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Back to Nature: Immunocapture and Related Methods for the Selective Analysis of Pharmaceutical and Biomedical Samples

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Article Highlights

- IAC can be used in sample pretreatment for the strong and selective capture of target compounds from complex clinical and biological samples.
- Advantages of IAC for sample pretreatment include the ability to produce heterogeneous or homogeneous antibodies against a wide variety of targets and with strong binding.
- Antibodies have been used for decades in sample pretreatment, with these agents and IAC being employed in many applications reported for affinity-based separations.
- Formats in which IAC may be used include off-line or on-line immunocapture, immunodepletion and ultrafast immunoextraction.
- Important factors to consider in IAC are the availability and selectivity of antibodies to the desired target, the application and elution conditions, and the availability or preparation of supports that contain the immobilized binding agent.
- Future developments in this field may include the development of new antibody-related binding agents and immobilization methods, supports or assay formats for IAC.

Modern pharmaceutical and biomedical analysis frequently involves complex samples such as blood, plasma, serum, urine, cerebrospinal fluid, cell cultures and tissues [1-4]. Analysis of components in these matrices has advanced significantly due to developments in mass spectrometry, liquid or gas chromatography, electrophoresis and combinations of these methods [1,2]. Examples include the use of LC-MS and LC-MS/MS in proteomics, metabolomics, lipidomics, clinical chemistry and the pharmaceutical industry [1-4].

Sample pretreatment is frequently a key component in such work [1-4]. Common non-specific procedures used for this purpose span from simple physical separation methods (e.g., filtration or precipitation) to phase-based methods (e.g., solid-phase extraction or various chromatographic steps using size-exclusion, ion-exchange and reversed-phase supports). The complexity of biological samples means their pretreatment may involve several of these procedures, especially when the goal is to examine a specific set of analytes. These multiple steps can add significant time, effort and cost to the overall analysis. The use of multiple steps for pretreatment will also tend to reduce reproducibility and analyte recovery, which can be important when examining trace compounds [4].

Immunoaffinity chromatography (IAC) is an alternative approach that may be used to overcome many of these issues with sample pretreatment, and especially when the goal is to selectively analyze trace analytes [5-8]. IAC is a form of liquid chromatography in which an antibody or related binding agent is employed as the stationary phase [5,8]. Immunocapture, also known as immunoextraction or immunoaffinity extraction, is a form of IAC that is used for compound isolation [5-8]. These methods make use of the specific, strong binding that is displayed by antibodies - a group of glycoproteins produced by the immune system in response to a foreign

agent, or antigen. Most natural antibodies contain one or more subunits with two identical antigen binding sites, or F_{ab} regions. Related agents include F_{ab} fragments, which can be generated through chemical or enzymatic treatment of intact antibodies, and recombinant or synthetic constructs of antibodies or their binding regions. The strong and highly selective interactions of these agents with their targets are determined by the arrangement of amino acid residues in these binding regions [8].

There are many reasons why IAC and immunocapture are valuable for the pretreatment and analysis of specific target compounds in complex biological samples. First, antibodies can be produced against a wide range of targets. These targets include both large analytes (e.g., proteins, viral particles, or bacterial cells) and, through coupling with a larger carrier agent, smaller targets such as metabolites, hormones and drugs [5,8]. Second, there are established techniques for producing either heterogeneous polyclonal antibodies (i.e., through the usage of host animals) or more homogeneous monoclonal antibodies (i.e., as obtained with hybridomas and cell cultures) [8]. Third, there is the high affinity that antibodies typically exhibit for their targets. These interactions often have association equilibrium constants in the range of 10^{5}-10^{12} M^{-1} or larger, or dissociation equilibrium constants of 10^{-5} to 10^{-12} M or lower [5,7,8]. This strong binding, along with the high selectivity of antibodies, means IAC and immunocapture can often be used even with trace compounds to recover and isolate a given target in a single step, thus greatly reducing the time and effort required for the pretreatment of complex biological samples [8].

The selectivity and strong binding of antibodies with their antigens have long been of interest in chemical separations and measurements [6,8,9]. It was shown as early as the mid-1930s to 1950s that materials such as charcoal, kaolin and cellulose could be used to adsorb or immobilize antigens to purify their respective antibodies. Following the development of activated agarose in

the mid-1960s, these supports were used by the early 1970s with antibodies to isolate targets such as peptides, proteins, enzymes and viruses [9]. A decade later, more rigid and higher-efficiency supports were used to create the technique of high-performance immunoaffinity chromatography [8,9]. Since then, IAC, immunocapture and related methods have continued to grow in popularity. For instance, a recent survey of the literature showed that antibodies have been employed in almost 60% of all reports that have used biological-related agents in chromatography for affinity-based separations [6]. Further discussion on the use of immunocapture over the last two decades is provided elsewhere in this issue [10].

Another reason immunocapture and IAC have been of interest is the variety of formats in which they may be used for sample pretreatment or analysis. For instance, immunocapture can be conducted either off-line or on-line with another analytical method [4-8,11-13]. Applications of off-line and on-line immunocapture have included the analysis of cytokines, steroids, hormones, toxins, proteins or protein variants and various classes of drugs [5,6,8,14]. Off-line immunocapture is more common and employs supports such as agarose, polyacrylamide, or polymethacrylate that are designed for use at low pressures and flow rates [4-8]. On-line immunocapture is typically faster, more precise and can be more easily automated but requires a support that is stable at the flow rates and pressures present in HPLC or LC-MS. Materials that may be used in this latter case range from particulate supports (e.g., derivatized silica or glass beads) to monolithic or perfusion-based media [5,7,8,14]. Two other forms of immunocapture are immunodepletion and ultrafast immunoextraction. In immunodepletion, immobilized antibodies are used to remove one or more undesired components from a sample [7,15,16]. This technique has been used in proteomics to remove high-abundance proteins (e.g., albumin) and allow the analysis of lower abundance components in samples [15,16]. In ultrafast immunoextraction, a specific target is rapidly removed

from a sample, as is accomplished by using a microscale column and flow rates that allow capture to occur within the millisecond-to-second time scale [3,17].

Some key factors to consider in immunocapture and IAC methods are the selectivity of the antibodies or binding agents and the availability of such agents for the desired target [5,8]. If the goal is to analyze a single, well-defined target, monoclonal antibodies may be a logical choice for immunocapture. However, even polyclonal antibodies can be used for this purpose, especially if any cross-reacting compounds can be separated or differentiated from the main target by a second analytical method [8,11,12]. Although cross-reactivity can limit the selectivity of antibodies and IAC, this can be an advantage in cases where multiple targets with similar structures are to be examined [11,12]. If a large set of targets with diverse structures are to be isolated or examined, a support that contains broad-specificity antibodies, a mixture of antibodies, or a combination of supports with different antibodies, can further be used to process the sample [8,11-13].

There are other factors, and potential limitations, which need to be considered when using immunocapture and IAC [5,7,8]. First, samples will generally need to be in a neutral pH aqueous buffer to allow strong binding to the antibodies. Second, elution conditions must be selected that will allow release of captured targets and possible regeneration of the antibodies for their reuse. These conditions may need to be evaluated for each application; however, guidelines and procedures for this process are available [5,8,15]. In addition, for new targets it may be necessary to create supports that contain antibodies for these targets. Various procedures and commercial preactivated materials are available for this purpose [5,7,8,14]. Columns containing immunoglobulin-binding agents such as protein A or protein G can also be purchased or prepared to adsorb antibodies for use in IAC and immunocapture methods [7,8,15].

The advantages of immunocapture and IAC should continue to make these important tools for the pretreatment and analysis of complex biological samples (e.g., see additional examples related to drug discovery and development that are provided in this issue) [10]. There are also many opportunities for future growth in this field. For instance, it is expected that more IAC methods that employ new types of antibody-related agents will appear as developments continue in biotechnology and biopharmaceuticals in the creation and design of antibody constructs or antibody mimics [5,8,18]. The implementation of improved supports, immobilization formats and mixed antibody supports should further lead to more commercial materials for this work, as well as greater use of immunocapture and IAC in miniaturized systems for the high-throughput analysis of large sets of targets in fields such as proteomics or metabolomics [3,4,6]. Moreover, additional IAC formats, such as chromatographic-based immunoassays for low-level targets, have the potential for even more applications in these areas [7,8,17,19,20]. Continued developments for each of these items are expected to offer even greater opportunities for the use of immunocapture, IAC and related methods in pharmaceutical and biomedical analysis.

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Disclosures

The author has no conflicts of interest to disclose.

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