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Analytical Method Development and Validation for Residual Solvent Test by Gas Chromatographic Technique

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Abstract: *The identification and quantification of volatile organic solvents from test samples subjected to analysis is performed by use of Gas Chromatography instrument. Gas chromatographic instrument parameters along with diluent, column and inert gases are necessary to optimize the analytical method. Analytical method to determine targeted residual solvent contents, is necessary for drug substances as well as drug products, for which methods are not published in pharmacopoeias, or not available from published data or literature for residual solvent present in drug substances or in combination formulated drug products or the available existing procedures need expensive solvents and methods available are cumbersome to extract the residual solvent contents present in samples along with proper separation. This research article intends to brief an appropriate as well as suitable simple approach for beginners in the field of chromatography to start development of residual solvent test and essential parameters to perform validation of method of analysis as per the guideline (ICH).*

Key words: *Gas chromatographic technique, GC, Method validation, Method development.*

1. INTRODUCTION:

Gas Chromatography is an analytical technique and gas chromatographic instrument is used to measure the content of various organic volatile components present in a sample. The chromatographic pattern of separation can be achieved due to partition and the analytes targeted to be separated (converted to vapour state), are mixed with gaseous mobile phase. The residual solvents present in the sample which are more soluble/adsorbed in stationary phase will elute slower and which are more soluble in mobile phase will travel faster and elute first. The partition coefficient for two analytes will not be same hence, elution of residual solvents, is based on their partition coefficient. Partition coefficient is nothing but the ratio of solubility of a substance to be examined, distributed between two immiscible phases at a constant temperature.

To analyze test sample using instrument of Gas Chromatography (chromatographic technique) has many advantages, which generates results within less time and timely planned analysis can be finished fast as well as the gas chromatographic instruments are efficient (e.g., high resolution, sensitive) and can detect the analytes at ppm (parts per million) or even at ppb (parts per billion) level concentration.

Instrument of GC, HPLC or UPLC are widely used in research field to accomplish the chromatographic separation between the analytes present in the sample for identification as well as for estimation i.e., to quantify or identify the analytes present in the sample. Both techniques have its own advantages, however for volatile materials, instrument of Gas Chromatography is usually preferred and in this article method development and validation by Gas Chromatography (GC) is discussed.

Published articles/books were searched and studied as a part of survey of literature for method development and validation. [1-8]. This paper briefs the information or procedure, as available from existing as well as accessible literature at the time of writing, as per references cited and to the best of my knowledge, as per my understanding as well as opinion to explain the method development as well as validation procedure in simple way.

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2. METHOD DEVELOPMENT BY GAS CHROMATOGRAPHIC TECHNIQUE:

Method development should be based on the knowledge of the test sample and targeted goals as well as resources available for optimization of chromatographic method. For the residual solvents (Class I to IV) as listed in USP [9], if compendial methods are available/published, then feasibility of these available methods should be performed and available test samples should be run in the compendial/phamacopoeial test method to evaluate for proper suitability of the method. In addition to this, the method of analysis described for residual solvents in Drug Master File or Common Technical Documents by manufacturer, if available should be checked and evaluated. Depending upon the nature of solvent and test sample (i.e., complex mixture, mixture of polar and non-polar, gas or liquid sample), the head space technique or direct injection technique should be applied (for optimization of method of analysis). The suitable diluent should be selected. When there are no official pharmacopeial published methods, then methods are planned to develop for the residual solvent test for the specific content's determination in new drug substances or new drug products.

Method development for optimization of the method of analysis is based on below mentioned parameters,

2.1 Test sample, properties and sample preparation
2.2 Instrumental conditions and method of analysis

2.1 Test Sample, properties and sample preparation: Gas chromatography is an analytical technique used to analyse the test samples. Prior to start of the Gas Chromatography method development, it is important to review the information available about the test sample. Proper selection of GC column along with instrument parameters are necessary for optimization of the method and also depends upon the nature of the test sample and its physicochemical properties. The proper study of all the related information gathered of the test sample is a very important step, and data such as all the residual solvent contents of the test sample, solubility of all contents and sample as a whole, in different solvents, exact boiling point of all the residual contents required to identify or quantify from the sample, etc. All the physicochemical data collected helps in optimization of preparation of sample. Maximum information should be obtained for sample and this information leads to achieve development fast and desired for the intended application of analytical method.

The proper suitable diluent should be selected. The most common diluents are water, dimethyl formamide and dimethyl sulfoxide. For extraction of residual solvent contents subjected for analysis from test sample in diluent should be set based on trials performed for time required for sonication, stirring or shaking or either of both. The concentration of analytes in the sample along with standard i.e., appropriate suitable concentration of standard and for contents of analytes in the sample to achieve optimum response in chromatography should be optimized.

2.2 Instrumental conditions and Method of analysis: The column planned to use in development trials and gas chromatographic instrument play an important role in getting the desired and proper separation as well as resolution between the analytes by way of optimizing the instrumental parameters (within the prescribed specification of the instrument given by the instrument manufacturer and within the calibrated range of the instrument performed for each parameter as per the procedure). The different options of various GC columns with different specifications available, allows the researcher to develop the desired and required parameters of method to obtain proper chromatographic results associated with GC column and GC instrument such as system suitability criteria for method as well as instrument to check performance of method and instrument to optimize within the limits.
Application of different columns: Two types of columns (capillary and packed) are generally used in gas chromatography. The coating of liquid as stationary phase along with inert material of solid support are employed in packed GC columns.

Wall-coated open tubular having a capillary tube coated with liquid as stationary phase or support-coated open tubular with inner line of support material on which are stationary phase, are used as capillary columns. In comparison to packed column, capillary columns are more effective for intended purpose of separation.

The option of choosing GC column for the purpose of optimization of method of analysis, is intended application along with properties of the column, as suitable to use in achieving the goal of separation. Different parameters such as operating range of column temperature and durability i.e., use of GC column to analyze for maximum number of samples, stationary phase, film thickness, diameter and length play important role in achieving proper chromatography.

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The interaction of test sample with mobile phase as well as stationary phase of the column leads to elution of analytes in gas chromatography. The separation is affected by the chemical properties (of the compounds) and is the basis for chromatographic separation, resolution or merger of peaks and different components in the sample show different retention time for each peak. Due to different stationary phases with different selectivities, merger of peaks observed on one column may get separation along with proper resolution on another column. Hence, to achieve proper/suitable separation test sample properties along with GC column properties should be studied prior to start of the method development. Stationary phase of the column, propensity to form hydrogen bonding, polarity and dipole moment are some of the factors that affect separation and these factors should be taken into consideration. Different types of columns from different manufacturers with different chemistry (with different stationary phases, column properties and different dimensions) are commercially available. Some of the column manufacturers for GC column with different types are PerkinElmer, Agilent, Restek, Thermo etc. and for details of chemistry of column, available catalogue/brochure containing all the scientific information should checked.

Carrier Gase: The inertness of mobile phase (carrier gas) is the basic criteria planned to use in the GC instrument. In the gas chromatography, hydrogen, helium or nitrogen gases are commonly used. The carrier gas (mobile phase) should be opted on its suitability in the method and the instrument as well as ready availability. Carrier gases intended to be used should be of high purity because impurities in the carrier gas may interfere in the chromatographic run and destroy the column. The commercially manufactured cylinders of high purity of gas are preferred to use.

Temperature of Column oven: The elution or separation pattern in the gas chromatographic instrument is affected by change/increase in the temperature and is an important step to incorporate in the method to achieve the proper elution as well as separation between different analyte peaks and can be set as per the requirement. Constant temperature generally does not work for elution or separation of different analytes to obtain different retention times and hence temperature programming (with ramp and hold time) is introduced for elution or separation of different analyte properly by increasing temperature at certain degree Celsius with time as specified and suitable from initial to end of the run time. Hold time along with increase in temperature (column oven) in the subsequent time points in the programme should be carefully chosen based on analyte boiling point. At the start of method development trials, firstly a linear temperature programme should be prepared and applied in the method to run to obtain chromatographic pattern to get knowledge essential (for chromatographic elution pattern) and next subsequent trails can be set through temperature gradient programme with ramp and hold time to achieve the proper separation for each component and can be set higher temperature in comparison to boiling point. Sometimes increase in temperature of column oven may not show proper elution or separation and gradual increase in temperature continuously may assist to achieve the separation goal.

Injector parameters: Different injection techniques (modes) based on requirement are used and these are direct injection, split or splitless or cold on-column. The temperature for injector set in the method functions to vaporize the test sample from liquid phase to gaseous phase and is carried to the column and based on polarity and interaction with stationary phase as well as mobile phase and separation is achieved. Injection volume for liquid samples should be chosen based on sample response from detector and if sample concentration is high then split injection can be selected in which defined amount (as in split mode opted) is taken for injection and remaining sample is discarded. The advantage of using split injection mode is that dilution of sample is avoided as small amount is injected and remaining is discarded however the disadvantage is that it cannot be used for trace/very low amount of sample. When quantity of residual solvent in test sample to determine its content i.e., its concentration is very low then splitless injection mode should be chosen and the advantage is its sensitivity for low concentration and other injection modes are direct, on column, cold on-column into capillary columns.

Types of detectors: Different detectors for specific detection such flame ionization detector, thermal conductivity detector, photo ionization detector, flame photometric detector, electron capture detector, mass selective detector or nitrogen-phosphorus detector etc. are used in the gas chromatographic instrument. Based on the suitability for the component detection based on contents to be analyzed, detector should be chosen. In most of the GC methods, flame ionization detector is used. Flow rate and change in chromatographic conditions alter the retention time and affects peaks. Temperature for detector should be set based on the detector type.

Optimization of the method: Following points are very important in finalization of optimized method,

A) Suitable concentration of sample as well as standard to achieve proper response from the instrument.

B) Linearity of analyte covering concentration range from LOQ to 150% of set specification limit.
C) Run time should be optimized in such a way that, as to avoid any co-elution of peaks or carryover from previous injection of analyte peak.
D) System suitability criteria for relative standard deviation, resolution between all of the nearly eluting peaks etc.
E) The analyte peak or any known solvent peak should not have any interference from any other solvent or diluent.

To check suitability of developed residual solvents test method following parameters may be suitably performed and it should comply with the predefined acceptance criteria.

A) System suitability parameter
B) Specificity (By spiking solvents at limit concentration).
C) Determination and precision of Limit of Quantification.
D) Reproducibility (Method precision)
E) Accuracy (i.e., recovery)

Once gas chromatographic method is optimized, method reproducibility should be checked. specificity, precision, and accuracy should be checked before planning final method validation as part of check for method during development and optimization. Finalized method should be subjected further for validation.

3. METHOD VALIDATION BY GAS CHROMATOGRAPHIC TECHNIQUE:

Validation of an analytical method of analysis, described in detail as stated in the test procedure, is the activity by which it is established from laboratory research studies endorsing the performance characteristics of analytical method mentioned to meet the requirements for the intended use of its analytical applications.

For non-compendial (non-phamacopoeial) test method, following described analytical method validation parameters should be performed as a part to evaluate the complete performance of the method.

A) System suitability of the test method
B) Specificity of the test method
C) LOD and LOQ of the test method
D) Linearity and range of the test method
E) Accuracy of the test method
F) Precision of the test method
G) Solution stability of sample solution and standard solution for the test method
H) Robustness for the test method

4. RESULT AND DISCUSSION:

When the method development by gas chromatography is completed, method development trials performed for optimization of the method should be documented as a summary along with complete details of optimized final method. Further validation of method of analysis should be planned and executed as per predefined prepared protocol. The data and results obtained from validation analysis should be documented in report with observation, discussion and conclusion in detail along with the acceptable limits.

5. CONCLUSION:

In method development document/report, finalized method should be reported in detail with all procedures necessary to perform analysis and is a reference document to prepare method validation protocol/document for analysis including final method details. Once planned method validation activities are complete, and obtained results are within the acceptable limit as per protocol, method can be used for its targeted content determination for further use.

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