International Journal of Advance and Applied Research
www.ijaar.co.in
ISSN – 2347-7075 Impact Factor – 7.328
Peer Reviewed Bi-Monthly
Vol.9 No.5 May – June 2022

SCIENTIFIC STUDY FOR CONTENT OF 1-BROMO-3-CHLOROPROPANE ,4-FLUORO-ANILINE AND 2-CHLORO-1-FLUORO-4-NITROBENZENE IN GEFITINIB BY GCMS TECHNIQUE

Mr.Vinayak Nachankar[1], Dr.Rama S Lokhande[2], Dr. Purnima Nag[3], Dr.Ravi Yadav[4]
[1] Research Scholar, Department of Chemistry, Jaipur National University, Jaipur, India.
[2] Research Guide, Head, School of Basic science, Head- Department of Chemistry, Jaipur National University, Jaipur,India.
[3] Research Co-guide ,Senior additional Director, School of Engineering and Technology, Jaipur National University, Jaipur, India.
[4] Additional Guide, Senior director, Cipla Limited, Vikroli R & D Centre, Mumbai, Maharashtra, India.
Corresponding Author- Mr.Vinayak Nachankar
Email- vinayaknachankar@gmail.com

Abstract
A Short run time, sensitive, stability-indicating GC-MS method has been developed for the quantitative estimation of 1-Bromo-3-Chloropropane ,4-Fluoro-Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene in Gefitinib. Effective chromatographic separation was achieved on 60 m x 0.32 mm I.D. 1.8 µ, VF-624 (6 % Cyanopropyl phenyl +polysiloxane fused silica analytical column of 94 % Di-methyl with helium mobile phases. The flow rate of the mobile phase was 1.5 mL min-1 with Injector Temperature: 160°C Interface temperature : 250°C column temperature Initially at 75°C and hold for 5.0 minutes then increase at the rate of 10°C/minute to 160°C and hold for 5.0 minute. Further increase at the rate of 25°C/minute to 250°C and hold for 10.0 minute. Further increase at the rate of 20°C/minute to 280°C and hold for 10.0 minute. The specificity of test results confirmed that the Gefitinib was homogenous in all API samples and the accuracy was found to be more than 96%, thus proving the stability-indicating power of the method.. The developed method was extensively validated as per ICH guidelines with respect to specificity, linearity, limit of detection and quantification, accuracy, precision and robustness. The developed GC-MS method was successfully applied for analysis of Gefitinib Active pharmaceutical ingredients samples.

Key words *Gefitinib API • GC-MS • Stability-indicating • Impurities •ICH guidelines*

Rationale of the study

A rationale for selecting impurity limits based nature of impurities of API substances on safety considerations has to be provided. Analytical technique should be able to separate all the impurities from each other and the method should be self capable to separate and quantify them in the presise forms. Such methods are to be validated demonstrating the accuracy, precision, and specificity, limit of detection, quantification, linearity range and interferences. These guidelines serve as a basis worldwide both for regulatory authorities and industry and bring the importance of a proper validation to the attention of all those involved in the process of submission of drug master files. The analytical research and development units in the pharmaceutical industry are responsible for preparation and validation of test methods. Every country has its own authorities agency to control ovrer product quality as it is directly related to human health on bulk drugs and their pharmaceutical formulations standards and obligatory quality indices for them The above discussion emphasizes the importance of analytical research for evaluation of safety and efficacy of the drug. Pharmaceutical Analysis generally involves two steps; a) separation of the compound of interest and b) quantitation of the compound. The field of the pharmaceutical analysis is stressed on the analysis of the drug substance and drug products.

Objective of the study

Evaluation of available literature implies that there are very few methods for estimation 1-Bromo-3-Chloropropane ,4-Fluoro-Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene in Gefitinib. still none of method specific process impurities are mentioned. 1-Bromo-3-Chloropropane ,4-Fluoro-Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene are the process impurities that can be formed during synthesis. Objective of method development would be to develop stability indicative, shorter method for related substances of Gefitinib which will be capable of quantifying process 1-Bromo-3-Chloropropane ,4-Fluoro-

Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene This method will be subsequently validated as per ICH guide lines.

Hypothesis of the study

A hypothesis is a statement of the researcher's expectation or prediction about relationship among study variables. The analytical research process starts and ends with the hypothesis. It is a core to the entire analytical procedure and, therefore, is of very importance. Hypothesis is heart of the research. In the research without hypothesis, research has to be done in scientific ways. The logical hypothetical question identifies the study concepts and asks how the concepts might be related a hypothesis is the predicted answer. The basic aspects important in the hypothesis are: difference that we are trying to find out, relationship, and the interactions. In relationship, we are trying to understand statistically significant relationship that means the difference in result did not occur by chance but due to statistical reasons. It means results are statistically significance. If the variation is occurred by chance, then results are not logically significant. Normally when we are stating a hypothesis there is an independent variable and dependent variable. Independent variable cause and dependent variable is effect. A hypothesis ensures the entire research process remains scientific and reliable. Though hypotheses are essential during the research process, it can produce complications with regards to probability, significance and errors. A hypothesis is predication based on observations. On this account researcher is in present paper mainly high kite the significance of the hypothesis and its importance in research methodology.

Methodology

Instrument used: Shimadzu GC-2010 plus with GCMS TQ8040

Instrument: A Gas chromatograph Mass Spectrometer capable of temperature programming,

Equipped with a capillary column, split / splitless injector, a Quadrapole Mass detector, a liquid auto sampler with a suitable software.

Chromatographic condition:

Column type: 60 m x 0.32 mm I.D. 1.8 µ, VF-624 (6 % Cyanopropyl phenyl +94 % Dimethyl polysiloxane fused silica analytical column is used.)

Carrier gas: Helium ; Flow rate: 1.5 ml/minute; Injection Mode: Split; Split Ratio: 1:10

Detector: Quadrupole Mass Detector;Detection mode: SIM;Ion Source temperature: 250°C

Ion voltage: Relative to tuning 0.5kV

Ion selection m/z : For 1-Bromo-3-chloropropane – 77 m/z

For 4-Fluoroaniline – 111 m/z; For 2-Chloro-1-fluoro-4-nitrobenzene – 129 m/z

Injector Temperature: 160°C; Interface temperature: 250°C

Oven temperature: For Standard –

Initially at 75°C and hold for 5.0 minutes then increase at the rate

of 10°C/minute to 160°C and hold for 5.0 minute. Further increase

at the rate of 25°C/minute to 250°C and hold for 10.0 minute

For Sample –

Initially at 75°C and hold for 5.0 minutes then increase at the rate

of 10°C/minute to 160°C and hold for 5.0 minute. Further increase

at the rate of 25°C/minute to 250°C and hold for 10.0 minute.

Further increase at the rate of 20°C/minute to 280°C and hold for

10.0 minute

Injection volume: 3.0 µl.

Liquid conditions (By Combipal):

Air Volume: 1.0 µl

Pre Clean with Solvent 1: 5

Pre Clean with Solvent 2: 5

Pre Clean with Sample : 5

Filling volume (l): 5

Fill speed (l/sec) : 2

Fill strokes: 5

Pull up Delay (ms): 400

Inject to: Appropriate.

Injection Speed (l/sec): 50

Pre Inject Delay (ms): 500

Post injection Delay (ms): 500

Post Clean with Solvent 1: 5

Post Clean with Solvent 2: 5

GC run time (sec): According to the program

Diluent (Blank): Methanol: Dichloromethane (1:1) v/v

Standard stock solution: Weigh accurately about 60 mg each of 1-Bromo-3-chloropropane standard,

4-Fluoroaniline standard and 2-Chloro-1-fluoro-4-nitrobenzene standard in 10 ml volumetric flask

containing about 2 ml of diluent. Dilute up to mark with diluent and shake well. Dilute 1.0 ml of above

solution to 200.0 ml with diluent.

Working solution: Dilute 1.0 ml of Standard stock solution to 100 ml with diluent.

Test solution: Weigh accurately and transfer about 500 mg of sample in 5 ml volumetric flask.

Add
about 4 ml of diluent, shake well to dissolve and dilute upto the mark with diluent.
NOTE: Prepare Standard stock solution, Working solution and Test solution in duplicate.
Procedure: Separately inject equal volumes of blank (diluent), working solution (1) (6 replicates),
working solution (2), blank (diluent), Test solution (1), Test solution (2) and working solution (1)
into the chromatographic system and record the chromatogram and measure the peak area
response and check for System suitability parameters. Disregard peak area due to blank interference.

System suitability parameters:

1. The Relative standard deviation for the peak area response of respective peaks for replicate injections of Working solution (1) and replicate injections of Working solution (1) including bracketing
standard is not more than 15.0 % each and Relative standard deviation for retention time for respective peaks for replicate injections of Working solution (1) and replicate injections of Working
solution (1) including bracketing standard is not more than 2.0 % each.
2. The Similarity factor between working solution (1) (6 replicates) and working solution (2)
(separately prepared and injected) is between 0.85 and 1.15. Calculate the Similarity factor using the following formula:

$$\text{Similarity factor} = \frac{\text{Average peak area of respective peaks in the Chromatogram obtained with working solution (1)}}{\text{Peak area of respective peaks in the Chromatogram obtained with working solution (2) (1) (g)}} \times \frac{\text{Wt. of respective standard in Standard stock solution (2) (g)}}{\text{Wt. of respective standard in Standard stock solution (1) (g)}}$$

Note: If the Similarity factor does not fall within 0.85 and 1.15, prepare fresh Standard stock solution in
duplicate, re-inject in single injection and calculate Similarity factor again as above. If the similarity factor falls within the limit, inject the re-prepared working solution (1) in replicate and continue the
sequence for Blank and sample.

Note: If blank interference is observed then take mean of triplicate blank injections for calculation. There should be no or not more than 10 % of blank interference.
Elution order: 1-Bromo-3-chloropropane, 4-Fluoroaniline and 2-Chloro-1-fluoro-4-nitrobenzene
Calculation: Content 1-Bromo-3-chloropropane, 4-Fluoroaniline and 2-Chloro-1-fluoro-4-nitrobenzene in ppm:

$$\text{Content (ppm)} = \frac{(A - B) \times W_1 \times 1 \times 1 \times 5 \times P \times 10^6}{(C - B) \times 10 \times 200 \times 100 \times W \times 100}$$

Where,
A = Peak area response of respective peaks obtained in the chromatogram of Test solution.
B = Mean peak area response of respective peaks interference obtained in the chromatogram of blank solution.
C = Mean peak area response of respective peaks obtained in the chromatogram of
working solution (1).
W 1 = Weight of respective standard taken for standard stock solution (1) in g.
W = Weight of sample taken in g.
P = Purity of respective standard on as such basis.
Limit:
1-Bromo-3-chloropropane : Not more than 3 ppm
4-Fluoroaniline : Not more than 3 ppm
2-Chloro-1-fluoro-4-nitrobenzene : Not more than 3 ppm

*Chemicals/Reagents used:

Reagent / Solvents used	Make	Grade
Methanol	Rankem	HPLC
Dichloromethane	Fischer scientific	HPLC

nitrobenzene in ppm:

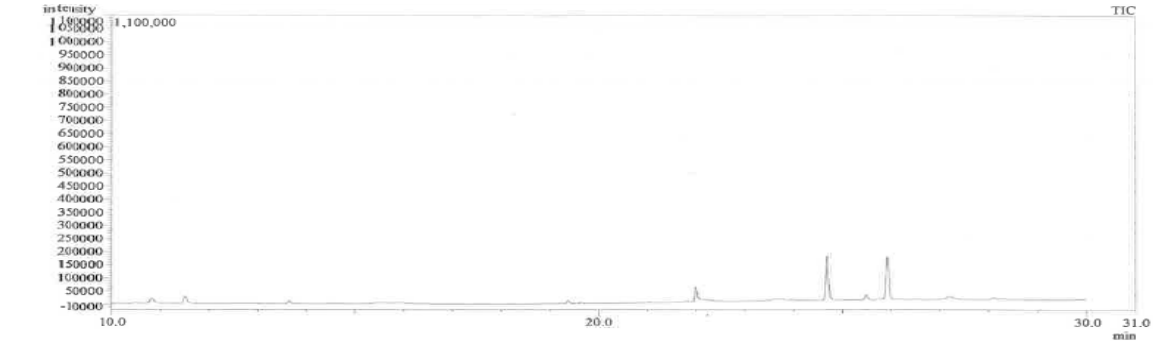
$$\text{Content (ppm)} = \frac{(A - B) \times W_1 \times 1 \times 1 \times 5 \times P \times 10^6}{(C - B) \times 10 \times 200 \times 100 \times W \times 100}$$

Where,
A = Peak area response of respective peaks obtained in the chromatogram of Test solution.
B = Mean peak area response of respective peaks interference obtained in the chromatogram of blank solution.
C = Mean peak area response of respective peaks obtained in the chromatogram of

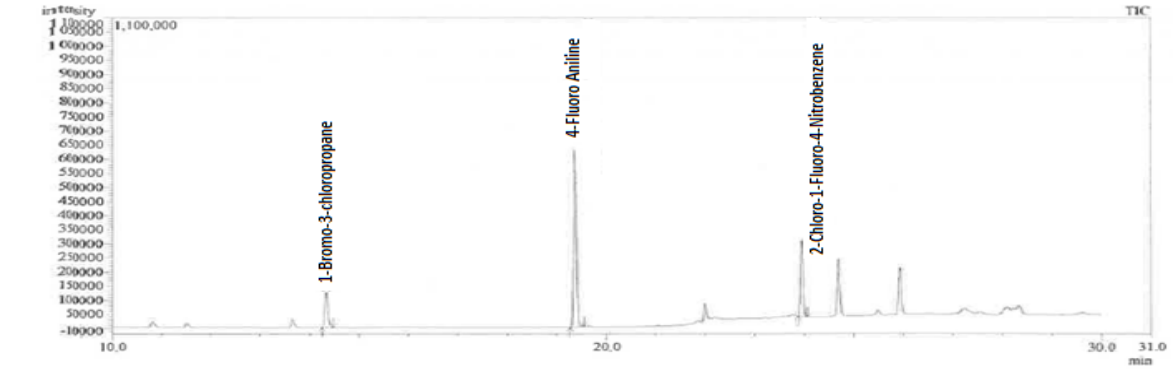
working solution (1).
W 1 = Weight of respective standard taken for standard stock solution (1) in g.
W = Weight of sample taken in g.
P = Purity of respective standard on as such basis.
Limit:
1-Bromo-3-chloropropane : Not more than 3 ppm
4-Fluoroaniline: Not more than 3 ppm
2-Chloro-1-fluoro-4-nitrobenzene: Not more than 3 ppm
*Chemicals/Reagents used:

Reagent / Solvents used	Make	Grade
Methanol	Rankem	HPLC
Dichloromethane	Fischer scientific	HPLC

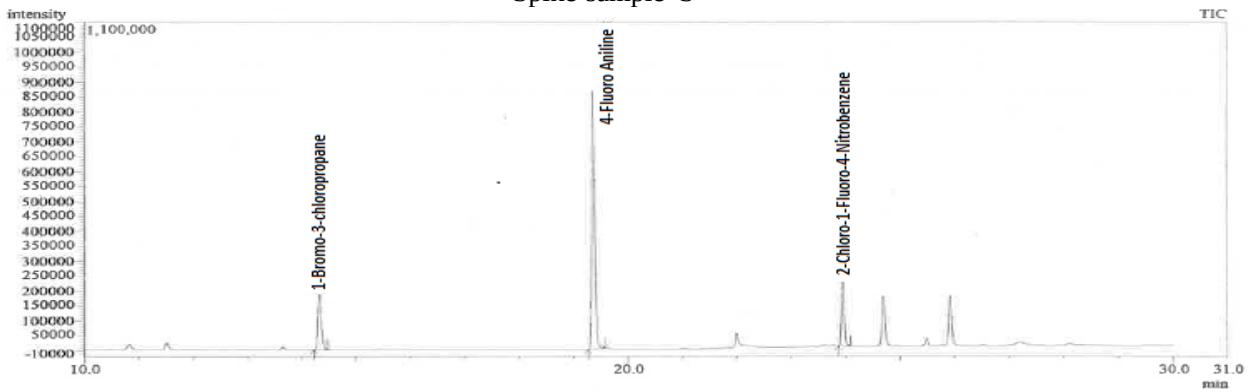
Blank



Standard



Spike-sample S



Sr.No.	In the chromatogram of Working solution.
1.	The Similarity factor between Standard solution A (six replicates) and Standard solution B (separately prepared and injected) for the peak of N-Nitrosodimethylamine should be between 0.85 and 1.15.
2.	The Relative standard deviation for the peak area response of N-Nitrosodimethylamine for six replicate injections of Standard solution A should not be more than 15.0 %.
3.	The Relative standard deviation for the retention time of N-Nitrosodimethylamine for six replicate injections of Standard solution A should not be more 2.0 % .

Conclusion

The GCMS method developed for 1-Bromo-3-Chloropropane ,4-Fluoro-Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene in Gefitinib Content is simple, specific, linear, sensitive, precise and efficient and is suitable for its intended purpose.

The method developed has shorter run time, thus ensuring optimum utilization of the GCMS Instrument system. It avoided use of any internal standard and hence it was cost effective as well as user friendly.

Run time of this method is very short due to shorter retention time of peak due to 1-Bromo-3-Chloropropane ,4-Fluoro-Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene in Gefitinib (i.e. all components are eluting withing 25 min & over all run time is 31 minute.)

Hence the proposed method is on GC MS with shorter run time obtained using advance and cost effective techniques is fulfil. Also shorter run time ensured lesser consumption of solvents in turn reducing further cost per analysis and also generating lesser solvent waste. The shorter run time also enabled analysis of multiple batches in short duration thus enhancing the out-put of batch analysis. The method was validated by critical principles stated in ICH guidelines, showing satisfactory data for all the method validation parameters tested. Evaluation of validation data confirmed that method had comparable system suitability parameters as well as precision as those specified in literature reference. Also its linearity range was established to confirm linear response across the concentration range. Further during robustness study, it was established that variations in temperature, flow, column length, column ID, did not have significant impact on Chromatographic pattern. Hence, the proposed method can be employed for assessing 1-Bromo-3-Chloropropane ,4-Fluoro-Aniline and 2-Chloro-1-Fluoro-4-Nitrobenzene Content of Gefitinib.This study provides an idea how to perform validation process to prove that the method is accurate for its intended purpose and to assure the capabilities of the test method. The definitions of method validation parameters are well explained. Document the experimental design study, the approach to validation is varied and opened to interpretation, and validation requirements differ during the development process of pharmaceuticals. Validation is an important procedure in the API Study and it is utilized to ensure that inbuilt quality is there in to the processes supporting drug development and manufacture to get expected outcome of research study.

Suggestion /Recommendations

The presence of an structural alert in a potential or actual impurity, most likely arising as a byproduct or carried-over reagent or starting material, in a drug substance or drug product is merely an indication that the compound may be a DNA-reactive genetoxic compound.. The correlation between structural alerts for direct or indirect electrophilic characteristics and relevant biological activity is highly imperfect. For virtually all actual or potential impurities that are structurally alerting there is likely to be a variety of possibilities for clarifying their genotoxicity status based on published data. A review of representative compounds from several classes of structurally alerting substances (epoxides, hydrazines, aromatic amines, halides and aldehydes) provides examples of different types of qualification strategies Overall, it seems prudent to obtain maximum "leverage" from toxicological approaches, which are likely to be relatively low cost, before making any significant process-related changes to an active pharmaceutical ingredient (API) synthesis.

Acknowledgments

I hereby sincerely thank Mr. Sandeep Bakshi Honorable Chancellor, Prof (Dr). K. L.Sharma Honorable Vice-Chancellor, Prof (Dr.) H. N. Verma Honorable Pro-Vice-Chancellor of Jaipur National University, Jaipur for their much valued support. It is my honour to express my most sincere gratitude and thanks to my Supervisor, Prof. (Dr.) Rama.S.Lokhande, Head, School of Basic science, Head Department of Chemistry, Jaipur National University, India and my Co-supervisor, Prof. (Dr.) Purnima Nag Senior additional Director, School of Engineering and Technology, Jaipur National University, Jaipur, India and Joint supervisor Dr. Ravi Yadav, Senior director, Cipla Limited,Vikroli R & D Centre, Mumbai,Maharashtra, India, for their support and guidance during the course of my research.

I am glad to express my thanks to Corvid pharmaceuticals Pvt. Ltd for providing the drug substance samples and support.

Not the least, I would like to acknowledge that my work would have not been concluded within schedule without unselfish support of my family members.

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