Before starting the GC method development, it's important to see what's known about the sample. Proper selection of the methods based upon the character of the sample (volatile or non-volatile molecule), its molecular mass, solubility and melting point.

As much as information should be collected about the samples including aggregation state, sample composition (analytes, matrix, solvent), information on GC relevant properties, like boiling point range, polarity, functional groups, solubility, reactivity, stability at room temperature within the presence of air. It should also be checked that the sample contains thermally or chemically labile components, aggressive substances (acid, bases), water or non-volatile residues [9].

Selection of Chromatographic Conditions:

Initial choice of column and supporting instrumentation features a positive influence on the chances for ultimate results of separation and optimization. Alteration or changing of column parameters (stationary phase, inner diameter, length, and film thickness) gives chromatographic control over column efficiency, resolution and speed of analysis. In GC, the elution order of analytes (solvents) is controlled by several factors like vapour pressure, solubility in stationary phase and tendency for molecular interaction within the stationary phase. All of these variables change with temperature and their cumulative effect ultimately determine the equilibrium distribution of solute molecules between the mobile and stationary phase [8,9].

Selection of Column:

A column is in fact, the starting and central piece for a GC chromatogram. An appropriate selection of a column can produce a chromatographic separation which provides accurate and reliable analytical results. An improper selection of a column can generate confusion, inadequate and poor separations which may lead to results that are invalid or complex to interpret. There are more than 10,000 compounds that can be analyzed by GC and more than 400 GC capillary columns. It's a challenge for a column manufacturer to give detailed column selection guidelines to satisfy such a vast application.

An optimized chromatographic separation starts with the column. The choice of the right capillary column for any application should be based on four factors which are stationary phase, column internal diameter, film thickness, and column length. The differences within the chemical and physical properties of injected organic compounds and their interactions with stationary phase are the basis of separation process. When strength of the analytes-phase interactions differs significantly for two compounds, one is retained longer than the other. How long they are retained within the column (retention time) is a measure of those analytes-phase interactions. Two compounds that co-elute (do not separate) on a specific stationary phase can be separated on another stationary phase of different chemistry column [10,11].

Selection of Carrier Gas (as Mobile phase):

Many inert gases are often used as a carrier gas or mobile phase for GC. Hydrogen, Helium and Nitrogen are all common carrier gases. Each carrier gas has its benefits and chromatographic systems that it is best suited. The selection of a gas to be used as a mobile phase introduced in gas chromatography is zeroed down by the requirements and considerations of a robust analytical method. The ideal characteristics of the mobile phases are inertness, purity, dryness, freedom from oxygen, safety, cost and ready availability [10,12].

Control of Column oven temperature program:

A column fitted in an oven mostly affects the effectiveness of the chromatographic separation in GC. In many cases, isothermal temperature is not the most effective temperature mode for sample separation. In such cases, a temperature program can be used. Most GC temperature programs have an initial temperature, a ramp (temperature increase per minute) and a final temperature. Using a linear temperature program, if any previous analysis information is not available to be used as a reference, would be an ideal start. To improve the resolution of earlier eluting peaks, decrease in the initial temperature or increase in the initial hold time can be worked on. Decreasing the initial temperature usually

results in the greater improvement in resolution but analysis time (Run time) is simultaneously increased. The resolution of peaks eluting in the middle of the chromatogram can be altered by changing the ramp rate temperature. If the peak resolution is more, the ramp rate temperature can be increased to reduce resolution and reduce the analysis time (run time). If there is no proper resolution, the ramp rate temperature can be decreased but this will then lead to an increase in the run time. For better resolution of late eluting peaks, decreasing the ramp rate temperature would be an ideal preference. Another option to alter the peak resolution in the middle region of the chromatogram is to use a mid-ramp hold. A mid ramp hold is a several minute isothermal temperature portion during a temperature ramp. Only, a final hold time has to be used if the temperature limit is reached [5,13].

Selection and Optimization of Injector type, temperature and injection volume:

Injection of samples into the GC system is a critical step in chromatographic separation. The reproducibility of the number of samples injected is an important indication to the reproducibility of method. A sample can be injected using an auto-sampler system. A major error in GC is poor injection technique. But now a day's modern GC have very remote chances of error with a very high degree of preciseness. The temperature of the injector is used to rapidly vaporize the liquid sample and convert into a gaseous phase that can be carried to the column for separation.

In capillary and micro packed gas chromatography (GC), there are two primary techniques for vaporizing a sample and transferring it onto the inlet of the analytical column: split and split less injections. The split and split less injections are the most commonly used techniques for the pharmaceutical sector. Split injector is selected for analysis of samples with high concentration level of solvents.

In the split injection mode, only a fraction of the vaporized sample is transferred onto the head of the column (where column is fitted into the injector part). The remaining of the vaporized sample is removed from the injection port via the split vent line. Split injections should be used only when sample concentrations are high enough to allow a portion of the sample to be discarded during the injection process, while maintaining a sufficient concentration of analytes at the detector to produce a signal [14,15].

Selection and Optimization of detector type and detector temperature:

A number of varieties of detector are commercially available to be used with GC, each having its own limitations and advantages. The most commonly used detector in GC is Flame Ionization Detector (FID). This is the most suitable detector used for pharmaceutical industries nowadays. Flame Ionisation Detector (FID) is typically used for organic compounds and used in quality control analysis of pharmaceutical compounds. Detector temperature and the relative flow rate of carrier gas, hydrogen and air into the detector are the key functioning parameters of a robust method. A series of standards is defined for evaluation of detector parameters such as drift, noise, sensitivity, linear range, dynamic range etc.

The response is inversely proportional to the retention time. Therefore, any change in chromatographic conditions which cause a change in the retention time will also affect the peak height. It follows that a decrease in the flow-rate results in reduced peak heights, however the peak area remains approximately constant [16].

Sample preparation:

It is the most critical part for the GC method development and validation. The sample that is injected into the GC following sample preparation must be either a liquid or a gas. The analytes must be volatile enough under the conditions of the inlet and column to get transferred through the column and at the same time not to contaminate the instrument or column. In most cases, liquid samples must be dissolved in a volatile organic solvent for auto sampler and headspace GC. The basic goal of sample preparation is to ensure that these conditions are met, with additional criteria that the preparation is reproducible to meet quantitative analysis requirements, if the analysis is to be performed routinely. For headspace sampler, sample preparation needs more precaution as we have to fill the headspace vial and crimp it with septa immediately to avoid contamination and further vaporisation of low boiling point solvents from the sample [10,16].

Method optimization:

Several steps are involved in gas chromatographic method development and validation. In the case of analytical procedures like GC, these steps might include review of information on samples to be analyzed, definition of separation goals, assurance of special procedure requirements, sample pre-treatment if any;

detector selection and setting, separation conditions optimization, check for problems or special procedure requirements, recovery of purified material, quantitative calibration and qualitative method development. The experimental conditions should be optimized to get desired separations and sensitivity after getting appropriate separations. This will be achieved through planned/systemic examination of parameters. During optimization one parameter is changed at a time and a set of conditions are isolated rather than using a trial and error approach [17].

Gas Chromatographic method validation:

Numerous novel drugs are being introduced which are constantly growing day by day. Therefore, it is absolutely necessary to evolve novel methods and introduce them for controlling their quality. Modern pharmaceutical analysis needs the following requirements - the analysis should take a minimal time and should be economical, the accuracy of the analysis must meet the acceptance criteria detailed in the guidelines / pharmacopoeia and the chosen method should be precise and selective.

The process of validation of analytical methods is adopted to confirm that the analytical procedure for a specific test meets the intended requirements. Guidelines from the United States Pharmacopeia (USP), ICH, Food & Drugs Administration (FDA) etc., can provide a framework for validations of pharmaceutical methods. Results from the method validation can be considered to judge its quality, reliability as well consistency pertaining to analytical results. Parameters of Analytical Method Validation are executed in accordance with ICH guidelines Q2 (R1). The typical validation parameters are System suitability, Specificity, Linearity and Range, Precision, Accuracy, LOD, LOQ and Robustness [17-20].

***System Suitability*:**

System suitability testing is the first and foremost parameter that needs to be evaluated during the validation as well as during the routine testing by the quality control department to ascertain whether the system selected for analysis is appropriate for meeting the specified acceptance criteria and the results can be generated can be release without any compromise on the quality of the product. The parameters used in the system suitability tests (SST) report are as follows; Number of theoretical plates or Efficiency (N), Resolution (Rs), Tailing factor (T) and Relative Standard Deviation (RSD).

Table 1. Acceptance criteria for system suitability.

Sl. No.	Parameter	Limit
1	Number of theoretical plates	> 2000
2	Resolution	> 1.5
3	Tailing factor	< 2.0
4	RSD	< 5.0 or <15.0

***Specificity*:**

Specificity of an analytical method is the ability to assess unequivocally the solvent in the presence of components which may be expected to be present. It can also be defined as the ability of a method to measure accurately a solvent in the presence of interference, such as synthetic precursors, excipients, other interfering solvents and known (or likely) degradation products that may be expected to be present in the sample matrix.

The definition has the following implications:

➢ Identification test: Identification tests should be able to differentiate compounds of closely related structures which are expected to be present i.e., to assure identity of an analyte.

➢ Purity test: To ensure that the analytical procedure performed allows an accurate statement of content of the impurity of an analyte i.e. related substances, residual solvents content, heavy metals, etc.

For GC, specificity is the most critical parameter to perform because there are many unknown impurities or other interfering materials present in the sample which are difficult to identify compared to solvents peaks.

***Linearity and Range*:**

Linearity is the ability of an analytical procedure to obtain a response that is directly proportional to concentration (amount) of solvent in sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of solvent in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line. Or, linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates to a straight line.

Linearity can be assessed by performing single measurements at several analyte concentrations. The data is then processed using a linear least-squares regression. The resulting plot slope, intercept and correlation coefficient provide the desired information on linearity.

***Precision*:**

The precision of an analytical procedure represents the nearness of agreement between a series of measurements generated from multiple sampling of the same homogenous sample under the similar analytical conditions and it is divided into 3 categories.

- Repeatability: precision under same operating conditions, same analyst over a short period of time.
- Intermediate precision: method is tested on multiple days, instruments, analysts etc.
- Reproducibility: inter-laboratory studies.

The ICH guidelines suggest that repeatability should be conformed duly utilizing at least 9 determinations with specified range for the procedure (e.g. three concentrations / three replicates each) or a minimum of 6 determinations at 100 % of the test concentration.

***Accuracy*:**

The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose "true value" is known) is analyzed and the measured value is identical to the true value. Typically, accuracy is represented and determined by recovery studies. There are three ways to determine accuracy that are comparison to a reference standard, recovery of the solvent spiked into blank or diluent and standard addition of the solvent.

***Limit of detection (LOD)*:**

LOD is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the solvent can reliably be detected, but not necessarily quantitated as precise value, under the stated experimental conditions. The detection limit is generally expressed in the concentration of solvent (ppm) in the sample. A number of approaches are recommended by the ICH for determining the detection limit of a sample, depending on the instrument used for analysis, nature of solvent and suitability of the method.

The acceptable approaches are visual evaluation, signal-to-noise ratio, standard deviation of the response and standard deviation of the slope of linearity plot.

The LOD can be calculated by using following formula;

$LOD = 3.3\ \delta/S$ ……. (1)

Where, δ is the standard deviation of intercepts of calibration curves and S is the slope of linearity plot.

***Limit of quantitation (LOQ)*:**

Limit of quantitation is the least concentration of drug in a sample which is estimated with appropriate precision and accuracy under the confirmed experimental conditions. Similar to LOD, ICH recommends the following methods for estimation of LOQ. The acceptable approaches are visual evaluation, signal-to-noise ratio, standard deviation of the response, standard deviation of the slope of linearity plot.

The LOQ can be calculated by using following formula;

$LOQ = 10.0\ \delta/S$ ……. (1)

Where, δ is the standard deviation of intercepts of calibration curves and S is the slope of linearity plot.

***Robustness*:**

Robustness is defined by the measure of the capability of an analytical method to stay unchanged by small deliberate changes in method parameters. The variable method parameters in GC technique may involve flow rate, column oven temperature, injector temperature and headspace temperature (Headspace oven, transfer line and needle).

CONCLUSION:

In the recent years, development of the analytical methods for identification, purity evaluation and quantification of drugs have received a great deal of attention in the field of separation approaches. In this review article, a general and very simple approach for the GC method development for the separation of compounds was discussed. The physicochemical properties of the primary compound are of utmost importance prior to any GC method development. Several steps are being considered for GC method development like column section (stationary phase and dimensions: column id, length, and film thickness), carrier gas selection (Nitrogen, Helium, flow rate), temperature programming (initial temperature, initial hold, ramp rate, final temperature, and final hold), injector selection, injector temperature, detector selection and detector temperature. Optimized methods need to be validated for various parameters (e.g. specificity, precision, accuracy, detection limit, linearity, etc.) as per ICH guidelines.

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