Indian J. Applied & Pure Bio. Vol. 40(3), 2406-2410 (2025). **ISSN: 0970-2091**
A web of Science Journal

Stability indicating HPLC method development and validation for Simultaneous estimations of some Combination Dugs: A Review

Mohd. Farooque[a*] and Gulam Javed Khan[b]

[a]U.B.K.W.T's D. Pharmacy College, Kunjkheda, Dist-Aurangabad - 431001 (India)
[b]Ali Allana College of Pharmacy, Akkalkuwa KBC NMU, Jalgaon - 425415 (India)

Abstract

One form of liquid chromatography is high performance liquid chromatography (HPLC). It can separate labile natural products, polymeric materials, ionic species, and macromolecules. The ICH criteria for the assessment of pharmaceutical formulations and medicines in bulk can be followed in the development and validation of the stability indicating HPLC method. An overview of the development and validation of the HPLC method for drug estimation can be found in the various published literature. Different experiments, such as altering the concentration of the mobile phase, the detecting wavelength, and the flow rate, can be used to develop the HPLC process. The generated approach will then undergo validation using various factors. Several metrics, including linearity, accuracy, precision, repeatability, robustness, LOD, and LOQ, can be used to validate the HPLC method.It is also necessary to carry out forced degradation studyto predict, evaluate, and ensure drug product safety. This stability indicating developed method can be used for routine analysis of drugs in bulk and pharmaceutical formulation.

Key words : HPLC, Method Development, Method Validation, Literature Review, Validation parameters.

High Performance Liquid Chromatography:

One kind of liquid chromatography is HPLC. Macromolecules, ionic species, polymeric materials, labile natural products, and a range of other high molecular weight polyfunctional groups can all be separated using high performance liquid chromatography (HPLC). High-resolution, high-speed liquid chromatography is known as HPLC.[18,19,21].

Basic Principles of HPLC:

Separation of compounds by HPLC depends on the interaction of analyte molecules with the stationary phase and the mobile phase. The stationary phase is a thin layer created on the surface of fine particles and the mobile phase is the liquid flowing over

the particles.

Types of Chromatography:

- Normal phase
- Reverse phase
- Ion exchange
- Size exclusion
- Gradient HPLC

Instrumentation of HPLC:

A. There is a solvent reservoir for the mobile phase.
B. The mobile phase must be delivered to the column by some type of pump.
C. Degasser to remove gas from the mobile phase/drug.
D. Sampling valves or loops are used to inject the sample in the flowing mobile phase.
E. The separation column contains the packing needed to accomplish the desired HPLC separation.
F. Ahead of the separation column there may be a guard or an in-line filter to prevent contamination of the main column.
G. Detector with some type of data handling device completes the basic instrumentation. (4-6) Current study includes literature survey about HPLC method development, validation of developed HPLC method, and forced degradation study.

HPLC method development:[10,12,20]

The wide variety of equipment, eluent, columns and operational parameters involved, makes high performance liquid chromatographic (HPLC) method development seem complex. The process is influenced by the nature of the analytes and generally follows the following steps:

Step 1 - Initial studies (Selection of HPLC method and initial conditions)
Step 2 - Selection of chromatographic conditions
Step 3 - Selectivity optimization
Step 4 - System parameters optimization

HPLC method Validation :

Validation of developed HPLC method can be carried out by using different parameters as per ICH guidelines. Validation can be defined as ‘‘The process of stabilizing documentary evidence demonstrating that a procedures, process or activity carried out in testing and then production maintains the desired level of compliance at all stages.’’

Typical validation characteristics :

These are as follows:

- ✓ System suitability
- ✓ Linearity and Range
- ✓ Accuracy
- ✓ Precision
- ✓ Robustness
- ✓ Limit of detection (LOD)
- ✓ Limit of quantitation (LOQ)

Forced Degradation Study :

Stability studies of drug substances via acid hydrolysis, oxidation, base hydrolysis, and thermal and photolytic stress testing are a part of development strategy under the ICH requirements. These studies provide information on a drug’s inherent stability and help to validate analytical methods to be used for evaluation stability. Therefore it is necessary

to conduct stability studies to predict, evaluate, and ensure drug product safety.[1,2,3,5,7,8,11,13,16,17]

Objectives :

Following are the objectives of current study:

- ✓ To search and study the literature on Stability Indicating HPLC method development and validation for simultaneous estimations of some combination drugs.

Literature review :

The review of literature for current study has been carried out. The scope of the review is to know and evaluate the existing methods in the literature derived from the guidelines of ICH for the aforementioned products. Literature review also evaluate the work published for stability indicating analytical procedure to estimate the active content, and majorly impurity profile in pharmaceutical dosage form which covers all process related impurities and degradation impurities.

Dimal A. Shah *et al*., Developed stability indicating HPTLC method for estimation of antihypertensive drug combination Nifedipine and valsartan.[7]

Ram Singh Bishnoi, Vishal Singh Solanki, Raviraj Baghel, Deepti Jain. RP-HPLC method development and validation for simultaneous estimation of Cilnidipine, Atenolol and Chlorthalidone.[5]

Mastanamma *et al*.[13] developed and validated stability indicating RP-HPLC method for simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and combined dosage form.[13]

Balasubramanian Narasimha *et al*.[3] developed Stability Indicating RP-HPLC Method and Validated for Oseltamivir API. The HPLC method validated for linearity, precision, ruggedness, and robustness.[3]

Ramaswamy developed and validated analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC.[16]

Kongkiatpaiboon *et al* developed and validated stability indicating HPLC method for determination of adrenaline tartrate.[11]

Shaikh *et al.*, developed and validated RP-HPLC Method for the Estimation of Cefpodoxime Proxetil in Bulk and Pharmaceutical Dosage.[17]

Ahmed *et al*. developed and validated RP-HPLC Methodfor simultaneous estimation of Metformin and Teneligliptin in Bulk and Pharmaceutical dosage form.[8]

Ansari *et al*. developed and validated Stability-Indicating Reverse Phase-High Performance Liquid Chromatography Method for Simultaneous Determination of Atenolol and Nifedipine in Bulk and Tablet Dosage Form.[1]

Shah *et al*., studied about stability indicating HPLC method development. Stability indicating HPLC methods are used to separate various drug related impurities that are formed during the synthesis or manufacture of drug product. This article discusses the strategies and issues regarding the development of stability indicating HPLC system for drug substance**.** A number of key chromatographic factors were

evaluated in order to optimize the detection of all potentially relevant degradants. The method should be carefully examined for its ability to distinguish the primary drug components from the impurities.[4]

Vasubabu Padarthi developed and validated Normal phase HPLC method for simultaneous analysis of Paracetamol and Ondansetron in fixed dose tablets.[15]

Methodology :

The methodology for developing and validating a stability-indicating HPLC method involves several systematic steps. Initially, appropriate HPLC methods and chromatographic conditions are selected, including mobile phase composition, detection wavelength, and flow rate. These parameters are optimized through trial and error to ensure efficient separation and peak resolution. Once the method is developed, it undergoes validation as per ICH guidelines, encompassing key parameters such as system suitability, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). Additionally, forced degradation studies are conducted under various stress conditions—acidic, basic, oxidative, thermal, and photolytic—to assess the drug's stability profile and ensure the method's specificity in detecting degradation products. This comprehensive approach ensures that the developed HPLC method is reliable, reproducible, and suitable for routine quality control of drugs in bulk and formulated products.

The literature survey shows that the stability indicating HPLC method can be developed and validated as per ICH guidelines for the estimation of drugs in bulk and pharmaceutical formulation. The HPLC method will be developed by different trials *i.e* by change in Mobile phase concentration, detection wavelength, and flow rate. Then, developed method can be validated with different parameters. The forced degradation study is also necessary to carry out to predict, evaluate, and ensure drug product safety. This stability indicating developed method can be used for routine analysis of drugs in bulk and pharmaceutical formulation.

References :

1. Ansari Yaasir Ahmed, Qazi Shoeb, Umme Rumana, Afroza Patel, Pathan Vahid Tajkhan, and Shaharukh Makrani. (2020). *International Journal of Pharmaceutical Quality Assurance. 11*(2): 219-223.
3. Balasubramanian Narasimhan, Abida Khan and Kona Srinivas. (2008). *Chem. Pharm. Bull. 56*(4): 413-417.
4. Shah Bhoomi P., Suresh Jain, K. Krishna Prajapati and Nasimabanu Y. Mansuri. (2012). *IJPSR, 3*(9): 2978-2988.
4. Chatwal Gurdeep R., and Sham K. Anand. (2015). Instrumental method of chemical analysis: Reprints-2015. Himalaya Publishing house; 2.624-2.639.
5. Bishnoi Ram Singh, Vishal Singh Solanki, Raviraj Baghel, and Deepti Jain. (2018). *Journal of Drug Delivery & Therapeutics. 8*(6s): 78-82.
6. Gary D. Christian. (2007). Analytical chemistry: 6th edition. Wiley India pvt. Ltd, New Delhi; 604-615.
7. Dimal A. Shah, Jaimin S. Patel, Priyanka Jadeja, Vandana B. Patel and K. Usmangani (2019). *Journal of Taibah University for Science. 13*(1): 722–730.

8. Ahmed Aejaz, G. J Khan, Tanveer Patel, K.I Molvi, and Yaasir Ansari (2019). *International Journal of Research in Advent Technology. 7*(4S): 33-37.
9. Kenneth A. Connors. (2011). A textbook of Pharmaceutical analysis: 3rd edition. Wiley India pvt. Ltd, New Delhi; 392, 411-412.
10. Kokil S.U. and M. S. Bhatia, (2009). *Indian J. Pharm. Sci., 71*(2): 111-114.
11. Kongkiatpaiboon Sumet, Nongnaphat Duangdee, Savita Chewchinda, Orapan Poachanukoon, and Kumpol Amnuaypattanapon. (2019). *Journal of King Saud University – Science. 31:* 48–51.
12. Madhavi *et. al*, (2014). *Int J Pharm Pharm Sci, 6*(1): 278-282.
13. Mastanamma, S.K., S.K. Chandini, S.K. Reehana, and P. Saidulu. (2018). *Future Journal of Pharmaceutical Sciences. 4:* 116-123.
14. Munson James W. (2001). Pharmaceutical analysis-modern methods: part-B, Reprint-2001. International medical book distributors, Mumbai; 15-85.
15. Padarthi Vasubabu (2016). *World Journal of Pharmaceutical Research. 5*(11): 756-764.
16. Ramaswamy Arun, Anton Smith and Arul Gnana Dhas. (2018). *Arabian Journal of Chemistry. 11:* 275–281.
17 Shaikh Siraj N, Sandeep S Kshirsagar, G J Khan, Patel M Siddik N, M Y Khalifa, Makrani Shaharukh I, Ansari Yasir A Razzaq, and Jayesh C Chaudhary (2021). *International Journal of Pharmaceutical Researeh* 13(2): 1513-1523.
18. Sharma, B.K. Instrumental method of chemical analysis: 26th edition. Goel publishing house, Meerut; C-286-385.
19. Skoog, D., J. Holler, T. Nieman. Principles of Instrumental analysis: 5th edition. Thomson information India pvt. Ltd, New Delhi; 725-739.
20. Siddartha, *et. al*. (2014). *Int J Pharm; 4*(1): 340-346.
21. Willard H., L. Merritt, J. Dean, and F. Settle. (1988). Instrumental methods of Analysis: 7th edit. CBS Publishers and Distributors Pvt. Ltd; 580-610.