Recent advances in various chromatographic techniques used for analysis of drugs in pharmaceutical products: A review

Chaitanya A Gulhane 1, Ojaswini A Fuladi 1*, Ravindrakumar L Bakal 1 and Jagdish V Manwar 2

1 IBSS Dr. Rajendra Gode Institute of Pharmacy, Mardi Road, Amravati-444602, MS, India.
2 IBSS Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati-444602, MS, India.

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Abstract

Chromatography is a vital biophysical method that allows for the separation, identification, and filtering of components in order to conduct quantitative research. High efficiency, quicker separation speed, high flow rate, increased detection sensitivity, and cost savings are all advantages of novel chromatography methods. The goal of using chromatography, which is a technique for quantitative investigation separated from its partition, is to accomplish a satisfactory separation in a reasonable amount of time. The objective of chromatography is to analyze a sample qualitatively and quantitatively and its primary function is to clean and separate at least one component of the sample. It examines new and emerging chromatography techniques, as well as main standards. Phospholipids can be determined qualitatively and quantitatively using a variety of analytical procedures. Current new chromatographic methods are presented in this review.

Keywords: Chromatography; Purification; Novel; Analytical; Identification; Application

1. Introduction

Chromatography is a scientific technique for separating components of a mixture into distinct entities. It is derived from Greek word, Chromo-Colour, and Graphic-Writing. Chromatography was first utilized in Russia in 1900 by Russian botanist Mikhail Tsvet [1]. The kind of connection between stationary phase, mobile phase and substances contained in the mixture is the fundamental part is effective and division of particles from one phase to another. Chromatography strategies based on partition are very effective. The purpose of applying chromatography is a quantitative analysis apart from its separation. Stationary phase in chromatography is solid phase and liquid phase; liquid phase is coated on surface of solid phase [2]. Mobile phase flowing over the stationary phase is gas or liquid phase. Liquid chromatography is used when the mobile phase is liquid, while gas chromatography is used when the mobile phase is gas. Apart from its separation, it is utilized as a method of quantitative analysis to accomplish sufficient separation within a molecular weight of protein. RNA, DNA particles, and viruses are purified using agar gel chromatography [3].

2. Classifications

Classification of chromatography is given in figure 1.
Figure 1 Classification of the Chromatography

2.1.1. Novel and recent chromatography techniques
- Paper chromatography Hybridization Assay (PACHA)
- Hydrophobic - Interaction chromatography
- Optical- force chromatography
- High performance and Immune- affinity chromatography
- Mixed - mode chromatography
- Dye- ligand chromatography

3. Paper Chromatography Hybridization Assay (Pacha)

3.1. Principle
It is a DNA hybridization method based on chromatographic relocation of DNA on a nitrocellulose strip that passes through an immobilized test area. PACHA is easier to utilize and has more strength. When PCR enhanced target DNA is introduced to one end of a nitrocellulose strip, narrow powers immobilized in the response zone allow DNA to migrate to the strip far edge [4]. It is located in the middle of a section of a small human papilloma infection that is in the process of hybridization. Hybridization proficiency is limited in this method by the hybridization arrangement stream speed and the volume of improved DNA migrating over immobilized test. This novel technique assures successful hybridization in a variety of tests and appears to be superior to the already available strong stage hybridization method (Fig.2) [5, 6].
4. Hydrophobic-Interaction Chromatography (HIC)

4.1. Principle

HIC works on the same principles as ion-exchange and size exclusion chromatography. When sample molecules with hydrophobic and hydrophilic districts are applied to an HIC section in a high salt buffer, the test solutes are less likely to survive. Hydrophobic regions that get revealed are absorbed by the media as salvation fades. Less salt is predicted to enhance binding as the particle becomes more hydrophobic. [7, 8].

4.2. The chain length parameter:

Shaltiel advocated varying the immobilizer alkyl chain length as a homologous sequence of hydrocarbon coated agarose as the first efficient technique to deal with protein purification. The important finding was that increasing the length of the chain by -CH2- units, increased the strength of protein restricting from impediment to reversible restricting all the way to extremely tight binding. In this approach, it was proposed that the features of hydrophobic agarose for protein purification may be optimally regulated by varying the length of the immobilizer alkyl chain and determining the optimal length for adsorbing and eluting the protein (Fig.3) [9, 10].
4.3. Factors
Ligand, Matrix, salt concentration, PH and temperature

4.4. Advantages
- Increase the detection – Sensitivity
- High flow-rated
- High efficiency
- Faster separation speeds.

4.5. Disadvantages
Significant baseline changes during gradient elution and a requirement for non-volatile mobile phases which complicates peptide isolation.

5. Optical force chromatography

5.1. PRINCIPLE
The balance between optical and liquid drag power acting on particles is required for optical force chromatography [11]. In small size study spanning several logical fields, the use of laser light as a tool for controlling tiny molecule suspension for natural, thermodynamic, and micro fluidic purposes has been realized. For molecular partition, optical chromatography involves freely centering a laser into a liquid stream flowing in the opposite direction of the laser propagation path (Fig.4) [12, 13].

![Figure 4 Optical Force Chromatography](image-url)
6. High performance and immuno-affinity chromatography (HPAC) AND (IAC)

6.1. Principle

6.1.1. High-performance affinity chromatography (HPAC)
In an HPAC system, it is a method in which an organically related ligand is used as the stationary phase. HPAC has been used in recent years to investigate the relationship between drugs, chemicals, and other substances and serum proteins. This method is used for detaching or quantifying specialists in complicated samples [14].

6.1.2. Immuno-affinity chromatography (IAC)
Immuno-affinity chromatography combines the use of liquid chromatography with the specific restriction of antibodies or other specialized agents. It is used for filtering and centralization of data prior to further analysis using another method. Antibody or antibody-related reagent is the stationary phase. Filtration, immune depletion, direct sample analysis, immunoassay, and joint examination procedures are all approaches that utilize it (Fig.5) [15].

![Figure 5 Immuno-affinity Chromatography for protein Purification](image)

7. Mixed-Mode Chromatography (MMC)

7.1. Principle
Multimodal chromatography is another name for mixed mode chromatography. Mixed mode media combine complementing chromatography methods inside a same medium, reducing the number of segment processes necessary throughout the purifying process. In a single ligand, mixed mode components contribute affinity, such as binding and selectivity (Fig.6) [16, 17].

![Figure 6 Types of Bimodal Mixed mode columns chromatography](image)
7.2. Elements
- Hydroxypepatite electrostatic and calcium co-ordination complexes.
- Hydrophobic ion exchange ligands.

7.3. Advantages
- Salt-tolerant adsorption
- Unique selectivity
- Cost savings
- Longer column-life times

7.4. Disadvantages
- Complicated
- Unpredictable
- Require extensive resources

8. Dye ligand chromatography
It is a purification method with a high degree of selectivity and purification rates. The capacity of multiple compounds to purine nucleotides for cibacton blue is required for improvement of this procedure [18]. The planar ring structure with negatively charged bunches resembles NAD design. The adsorbed proteins are isolated from the column under suitable PH conditions, elution with high ionic strength arrangements and using particles that trade adsorbent properties (Fig. 7) [19, 20].

![Dye Ligand Chromatography](image)

**Figure 7** Dye Ligand Chromatography

9. Application areas of chromatography in medicine
- In pharmaceutical analysis, there are a variety of applications.
- In the food and beverage sectors, there are applications.
- In chemical industry applications.
- Applications in the field of forensic science.
- Environmental science applications

10. Results and discussion
Novel and current chromatographic methods are presented in this review. As a result, novel chromatography techniques have a high efficiency as well as a faster separation speed. Amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, medicines, antibiotics, and steroids have all been purified using this chromatography technology. In effluent and surface water samples, new chromatographic methods aid identification and quantification.
11. Conclusion
A new chromatographic technique has been devised that is simple, precise, quick, accurate, and repeatable. As in the case of herbal pigments, chromatographic methods were utilized to separate compounds depending on their colour. The new chromatographic approach enhances productivity, resolution speed, time consumption, and sensitivity. In the future, three-dimensional chromatographic separation will be possible, which will be useful in a variety of industries. Three-dimensional separation methods may provide a route to large peak capacities. Multidimensional separation is attractive and is commonly employed to separate complicated sample.

Compliance with ethical standards

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Nil.

References
[1] Nielsen SS. Food Analysis. Food Science Text Series, Springer, USA. 2010; 602(4): 4419-1478.
[2] Hostettmann K, Marston A, and Hostettmann M. Preparative chromatography techniques: Application in natural product isolation. Berlin: springer. 2011; (2): 653-657.
[3] Gerberding SJ, Byers CH. Preparative ion- exchange chromatography of proteins from dairy whey J. Chromatography A. 1998; 808: 141-51.
[4] World health organization expert committee on onchocerciasis.Third report world health Organization technical representative series.1987; 753: 167.
[5] Boatin BA, Toe L, Alley ES, Dembele N, Dadzie KY. Diagnostics in onchocerciasis: Future challenges. Ann Tropical Medicine and international health parasitol. 1998; 92: 541-545.
[6] Maizels RM, Bradley JE, Helm R, Karam M. Immuno diagnosis of onchocerciasis: Circulating antigens and antibiotics to recombinant peptides. Acta Leiden. 1990; 59: 261-270.
[7] Amsterdam A, Er-El z and shaltiel S. Ultrastructural identity of hydrocarbon agaroses Israel Journal of medical science. 1974; 10: 1580.
[8] Demiroglou A and Jennissen HP. synthesis and protein binding properties of spacer free thioalkyl agaroses. Journal of chromatography. 1990; 520: 1-7.
[9] Jennison HP. cyanogen bromide and tresyl chloride chemistry revisited: the special reactivity of agaroses as a chromatographic and biomaterial support for immobilizing novel chemical group. Journal of molecular recognmition. 1995; 8: 116-124.
[10] Kohn J and Wilchek M. procedures for the analysis of cyanogens bromide Activated sepharose or sephadex by quantitative determination of cyanate esters and imidocarbonates. Analytical biochemistry. 1981; 115: 375-382.
[11] A Terray, J Oakey, DWM Marr. Applied physics letters. 2002; 81: 1555-1557.
[12] SJ Hart, AV Terray. Applied physics letters. 2003; 83: 5316-5318.
[13] T Imasaka, Y Kawaabata, T Kaneta, Y Ishidzu. Analytical chemistry. 1995; 67: 1763-1765.
[14] Martin M. Guichon, G. Effects of high pressures in liquid chromatography. J. Chromatog. A. 2005; 7(1-2): 16-38.
[15] Hage DS, Phillips TM. Chapter 6 Immunoaffinity chromatography. In: Hage DS, editor. Handbook of affinity chromatography. NY, USA: Taylor and Francis. 2006; 715(1): 3-28.
[16] DR Nau, in: M.T.W. Hearn. HPLC of proteins, peptides and polynucleotides: contemporary topics and applications, VCH Publications, New York, NY. 1991; 70(11): 331.
[17] Jandera P, Urban J, Skerkovi V, Langmaier P, Kubickova R, Planeta J, J Chromatogr A. 2010; 1217: 22-23.

[18] Amicon, Dye ligand chromatograph. Applications method. Theory of matrix gel media, Am icon Division, N.R. Grace and company - conn. cherry hill drive. 1923; (24): 1989.

[19] Scopes R.K. Use of dierncial dye ligand chromatography with affinity elution for enzyme Purification: 2-keto-3-deoxy-6-ophospho-glucolate aldolase from zymomonas mobilis. Anal Biochemistry. 1984; 136: 525-9.

[20] Cutter p. Methods in molecular biology, dye ligand affinity chromatography, second edition. Humana press. 2004; (2): 481.