HHS Public Access

Author manuscript

Analyst. Author manuscript; available in PMC 2019 January 15.

Published in final edited form as:

Analyst. 2018 January 15; 143(2): 374–391. doi:10.1039/c7an01469d.

High Performance Affinity Chromatography and Related Separation Methods for the Analysis of Biological and Pharmaceutical Agents

Chenhua Zhang, **Elliott Rodriguez**, **Cong Bi**, **Xiwei Zheng**, **Doddavenkatana Suresh**[a], **Kyungah Suh**, **Zhao Li**, **Fawzi Elsebaei**[b], and **David S. Hage**[*]

Department of Chemistry, University of Nebraska, Lincoln, NE 68588-0304 (USA)

Abstract

The last few decades have seen the development of many high performance separation methods that use biologically-related binding agents. The combination of HPLC with these binding agents results in a technique known as high performance affinity chromatography (HPAC). This review will discuss the general principles of HPAC and related techniques, with an emphasis on their use for the analysis of biological compounds and pharmaceutical agents. Various types of binding agents for these methods will be considered, including antibodies, immunoglobulin-binding proteins, aptamers, enzymes, lectins, transport proteins, lipids, and carbohydrates. Formats that will be discussed for these methods will range from the direct detection of an analyte to indirect detection based on chromatographic immunoassays, as well as schemes based on analyte extraction or depletion, post-column detection, and multi-column systems. The use of biological agents in HPLC for chiral separations will also be considered, along with the use of HPAC as a tool to screen or study biological interactions. Various examples will be presented to illustrate these approaches and their applications in fields such as biochemistry, clinical chemistry, and pharmaceutical research.

1 Introduction

HPLC is an important method for the separation, analysis and characterization of biological, clinical and pharmaceutical samples.[1–4] The combination of biologically-related binding agents, or “affinity ligands”, with HPLC has been of great interest in recent years for the creation of selective separation methods for the analysis of targets such as drugs, disease biomarkers, and biopharmaceuticals.[1,2,4–6] This has given rise to a technique known as high performance affinity chromatography (HPAC).[1–3,5,6] This review will discuss the general principles of HPAC and related techniques, with an emphasis on their use for the analysis of biological compounds and pharmaceutical agents. Various types of binding agents for these methods will be considered, including antibodies, antibody mimics, immunoglobulin-

[*]Author for correspondence: 704 Hamilton Hall, Chemistry Department, University of Nebraska, Lincoln, NE 68588-0304 USA. Phone: 402-472-2744; FAX: 402-472-9402; dhage1@unl.edu.

[a]Home Department: Department of Chemistry, Tumkur University, Tumkur, Karnataka 572103, India;

[b]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

binding proteins, carbohydrates, carbohydrate-binding agents, enzymes, transport proteins, and lipid-based agents(see Table 1).

2 General principles of affinity-based methods in HPLC

Affinity chromatography is a type of liquid chromatography in which an immobilized biologically-related binding agent, or "affinity ligand", is used as the stationary phase(see Figure 1).[7] The merits of this method include its high selectivity and ability to provide relatively simple separations for specific target compounds, even when this approach is used with complex mixtures such as cell cultures, food samples, and clinical specimens like serum or urine. [1–7] One limitation or requirement for this method is the need for an appropriate binding agent for the given target.[4–7] Another requirement is the need for adequate techniques for immobilization of the binding agent and conditions that can be used with this agent for the application and elution of the target for either its purification or analysis.[7] The following sections of this review will discuss the various categories of binding agents that have been employed in this method, the types of compounds that bind to these agents, and the conditions under which these binding agents have been used in chromatographic systems for target isolation, analysis or characterization.

Traditional affinity chromatography is commonly used for biomolecule purification and generally employs low-performance supports such as agarose or carbohydrate-based materials.[1,7,8] HPAC is a type of affinity chromatography that instead uses supports that are suitable for work in HPLC, such as narrow-diameter silica or glass particles, perfusion-based materials, and monolithic supports.[4,7,8] This combination provides HPAC with greater speed, precision and ease of automation than traditional affinity chromatography, making HPAC more suitable for use in analytical applications.[1,5,8,9] The retention of a solute or analyte in either traditional affinity chromatography and HPAC is based on the specific and reversible interactions that occur between many biological binding agents and their targets.[8,9] An example would be the use of an immobilized antibody within a column to bind and capture its given target, or antigen, from applied samples.[1,5,7,8,10]

There are various ways that affinity ligands can be used in HPAC and related methods. One common format is the on/off elution mode that is shown in Figure 1. In this format, a sample containing the target analyte is applied to an affinity column in the presence of an application buffer.[11–13] During this application step, non-retained components will pass through the column, while the analyte is retained by the immobilized binding agent. The analyte is then later eluted by applying an elution buffer, followed by column regeneration.[11,12] This format is often used for binding agents that have strong retention for their targets (i.e., association equilibrium constants of roughly 10^6 M^{-1} or higher).[14] The presence of this strong binding means that an elution buffer that involves a change in the pH, ionic strength, polarity or mobile phase content (e.g., the use of chaotropic agents) is needed to weaken the interaction of the target with the column and to promote the target's elution.[15]

It is sometimes possible to use affinity ligands in HPAC under isocratic conditions. The resulting method is known as weak affinity chromatography.[16] This situation allows the same mobile phase to be used for both sample application and elution. This approach can be

practical when the association equilibrium constant for the target with the immobilized binding agent is less than 10^5–10^6 M^{-1}.[16] This approach is typically used with affinity columns that are employed in chiral separations[17,18] or in studies of biological interactions that have weak-to-moderate binding strengths.[1,2,5]

3 Affinity methods in HPLC using antibodies

Antibodies, or immunoglubulins, have been used in a variety of ways in HPAC and related affinity methods. The use of antibodies or related agents as stationary phases in chromatography is often referred to as immunoaffinity chromatography (IAC), and the use of these stationary phases in HPLC is known as high performance immunoaffinity chromatography (HPIAC).[13,14,19] Antibodies are glycoproteins that are produced by the body in response to a foreign agent, or antigen (e.g., bacteria or a virus).[13,14,19] The structure of a typical antibody, using immunoglobulin G (IgG) as an example, consists of a "Y"-shaped structure (e.g., see Figure 2).[13,14,19] The lower stem of such an antibody is known as the F_c region and is highly conserved from one type of antibody to the next.[13] The upper arms contain two identical antigen-binding sites and are known as the F_{ab} regions; the amino acid sequences of these two regions can be highly variable between different antibodies.[13,19] It is this variability that allows the body to produce antibodies against a wide range of targets and to produce antibodies that have strong and selective binding to these targets, with association equilibrium constants that are often in the range of 10^8–10^{12} M^{-1}.[19]

One format in which HPIAC, IAC and related methods can be used is for the purification or direct detection of an analyte. This type of work is usually done by utilizing the on/off format that was illustrated in Figure 1.[11–13] Purification of many pharmaceutical agents and biomolecules have been performed by using IAC with the on/off elution mode, including proteins, glycoproteins, drugs, and environmental agents.[1,13,14] A wide variety of analytes have also been analyzed by IAC with direct detection, such as antibodies, hormones, fibrinogen, albumin, β_2-microglobulin, transferrin, cytokines, cell receptors and carbohydrates.[20] This direct detection approach has further been used to investigate the thermodynamics and kinetics of binding by immobilized antibodies against targets such as the herbicide 2,4-dichlorophenoxyacetic acid and the hormone L-thyroxine.[11,12] In addition, antibodies have been used in HPLC columns as chiral stationary phases and with direct detection for such analytes as *R/S*-abscisic acid, various D/L-amino acids, (*R, R*)- or (*S, S*)-finrozole, D/L–hydroxy acids, D/L-lactic acid, *S*-methamphetamine, and L-triiodothyronine.[17,18,21,22]

Indirect analysis can also been achieved by using antibodies in HPAC or related methods. The resulting combination is often known as a chromatographic immunoassay or flow-based immunoassay.[1,13,14,19] As is demonstrated in Figure 2, these techniques may involve the use of either a competitive binding format (e.g., the simultaneous injection, sequential injection or displacement methods) or a non-competitive format (e.g., a sandwich immunoassay or one-site immunometric assay). In addition, these methods can make use of labels for detection that range from enzymes or fluorescent tags to chemiluminescent agents, liposomes, and radioisotopes.[23]

A chromatographic competitive binding immunoassay usually involves competition between a target analyte and a labeled analog for immobilized antibodies in a column.[13] There are several types of methods that fall in this category.[23,24] One of these is the simultaneous injection format, in which a mixture of the target and a fixed amount of a labeled analog of this target are injected together onto a column that contains a small amount of antibodies for the target. Due to competition between the labeled analog and the target for the immobilized antibodies, the amount of labeled analog that is non-retained will increase as the concentration of target in the sample is increased.[25] This approach has been employed with fluorescent, chemiluminescent, thermometric and electrochemical detection; examples include the use of labels based on fluorescein, Lucifer yellow, Texas red, Cy5 and acridinium ester or the enzymes horseradish peroxidase (HRP), alkaline phosphatase and catalase.[23] Some analytes that have been measured by this format are drugs and hormones like testosterone, thyroxine, cortisol, theophylline, digitoxin and gentamicin or peptides and proteins such as insulin, human serum albumin (HSA), transferrin, and IgG.[23] This method has also been employed with the targets carbaryl, isoproturon, and atrazine.[23]

The sequential injection format is another type of chromatographic competitive binding immunoassay. This method has the target and sample being applied first to the immobilized antibody column, followed later by injection of the labeled analog.[26] This approach again gives a response in which the amount of non-retained label increases as the target concentration increases; however, this method avoids the need for placing any label into the sample and can give lower detection limits than the simultaneous injection method.[13,19] The sequential injection format has been used for the analysis of imazethapyr, IgG, digoxin, atrazine, and HSA.[19,23] Labels that have been utilized in this technique have included fluorescent tags (e.g., fluorescein), enzymes (e.g., β-galactosidase, alkaline phosphatase, and HRP), and liposomes that incorporate such tags or labels.[19,23]

A displacement immunoassay, which is shown in Figure 2(a), is another type of chromatographic competitive binding technique.[19,23] In this method, the labeled analog is first injected onto an antibody column; this is followed by an injection of the target, which can displace some of the bound labeled analog. The size of the displacement peak for the labeled analog will be related directly to the concentration of target in the sample.[13,14] This type of assay has been combined with fluorescent labels for the detection of cocaine, benzoylecgonine, and 2,4-dinitrophenol, and has been used with absorbance detection for the analysis of transferrin and HSA.[27–29] In addition, this method has been adapted for use with ultrafast affinity extraction for the analysis of free drug and free hormone fractions.[30,31] A related method based on a reverse displacement assay has been described (i.e., using the drug phenytoin as a model analyte), in which an analog of the target was immobilized and used to bind a labeled binding agent such as an antibody or F_{ab} fragment.[32] In this latter technique, the target was able to bind to some of the labeled agent and cause it to be displaced from the column, giving a signal that was proportional to the target's concentration.[32]

Two types of non-competitive chromatographic immunoassays are the one-site immunometric assay and the two-site immunometric assay. In a one-site immunometric assay, a fixed amount of labeled antibodies or antibody fragments is mixed with the sample

and target. This mixture is then injected onto a column that contains an immobilized analog of the analyte, which is used to remove any excess labeled antibodies or antibody fragments.[13,33] Either the amount of the non-retained or retained labeled agent can then be used to determine the concentration of target that was present in the original sample.[1,13] This technique has been used to measure granulocyte colony-stimulating factor, interleukin-10, digoxigenin, α-(difluoromethyl)ornithine, fatty acid binding protein, 17-estradiol, α-fetoprotein, digoxin, and thyroxine.[23] These assays have used labels based on fluorescein, HRP, alkaline phosphatase, β-galactosidase, and liposomes containing fluorescent agents.[23]

The two-site immunometric assay, or sandwich immunoassay, utilizes two different antibodies against the same target, with one type of antibody being immobilized for target extraction and the other being labeled for detection.[1,13] The general scheme for this type of assay is shown in Figure 2(b). The amount of target in a sample can be determined by looking at the amount of labeled antibodies that are captured along with the target by the column.[1,13] Examples of targets that have been examined by using a chromatographic sandwich immunoassay have included thyroid-stimulating hormone, parathyroid hormone, HSA, IgG, human chorionic gonadotropin, interleukin-5, and anti-IgG antibodies.[34–37] These methods have employed labels that make use of absorbance, fluorescence, chemiluminescence orelectrochemical detection.[34–37]

Immunoextraction is another common application for HPIAC or IAC in pharmaceutical and clinical analysis. This method uses an immobilized antibody column to isolate one or more targets prior to their analysis by a second method. Immunoextraction has been coupled off-line with liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) for the analysis of atrazine, bovine serum albumin (BSA), cortisol, clenbuterol and phenytoin in urine, food and water.[14,19,38] For instance, an off-line immunoextraction column has been used to bind testosterone and epitestosterone from urine, followed by analysis of the extracted fraction by micellar electrokinetic chromatography.[38] On-line immunoextraction has also been coupled with LC, CE and mass spectrometry (MS) for the analysis of drugs, proteins and other targets.[19,33,38–40] As an example, one recent study used on-line immunoextraction with HPAC to capture normal or modified forms of HSA and to examine the binding by various drugs with this captured protein.[41]

Immunodepletion is a method related to immunoextraction that instead uses antibody columns to remove a possible interfering component from a sample.[19] This method has often been used in proteomics and the discovery of low-abundance proteins as biomarkers.[1,19,42] In this application high abundance proteins (e.g., HSA and IgG in human serum) can be removed by a column that contains antibodies against these proteins. The use of immunodepletion in this manner can make it easier to study and examine low abundance proteins and to analyze the simplified sample by a second method, such as two-dimensional electrophoresis or reversed-phase chromatography.[42–45]

Post-column immunodetection is an approach in which columns containing antibodies or related binding agents are utilized to monitor specific solutes that are eluting from an HPLC column.[20,46] This technique typically uses a post-column reactor to combine and mix the separated components with a labeled agent, which is used for indirect target detection in a

chromatographic immunoassay. The post-column reactor is followed by a column that contains an immobilized antibody or antigen that is used to measure an eluting target through the effect this target has on the ability of the labeled agent to bind to this second column.[20,46] Post-column immunodetection has been performed with sandwich immunoassays and one-site immunometric assays.[14] For instance, a sandwich immunoassay format has been used in a post-column scheme to measuring growth-hormone-releasing factor as it eluted from a reversed-phase column.[47] One-site immunometric assays have been employed in post-column methods to monitor digoxin, digoxigenin and human methionyl granulocyte colony-stimulating factor.[14] The use of an antibody column for direct detection in a post-column system is also possible if the eluting target is capable of generating enough signal for detection. This last approach has been used to monitor acetylcholinesterase (AChE) that eluted from a size-exclusion column by capturing this target with an anti-AChE antibody column and detecting the AChE by adding a coloring-forming substrate for this enzyme.[47]

4 Immunoglobulin-binding proteins

Immunoglobulin-binding proteins are bacterial proteins that can be used to bind, purify or capture immunoglobulins from various samples and sources.[48] A common example is protein A from *Staphylococcus aureus*, which is often used to isolate and bind many subclasses of IgG antibodies[49] and a number of other immunoglobulin classes (e.g., IgA and IgM from some species).[48] Another example is protein G, which is from group G streptococci and which can bind IgG-class antibodies from a wide variety of species.[50,51] Protein L, which is obtained from *Peptostreptococcus magnus*, is another immunoglobulin-binding protein and has strong binding with human IgG, IgA, IgM, and IgE class antibodies.[52] In addition, recombinant hybrid proteins such as protein A/G and protein G/L have been developed to expand the types of immunoglobulins that can bind to a single agent in this group.[48]

Immunoglobulin-binding proteins have been used not only for isolating various types of immunoglobulins and antibodies but also for detecting and measuring these targets in various samples.[53–58] This latter application has been of particular interest in recent years with the rapid growth in the market for monoclonal antibodies as biopharmaceutical products.[59,60] For instance, protein A has been used in affinity chromatography for the measurement of both monoclonal antibodies and polyclonal antibodies.[53,54] Protein G affinity chromatography has also been used to measure monoclonal and polyclonal antibodies.[55,58,61] Such applications have involved both particulate supports and perfusion media or monoliths.[54–56]

A number of reports have used columns containing immunoglobulin-binding proteins in combination with other types of columns. For instance, protein A has been combined with size exclusion chromatography for the simultaneous analysis of host cell proteins, monoclonal antibodies and their aggregates (see Figure 3).[62–65] In this approach, a size separation of the sample components that are not retained by a protein A column (e.g., feed impurities or host cell proteins) can be used to provide information that can aid in the

consistent production of monoclonal antibodies,[66] while a size separation of the retained components can be used to separate and measure antibodies versus antibody aggregates.[67]

Protein G has been used in affinity columns in a sandwich immunoassay to detect antibodies in human serum.[57] This method has been applied to the analysis of anti-human growth hormone antibodies, anti-BSA antibodies and human IgG.[57,68,69] Protein G-based immunoassays have also been carried out in other formats. One example is a dual column immunoassay that combined a protein G column with a reverse-phase column to measure both the titer of antibodies against a specific antigen and the amount of the antigen in injected samples.[61]

Immunoglobulin-binding proteins can be further employed as secondary binding agents to capture and immobilize antibodies in columns. This approach has been used with protein G columns to immobilize antibodies that could then be employed to bind and measure fluoroquinolones or insulin in serum.[70,71] Protein G columns have also been used to adsorb antibodies for use in competitive binding immunoassays.[72,73] This latter work has utilized such columns in a simultaneous injection competitive immunoassay to measure HSA[72] and in sequential injection-based methods to analyze HSA or transferrin.[72,73]

5 Aptamers

Aptamers are single strands of DNA or RNA with three-dimensional structures that are capable of binding to specific target compounds.[74–77] These binding agents can have relatively large association equilibrium constants, with values that may be as high as 10^6 to 10^9 M^{-1}.[74,76] The degree and specificity of this binding has made aptamers of interest as antibody mimics for both small and large targets (e.g., proteins, low mass biomolecules, and drugs).[74,78] Aptamers have been used within biosensors and/or in columns for the detection of biomarkers or for the purification of various compounds of interest in pharmaceutical and clinical applications. [74,76,77,79]

Aptamers can be generated by using a technique known as the systematic evolution of ligands by exponential enrichment, or SELEX.[79] SELEX is a multi-step cycling technique in which a large pool of oligonucleotides is screened to eventually acquire sequences which bind to the desired target.[80] These sequences are amplified by using the polymerase chain reaction and the screening process is repeated until an aptamer is obtained with both the desired specificity and binding strength for the target.[80]

A few studies have examined the use of aptamers in columns that can bind to small targets. Early work in this area explored the use of immobilized aptamers in capillary columns for the separation of adenosine and other nucleotides.[81,82] These columns were also used for the analysis of adenosine *in vivo* in rat brains, and demonstrated good reproducibility, stability and efficiency in such an application.[82] In addition, aptamers have been utilized with a perfusion support or silica particles and used as chiral stationary phases for the separation of D- and L-arginine-vasopressin, D- and L-adenosine, and the enantiomers of tyrosine and various related analogs (see Figure 4).[83–86]

Aptamers have also been employed in columns for the separation and analysis of biomacromolecules. For example, a biotinylated DNA aptamer has been bound to immobilized streptavidin on beaded polyacrylamide and used to purify a recombinant human L-selectin–receptor globulin fusion protein from a Chinese hamster ovary cell culture.[87] Two aptamer sequences were developed as affinity ligands for thrombin and used in a chromatographic sandwich assay for this target, giving a detection limit of 0.1 nM without the need for labeling or derivatization.[88] A biotinylated aptamer against cyctochrome C was bound to a glycidyl methacrylate/trimethylolpropane trimethacrylate (GMA/TRIM) monolith containing immobilized streptavidin and used to separate cytochrome C and thrombin from a mixture of several proteins; this technique was then used to detect cytochrome C in a liver tissue lysate and thrombin in serum and blood.[89] Another report used an aptamer that was covalently immobilized in a GMA/ethylene dimethacrylate (GMA/EDMA) monolith for the extraction and screening of lysozymes in samples such as chicken egg white.[90] In addition, an organic-inorganic hybrid silica monolith was used to immobilize an aptamer for the binding and detection of human α-thrombin.[91]

Aptamer affinity chromatography has been used in several studies with capillary columns[81,82,88,89,91,92] and microfluidic devices.[93,94] For instance, aptamers that could bind to thrombin were covalently attached to the inner surface of a bare fused silica capillary and used to extract thrombin from a protein mixture, with the thrombin being detected and identified by fluorescent labeling and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).[92] A microfluidic device has been used with aptamer affinity chromatography for the thermally-controlled extraction and elution of adenosine monophosphate.[93] Another group developed a microfluidic device capable of photolytic elution by using a photo-cleavable linker to immobilize an RNA aptamer.[94] This RNA aptamer was used to retain several proteins, which were then released and eluted after exposure of the aptamer to UV light.[94]

6 Enzymes

There have been a number of applications for affinity chromatography involving work with enzymes as either targets or immobilized binding agents. For instance, the use of enzymes in therapeutics has created a demand for improved methods for separating and purifying these agents.[95–97] One advantage of using enzymes as therapeutics is their ability to often bind their substrate or target with great affinity and selectivity.[95,97] Another advantage of enzymes is their capability to catalytically convert their substrate into a product in an efficient manner.[95,97] Affinity separations for enzymes have often used immobilized substrates, inhibitors or cofactors as the binding agents.[1] Specific examples are the use of immobilized flavin nucleotides for the extraction of flavin adenine dinucleotide synthetase from rat liver[98] and the use of furin-specific inhibitors for purification of the proprotein/prohormone convertase furin from ovarian cells.[99]

There have been a few cases in which immobilized enzymes have been used directly for the isolation or extraction of targets. An example is work in which carboxypeptidase A was immobilized and used for the isolation of carboxypeptidase inhibitor.[100] Another study used immobilized pepsin to isolate major and minor forms of a pepsin inhibitor from a

parasite.[101] The immobilization of enzymes in columns can often be done in a manner that does not drastically affect the activity or selectivity of the enzyme.[96,102–104] An additional advantage of using immobilized enzymes is their good stability and ability to be reused multiple times, while providing a format that allows easy removal of the bound targets or products from the enzyme.[102,104]

Another application of immobilized enzymes has been their utilization as chiral stationary phases.[105–114] This approach makes use of the ability of some enzymes to select between the enantiomers of solutes that interact with their binding sites.[106] For example, immobilized penicillin G acylase has been employed in chiral separations for several acidic aryl agents and non-steroidal anti-inflammatory drugs,[107–109] while glucoamylase G1 and G2 have been used as chiral stationary phases for amino alcohols.[110,111] Other enzymes that have been used as chiral selectors are cellobiohydrolase I, lysozyme, trypsin, α-chymotrypsin, and pepsin.[105,112–114]

A related field to affinity chromatography is that of immobilized enzyme reactors, or IMERs.[115–119] Analytical applications of IMERs have included their used for screening potential enzyme inhibitors, examining drug metabolism pathways, and digesting proteins for peptide mapping.[1,115–119] IMERs for chromatographic systems have been made using silica, agarose and various types of monoliths as the support.[120–124] In addition, IMERs have been used in such formats as HPLC, CE and microfluidics.[1,115–119,121,125–127]

7 Lectins

Lectins are another group of binding agents that have been used in affinity chromatography and HPAC. Lectins are non-immune system proteins that can bind reversibly to specific carbohydrate groups.[128–131] This property has made lectins important as tools for capturing and studying glycoconjugates such as glycoproteins.[129] One lectin that has often been used in affinity columns is concanavalin A (Con A) from *Canavalia ensiformis*, which can bind to high-mannose type glycans, hybrid type glycans with mannose branching, and complex type biantennary glycans.[132–134] Another lectin that has frequently been used as an affinity ligand is wheat germ agglutinin (WGA) from *Triticum vulgare*, which binds to sialic acid and the core portion of *N*-glycans, such as *N, N*-diacetylchitobiose (also known as $(GlcNAc)_2$), and the tetrasaccharide GlcNAcβ1–4Manβ1–4GlcNAcβ1–4GlcNAc which is present in bisected *N*-glycans.[135] Other examples of lectins that have been employed in affinity methods are *Aleuria aurantia* lectin (AAL) and *Sambucus nigra* agglutinin (SNA), which bind to α1–6, α1–2, α1–3 linked fucoses and α2–6 linked sialic acids.[136,137] *Artocarpus* lectin (AIL or jacalin) has also been used and binds Galβ1-3GalNAcor high mannose type glycan s.[138,139]

The selective enrichment of carbohydrate-containing targets like glycoproteins or glycopeptides by lectins has become important in studying complex biological mixtures.[42,138,140,141] For instance, Con A and WGA both bind to asparagine-linked glycans, while jacalin can bind to serine- or threonine-linked glycans.[138,140] These binding specificities have been combined in a multi-lectin column where Con A, WGA and jacalin were each immobilized to agarose and used to capture a wide range of glycoproteins from

human serum.[138] Multi-lectin affinity chromatography was also used with SDS-PAGE and LC-MS/MS to identify biomarkers for diabetic nephropathy in human plasma,[141] and multi-lectin columns have been utilized in the analysis of cellular lysates.[142,143]

Several reports have combined immunodepletion with lectin columns. For instance, the immunodepletion of twelve high-abundance proteins was used along with a column containing immobilized Con A and WGA and detection based on quadrupole TOF-MS to measure low abundance glycoproteins in human serum.[42] An automated platform was developed by coupling an immunodepletion column on-line with a multi-lectin column;[144] this platform was used to deplete abundant serum proteins prior to the enrichment and analysis of lower-abundance glycoproteins.[145,146] The fractions collected from this multi-dimensional system have been subjected to isoelectric focusing, followed by with-in gel digestion and LC-MS analysis.[147]

When several lectin columns are coupled together, the resulting technique is known as serial lectin affinity chromatography (SLAC).[139] For instance, high-mannose N-linked glycan-containing glycoproteins have been depleted by using a Con A column, followed by use of a jacalin column to capture O-linked glycan-containing glycoproteins.[139] Serial lectin affinity chromatography has been used to characterize co-existing glycans on the same glycoconjugates by altering the order.[148] SLAC can also be used to assess the degree of a specific type of glycosylation. For instance, SLAC with Con A and SNA has been used in parallel with Con A affinity chromatography and stable isotope coding to compare the degree of α2–6 sialylation on hybrid, high-mannose and complex biantennary glycans.[149] In another example, sialic acid-bearing glycoforms with complex-type glycans were fractionated into bi-antennary and tri-, tetra-antennary glycoforms by utilizingserial SNA/Con A affinity chromatography. [150]

Lectins have been employed with various high performance materials and column formats. One report used a polystyrene-divinylbenzene perfusion support with immobilized Con A, WGA and jacalin to make a multi-lectin column.[151] This column was combined with SDS-PAGE, LC-MS, isoelectric focusing and an antibody microarray to identify breast cancer-related glycoproteins in human blood.[152] A silica-based microcolumn was used to immobilize Con A SNA, *Ulex europaeus* lectin (UEA-I) and *Phaseolus vulgaris* agglutinin (PHA-L), enabling work with microscale samples of human serum.[137,153,154] Macroporous silica particles with a diameter of 1.6 μm were used with Con A and AAL to enrich glycoproteins from human serum and to perform glycomic and glycoproteomic studies.[155]

Several monolith supports have also been employed with lectins. One study compared the use of immobilized Con A and WGA on a cationic monolith based on 2-(methacryloyloxy)ethyl]trimethyl ammonium chloride (MAETA) or based on a neutral GMA/EDMA monolith, with the cationic monolith allowing the concentration and detection of glycoproteins at levels down to roughly 10^{-8} M.[156] A capillary monolith was developed in which Con A was immobilized onto GMA/EDMA that had been modified with Cu^{2+}-iminodiacetic acid; this type of column doubled the amount of active binding sites and was used to enrich and compare glycoproteins in samples such as urine.[157] Another study placed gold nanoparticles onto an EDMA-based monolith, which were then used to immobilize

Erythrina cristagalli lectin (ECL) for the enrichment of galactosylated proteins.[158] In addition, Con A has been immobilized onto a nanoporous gold monolith and used to capture ovalbumin from a mixture of this protein and BSA.[159]

8 Transport proteins

Serum transport proteins and related agents have been used for a number of applications in affinity chromatography and HPAC.[2] One example of this type of agent is HSA. HSA is the most abundant protein in human plasma, with a normal concentration of 35–50 g/L. HSA and the closely-related protein BSA can interact with various solutes that include many drugs, fatty acid s, and low mass hormones.[160,161] The two major binding sites on HSA for drugs are often referred to as Sudlow sites I and II.[162] A second example of a serum transport protein is alpha$_1$-acid glycoprotein (AGP). AGP is an acute phase glycoprotein that has a normal plasma concentration of 0.5–1.0 g/Land a carbohydrate content as high as 45% (*w/w*).[163–165] AGP is an acidic protein, with an isoelectric point of 2.8–3.8, and can bind to a number of basic, neutral or cationic drugs.[2,166] Other examples of serum binding agents for small solutes are low density lipoprotein (LDL) and high density lipoprotein (HDL).[167–175] These lipoproteins are complex particles of proteins and lipids that transport substances such as triglycerides and cholesterol esters,[172–175] as well as some drugs.[167–175]

Serum proteins have been widely used as chiral stationary phases.[166] For instance, HSA and BSA-based columns have been used to separate many acidic and neutral chiral compounds that have included coumarins (e.g., warfarin), benzodiazepine derivatives, barbiturates, benzothiadiazeines, α-arylpropionic acids (e.g., ibuprofen), and some amino acids (e.g., tryptophan).[176–181] Factors that can be varied to optimize these separations are the pH of the mobile phase[166,179,182], the use of organic modifiers in the mobile phase (e.g., 1-propanol) and the ionic strength of the mobile phase[166,183] Many chiral HPLC methods have used these binding agents with silica particles,[176–181] although some work has also employed silica monoliths or organic monoliths.[183–186]

AGP has also been used as a chiral stationary phase for a variety of compounds.[187–194] A few examples are the chiral separation and analysis of atenolol, citalopram, warfarin, verapamil, norverapamil, atropine, and vinca alkaloid analogs.[187–191] Liquid chromatography-tandem mass spectrometry has been used with AGP columns to separate and quantitate racemic drugs such as methadone, ornidazole, azelnidipine, ondansetron, and doxazosin.[192–196] These efforts have used AGP with both silica particles and silica monoliths.[197,198] Factors that can be used to adjust these separations have again included temperature and the pH or composition of the mobile phase (e.g., the use of organic modifiers such as methanol, ethanol, propanol or a tertiary alcohol).[199,200]

Several types of columns containing serum proteins have been used as tools to study solute-protein interactions.[2] One way this can be done is through zonal elution experiments, as shown in Figure 5(a). In a zonal elution experiment, a narrow plug of an analyte solution is injected onto a column that contains an immobilized agent (e.g., a serum transport protein). The elution of this sample plus is then monitored, with the retention time of the analyte being related to the equilibrium constant for this analyte to the immobilized binding agent.

For instance, the retention time for an injected probe compound that has a known binding site on an immobilized protein can be monitored while various concentrations of a possible competing agent are placed into the mobile phase.[201] This type of experiment can make it possible to determine how the injected and added solutes compete for a given site on the protein and to measure the affinities of these solutes at this site. For instance, this method has been used to examine site-specific interactions of drugs such as acetohexamide and tolbutamide with HSA[202] and to compare the binding sites of carbamazepine and propranolol on AGP.[203] Zonal elution can also be employed to examine the effects on this binding when there is a change in the temperature or the solvent pH and composition.[204–206]

Frontal analysis is another method that has been used with immobilized serum proteins to investigate solute-protein interactions. In this technique, a known concentration of a solute is continuously applied to a column to titrate binding sites on an immobilized agent. A breakthrough curve is then obtained, as shown in Figure 5(b), in which the mean position of this curve is related to the concentration of the applied solute, the number of binding sites for the solute in the column, and the equilibrium constants for the solute at these sites. This technique has been used to examine the interactions of HSA with *R/S*-warfarin and D/L-tryptophan (as well as coumarin or indole derivatives), thyroxine, acetohexamide, carbamazepine, imipramine, lidocaine, phenytoin, tolbutamide, and verapamil.[180,181,202,207–215] Frontal analysis has also been used to examine the binding of carbamazepine and lidocaine with AGP[203,211] and the interactions of *R*- and *S*-propranolol with HDL, LDL and very low density lipoprotein.[216,217] This method can also be employed to examine the effects of temperature and solvent competition on these interactions, as well as solute-solute competition or displacement.[204,205]

Another way serum proteins have been used in HPAC is in the method of ultrafast affinity extraction, as is illustrated in Figure 5(c).[218,219] In this technique, a sample of the solute is prepared in the presence or absence of a soluble binding agent such as a serum protein. This sample is then applied onto an affinity microcolumn for extraction of the solute, which is often done at a high flow rate.[218] One way this method can be used is to capture the non-protein bound (or free) form of the solute as the sample passes through the column.[220–222] This method has been used to measure free drug and hormone fractions in clinical samples and to measure the equilibrium and rate constants for drug or hormone interactions with proteins in solution.[30–32,220–227] Affinity ligands that have been used in microcolumns for this purpose have included both antibodies and serum proteins such as HSA or AGP.[30–32,220–227] Ultrafast affinity extraction has also been employed in multi-dimensional HPAC to study drug interactions in clinical samples[222,227] and to examine protein interactions with multiple solutes (e.g., chiral drug mixture, such as *R/S* -warfarin)[223] or for solutes that may have multiple binding agentsin a sample.[226]

9 Lipids

Lipids have explored for use in some applications of HPAC and affinity-based separations. One reason this area has been of interest is because drug lipophilicity directly affects the degree to which pharmaceutical agents can undergo partitioning into cell membranes and passive membrane transport.[228] Immobilized artificial membrane (IAM) chromatography is

a technique that been employed with lipids to estimate the partition coefficients of drugs for cell membranes[229] and to study the oral absorption, blood brain barrier permeability, skin permeability, and pharmacokinetics of various drugs.[230] The artificial membranes that are used in these columns consist of monolayers of phospholipid analogs that are covalently attached to a support (see Figure 6).[231,232] Phosphatidylcholine, which is a major component of biological membranes, is the most common agent that has been immobilized for this purpose.[233] Supports containing sphingomyelin and cholesterol have also been recently created and tested for use as stationary phases in IAM chromatography.[234]

Another application for the columns in IAM has been as platforms for the immobilization of receptors or transporters that are normally found in cell membranes.[235–239] The resulting columns have been used to screen the binding of drug candidates to these receptors and transporters.[235] These columns can also be used with techniques such as frontal analysis and zonal elution, as described in Section 8, to determine the equilibrium constant sand rate constants for these interactions. For instance, nicotinic acetylcholine receptors have been placed on IAM supports and used with frontal analysis to determine the binding constants of these receptors for (±)-epibatidine, 3-(2(s)-azetidinylmethoxy) pyridine, (−)-nicotine, carbachol and atropine.[236,237] P-Glycoprotein has been immobilized onto IAM supports to examine the allosteric interactions and binding constants of this binding agent with doxorubicin, vinblastine, cyclosporin A and verapamil.[238,239]

Immobilized liposome chromatography (ILC) is a related technique that has been employed in characterizing drug-membrane interactions.[240] In ILC, liposomes or lipid bilayers are immobilized onto a support by methods such as entrapment, the binding by these agents to immobilized hydrophobic ligands, or the adsorption of a biotinylated from of such an agent onto immobilized avidin.[241–243] Several studies have used ILC to screen the bioactive ingredients or membrane penetrable components in traditional Chinese medicine[244–249] and to estimate the affinities of these interactions[245,247,248]

Monoliths have been used with immobilized lipids, as has been demonstrated with a cholesterol-containing polymeric support.[250] This stationary phase has been used with compounds such as alkylbenzenes, steroid hormones and polycyclic aromatic hydrocarbons, giving retention properties that are distinct from both an IAM stationary phase and stationary phases that are commonly used in reversed phase chromatography.[251,252] A method for immobilizing phosphatidylcholine in a GMA/EDMA monolith has also been described[253,254] and applied to the study of drug-membrane interactions.[254] In addition, liposomes have been immobilized within a silica monolith and used to test the interactions of these liposomes with a variety of neutral, acidic or basic drugs. [246]

10 Carbohydrates

A variety of carbohydrate-based agents have been used in HPAC and affinity chromatography for pharmaceutical and biomedical analysis. One important application of these ligands has been their use in the separation and analysis of chiral drugs.[255] Polysaccharides such as cellulose and amylose are commonly used in chiral stationary phases for HPLC and many such columns are commercially available.[256,257,258] Examples

are 3,5-dimethylphenyl carbamate phases that make use of cellulose or amylose, as well as 4-methyl benzoate-based chiral stationary phases.[256]

Cyclodextrinsare another group of carbohydrate-based agents that have been widely used as chiral stationary phases. Cyclodextrins have a torus-like arrangement that contain six, seven or eight glucopyranose units, giving agents known as α-cyclodextrin, β-cyclodextrin, or γ-cyclodextrin, respectively.[259,260] The chiral recognition of a solute by a cyclodextrin is related to the relatively hydrophobic cavity and a hydrophilic exterior of the cyclodextrin, which contains a specific arrangement of alcohol groups about the mouth of the cavity. The process of chiral selection involves formation of inclusion complex between a solute and cavity of the cyclodextrin, along with differential binding of the analyte to 2- and 3-hydroxyl groups at the mouth of the cavity.[255,261]

Cyclodextrin columns have been used in various ways for chiral separations.[262–278] This has included work with both reversed-phase and HILIC type mobile phases.[262,263] A combination of the reversed-phase and HILIC modes has been used on a β-cyclodextrin stationary phase for the analysis of traditional Chinese medicine.[263] In addition, a restricted access β-cyclodextrin column has been used for the direct injection and analysis of chlorthalidone, aminoglutethimide, amlodipine and chlorpheniramine in samples containing BSA.[264] Cationic cyclodextrin derivatives have been immobilized to silica for use in the separation of acidic enantiomers[265–267] such as dansyl amino acid, carboxylic aryl compounds and flavonoids.[266] Phenylcarbamoylated cyclodextrin derivatives that can undergo π-π and dipole-dipole interactions with chiral analytes have been developed as well;[268] these agents were used in a bilayer stationary phase that contained both non-derivatized and phenylcarbamoylated-β-cyclodextrin to separate isoxazolines, flavonoids and dansyl amino acids.[269] A number of recent studies have examined the use of ‘click’ chemistry for the immobilization of cyclodextrins onto chromatographic supports.[270–273] The use of both silica monoliths and organic monoliths have also been considered for use with immobilized cyclodextrins in chiral separations.[274–278]

Aliphatic and aromatic functionalized cyclofructans (CFs) are another set of carbohydrate-related agents that have been examined for use in chiral separations.[279] Cyclofructans are macrocyclic oligosaccharides that contain six or more molecules of D-fructofuranose that are coupled through a β2-1 linkage. Cyclofrutans can be classified based on the number of fructose units they contain, giving compounds such as cyclofructan 6 (CF6), cyclofructan 7 (CF7), and cyclofructan 8 (CF8).[279] Nearly 120 racemic primary amine-containing compounds have been separated on an isopropyl-carbamate functionalized CF6 stationary phase.[280] Stationary phases containing *R*-naphthylethylcarbamate CF6 or dimethylphenyl-carbamate CF7 were also evaluated for their chiral recognition of a wide range of chiral acids, amines, metal complexes and neutral compounds. A total of 94 chiral compounds were resolved by either column, which accounted for 43% of compounds in a set of randomly-chosen potential analytes.[281]

11 Conclusion

This review has discussed how various biologically-related binding agents have been employed in HPAC and related methods as tools for a number of pharmaceutical and biomedical applications. It was shown that many binding agents are available for this work, ranging from antibodies and antibody mimics (e.g., aptamers) to enzymes, transport proteins, immunoglobulin-binding proteins, lectins, carbohydrates, and lipids. The specific and reversible interactions of these agents with their targets have made these binding agents useful as stationary phases in various separation formats. One common example is the on/off elution mode, which can be used for direct analyte detection, affinity-based extraction, or the depletion of a given target from a sample (e.g., as occurs in immunodepletion). Various techniques for indirect detection are also possible in HPAC and related methods, as might occur in chromatographic immunoassays that employ competitive binding or non-competitive binding formats. Other uses described for these binding agents in chromatographic systems have included post-column detection, multi-column systems, chiral separations, and the study of biological interactions. These efforts have also explored the use of various supports with these agents, such as particulate supports, monoliths, and mixed-bed materials. As additional types of supports, binding agents, and formats are developed for these binding agents in HPAC, it is expected that an even greater range of applications will appear for these methods in the analysis and characterization of samples in fields such as biochemistry, clinical chemistry and pharmaceutical research.

Acknowledgments

This work was supported by the National Institutes of Health under grants R01 GM044931 and R01 DK069629, and the National Science Foundation (NSF) under grant CHE 1309806.

References

1. Hage DS, Anguizola JA, Bi C, Li R, Matsuda R, Papastavros E, Pfaunmiller E, Vargas J, Zheng X. J Pharm Biomed Anal. 2012; 69:93–105. [PubMed: 22305083]
2. Hage DS, Anguizola J, Barnaby O, Jackson A, Yoo MJ, Papastavros E, Pfaunmiller E, Sobansky M, Tong ZH. Curr Drug Metab. 2011; 12:313–328. [PubMed: 21395530]
3. Moser AC, Hage DS. Electrophoresis. 2008; 29:3279–3295. [PubMed: 18646279]
4. Pfaunmiller, EL., Bas, J., Brooks, M., Milanuk, M., Rodriguez, E., Vargas, J., Matsuda, R., Hage, DS. Analytical Separation Science. Anderson, JL.Berthod, A.Pino, V., Stalcup, AM., editors. WILEY-VCH; Weinheim: 2015. p. 461-482.
5. Hage DS, Anguizola JA, Jackson AJ, Matsuda R, Papastavros E, Pfaunmiller E, Tong Z, Vargas-Badilla J, Yoo MJ, Zheng X. Anal Methods. 2011; 3:1449.
6. El Deeb S, Wätzig H, El-Hady DA. Trends Anal Chem. 2013; 48:112–131.
7. Hage, DS., Ruhn, PF. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 3-13.
8. Hage, DS. Encyclopedia of Chromatrography. 3. Cazes, J., editor. CRC Press; Boca Raton: 2010. p. 17-23.
9. Yoo, MJ., Hage, DS. Monolithic Chromatography and its Modern Applications. Wang, PG., editor. ILM; Glendale: 2010. p. 3-25.
10. Hage DS, Jackson A, Sobansky MR, Schiel JE, Yoo MJ, Joseph KS. J Sep Sci. 2009; 32:835–853. [PubMed: 19278006]
11. Nelson MA, Moser A, Hage DS. J Chromatogr B. 2010; 878:165–171.

12. Pfaunmiller E, Moser AC, Hage DS. Methods. 2012; 56:130–135. [PubMed: 21907805]
13. Pfaunmiller, EL., Bi, B., Li, Z., Vargas-Badilla, J., Zheng, X., Hage, DS. Advances in Liquid Chromatography: New Developments in Stationary Phases and Supports for Drugs and Bioanalytical Applications. Hage, DS., editor. Future Medicine Ltd; London: 2015. p. 60-74.
14. Hage, DS., Phillips, TM. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 127-172.
15. Walters RR. Anal Chem. 1985; 57:1099A–1114A.
16. Hage, DS., Anne Nelson, M., Xuan, H. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 79-97.
17. Hofstetter H, Hofstetter O. Trends Anal Chem. 2005; 24:869–879.
18. Koidl J, Hodl H, Schmid MG, Konrad M, Petschauer S, Kostner GM, Gubitz G. J Biochem Biophys Methods. 2006; 69:33–42. [PubMed: 16697467]
19. Moser AC, Hage DS. Bioanalysis. 2010; 2:769–790. [PubMed: 20640220]
20. Hage DS. J Chromatogr B. 1998; 715:3–28.
21. Franco EJ, Hofstetter H, Hofstetter O. J Pharm Biomed Anal. 2009; 49:1088–1091. [PubMed: 19250789]
22. Zeleke JM, Smith GB, Hofstetter H, Hofstetter O. Chirality. 2006; 18:544–550. [PubMed: 16642491]
23. Hage, DS., Moser, AC. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 789-836.
24. Matsuda R, Rodriguez E, Suresh D, Hage D. Bioanalysis. 2015; 7:2947–2966. [PubMed: 26571109]
25. Hage DS, Thomas DH, Chowdhuri AR, Clarke W. Anal Chem. 1999; 71:2965–2975. [PubMed: 10450148]
26. Hage DS, Thomas DH, Beck MS. Anal Chem. 1993; 65:1622–1630. [PubMed: 8328676]
27. Hage DS, Taylor B, Kao PC. Clin Chem. 1992; 38:1494–1500. [PubMed: 1643722]
28. Wemhoff GA, Rabbany SY, Kusterbeck AW, Ogert RA, Bredehorst R, Ligler FS. J Immunoligical Methods. 1992; 8:223–230.
29. Whelan JP, Kusterbeck AW, Wemhoff GA, Bredehorst R, Ligler FS. Anal Chem. 1993; 5:3561–3565.
30. Clarke W, Schiel JE, Moser A, Hage DS. Anal Chem. 2005; 77:1859–66. [PubMed: 15762597]
31. Ohnmacht CM, Schiel JE, Hage DS. Anal Chem. 2006; 78:7547–7556. [PubMed: 17073425]
32. Schiel JE, Tong Z, Sakulthaew C, Hage DS. Anal Chem. 2011; 83:9384–9390. [PubMed: 22122161]
33. Pfaunmiller EL, Anguizola JA, Milanuk ML, Papastavros E, Carter N, Matsuda R, Zheng X, Hage DS. J Chromatogr A. 2014; 1366:92–100. [PubMed: 25263063]
34. Karube I, Matsunaga T, Satoh T, Suzuki S. Anal Chim Acta. 1984; 156:283–287.
35. Hage DS, Kao PC. Anal Chem. 1991; 63:586–595. [PubMed: 2031560]
36. Johns MA, Rosengarten LK, Jackson M, Regnier FE. J Chromatogr A. 1996; 743:195–206. [PubMed: 8817882]
37. Wang Q, Wang Y, Luo G, Yeung WSB. J Liq Chromatogr Relat Technol. 2001; 24:1953–1963.
38. Chen HX, Deng QP, Zhang LW, Zhang XX. Talanta. 2009; 78:464–470. [PubMed: 19203610]
39. Chen HX, Huang T, Zhang XX. Talanta. 2009; 78:259–264. [PubMed: 19174235]
40. Jiang T, Mallik R, Hage DS. Anal Chem. 2005; 77:2362–2372. [PubMed: 15828768]
41. Matsuda R, Jobe D, Beyersdorf J, Hage DS. J Chromatogr A. 2015; 1416:112–120. [PubMed: 26381571]
42. Hao P, Ren Y, Xie Y. J Chromatogr B. 2009; 877:1657–1666.
43. Chromy BA, Gonzales AD, Perkins J, Choi MW, Corzett MH, Chang BC, Corzett CH, McCutchen-Maloney SL. J Proteome Res. 2004; 3:1120–1127. [PubMed: 15595720]
44. Dowling P, O'Driscoll L, Meleady P, Henry M, Roy S, Ballot J, Moriarty M, Crown J, Clynes M. Electrophoresis. 2007; 28:4302–4310. [PubMed: 18041032]

45. Cellar NA, Karnoup AS, Albers DR, Langhorst ML, Young SA. J Chromatogr B. 2009; 877:79–85.
46. Irth H, Oosterkamp AJ, Tjaden UR, van der Greef J. Trends Anal Chem. 1995; 14:355–361.
47. Vanderlaan M, Lotti R, Siek G, King D, Goldstein M. J Chromatogr A. 1995; 711:23–31. [PubMed: 7496493]
48. Phillips, TM. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 367-397.
49. Kronvall G, Frommel D. Immunochemistry. 1970; 7:124–127. [PubMed: 4984589]
50. Björck L, Kronvall G. J Immunol. 1984; 133:969–974. [PubMed: 6234364]
51. Akerström B, Björck L. J Biol Chem. 1986; 261:10240–10247. [PubMed: 3733709]
52. Akerström B, Björck L. J Biol Chem. 1989; 264:19740–19746. [PubMed: 2479638]
53. Compton BJ, Lewis M, Whigham F, Gerald JS, Countryman GE. Anal Chem. 1989; 61:1314–1317. [PubMed: 2774191]
54. Paliwal SK, Nadler TK, Wang DIC, Regnier FE. Anal Chem. 1993; 65:3363–3367. [PubMed: 8297024]
55. Gadowski L, Abdul-Wajid A. J Chromatogr A. 1995; 715:241–245. [PubMed: 8520669]
56. Tscheliessnig A, Jungbauer A. J Chromatogr A. 2009; 1216:2676–2682. [PubMed: 18703200]
57. Riggin A, Regnier FE, Sportsman JR. Anal Chem. 1991; 63:468–474. [PubMed: 2064010]
58. Blank GS, Vetterlein D. Anal Biochem. 1990; 190:317–320. [PubMed: 2127161]
59. Ecker DM, Jones SD, Levine HL, Ecker DM, Jones SD, Levine HL. MAbs. 2015; 7:9–14. [PubMed: 25529996]
60. Rathore AS. Trends Biotechnol. 2009; 27:546–553. [PubMed: 19647883]
61. Janis LJ, Regnier FE. Anal Chem. 1989; 61:1901–1906. [PubMed: 2802148]
62. Horak J, Ronacher A, Lindner W. J Chromatogr A. 2010; 1217:5092–5102. [PubMed: 20580008]
63. Lemmerer M, London AS, Panicucci A, Gutierrez-Vargas C, Lihon M, Dreier P. J Immunol Methods. 2013; 393:81–85. [PubMed: 23612045]
64. Gjoka X, Schofield M, Cvetkovic A, Gantier R. J Chromatogr B. 2014; 972:48–52.
65. Williams A, Read EK, Agarabi CD, Lute S, Brorson KA. J Chromatogr B. 2017; 1046:122–130.
66. Birch JR, Racher AJ. Adv Drug Deliv Rev. 2006; 58:671–685. [PubMed: 16822577]
67. Berkowitz SA, Engen JR, Mazzeo JR, Jones GB. Nat Rev Drug Discov. 2012; 11:527–540. [PubMed: 22743980]
68. Wang Q, Luo G, Ou J, Yeung WSB. J Chromatogr A. 1999; 848:139–148. [PubMed: 10427754]
69. de Frutos M, Paliwal SK, Regnier FE. Anal Chem. 1993; 65:2159–2163. [PubMed: 8372972]
70. Shen H, Aspinwall CA, Kennedy RT. J Chromatogr B. 1997; 689:295–303.
71. Holtzapple CK, Pishko EJ, Stanker LH. Anal Chem. 2000; 72:4148–4153. [PubMed: 10994977]
72. Pfaunmiller EL, Anguizola JA, Milanuk ML, Carter N, Hage DS. J Chromatogr B. 2016; 1021:91–100.
73. Cassidy SA, Janis LJ, Regnier FE. Anal Chem. 1992; 64:1973–1977. [PubMed: 1416045]
74. Smuc T, Ahn IY, Ulrich H. J Pharm Biomed Anal. 2013; 81–82:210–217.
75. Ma H, Liu J, Ali MM, Mahmood MAI, Labanieh L, Lu M, Iqbal SM, Zhang Q, Zhao W, Wan Y. Chem Soc Rev. 2015; 44:1240–1256. [PubMed: 25561050]
76. Proske D, Blank M, Buhmann R, Resch A. Appl Microbiol Biotechnol. 2005; 69:367–374. [PubMed: 16283295]
77. Balamurugan S, Obubuafo A, Soper SA, Spivak DA. Anal Bioanal Chem. 2008; 390:1009–1021. [PubMed: 17891385]
78. Zhao Q, Wu M, Chris Le X, Li XF. Trends Anal Chem. 2012; 41:46–57.
79. Bouchard PR, Hutabarat RM, Thompson KM. Annu Rev Pharmacol Toxicol. 2010; 50:237–257. [PubMed: 20055704]
80. Wu YX, Kwon YJ. Methods. 2016; 106:21–28. [PubMed: 27109056]
81. Deng Q, German I, Buchanan D, Kennedy RT. Anal Chem. 2001; 73:5415–5421. [PubMed: 11816567]
82. Deng Q, Watson CJ, Kennedy RT. J Chromatogr A. 2003; 1005:123–130. [PubMed: 12924787]

83. Scriba GKE. Chromatographia. 2012; 75:815–838.
84. Ruta J, Ravelet C, Desire J, Decout JL, Peyrin E. Anal Bioanal Chem. 2008; 390:1051–1057. [PubMed: 17899039]
85. Michaud M, Jourdan E, Villet A, Ravel A, Grosset C, Peyrin E. J Am Chem Soc. 2003; 125:8672–8679. [PubMed: 12848575]
86. Ravelet C, Boulkedid R, Ravel A, Grosset C, Villet A, Fize J, Peyrin E. J Chromatogr A. 2005; 1076:62–70. [PubMed: 15974070]
87. Romig TS, Bell C, Drolet DW. J Chromatogr B. 1999; 731:275–284.
88. Zhao Q, Li XF, Shao Y, Le XC. Anal Chem. 2008; 80:7586–7593. [PubMed: 18759461]
89. Zhao Q, Li XF, Le XC. Anal Chem. 2008; 80:3915–3920. [PubMed: 18363332]
90. Han B, Zhao C, Yin J, Wang H. J Chromatogr B. 2012; 903:112–117.
91. Deng N, Liang Z, Liang Y, Sui Z, Zhang L, Wu Q, Yang K, Zhang L, Zhang Y. Anal Chem. 2012; 84:10186–10190. [PubMed: 23137349]
92. Connor AC, McGown LB. J Chromatogr A. 2006; 1111:115–119. [PubMed: 16569569]
93. Nguyen TH, Pei R, Stojanovic M, Lin Q. Microfluid Nanofluidics. 2009; 6:479–487.
94. Chung WJ, Kim MS, Cho S, Park SS, Kim JH, Kim YK, Kim BG, Lee YS. Electrophoresis. 2005; 26:694–702. [PubMed: 15690422]
95. Vellard M. Curr Opin Biotechnol. 2003; 14:444–450. [PubMed: 12943856]
96. Moehlenbrock, MJ., Minteer, SD. Enzyme Stabilization and Immobilization. Minteer, SD., editor. Springer; New York: 2010. p. 1-9.
97. Maximov V, Reukov V, Vertegel AA. J Drug Deliv Sci Technol. 2009; 19:311–320.
98. Bowers-komro DM, Yamada Y, Mccormick DB. Biochemistry. 1989; 28:8439–8446. [PubMed: 2557903]
99. Kuester M, Becker GL, Lindberg I, Steinmetzer T, Than ME. Biol Chem. 2011; 392:973–981. [PubMed: 21875402]
100. Homandberg GA, Litwiller RD, Peanasky RJ. Arch Biochem Biophys. 1989; 270:153–161. [PubMed: 2648995]
101. Martzen MR, McMullen BA, Smith NE, Fujikawa K, Peanasky RJ. Biochemistry. 1990; 29:7366–7372. [PubMed: 2223768]
102. Zaborsky OR. Methods Enzymol. 1976; 44:317–332. [PubMed: 1021688]
103. Friedberg, F., Rhoads, AR. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 313-346.
104. Hage, DS., Bian, M., Burks, R., Karle, E., Ohnmacht, C., Wa, C. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 101-126.
105. John Lough, W., Wainer, IW., Patel, S. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 571-592.
106. Haginaka J. J Chromatogr B. 2008; 875:12–19.
107. Calleri E, Massolini G, Loiodice F, Fracchiolla G, Temporini C, Félix G, Tortorella P, Caccialanza G. J Chromatogr A. 2002; 958:131–140. [PubMed: 12134810]
108. Calleri E, Massolini G, Lubda D, Temporini C, Loiodice F, Caccialanza G. J Chromatogr A. 2004; 1031:93–100. [PubMed: 15058571]
109. Massolini G, Calleri E, Lavecchia A, Loiodice F, Lubda D, Temporini C, Fracchiolla G, Tortorella P, Novellino E, Caccialanza G. Anal Chem. 2003; 75:535–542. [PubMed: 12585481]
110. Stoffer B, Frandsen TP, Busk PK, Schneider P, Svendsen I, Svensson B. Biochem J. 1993; 292:197–202. [PubMed: 8503847]
111. Strandberg A, Nyström A, Behr S, Karlsson A. Chromatographia. 1999; 50:215–222.
112. Zheng Y, Wang X, Ji Y. Talanta. 2012; 91:7–17. [PubMed: 22365673]
113. Fanali S, Caponecchi G, Aturki Z. J Microcolumn Sep. 1997; 9:9–14.
114. Ståhlberg J, Henriksson H, Divne C, Isaksson R, Pettersson G, Johansson G, Jones TA. J Mol Biol. 2001; 305:79–93. [PubMed: 11114249]
115. Bartolini M, Cavrini V, Andrisano V. J Chromatogr A. 2007; 1144:102–110. [PubMed: 17134713]

116. Forsberg EM, Green A JR, Brennan JD. Anal Chem. 2011; 83:5230–5236. [PubMed: 21591743]
117. Forsberg EM, Sicard C, Brennan JD. Annu Rev Anal Chem. 2014; 7:337–359.
118. Kim HS, Wainer IW. J Chromatogr B. 2005; 823:158–166.
119. Hodgson RJ, Besanger TR, Brook MA, Brennan JD. Anal Chem. 2005; 77:7512–7519. [PubMed: 16316156]
120. Luckarift HR. J Liq Chromatogr Relat Technol. 2008; 31:1568–1592.
121. Matosevic S, Szita N, Baganz F. J Chem Technol Biotechnol. 2011; 86:325–334.
122. Girelli AM, Mattei E. J Chromatogr B. 2005; 819:3–16.
123. Hong T, Chen X, Xu Y, Cui X, Bai R, Jin C. J Chromatogr A. 2016; 1456:249–256. [PubMed: 27334417]
124. Yuan H, Zhang L, Zhang Y. J Chromatogr A. 2014; 1371:48–57. [PubMed: 25456586]
125. Liang W, Hou Z, Wang H, Xu W, Wang W. Chromatographia. 2015; 78:763–773.
126. Wang X, Li K, Adams E, Van Schepdael A. Electrophoresis. 2014; 35:119–127. [PubMed: 24170447]
127. Scriba GKE, Belal F. Chromatographia. 2015; 78:947–970.
128. Peumans WJ, Van Damme EJ. Plant Physiol. 1995; 109:347–352. [PubMed: 7480335]
129. Alley WR, Mann BF, Novotny MV. Chem Rev. 2013; 113:2668–2732. [PubMed: 23531120]
130. Van Damme, EJM. Handbook of Plant Lectins : Properties and Biomedical Applications. John Wiley; Chichester: 1998.
131. Kobayashi, Y., Tateno, H., Ogawa, H., Yamamoto, K., Hirabayashi, J. Lectins. Hirabayashi, J., editor. Springer; New York: 2014. p. 555-577.
132. Bergström M, Nilsson M, Isaksson R, Rydén I, Påhlsson P, Ohlson S. J Chromatogr B. 2004; 809:323–329.
133. Bedair M, El Rassi Z. J Chromatogr A. 2004; 1044:177–186. [PubMed: 15354437]
134. Luo R, Archer-Hartmann SA, Holland LA. Anal Chem. 2010; 82:1228–1233. [PubMed: 20078030]
135. Okanda FM, El Rassi Z. Electrophoresis. 2006; 27:1020–1030. [PubMed: 16470784]
136. Fukushima E, Yagi Y, Yamamoto S, Nakatani Y, Kakehi K, Hayakawa T, Suzuki S. J Chromatogr A. 2012; 1246:84–89. [PubMed: 22410158]
137. Madera M, Mechref Y, Novotny MV. Anal Chem. 2005; 77:4081–4090. [PubMed: 15987113]
138. Yang Z, Hancock WS. J Chromatogr A. 2004; 1053:79–88. [PubMed: 15543974]
139. Durham M, Regnier FE. J Chromatogr A. 2006; 1132:165–173. [PubMed: 16919642]
140. Yang Z, Hancock WS. J Chromatogr A. 2005; 1070:57–64. [PubMed: 15861788]
141. Ahn J-M, Kim B-G, Yu M-H, Lee I-K, Cho J-Y. PROTEOMICS – Clin Appl. 2010; 4:644–653. [PubMed: 21137083]
142. Lee LY, Hincapie M, Packer N, Baker MS, Hancock WS, Fanayan S. J Sep Sci. 2012; 35:2445–2452. [PubMed: 22997032]
143. Dai L, Liu Y, He J, Flack CG, Talsma CE, Crowley JG, Muraszko KM, Fan X, Lubman DM. Proteomics. 2011; 11:4021–4028. [PubMed: 21898824]
144. Kullolli M, Hancock WS, Hincapie M. Anal Chem. 2010; 82:115–120. [PubMed: 19957969]
145. Gbormittah FO, Hincapie M, Hancock WS. Bioanalysis. 2014; 6:2537–2548. [PubMed: 25411697]
146. Gbormittah FO, Lee LY, Taylor K, Hancock WS, Iliopoulos O. J Proteome Res. 2014; 13:4889–4900. [PubMed: 25184692]
147. Zeng Z, Hincapie M, Pitteri SJ, Hanash S, Schalkwijk J, Hogan JM, Wang H, Hancock WS. Anal Chem. 2011; 83:4845–4854. [PubMed: 21513341]
148. Jung K, Cho W. Anal Chem. 2013; 85:7125–7132. [PubMed: 23758413]
149. Qiu R, Regnier FE. Anal Chem. 2005; 77:2802–2809. [PubMed: 15859596]
150. Qiu R, Regnier FE. Anal Chem. 2005; 77:7225–7231. [PubMed: 16285669]
151. Kullolli M, Hancock WS, Hincapie M. J Sep Sci. 2008; 31:2733–2739. [PubMed: 18693314]

152. Zeng Z, Hincapie M, Haab BB, Hanash S, Pitteri SJ, Kluck S, Hogan JM, Kennedy J, Hancock WS. J Chromatogr A. 2010; 1217:3307–3315. [PubMed: 19782370]
153. Madera M, Mechref Y, Klouckova I, Novotny MV. J Chromatogr B. 2007; 845:121–137.
154. Madera M, Mechref Y, Klouckova I, Novotny MV. J Proteome Res. 2006; 5:2348–2363. [PubMed: 16944947]
155. Mann BF, Mann AKP, Skrabalak SE, Novotny MV. Anal Chem. 2013; 85:1905–1912. [PubMed: 23278114]
156. Bedair M, El Rassi Z. J Chromatogr A. 2005; 1079:236–245. [PubMed: 16038310]
157. Feng S, Yang N, Pennathur S, Goodison S, Lubman DM. Anal Chem. 2009; 81:3776–3783. [PubMed: 19366252]
158. Alwael H, Connolly D, Clarke P, Thompson R, Twamley B, O'Connor B, Paull B. Analyst. 2011; 136:2619–2628. [PubMed: 21552581]
159. Alla AJ, d' Andrea FB, Bhattarai JK, Cooper JA, Tan YH, Demchenko AV, Stine KJ. J Chromatogr A. 2015; 1423:19–30. [PubMed: 26554297]
160. Peters, T. All About Albumin: Biochemistry Genetics and Medical Applications. 1. Academic Press; San Diego: 1996.
161. Spector AA, John K, Fletcher JE. J Lipid Res. 1969; 10:56–67. [PubMed: 5773785]
162. Arroyo V, García-Martinez R, Salvatella X. J Hepatol. 2014; 61:396–407. [PubMed: 24751830]
163. Haginaka J. J Chromatogr A. 2001; 906:253–273. [PubMed: 11215891]
164. Fournier T, Medjoubi N, Porquet D. Biochim Biophys Acta. 2000; 1482:157–171. [PubMed: 11058758]
165. Kremer JM, Wilting J, Janssen LH. Pharmacol Rev. 1988; 40:1–47. [PubMed: 3064105]
166. Patel, S., Wainer, IW., Lough, WJ. Handbook of Affinity Chromatography. 2. Hage, DS., editor. CRC Press; Boca Raton: 2005. p. 663-684.
167. Harmony, JAK., Aleson, AL., McCarthy, BM. Biochemistry and Biology of Plasma Lipoproteins. Scanu, AM., Spector, AA., editors. Marcel Dekker Inc; New York: 1986. p. 403-430.
168. Mbewu AD, Durrington PN. Atherosclerosis. 1990; 85:1–14. [PubMed: 2149271]
169. Durrington, PN. Lipoproteins and lipids. Wright; London: 1989.
170. Havel, RJ., Kane, JP. The Metabolic and Molecular Basis of Inherited Disease. 7. Scriver, CR.Beaudet, AL.Sly, WS., Valle, D., editors. McGraw-Hill; New York: 1995. p. 1129-1138.
171. Skipski, VR. Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. Nelson, GJ., editor. John Wiley; New York: 1972. p. 471-583.
172. Barklay, M. Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. Nelson, GJ., editor. John Wiley; New York: 1972. p. 587-603.
173. Jonas, A. Biochemistry of Lipids, Lipoproteins and Membranes. Vance, JE., Vance, D., editors. Elsevier; Amsterdam: 2002. p. 483-504.
174. Fruchart, JC. Human plasma lipoproteins. Walter De Gruyter Inc; Berlin: 1989.
175. Wasan KM, Cassidy SM. J Pharm Sci. 1998; 87:411–424. [PubMed: 9548892]
176. Domenici E, Bertucci C, Salvadori P, Felix G, Cahagne I, Motellier S, Wainer IW. Chromatographia. 1990; 29:170–176.
177. Kaliszan R, Noctor TA, Wainer IW. Mol Pharmacol. 1992; 42:512–517. [PubMed: 1406602]
178. Noctor TA, Pham CD, Kaliszan R, Wainer IW. Mol Pharmacol. 1992; 42:506–511. [PubMed: 1406601]
179. Allenmark S, Andersson S. Chirality. 1989; 1:154–160.
180. Loun B, Hage DS. J Chromatogr B. 1992; 579:225–235.
181. Yang J, Hage DS. J Chromatogr A. 1993; 645:241–250.
182. Chu Y-Q, Wainer IW. Pharm Res. 1988; 5:680–683. [PubMed: 3244624]
183. Pfaunmiller EL, Hartmann M, Dupper CM, Soman S, Hage DS. J Chromatogr A. 2012; 1269:198–207. [PubMed: 23010249]
184. Mallik R, Hage DS. J Pharm Biomed Anal. 2008; 46:820–830. [PubMed: 17475436]

185. Lu J, Ye F, Zhang A, Wei Z, Peng Y, Zhao S. J Sep Sci. 2011; 34:2329–2336. [PubMed: 21548087]
186. Hong T, Zheng Y, Hu W, Ji Y. Anal Biochem. 2014; 464:43–50. [PubMed: 25062851]
187. Breton D, Buret D, Clair P, Lafosse M. J Chromatogr A. 2005; 1088:104–109. [PubMed: 16130737]
188. Enquist M, Hermansson J. Chirality. 2004; 1:209–215.
189. Locatelli I, Kmetec V, Mrhar A, Grabnar I. J Chromatogr B. 2005; 818:191–198.
190. Sandström R, Lennernäs H, Öhlén K, Karlsson A. J Pharm Biomed Anal. 1999; 21:43–49. [PubMed: 10701911]
191. Soares R, Singh AK, Kedor-Hackmann ERM, Santoro MIRM. J AOAC Int. 2009; 92:1663–1672. [PubMed: 20166584]
192. Etter ML, George S, Graybiel K, Eichhorst J, Lehotay DC. Clin Biochem. 2005; 38:1095–1102. [PubMed: 16298356]
193. Du J, Ma Z, Zhang Y, Wang T, Chen X, Zhong D. J Pharm Biomed Anal. 2013; 86:182–188. [PubMed: 24004635]
194. Kawabata K, Samata N, Urasaki Y, Fukazawa I, Uchida N, Uchida E, Yasuhara H. J Chromatogr B. 2007; 852:389–397.
195. Liu K, Dai X, Zhong D, Chen X. J Chromatogr B. 2008; 864:129–136.
196. Liu K, Zhong D, Chen X. J Chromatogr B. 2010; 878:2415–2420.
197. Xuan H, Hage DS. J Sep Sci. 2006; 29:1412–1422. [PubMed: 16894786]
198. Mallik R, Xuan H, Hage DS. J Chromatogr A. 2007; 1149:294–304. [PubMed: 17408678]
199. Karlsson A, Aspegren A. Chromatographia. 1998; 47:189–196.
200. Loeser E, Yowell G, Drumm P. J Liq Chromatogr Rel Technol. 2006; 29:2625–2640.
201. Matsuda R, Li Z, Zheng X, Hage DS. J Chromatogr A. 2015; 1408:133–144. [PubMed: 26189669]
202. Joseph KS, Hage DS. J Chromatogr B. 2010; 878:1590–1598.
203. Xuan H, Joseph KS, Wa C, Hage DS. J Sep Sci. 2010; 33:2294–2301. [PubMed: 20574961]
204. Hage DS. J Chromatogr B. 2002; 768:3–30.
205. Hage DS, Jackson A, Sobansky MR, Schiel JE, Yoo MJ, Joseph KS. J Sep Sci. 2009; 32:835–53. [PubMed: 19278006]
206. Loun B, Hage DS. Anal Chem. 1994; 66:3814–3822. [PubMed: 7802261]
207. Yang J, Hage DS. J Chromatogr A. 1996; 725:273–285. [PubMed: 8900576]
208. Tweed SA, Loun B, Hage DS. Anal Chem. 1997; 69:4790–4798. [PubMed: 9406530]
209. Kim HS, Hage DS. J Chromatogr B. 2005; 816:57–66.
210. Yoo MJ, Smith QR, Hage DS. J Chromatogr B. 2009; 877:1149–1154.
211. Soman S, Yoo MJ, Jang YJ, Hage DS. J Chromatogr B. 2010; 878:705–708.
212. Chen J, Ohnmacht C, Hage DS. J Chromatogr B. 2004; 809:137–145.
213. Mallik R, Yoo MJ, Chen S, Hage DS. J Chromatogr B. 2008; 876:69–75.
214. Joseph KS, Moser AC, Basiaga SBG, Schiel JE, Hage DS. J Chromatogr A. 2009; 1216:3492–3500. [PubMed: 18926542]
215. Conrad ML, Moser AC, Hage DS. J Sep Sci. 2009; 32:1145–1155. [PubMed: 19296478]
216. Sobansky MR, Hage DS. Adv Med Biol. 2012; 53:199–216. [PubMed: 25392741]
217. Sobansky MR, Hage DS. Anal Bioanal Chem. 2014; 406:6203–6211. [PubMed: 25103529]
218. Zheng X, Li Z, Beeram S, Podariu M, Matsuda R, Pfaunmiller EL, White CJ, Carter N, Hage DS. J Chromatogr B. 2014; 968:49–63.
219. Beeram S, Bi C, Zheng X, Hage DS. J Chromatogr A. 2017; 1497:92–101. [PubMed: 28366566]
220. Mallik R, Yoo MJ, Briscoe CJ, Hage DS. J Chromatogr A. 2010; 1217:2796–2803. [PubMed: 20227701]
221. Zheng X, Li Z, Podariu MI, Hage DS. Anal Chem. 2014; 86:6454–6460. [PubMed: 24911267]
222. Bi C, Zheng X, Hage DS. J Chromatogr A. 2016; 1432:49–57. [PubMed: 26797422]

223. Zheng X, Yoo MJ, Hage DS. Analyst. 2013; 138:6262–5. [PubMed: 23979112]
224. Clarke W, Chowdhuri R, Hage DS. Anal Chem. 2001; 73:2157–2164. [PubMed: 11393835]
225. Zheng X, Matsuda R, Hage DS. J Chromatogr A. 2014; 1371:82–89. [PubMed: 25456590]
226. Zheng X, Bi C, Brooks M, Hage DS. Anal Chem. 2015; 87:11187–11194. [PubMed: 26484387]
227. Zheng X, Podariu M, Matsuda R, Hage DS. Anal Bioanal Chem. 2016; 408:131–140. [PubMed: 26462924]
228. Stewart BH, Helen Chan O. J Pharm Sci. 1998; 87:1471–1478. [PubMed: 10189252]
229. Ong S, Liu H, Pidgeon C. J Chromatogr A. 1996; 728:113–128. [PubMed: 8673230]
230. Tsopelas F, Vallianatou T, Tsantili-Kakoulidou A. Expert Opin Drug Discov. 2016; 11:473–488. [PubMed: 26966996]
231. Yang CY, Cai SJ, Liu H, Pidgeon C. Adv Drug Deliv Rev. 1997; 23:229–256.
232. Ong S, Liu H, Qiu X, Bhat G, Pidgeon C. Anal Chem. 1995; 67:755–762. [PubMed: 7702190]
233. Pidgeon C, Venkataram UV. Anal Biochem. 1989; 176:36–47. [PubMed: 2712289]
234. De Vrieze M, Verzele D, Szucs R, Sandra P, Lynen F. Anal Bioanal Chem. 2014; 406:6179–6188. [PubMed: 25124450]
235. Moaddel R, Lu L, Baynham M, Wainer IW. J Chromatogr B. 2002; 768:41–53.
236. Zhang Y, Xiao Y, Kellar KJ, Wainer IW. Anal Biochem. 1998; 264:22–25. [PubMed: 9784183]
237. Wainer IW, Zhang Y, Xiao Y, Kellar KJ. J Chromatogr B. 1999; 724:65–72.
238. Zhang Y, Leonessa F, Clarke R, Wainer IW. J Chromatogr B. 2000; 739:33–37.
239. Lu L, Leonessa F, Clarke R, Wainer IW. Mol Pharmacol. 2001; 59:62–68. [PubMed: 11125025]
240. Beigi F, Qing Y, Lundahl P. J Chromatogr A. 1995; 704:315–321. [PubMed: 7670628]
241. Lundahl P, Beigi F. Adv Drug Deliv Rev. 1997; 23:221–227.
242. Liu X-Y, Nakamura C, Yang Q, Kamo N, Miyake J. J Chromatogr A. 2002; 961:113–118. [PubMed: 12186381]
243. Mao X, Kong L, Luo Q, Li X, Zou H. J Chromatogr B. 2002; 779:331–339.
244. Zhang C, Li J, Xu L, Shi ZG. J Chromatogr A. 2012; 1233:78–84. [PubMed: 22381893]
245. Wang Y, Kong L, Lei X, Hu L, Zou H, Welbeck E, Bligh SWA, Wang Z. J Chromatogr A. 2009; 1216:2185–2191. [PubMed: 18585729]
246. Moravcová D, Planeta J, Wiedmer SK. J Chromatogr A. 2013; 1317:159–166. [PubMed: 23978749]
247. Chen X, Xia Y, Lu Y, Liang J. J Pharm Biomed Anal. 2011; 54:406–410. [PubMed: 20880649]
248. Chen X, Deng Y, Xue Y, Liang J. J Pharm Biomed Anal. 2012; 70:194–201. [PubMed: 22789901]
249. Sheng L-H, Li S-L, Kong L, Chen X-G, Mao X-Q, Su X-Y, Zou H-F, Li P. J Pharm Biomed Anal. 2005; 38:216–224. [PubMed: 15925211]
250. Szumski M, Grzywiński D, Buszewski B. J Chromatogr A. 2014; 1373:114–123. [PubMed: 25465372]
251. Al-Haj MA, Haber P, Kaliszan R, Buszewski B, Jezierska M, Chilmonzyk Z. J Pharm Biomed Anal. 1998; 18:721–728. [PubMed: 9919974]
252. Grzywiński D, Szumski M, Buszewski B. J Chromatogr A. 2015; 1408:145–150. [PubMed: 26187765]
253. Moravcová D, Carrasco-Correa EJ, Planeta J, Lämmerhofer M, Wiedmer SK. J Chromatogr A. 2015; 1402:27–35. [PubMed: 26024990]
254. Wang Q, Peng K, Chen W, Cao Z, Zhu P, Zhao Y, Wang Y, Zhou H, Jiang Z. J Chromatogr A. 2017; 1479:97–106. [PubMed: 27974157]
255. Armstrong DW, Ward TJ, Armstrong RD, Beesley TE. Science. 1986; 232:1132–1135. [PubMed: 3704640]
256. Ward TJ, Ward KD. Anal Chem. 2012; 84:626–635. [PubMed: 22066781]
257. Shen J, Ikai T, Okamoto Y. J Chromatogr A. 2014; 1363:51–61. [PubMed: 24997110]
258. Chankvetadze B. J Chromatogr A. 2012; 1269:26–51. [PubMed: 23141986]
259. Szejtli J. Chem Rev. 1998; 98:1743–1754. [PubMed: 11848947]
260. Crini G. Chem Rev. 2014; 114:10940–10975. [PubMed: 25247843]

261. Hinze WL, Riehl TE, Armstrong DW, DeMond W, Alak A, Ward T. Anal Chem. 1985; 57:237–242.
262. Risley DS, Strege MA. Anal Chem. 2000; 72:1736–1739. [PubMed: 10784135]
263. Liu Y, Xue X, Guo Z, Xu Q, Zhang F, Liang X. J Chromatogr A. 2008; 1208:133–140. [PubMed: 18778835]
264. Wang H, Xu D, Jiang P, Zhang M, Dong X. Analyst. 2010; 135:1785–1792. [PubMed: 20498907]
265. Zhou J, Tang J, Tang W. Trends Anal Chem. 2015; 65:22–29.
266. Yao X, Tan TTY, Wang Y. J Chromatogr A. 2014; 1326:80–88. [PubMed: 24411138]
267. Wang R-Q, Ong T-T, Tang W, Ng S-C. Anal Chim Acta. 2012; 718:121–129. [PubMed: 22305907]
268. Lin Y, Zhou J, Tang J, Tang W. J Chromatogr A. 2015; 1406:342–346. [PubMed: 26138603]
269. Zhao J, Lu X, Wang Y, Lv J. J Chromatogr A. 2015; 1381:253–259. [PubMed: 25614192]
270. Wang Y, Chen H, Xiao Y, Ng CH, Oh TS, Tan TTY, Ng SC. Nat Protoc. 2011; 6:935–942. [PubMed: 21720308]
271. Kolb HC, Finn MG, Sharpless KB. Angew Chemie Int Ed. 2001; 40:2004–2021.
272. Guo Z, Lei A, Zhang Y, Xu Q, Xue X, Zhang F, Liang X. Chem Commun. 2007:2491–2493.
273. Jin G, Yu D, Guo Z, Yang D, Zhang H, Shen A, Yan J, Liang X. RSC Adv. 2016; 6:8584–8587.
274. Lubda D, Cabrera K, Nakanishi K, Lindner W. Anal Bioanal Chem. 2003; 377:892–901. [PubMed: 13680065]
275. Ai F, Wang Y, Chen H, Yang Y, Yang Tan TT, Ng S-C. Analyst. 2013; 138:2289–2294. [PubMed: 23446341]
276. Barhate CL, Breitbach ZS, Pinto EC, Regalado EL, Welch CJ, Armstrong DW. J Chromatogr A. 2015; 1426:241–247. [PubMed: 26643720]
277. Zhang Z, Wu M, Wu R, Dong J, Ou J, Zou H. Anal Chem. 2011; 83:3616–3622. [PubMed: 21456603]
278. Zhang Q, Guo J, Wang F, Crommen J, Jiang Z. J Chromatogr A. 2014; 1325:147–154. [PubMed: 24377739]
279. Sun P, Wang C, Breitbach ZS, Zhang Y, Armstrong DW. Anal Chem. 2009; 81:10215–10226. [PubMed: 20000643]
280. Sun P, Armstrong DW. J Chromatogr A. 2010; 1217:4904–4918. [PubMed: 20561631]
281. Sun P, Wang C, Padivitage NLT, Nanayakkara YS, Perera S, Qiu H, Zhang Y, Armstrong DW. Analyst. 2011; 136:787–800. [PubMed: 21140011]

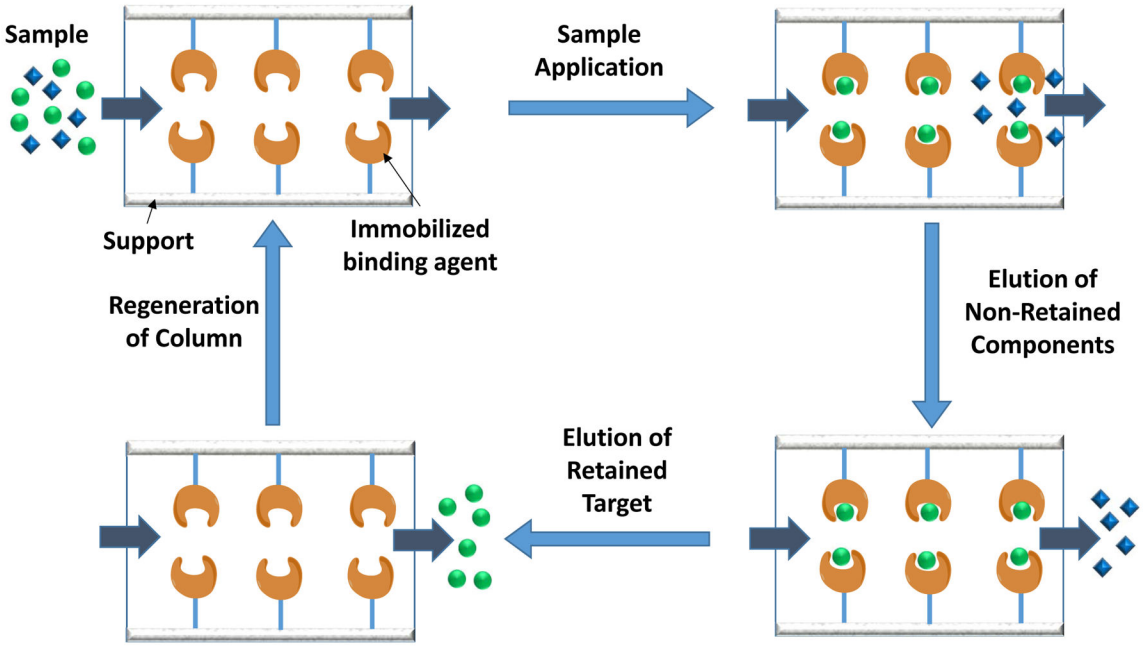

Figure 1.

Steps involved in the on/off elution mode of affinity chromatography. The analyte, or target, that can be retained by the immobilized binding agent is represented by the circles, while non-retained sample components are represented by the diamonds.

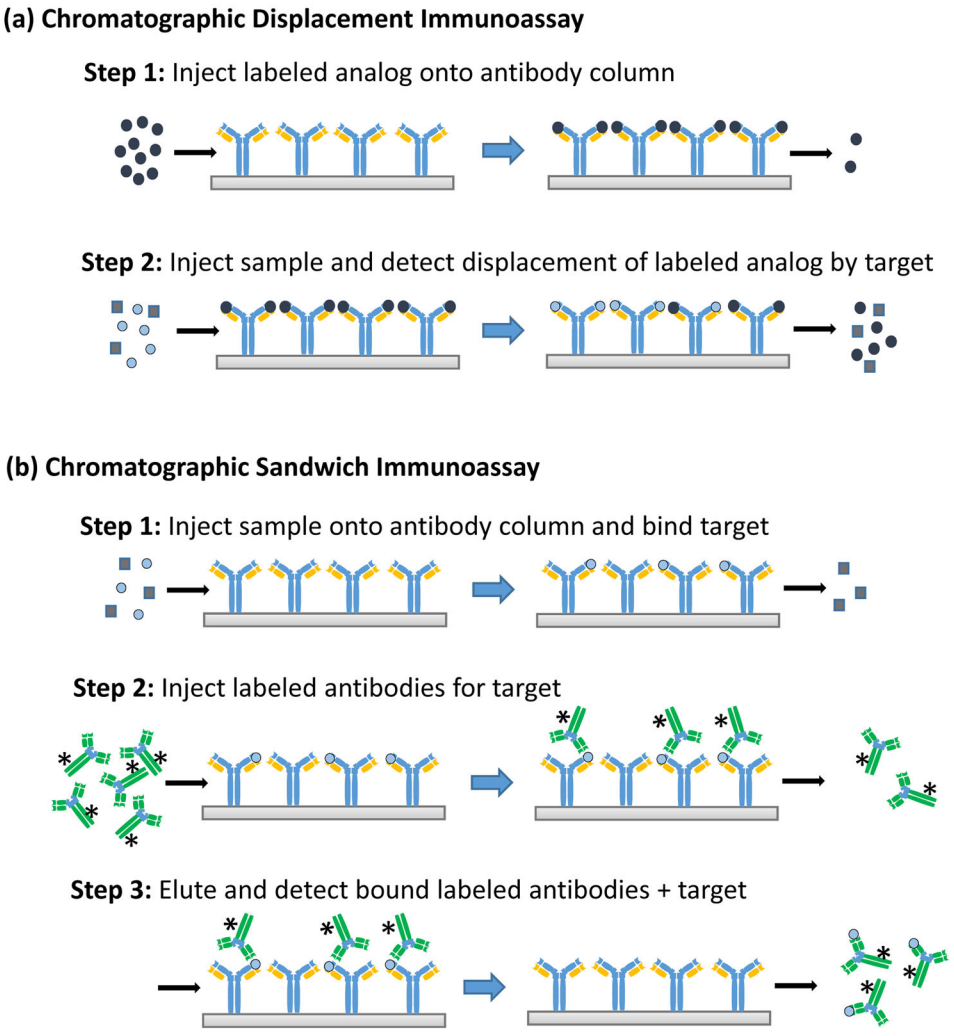

Figure 2.
General schemes for (a) a chromatographic displacement immunoassay and (b) a chromatographic sandwich immunoassay.

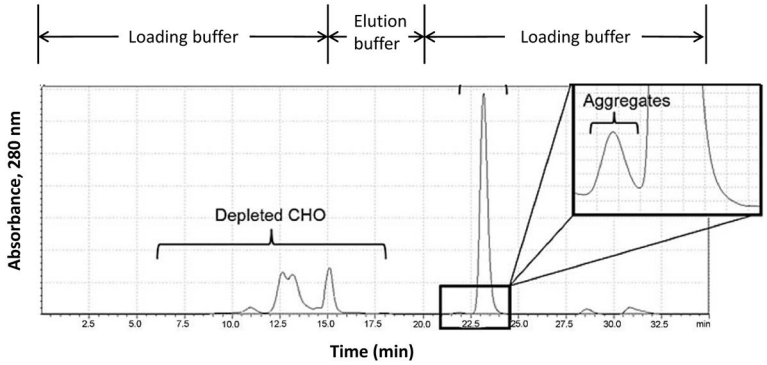

Figure 3.

Chromatogram obtained by combining protein A and size-exclusion HPLC columns for the analysis of monoclonal antibodies versus other components in a Chinese hamster ovary (CHO) cell culture. The peaks for the monoclonal antibodies and their aggregates are shown to the right, after their elution from the protein A column and loading onto the size-exclusion column, and the results for the other sample components (e.g., DNA fragments, host cell proteins, amino acids, nucleotides, etc.) that were non-retained by protein A column are shown to the left, after the first application of the loading buffer and passage of these components through the size-exclusion column. Adapted with permission from Ref. 64.

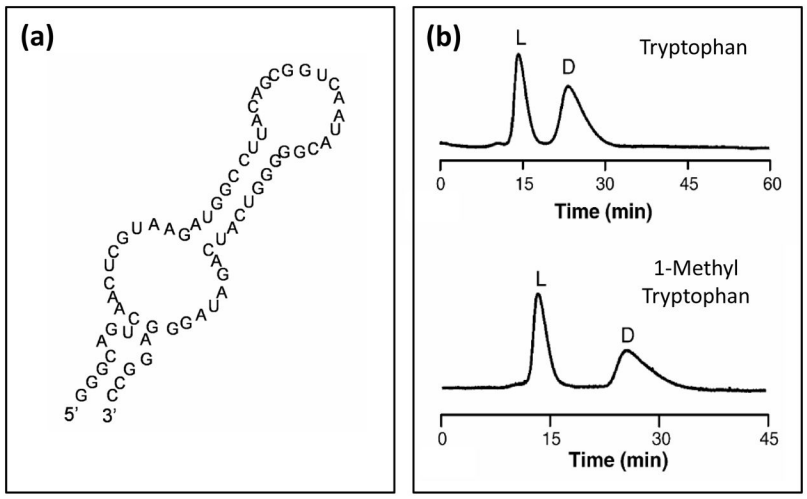

Figure 4.

(a) Sequence of an L-RNA aptamer that was used as a binding agent for L-tyrosine and related compounds and (b) chromatograms that were obtained when using this aptamer in an HPLC column for the chiral separation of D/L-tryptophan (top) and D/L-1-methyl tryptophan (bottom). Adapted with permission from Ref. 86.

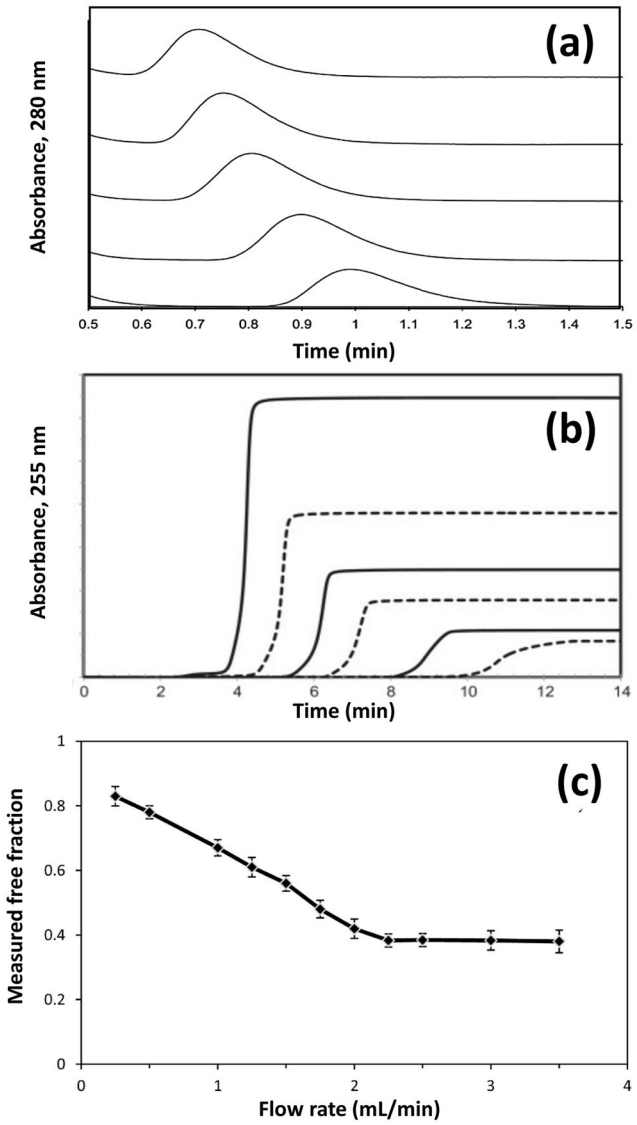

Figure 5.

Examples showing the use of HPAC to study biological interactions based on (a) competition studies in zonal elution experiments, (b) frontal analysis, or (c) ultrafast affinity extraction. The results in (a) are for the injection of L-tryptophan as a site-selective probe onto a 2 cm × 2.1 mm I.D. column containing immobilized HSA columns and in the presence of various concentrations of tolbutamide in the mobile phase (left-to-right: 20, 15, 10, 5, or 1 μM). The chromatograms in (b) were obtained for glimepiride that was applied to a 2 cm × 2.1 mm I.D. column containing HSA; the glimepiride concentrations were 50, 30, 20, 15, 10, or 5 μM (top-to-bottom). The data in (c) shows the effect of the injection flow rate on the measured free fraction for verapamil in a sample that contained a mixture of 10 μM verapamil and 20 μM AGP that was applied to a 0.5 cm × 2.1 mm I.D. AGP microcolumn. These figures are adapted with permission from Refs. 201, 202, and 219.

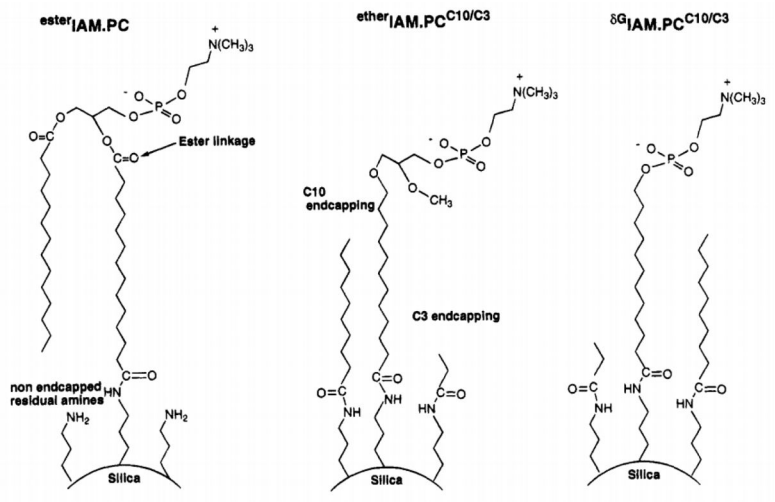

Figure 6.

Examples of three stationary phases used in immobilized artificial membrane (IAM) columns based on phosphatidylcholine (PC). Reproduced with permission from Ref. 232.

Table 1

Examples of biological binding agents that have been used in high performance affinity chromatography and related methods

Type of binding agent	Applications
Antibodies	Sample purification Chromatographic immunoassays Immunoextraction and immunodepletion Post-column immunodetection
Immunoglobulin-binding proteins	Antibody measurement of characterization Secondary binding agents for antibody immobilization
Aptamers	Small targets separation (nucleotides) Biomacromolecule analysis (thrombin, cyctochrome C, lysozymes).
Enzymes	Inhibitor isolation Chiral separations Immobilized enzyme reactors Screening of enzyme targets and inhibitors
Lectins	Glycoproteomic anlaysis Glycomic analysis
Transport proteins	Chiral separations Solute-protein interaction studies Free drug fraction analysis
Lipids	Drug lipophilicity measurements Ligand screening for membrane receptors
Carbohydrates	Chiral separations