A REVIEW ON REVERSE PHASE GREEN LIQUID CHROMATOGRAPHY USING ALTERNATIVE SOLVENTS FOR ANALYSIS

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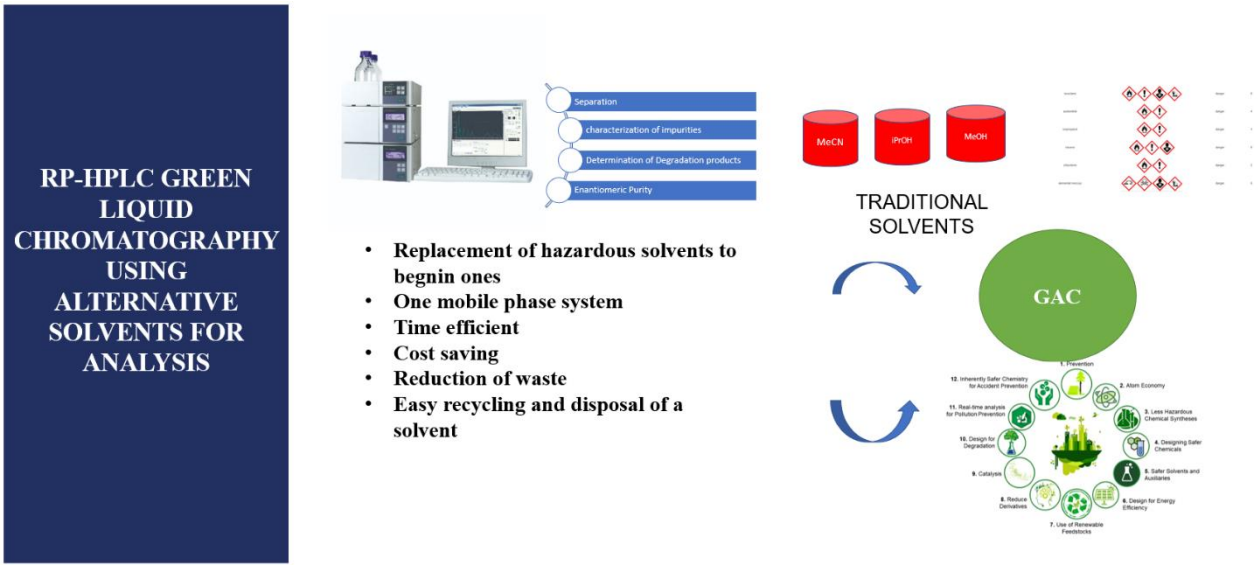
ABSTRACT:

One of the main aims of green analytical chemistry (GAC) is the reduction of solvents and chemicals consumed. Recycling the mobile phase in chromatographic techniques provides an efficient way to implement GAC principles. However, this is not an easy job, particularly in the case of the gradient mode. Analysis of multi-pharmaceuticals for the same manufacturer using one mobile phase system dramatically reduces consumed solvents, time, and cost for pharmaceuticals analysis in quality control laboratories. This work is an attempt to reduce time, cost and effort needed for quality control analysis of several dosage forms produced by the same manufacturer. Our novel and green RP-HPLC method is able to separate and quantify a mixture of components produced by the same manufacturers.

The quality of a pharmaceutical product is directly related to health of patients. During the development of a new analytical method, as liquid chromatography, must be considered various factors, such as reliability, detection and separation of all compounds of interest, speed analysis, reduced need for pretreatment of the sample, low final cost analysis, and use of non-toxic reagents neither for the operator or for the environment. The use of simple and easy methods of execution, fast, precise, accurate and environmentally friendly becomes more and more interesting for the pharmaceutical industry in Quality Control of drugs and medicines. The lack of environmental friendly methods for the analysis of pharmaceuticals with minimum generation of toxic waste and the consciousness of the analytical decisions is a gap that currently drives the research groups.

KEYWORDS: Green liquid chromatography; Reversed-phase chromatography; alternative solvents; ethanol; micellar liquid chromatography.

GRAPHICAL ABSTRACT



INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

MATERIAL AND METHODS

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. Column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance. [6]

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

Based on Principle of Separation

Liquid/solid chromatography (Adsorption): LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.[3]

Liquid/Liquid chromatography (Partition Chromatography): LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.[1,2]

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions. [3]

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers. [3]

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity. [3]

Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength. [4]

Based on the scale of operation

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC.[5]

Based on type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample.[6]

Greening Reversed-Phase Liquid Chromatography

High-performance liquid chromatography (HPLC) is the most widely used analytical tool for pharmaceutical analysis. Indeed, it is the most important technique involved in the quality

control of bulk drugs and pharmaceutical formulations (i.e., analysis of active pharmaceutical ingredients (API), characterization of impurities, determination of degradation products to test product stability, and determination of enantiomeric purity), and also in the determination of drugs and metabolites in biological samples[7]. Most HPLC methods developed in pharmaceutical laboratories are based on the reversed-phase (RP) mode, using a hydrophobic stationary phase and a polar mobile phase. In quality control, the primarily used detection mode is ultraviolet (UV)/Visible detector. Therefore, mobile phase compatibility with this detection is a parameter often taken into account in pharmaceutical analysis development. The mobile phase of RP-HPLC is usually a mixture of water (containing additives to adjust pH and ionic strength) and organic solvent, such as acetonitrile (ACN) and methanol (MeOH)[8]. These two solvents are by far the preferred organic solvents used in RP-HPLC because of their remarkable combination of properties favorable for RP-HPLC applications. Among them include complete miscibility with water, relatively low viscosity of their aqueous solutions (especially in the case of ACN), low UV cut-off wavelength (190 nm and 205 nm for ACN and MeOH, respectively), availability in the high purity required for HPLC, and low chemical reactivity with most sample species, as well as with HPLC instrument and column surfaces[9].

Despite these remarkable properties, ACN and MeOH present some issues in terms of environmental impact and health safety. Indeed, ACN is flammable, volatile, and toxic. Even if MeOH is less toxic and more easily biodegradable than ACN, it is also ranked as a hazardous solvent due to its inherent toxicity and the great requirements of its waste disposal[10]. Unfortunately, the amount of waste generated by RP-HPLC analyses cannot be neglected. In fact, one continuously operating liquid chromatograph equipped with a conventional LC column (15–25 cm length, 4.6 mm i.d., packed with 5 µm particles) and a mobile phase flow rate of 1 mL/min produces about 1.5 L of waste per day, meaning about 500 L of effluent per year [11]. Although this volume of waste is small compared to the amount of waste generated by large industrial manufacturing companies, some big pharmaceutical companies use hundreds of liquid chromatographs in their research and development, and quality control laboratories, resulting in thousands of liters of toxic waste produced every day. Moreover, the use of HPLC is becoming more and more intensive due to the technological advances allowing high-throughput analysis, which also increases, at the same time, the amount of waste produced. These HPLC waste streams containing ACN and MeOH must be disposed as chemical waste, which is costly and adds to the environmental waste-disposal burden of the laboratory.

Regarding the health and environmental issues of organic solvents commonly used in RP-HPLC, the greening of RP-HPLC methods has received great interest in the analytical community, whose aim is to search for new alternatives to replace polluting analytical methods with cleaner ones. Green analytical chemistry (GAC) emerged from green chemistry in the 2000s [12], and has gained increasing attention and acceptance among researchers. Its concept refers to eliminating or reducing hazardous chemicals from analytical processes to improve environmental and health friendliness, without compromising method performance [13].

Based on the 12 principles of green chemistry, some strategies are commonly implemented to achieve greener liquid chromatography methods. They focus on a reduction in solvent consumption through a decrease in column length, internal diameter, and/or column particle; the replacement of toxic and hazardous solvents, such as ACN and MeOH, with less toxic and more environmentally friendly alternatives; and by increasing the importance of recycling in larger scale preparative separation technologies[14]. Chromatographic techniques have the potential to be greener at all steps of the analysis, from sample collection and preparation, to separation and final determination. Some strategies for greening chromatographic methods are more efficient than others; therefore, evaluation methods are needed to assess the greenness of analytical methods. Some tools have already been developed; the two best known are the NEMI labelling and analytical Eco-scale methods . NEMI labelling results in an easy-to-read pictogram stating if hazardous or corrosive reagents are used or if the procedure generates significant amounts of waste. The analytical Eco-scale is a more quantitative approach, based on subtracting penalty points from a total of 100, based on the amount and hazard of reagents, energy consumption, occupational hazards, and amount of waste generated [15].

Over the past years, several reviews have covered the application of GAC principles to chromatography analysis in general, while some focused especially on pharmaceutical analysis. Since it is difficult to eliminate the use of organic solvents in RP-HPLC, the better way to make this technique greener is to replace hazardous solvents with more benign ones. Solvent-reduction strategies can also be successfully followed to achieve a method even more ecological. However, an overall reduction in the amount of solvent used, and consequently the waste generated, often implies the purchase of expensive ultra-high-performance liquid chromatography (UHPLC) instruments or the development of new technologies. This review will present recent advances in greening RP-HPLC methods dedicated to pharmaceutical analysis through the use of alternative solvents.[16]

Alternative Organic Solvents in RP-HPLC Mobile phases in RP-HPLC are classically mixtures of water, containing additives to adjust pH and ionic strength, and organic solvent. Acetonitrile and MeOH are the two organic modifiers most widely used by HPLC users in the RP. Unfortunately, both solvents are ranked hazardous due to their toxic effect and the great importance placed on the safe detoxification of their waste, even though MeOH is considered more environmentally friendly than ACN, and therefore, should be preferred whenever possible. Since it appears difficult to develop a RP-HPLC method without an organic solvent, a strategy to make this technique greener is to replace ACN and MeOH with other less toxic organic solvents to minimize the environmental and health impacts. The greenness degree of an organic solvent is assessed based on its environmental, health, and safety (EHS) criterion and life-cycle assessment (LCA). The EHS assessment is composed of environmental indicators (e.g., water and air hazards, and persistency), as well as indicators related to human health (e.g., chronic and acute toxicity, and irritation) and safety (e.g., stability, reactivity, flammability, explosion, and release

potential) hazards. It allows the assessment of possible hazards inherent to the solvent properties. The LCA method is used for a detailed assessment of emissions into the environment, as well as resource use over the full life cycle of a solvent. It includes the production, use, potential recycling, and disposal of a solvent. In other words, LCA allows the quantification of environmental impacts that are indirectly attributable to the use of solvents. An organic solvent will be preferable in terms of greenness if it combines good EHS and LCA assessments. Several general solvent selection guides (SSGs), based on EHS only or EHS combined with LCA, have been published[17]. The SSGs have mainly been developed by pharmaceutical industry companies, and they present some differences in the classification of solvents which can be considered as green. These guides have been largely developed to be applied to organic synthesis, and thus, are not adapted to solvent selection for analytical chemistry applications. The organic solvents commonly accepted as green, and which can be used in RP-HPLC, are ethanol, isopropanol, n-propanol, acetone, ethyl acetate, ethyl lactate, and propylene carbonate.[18]

CONCLUSION

The main target is to reduce the consumption of hazardous solvent and to replace toxic and environmentally hazardous solvents with more benign alternatives.

In addition, reducing the flow rate in order to miniaturizing the waste generation is also within the field of green chromatography. On the other hand, the most effective technique for the "Green" approach can be miniaturized completely. Flow rate decreases from mL to microliter per minute. Thus, waste production and solvent consumption are significantly reduced. Recently, studies without organic solvents such as SFC, SBWC are frequently encountered.

This review discusses the approaches used to achieve the goals of green chromatography, aimed at protecting the environment and the analyst in pharmaceutical analysis. While one cannot completely convert the analysis to the green, the steps taken to green are very valuable, particularly in wide range used HPLC both in drug quality control laboratories in industry and in research studies.

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