

Selective extractions using molecularly imprinted polymers

Laboratories are routinely challenged to achieve better sensitivities, increase throughput, improve productivity and reduce costs. With continual improvements in analytical instrumentation, sample preparation is increasingly becoming the rate-limiting step in most laboratories.

Traditionally, liquid-liquid extraction (LLE) is used to remove organic analytes from aqueous samples. Solid-phase extraction (SPE) techniques provide some relief to the labour intensiveness and high-solvent use of LLE. However, SPE suffers its own set of limitations. While SPE sorbents are available in different materials (silica, resins), with different functional groups bound to the solid support (C-8, C-18, ion-exchange, etc), these materials often lack selectivity for the target compounds being extracted. This lack of selectivity can result in excessive background interferences and/or require additional sample clean-up steps. Additional steps reduce the throughput and increase the cost of the analysis.

Affinity chromatography, using immobilized antibodies or other proteins, can greatly improve the selectivity of the extraction. However, these protein-based sorbents typically have limited stability and can be expensive, reducing their usefulness on a routine basis.

Now a new separation technique using molecularly imprinted polymers (MIPs) is commercially available in Canada.

What are MIPs?

Molecular imprinting is a technique for introducing selectivity towards a specific compound, or a class of compounds, during the creation of the SPE sorbent. This is achieved by using a template molecule chosen to be representative of the 3-dimensional shape and functional chemistries of the target compound or class of interest. Functional monomers are added, which form complexes with the template mole-

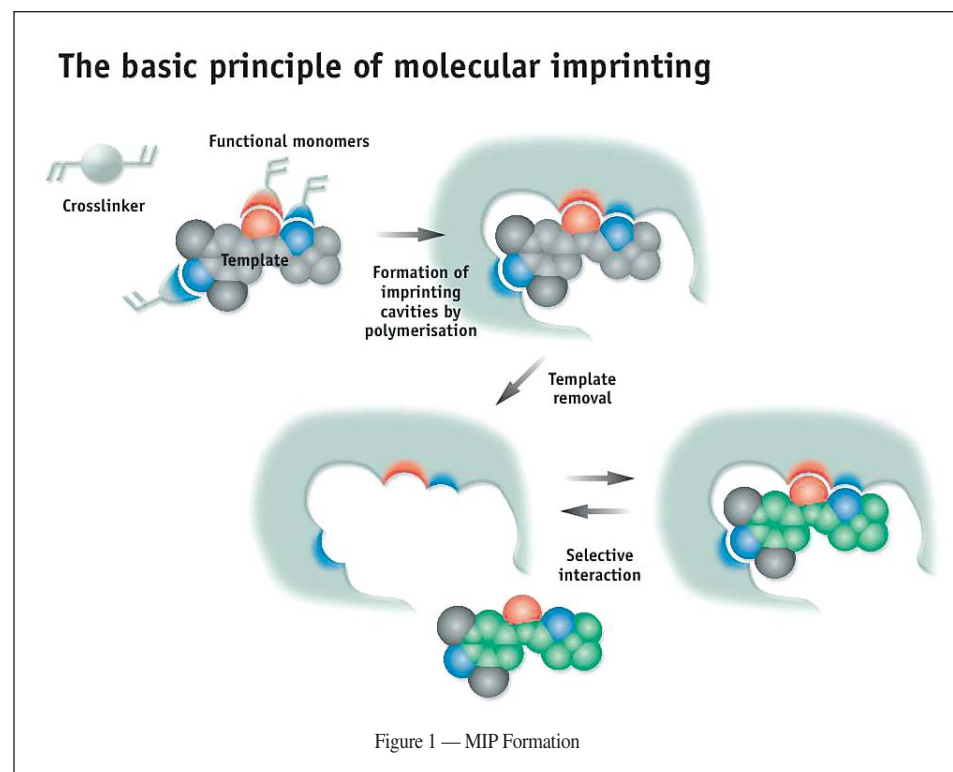
cule. A polymer is then formed to include the functional monomers, creating a mould around the template. Once the template molecule is removed, the polymer is left with imprints of the molecule (binding sites) that complement the 3-D shape and functional groups of its intended target molecules. The MIP is comparable to a man-made antibody or receptor in terms of selectivity, but has the stability of a polymer material. Because MIPs are highly cross-linked polymers, they are very stable, even when used with extreme pHs, a wide variety of organic solvents and various temperatures. Figure 1 shows a schematic of MIP formation.

Advantages include extreme stability, high selectivity, and strong binding affinity with their target compounds/classes, leading to more simplified extraction procedures and much cleaner extracts.

Figure 2 provides a comparison of chromatograms obtained using MIP extraction versus conventional mixed-phase SPE extraction. It is easy to see the reduced background and the resulting improvement in sensitivity of the analysis.

Other advantages include simplified extraction procedure; reduced sample preparation time; reduced labour cost and solvent use; reduced sample size requirement; consistently high recoveries of target compound(s); and cleaner extracts, which enable improved sensitivity, minimized ion-suppression with LC/MS and LC/MS/MS techniques, and potential use of HPLC rather than LC/MS or LC/MS/MS.

MIPs can be used for numerous analytes or analyte classes in a variety of sample types. Several MIPs have been produced and prepared as SPE columns on a



commercial scale by MIP Technologies in Sweden.

In addition to analytical-scale sample clean-up, MIPs have been used successfully for HPLC columns, chiral enantiomer separations, scale-up and industrial process-scale purification. Due to the high degree of selectivity, MIPs can improve significantly the productivity of industrial-scale purifications compared with conventional resins. They have shown some utility for purification of peptides, although research is ongoing¹. Customized MIP sorbents can be developed easily for any small molecule for the most demanding separation applications.

Some specific examples of applications, and advantages, include:

Simplified extraction, time/labour saving and improved sensitivity:

A tobacco-specific nitrosamine metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), is a potential carcinogen and a useful biomarker of exposure to carcinogenic tobacco nitrosamines. NNAL may be measured at very low levels in the urine of non-smokers exposed to second-hand smoke. A MIP specific for NNAL was used by the US Centers for Disease Control to develop a simplified sample preparation method for LC/MS/MS analysis². Using the MIP-based extraction method reduced the sample preparation from over 20 steps and a two-day process to a much simplified and faster process taking less than one hour. In addition to the tremendous time- and labour-saving benefits, the MIP method enabled a reduction of sample size from 100 mL to 10 mL while still delivering improved sensitivity from ppb levels to low-ppt levels.

Suitability for an entire class of compounds:

Beta-agonist drugs have been used for their growth promotion activity in cattle and other farm animals. Regulatory control programs are active in Canada to detect the presence of illegal veterinary

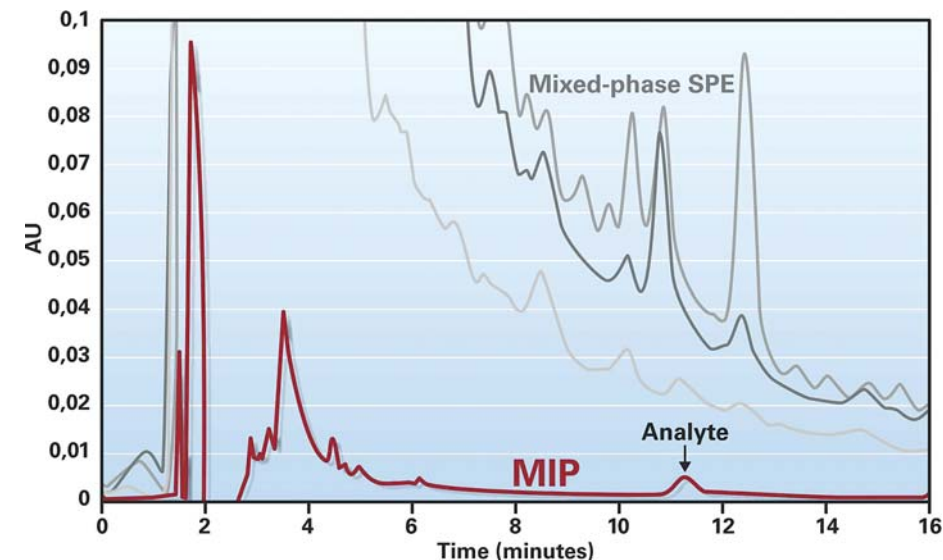


Figure 2 — Comparison of HPLC chromatogram of Clenbuterol extraction from a 5 mL urine sample using MIP and conventional mixed-phase SPE column

drug residues. The class-specific MIP for beta-agonists has been used successfully for the quantification of low levels of up to 15 beta-agonist drugs in calf urine. Since MIP extractions are extremely selective, they reduce significantly the background due to the sample matrix. Consequently, this technique has been shown to reduce the incidence of ion-suppression traditionally observed in LC/MS/MS analyses, thereby reducing the incidence of false negatives³. Also, since matrix components in sample extracts are significantly reduced, methods are easier to use for multiple matrices and multiple species. The beta-agonist MIP has been used successfully for liver samples (bovine, porcine, and chicken)⁴, muscle tissue (bovine⁵, fish⁴), urine (bovine)⁴, and hair (bovine)⁶.

Efficient extraction of a class of drugs from environmental samples:

Beta-blocker drugs are widely described for indications such as angina, hypertension, arrhythmia and other cardiac conditions. After being consumed by patients, these drugs are excreted in the urine, thereby entering wastewater treat-

ment plants (WWTP). These compounds are incompletely removed by traditional WWTPs and, therefore, have been detected in environmental waters at low concentrations (parts per trillion). MIPs specific for a class of beta-blocker drugs have been used in conjunction with LC/MS/MS analysis to quantify successfully low-ppt levels of these drugs from only 100 mL of water⁷.

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Footnotes:

1. see www.miptechnologies.com
2. Y Xia et al, *Anal Chem*, 77, 7639-7645 (2005).
3. N Van Hoof et al, *Rapid Comm Mass Spec*, 19, 2801-2808 (2005)
4. S Söderlund et al, National Food Administration, Uppsala, Sweden (poster presentation)
5. P Kootstra et al, *Anal Chim Acta*, 529, 75-81 (2005)
6. K Wubs et al, Laboratory for Food and Residue Analyses, Bilthoven, the Netherlands (poster presentation)
7. T Pizzolato and M Gros et al, Instituto de Quimica, Porto Alegre, Brasil and Dept of Environmental Chemistry, Barcelona, Spain (poster presentation — see pdf on www.miptechnologies.com)