ELSEVIER

Journal of Chromatography B, 745 (2000) 39–48

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Immuno-affinity solid-phase extraction

D. Stevenson*

School of Biological Sciences, Robens Analytical Centre, University of Surrey, Guildford, GU2 5XH, UK

Abstract

The measurement of trace organics such as drugs and pesticides at low concentration in biological and environmental samples is a challenging analytical task. Despite recent advances in instrumentation most analysts regard sample preparation as the rate-limiting step in the overall analytical method. In recent years there has been a lot of interest in immobilising antibodies onto solid supports such as silica to provide highly selective solid-phase extraction. This paper reviews the use of immuno-affinity for solid-phase extraction. It uses as examples extraction of chlortoluron and isoproturon from water and morphine and clenbuterol in urine and plasma respectively. An extensive list of other examples is given. Optimisation procedures are discussed in detail. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Immuno-extraction; Morphine; Clenbuterol; Isoproturon; Chlortoluron

Contents

1. Introduction 39
 1.1. Preparation of immuno-affinity columns 40
 1.2. Optimisation of immuno-extraction 41
 1.3. Specificity of binding 43
2. Applications 43
3. Future possibilities 47
References 47

1. Introduction

The measurement of trace organics such as drugs, pesticides, food additives, and other environmental contaminants at low concentration in a range of complex matrices is a challenging analytical task. Such measurements though are very important and very large numbers of analyses are carried out. The purpose of many of these analyses is to monitor human and environmental exposure to chemicals or testing the safety and efficacy of new products. Many of these types of measurements use sophisticated, modern instrumental separation methods such as high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). Recent advances in instrumentation and the range of detectors available means that analytical scientists can measure and identify analytes at lower and lower concentrations.

However despite the recent advances in instruments and the ability to achieve higher and higher

*Tel.: +44-1483-879-220; fax: +44-1483-300-374.
E-mail address: d.stevenson@surrey.ac.uk (D. Stevenson).

0378-4347/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(00)00204-8

theoretical plates, most analysts still regard sample preparation as the rate limiting step in the overall analytical procedure when dealing with the complex matrices mentioned earlier. Many different matrices are encountered such as blood, plasma, serum, erythrocytes, urine, tissue, saliva, hair, bone, air, water foods, soil, sediment etc. Sample preparation approaches include liquid–liquid extraction, solid-phase extraction (SPE), protein precipitation, soxhlet extraction, microwave digestion, supercritical fluid extraction, filtration, homogenisation, dialysis, sonication, and many others. Such steps may be the only sample preparation or quite often they are used in combination with other methods.

In recent years SPE has become the method of first choice for many applications [1]. A wide range of phases is commercially available and the technique can be automated on a number commercial instruments both off-line and on-line. An idealised SPE protocol involves trapping analyte on a solid-phase, washing off interferences less attracted to the sorbent, and then eluting analyte(s) leaving interferences with greater attraction bound to the sorbent. The challenge is thus to optimise choice of washing and elution solvent as well as solid-phase chemistry to match analyte properties. Other considerations such as toxicity and disposal of solvents and sorbents are also of concern, as well as cost.

Although it has undoubtedly grown in popularity SPE, certainly in earlier years, has suffered from questions as to the batch-to-batch reproducibility of phases. This has been mostly overcome with a better understanding of surface chemistry and the complex interactions involved in analyte retention and elution. A further consideration in many laboratories is the time involved in method development and the desire for essentially generic protocols.

Affinity chromatography utilises biological interactions for the separation and detection of selected analytes [2]. One of the most successful applications of affinity chromatography has been the use of antibodies as the selector ligand. Immuno-affinity chromatography has found use in both chromatography and detection systems [3,4].

Antibody methods particularly enzyme linked immunosorbent assays (ELISA) have become popular particularly for pesticides in water [5,6]. However their use has been limited by the fact that many antibodies are not specific, and cross-react with other closely related analytes. This can make quantitation of an individual analyte difficult although it is undoubtedly an advantage when screening many samples, which turn out to be below action levels. ELISA type methods are also better suited to measurement in biological matrices such as blood and urine, rather than complex environmental matrices.

In the last few years there has been a lot of interest in immobilising antibodies onto solid supports such as silica to provide highly selective SPE. This has been successfully utilised for pesticides and other trace organics in environmental samples [7,8] as well as for drugs, metabolites and endogenous compounds in biological fluid [9,10]. This approach uses the specificity of antibodies along with the ability of HPLC and GC to separate structurally closely related analytes that might cross-react. In some examples (see below) it has been possible to use immuno-affinity chromatography as the only sample preparation step necessary. In others the immuno-affinity chromatography has been used in combination with other sample preparation steps. In this laboratory attempts have been made to develop a generic protocol optimised to allow processing of samples of both drugs and pesticides in biological samples and environmental matrices respectively [7–11]. Much of the immuno-affinity work reviewed in this paper has used antibodies already raised and used in traditional antibody tests before investigating them as possible immuno-extraction columns.

1.1. Preparation of immuno-affinity columns

The key reagent for immuno-extraction is the antibody. A detailed description of antibody production, purification and assessment is outside the scope of this review but some general comments are relevant. Antibodies have been raised in a variety of species but the most common approach is to use sheep, rabbits or mice. A compound capable of producing an immune response is known as an antigen. As many of the compounds of interest, particularly drugs and pesticides are of small molecular mass (less than 1000) it is necessary to couple them to a carrier protein in order to illicit an immune response. The target analyte that is coupled to the carrier protein is known as the hapten. Samples of the animal's blood are taken and screened for the presence of antibodies. It can take several months

and even over a year before antibodies are produced. If this is successful it is hoped that some of the antibody will recognise the target analyte and not only the whole complex. This does not always happen and is one of the disadvantages of the immuno-affinity approach.

This can be illustrated from an attempt to produce a class specific antibody. In this laboratory antibodies were successfully raised (separately) to both the phenylurea herbicides chlortoluron and isoproturon. The antibodies were raised to a structural analogue of each. However when further structural analogues were synthesised with the aim of recognising the phenylurea group by bonding to the opposite end of the molecules no antibodies were produced in sheep, (see Fig. 1 for structures). It should be noted that this attempt to achieve group recognition was mostly overcome by using a mixture of the antibodies, combined with their cross-reactivity to closely related structure [11].

In the examples from this laboratory (currently chlortoluron, isoproturon, clenbuterol and morphine) antibodies have been raised for classical immunoassay use and have been assessed for that purpose. They have in all cases proven suitable for subsequent immuno-extraction.

Immuno-affinity columns are usually prepared on silica, controlled pore glass, agarose or other soft gels [12]. Support materials need to be easily activated to allow coupling, chemically and mechanically stable, hydrophilic to minimise non-specific interactions and have a uniform particle size. Support materials in common use are listed in Table 1.The reactions used to couple the antibodies to the support usually involve reaction with the carboxyl or amino groups on the antibodies. Supports are usually activated with reagents such as *N*,*N*-carbonyl diimidazole, cyanogen bromide, *N*-hydroxysuccinimide and tresyl or tosyl chloride. In this laboratory glutaraldehyde activated silica or controlled pore glass has been the preferred approach [13].

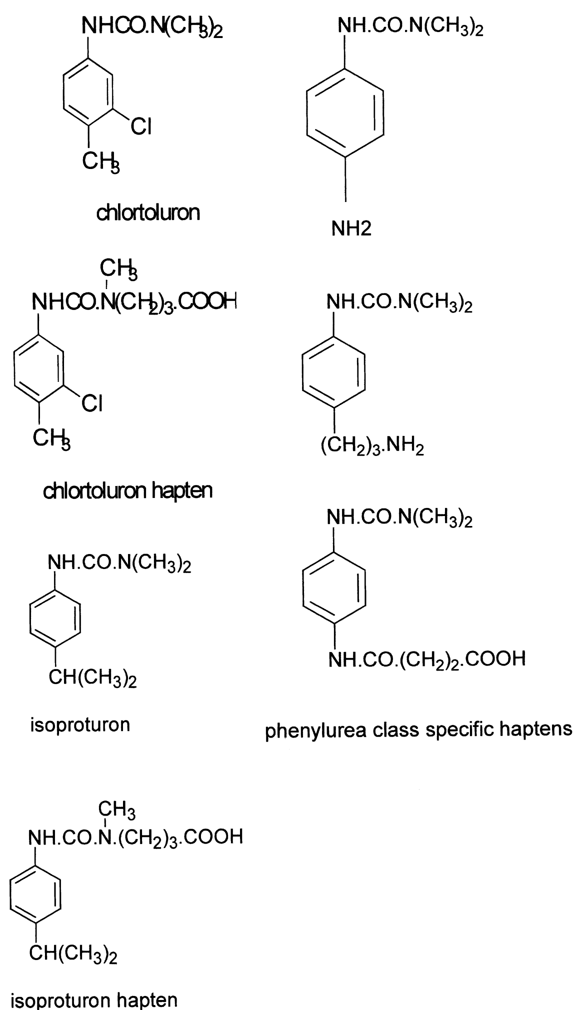

Fig. 1. Structures, chlortoluron (a), chlortoluron hapten (b), isoproturon (c), isoproturon hapten (d), candidate haptens for phenylurea group (e).

1.2. Optimisation of immuno-extraction

Once an immuno-column has been prepared much effort has been devoted to optimising of washing and elution protocols. When using the columns in an off-line SPE mode desorption in the smallest volume possible is the aim. This means that further pre-concentration steps are often unnecessary. Extensive recovery and pre-concentration experiments in this laboratory have shown that elution can be achieved in a one ml fraction, though slightly larger volumes are common. Columns are conditioned with phosphate buffered saline (PBS) at neutral pH or in some reports water. Samples need to be applied at a pH close to neutral, typically pH 5–8 [7–11].

The most suitable washing solvent has been PBS at pH 7. Once the clenbuterol, morphine, isoproturon or chlortoluron had been applied, in our studies, it could be washed with at least 20 ml of PBS or water

Table 1
Support materials for immuno-affinity

Type of support	Supplier
AvidGel and AvidGel CPG	Bioprobe
BioGel/Affi-Gel	BioRad
Fractogel	EM Separatins
HEMA–AFC	Alltech
Reactigel	Pierce
Sepharose/Superose/Sephacryl	Pharmacia
Trisacryl/Ultrogel	IBF
TSK Gerl Toyopearl	TosoHaas
Emphaze	Pierce
HiPAC	ChromatoChem
POROS	Perseptive
Protein-Pak Affinity Packings	Waters
Ultraaffinity–EP	Bodman

without any elution of analyte. A very similar elution solvent was used in all cases, low pH and PBS in approximately equal concentration with ethanol or methanol [7–10]. This generic protocol decreases the time needed for future method development. A typical scheme is shown in Fig. 2. Such a protocol does assume that the analyte will be essentially irreversibly bound during loading and washing steps. For elution a completely different solvent is used, but the mildest conditions possible are used in order to allow regeneration of the column for processing further samples.

A similar protocol (using 70% methanol for desorption) has been developed for desorption of triazines [14]. A similar procedure was also developed for the selective extraction of polyaromatic hydrocarbons from river water, sludge and tissues. The antibody had been raised to pyrene and desorption was achieved with 5 ml of acetonitrile–water (70:30) [15]. An anti-methyltestosterone column was used to extract metabolites of metandione and stanozolol with a desorption solvent comprising 3 ml of 60% methanol in water. This procedure incorporated a wash step with 15% methanol in water rather than the PBS favoured in many methods [16]. An anti-salbutamol column was used for extraction of beta-2-agonists. Desorption of the immuno-affinity column used 80% ethanol in pH 4 acetate buffer [17]. For the immuno-extraction of morphine and its glucuronide metabolites 10 ml of 10% pH 2 glycine buffer in methanol was used [18].

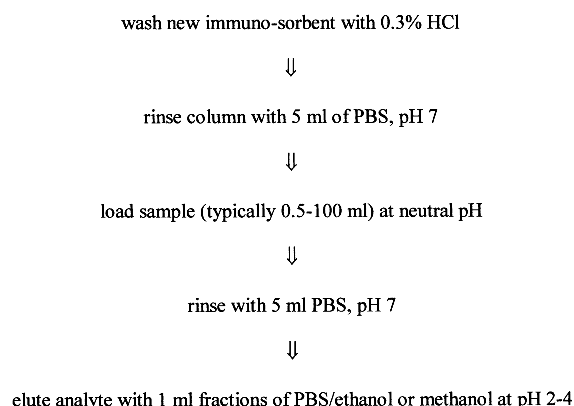

Fig. 2. Typical immuno-extraction scheme.

The stability of antibodies does vary, as exemplified in a method to determine ochratoxin A in coffee. In this procedure the immuno-affinity column was eluted using pure methanol [19]. A procedure to extract zearalone from corn extracts also used pure methanol for desorption [20]. The immuno-extraction of bufuralol from plasma used 95% methanol in pH 5 ammonium acetate buffer [21]. Several other procedures used 2% acetic acid in water as elution solvent without adding organic modifier, for example for on-line measurement of LSD [22], and fluoroquinolones [23]. This solvent was also used for a method using an anti-benzodiazepine column to screen combinatorial libraries [24]. The determination of Aflatoxin M(1) in milk used a monoclonal antibody column and an elution solvent of just 10% methanol in water [25].

In the case of the two phenylurea herbicides [7,8] up to 1 l of water containing low concentration could be passed through the immuno-column without breakthrough of analyte. This could still be quantitatively recovered in a single 1 ml fraction, exemplifying the pre-concentration that can be achieved.

Column capacity has been assessed in terms of analyte mass breakthrough. This has varied from antibody to antibody. It should be noted that such figures are only a guide, as describing a volume of antibody does not account for dilution that will occur during purification of antiserum. An estimate of column capacity for four immuno-affinity columns when first prepared is shown in Table 2. Limited column capacity is not particularly a disadvantage for trace analysis as the mass for each analyte in

Table 2
Capacity of immuno-affinity columns

Immuno-column	Capacity (ng)	Ref.
Chlortoluron	500	[7]
Isoproturon	200	[8]
Clenbuterol	300	[10]
Morphine	45	[9]

Table 1 is easily within the range of modern methods of analysis.

Limited availability of antiserum for some analytes is the main criterion hence the attempts to re-use columns as many times as possible. Columns have been used up to at least 50 times even for processing biological samples both in the off-line and on-line mode [26].

1.3. Specificity of binding

The specificity of analyte antigen binding has been demonstrated a number of times. This is best illustrated when using the anti-clenbuterol and anti-morphine columns to demonstrate the recovery of each analyte on the column composing the antibody to the other [9,10]. Immuno-affinity columns can retain analytes structurally closely related to the target compound via cross-reactivity. It should be noted that the cross-reactivity observed in classical immunoassay does not necessarily mimic that seen when using the same antibody in immuno-affinity mode. Table 3 shows the cross-reactivity of our isoproturon and chlortoluron antibodies to other phenylureas, as tested in the ELISA format [5,6]. Fig. 3a and b show the recovery of various phenylureas from the anti-isoproturon and anti-chlortoluron columns. Despite the fact that these (particularly chlortoluron antibodies) showed little cross-reactivity in ELISA format there is some retention of the other phenylureas. In terms of a class specific antibody fraction 13 (the second elution fraction) shows a significant level of all phenylureas except metoxuron. It has been suggested [26], that it is the capacity of the immuno-sorbent that is exceeded at a lower mass by closely related species. Although cross-reactivity arises through serendipity rather than design (see earlier) it can be extremely useful for extracting a group of compounds which are then subsequently separated and measured by chromatography, which is of course well suited to that task. In order to maximise the class of compounds that could be trapped we reported a procedure based on mixing both a chlortoluron and an isoproturon column together in a single column [27]. Other examples include triazines, phenylureas and polyaromatic hydrocarbons [28–30].

Table 3
Cross-reactivity of antibodies to other phenylureas, shown in ELISA assay

Compound	Anti-chlortoluron	Anti-isoproturon
Chlortoluron	100	0.2
Isoproturon	47	100
Chlorbromuron	71	0.1
Metoxuron	8.8	0.1
Chlorsulphuron	1.3	0

2. Applications

The growing popularity of immuno-affinity solid-phase extraction is shown by the increased literature in this area. A wide range of applications of solid-phase immuno-affinity extraction has been developed, both on-line and off-line. The most common analytes are the phenylurea and triazine herbicides and growth promoters. Table 4 shows some examples of immuno-affinity methods described recently. This includes both on-line and off-line procedures. It can be seen that pesticides in water and drugs (both therapeutic and recreational)in biological samples are the most common examples. Other applications include steroids and other endogenous compounds, toxins and environmental pollutants. Matrices include water, soil, sludge, food, drinks, crops, plasma, urine and tissue Further examples are given in a recent review article [4].

Two examples of the clean traces that can be obtained for immuno-extraction are shown in Figs. 4 and 5. Fig. 4 shows the clean up obtained from immuno-extraction of chlortoluron from river water. A 10 ml sample was spiked with chlortoluron at 30 ng/ml. The immuno-column was washed with 5 ml of PBS and then eluted with 1 ml of 50% ethanol in pH 2 PBS. No further clean up was carried out. HPLC was on ODS with UV detection. Full details

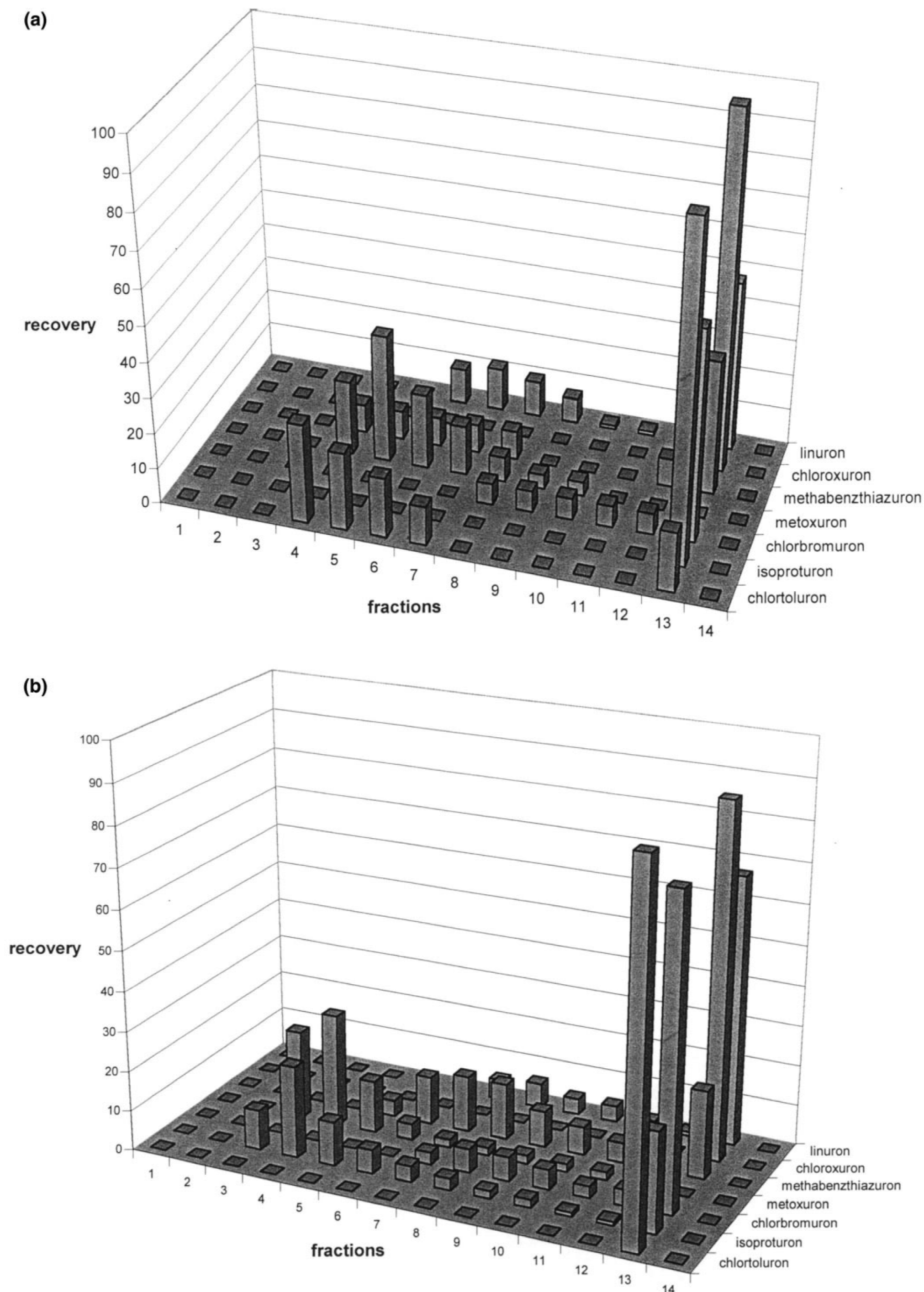

Fig. 3. (a) Cross-reactivity of isoproturon immuno-affinity column to other phenylureas. Fraction 1=sample loading, fractions 2-11=PBS wash, fractions 12–14=elution fractions. All fractions are 1 ml. One ml of 100 ng/ml of each phenylurea added. (b) Cross-reactivity of chlortoluron immuno-affinity column to other phenylureas. Fraction 1=sample loading, fractions 2–11=PBS wash, fractions 12–14=elution fractions. All fractions are 1 ml. One ml of 500 ng/ml of each phenylurea added.

Table 4
Some examples of methods using immuno-affinity extraction

Analyte(s)	Matrix	Ref.
Aflatoxins	Nuts	[31]
Aflatoxins	Milk	[25]
Algal toxins	Shellfish	[32]
Anabolic steroids	Urine, faeces	[33]
Anabolic steroids	Urine	[16]
Atrazine	Water	[34]
Atrazine	Water	[35]
Bufuralol	Plasma	[21]
Carbendazim	Water	[36]
Chlortoluron	Water	[7]
Clenbuterol	Plasma	[10]
Clenbuterol, salbutamol	Urine	[17]
Corticosteroids	Liver, milk, urine, faeces	[37]
Corticosteroids	Urine	[38]
Dexamethasone	Urine	[39]
Dexamethasone, Betamethasone	Urine	[40]
Fluoroquinones	Liver	[23]
Floroqinolones	Milk	[41]
Isoproturon	Water	[8]
LeukotrieneE-4	Urine	[42]
LSD	Urine	[22]
LSD	Urine	[43]
Morphine	Urine	[9]
Morphine, and metabolites	Blood	[18]
Mycotoxins	Cereals	[44]
Ochratoxin A	Coffee	[19]
Ochratoxin A	Beer	[45]
Ochratoxin A	Wheat	[46]
PAHs	Water, sludge, tissue	[15]
PAHs	Urine	[47]
S-phenylmercapturic acid	Urine	[48]
Phenylureas, triazines	Water, soil	[28]
Phenylureas, triazines	Waters, sediments	[30]
Phenylureas, triazines	Water, soil, sludge	[26]
Phenylureas	Potatoes, carrots, peas	[27]
Phenylureas	Foods	[49]
TCDD	Serum	[50]
TCDD	Milk	[51]
Tetradotoxin	Urine	[52]
TXB1, TXB2	Urine	[53]
Tetracylines, Sulphathiazole	Honey	[54]
Triazines	Water	[55]
Triazines, phenylureas	Water, fruit juice	[56]
Triazines	Plants	[57]
Zearalenone	Food	[58]
Zearalenone	Corn	[20]

are in Ref. [7]. Fig. 5 shows the clean up obtained with immuno-extraction of morphine from urine. HPLC was used with electrochemical detection. Urine was diluted in PBS pH 7, applied to the immuno-column. It was washed with 15 ml of PBS and eluted with 1 ml of 40% ethanol in pH 4 PBS. No further sample preparation was carried out. It should be emphasised that much of the blank signal arises from the direct injection of the immuno-column elution buffer which is processed directly. Full details are in Ref. [9].

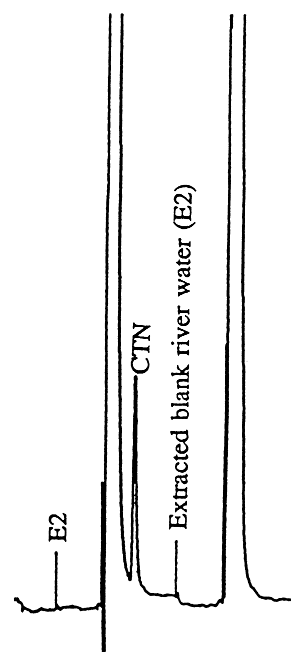

Fig. 4. HPLC traces obtained from immuno-extraction of chlortoluron from river water. Column Bondaclone C_{18}, eluent 70% methanol, detection UV at 244 nm. Chlortoluron retention time 5 min.

Another example of the performance of immuno-extraction columns showed their ability to extract the environmentally important polyaromatic hydrocarbon class from water using an anti-pyrene antiserum. The same paper [26] describes the extraction of polar metabolites along with atrazine using a mixed bed immuno-sorbent, as well as describing phenylurea immuno-extraction.

As mentioned earlier the extraction of drugs from biological fluids is the other main area. Morphine, morphine 3-glucuronide and morphine 6-glucuronide were extracted from whole blood on an immuno-column consisting of a mixture of the three anti-bodies [18]. The column capacity was approximately 250 ng per ml of column for each. Columns could be

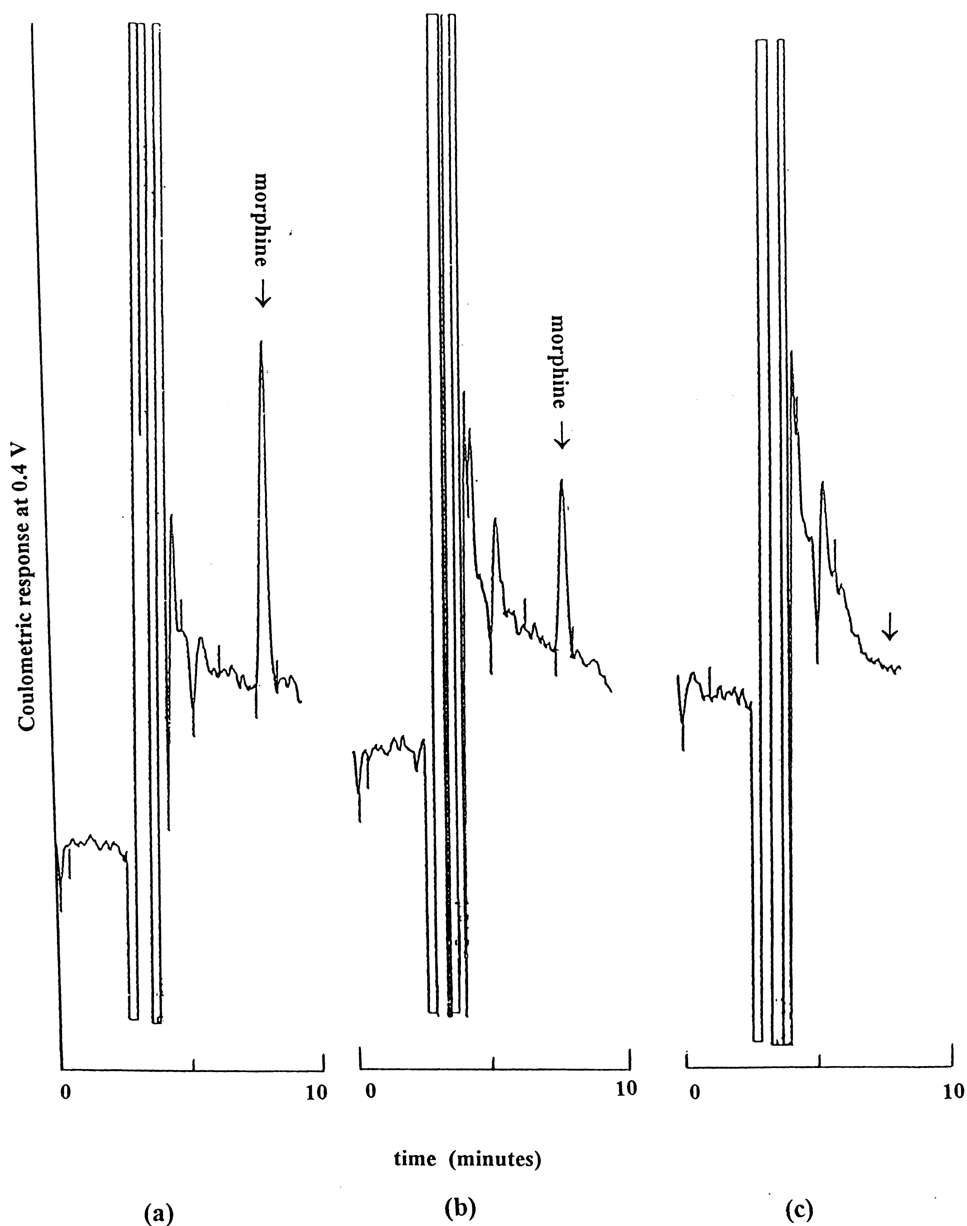

Fig. 5. HPLC traces obtained from immuno-extraction of morphine from urine. Column Hypersil CPS, eluent 13% acetonitrile in pH 2.5 phosphate (0.065 *M*) containing 1.5 m*M* sodium lauryl sulphate. Detection ECD at +0.45 V.

reused 20 times. The method has been used to screen overdose cases. Several other examples are shown in Table 4.

Although there are many examples where the selectivity of immuno-affinity provides sufficiently clean chromatograms to obviate the need for further sample processing it has been used advantageously in combination with other approaches. In the analysis of plant materials for phenylureas on a mixed immuno-affinity column it was necessary to use this approach in combination with clean up an anion-exchange or gel filtration column [27]. Also common

is the use of traditional SPE or liquid–liquid extraction before application to the immuno-affinity column. When using an immuno-column to process clenbuterol from plasma [10] better reproducibility was obtained when using a simple protein precipitation step with acetonitrile. There was no interference with the HPLC trace in the clenbuterol region. This suggests that non-specific interaction of plasma proteins with the immuno-sorbent was interfering with analyte binding. Immuno-affinity clean up has also been used in combination with supercritical fluid extraction (SFE). Triazines and polar metabolites, and polyaromatic hydrocarbons were immuno-extracted from soils and soot after initial SFE [29].

Most immuno-extraction procedures are used in combination with HPLC. This is not surprising as the immuno-column elution buffer is usually an aqueous buffer often with an organic modifier and is thus compatible with reversed-phase HPLC mobile phases. However procedures involving immuno-extraction of steroids have been described [33,37]. These were a multi step clean up methods with the final determination by GC–MS.

The possibility offered by immuno-extraction for the analysis of some of the most difficult environmental analytes is exemplified by the use of an anti-1,3,7,8 tetrachlorodibenzo-*p*-dioxin column. This has been used for residue analysis in milk, itself one of the more difficult matrices with which to work [50,51].

3. Future possibilities

Immuno-affinity extraction has been shown as another feasible procedure for trace analysis of biological and environmental samples. As more antibodies become available and as procedures to develop them become more sophisticated it is likely that more methods will be developed. To date the lack of commercial columns has meant that most work has come from research laboratories. Now that our understanding of how to optimise antibody based extraction has matured attention may focus more on analytes that are very difficult to extract by other procedures, such as large molecular weight water soluble proteins and polypeptides unstable to conditions used in traditional SPE or liquid–liquid extraction.

A further development is the use of molecular imprinted polymers as antibody mimics [59–64]. These to-date have not been as specific as biological antibodies. They are however much easier to synthesise and consequently much less expensive. As protocols become available procedures based on MIP extraction are expected to increase.

References

[1] L.A. Berrueta, B. Gallo, F. Vicente, Chromatographia 40 (1995) 474.
[2] D.S. Hage, Clin. Chem. 45 (1999) 593.
[3] D.S. Hage, J. Chromatogr. B 715 (1998) 3.
[4] J.M. Van Emon, C.L. Gerlath, K. Bowman, J. Chromatogr. B 715 (1998) 211.
[5] M.F. Katmeh, G.W. Aherne, G. Frost, D. Stevenson, Analyst 119 (1994) 431.
[6] M.F. Katmeh, G.W. Aherne, D. Stevenson, Analyst 121 (1996) 1699.
[7] S.J. Shahtaheri, M.F. Katmeh, P. Kwasowski, D. Stevenson, J. Chromatogr. A 697 (1995) 131.
[8] S.J. Shahtaheri, P. Kwasowski, D. Stevenson, Chromatographia 47 (1998) 453.
[9] B.A. Rashid, G.W. Aherne, M.F. Katmeh, P. Kwasowski, D. Stevenson, J. Chromatogr. A 797 (1998) 245.
[10] B.A. Rashid, P. Kwasowski, D. Stevenson, J. Pharm. Biomed. Anal. 21 (1999) 635.
[11] A. Martin-Esteban, P. Kwasowski, D. Stevenson, Chromatographia 45 (1997) 356.
[12] M.A.J. Godfrey, Analyst 123 (1998) 2501.
[13] M.F. Katmeh, G.W. Aherne, M.A.J. Godfrey, D. Stevenson, Analyst 121 (1997) 481.
[14] V. Pichon, L. Chen, M-C. Hennion, R. Daniel, A. Martel, F. Le Goffic, J. Abian, D. Barcelo, Anal. Chem. 67 (1995) 2451.
[15] M. Bouzige, V. Pichon, M-C. Hennion, Environ. Sci. Tech. 33 (1999) 1916.
[16] W. Schanzer, P. Delahaut, H. Geyer, M. Machnik, S. Horning, J. Chromatogr. B 687 (1996) 93.
[17] A. Koole, J. Bosman, J.P. Franke, R.A. de Zeeuw, J. Chromatogr. B 726 (1999) 149.
[18] J. Beike, H. Kohler, B. Brinkman, G. Blaschke, J. Chromatogr. B 726 (1999) 111.
[19] A. Pittet, D. Tornare, A. Huggett, R. Viani, J. Agric. Food. Chem. 44 (1999) 3564.
[20] A. Visconti, M. Pascale, J. Chromatogr. A 815 (1998) 133–140.
[21] S. Ikegawa, K. Matsuura, T. Sato, N.M.R. Isriyanthi, T. Niwa, J. Pharm. Biomed. Anal. 17 (1998) 1.
[22] J. Cai, J. Henion, Anal.Chem. 68 (1996) 72.

[23] C.K. Holtzapple, C.K. Buckley, L.H. Stanker, J. Agric. Food Chem. 47 (1999) 2963.
[24] M.L. Nedved, S. Habibigoudarzi, B. Ganem, J.D. Henion, Anal. Chem. 68 (1996) 4228.
[25] E. Ioannoukakouri, M. Christodoulidou, E. Christou, E. Constantinidou, J. Food Agric. Immun. 7 (1995) 131.
[26] V. Pichon, M. Bouzige, C. Miege, M-C. Hennion, Trends Anal. Chem. 18 (1999) 219.
[27] S. Herrera, A. Martin-Esteban, A. Fernandez, D. Stevenson, C. Camara, Fren. J. Anal. Chem. 362 (1998) 547.
[28] V. Pichon, H. Rogniaux, N. Fischer-Durand, S. Benrjeb, F. Le Goffic, M-C. Hennion, Chromatographia 45 (1997) 289.
[29] V. Pichon, H. Auchard-Macler, P. Oubihi, P. Sassiat, M-C. Hennion, M. Caude, Chromatographia 46 (1997) 529.
[30] I. Ferrer, M-C. Hennion, D. Barcelo, Anal. Chem. 69 (1997) 4508.
[31] S.M. Pearson, A.A.G. Candlish, K.E. Aidoo, J.E. Smith, Biotech. Techs. 13 (1999) 97.
[32] L. Puech, S. Dragacci, E. Gleizes, J.M. Fremy, Food Add. Contam. 16 (1999) 239.
[33] M. Dubois, X. Taillieu, Y. Colemonts, B. Lansival, J. De Graeve, P. Delahaut, Analyst 123 (1998) 2611.
[34] J.G. Rollag, M. Beck-Westermeyer, D.D. Hage, Anal. Chem. 68 (1998) 3631.
[35] D.H. Thomas, M. Beckwestermeyer, D.S. Hage, Anal. Chem. 66 (1994) 3823.
[36] D.H. Thomas, V. LopezAvila, L.D. Betowski, J. VanEmon, J. Chromatogr. A 724 (1996) 207.
[37] P. Delahaut, P. Jacquemin, Y. Colemonts, M. Dubois, J. De Graeve, H. Deluyker, J. Chromatogr. B 696 (1997) 203.
[38] C.S. Creaser, S.J. Feely, S.J. Houghton, M. Seymour, J. Chromatogr. A 794 (1998) 37.
[39] C.S. Creaser, S.J. Feely, E. Houghton, M. Seymour, P. Teale, Anal. Commun. 33 (1996) 5.
[40] R. Bagnati, V. Ramazza, M. Zucchi, A. Simonella, F. Leone, A. Bellini, R. Fanelli, Anal. Biochem. 235 (1996) 119.
[41] C.K. Holtzapple, S.A. Buckley, L.H. Stanker, J. Ass. Off. Anal. Chem. 82 (1999) 607.
[42] J.Y. Wescott, K.M. Maxey, J. McDonald, S.E. Wenzel, Prost. Oth. Lip. Med. 55 (1998) 301.
[43] K.S. Webb, P.B. Baker, N.P. Cassells, J.M. Francis, D.E. Johnston, S.L. Lancaster, P.S. Minty, G.D. Reed, S.A. White, J. Foren. Sci. 41 (1996) 938.
[44] R. Krska, R. Schuhmacher, J. Weingarner, M. Grasserbauer, Cereals Res. Commun. 25 (1997) 327.
[45] P. Degelmann, M. Becker, M. Herderich, H.U. Humph, Chromatographia 49 (1999) 543.
[46] K.A. Scudamore, S.J. Macdonald, Food Add. Contam. 15 (1998) 401.
[47] R. K Beensenfarmen, R.K. Botnen, I.V. Noto, H. Noto, J. Jacob, S. Ovrebo, Int. Arch. Occ. Environ. Hlth. 72 (1999) 161.
[48] L. Ball, A.S. Wright, N.J. Van Sittert, P. Aston, Biomarkers 2 (1997) 29.
[49] J.F. Lawrence, C. Menard, M-C. Hennion, V. Pichon, F. LeGoffic, N. Durand, J. Chromatogr. A 732 (1996) 277.
[50] W.L. Shelver, G.L. Larsen, J.K. Huwe, J. Chromatogr. B 705 (1998) 261.
[51] W.L. Shelver, J.K. Huwe, J. Liq. Chrom. Rel. Tech. 22 (1999) 813.
[52] K. Kawatsu, T. Shibata, E. Hamano, Toxicon 37 (1999) 325.
[53] B. Chiabrando, C. Corada, A. Bachi, R. Fanelli, Prostaglandins 47 (1994) 409.
[54] W. Heering, E. Usleber, R. Dietrich, E. Martlbauer, Analyst 123 (1998) 2759.
[55] A. Marx, T. Giersch, B. Hock, Anal. Letts. 28 (1995) 267.
[56] J. Dalluge, T. Hankemeier, R.J.J. Vreuls, U.A.T. Brinkman, J. Chromatogr. A 830 (1999) 377.
[57] J.N. Lawrence, C. Menard, M-C. Hennion, V. Pichon, F. Le Goffic, N. Durand, J. Chromatogr. A 752 (1996) 147.
[58] E. Rosenberg, R. Krska, R. Wissiack, V. Kmetov, R. Josephs, E. Razzazi, M. Grasserbauer, J. Chromatogr. B 819 (1999) 277.
[59] G. Wulff, Angew. Chem. Int. Ed. Engl. 34 (1995) 1812.
[60] K. Mosbach, Trends Biochem. Sci. 19 (1994) 9.
[61] D. Stevenson, Trends Anal. Chem. 18 (1999) 154.
[62] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatographia 46 (1996) 57.
[63] B. Sellegren, Anal. Chem. 66 (1994) 1578.
[64] J. Olsen, P. Martin, I.D. Wilson, G.R. Jones, Analyst 124 (1999) 467.