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Experimental comparison of three monoclonal antibodies for the class-selective immunoextraction of triazines Correlation with molecular modeling and principal component analysis studies

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Abstract

The specificity of three immunosorbents (ISs) based on different monoclonal anti-triazine antibodies has been characterized by extraction recoveries studies and with step elution experiments. Both indicated that the anti-dichloroatrazine IS is specific of terbutylazine and cyanazine. The anti-atrazine IS is specific of the chlorotriazines, whereas the anti-ametryn IS can trap all the triazines. This confirms the great influence of the hapten design on the specificity of the resulting antibodies, even if the target molecules are small. Moreover, the anti-ametryn IS is suitable for class-selective extraction of triazines contained in complex matrices. An approach designed to learn more about the specificity for a group of structurally related compounds of antibodies produced with a given compound is proposed and evaluated. Molecular modeling followed by principal component analysis has been used to obtain distribution maps with the relative position of each immunoconjugate and all the triazines. In all three cases, conclusions on specificity made with the analysis of the maps fit well with the experimental results. Consequently, molecular modeling coupled with principal component analysis seems to be a unique, inexpensive, and rapid tool to select the appropriate hapten providing highly specific or class-specific antibodies according to the given problem.

Keywords: Molecular modeling; Principal component analysis; Affinity sorbents; Solid-phase extraction; Immunoextraction; Monoclonal antibodies; Triazines

1. Introduction

Despite advances in instrumentation, a pre-treatment step is usually necessary to extract and isolate analytes from complex matrices. Solid-phase extraction (SPE) is a powerful method for sample preparation. However, retention on classical supports such as octadecyl silicas or polymers is based on hydrophobic interactions and co-extraction of many interfering compounds may occur, preventing the identification and quantitation of the target compounds. An approach to develop more selective SPE sorbents consists of producing antibodies against a target molecule and bonding them to a solid-support to form an immunosorbent (IS) [1]. These sorbents offer three main properties: high selectivity based on

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structure recognition, high affinity of the antibodies for the antigens, and the ability of the antibodies to recognize compounds with a structure similar to the antigen, so-called cross-reactivity. ISs represent a unique tool to selectively extract traces of antigen or structurally related compounds from various matrices [2].

Pesticides and common organic pollutants are low-molecular-mass molecules that are unable to induce an immune response. Therefore, the target molecule is derivatized into a hapten, by introducing a functional group, in order to link it to a carrier protein. The immunoconjugate thus formed is immunogenic and a mammal can be immunized to produce specific antibodies to the antigen. As many pesticides and other pollutants in a given family have closely related structures, the antibodies are able to trap several compounds belonging to the family. This property has allowed the development of, among others, class-specific immunosorbents for phenylurea and triazine herbicides [3–15], polycyclic aromatic hydrocarbons (PAHs) [16–18], and BTEX (benzene, toluene, ethylbenzene, and xylene isomers) [19].

To obtain antibodies able to recognize a whole class of structurally related compounds, the hapten design is fundamental. Currently, hapten design is often based on trial-and-error assays: the conclusions are drawn after having produced and characterized the antibodies [20–22]. Our group showed that antibodies produced with an isoproturon-based hapten were less class-specific for phenylureas than antibodies obtained with a chlortoluron-based hapten [5]. It would be less costly and more attractive to determine a priori the preponderant parameters that influence antigen–antibody recognition. Molecular modeling yields volumes and charge distribution within a molecule. These two features are important, as it was pointed out in previous studies that links exist between the structural properties of the target compounds and those of the hapten [23,24]. This approach has been successfully used by characterizing four haptens and selecting one to develop anti-trichlorophenols antibodies [24]. However, trichlorophenols are simple molecules: only carbon charges within the aromatic cycle were considered. When molecules are more complex, such as triazines or phenylureas, the number of electronic parameters quickly increases and the steric parameters may significantly vary.

The objective of this study is to provide tools for a better selection of the hapten design according to the desired specificity of antibodies for a group of structurally related compounds. Triazine herbicides have been chosen here. First, three immunosorbents created with monoclonal anti-dichloroatrazine, anti-atrazine, and anti-ametryn antibodies were characterized to accurately determine their specificity. Second, the steric and functional parameters of triazines, metabolites, and immunoconjugates were calculated using molecular modeling. Third, principal component analysis (PCA) was used to analyse the results and point out correlations between the theoretical and experimental characterization of the specificity of three batches of antibodies for triazines and their metabolites.

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile and methanol were from Mallinckrodt Baker (Noisy-le-sec, France). Ultrapure water was obtained by purifying demineralized water in a Milli-Q filtration system (Millipore, Saint-Quentin en Yvelines, France). Triazines were purchased from Cluzeau Info Labo (CIL, Saint-Foy-la-Grande, France). Metabolites are abbreviated as follows: diaminoatrazine (DAA), deethylatrazine (DEA), deisopropylatrazine (DIA), deethylterbutylazine (DET), hydroxyatrazine (OHA), hydroxydeethylatrazine (OH-DEA), hydroxydeisopropylatrazine (OH-DIA), and hydroxyterbutylazine (OHT). Stock solutions of selected solutes were prepared by weighing and dissolving in methanol or a mixture of methanol–water (1:1, v/v) for some metabolites. They were stored at 4 °C. Phosphate-buffered saline (PBS) solution was 0.01 *M* sodium phosphate buffer containing 0.15 *M* sodium chloride (pH 7.4).

2.2. Immunosorbents

Giersch et al. generously gave us three batches of monoclonal anti-triazine antibodies produced with three different kinds of haptens linked to bovine serum albumin (BSA), the carrier protein. Their structures are given in Fig. 4. They were based on

dichloroatrazine, atrazine, and ametryn, and gave the corresponding antibody batches P6A7, K4E7, and K1F4 [25,26]. Then, 1.7, 1.8, and 1.8 mg of these antibodies were, respectively, immobilized on 100 mg of glutardialdehyde-activated silica, 30 nm pore size (Mallinckrodt Baker) using the procedure described elsewhere [1]. The resulting sorbents were introduced into 1-ml disposable cartridges.

2.3. Immunoextraction procedure

The first step of the procedure consisted of conditioning the immunosorbent with 5 ml of PBS, then 10 ml of ultra-pure water. The sample was percolated through the immunosorbent. In some cases, a washing step with 2 ml of ultra-pure water was used to remove interfering compounds non-specifically retained on the solid support. The test compounds were then eluted from the cartridge with methanol–water (7:3). The cartridge was regenerated with 10 ml of ultra-pure water, then 5 ml of PBS containing 0.1% azide, and was stored for at least 2 days at 4 °C before use again [4].

Step elution was performed by percolation of 3 ml of water spiked with 400 ng of one of the triazines. Elution was performed with successive fractions containing an increasing content of methanol from 10 up to 80%.

2.4. Apparatus

Liquid chromatography (LC) was performed with a Varian LC system workstation comprising a Varian ProStar 400 autosampler, a Varian ProStar 230 solvent-delivery system, set at a flow-rate of 1 ml/min, and a Varian ProStar 330 diode-array detection (DAD) system (Varian, Les Ulis, France). Compounds were separated on a 250×4.6 mm I.D. column packed with 5 μm Hypersil ODS (Colochrom, Gagny, France).

2.5. LC conditions

The gradient used for the triazines separation was as follows: a mixture of acetonitrile–0.005 *M* phosphate buffer at pH 7 (5:95) from 0 to 2 min, 5 to 25% acetonitrile from 2 to 6 min, 25 to 30% from 6 to 20 min, and 40% at 50 min. The gradient used for the separation of the metabolites of triazines was as follows: a mixture of acetonitrile–0.005 *M* phosphate buffer at pH 7 (4:96) from 0 to 8 min, 4 to 10% from 8 to 20 min, 10 to 40% from 20 to 50 min, and 95% at 70 min.

2.6. Molecular modeling

Molecular modeling was performed using the HyperChemPro 6.0 software package (Hypercube, Gainesville, FL, USA). Conformations of low energies for each compound were evaluated using the molecular mechanic (MM+) method and then refined using the semi-empirical mechanic (PM3) method. Finally, the conformation of lowest energy was refined with ab-initio quantum mechanic (3-21G basis set) calculations and the electronic distribution was obtained.

2.7. Principal component analysis

PCA was performed using The Unscrambler 7.5 software package (CAMO, Corvallis, OR, USA). As the data values are quite different, standardization has been carried out to give all variables the same variance, that is to say to give all variables the same chance to influence the estimation of the components.

3. Results and discussion

3.1. Determination of the specificity of the antibodies towards the triazine group by experimental characterization of the immunosorbents

The first step of the study consisted of the experimental characterization of the specificity of monoclonal antibodies, obtained with haptens based on dichloroatrazine, atrazine, or ametryn. They have been immobilized on silica to form immunosorbents. Two approaches were possible. The first one consisted of the determination of the extraction recoveries (defined as the ratio between the bound amount and the introduced amount) after percolation of the analytes in a given volume of water [27]. The higher the extraction recovery, the stronger the affinity of the antibodies for an analyte. Thus, extraction recoveries indicate the antibodies' spe-

cificity and, moreover, the possibility of analyte extraction from real matrices. The second approach consisted of percolation of the analytes dissolved in a small volume of water and in their elution with small successive fractions containing an increasing percentage of organic solvent. In this case, the higher the percentage of the organic solvent required to elute the analyte, the stronger the affinity of the antibodies. In general, this method gives more accurate results but it is not really helpful for the development of extraction procedures. Thus, the first approach is usually preferred.

3.1.1. Determination of the extraction recoveries

Once the desorption step has been optimized, incomplete recovery in a solid-phase extraction process is due to analyte breakthrough, caused either by insufficient retention or overload of the sorbent capacity (maximum amount of analytes that can be bound by the sorbent). Thus, to rule out the role of sorbent capacity and only focus on retention linked to the affinity of the antibodies, the capacity of each immunosorbent has to be determined first.

This has been carried out by measuring the amount of adsorbed analyte as a function of the amount of analyte introduced onto the IS. To estimate correctly the capacity, one has to use the triazine-antigen or a triazine that has a great affinity for the immobilized antibodies [28]. With the anti-dichloroatrazine IS, terbutylazine has been used because Giersh et al. have shown a high affinity of the anti-dichloroatrazine antibodies for this compound [29]. With the anti-atrazine IS, the antigen has been used. With the anti-ametryn IS, terbutryn has been selected because previous results have indicated a strong affinity of the anti-ametryn antibodies for this compound [26]. The sorbent capacity was 1.8 ± 0.1 μg per 100 mg for the anti-atrazine and anti-ametryn ISs, and 750 ± 50 ng per 100 mg for anti-dichloroatrazine IS.

Tables 1–3 present the average extraction recoveries measured on the three ISs, after percolation of various volumes of ultra-pure water spiked with triazines. For the anti-dichloroatrazine IS (Table 1), extraction recoveries are quite low for all triazines. Indeed, after percolation of 25 ml of spiked water, only terbutylazine and cyanazine gave a recovery higher than 90%. Thus, this IS is not class-selective, due to the weak cross-reactivity of the antibodies. It is difficult to classify the various triazines by an affinity order using these experiments. Affinity seems to be highest for terbutylazine and cyanazine, then similar for sebutylazine, atrazine, and propazine, and lower for simazine and the other triazines.

Table 1
Average extraction recoveries (%) obtained after percolation of 5, 10, and 25 ml of ultra-pure water spiked with 100 ng each of the triazines on the anti-dichloroatrazine IS

	5 ml* ($n=2$)	10 ml	25 ml* ($n=2$)
Atrazine	83	62	23
Cyanazine	99	84	93
Propazine	44	35	25
Sebutylazine	74	71	35
Simazine	31	1	3
Terbutylazine	109	86	90
Prometon	0	0	2
Prometryn	0	21	0
Terbutryn	0	0	0

* Mean value of two measurements.

Table 2 indicates that the anti-atrazine antibodies show a less limited specificity. In fact, after the percolation of 25 ml of spiked water, which is a volume equivalent to the 10 ml percolated on the anti-dichloroatrazine IS (cf. ratio of their respective capacity) [27], extraction recoveries were close to or higher than 70% for six chlorotriazines (with a chlorine substituent in the 2-position on the heterocycle). When the substituent in the 2-position was a methoxy (prometon) or a thiomethyl (terbutryn and

Table 2
Average extraction recoveries (%) and standard deviation (in parentheses) obtained after percolation of 5, 10, 25, and 50 ml of ultra-pure water spiked with 100 ng of each of the triazines on the anti-atrazine IS

	5 ml* ($n=2$)	10 ml ($n=3$)	25 ml ($n=3$)	50 ml ($n=3$)
Atrazine	100	87 (10)	91 (6)	102 (10)
Cyanazine	95	84 (14)	96 (12)	95 (11)
Propazine	81	83 (9)	75 (8)	81 (10)
Sebutylazine	80	94 (7)	100 (4)	80 (6)
Simazine	92	87 (9)	93 (5)	98 (10)
Terbutylazine	93	58 (8)	67 (6)	55 (5)
Prometon	46	50 (9)	25 (5)	6 (4)
Prometryn	31	29 (11)	0 (0)	0 (0)
Terbutryn	0	3 (2)	0 (0)	0 (0)

*: Mean value of two measurements.

Table 3
Average extraction recoveries (%) obtained after percolation of 5, 10, 25, 50, 100, and 150 ml of ultra-pure water spiked with 100 ng each of the triazines on the anti-ametryn IS

	5 ml* (n=2)	10 ml* (n=2)	25 ml* (n=2)	50 ml* (n=2)	100 ml	150 ml
Atrazine	95	97	90	89	82	94
Cyanazine	97	94	91	102	98	112
Propazine	89	84	105	85	84	90
Sebutylazine	97	103	100	93	96	95
Simazine	97	96	114	106	91	98
Terbutylazine	96	109	85	88	84	90
Prometon	96	90	99	90	89	90
Prometryn	115	93	107	101	85	105
Terbutryn	101	88	92	95	66	88

*: Mean value of two measurements.

prometryn), extraction recoveries decreased dramatically.

Table 3 shows that high extraction recoveries were obtained for all the triazines with the anti-ametryn IS with percolated volumes up to 150 ml. The specificity of these antibodies seems to be independent of the nature of the substituent in the 2-position. Moreover, high breakthrough volumes were obtained indicating that this IS is suitable for class-selective extractions.

Table 4 presents the extraction recoveries of eight triazine metabolites (dealkylated and hydroxylated) on the three ISs. Some extraction recoveries have not been measured for percolated volumes higher than 5 ml because they were already close to 0% for this volume. The metabolites are less recognized than parent molecules, even with the anti-ametryn IS. The anti-dichlorotriazine IS only retains DET to a small extent. Anti-atrazine and anti-ametryn antibodies have a moderate affinity for OHA, DEA, which are the two main metabolites of atrazine, and for DET.

These results indicate that the hapten design has a great influence on the specificity of the obtained antibodies. The three batches of antibodies bind the various triazines and their metabolites differently. Table 3 shows that with the anti-ametryn antibodies, breakthrough volumes have not been reached and no affinity differences were observed from the recovery experiments. Moreover, with the anti-dichloroatrazine and anti-atrazine ISs, several triazines had extraction recoveries equal to 0% and could not be classified by affinity order. Thus, step elution experiments have been carried out to study more precisely the anti-triazine antibodies specificity.

Table 4
Average extraction recoveries (%) obtained after percolation of 5, 10, and 25 ml of ultra-pure water spiked with 100 ng each of the metabolites on the anti-dichloroatrazine, anti-atrazine, and anti-ametryn ISs

	Anti-dichloroatrazine IS			Anti-atrazine IS			Anti-ametryn IS		
	5 ml* (n=2)	10 ml* (n=2)	25 ml	5 ml	10 ml* (n=2)	25 ml	5 ml	10 ml* (n=2)	25 ml
DEA	11	0	0	100	44	59	65	44	44
DIA	0	nd	nd	26	22	13	15	6	0
DAA	0	nd	nd	0	0	nd	7	0	nd
DET	5	22	5	83	50	0	32	25	17
OHA	0	nd	nd	88	91	44	73	107	52
OH-DEA	0	0	0	0	0	0	0	0	0
OH-DIA	0	0	nd	1	0	nd	0	0	nd
OHT	8	0	0	100	65	0	29	17	0

nd: Not determined.
*: Mean value of two measurements.

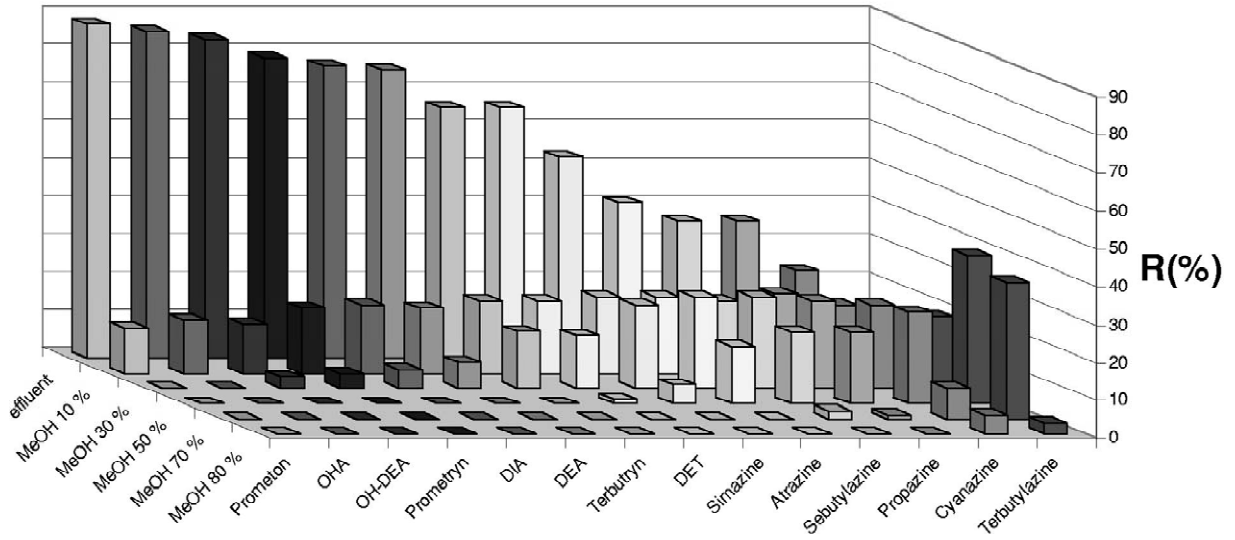

Fig. 1. Step elution of triazines and metabolites from the anti-dichloroatrazine IS, after percolation of 400 ng of one compound in 3 ml of ultra-pure water and elution with successive fractions of 0.5 ml containing an increasing percentage of methanol.

3.1.2. Step elution procedures

Step elution procedures have been carried out on the three immunosorbents. Each triazine has been studied separately to avoid competition. The first step consisted of the percolation on an IS of a small amount of a triazine dissolved in 3 ml of ultra-pure water. The triazine was then eluted with successive water fractions that contained an increasing percentage of methanol. The resulting elution profiles obtained with triazines and metabolites on the anti-dichloroatrazine, anti-atrazine, and anti-ametryn ISs are presented in Figs. 1–3, respectively. Affinity of the antibodies for the compounds increases from left to right in Figs. 1–3.

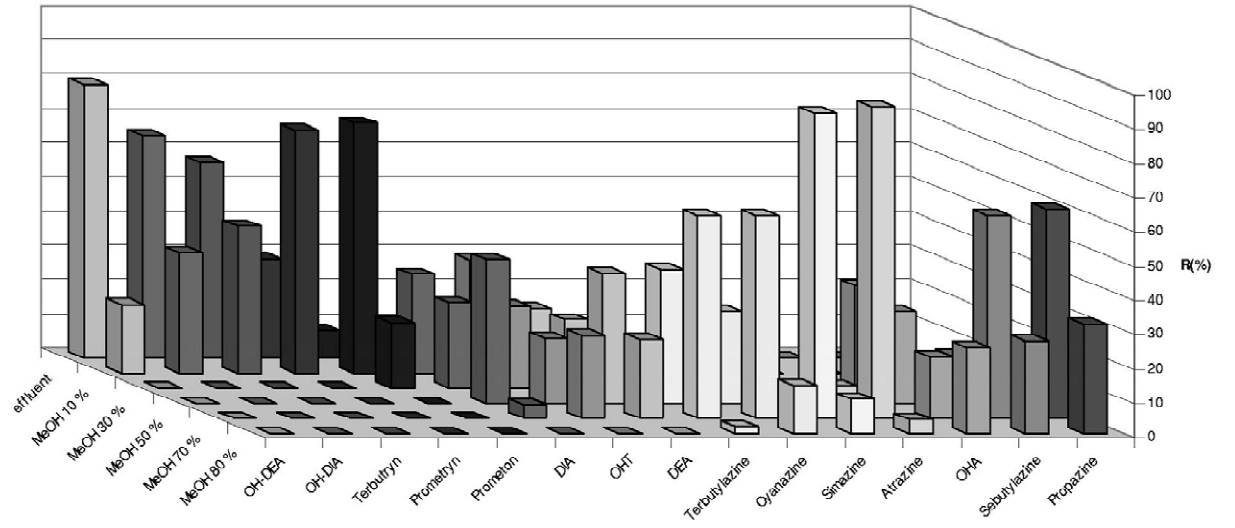

Fig. 2. Step elution of triazines and metabolites from the anti-atrazine IS, after percolation of 800 ng of one compound in 3 ml of ultra-pure water and elution with successive fractions of 3 ml containing an increasing percentage of methanol.

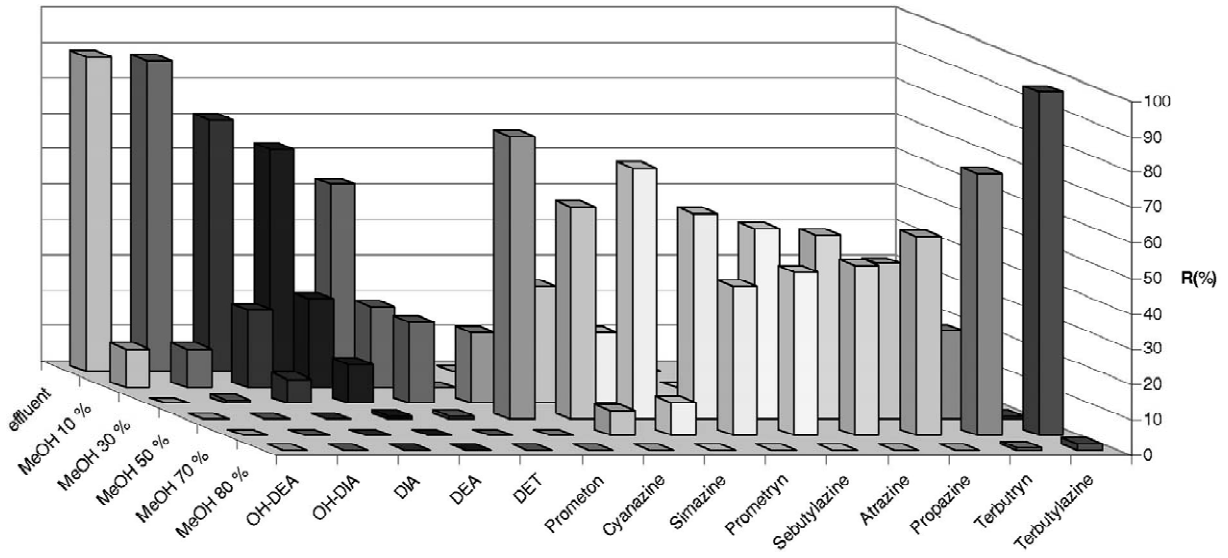

Fig. 3. Step elution of triazines and metabolites on the anti-ametryn IS after percolation of 800 ng of one compound in 3 ml of ultra-pure water and elution with successive fractions of 0.5 ml containing an increasing percentage of methanol.

The anti-dichloroatrazine antibodies have the strongest affinity for terbutylazine, and then for cyanazine because a fraction containing 80% of methanol is required to elute these compounds (Fig. 1). These results match the conclusions drawn from the extraction recoveries, and permit classification of atrazine, propazine, and sebutylazine. Moreover, it is also possible to classify compounds that show low recoveries such as prometon, prometryn, terbutryn, OHA, DEA, DIA, and OH-DEA.

The elution profiles presented in Fig. 2 have been obtained with the anti-atrazine IS. The chlorotriazines have a high affinity for these antibodies, though some differences were observed as a function of the amino substituents. This confirms the results obtained with the extraction recoveries. These antibodies also have a strong affinity for OHA, which did not appear previously. Some compounds such as prometon, DEA, DIA, and OHT form another group characterized by a moderate affinity for the antibodies. Finally, thiomethyl compounds and dealkylated or hydroxylated metabolites have quite a low affinity for these antibodies.

With the anti-ametryn IS, the elution profile presented in Fig. 3 is rather different from the ones of Figs 1 and 2. This profile shows two groups of compounds. The first one contains triazines and DET, which have a strong affinity for the antibodies. The second one consists of the remaining metabolites which have a weak affinity for the antibodies, but still stronger than the one observed in Fig. 2.

3.2. Specificity study of the antibodies with molecular modeling of immunoconjugates and triazines and correlation with experimental results

3.2.1. Determination of steric and electronic parameters of the triazines, their metabolites, and the immunoconjugates

The extraction recoveries and the step elution experiments are efficient experimental tools to determine the respective specificity of the anti-dichloroatrazine, anti-atrazine, and anti-ametryn antibodies. However, it would be more attractive to be able to predict the specificity of the antibodies obtained with a given hapten. As antigen–antibody recognition is based on steric criteria and interactions resulting from the electronic properties of the molecules, molecular modeling may be helpful as it allows the determination of volumes and charges of the compounds.

Molecular modeling of triazines, metabolites, and immunoconjugates regions that determine a priori the antibodies recognition sites (surrounded by a dotted line in Fig. 4) has been carried out. The resulting

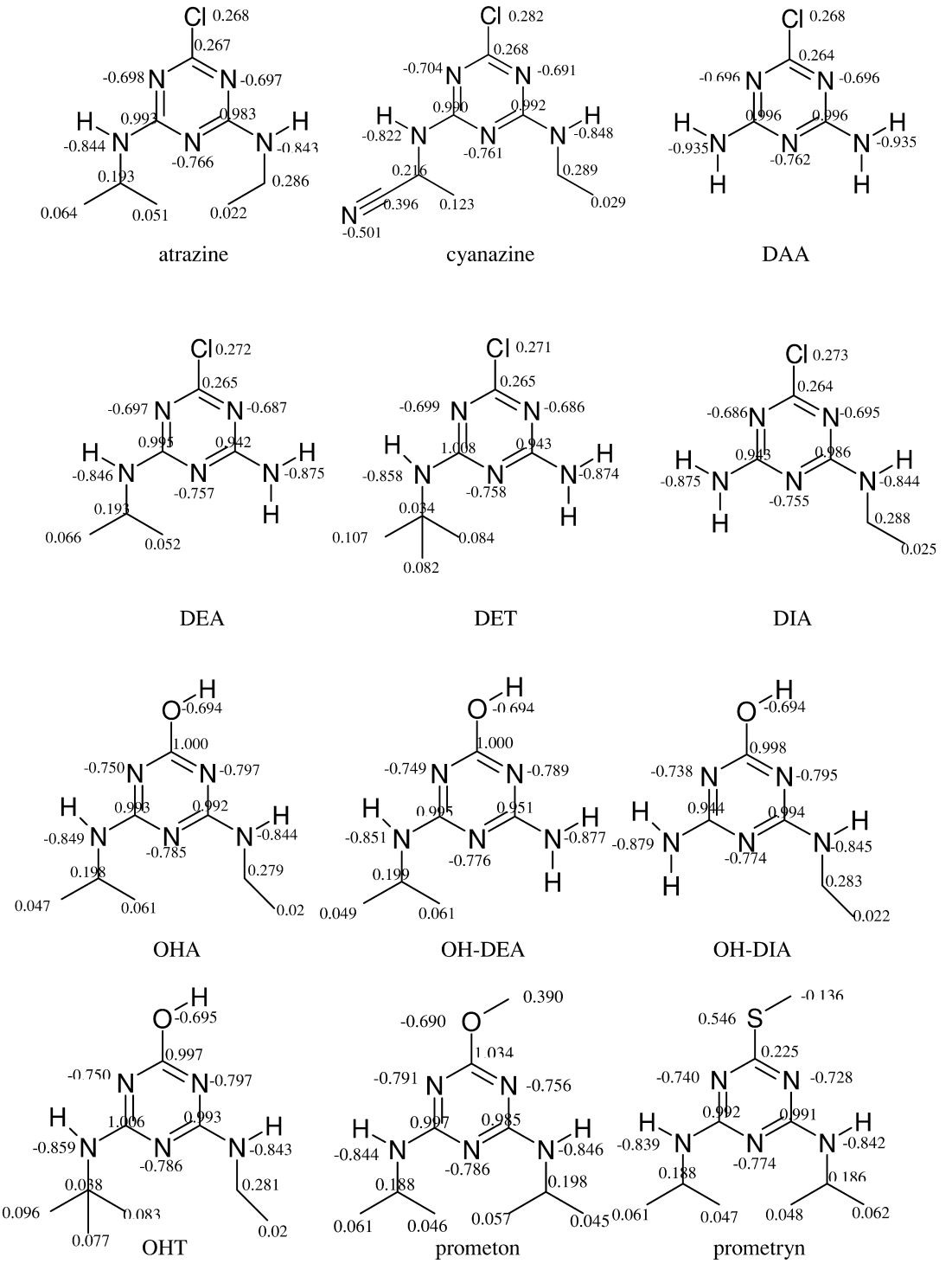

Fig. 4. Charges, obtained by molecular modeling, of atoms and of groups belonging to the nine triazines, eight metabolites, and the three immunoconjugates (I.s). The hydrogen charges of the amino groups are always close to 0.360 except for DAA (0.376). The hydrogen charges of the hydroxy groups are always close to 0.416. (---) Region of the immunoconjugates regarded as specific and accessible by the antibodies during their production.

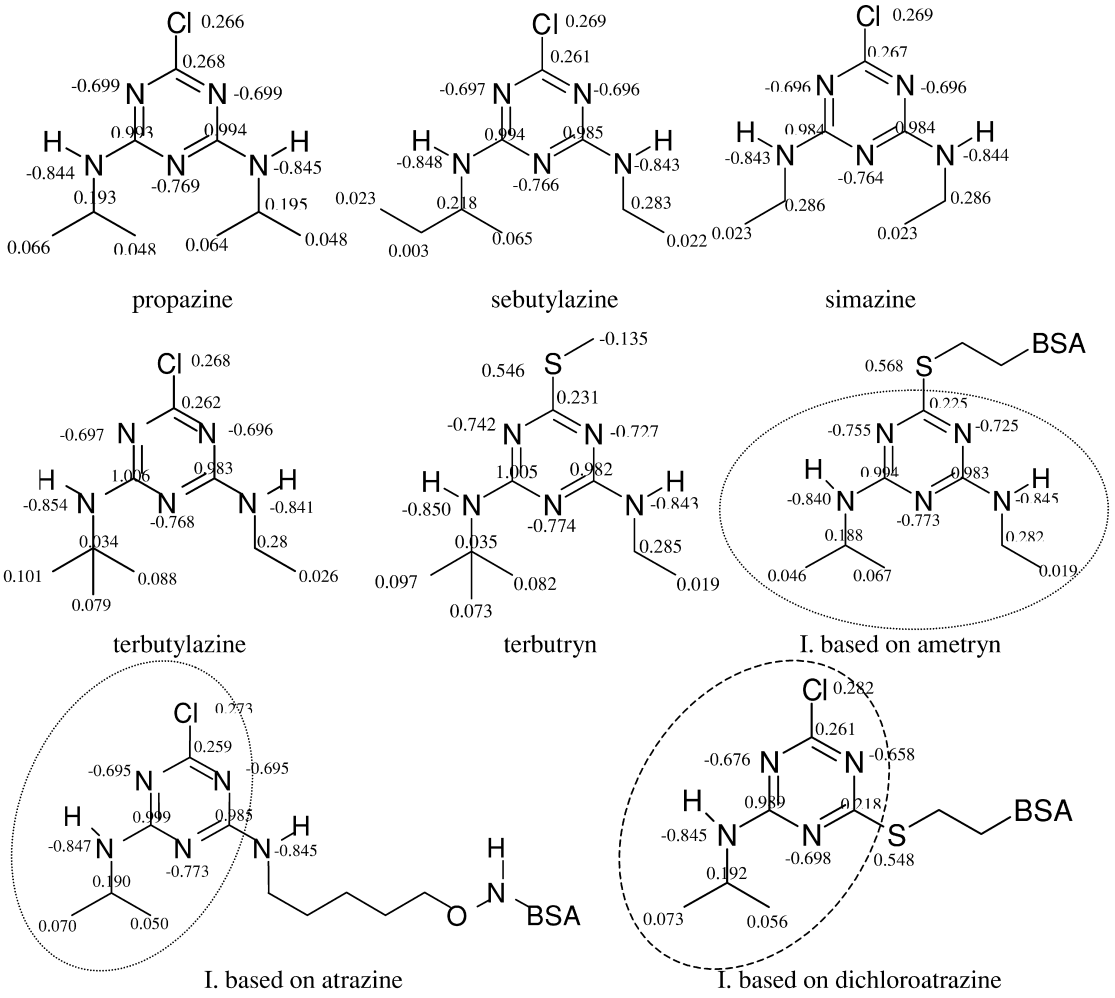

Fig. 4. (*continued*)

steric parameters are presented in Table 5. As expected, they markedly vary from one compound to the next. The charges of each atom or group that constitutes the compounds are presented in Fig. 4. A detailed analysis of these values indicates an increasing mesomeric effect of the substituent in the 2-position on the heterocycle from the chlorine, to the thiomethyl, methoxy, and then hydroxy, as the relocation of the electrons increases. For example, the global negative charge of the cycle increases from propazine (−2.167) to prometryn (−2.242), and prometon (−2.333), while the nature of the amino substituents remains constant indicating the coherence of the results obtained with the molecular modeling software.

To draw conclusions about the specificity of antibodies produced with a given immunoconjugate for the triazine family, a reasonable hypothesis has been used: the more numerous the similarities of a compound with the immunoconjugate region that determines a priori the recognition sites of the antibodies, the higher the affinity of the resulting antibodies for this analyte. Thus, one has to compare the steric and electronic data of the specific immunoconjugate region with the corresponding region of the target compounds.

Table 5
Volumes, obtained with molecular modeling, of the amine substituents and of the substituent in the 2-position for triazines and the regions of the immunoconjugates that determine, a priori, the specificity of the antibodies

	Volume (Å^3)	
	Amine substituents	Substituent in the 2-position
Atrazine	255, 305	98
Cyanazine	255, 329	98
Propazine	305, 305	98
Sebutylazine	255, 349	98
Simazine	255, 255	98
Terbutylazine	255, 349	98
DIA	127, 255	98
DEA	127, 305	98
DET	127, 349	98
DAA	127, 127	98
Prometryn	305, 305	144
Terbutryn	255, 349	144
Prometon	305, 305	133
OHA	255, 305	75
OHT	255, 349	75
OH-DIA	127, 255	75
OH-DEA	127, 305	75
I.[a] (dichloroatrazine)	305, nd[b]	98
I. (atrazine)	305, nd	98
I. (ametryn)	255, 305	nd

[a] I.: Immunoconjugate.
[b] nd: Not determined because corresponding to the group linked to the carrier protein.

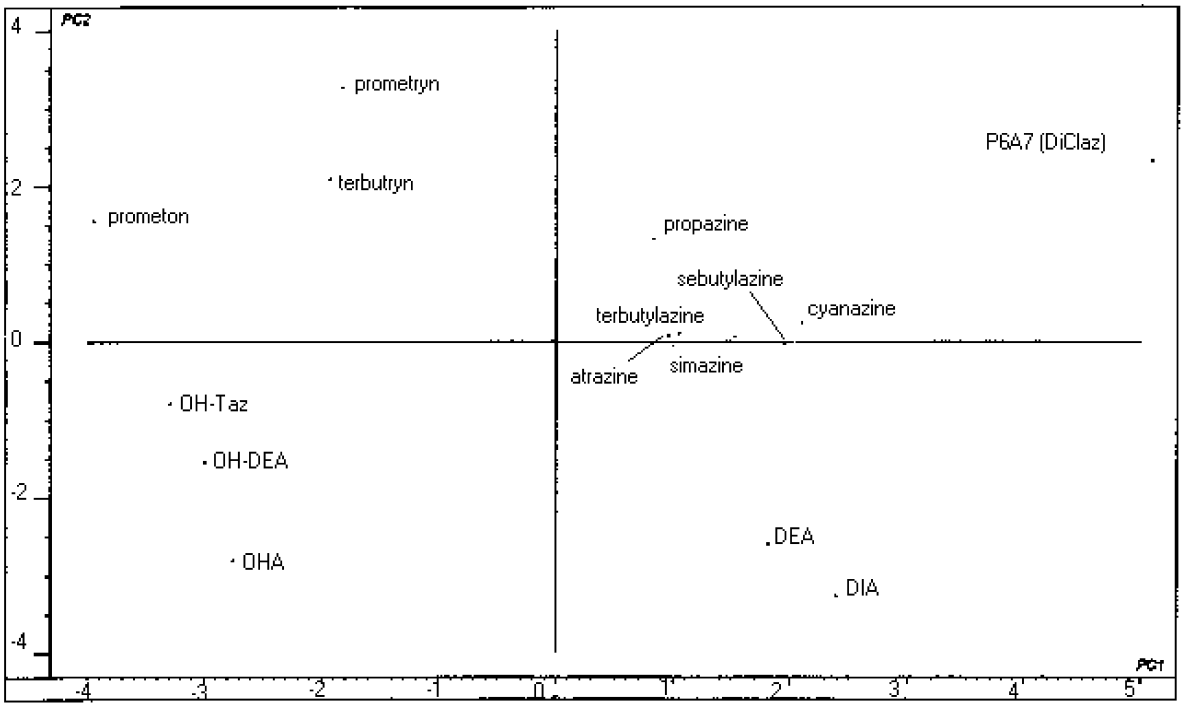

Fig. 5. Distribution obtained by PCA of the dichloroatrazine-based immunoconjugate (P6A7), triazines, and metabolites in the plane of the first two principal components.

3.2.2. Principal component analysis of the data and correlation with measured specificity

Numerous data have been obtained with molecular modeling of triazines, metabolites, and the specific immunoconjugate regions. Therefore, a straightforward parameter-by-parameter comparison is inadequate. To reduce the number of dimensions of the parameter space, PCA has been carried out. The horizontal axis of the representation is always chosen to be the most informative.

Fig. 5 presents the distribution of triazines, metabolites, and the dichloroatrazine-based immunoconjugate in the plane of the first two principal components that explain 67% of the variance. If more components are considered, the variance explained increases slightly but the relative distribution of the compounds is not modified. As the immunoconjugate is located far from all triazines in Fig. 5, one can conclude that the steric and electronic parameters of the immunoconjugate strongly differ from those of all the triazines and metabolites. Thus, the antibodies produced with this immunoconjugate would not present a high affinity for the triazines and their metabolites. As low extraction recoveries have been measured with the resulting immunosorbent, there is a good correlation between the conclusion drawn with molecular modeling and principal component analysis, and the measured specificity of the antibodies.

Moreover, in Fig. 5, cyanazine and terbutylazine are closer to the immunoconjugate than prometon, OHA, and OH-DEA. Thus, the relative position of the immunoconjugate and the compounds agrees with the classification obtained by the step elution experiments (cf. Fig. 1) indicating that steric parameters and partial charges of atoms or groups of atoms representing dipole–dipole interactions are sufficient to describe the antigen–antibody interactions. Thus, this approach may be used to choose between several haptens.

A PCA has been similarly carried out with the data of the atrazine-based immunoconjugate specific region and the corresponding regions of the triazines and their metabolites. Fig. 6 presents the obtained

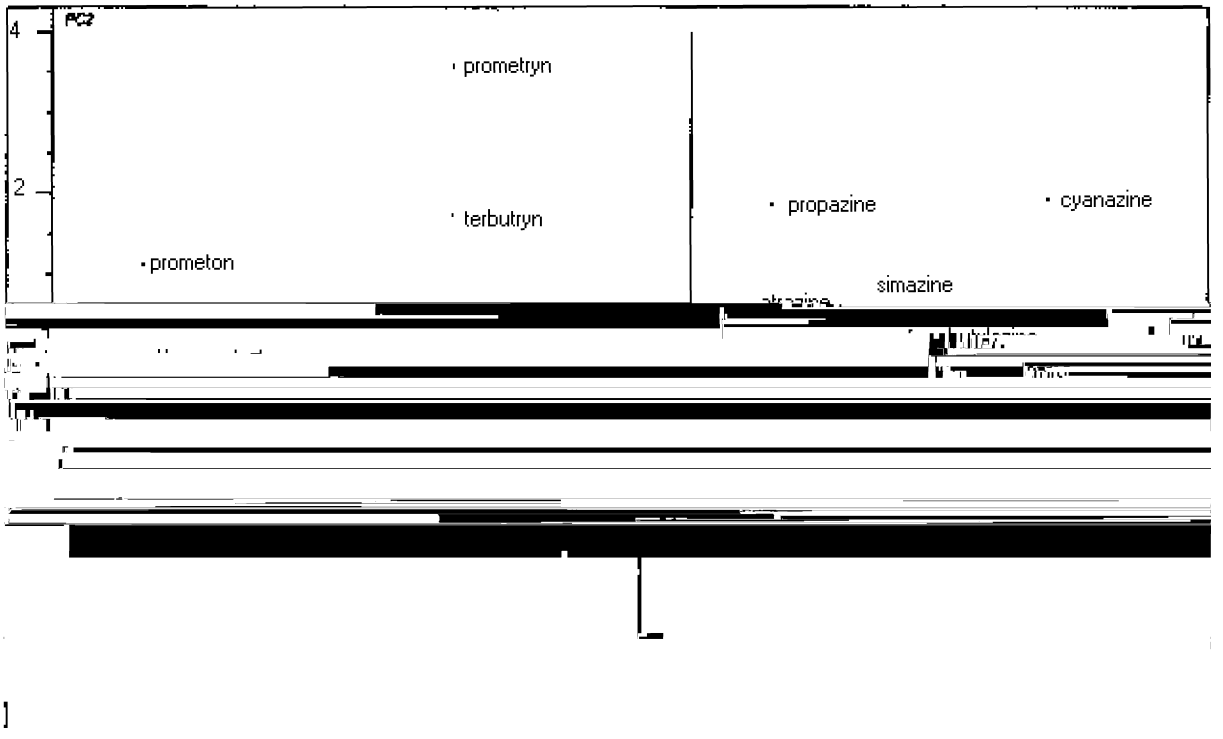

Fig. 6. Distribution obtained by PCA of the atrazine-based immunoconjugate (K4E7) triazines, and metabolites in the plane of the first two principal components.

distribution in the plane of the first two principal components. The parameters of the atrazine-based immunoconjugate are close to the values of chlorotriazines, while prometon, prometryn, terbutryn, and metabolites are rather distant. Thus, the resulting antibodies should have a strong affinity exclusively for the chlorotriazines. This conclusion is confirmed by the extraction recoveries and the step elution results. OHA showed a high affinity for the anti-atrazine antibodies an unexpected result according to Fig. 6. This may be explained by the existence of a strong hydrogen-bonding interaction with the donor and acceptor hydroxy group of OHA and the recognition site. Potential hydrogen-bonding has not been taken into account in the data.

Finally, similar a PCA has been carried out for the parameters of the ametryn-based immunoconjugate specific region and the corresponding regions of the triazines and their metabolites. Fig. 7 presents the obtained distribution in the plane of the first two principal components, indicating that the parameters of the immunoconjugate are near to those of numerous triazines. Thus, the anti-ametryn antibodies should have a strong affinity for these compounds. OH-DEA, DEA, and DIA are the farthest from the immunoconjugate and should have the lowest affinity with for the anti-ametryn antibodies. These observations match very well the experimental results obtained with step elution procedures (cf. Fig. 3).

Since the resulting compound distributions correlated well with the measured specificities in all three cases, it is possible to conclude that molecular modeling coupled to principal component analysis of the data can be a unique tool to select the most appropriate hapten as a function of a desired specificity of some antibodies.

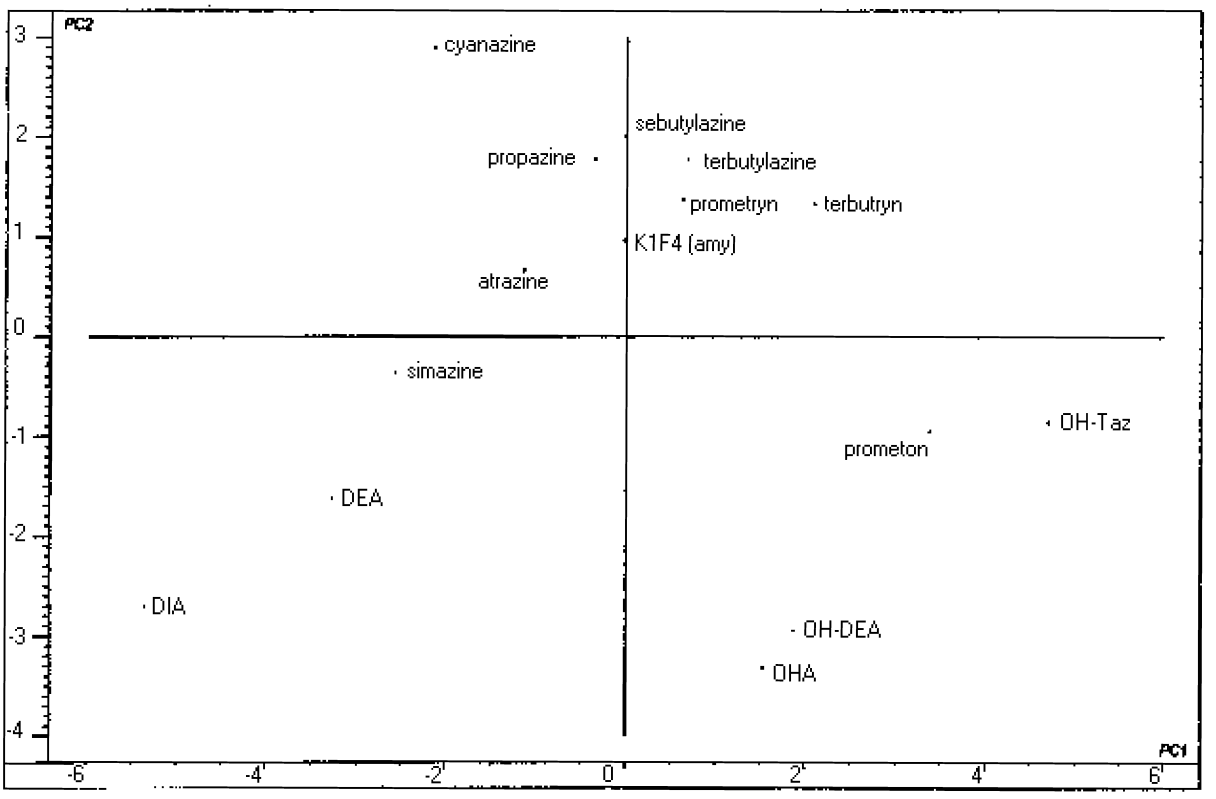

Fig. 7. Distribution obtained by PCA of the ametryn-based immunoconjugate (K1F4), triazines, and metabolites in the plane of the first two principal components.

4. Conclusion

Based on the knowledge of the interactions between antigen and antibodies that occur in immmunoextraction, this study has demonstrated that it is possible to optimize the hapten design for good trapping of only one analyte or a group of structurally related analytes. The results obtained with molecular modeling and principal component analysis fit well the experimental data observed using three different monoclonal antibodies. It should be interesting to apply this tool to other groups of pollutants as well. The approach of using molecular modeling and principal component analysis seems to be a unique, inexpensive, and rapid tool for the selection of the appropriate hapten according to the needs of each problem. The major advantage of this approach is that it is not dependent on the structure of the molecules and remains the same independently of the targeted compound family.

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