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Research

Isolation of Natural Products by Ion-Exchange Chromatography

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Abstract:
Ionizable compounds can be separated based on changes in charge characteristics using Ion-exchange chromatography (IEC). Liquid chromatography (LC) is a method that is very adaptable and frequently utilized due to its enormous sample-handling capacity, broad application (especially to proteins and enzymes), reasonable cost, powerful resolving ability, and simplicity of scale-up and automation. Extraction, isolation, and characterization of certain analytes from complicated plant, animal, microbial, and food matrices is the main objective of many natural products chemists. They heavily rely on extremely complex and highly hyphenated current instruments to accomplish this purpose. Nonetheless, the great bulk of contemporary equipment commonly seen in natural product chemists' labs is based on the elementary ideas of intermolecular interactions to accomplish separation. Fundamentally, the most basic and potent of these interactions is ion-exchange chromatography, which is regarded as a reasonably priced and efficient method for "cleaning up" a sample. High recoveries of important analytes are another benefit of IEC, as is the option to alter the stationary and mobile phases to "catch and release" molecules of interest with specificity.

Keywords: Ion-exchange, chromatography, natural products, isolation, characterization.

1. Introduction

Ion chromatography, which includes ion-exchange chromatography (IEC), ion-partition / interaction chromatography, and ion-exclusion chromatography, is an essential analytical method for the separation and identification of ionic compounds [1]. Particle chromatography separation relies on ionic (or electrostatic) interactions between polar and ionic separators, particles in the eluent, and ionic utility groups set up for chromatographic support. Ion chromatography differentiation is influenced by two distinct processes: ion extraction from charged ions that are comparable to ions concentrated on chromatographic support, and ion exchange from competition for ionic binding (attraction).

Up until now, ion exchange has been the most widely used ion chromatography technique [2]. One of the most significant advertising techniques for differentiating between peptides, proteins, nucleic acids, and similar biopolymers that are charged by molecules with varying molecular sizes and cellular composition is chromatography [3-6]. Ionic bonds

between charged groups of biomolecules and ion-exchange/support-bearing gel provide the basis for the split [7].

Biomolecules exhibit different degrees of contact with charged chromatography media due to their various charging properties [8]. The first report of ion-exchange chromatography dates back to 1850, Thompson studied the insertion of ammonium ion into the soil [9 - 11]. Spedding and Powell published a series of papers describing the practical methods of the preparation of the unusual earth by the removal of ion exchange chromatography in 1947.

Since the 1950s, Kraus and Nelson have published various analytical techniques that depend on metal particles and depend on the anionic chromatographic separation of their chloride, fluoride, nitrate, or sulfate [12]. In 1956, Peterson and Sober revealed utilizing ion exchange chromatography to isolate proteins. Small introduced ion exchange chromatography in its current form, Stevens and Bauman in 1975 [3]. Gjerde et al. distributed an anion chromatography strategy in 1979 and this was trailed by a comparable technique for cation chromatography in 1980 [12].

Ion- exchange chromatography has been used for many years to distinguish various ionic substances; cions and anions and is still in use. The popularity of ion exchange chromatography has increased in recent years because this process allows for the analysis of molecules in medicine, biotechnology, environment, agriculture, and other industries [2].

1.1. Ion exchange mechanism

Ion-exchange chromatography designed for the separation of charged or separately charged chemicals contains from moving and vertical phases similar to other types of liquid chromatography column techniques [9 - 11]. Mobil sections have an aqueous buffer system in which the mixture will be dissolved. The vertical phase is usually formed from an inert organic matrix derived from chemical and active ionizable groups (modified ions) that carry the opposite charged ion [11]. Ions exist in a state of equilibrium between the moving phase and the vertical phases that create the two potential types, the anion and the cation exchange are called counter ions [1,13]. The alternating matrix counter ions may include protons (H +), hydroxide groups (OH-), mono-charged atomic ions (Na +, K +, Cl-), double charged atomic ions (Ca2 +, Mg2 +), and polyatomic inorganic ions (SO4 2 -, PO4 3-) and organic compounds (NR2H +) and acids (COO-) [11]. Cations are separated in the cation-exchange resin column and the anions in the anion exchange resin column [10]. Based on binding analysts to positive or negative charge groups, the classification is set to a fixed and moderate level of free ion counters in the cellular phase according to their net charge variables [13 - 14].

Ion exchange chromatography involves the separation of polar ionic analyzes using chromatographic support extracted by active ionic groups with charges against the ion analyzes. Analytical ions and equally charged ions of eluent compete with binding in the ionic charge group with the upper surface of the standing phase. By assuming that exchange ions (analysts and ions in the cell phase) are cations, competition can be defined using the following equation;

S-X-C + + M + ↔ S-X-M ++ C +

In this process the cent M + of the eluent is replaced by the analyte cation C + bound to the X-anion-focused surface of the chromatographic (S) support surface.

In anion exchange chromatography, ion exchanges anions and the number is represented as follows;

SX + A- + B-↔ S-X + B- + A

The B-anion of the eluent is replaced by an analyte cation A- attached to ion X + well-charged on the surface of the standing phase. The adsorption of the analyte in the vertical phase and the reduction of eluent ions are repeated during their movement in the column, leading to separation due to ion exchange [2].

Cells are totally different in their charging state and will show various levels of interaction with charged chromatography support depend upon contrasts in their general coefficient, charge thickness and appropriation of above charge. The total surface charge of all molecules with visible groups is highly dependent on pH [13]. The pH of the cell phase should therefore be selected in accordance with the total charge of the fermented protein within the opposite mixture of that of the active-matrix group, that it will remove the ion counter of the active group and bind the matrix. On the other hand, protein in

contrast will not be stored. Analyte protein adsorbed can be stimulated by altering the pH of the cellular phase resulting in a complete charge of the adsorbed protein, hence its ability to bind to matrix. In addition, increasing the concentration of similarly charged species within the moving phase may result in the release of bound proteins. During ion exchange chromatography for example in anion exchange, poorly charged protein analysts can be competitively eliminated with the addition of badly charged ions. The closer the interaction between the salt ions and the active groups will ultimately exceed the fact that the interaction exists between protein cases and the active groups, resulting in protein exchange and elution by gradually increasing the salt concentration in the moving phase [11].

Complex mixtures of anions or cations can usually be separated along with the estimated amount of each ion measured in a short period of time by ion chromatography exchange [10]. In classical ion-exchange chromatography the separation is done in open column mode.

Fully filled column with a standing section like small particles made of glass 1-2 cm wide. The cell phase or eluent contains a competitive ion and is continuously transferred to the column and broken down into it under gravity. The sample mixture is applied at the top of the column and allowed to pass through the ion-exchange material bed. A simple flow is then initiated and the pieces of eluent are collected periodically from the column.

Chromatography of open column exchanges is very slow due to the low flow rates of eluent.

Increased flow rate may result in a decrease in the efficiency of the separation. In modern ion-exchange chromatography the use of high ion exchange materials is combined with

flow detection has passed these challenges. Separation is performed in a full ion-exchanger column as particles of the same size. Particles of ion-exchange material are generally much smaller than those used in the open column of ion-exchange chromatography [1]. However, the ion exchange frames used in modern chromatography are less powerful than the old frames [10]. The eluent should be cast into a column due to the size of the small particles of the vertical phase. The sample mixture is applied to the eluent by the injection hole. Finally, the ions are separated by a flowing stream visual aids [1].

This method was used for the analysis of anions and cations, including iron ions, mono- and oligosaccharides, alditols and other polyhydroxy compounds, aminoglycosides (antibiotics), amino acids and peptides, organic acids, amines, alcohol, phenols, thiols, nucleotides and nucleosides and other tropical molecules. It has been used successfully in the analysis of raw materials, multi-functional ingredients, counter ions, impurities, and reduction products, bonding materials, mixtures and various stages of the production process and in the analysis of cleaning equipment solutions, waste streams, container compliance and other applications [2]. Extensive performance including high performance and high input formats, medium cost, robust resolution capability, large power management sample and simplicity and automation allow ion exchange chromatography to be one of the most important and widely used in all water chromatographic process [11].

Although the widespread use of ion exchange chromatography method of differentiation has not been fully elucidated. A great deal of effort has been made to explain the process of excessive ion exchange [3,9]. Another important disadvantage of this method is that this method does not provide specific details of events occurring on the surface of the standing phase, because ion-exchange equilibrium is always determined by the balance between solute interactions and active eluent interactions in resin areas [3]. Ion exchange is similar to witchcraft, because in both solid cases it takes a dispersed sample. The most important difference between them is in the stoichiometric nature of ion exchange. Each ion extracted from the solution is replaced by an equal amount of another ion of the same charge, while the solute is usually taken stoichiometrically without a change in sorption [15]. Stoichiometric removal is based on the law of mass action and defines the retention of solute ion as an exchange process with a ion counter attached at the top [9].

According to this model, the storage of protein under theological conditions, the corresponding conditions

are related to ion concentration and can be equally represented as follows;

log k = - (Zp / Zs) log Cm + log (φQ)

K is the last element and Cm is the focus of the counter ion in the cellular phase. Zp / Zs (= Z) is the ratio of the protein element charge to the counter ion value and reflects the statistical estimate of the electrostatic interaction of the protein with the standing phase as it moves between columns.

The behavior of the ion exchange chromatographic system can be explained by stoichiometric models. However, the method of ion exchange separation is very complex and stiochiometric considerations do not apply to long range methods, as electrostatic interactions due to the distribution of ions in the solution are also influenced by electrostatic forces [3,6]. Other interactions between solute-solute, solute-solvent and solvent-solvent also contribute to the retention and selectivity of ion exchange.

For example, ion-dipole interactions and distribution, should be included as important means.

In addition to the entropic contribution from solvent, such as water, structures around ion exchange sites should also be considered important [3]. In addition to this key to differentiating electrostatic interactions between ion-exchange sites as well

counter ions in ion exchange chromatography [6].

An important distinguishing feature of ion exchange frames from other types of gels is the presence of active groups. The groups are attached to the matrix. The process of ion exchange between solution ions occurs in these functional groups. The exchange of ions between ion resin and solution is governed by two principles:

1. The process is reversed, only known abnormal variations are known
2. The exchange reaction occurs on the basis of equality according to the principle of electro neutrality.

The number of ions exchanged by ion exchanges should be in line with the number of millions of charged ions released from ion exchange [16].

Equilibrium is established in each sample component between clear and vertical segments when the sample is presented in ion-exchange chromatography. The distribution of part (A) between the two categories is expressed by the distribution coefficient, “DA”.

DA = [A] r / [A] m

The value of the DA depends on the size of the population of the part A molecules in the fixed and known phases [1]. As equilibrium fluctuates, there is a continuous, rapid exchange of part A molecules between two phases. The fraction of time, fm, that the middle A molecule spends on the cellular phase is given by:

fm = A value for A in the mobile category / Total A

fm = [A] m Vm / [A] m Vm + [A] r w

= 1 / 1+ DA (w / Vm)

k '= DA (W / Vm)f_m = 1 / 1 + k’

w: Weight of the stationary phase

V_m: Volume of the mobile phase [1]

The method of anion and cation exchange is very similar. When analyzes enter the ion exchange column, they first bind ionic sites illegally charged in the Coulombic magnetic field [2]. According to Coulomb's law, the interaction between ions in the solute and the ligands charged in contrast to the matrix in ion-exchange chromatography is due to electrical energy. Coulomb's law is given by equation as follows;

f = q1q2 / er2

f: Electrical interaction

q1q2: Charge on ions

e: Continuous dielectric medium

r: The distance between costs.

When charging on both ions is the same (both positive or negative) the power is disgusting, when different (one positive and the other negative) the energy is attractive. As the ion charge of animal species increases (Divalent ion must have a stronger affinity than the flexible ion) and when the constant dielectric drop (two opposing molecules rise higher in the living solvent than in water), the interaction increases. On the other hand, the distance between cases increases cooperation and decreases. In addition, other links, in particular, are van der Waals forces participating in Coulombic forces [2,17].

Ion chromatography, also known as adsorption chromatography, is a useful and popular method for it;

• large capacity,
• high resolution capacity,
• different forms of separation,
Multiple scenarios and wide scope,
• the tendency to concentrate the sample
• low cost [17].

The typical components of ion-exchange chromatography are presented below:

• High pressure pump with pressure indicator and flow, to deliver the event
• Sample injection into an unknown stream and column
• Column, to separate sample mixture into portions
• The oven, if you prefer
• Detector, measuring analytical peaks as eluent from column
• Data system for collecting and arranging chromatograms and data

In ion-exchange chromatography, adsorption and desorption processes are determined by the characteristics of three cooperating entities;
• Stability phase,
• Parts of the moving phase
• Solute [18].

2.Ion exchange chromatography applications

Ion-exchange chromatography can be utilized in the separation and purification of many charged or imperceptible atoms like proteins, peptides, chemicals, nucleotides, DNA, anti-microbials, nutrients, etc from natural sources or engineered origin. Instances of where ion-exchange chromatography has been utilized as a chromatographic fluid strategy for the separation and purification of bioactive atoms from regular sources can be given underneath.

Sample 1:

Source: Nigella sativa Linn.

Extraction Process: Sativa was produced, dried, and processed into powder for a water extract. The powder was soaked at 10,000 rpm for 30 minutes at 4ºC after being dissolved in phosphate buffer saline (pH 6.4). By eliminating a layer of fat and an insoluble pellet, the supernatant was recovered for use as a solvent. The Bradford method is used to calculate the soluble extract's protein content. The proteins are then sold using a 3500 MW cut dial and centrifuged bagged in a 0.05 M phosphate buffer (pH 6.4).

Standard Set: XK50 / 30 (5 x 15 cm) DEAE column for paddle A50.

Eluent: 0.05 M phosphate buffer (pH 6.4) containing 0.01 M NaCl. The individual components were present compiled with a growing concentration of NaCl

Discovery: UV detector at 280 nm

Analytical (s): Number of protein groups ranging from 94–10 kDa molecular mass [19].

Sample 2:

Source: Olea europea L.

Extraction process: Extraction was made using water that was at normal temperature and the leaves and roots of two-year-old olive trees. Internal substances like D-3-O-methylglucopyranose (MeGlu) were employed in the right quantity. The discharge took place during the course of a 15-minute motion, and the suspension was then set for 10 minutes at 3000 rpm. To remove anion impurities, the aqueous phase was filtered before being passed to the OnGuard A (Dionex) cartridge.

Standing Phase: Two columns of the Dionx CarboPac PA1 anion exchange and a monitoring column and a CarboPac MA1 column with the monitoring column used for the High Performance Anion Exchange Chromatography process.

Eluent: Eluent contained 12 mM NaOH containing 1 mM barium acetate. Flow rate was 1 mL / min.

Detection: Detected amperometric stroke

Analyte (s): myo-inositol, galactinol, mannitol, galactose, glucose, fructose, sucrose, raffinose and stachyose [20].

Sample 3:

Source: Soybeans

Extraction process: After 30 minutes of petroleum ether soaking, soybeans are centrifuged twice. A 0.03 M Tris-HCL bath containing 0.01 M 2-mercaptoethanol (pH 8) was used to extract the protein, which was subsequently centrifuged (16.250 x g, 20 min, 20 oC) after extraction for an hour. At

pH 6.4, 2 M HCl was used to prepare the supernatant, which was then centrifuged (16.250 x g, 20 min, 2–5 oC). To achieve a purified fraction that included 11S globulin, the precipitate was dissolved in the Tris-HCl buffer and the procedure was repeated. After the first shower of the 11S fraction, the supernatant was collected and centrifuged (16.250 x g for 20 minutes at 2-5 oC) at pH 4.8. The precipitate was dissolved in the Tris-HCl buffer (pH 8) while the supernatant was maintained at low temperatures. To get a purified fraction of rain that contains 7S globulin, the procedure was repeated.

Standing Phase: Anion Anion column of POROS HQ / 10 packed with polystyrene-divinylbenzene beads attached.

Simple: The initial method of soy protein separation by HPIEC involved the use of a binary gradient, in which the mobile phase was a buffer solution containing sodium chloride and the cellular phase was a buff solution at a certain pH (always pHs higher than the isoelectric pH of soy protein, pI = 4.8–6.4).

Sample 4:

Source : Phaseolus vulgaris

Extraction procedure: The seeds were crushed and steeped in a buffer solution of 20 mM Tris-HCl (pH 7.6) at 4 oC for 24 hours. To extract the proteins from the seeds, they were put in a blender, and then they underwent 30,000 g of centrifugation at 4 oC. The supernatant was then mixed with 450 g/l of ammonium sulphate until it reached 70% saturation. Centrifugation was used to remove the precipitate, and prolonged dialysing with distilled water followed. After being freeze dried, the dialyzed protein extract was employed for chromatographic separation.

Stationary Phase: Q-Sepharose Column (3 cm x 7 cm), anion-exchange

Eluent: 20 mM Tris-HCl (pH 7.6) was used to initially elute the column after it had reached equilibrium. Using 1 M NaCl in the equilibration buffer, the bound fraction was eluted. The flow rate used for all chromatographic stages was 100 ml/h. At a flow rate of 0.5 ml min-1, selected fraction Q1 underwent further separation after being lyophilized and dissolved in 100 mM Tris-HCl (pH 7.6) buffer.

Detection: UV detector, 280 nm

Analyte(s): A 5447 Da antifungal peptide [24].

Sample 5:

Source: Sweet dairy whey

Extraction procedure: Once the sweet whey from the cheese-making process has been created, it is subjected to additional reverse osmosis processing to raise the solids content from about 5.5% (w/w) to 14.6% (w/w).

Stationary phase: Pharmacia's Q- and S-Sepharose anion- and cation-exchange resins

Eluent 1: It was discovered that two simultaneous pH and salt concentration step adjustments were required for the anion-exchange process in order to carry out the anion-exchange separation. The initial state or feed loading buffer was a buffer containing 0.01 M sodium acetate with a pH of 5.8. One column volume of this buffer was added to the column after the whey feed was added to remove any components, including the IgG, that did not bind to the resin. In order to desorb the proteins whose pI values were higher than 5.0, two column volumes of 0.05 M sodium acetate, pH 5.0, were then poured through the column.This comprises bovine serum albumin and -lactoglobulin. Two column volumes of 0.1 M sodium acetate, pH 4.0, were added after that to eventually desorb the - lactalbumin, whose pI range is above that of the passing pH wave at 4.0 and lies between 4.2 and 4.5. The cleaning cycle was then conducted to get the column ready for the following run following this second step change.

Eluent 2: One stage of pH alteration was found to be sufficient to carry out the cation-exchange separation in the cation-exchange process. As the beginning state or feed loading buffer, 0.05 M sodium acetate, pH 5.5, was employed. It was best to put the anion-exchange breakthrough curve fraction onto the cation-exchange column in a single column volume. One column volume of the initial buffer was then run through the column to wash off any material that did not bind to the resin after the anion-exchange breakthrough curve fraction was put onto it. The bound IgG was then released using a step change in pH. Two column contents of the buffer—0.05 M sodium acetate, pH 8.5—were passed to achieve this. Because the highest limit of this buffer's pI range is 8.3, the IgG began to elute as the pH wave passed

across the cation bed. This pH step adjustment was followed by the start of the cleaning cycle.
Detection: UV Detector
Analyte(s): α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulin G and lactose[25].

Sample 6:
Source: Morus alba (mulberry) leaves
Extraction procedure: In ice-cold 50 mM Tris-HCl, pH 7.5, containing 0.3 M NaCl, 20 mM diethyldithiocarbamic acid, 5% glycerol, and 2% polyvinylpyrrolidone, fresh leaves were homogenised. Fresh leaves in the amount of 3 ml g-1 served as the buffer. The homogenate was held at 20°C for 24 hours after being filtered through a layer of cheesecloth. It was centrifuged at 8000xg for 40 min at 4°C after thawing. Ammonium sulphate was added to the supernatant until it reached 70% saturation. The precipitate that resulted from this process was recovered by centrifugation at 8000xg for 40 min, redissolved in 50 mM Tris-HCl, pH 7.5, containing 0.3 M NaCl, and dialyzed against the buffer overnight at 4°C. After centrifuging the solution at 13,000 x g for 15 min, the supernatant was collected and kept at -20 °C. A portion of the protein-containing dialyzed ammonium sulphate fraction was added to the N-acetylgalactosamineagarose column for affinity chromatography. Then, additional separation was carried out on a Sephacryl S-200 column, and chromatography with anion exchange came next. Gel filtration chromatography and anion exchange were used for further purification..
Stationary Phase: Anion-exchange chromatography, a DEAE-Sephacel column (2x9 cm)
Eluent: Equilibrated with 20 mM Tris–HCl, pH 7.5 at flow rate 20 ml min-1 and then eluted
stepwise with the buffer containing NaCl.
Detection: UV Detector, 280 nm
Analyte(s): Lectins, MLL 1 and MLL 2 [26]

Sample 7:
Source: Lycium ruthenicum Murr.
52 Column Chromatography
Extraction: Using hot water to remove the plant's fruits produced a sample of crude polysaccharide called CLRP. CLRP had a 66.2% carbohydrate and a 7.3% protein composition. CLRP was a black polysaccharide sample in which column chromatography was unable to eliminate the pigment. Decolorization was carried out using 30% H2O to prevent the impact of pigment on the structure analysis. Following decolorization, the CLRP had a carbohydrate content of 93.2% and a protein content of 4.4%. Anion exchange chromatography was used to purify the decolored CLRP, producing the five polysaccharide subfractions LRP1, LRP2, LRP3, LRP4, and LRP5.
Stationary Phase: DEAE-cellulose column
Eluent: Distilled water, 0.05–0.50 mol/L NaHCO3 - solution
Detection: UV Detector, 280 nm
Analyte(s): Glycoconjugate polysaccharide (LRGP1) [27]

Sample 8:
Source: Coprinus comatus
Extraction: In order to remove fat, 100 g of C. comatus stipe powder was extracted three times with 1 L of 95% ethanol at reflux for two hours. The residue was then extracted three times with 2 L of distilled water for two hours at 80 °C with intermediate centrifugation (2000 g, 15 minutes). The collected aqueous supernatants were concentrated to 400 mL (reduced pressure at 40 °C) and then precipitated with three volumes of 95% ethanol. Crude polysaccharide material was obtained after drying the precipitate at 40 °C after being cleaned with ethanol and acetone. After centrifuging the solution, which was made up of 100 mL of 0.2 M sodium phosphate buffer (pH 6.0) and crude polysaccharide material, the solution was added to a DEAE-Sepharose CL-6B column.
Stationary Phase: DEAE-Sepharose CL-6B column (3.5 cm × 30 cm).
Eluent: 0.2 M sodium phosphate buffer (pH 6.0), and linear gradient of 0.3–1.5 M NaCl in 0.2
M sodium phosphate buffer (pH 6.0).
Detection: UV Detector, 490 nm (phenol–H2SO4) and 500 nm (Folin–phenol)
Analyte(s): Polysaccharides; disaccharide α,α-trehalose, α-D-glucan, β-D-glucan, α-L-fuco-α-Dgalactan [28].

Sample 9:
Source: Physalisalkekengi var. francheti

Extraction: The dried and defatted fruit calyx were each extracted using a separate enzyme—Neutral proteinase, Papain, and alkaline protease—at the proper pH and temperature, and then centrifuged at 5000 rpm for 10 minutes for each extract. At room temperature, the supernatant was concentrated before being precipitated with the addition of ethanol in a 1:4 (v/v) ratio. The precipitate was dissolved in distilled water, the mixture was then washed with sevag reagent (1:4 isoamyl alcohol to chloroform), centrifuged at 5000 rpm for 15 minutes, and the protein was extracted. Before concentration in a vacuum evaporator at 55 °C, the supernatant was dialyzed for 24 hours against deionized water. At room temperature, the mixture was precipitated by adding ethanol in a ratio of 1:4 (v/v), and the precipitate was then freeze dried. Using glucose as a reference, total sugars were identified using the phenol-sulfuric acid assay.
Stationary Phase: DEAE anion-exchange column
Eluent: At a flow rate of 0.6 mL/min, gradient solutions (0.1 M, 0.25 M, 0.5 M NaCl, and 0.5 M NaOH) were first used to elute the column, followed by distilled water. Remains were put onto a Sephadex G-200 gel column (2.5 65 cm) after the principal polysaccharide fractions were collected using a fraction collector, condensed using a rotary evaporator at 55 °C, and loaded. At a flow rate of 0.3 mL/min, 0.1 M NaCl was used to elute the column. The majority of the portion was gathered and freeze dried. Using glucose as the reference material, the phenol-sulfuric acid technique was used to determine the sugar content of each of these fractions.
Detection: UV Detector, 490 nm
Analyte(s): Polysaccharides [29].

Sample 10:
Source: Ornithogalum caudatum Ait.
Extraction: The entire dried plant was immersed in 95% ethanol to dissolve the lipids, inactivate the enzymes, and remove the colours before being refluxed in hot distilled water for four hours at 90 °C. Under reduced pressure in a rotary evaporator, the aqueous extract was concentrated to 30% of its original volume, and proteins were eliminated using the Sevag method. With 40% ethanol, the resulting solution was precipitated. Ethanol was used to add the supernatant up to 60%, and it was then left at 4 °C overnight. The polysaccharide pellets were created by centrifuging at 4000 rpm for 15 minutes, thoroughly dissolving them in the required volume of distilled water, and then performing intense dialysis for two days against distilled water (cut-off M w 3500 Da). After centrifuging the retentate part to remove the insoluble material, it was concentrated. To produce crude extract, the supernatant was lyophilized in the end. After being dissolved in buffer solution containing 0.2 mol/L tris (hydroxymethyl) aminomethane hydrochloride, the crude extract was filtered through filter paper. A column for anion-exchange chromatography was used to process the solution. For additional separations after ion exchange chromatography, various chromatographic techniques were applied.

Stationary Phase: DEAE-Sepharose fast flow anion-exchange chromatography column (10 × 300 mm)
Eluent: The polysaccharides were eluted with Tris–HCl buffer solution, followed with gradient
elution of 0.1–0.8 mol/L NaCl at a flow rate of 0.8 ml/min.
Detection: UV Detector, 486 nm (phenol–sulfuric acid method)
Analyte(s): Water soluble polysaccharides [30].

Sample 11:
Source: Paecilomyces variotii
Extraction: Ammonium sulphate was added to the supernatant after the fermentation process to reach a final concentration of 80% saturation. The liquid was heated to 4oC and then ammonium sulphate was continuously stirred in. Centrifugation was used to separate the precipitated proteins for 30 min. at 10,000 rpm and 5 °C. The separated proteins were then re-suspended in a small amount of distilled water, dialyzed for 24 hours against distilled water using cellulose dialysis tubing, and concentrated by freeze-drying. Acetate buffer (20 mM, pH 6.0) was used to dissolve the partially purified enzyme before it was run down a column.
Stationary Phase: Diethylaminoethyl (DEAE) Sepharose column (0.7 x 2.5 cm)
Eluent: The same buffer was able to equilibrate with acetate buffer (20 mM, pH 6.0). A linear gradient from 0-1M NaCl in the acetate buffer was then applied after the solution was passed through the

column at a rate of 1 mL.mim-1. In an automated fraction collector (Pharmacia Biotech), the eluted fractions were collected, and the absorbance of the fractions was determined at 280 nm. The tannase activity of the primary peak fractions was then determined, and only the fractions with tannase activity were pooled.
Detection: UV Detector, 280 nm
54 Column Chromatography
Analyte: Tannase [31]

Sample 12:
Source: Castanospermum australe
Extraction: 50% MeOH extract of seeds
Stationary Phase: (1) Amberlite IR-120B (500 mL H+ form), (2) Dowex 1-X2 column (3.8×90 cm, OH−form), (3) Amberlite CG-50 column (3.8×90 cm, NH4 + form), (4) Dowex 1-X2 column (3.8×90 cm, OH− form) (Repeated separation on different ion exchange columns).
Eluent: 0.5 M NH4OH, H2O
Detection: UV Detection by HPTLC
Analyte(s): Pyrolizidine alkaloids; fagomine; 6-epi-castanospermine; castanospermine; australine; 3-epi-fagomine; 2,3-diepi-australine; 2,3,7-triepi-australine; 3-epi-australine; 2Rhydroxymethyl- 3S-hydroxypyrrolidine; castanospermine-8-O--D-glucopyranoside; 1-epi-australine-2-O--D-glucopyranoside and 1-epi-australine [32].

3. Recent Advancement in Ion-exchange Chromatography

a. Stationary-Phase Architecture
Simlane-based modification of porous silica substrates, electrostatic-agglomerated films on nonporous substrates, electrostatic-agglomerated films on ultrawide-pore substrates, polymer-grafted films on porous substrates, chemically derivatized polymeric substrates, polymer-encapsulated substrates, ionic molecules adsorbed onto chromatographic substrates, and step-growth porosity are the nine basic architectures that make up stationary-phase construction for Five of them: step-growth polymers on polymeric substrates, chemically derivatized polymeric substrates, electrostatic-agglomerated films on ultrawide pore substrates, and films grafted with polymers on porous substrates.

b. Electrostatic agglomerated films on ultrawide-pore substrates:
The majority of greater capacity variants using ultrawide-pore substrates have mainly replaced electrostatic agglomerated films on nonporous surfaces. It is conceivable to create materials with noticeably larger capacities by employing an architecture similar to that based on nonporous substrates but using substrates with pore diameters in the 100-300 nm range [33]. The ultrawide-pore substrate's pore size and the colloidal ion-exchange material's particle size are selected so that the pore size is sufficient to support a coating of ion-exchange colloid on the porous substrate's interior and exterior surfaces. The capacity of the resulting material, which is 30-150 Eq/mL for materials using an ultrawide pore format as opposed to 5-30 Eq/mL for materials using a nonporous format, can be 6-8 times that of an identical particle size non-porous substrate when the substrate pore size and colloidal particle size are optimally matched. This stationary phase architecture has been widely used recently due to the growing significance of high capacity chromatographic materials in IC and the accessibility of high capacity suppressor devices.
c. Polymer-grafted films on porous substrates:
In situations when crosslinking is not necessary for selectivity control, this sort of material is frequently utilised to construct high capacity packings. These kinds of chromatographic materials are made by attaching polymer strands to a substrate's surface[34,35]. Such materials are made by either preparing the substrate with polymerizable groups on the surface, altering the surface to add polymerizable groups, or altering the surface to add an initiator species. After allowing the resin, monomer (or monomers), and initiator to react, a composite polymer graft with polymer strands poking out from the substrate surface is created. This synthesis method forbids the use of crosslinking monomers since adding one to the reaction mixture will result in the formation of a gel with the substrate particles suspended in the gel. Controlling selectivity in such grafted films is limited by the inability to use crosslinkers in grafted polymer films. This architecture is primarily utilised in applications that call for a stationary phase with a sizable water content and high capacity. However, in reality,

almost all such materials are made utilising polymeric substrates. Such materials can be prepared from either polymer-based or silica-based substrates.

d. Chemically derivatized polymeric substrates:

The actual chemistry employed for the derivatization reaction in commercial products is typically unclear because this sort of substance frequently involves specialised synthesis methods. These kinds of chromatographic materials typically have a large capacity since functional groups are not always restricted to the substrate's surface. The popularity of these materials has increased recently as column capacities have migrated upward. Because good chromatographic performance depends on the derivatization being restricted to the surface, this stationary-phase synthesis approach has a fundamental weakness. Slow mass transport and subpar chromatographic performance will be displayed by reactions that occur within the substrate's dense polymer matrix below the surface.

e. Polymer-encapsulated substrates:

A substrate, a preformed polymer with residual double bonds, and a suitable free radical initiator are combined, dissolved in an appropriate solvent, and the solvent is removed to leave a polymer film on the surface of the substrate. The film is then cured at a high temperature to produce a crosslinked film that permanently encapsulates the substrate. This architecture's advantage is that it can be employed with inorganic substrates that are not amenable to covalent alteration because chemical attachment to the substrate's surface is not necessary. Although the approach was first designed to create a reversed-phase material based on alumina, it was later modified to create a weak cation-exchange phase utilising a premade butadiene-maleic acid copolymer as the encasing polymer[36]. A significant change in stationary-phase design for the separation of inorganic cations resulted from the first commercial introduction of stationary phases based on this methodology. Nearly all separation products prior to the launch of this novel synthesis technique were built on strong-acid cation-exchange stationary phases. Since then, almost all stationary phases used for the separation of inorganic cations have been carboxylic acid-based weak cation-exchange stationary phases. The potential for phase swelling and shrinkage during gradients or temperature programming, depending on the film's curing conditions, is a drawback of this synthetic technique. Alkaline reagents can still attack the underlying silica by penetrating to the surface coating, resulting in bed collapse, even if the coating is free of surface flaws.

4. Conclusion

Since the early 19th century, when this work's classification of medicinal substances took place, the field of drug development research has expanded tremendously [37]. As a result, during the past ten years, there has also been an increase in interest in liquid chromatographic procedures as a result of the expanding pharmaceutical sector, its demands, and the specialization of highly specialised and effective classification methods. Bioactive compounds are categorised into several sources using a variety of liquid chromatography techniques [25]. The oldest and most potent type of liquid chromatography is called ion exchange chromatography. It is still commonly used today to analyse and categorise molecules that can be ionised or have separate charges, such as proteins, enzymes, peptides, amino acids, nucleic acids, carbohydrates, polysaccharides, lectins, or any other compounds. colorimetric [38]. Additionally, natural compounds that are protonated bases, such as alkaloids, or deprotonated acids, such as fatty acids, or from amino acids can be separated and purified using ion exchange chromatography [39]. Ion exchange chromatography offers numerous benefits. Numerous high-energy compounds are analysed using this technology often. The process is inexpensively and simply scaled up for production. With the ion exchange stage, the target molecule can be produced with high degrees of purity. This method can be used to track soluble natural compounds [17,39]. Because of this, ion exchange chromatography, which has been used for more than 50 years to separate ionic atoms, is still a viable and well-liked technique for categorising natural products in contemporary drug discovery, and it keeps expanding as new technologies are created.

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