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ISOLATION OF PHYTOPHARMACEUTICALS BY FLASH CHROMATOGRAPHY.

[1*]Ms. Akshada Kale, [1]Dr. Anilkumar Aher, [2]Dr. Jitendra Nehete, [3]Ms. Nutan Navale, [4]Ms. Mayuri Morade

[1*, 2] Department of Pharmacognosy, MGV's College of Pharmacy, Nashik.

[1, 3, 4] Department of Pharmacognosy, MVP's College of Pharmacy, Nashik.

Abstract: The most traditional technique for isolating phytoconstituents from plant extract is column chromatography. Column chromatography is a very inefficient method that can cause any process to stall out rapidly. This led to the invention of flash chromatography, a revolutionary preparative liquid chromatography method in which the mobile phase flows downward under positive air pressure. Flash chromatography is based on an air pressure system that combines short and medium column chromatography, and is designed to make the purification of synthetic products quick and simple. This method of preparative liquid chromatography is straightforward, quick, and affordable. In previous years, the use of such systems for the separation of complicated natural product combinations, like extracts, was disregarded; however, these days, flash chromatography is becoming more and more popular for the separation and isolation of phytopharmaceuticals from plant extract. For such ingredients, it has proven particularly challenging to purify a number of phytopharmaceuticals using traditional methods. The preferred technique is flash chromatography. This article focuses on the new Flash Chromatography method for isolating phytopharmaceuticals.
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Index Terms - Flash chromatography, Phyto-pharmaceuticals, Separation and isolation, Natural products.

I. INTRODUCTION

Two types of column chromatography are distinguished based on how the solvent descends the column. Gravity column chromatography is the process of allowing the solvent to percolate, or flow, down the column. Flash chromatography is the process where the solvent is driven down the column by positive air pressure. (ROGE). Essentially, flash chromatography is a mix of shorter column chromatography and medium-pressure chromatography driven by air pressure, specifically designed for extremely quick separation. A common method in the drug-discovery process is flash chromatography, which divides mixtures of compounds into their components (1)

Two ways flash chromatography varies from the traditional method:

1. The use of somewhat smaller Silica gel particles (250–400 mesh)
2. Pressurized gas (about 10-15 psi) is utilized to force the solvent through the stationary phase column because the tiny gel particles are obstructing the solvent's passage. Quick and high-resolution chromatography is the end product.

A gradient pump, sample injection ports, a UV detector, and a fraction collector to collect the eluent are among the parts of automated flash chromatography systems that are typically found on more costly HPLC systems. These automated systems often offer a faster and less expensive alternative to performing several injections on prep-HPLC systems, and they can separate samples ranging from a few milligrams to an industrial kilogram scale. A user can only gather factions that contain their target compound, and the software that runs the automated system helps the user locate the purified material inside the fraction collector. For preservation and/or future recall needs, the program additionally stores the process's final chromatograph.

The basic concept of operation is that the eluent is quickly forced through a short glass column with a wide inner diameter while under gas pressure—typically nitrogen or compressed air. An adsorbent with a specified particle size is put inside the glass column [Vogel]. Silica gel with a particle size of 40–63 µm is the most commonly employed stationary phase, although packing with other sizes is also an option. If you were to employ particles smaller than 25 µm, you would only be able to achieve very low flow rates with very low viscosity mobile phases. Gel beds typically have a height of 15 cm and operate between 1.5 and 2.0 bars. Originally, normal phase chromatography could only be performed using unmodified Silica gel as the stationary phase. However, in the interim and in addition to HPLC, flash chromatography is increasingly using reversed-phase materials. (2)

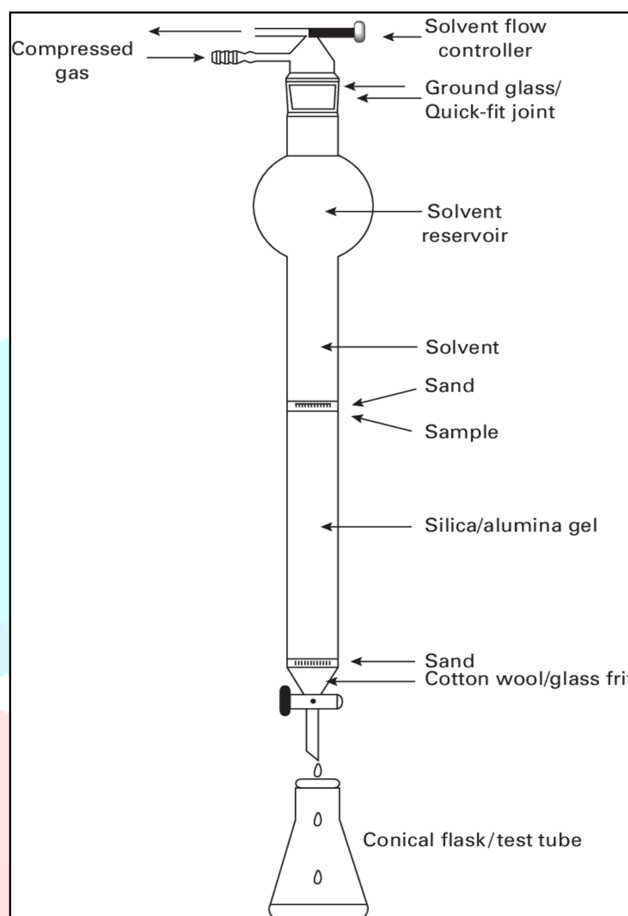

fig no 1: instrumentation of flash chromatography

Components of Flash Chromatographic System:

- **Sorbent Selection**:

Using the appropriate adsorbent is one of the fundamental requirements for effective separations. Silica gel is the most significant stationary phase used in column chromatography. Alumina (Al_2O_3) and silica gel (SiO_2) are two adsorbents that are frequently used in column chromatography. The size of the adsorbed particles influences the solvent's flow through the column. Flash chromatography uses smaller particles (higher mesh values), while gravity chromatography uses larger particles (lower mesh values). The Rf difference of the substances to be separated determines how much silica gel is needed, and the volume of sample determines how long the chromatography process must take.

The following adsorbents are frequently employed in flash chromatography:

- Silica: Good separation is accomplished in a slightly acidic media.
- Florisil: A neutral, gentle medium. 200 mesh can work well for simple separations.
- Alumina: A neutral or basic media. can work well for simple purification and separation processes.
- In the reverse phase of silica, the most polar molecules elute first while the least polar ones do so last.

➢ **Solvent Systems:**

The two solvents used in flash column chromatography are typically a combination of one polar and one nonpolar component. Sometimes only one solvent needs to be used.(3)

Table 1: An overview of the characteristics of popular flash solvents.

Solvent	Density (g/ml)	Elution Strength	Boiling Point (°C)
n-Hexane	0.66	0.01	69
2 2 4-Trimethylpentane	0.69	0.02	99
Cyclohexane	0.77	0.03	81
1 1 2-Trichloromethane	1.48	0.31	61
Toluene	0.87	0.22	110
Dichloromethane	1.33	0.30	40
Ethyl Acetate	0.90	0.45	77
Methyl-t-butyl ether	0.74	0.48	55
Acetone	0.79	0.53	56
Tetrahydrofuran	0.89	0.35	66
Acetonitrile	0.78	0.50	82
Isopropanol	0.79	0.60	82
Ethanol	0.79	0.88	78
Methanol	0.79	0.70	65
Water	1.00	0.073	100

➢ **Column Selection**

Choose a column with an ID of 10, 20, or 40 mm depending on the prerequisites.

Table 2: Average amount of eluant needed for elution and packaging.

Column Diameter (mm)	Volume of eluant* (ml)	Sample Load (mg)		Fraction Size (ml)
		Rf > 0.2	Rf >0.1	
10	100	100	40	5
20	200	400	160	10
30	400	900	360	20
40	600	1600	600	30
50	1000	2500	1000	50

➢ **Packing the Column**:

To block the silica gel from exiting the glass column through the stopcock, there is either a glass frit or a cotton wool plug just above it. Add the 230–400 mesh dry silica gel adsorbent. The column is filled by inverting it into the silica gel jar, scooping out material, tamping it down, and then scooping out additional material. Using a beaker, pour the gel into the column as an additional method of filling it. To pack the silica gel and drive air into the column, we can alternatively use a pipette bulb.

Load the sample onto the Silica gel column(3)**:** Two different methods are used to load the column

a. Wet Loading Method: The material to be purified (or divided into components) is dissolved in a tiny amount of solvent, such as acetone, hexanes, or another solvent, in the wet process. Onto the column is loaded this solution. In certain cases, the preferred solvent for loading the sample onto the column has a higher polarity than the eluting solvents. In this instance, it is crucial that we load

the sample with a small number of solvent droplets while using the wet method of column loading. The loading solvent will obstruct the elution and, thus, the purification or separation of the mixture if we utilise an excessive amount of solvent. It is advised to load columns using the dry approach in such circumstances.

b. **Dry Loading Method:** After removing as much solvent as possible from the sample to be examined, add roughly 100 mg of silica gel. Until the solvent has evaporated and only a dry powder is left, swirl the mixture. After folding a piece of weighing paper, place the dry powder on it and place it on top of the prepared column. Now that the eluting solvent is fresh, we may start the elution process.

- **Elute the column:**

Using a pipette bulb, apply pressure to the Pasteur pipette's top to drive the solvent through the column. Don't allow the silica to dry out; only push the solvent to the very top of the silica. If needed, add more solvent. The pressure ought to be as low as is required to maintain a constant stream exiting the column. If the compound is not coloured, the procedure becomes more difficult. These investigations involve the sequential collection of equal-sized fractions that are meticulously marked for further analysis.

- **Analyse the fractions:** If the fractions are coloured, we can just mix like-colored fractions together, however it's normally best to take precautions first. TLC analysis is typically used to evaluate fractions that are not coloured. Following the determination of each fraction's composition, the fractions containing the desired component or compounds are mixed.

- **Cleaning the Column:** Using pressurised gas, completely empty the column of any leftover solvent. A two-hour air flow through the column will produce dry, freely flowing silica gel. Empty the column's contents into the garbage container marked "Silica." Generally, it is enough to just wash the column with acetone and water. You can apply a tiny bit of liquid soap if needed. Once the reservoir has been completely empty of liquid solvent, use an aspirator to create a vacuum to remove any remaining solvent from the bottom of the column. Steer clear of using soaps or abrasive brushes on the columns to prevent scratches.

- **General procedure**(4):
  - The mixture is first separated and the desired component is moved on analytical TLC to an Rf using a low viscosity solvent solution (such as ethyl acetate/30–60°C petroleum ether).
  - 40–63 mm dry A single quantity of silica gel is put into the column to provide a 5.5–6-inch depth. The column is secured for pressure packing and elution, and the gel is packed by gently tapping the column vertically on the bench top with the stopcock open.
  - To fully fill the column, gently pour the above-mentioned solvent over the sand (provide air pressure by holding the cork with the air line on top of the column). Due to the solvent being quickly driven through the column, this causes the pressure above the absorbent bed to rise quickly and compress the silica gel. If the pressure is not maintained until all of the air has been released and the lower portion of the column has cooled, the column may break and needs to be repacked unless the desired separation is very slight.
  - Excess eluant is driven out of the column as the pressure is removed. It is important to keep the top of the silica gel from drying out. The sample is then pipetted to the top of the adsorbent bed as a 20–25% solution in the eluant, and the flow controller is momentarily put on top of the column to force the entire sample into the Silica gel. Usually, the column's packing solvent is also utilised to elute the column.
  - In order to avoid disturbing the adsorbent bed, the column walls are cleaned down with a few millilitres of new eluant, the washings are put onto the column as before, and the column is gently filled with eluant.
  - Until the solvent is completely used, fractions are gathered. The column should not be allowed to run dry because further elution may occasionally be required.

- To obtain the necessary substance, the purified components are identified using TLC, the relevant fractions are mixed, and the solvent is eliminated using rotary evaporation.

Flash chromatography's function in extracting the active ingredient from complicated plant extracts:

Natural substances are being considered as potential replacements for pharmaceuticals, and there is an increasing demand for these complicated mixtures to be separated. A quick and affordable method for separating the essential ingredients in complicated plant extracts is flash chromatography.

I. Using flash chromatography to isolate methyl gamma linolenate from Spirulina platensis, it was found to have an apoptosis-inducing activity in human lung cancer A- 549 cell lines. Using a flash chromatography technology (Isolera system), gamma linolenic acid, an essential Omega-6 Polyunsaturated Fatty Acid (PUFA) with potential medical applications, was extracted as a methyl ester from microalgae called Spirulina platensis. Comparing the isolated methyl gamma linolenate percentage yield to other standard methods, it is determined to be 71% w/w, which is an extremely good yield. It was tested utilising the SRB assay for in vitro cytotoxic screening on A-549 lung cancer cell lines, and the outcome was compared to that of regular rutin. It was determined that the methyl gamma linoleate that was recovered from Spirulina platensis may have been isolated more readily thanks to the Flash chromatography technique, and that this isolated molecule was highly lethal to human lung cancer cell lines[5]

II. Liquid-liquid extraction has been used to separate marjoram extracts into polar and nonpolar components. The extracts' polar and nonpolar components were further separated using flash chromatography. A binary solvent system consisting of methanol (mobile phase B) and ethyl acetate (mobile phase A) was used for the separation, which was done at a flow rate of 20 mL/min and with a stepwise gradient that took 45 minutes to go from 10% to 90% methanol. There were roughly 90 polar and 45 nonpolar fractions found. The eluates' UV absorptions were observed at 280, 320, and 360 nm in wavelengths. Using the Ferric ion reducing antioxidant power (FRAP) and DPPH assays, the antioxidant activity of each fraction was examined, and the Folin-Ciocalteau method was used to calculate the total phenolic content. The polar and nonpolar sets' top three antioxidant fractions were chosen so that the principal polyphenols could be identified and measured using the LC-ESI-MS technology. NMR spectroscopy helped to clarify the structure of the purified fraction of rosmarinic acid [6].

III. Using flash chromatography to extract solanesol from tobacco leaves based on molecularly imprinted Suspension polymerization was used to create the spherical MIP particles of Solanesol, which measured 250–350 μm. The stationary phase of the MIP particles was packed in a standard Teflon column. As the sample solution, methanol was eluted using both methanol and an 80/20, v/v methanol/acetic acid solution. In each process, 370.8 mg of purified Solanesol (98.4%) could be obtained from the extract (20 mM, 40 ml) of tobacco leaves (14.7 g), and the yield of Solanesol was 2.5% of the weight of dry tobacco leaves. The MIP-Flash column's adsorption capacity was found to be 107.3 μmol/g under ideal chromatographic conditions. The findings presented here support the viability of using MI to directly extract extremely pure active chemicals from natural goods on a wide scale.[7]

IV. It was shown that several citrus limonoid glycosides are particularly challenging to purify using standard methods.Nomilin 17-B -D-glucopyranoside and Nomilinic acid 17-β -D-glucopyranoside, two closely related limonoid glucosides, can be separated and isolated using a reversed-phase flash chromatographic process. The Biotage Flash-75 system was utilised to load the crude glucoside extract. The glucosides were then isocratically eluted using a mobile phase consisting of water, methanol, and acetonitrile (75:10:15) in Potassium Dihydrogen Phosphate buffer, at a flow rate of 50 mL/min. For a duration of one hour, fractions (1 min) were collected, and each fraction was subjected to analytical HPLC. The same chemicals were present in fractions that were combined, concentrated, and freeze-dried. The identities of the eluted fractions were further verified by electrospray ionisation mass spectrometry. A excellent yield and 93% purity were obtained by successfully separating the closely eluting glucosides.[8]

V. A subclass of flavonoids found in citrus species are called polymethoxyflavones, or PMFs. A quick, repeatable process is essential for purifying PMFs since in vitro investigations suggest that they could be used as chemopreventive agents. In the current investigation, flash chromatography is used to quickly separate PMFs from Cleopatra mandarin (Citrus reshni Hort. ex Tan.) and Marrs sweet orange (Citrus sinensis L. Osbeck). In a Soxhlet extractor, dried peels were ground into a powder and extracted with hexane. The extract underwent concentration, silica gel impregnation, and flash chromatography utilising a silica gel column. The separations were performed using a gradient solvent system consisting of acetone and hexane. At wavelengths of 254 nm, 340 nm, and throughout the entire wavelength scan, the PMF separation was seen. Following HPLC analysis, the separated peaks were gathered as separate fractions and combined. In terms of gramme level quantity, four PMFs were isolated with excellent purity. These were determined to be tangeretin, nobiletin, tetramethoxyflavone, and sinensitin based on mass spectral and NMR analyses. The devised approach was shown to be robust and to have a low RSD (%) during testing for repeatability. To the best of our knowledge, this is the first study on PMF isolation from Marrs sweet orange and Cleopatra mandarin.(9)

VI. This work devised a straightforward and effective method for preparing the three flavone aglycones (luteolin, apigenin, and genkwanin) from Equisetum palustre L. by employing gel resin flash chromatography after macroporous resin. D101 resin was selected to enrich three flavone aglycones out of six commonly used macroporous resins due to its good enrichment efficiency in both static and dynamic tests. Luteolin, Apigenin, and Genkwanin concentrations increased 13.3-fold, 12.5-fold, and 12.9-fold after one run treatment with D101 resin, with recovery yields of 83.6%, 78.8%, and 81.2%, respectively. On a Toyopearl HW-40S gel resin column, flash chromatography was immediately applied to the enriched sample. One gel resin flash chromatography run yielded luteolin, apigenin, and genkwanin with purities of greater than 97% and recovery yields of 91.8%, 92.3%, and 93.1%. The invented process produces high-purity products with good recoveries, has readily reusable solvents, and is easily scaleable up. As such, it presents a viable platform for the large-scale synthesis of flavone aglycones from plant extracts, such as Equisetum palustre(10)

VII. Flash chromatography has been used to isolate the flavonoids of Chenopodium album L. from acetone extract. The herb has been utilised for its diuretic, moderately laxative, sedative, hepatoprotective, antiparasitic, antiphlogistic, antirheumatic, and odontalgic effects in traditional medicine. A flavonoid found in C. album exhibits strong anti-inflammatory, NF-kappa B inhibition, free radical scavenging, and anti-inflammatory properties, which may have an antirheumatic effect. The Teledyne Isco CombiFlash® CompanionTM 4x equipment was utilised to accomplish flavonoid isolation. A 24 g, C-18, Reversed Phase RediSep spherical silica gel, 40-63μ, was placed in a glass column with a 270-minute flow rate of 21 ml/min. With solvent A being 0.1% formic acid in water (v/v) and solvent B being acetonitrile, a gradient elution was carried out. Flash Chromatography yielded a maximum yield of 7.335 mg/g flavonoid from acetone extract, as demonstrated by fractions collected in test tubes. Subsequently, the separated flavonoid fraction underwent structural clarification using UV, IR, 1H, and 13C NMR, as well as MS (11)

VIII. Vanillin is isolated from Decalepis hamiltonii roots using flash chromatography and validated and quantified using high-performance liquid chromatography (HPTLC). Its roots have anti-inflammatory, antipyretic, antioxidant, hepatoprotective, neuroprotective, anxiolytic, and antifungal properties in Ayurvedic medicine. Using Isolera Flash Chromatography (Biotage), a quick method of preparative column chromatography, 50 mg of ether extract was chromatographed. The KP-Sil 10 gm column from a normal phase SNAP cartridge was utilised. The mobile phase was a binary gradient of ethanol and toluene with a 10 mL/min flow rate that was eluted, yielding a 15 mL column volume. Eluting pure vanillin took two to four column volumes. Ten millilitres of each fraction were collected in collecting tubes using a 254 nm wavelength for recording. Afterwards, HPTLC was used to verify the fractions' purity, and it was discovered that both the purity and the percentage yield were 1.126%. The DPPH Method was used to assess D. hamiltonii's ability to scavenge free radicals in methanol extract, and the antioxidant activity was verified by comparison with ascorbic acid, a standard (12)

II. CONCLUSION

A quick, easy, economical, and time-saving method for preparative liquid chromatography is flash chromatography. Extrapolating conventionally acquired TLC results to preparative scale is the basis for separations. The yield of active components as a percentage is higher than in column chromatography. It is advised to explore the potential of flash chromatography in the field of phytochemical research.

REFERENCES

1. Shu Y, Liang Y, Liang Z, Zhao X. Journal of Liquid Chromatography & STUDIES ON A SIMPLE AND EFFICIENT METHOD FOR LARGE-SCALE PREPARATION OF GENKWANIN FROM DAPHNE GENKWA SIEB . ET ZUCC . USING NORMAL-PHASE. 2014;(November):37–41.
2. Chaudhari H, Chaudhari F, Patel M, Pradhan PK, Upadhyay UM. International Journal of Pharmaceutical Development & Technology. 2012;2(2):80–4.
3. Pressure PA, Roge AB, Nagar S. Roge et al. ,. 2011;2(8):1930–7.
4. Stevens WC, Daniel J. General methods for flash chromatography using disposable columns. 2009;247–52.
5. Jubie S, Dhanabal SP, Chaitanya MVNL. Isolation of methyl gamma linolenate from spirulina platensis using flash chromatography and a apoptosis inducing effect. BMC Complement Altern Med [Internet]. 2015;4–11. Available from: http://dx.doi.org/10.1186/s12906-015-0771-8
6. Camphuis G, Aguil I, Gangopadhyay N, Rai DK. Antioxidant activity guided separation of major polyphenols of marjoram (Origanum majorana L .) using flash chromatography and their identification by liquid chromatography coupled with electrospray ionization tandem mass spectrometry †. 2014;3205–13.
7. Meng Z, Qiu L, Chen J, Guo Y. Accepted Manuscript. 2016;
8. Raman G, Cho M, Brodbelt JS, Patil BS. Isolation and Purification of Closely Related Citrus Limonoid Glucosides by Flash Chromatography. 2005;160(May 2004):155–60.
9. Uckoo RM, Jayaprakasha GK, Patil BS. Rapid separation method of polymethoxyflavones from citrus using flash chromatography. Sep Purif Technol [Internet]. 2011;81(2):151–8. Available from: http://dx.doi.org/10.1016/j.seppur.2011.07.018
10. Wei Z, Luo M, Zhao C, Wang W, Zhang L, Zu Y, et al. An efficient preparative procedure for main flavone aglycones from Equisetum palustre L . using macroporous resin followed by gel resin flash chromatography. Sep Purif Technol [Internet]. 2013;118:680–9. Available from: http://dx.doi.org/10.1016/j.seppur.2013.07.045
11. Arora S, Itankar P, Extraction, isolation and identification of flavonoid from Chenopodium album aerial parts, Journal of Traditional and Complementary Medicine (2018), https://doi.org/10.1016/j.jtcme.2017.10.002
12. Shashi Shankar Tiwari, Amit Srivastava, Sharad Srivastava & A. K. S. Rawat, Isolation and quantification of vanillin through flash & HPTLC chromatographic techniques from Decalepis hamiltonii Wight and Arn Root and their antioxidant Studies, Journal of Liquid Chromatography & Related Technologies (2012) http://dx.doi.org/10.1080/10826076.2011.633675