

## PHYSICAL AND ANALYTICAL CHEMISTRY

Review

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### Flash Chromatography and Semi-Preparative HPLC: Review on the Applications and Recent Advancements over the Last Decade

Flash chromatography and Semi-Preparative High Performance Liquid Chromatography have showed themselves as promising separation tools. These techniques assist in the process of isolation, fractionation and purification of chemical, biological and pharmaceutical substances. The present article describes the recent advancements made in flash chromatography and semi-preparative HPLC techniques in the last decade with specific focus on natural products. This article highlights the basics, instrumentation, current advancements made to facilitate the separation, advantages and applications of these two techniques. Flash chromatography is a versatile tool for the rapid but efficient separation and purification of analytes in relatively pure form. Nowadays, there are even functionalized silica pre-packed cartridges with different silica weights and particle sizes that are available to use. Flash separation using dual columns can be used to enhance separation of complex mixtures and can be applied to numerous classes of compounds. The semi-preparative techniques, despite their high cost, can be helpful in high purity separation and production of reference standards for the pharmaceutical industry. This comprehensive review presents a brief analysis of all the recent research employing these two techniques for varied applications. This review will help chromatography specialists to make the decision of applying these two techniques in order to accelerate their research and development journey.

*Keywords:* Flash chromatography, Semi-preparative HPLC, Isolation, Fractionation, Purification, Recent advancements, Evolution, Applications.

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#### List of abbreviations

MIP: Molecular Imprinting Polymers  
SPE: Solid phase extraction  
FA: Formic acid

GLA-ME: Gamma-linolenic acid methyl ester  
FG and F2G: Mono- and diferuloyl glycerol,  
TF1: Theaflavin  
TF2A: Theaflavin-3-gallate  
TF2B: Theaflavin-3'-gallate  
TF3: Theaflavin-3,3'-digallate  
RP-HPLC: Reverse phase — High Performance Liquid Chromatography  
HSCCC: High speed counter current chromatography  
EA: Ethyl acetate  
YMC: Octadecylsilane semi-preparative column  
YMC ODS-AQ: Semi-preparative column  
LDH: Lactate dehydrogenase  
ALA:  $\alpha$ -linolenic acid  
LA, 18:2n6: Linoleic  
BHP: Biohydrogenation products  
RA: Rumenic acid,  
VA: Vaccenic acid

### *Review plan*

*Inclusion and Exclusion criteria:* The present review focuses on some of the recent advancements in flash chromatography and semi-preparative HPLC over the last decade and also highlights various applications and advantages of these two chromatographic methods. The data reported in recent research publications is summarized to make better understanding of the applications and improvements made in these two chromatographic techniques.

Various web search engines, including Google Scholar, PubChem, Science Direct Database, and others, were used to collect data from researches in which both flash and semi-preparative HPLC are applied. The data was gathered through papers published the last decade, from 2011 to 2021. The key words used in search of the data included: liquid chromatography, flash chromatography, semi-preparative HPLC, evolution, applications, recent advancements, purification, fractionation. Few manufacturer application notes are also summarized here. Collected data was further sorted on the basis of specific inclusion-exclusion criteria. In this present review, we summarized research papers based on flash chromatography and semi-preparative HPLC after 2010. The articles published before 2010 are excluded from our review.

### *Introduction*

In the pharmaceutical industry, all manufactured products need to be of highest quality to ensure least risk to the patients. To confirm that the pharmaceutical products meet the required standards, researchers, manufacturers and developers across the globe apply numerous analytical tools and techniques during the development process, for the qualitative or quantitative estimations and for elucidation of structures of components in complex mixtures. Chromatography has a vital position in global research for its wide field of applications. The last few decades presented tremendous technological advancements in chromatographic instrumentation and techniques. Among these, the techniques of flash chromatography and semi-preparative HPLC continue to grow, providing solutions to many analytical problems across varied research scopes. The present review attempts to focus on some of the recent advancements in flash chromatography and semi-preparative HPLC over the last decade and also highlights various applications and advantages of these two chromatographic methods.

### *Flash chromatography*

It is a modified version of column chromatography first proposed in 1978 by W.C. Still in order to purify desired products during chemical synthesis [1]. Due to its several advantages, including low cost, lesser time consumption and high yield, it was later used in natural products extraction [2, 3], sample enrichment, and purifying [4, 5] procedures. This technique is analogous to the traditional column chromatography, however with positive pressure being applied to drive the solvent through the column [6]. As this chromatographic technique operates at lower pressures compared to HPLC, it is also referred to as low or medium pressure chromatography. It is now the most widely used approach for macroscale purifications in academic, research and development laboratories [7]. Flash chromatography generally is an air-driven fusion of medium-

pressure and short-column chromatography which has been successfully used for quick separations. It can be applied to both reverse and normal phase separation [8]. Some of the recent advancements in flash chromatography include the optimization of numerous operating parameters such as flow rate, mass of sample load, and separation time using the binary gradient, binary solvent gradient slope, silica and modified silica based stationary phases in order to isolate and purify milligram to few hundredth of gram amounts of analytes in a relatively purified form [9]. The chromatogram obtained from a flash chromatographic run is represented in Fig. 1.

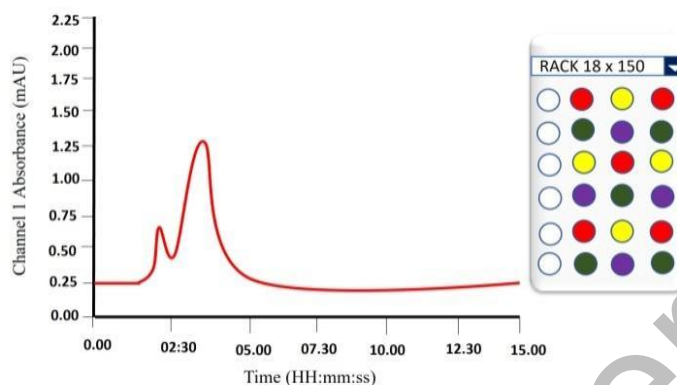


Figure 1. Flash chromatogram with fraction collection

#### Current reports using flash chromatographic separations

Selman *et al.* has reported the development of functionalized silica materials which allow the purification of fullerenes [10]. Nowadays flash purification systems are equipped with pre-packed cartridges with different silica weights and particle sizes, that are commercially available to assist versatile separation processes [11]. Duckworth *et al.* (2017) utilized dual-column flash chromatography approach (i.e. reverse phase absorption and size exclusion) to purify pyoverdine from *Pseudomonas putida* GB1 [12]. In modern flash chromatography systems, the solvent is pumped through the cartridges which can also be bought pre-packed. Preliminary purification and concentration of organic compounds [13], separation and purification of bioactive compounds [14] are the two major applications of flash chromatography. Today, a wide range of industrial flash chromatographic equipment is available, each featuring manually packed columns or pre-packed cartridges, high-efficiency solvent pumps, automatic fraction collectors and microcomputer-controlled detectors [15]. Reversed phase flash chromatography has been used to purify oregonin from aqueous solution of spray-dried alder extract [16]. With the benefits of the improved instrumentation, nowadays flash separation offers high loading capacity, increased speed of separation and ease of use, thereby making it viable for large-scale separations.

There is also a report on the use of Molecular Imprinting Polymers (MIPs) for flash separation techniques. In this study, Xue *et al.*, 2013 extracted pure shikimic acid from Chinese star anise by simple flash column chromatography using MIPs [21, 22] as the adsorbent produced by dispersion polymerization technique [23, 24]. MIPs were packed onto HPLC or Solid phase extraction (SPE) micro-columns [7] in order to extract active ingredients from herbal plants. The extraction on an industrial scale, however, failed due to the poor mechanical stability of MIP in the columns, complexity and discontinuity in SPE micro-columns. By combining molecular imprinting technology and flash chromatography, Xue *et al.*, 2014 developed a highly selective flash chromatography method for separation of shikimic acid. Researchers used columns with MIP-containing micropore filters, pre-equilibrated with acetic acid: water (5:95 %v/v) before loading the sample. They loaded non-purified ethyl acetate extract onto the MIP-flash column with elution at 1.7 ml/min with EtOH: water (40:60 %v/v), that led to the successful separation of shikimic acid enriched fraction [25]. The fractions obtained were then evaporated to dryness using a rotary evaporator. The study reported that the MIP-flash column provided better capability and reusability than the HPLC columns packed with MIPs. This method allows the large-scale isolation of highly purified active compounds from any herbal plant; is easy, repeatable, and cost-effective.

In another study, Hossain *et al.*, 2018 used the flash chromatography technique to separate polyphenols from pure red onion extract. The 80 % methanolic extract of powdered red onion was loaded on a Telos C18

reverse flash column and separated using water (A) : 0.5 % FA (formic acid) in ACN(B) as the mobile phase at 40 ml/min flow rate. Polyphenols were isolated using a sequential gradient of 0–100 % B over 35 minutes, with over 70 fractions recovered at a time scale of 0.5min/fraction. This study established the utility of flash chromatographic fractionation for the effective separation of polyphenols [26].

Ma Xiaoqin *et al.*, 2016, has developed the flash chromatography method with large spherical MIPs used as stationary phase for the extraction of highly purified solanesol from tobacco leaves. MIP activated flash cartridges were loaded with methanolic extract of tobacco leaves and eluted at different flow rates (2, 4, 6 and 8 ml/min). Next, the un-absorbed solanesol was removed by leaching the column with MeOH at 2 ml/min flow rate for 20 min. Finally, the adsorbed solanesol was extracted with acetic acid/methanol mixtures in different ratios (3/7, 2/8, 1/9, 0/10 v/v) at specific flow rates for 1 h, and the highly purified solanesol was obtained [27]. The reusability of these MIP-flash columns was tested by repetitive loading ( $n = 5$ ) on the same column under the optimal flash chromatographic condition. It was observed that these columns are reversible during five rounds of regeneration, with a standard deviation of 4 % for five separate adsorption rate assessments, indicating that the MIP-flash column has excellent reusability capacity [27].

In other research, Jubie *et al.*, 2015 utilized flash chromatography system to isolate gamma-linolenic acid methyl ester (GLA-ME) from *Spirulina platensis*, a commercially well-known blue-green micro algae [28] rich in phenolic acids, tocopherol and GLA [29, 30]. Using a high-performance flash chromatography approach (Isolera system), omega-6 PUFA i.e., GLA was isolated as its methyl ester (GLA-ME), which acts as a molecular target for lung cancer treatment. GLA-ME was isolated by loading the vacuum dried enhanced GLA fraction (0.5 g) in a 50 g KPSilBiotage SNAP cartridge, using 60 % hexane and 40 % acetone as the mobile phase at a constant flow rate (50 ml/min). A total of 105 fractions, each of 22 ml, were collected at 254 nm, then each fraction was proceeded to TLC for additional analysis. GLA-ME was found to be present in fractions 6-17. This high-performance flash chromatography method improved the productivity of GLA-ME extraction from *Spirulina platensis* [31].

Compton *et al.*, 2020 developed and optimized a flash chromatography method using silica stationary phase with a binary gradient of acetone and hexane as the mobile phase to isolate and purify naturally occurring phenylpropanoids i.e., mono- and diferuloyl glycerol, (FG and F2G respectively) which provide protection from UV radiations and oxidation in plants. Flash chromatography system (Teledyne, CombiflashRF200) was used to purify the crude precipitate and Redisep column was used for the following separation. The method was optimized with consideration to the size of used Redisep column, flow rate of solvent, solvent gradient program and volume of the sample load. For purification of FG and F2G, the 3.0 g crude precipitate dissolved in acetone was loaded onto the Redisep column (12 g) using syringe injection, which was then aspirated to dryness for 1 hour before being pumped to the the Redisep separation load column (120 g) at 57 ml/min flow rate. Separation was performed in “liquid load” mode with 0-100 % acetone in hexane gradient. The fractions that included target peaks were combined and evaporated to dryness to obtain purified FG (yellow, oily liquid) and F2G (white solid) [32].

This solid sample loading provided great consistency with no pumping issues and leakages, assisting in rapid purification and obtaining low concentrations of FG and F2G.

RediSepRf Gold® C18Aq columns are reported to be useful for removal of salts and buffers from solvents that are commonly used to purify chemicals. The ability of these columns to adsorb chemicals under highly aqueous conditions without phase collapse makes them excellent for desalting samples. This approach was used to desalt brilliant blue using a methanol water gradient [33].

The application of CombiFlash® Rf-200 columns to purify C60 fullerene from soot is also reported. As most solvents make it difficult to purify fullerenes, the isolation of C60 fullerene was accomplished by dissolving 25 mg sample of soot in carbon disulphide loaded on a CombiFlash® Rf 200 column using a 2-propanol/toluene mixture with ultraviolet detector at 310 nm [34].

A very practical and successful method for the purification of primary amines from a complex mixture was achieved using a ledyne C-18 IscoRediSep reversed phase column with water/ACN as the mobile phase [35]. A large number of the manufacturers of flash chromatographic systems are providing several advancements in their instrumentation including multiple column ports, automated injections with multiple stationary phase chemistries and solvent systems, multiple channel detections using UV, fluorescence and mass guided separation in order to provide more effective and accurate fractionations and increase the purity of the fractions collected through these separations. Robust method transfer from TLC to flash separation through various computer controlled softwares are also being applied today [36].

### Semi-preparative HPLC

Since its introduction several decades ago, HPLC continues to remain as one of the most versatile tools in chemical analysis. Semi-preparative HPLC, which evolved in the early 1970's, is now being used for numerous applications including the isolation and purification of compounds from natural product extracts [17], purification of drugs and their by-products during manufacturing process and impurity analysis. The capability to scale-up from analytical operations is one of the key benefits of semi-preparative separation. In the 1970s, the first preparative HPLC system was created to enhance separation power. Semi-preparative HPLC falls under the small scale preparative HPLC and is being used for the separation of small quantities of sample [19]. Using the optimized HPLC parameters (flow rate, mobile phase, stationary phase, maximum amount of sample load, separation temperature), analysis specialists can improve preparative operations thereby saving time and resources [20]. Semi-preparative HPLC allows the separation of high purity compounds [18] which can be used as reference standards in pharmaceutical industries. A typical semi-preparative high performance liquid chromatographic result graph is depicted in Fig. 2.

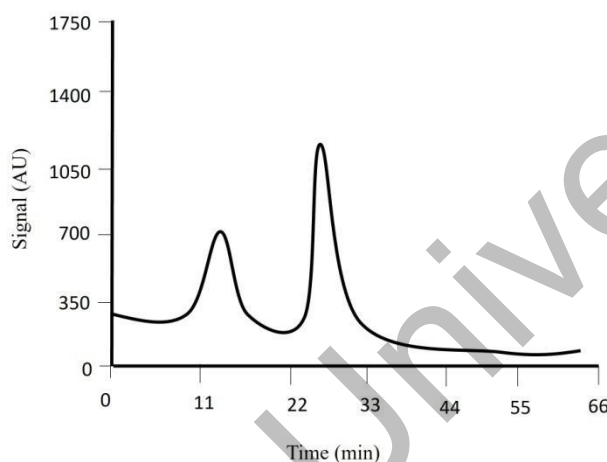


Figure 2. Chromatogram showing isolation of compounds in semi-preparative HPLC

#### Scale-up process of Analytical HPLC to Semi-preparative HPLC

In the scale-up process, analytical runs are initially performed to establish the separation efficiency, which includes determination and optimization of the appropriate mobile phase, flow rate, stationary phase, separation temperature, and other HPLC parameters. Using the optimized HPLC parameters, this method can be effectively scaled up from analytical to semi-preparative and preparative analysis [37].

The major steps involved in semi-preparative HPLC scale-up and optimization include the modification of the analytical approach in terms of resolution, overcharging on the analytical column and extension of the column size to semi-preparative and preparative scale. The prime parameters that need to be scaled up are the flow rate of separation and the column loading.

The equations that are used to scale up the flow rate (1) and volume (2) are:

$$V_1/V_2 = r_1^2/r_2^2, \quad (1)$$

where  $V_1$  is the flow rate column 1;  $V_2$  is the flow rate column 2.

$$X_1/(\pi \times r_1^2) = X_2/(\pi \times r_2^2) \times 1/C_L, \quad (2)$$

where  $r_1$  is the radius of column 1;  $X_1$  is the max. volume column 1;  $r_2$  is the radius of column 2;  $X_2$  is the max. Volume column 2;  $C_L$  is the ratio lengths of columns = 1; Column 1 is the Analytical Column; Column 2 is the Preparative Column.

#### Current reports using Semi-preparative HPLC separations

In this study, Xu *et al.* (2010) applied the semi-preparative HPLC to isolate and purify four major theaflavins found in black tea, including theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B), and theaflavin-3,3'-digallate (TF3). Black tea is at about 80 % of global tea output and has several therapeutic effects, including antioxidant properties [38–40], inhibition of carcinogenesis [41], suppression of lipid peroxidation [42] and protection against cardiovascular diseases [43] due to the action of these four

primary theaflavins [44]. In this study, 35 g of the black tea extract TF50 was loaded on a Mitsubishi SP-207 resin filled column (highly porous modified adsorbent material). Progressive elution with a gradient of 20 %, 30 %, 40 %, 50 %, and 70 % aq. EtOH at a flow rate of 33 ml/min, generated an 80 % pure mixture of these theaflavins (TF80). Further separation on a C18 preparative column with ACN / dist. water / GAA (26:73.5:0.5, v/v/v) as mobile phase at 5 ml/ min flow rate resulted in isolation of the pure theaflavins. Compared to the traditional isolation processes that lasted several hours, this semi-preparative HPLC method required only 45 minutes enabling effective separation of the theaflavins [45].

In another study, Basar *et al.* (2014) developed and validated a simple semi-preparative-RP-HPLC/PDA method of isolation and quantification of glycyrrhizin from nine distinct samples of *Glycyrrhiza glabra* root collected from different geographical regions. The isolation and quantification were proceeded on reversed phase semi-preparative column with octadecylsilane guard column using binary gradient elution with 30–100 % solvent B [0.1 % v/v trifluoroacetic acid in MeOH] in solvent A [0.1 % v/v trifluoroacetic acid in water] at 3.00 mL/min flow rate at 25–28 °C for 30 min. Prior to loading, the residue obtained from the extracts of each sample was re-dissolved in methanol and filtered. For the analysis, 100 µl sample was injected into the system and the runs were monitored at 254 nm. The study report also demonstrated the validation of the method using nine different samples of *Glycyrrhiza glabra* roots. This method allowed the efficient and quick isolation and quantification of glycyrrhizin with high accuracy and low cost [46].

High speed counter current chromatography (HSCCC) is a continuous liquid-liquid partition chromatography method that works by isolating substances between two immiscible liquid phases. It has numerous benefits, including irreversible adsorption, minimal potential of sample denaturation, complete sample recovery, and relatively low cost [47, 48]. In this study, for the semi-preparative isolation of 10 isomers of caffeoylquinic acid derivatives obtained from the roots of Burdock (*Arctiumlappa* L.), Zheng *et al.* (2018) developed and applied semi-preparative HPLC in combination with HSCCC. These 10 isomers were isolated from the ethyl acetate (EA) extract of Burdock roots which was further fractionated using macroporous resin chromatography yielding three fractions (Fraction 1–3) from the elution of 40 % MeOH. Fractions were collected and purified by HSCCC using EA-petroleum ether-MeOH-water as a solvent system at different ratios as shown in [49]. This revealed that Fraction 1 mainly contains compounds A–C, Fraction 2 mainly contains compound D, and Fraction 3 mainly contains compounds E–J. So, After the separation of derivatives by HSCCC, these collected fractions (i.e., blend of the derivatives) were further proceeded for the purification and identification by YMC octadecylsilane semi-preparative column with mobile phase ACN – 0.1 % FA in water (25:75, v/v) and MeOH – 0.1 % FA in water (25:75, v/v) at 3.0 mL/min flow-rate, observed at 280 nm. This research showed that combination of semi-preparative HPLC and HSCCC is a highly effective method for isolation of ten bioactive caffeoylquinic acid derivatives from natural products [49].

Using semi-preparative HPLC system, Li Aifeng *et al.* (2014) extracted seven known flavonoids (mainly quercetin, apigenin, diosmetin, luteolin and their glycosides) from the dried *T. kirilowii Maxim* peel, which is broadly used in traditional Chinese medication as a cure to a cardiovascular, cerebrovascular, and respiratory diseases [50]. The EA fraction of dried peel of *T. kirilowii Maxim* was concentrated by elution through a glass column wet-packed with polyamide resins with a gradient elution program consisting of aqueous ethanol 10 % – 100 % EtOH. Fractions, eluted with 70 % and 90 % EtOH, yielded two subfractions (I and II), which were injected into the semi-preparative column (YMC ODS-AQ) for further isolation of seven flavonoids (I to VII) using isocratic elution mode with MeOH-water as mobile phase in different ratios [for subfraction-I (46:54, v/v) and subfraction-II (66:34, v/v)] at a flow rate of 3.5 ml/min. The column eluent was checked with the UV detector set to 350 nm and manually collected according to the chromatograms. The study suggests the suitability of this procedure for the large-scale isolation of flavonoids from plant extracts [50].

In this study, Zhao Xu *et al.* (2020) applied semi-preparative HPLC to isolate high-purity anthocyanin monomers from grape pomace [51]. To extract anthocyanin from grape pomace, ultrasonification (59 kHz) with acidified methanol (500 ml methanol solution containing 2 % formic acid) was conducted for 10 min. Obtained concentrated crude extract was purified by Amberlite XAD-7HP resin (28 mm×400 mm) column until the resin was saturated. High-purity anthocyanin monomers were isolated from the purified crude extract using semi-preparative HPLC system with gradient elution using ACN/ water/FA (6/92/2, v/v/v) (A) and ACN/water /FA (54/44/2, v/v/v) (B) as the solvent system. Purified fractions of anthocyanin were collected by the automatic fraction collector. This study is the first time report of the effective separation of anthocyanin monomers using a single-step semi-preparative HPLC technique [51].

When a patient suffers from ischemic stroke, the level of LDH in their blood increases quickly, which leads to organ damage. Exploration of LDH inhibitors derived from medicinal plants could be a promising approach for invention of therapeutic drugs for those suffering from ischemic stroke [52, 53]. Isoflavonoids, which have been mainly used to treat ischemic stroke, are powerful LDH inhibitors [54, 55]. So here, in this study, Senlin Li *et al.* (2016) employed ultrafiltration HPLC coupled with PDA detection, and ESI-MS to screen and identify sixisoflavonoids-I to VI (i.e., LDH inhibitors Tectoridin, Iristectorin A, Iridin, Tectorigenin, Iriogenin and Irisfloreutin) which are the primary active compound of *Belamcandaechinensis* [56]. Further semi-preparative HPLC was performed with the primal optimization of its conditions, including composition of solvent system, flow rate, and injection volume, to separate and isolate these d LDH inhibitors from the extract of *B. chinensis* rhizome. As per this study report, the optimized solvent system contained solvent A (0.5 % acetic acid in water) and solvent B (ACN), with gradient elution times of 0–3 minutes for 10 to 20 % B; 3–6 minutes for 20 to 23 % B; 6–9 minutes for 23 to % B; 9–15 minutes for 30 to 50 50 % B; and 15–20 minutes for 50 % B. The optimal separation was achieved using 10 ml/min flow rate and 1.0 ml injection volume. This method proposed the isolation of the LDH inhibitors (% purity > 92 %) by applying ultrafiltration-LC-MS in combination with Semi-preparative HPLC, that proved to be of low-cost and high-efficiency, indicating its significant potential for commercial applications [57].

The traditional process for identification of natural active compounds includes purification followed by one-to-one screening, which takes longer time, affects the stability and sensitivity of the method [58]. Zhang *et al.* (2018) developed a method for the rigorous screening, preparation, and evaluation of antioxidant potential of natural products [59] by utilizing semi-preparative HPLC with two step separation approach followed by on-line HPLC-radical scavenging detection. Rigol semi-prep-HPLC system was applied to separate and purify seven representative antioxidants from *Magnolia officinalis* using a two-step separation procedure that included both gradient and isocratic elution. The separation of compounds was achieved under specific conditions, for compounds 1–4: injection volume (200  $\mu$ l), 3.0 ml/min flow rate, detection wavelength of 275 nm with mobile phase solvent A (0.2 % CH<sub>3</sub>COOH in water, v/v) and solvent B (MeOH) in gradient elution mode. Compounds 5–7 were obtained at flow rate (3.0 ml/min), injection volume 300  $\mu$ L at 275 nm with mobile phase composed of solvent A (0.2 % CH<sub>3</sub>COOH in water, v/v) and B (MeOH) in isocratic elution mode [58].

Turner *et al.* (2014) used Silver ion solid-phase extraction (Ag<sup>+</sup>-SPE) and semi-preparative Ag<sup>+</sup>-HPLC to isolate and collect pure isomers, including several t-monoenes (t12, t13, t14, t15, and t16-18:1) as well as non-conjugated dienes (i.e. t10, c15, t11, t15-, and t11, c15-18:2) unique to  $\alpha$ -linolenic acid ALA [57]. Linoleic (LA, 18:2n6) and  $\alpha$ -linolenic acid (ALA, 18:3n3) [60] are two polyunsaturated fatty acids (PUFAs) found in bovine feed that are metabolized by rumen microbes, leading to generation of various biohydrogenation products (BHP) that can be found in milk and meat. Biological functions of LA-BHP, which include conjugated linoleic acid [cis (c) 9, trans (t) 11-18:2 (Rumenicacid, RA) and t10, c12-18:2], as well as trans fatty acid isomers [t9, t10, and t11-18:1 (Vaccenic acid, VA)], have been investigated, but the influence of many BHP specific to ALA still has not been thoroughly researched, taking into consideration that most ALA-BHP are not available in the marketplace. In this reported study, a semi-preparative silver ion (Ag<sup>+</sup>) HPLC approach was developed for the purification and collection of ALA-BHP. Researchers used Ag<sup>+</sup>-solid phase extraction approach to fractionate the sample and collected the t-18:1, 18:2, and 18:3 fractions. Collected fraction were further isolated using semi-preparative Ag<sup>+</sup>-HPLC, and the t-18:1 isomer, 18:2 isomer, and 18:3 isomers were obtained [57].

### Conclusions

This review summarizes the effective application of flash chromatography and semi-preparative HPLC for the fractionation, isolation and purification process. Enlisted research papers specifically discuss their applications in the area of natural compounds. The flash chromatography with its ease of use, varied stationary phase chemistries, multiple columns and detectors, automated fraction collectors represents a very viable tool for fractionation and purification. Semi-preparative separation can assist in obtaining highly purified compounds which expands its applicability in diverse scopes. This review will help chromatography specialists to make decision of applying these two techniques to improve their research and development studies.

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