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Review article

ADVANCES IN CHROMATOGRAPHIC SEPARATION METHODS FOR FORCED DEGRADATION PRODUCT ANALYSIS

Mishra Shweta*, Chauhan Ashlesha

Department of Pharmaceutical Chemistry, K.B. Institute of Pharmaceutical Education and Research, a constituent college of Kadi Sarva Vishwavidyalaya, Sector-23, Gandhinagar, Gujarat, India

Abstract

Putting new pharmaceutical compounds and finished pharmaceutical goods through a process of "forced degradation" involves subjecting them to more extreme conditions than "accelerated degradation." Procedures that indicate stability must be shown to be particular. It also helps in understanding the structure of the degradation products and provides insight into the paths and products of the therapeutic substance's deterioration. In order to help with the development of formulation and packaging, forced degradation studies may be used to ascertain the molecule's chemical behaviour. To add insult to injury, the regulatory guidance is very nebulous and offers zero details about how forced degradation experiments should be conducted. Hence, the present trends in forced degradation research performance are the focus of this work. In order to achieve this goal, a study design is provided, the reasons of deterioration are detailed, and analytical approaches that aid in the development of stability indicating methods are detailed.

Keywords: Conditions for degradation, Component that breaks out, Degradation by force, Method for showing stability, Evacuation evaluation

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*Corresponding Author

Introduction

The substance steadiness of drug atoms is a significant subject since it influences the helpful item's viability and wellbeing. Guidance from the FDA and the ICH states that information from steadiness tests is important to comprehend how the nature of a medication substance or medication item has changed after some time because of the effect of different ecological elements. Since it helps in choosing the right plan and bundling, as well as giving the right stockpiling conditions and time span of usability if necessary, realizing a particle's security is vital with regards to administrative desk work. Degradation products are an outcome of forced degradation, which entails breaking down medicinal items and compounds under conditions that are more harsh than rapid deterioration. The stability of the molecule may be determined by analysing these breakdown products. As per the ICH standard, stress testing is done to find out what degradation products to expect. This helps with finding out how stable the molecule is intrinsically, figuring out how degradation works, and making sure the stability indicating protocols are valid [1]. However, when it comes to the mechanics of stress testing, these regulations are rather nebulous and provide no guidance on how to implement forced deterioration. Official stability programmes do not entail forced degradation studies, despite the fact that they are necessary for scientific purposes and are required by regulators throughout the pharmaceutical development process.

Prior to submitting the registration dossier, stability investigations of the new medicinal moiety must be completed. It is now mandatory to comply with this rule. A twelve-month long-term study and a six-month accelerated stability study are the two main varieties of stability studies. The six-month-long intermediate studies, on the other hand, may use less rigorous conditions than the expedited trials. Consequently, a lot more time would be required to conduct the examination of degradation products, which involves separation, identification, and quantification. Forced degradation studies, in contrast to stability studies, are crucial in rapidly producing degradants, often within a few weeks. A stability indicating approach might be developed using samples generated by induced degradation. In the future, this technology may be used to analyse samples that are generated from rapid and long-term stability research [2]. With this evaluation, we want to provide some advice about the efficiency and effectiveness of forced deterioration in the development of a stability indicator method.

Flask Chromatography

Only thin-layer chromatography (TLC) does not use columns to separate compounds. This method is also known as chromatography at medium pressure. As a rule, positive gaseous tension is utilized to drive the dissolvable down the segment in streak chromatography. Since the dissolvable's stream is so limited, compressed gas is utilized to push the dissolvable down the fixed stage section. The strategy's capacity to work with a quicker pace of dissolvable stream is one of its numerous important elements. Since it considers the quick partition of particles from blends, streak chromatography is utilized in the medication advancement process [3]. This varies from the customary methodology that depends on gravity stream. Flash chromatography differs from conventional procedures in many aspects, some of which are:

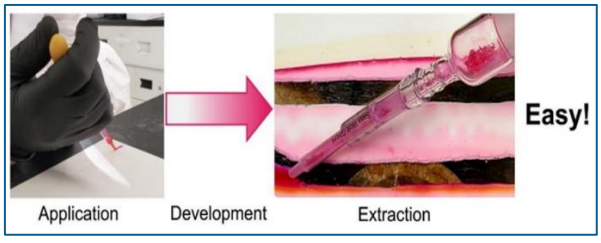
- It makes use of silica gel particles that are able to run through 250-400 mesh openings.
- The solvent is driven through the stationary phase column by means of pressurized gas at a pressure of about 10 to 15 pounds per square inch.

Flash chromatography is a powerful tool for simplifying complex mixtures into their component parts. The refining of synthetic goods, the separation of pure chemicals for more research, this separation, and the isolation of selected molecules from natural products are among the other uses. Silica is one of the most used stationary phase materials, and SiO_2 and Al_2O_3 are the two main adsorbent kinds. There is a relationship between the adsorbent particle size and the solvent flow [4]. No extra materials like cotton, glass, fleece, or sand are required when a permeable plate is secured to the section. Makers are presently dealing with mechanized streak chromatographic gadgets. Robotized streak chromatographic frameworks for the most part come in two flavours: low strain fluid chromatography (LPLC) and medium tension fluid chromatography (MPLC).

Technique for Thin-Layer Preparation

An often-used purification technology, preparative flimsy layer chromatography (prep TLC) is appropriate for limited scope response execution. Preparative meagre layer chromatography (prep TLC) is an essential piece of the "covered up educational program" of research facility practice because of the changed and non-standard clarifications of the recommended system for stacking, running, and recuperating tests. Our simple and cost-effective methods for loading and collecting plate samples are detailed in this article. These methods help make this technology more user-friendly and improve its speed and accuracy [5].

Figure 1: Technique for Thin-Layer Preparation



HPLC for Preparation

Large columns and high flow rates are often associated with preparative HPLC. Conversely, the success or failure of a preparative HPLC experiment is determined by the separation's intended use [6]. This is valid irrespective of the system's capacity or the amount of mobile phase being pushed through it. The purpose of an analytical HPLC run is to identify the molecule's quantitative and qualitative properties. Isolating and purifying a valuable product is what a preparative HPLC run is all about (Table 1). Preparative high-performance liquid chromatography (HPLC) is on the pricier side when compared to other standard purification methods like distillation, crystallization, or extraction. Therefore, it has only been used to rare or costly things. The increasing need for the synthesis of very pure compounds in a range of amounts for use in activity, toxicological, and medicinal screens is causing shifts in the operational field of preparative high-performance liquid chromatography (HPLC) [7].

Table 1: Analytical and Preparative HPLC: A Definition

Analytical HPLC	Preparative HPLC
Sample goes from detector into waste	Sample goes from detector into fraction collector
Goal: Quantification and/or identification of compounds	**Goal:** Isolation and/or purification of compounds

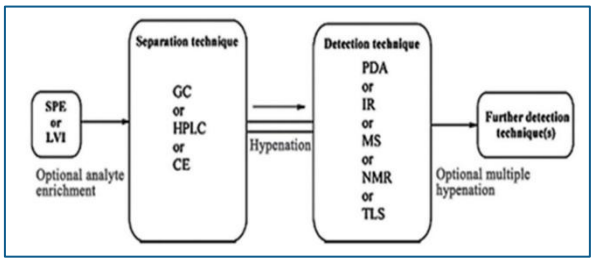
A Critical Assessment of Hybrid Methods

Using chromatography, one may isolate chemically pure or almost pure fractions from a mixture. Spectrophotometry may give some useful information for identification by comparing the sample to a database of known spectra. At the point when a partition method and an internet based spectroscopic identification innovation are consolidated, a joined methodology is created.1. A "hyphenated technique" is two separate analytical procedures that are combined or linked via an acceptable interface [8]. The term "hyphenated techniques" refers to a broad category of methods that include separation-separation, identification-identification, and identification-separation processes [9]. Hirschfeld first used the term "hyphenation" in 1980 to describe the potential for merging many instrumental analytical processes into one run (Hirschfeld, 1980). As one would expect, the goal of this coupling is to improve upon the detection capabilities of each individual analytical technique in order to provide more comprehensive identification and quantification data [10].

The advantages of hyphenated technique are mention below:

- Hyphenated Methods' Benefits
- Quick and precise evaluation
- Increased level of mechanization
- Efficient sample processing
- Enhanced repeatability
- Lessening of pollution because to its sealed design
- Concurrent separation and quantification accomplished.

Figure 2: A schematic depicting the hyphenation of spectroscopic and chromatographic methods

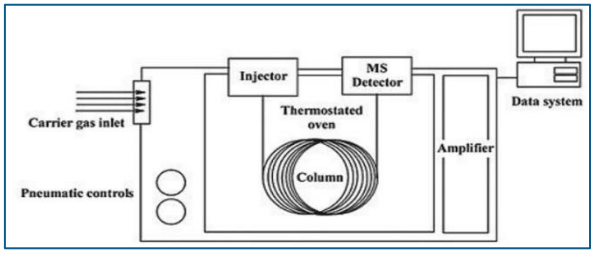


The list of hyphenated techniques is mention below:

GC-MS:

Vapour and semi-volatile chemicals may be separated using gas chromatography (GC), but their identities cannot be determined by this method. Mass spectrometry (MS) differs in that it can identify chemicals by revealing their molecular structures, but it can't separate chemical compounds. These two approaches eventually merged with the development of GC algorithms [11]. The principal joined technique was gas chromatography-mass spectrometry (GC-MS), which can convincingly demonstrate the presence of natural unpredictable semi-unstable mixtures and leftover solvents. Some characteristics, such thermal stability and the ability to be volatile, are required for GC-MS analysis of the chemical [12]. These processes are very complementary as they both involve the sample when it is in the vapour phase. But since the carrier gas is present in gas chromatography (GC), which works at a high strain (760 torr), the two methodologies are totally unrelated. Mass spectroscopy, interestingly, requires a vacuum with a strain between 10-6 and 10-5 torr [13].

Figure 3: Schematic Presentation of GC-MS

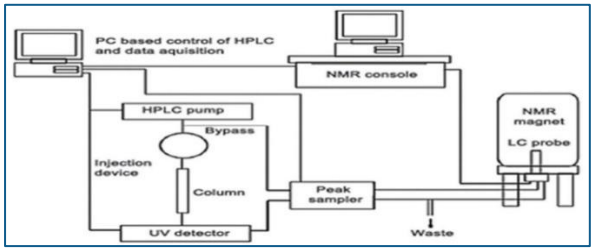


LC-MS:

Liquid chromatography-mass spectrometry is a technology that can separate compounds by combining the advantages of mass spectrometry with liquid chromatography. A combination of high-performance liquid chromatography (HPLC) for impurity and degradation product separation and mass spectrometry for item identification and molecular weight allows for

comprehensive analysis. Long-lived mass spectrometry (LC-MS) is a highly selective method. This is why LC-MS is considered specific; it can detect and identify molecules even in the presence of other substances. The high-performance liquid chromatography (HPLC) system is difficult to accommodate in a mass spectrometry vacuum since its flow rate is around one millilitre per minute. Also, the diluent has to be vaporised, which might harm the thermally labile compounds due to the high heat [14]. Both approaches were able to have their capabilities enhanced by increasing the hyphenation.

Figure 5: Schematic Presentation of LC-NMR

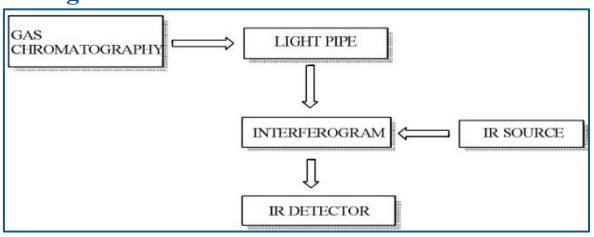


EC-MS:
In order to create EC-MS, two instruments are combined: electrochemistry (EC) and mass spectrometry (MS). In this way, electrochemical oxidation may be carried out, and the resulting product can be sent straight to the ESI interface so that nuclear magnetic resonance (MS) can track intermediates with short half-lives.

CE-MS:
Chemical elution mass spectrometry (CE-MS) is a method for online separation that distinguishes molecules according to their structural information and electrophoretic mobilities [16]. By using capillary electrophoresis (CE), the components may be separated, and their mass can be used to identify them. In addition to being fast and efficient, this method only needs a little quantity of sample and solvent for analysis. The CE is connected to the MS with the use of lengthy capillaries. The time needed to complete the analysis will rise as a consequence of this. Also, there isn't enough volatile buffer, which is incompatible with the MS, to go around.

GC-IR:
By integrating gas chromatography with infrared spectroscopy, a technique called GC-IR is created. Infrared spectroscopy is a non-destructive technique, which makes this procedure both incredibly sensitive and expensive [17]. It also makes sample recovery possible. Here, infrared spectroscopy (IR) serves as the identification tool, while gas chromatography (GC) handles the separation part. Gas chromatography allows for the separation of the analyte's components. These parts will be carried along by the column. Both of these procedures are set up using vacuum tubes or a glass column. A small glass pipe with an internal gold coating and a narrow tube connecting it to the column serves as the interface in this approach [18]. By heating the light pipe, condensation may be eliminated and the channel length can be maximized. Enhanced sensitivity is the end consequence of this.

Figure 6: Schematic Presentation of GC-IR



LC-MS/MS:
With LC-MS/MS, you can recognize in excess of 300 unique compound classes with just a little infusion volume. Time burned through dissecting and energy put into pre-treating tests are both cut down. It all beginnings with LC-MS in the LC-MS/MS method. This approach is undeniably more exact and delicate than the LC-MS [19]. Contrasted with the LC-MS gear, its awareness is 20-100 times higher. There is a second separating process in this strategy, making it more careful than others.

GC-MS/MS:
This approach achieves its goals by combining gas chromatography with tandem mass spectrometry. Among its potential applications is ultra-trace analysis, thanks to the method's sensitivity and specificity. Qualitative identification using mass spectrometry and ion traps is possible by a variety of approaches, including product ion scans, precursor ion scans, neutral loss using a triple quadrupole, and product scans. Recent years have shown an increase in scanning speed and a rise in quadrupole sensitivity rather than a decline [20].

GC×GC-MS:
In this approach, two-dimensional gas chromatography and mass spectroscopy work together. Because of this link, the GC peaks will be more precisely resolved. The analyte may not always be evenly distributed during the whole retention time in one-dimensional gas chromatography. Using the two-dimensional GC improved the resolution. Mass spectrometry is able to detect trace levels of analyte components because two-dimensional gas chromatography successfully separates all of the analyte components [21].

GC-NMR:
This approach combines gas chromatography with nuclear magnetic resonance. Gas chromatography (GC) is used for component separation, whereas nuclear magnetic resonance (NMR) is employed for component identification. Hyphenation is the mechanism that gives the molecules the structural details of the separated components [22].

GC-AES:

This approach aims to achieve a synergy between gas chromatography and atomic emission spectroscopy. Element analysis makes use of a variety of techniques, one of which is atomic emission spectroscopy [23]. GC is in charge of the component separation, and elemental identification is done all through the separation process with the help of AES. By using GC, the elemental composition of each isolated peak may be determined.

Conclusion

Forced degradation tests provide information on the active components' likely degradation pathways and products, which may help us understand the degradants' structures. By using degradation products generated by forced degradation research, stability indicating techniques may be devised. The development of these procedures is aided by these degradation products, which are hypothetical byproducts that may be created under suitable storage conditions. Starting degradation studies early in the medication development process is preferred. There is enough time to learn more about the molecule's stability throughout this period. As a result, the formulation's manufacturing process and storage conditions may be fine-tuned using the right data. The experimenter should utilize presence of mind to direct this exploration since there is nobody set of conditions that can be applied to all medication items and medication substances and in light of the fact that the administrative direction doesn't determine the circumstances that ought to be utilized. The ideal level of decay, which might be somewhere in the range of 5% to 20%, is the end point of a technique utilized for constrained disintegration. A well-planned and executed forced degradation study is required to provide a sufficient sample for the development of a stability signalling method.

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Conflict of Interest

The author declares no conflict of interest, financial or otherwise.

References

1. International Conference on Harmonization (ICH). Q1A(R2): Stability testing of new drug substances and products (revision 2). 2003. Available from: http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm128204.pdf.
2. Reynolds DW, Facchine KL, Mullaney JF, et al. Available guidance and best practices for conducting forced degradation studies. Pharm Technol. 2002;26(2):48-56.
3. Still WC, Kahn M, Mitra A. Flash chromatography. J Org Chem. 1978;43(14):2923-5.
4. Kaushal C, Srivastava B. A process of method development: a chromatography approach. J Chem Pharm Res. 2010;2(2):519-45.
5. Leonard J, Lygo B, Procter G. Advanced practical organic chemistry. 3rd ed. Boca Raton, FL: CRC Press; 2013.
6. Reynolds MR, Piazza S, Chiaramonte J, Chapa-Villarreal FA, Trant JF. New J Chem. 2021.
7. Unger KK. Handbuch der HPLC. Darmstadt: GIT Verlag; 1994.
8. Merrifield RB. Solid phase peptide synthesis. J Am Chem Soc. 1963;85:2149.
9. Guo X, Lankmayr E. Hyphenated techniques in gas chromatography. Graz University of Technology, Austria; 14-19.
10. Duncan WP, Kirk O. Encyclopedia of chemical technology. Vol. 2. California: John Wiley & Sons; 1992.
11. Hites RA. Gas chromatography-mass spectrometry. In: Handbook of instrumental techniques for analytical chemistry. New Jersey: Prentice Hall; 1997. p. 609-10.
12. Settle FA. Handbook of instrumental techniques for analytical chemistry. Delhi: Pearson Education; 2004.
13. Premstaller A, Oberacher H, Walcher W, Timperio AM, Zolla L. Chem Anal JP et al. 2001;73:2390.
14. Barroso B, Lubda D, Bischoff R. J Proteome Res. 2003;2:633.
15. Lianghai H, Mingliang Y, Xiaogang J, Shun F, Hanfa Z. Advances in hyphenated analytical techniques for shotgun proteome and peptidome analysis—a review. Anal Chim Acta. 2007;598:193-204.
16. Pasch H, Heinz LC, Macko T, Hiller W. Pure Appl Chem. 2008;80(8):1747.
17. Claridge TDW. High-resolution NMR techniques in organic chemistry. Elsevier Science Ltd; 1999. p. 228.
18. Vogel M, Karst U. Electrochemistry–mass spectrometry: an emerging hyphenated technique for bioanalysis. Springer-Verlag. Anal Bioanal Chem. 2012;403:333-4.
19. Cai J, Henion J. Review: capillary electrophoresis-mass spectrometry. J Chromatogr A. 1995;703:667-92.
20. Ghulinyan M, et al. Gas chromatography-infrared spectrometry (GC/IR). Appl Phys Lett. 2006;88:241103.
21. Liu Z, Phillips J. Comprehensive two-dimensional gas chromatography using an on-column thermal desorption modulator interface. J Chromatogr Sci. 1991;29(6):227-31.
22. Buddrus J, Herzog H. Coupling of chromatography and NMR: study of flowing gas chromatographic fractions by proton magnetic resonance. Org Magn Reson. 1981;15(2):211-3.
23. Herzog H, Buddrus J. Coupling of chromatography and NMR. Part 5: analysis of high-boiling gas-chromatographic fractions by on-line nuclear magnetic resonance. Chromatographia. 1984;18(1):31-3.