REVIEW ARTICLES

Molecularly imprinted polymers

Polyméry s molekulovými odtlačkami

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Summary

Nowadays about 28 million different simple or complex chemical entities are known. Today we cannot imagine the life without different kinds of drugs, cosmetics, pesticides, food additives or stimulants. In the recent period in the field of analytical chemistry or pharmacy more modern techniques or methodologies are required which will allow selective determination of different kinds of analytes, especially in complex biological matrices. The imprinted polymers are very often used for the preparation of samples before analysis and this procedure can reduce the possibilities of interferences. This paper deals with the characterization, preparation, properties and application of imprinted polymers in the field of drugs, cosmetics, food and biological materials.
Keywords: molecularly imprinted polymers • chromatography • extraction • drugs

Súhrn

V súčasnosti je známych približne 28 miliónov rôznych jednoduchých alebo komplikovaných zlúčenín. Dnes si ťažko môžeme predstaviť život bez rôznorodých druhov liečiv, kozmetických prípravkov, pesticídov, prísad do potravín, alebo povzbudzujúcich prostriedkov. V poslednom čase v oblasti analytickej chémii, alebo farmácie stále viac sa vyžaduje použiť moderné techniky alebo nové metodológie, ktoré umožňujú selektívne stanovenie rôznych analytov, zvlášť v zložitých biologických vzorkách. Stále častejšie na prípravu vzoriek k analýze sa využívajú polyméry s molekulovými odtlačkami, pomocou ktorých možno značne zabrániť interferenciám pri stanovení stopových koncentrácií analytov. Tato práca sa zaoberá charakterizáciou, prípravou, vlastnosťami a aplikáciou polymérov s molekulovými odtlačkami v oblasti analýzy vzoriek liečiv, kozmetických prípravkov, potravín a biologických materiálov.
Klíčová slova: polyméry s molekulovými odtlačkami • hromatografia • extrakcia • liečiva

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Introduction

The most frequently used methods for sample preparation are liquid-liquid extraction (LLE) or solid-phase extraction (SPE). Due to a large waste of solvents, the LLE method is not employed today so often as its good alternative, the SPE method. The SPE is used in food, environmental, clinical or pharmaceutical chemistry to preconcentrate, cleanup complex matrices, analyte storage with high volatility or those not stable in a liquid medium or to carry out derivatization reactions between the reactive groups on the sorbent surface and those in the analyte[1–3].

The most common coupling of the SPE method is with high performance liquid chromatography (HPLC), capillary electrophoresis (CE) or gas chromatography (GC) where the solid phases are often based on silica or bonded silica. The SPE method is simple and fast, it does not require a high quantity of a solvent, it is cheap, effective and recoverable, and it can be automated in a simple way[4].

However, despite the many qualities, this method has still limitations which play an important role in the extraction mechanisms. First of all the SPE is not so selective recording to requirements in modern biological or environmental laboratories. Mostly, the problem is in incomplete end-capping processes or with the presence of interfering groups in complicated matrices which have similar or the same sorption mechanisms as the analyte of interest[5, 6].

For this reason the SPE has undergone changes, which have allowed a wider application in a sample preparation.

First, there was an application of the molecularly selective immunosorbents. Immunosorbents make use of special molecular recognition between antibodies and antigens. In spite of a big selectivity to the target molecules, they are very expensive to prepare, time-consuming, fragile and less stable. Moreover, antibodies are easily denatured in the presence of organic solvents and consequently difficult to isolate[7–10].

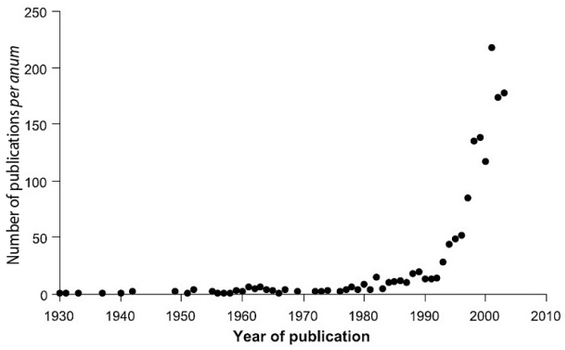

Fig. 1. The number of publications within the field of molecular imprinting science and technology per annum for the period 1931–2003[14]

All these obstructions can be omitted by using molecularly imprinted polymers (MIPs). The MIPs are synthetic highly cross-linked polymers prepared in the presence of the target molecule, named the template[11, 12]. In the presence of this specific analyte special cavities are formed, tailor-made by copolymerization of functional and cross-linking monomers. After polymerization, the print molecule is removed leaving three-dimensional binding sites. Consequently, the resultant polymer possesses the abilities to recognize any molecule or groups of molecules on which it was designed[13].

Over many years, since Polyakov [14] in the 1930s published his first report about molecular imprinting, the interest for the MIPs was insignificant. The true growth started at the beginning of the 1990s and has been rapidly increasing since. In spite of their great similarity to the biological systems, they are still inexpensive and simple to prepare.

Schematically, the growing interest in the MIPs is shown in Figure 1.

Characterization of MIPs

It should be noted that simultaneously with MIP preparation, a non-imprinted polymer (NIP, a polymer without the presence of the template) is always prepared to compare nonspecific interactions of the target molecules with the non-imprinted cavities.

The MIPs, as very selective and sensitive materials, are perfect tools for pre-concentration or extraction of an analyte of interest, mainly in environmental or biological laboratories and even in cosmology[15, 16].

Izenberg et al.[16] suggested employing of the MIPs in astrobiology missions as an excellent device for biological samples detection in multi-sensor microlaboratories.

However, another and more ordinary application of MIPs can include stationary phases and sorbents for HPLC, thin layer chromatography (TLC) or capillary electro-chromatography (CEC) in analytical chemistry, catalysts, biomimetic sensors, binding assays, reusable protecting groups or polymer-supported reagents in organic synthesis[17–19].

Molecular imprinting techniques

Generally MIPs can be synthesized by three different procedures[10, 20, 21]:

A. *The non-covalent imprinting* is the most commonly used procedure for the MIPs synthesis. This technique exploits *in situ* forming of the template and functional monomer complex by nonspecific interactions such as hydrogen bonding, van der Waals or electrostatic forces, ionic or hydrophobic interactions. The main advantage of this method is its simplicity, low costs of preparation, fast binding of the template, easy removal of the template from the polymer by Soxhlet extraction, for instance, and its potential application to a wide range of target molecules. However, the polymerization conditions have to be carefully chosen to minimize the nonspecific binding sites.

B. *The covalent imprinting* depends on covalent-linkage of the template with the functional monomer prior to the polymerization process. The template removal takes place in the way of a chemical reaction. The binding of the target molecules is proceeding in the same way and via the same covalent interactions as with the template. The main advantage of this approach is that a wider spectrum of polymerization conditions can be used and the template/monomer complex is stable and stoichiometric. In comparison with the non-covalent approach this method is much more exacting, more extensive, the template binding and its releasing is slower and due to the polymerization conditions there are some limits for the use of different molecules as a template.

C. *The semi-covalent imprinting* is the hybridization of both approaches, covalent and non-covalent. The polymerization process takes place on a covalent way but the following template binding is non-covalent. The combination of these two methods provides advantages from both of these. Firstly, during of the quick binding process of the template the production of stable and stoichiometric complex takes place and secondly, the target molecules are fast binding in a non-covalent way.

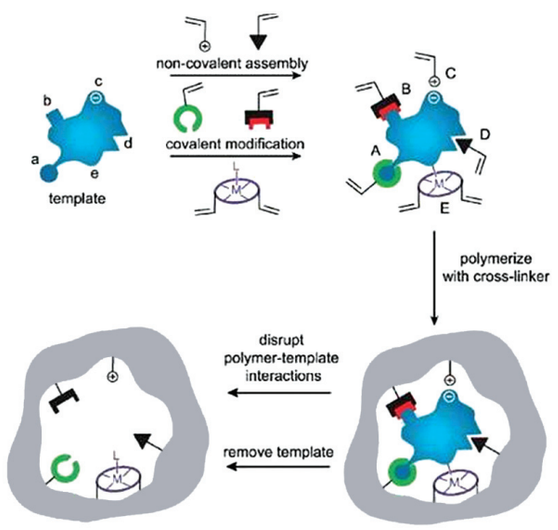

Fig. 2. The molecular imprinting process[14]

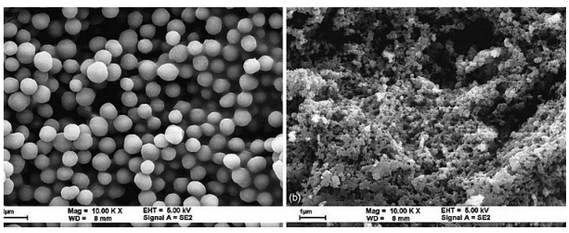

Fig. 3. The morphology of MIP particles prepared by (a) precipitation polymerization, (b) traditional bulk polymerization[26)]

The scheme of the imprinting process is shown in Figure 2.

Production methodologies of MIPs

Presently the MIPs are prepared by the six following techniques:

A. *Bulk polymerization* is the most widely used procedure for the MIPs preparation[22–24)]. This method does not require any complicated devices or particular skills. However, in spite of its simplicity and universality, this method is time-consuming, wasteful, needs the crushing, grounding and sieving processes, which affects the large loss of the product. Moreover, the particles produced by this method have low capacity, are irregular in shape and size, which causes peaks tailing and broadening because of theirs heterogeneity.

B. *Precipitation polymerization* is a modification of bulk polymerization and employs the largest amount of porogen, usually more than 95% (w/v). From this reason, precipitation polymerization disables particles aggregation and provides the microspheres with diameters in scales ranging from 0.3 to 10 μm, suitable for chromatographic applications. Except the limitations typical of bulk polymerization, this approach uses the largest volume of porogen and the MIP particles have the binding sites which are inside their networks, causing a slow mass transfer of target molecules[14, 25)]. The morphology of MIPs beads prepared by bulk polymerization and precipitation polymerization is shown in Figure 3.

C. *Suspension polymerization* permits to obtain spherical beads in a fast and simple way and is mainly used for chromatography and electrochromatography-grade imprinted materials. This methodology has been carried out in liquid organic solvents or in water as the continuous phase and provides highly reproducible results. Suspension polymerization has proceeded in the UV irradiation only by less than 2 h. The particles diameter achieved by this method depends on the amount of the surfactant and the stirring speed. The presence of the surfactant in the mixture can cause some problems because of its interfering with the template-monomer interactions. Moreover, when water as a suspension phase is used, the non-covalent approach is not possible[22, 27, 29)].

D. *Two-steps swelling polymerization* method has used water as the suspension medium. This technique offers monodisperse beads ranging from 2 to 50 μm, ideal for HPLC use. These particles possess good separating abilities and yielded better column efficiencies and peak shapes than the particles achieved by bulk polymerization. Main limitations of this method are complications due to the reaction conditions and procedures[21, 28, 30, 31)].

E. *Surface imprinting polymerization* is the method where the MIP layers are grafting onto the surface of preformed beads. The surface imprinting polymerization takes place in the presence of common substrates, used in other types of polymerization and in the presence of an emulsion stabilizer with a polymer matrix-forming comonomer. This method has found an application in separation, medical uses or sensing. The methodology is easy, yields monodisperse, thin imprinted layers but needs a complicated system and is time-consuming[21, 32)].

F. *In situ polymerization* is achieved by a direct one-step polymerization of a polymer mixture in stainless steel columns. After the polymerization, the template and porogen are washed out by a methanol-acetic acid mixture. The monolithic-MIP preparation is simple and the results demonstrate high selectivity, sensitivity, reproducibility and fast mass transport. The time consuming character of the preparation of each new template system is the main limitation of this approach[21)].

MIPs preparation

Briefly, the procedure for the MIP synthesis is as follows. The template molecule with a functional monomer, cross-linking monomer, porogenic solvent (porogen) and an initiator have to be mixed. In order to initiate polymerization, the mixture is heated or irradiated with UV light. During the polymerization process a stable complex develops between the functional groups of the template and the functional monomer. In order to maximize interactions, the functional monomer has to possess complementary groups with the template as much as is possible. Finally the product in the form of a rigid and highly cross-linked polymer is crushed, sieved and submitted to an extraction process in order to remove the template. After that, the three-dimensional cavities can recognize any target molecules with a complementary shape and chemical properties to those of the template.

The most common functional monomers described in the literature are: methacrylic acid (MAA)[17, 33–42)], acrylamide (AA)[35, 43–46)], 2-vinylpyridine (2-VP)[38–40)], 4-vinylpyridine (4-VP)[17, 35, 43, 44, 47)], trifluoromethacrylic acid (TFMAA)[14, 31, 41)], styrene[17)] and methacrylamide (MAAM)[48, 49)].

Another essential element of MIP polymerization is a cross-linker which has three major functions: firstly, it controls the morphology of the polymer matrix. Secondly, it is necessary to stabilize the imprinted binding sites, so the amount of the cross-linker should be sufficient to keep the stability of them. Finally, its presence provides the stability of the polymer matrix. Usually, an 80% excess of a cross-linker is used in the polymerization mixture.

The commonly used cross-linking monomers are: ethylene glycol dimethacrylate (EGDMA)[17, 31, 33–35, 37, 40–43)], trimethylolpropane trimethacrylate (TRIM)[42, 50, 51)],

N,N'-1,3-phenylene bismethacrylamide (PBMA)[52] and bisacrylamide (BAAM)[52].

The role of a porogen is to dissolve all components present in a polymerization mixture and to make it possible to produce large pores in order to allow an access to the binding sites, which directly influences the imprinted polymer performance. The nature and level of porogenic solvents determines the strength of the non-covalent interactions. The porogens should have relatively low polarity, in order to reduce the interferences during the template-monomer complex formation.

The most widely used organic porogens are: methanol, acetonitrile, toluene, dodecanol, dichloromethane or chloroform. All of them enhance ionic interactions between the template and the functional monomer. Also water can be used as a porogen, which can support the formation of hydrophobic interactions[14, 21].

Advantages and drawbacks of MIPs

Molecularly imprinted polymers in comparison with biomolecules (receptors, enzymes) have many advantages but also a few properties which need to be improved or completely eliminated. Receiving the best polymer may take a few years and hundreds bad sorbents but they are still the most universal materials in modern laboratories.

The advantages of MIPs include[8, 19, 33, 52–57]:

- Low cost and simple preparation;
- High selectivity;
- Possibility of use in aggressive media (concentrated bases or acids, organic solvents);
- Mechanical strength;
- Durability to heat and pressure;
- Possibility to repeat analyses without loss of their activities;
- Potential applications for a wide range of target molecules.

The disadvantages of MIPs can be enumerated as follows[58, 59]:

- The imprinted polymers are insoluble;
- It is difficult to completely remove the template from the polymer;
- Cavities which are non-imprinted are always present in the polymer;
- There is no ideal effective procedure for the design of MIPs.

Application of MIPs

Many papers describe an application of MIPs as sorbents in SPE, SPME or TLC, stationary phases in LC, the chiral selective matrix in CE and CEC, membranes, drug delivery systems, sensors, in immunoassays or catalysis[7, 20, 60–62].

The MIPs have found a wide application in analyses of different kinds of samples. The most common investigative matrices are:

• *Food samples*: caffeine in beverages and coffee[63], simultaneous determination of caffeine and theophylline in green tea and human plasma[36], quercetin in red wine[64], sulfamethazine in milk[65], triazines in food samples[60, 66], clenbuterol in animal feeds[67, 68], tetracycline antibiotics in egg sample[23] or nerve agent degradation products in rice samples[12].

• *Environmental samples*: triazines and their metabolites[69], anti-inflammatory drugs[70], catechol[71] in river water, benzo(a)pyrene in tap water, lake water or instant coffee samples[72], ß-blockers[73], bisphenol A[51, 74–76], ciprofloxacin[77], triazines[78] and naphthalene mono- and disulfonates[79] in water samples, 4-chlorophenols and 4-nitrophenol in river water[80], nerve agent degradation products in aqueous soil extracts[81], organophosphorus pesticides in water and soil[82] or sulfonylurea in water and soil samples[83].

• *Drugs and biological samples*: caffeine in human urine[63], ß-agonists in the porcine[67] and bovine muscle[82, 85] and the liver[67], calves urine[86], and biological materials[68] or in the pork liver[87], propranolol[88], albuterol[89], sulpiride and atenolol[90], Cd(II)[91], Fe(III)[92], trimethoprim[93], phenytoin[94], ropivacaine, mepivacaine and bupivaciaine[95] and derivatives of phenylcarbamic acid[96] in human plasma, methotrexate[97], clenbuterol in calves urine[98], theophylline[99] and degradation products of nerve agents[11] in human serum, tramadol in human plasma[100, 101] and urine samples[100], cotinine[101] and naproxen[103] from urine samples, morphine[104], quercetin from rats plasma[105], verapamil and its metabolites in urine, plasma and cell culture[106], 17ß-estradiol[45], pentamidine[107], L-theanine from plant material[108], mycophenolic acid in maize[109], phenylcarbamic acid derivatives in rat serum and human plasma[110, 111], diphenyl phosphate[112] and tamoxifen[113] in human urine, scopolamine in urine and serum samples[113], atropine and scopolamine in pharmaceutical preparations containing *Scopolia* extract[115], estrogens in fishery samples[116], phenobarbital in human urine and medicines[117], nateglinide and its enantiomer[118], ciprofloxacin and enrofloxacin in urine and tissues samples[119], cholesterol[24], tetracycline antibiotics in pig kidney tissue extract[120], ceramides in yeast lipid extracts[121], (–)-ephedrine in herbal ephendra[122], anti-EGFR inhibitors in extract and whole *Caragana jubata* plant[123], chloramphenicol in ophthalmic solutions and spiked milk[124], (S)-nicotine in cigarette smoke extract[125].

Conclusion

Presently molecularly imprinted polymers are the most promising and popular research objects in chemistry. They are used as sorbents in SPE and SPME, as stationary phases in HPLC or CEC, as sensors, catalysts, binding assays, reusable protecting groups or polymer-supported reagents in organic synthesis. Due to their properties they are excellent materials for sample pre-concentration, cleaning or extraction, especially for complex matrices.

Among all available sorbents, they are distinguished by their durability on harsh media, heat, pressure or mechanical strength. They are highly selective for different target molecules, nature friendly because of a high level of regeneration, and the big advantage is also

low costs of MIPs preparation. Working with MIPs, in most cases, does not require complicated instruments or special skills of the operator.

The MIPs, depending on the necessity or laboratory equipment, can be prepared by different ways: by the most popular bulk polymerization, suspension polymerization, precipitation polymerization, two-steps swelling polymerization, *in situ* polymerization or by surface imprinting polymerization.

Conflict of interest: none.

References

1. **Poole C. F.:** New trends in solid-phase extraction. Trends Anal. Chem. 2003; 22, 362–373.
2. **Altun Z., Abdel-Rehim M., Blomberg L. G.:** New trends in sample preparation: on-line microextraction in packed syringe (MEPS) for LC and GC applications Part III: Determination and validation of local anaesthetics in human plasma samples using a cation-exchange sorbent and MEPS-LC-MS-MS. J. Chromatogr. B 2004; 813, 129–135.
3. **Huck C. W., Bonn G. K.:** Recent developments in polymer-based sorbents for solid-phase extraction, Review. J. Chromatogr. A 2000; 885; 51–72.
4. **Bielicka-Daszkiewicz K., Voelkel A.:** Theoretical and experimental methods of determination of the breakthrough volume of SPE sorbents. Talanta 2009; 80, 614–621.
5. **Stevenson D.:** Molecular imprinted polymers for solid-phase extraction. Trends Anal. Chem. 1999; 18, 154–158.
6. **Picó Y., Fernández M., Ruiz M. J., Font G.:** Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment, Review. J. Biochem. Bioph. Methods 2007; 70, 117–131.
7. **Xu Z. X., Gao H. J., Zhang L. M., Chen X. Q., Qiao X. G.:** The Biomimetic Immunoassay Based on Molecularly Imprinted Polymer: A Comprehensive Review of Recent Progress and Future Prospects. J. Food Sci. 2011; 76, R69–R75.
8. **Bui B. T. S., Haupt K.:** Molecularly imprinted polymers: synthetic receptors in bioanalysis, Review. Anal. Bioanal. Chem. 2010; 398, 2481–2492.
9. **Shi Y., Zhang J., Shi D., Jiang M., Zhu Y., Mei S., Zhou Y., Dai K., Lu B.:** Selective solid-phase extraction of cholesterol using molecularly imprinted polymers and its application in different biological samples. J. Pharm. Biomed. Anal. 2006; 42, 549–555.
10. **He Ch., Long Y., Pan J., Li K., Liu F.:** Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples, Review. J. Biochem. Bioph. Methods 2007; 570, 133–150.
11. **Valtchev M., Palm B.S., Schiller M., Steinfeld U.:** Development of sulfamethoxazole-imprinted polymers for the selective extraction from waters. J. Hazard. Mater. 2009; 170, 722–728.
12. **Zi-Hui M., Qin L.:** Determination of degradation products of nerve agents in human serum by solid-phase extraction using molecularly imprinted polymer. Anal. Chim. Acta 2001; 435, 121–127.
13. **Tom L. A., Foster N.:** Development of a molecularly imprinted polymer for the analysis of avermectin. Anal. Chim. Acta 2010; 680, 79–85.
14. **Alexander C., Andersson H. S., Andersson L. I., Ansell R. J., Kirsch N., Nicholls I. A., O'Mahony J., Whitcombe M. J.:** Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003, Review. J. Mol. Recognit. 2006; 19, 106–180.
15. **Theodoridis G., Manesiotis P.:** Selective solid-phase extraction sorbent for caffeine made by molecular imprinting. J. Chromatogr. A 2002, 948, 163–169.
16. **Izenberg N. R., Murrray G. M., Pilato R. S., Baird L. M., Levin S. M., Van Houten K. A.:** Astrobiological molecularly imprinted polymer sensors. Planet. Space Sci. 2009; 57, 846–853.
17. **Luliński P.:** Polimery ze śladem molekularnym w naukach farmaceutycznych. Cz. I. Podstawy procesu tworzenia śladu molekularnego. Zastosowanie w syntezie leków i technologii postaci leku. Polimery 2010; 55, 799–805.
18. **Whitcombe M. J., Vulfson E. N.:** Imprinted Polymers. Adv. Mater. 2001; 13, 467–468.
19. **Ansell R. J., Kriz D., Mosbach K.:** Molecularly imprinted polymers for bioanalysis: chromatography, binding assays and biomimetic sensors. Curr. Opin. Biotechnol. 1996; 7, 89–94.
20. **Turiel E., Martin-Esteban A.:** Molecularly imprinted polymers for sample preparation: A review, Review. Anal. Chim. Acta 2010; 668, 87–99.
21. **Yan H., Row K. H.:** Characteristic and synthetic approach of molecularly imprinted polymer. Int. J. Mol. Sci. 2006; 7, 155–178.
22. **Pan J., Xue X., Wang J., Xie H., Wu Z.:** Recognition property and preparation of Staphylococcus aureus protein A-imprinted polyacrylamide polymers by inverse-phase suspension and bulk polymerization. Polymer 2009; 50, 2365–2372.
23. **Jing T., Gao X. D., Wang P., Wang Y., Lin Y. F., Zong X. Ch., Zhou Y. K., Mei S. R.:** Preparation of high selective molecularly imprinted polymers for tetracycline by precipitation polymerization. Chin. Chem. Lett. 2007; 18, 1535–1538.
24. **Kitahara K., Yoshihama I., Hanada T., Kokuba H., Arai S.:** Synthesis of monodispersed molecularly imprinted polymer particles for high-performance liquid chromatographic separation of cholesterol using templating polymerization in porous silica gel bound with cholesterol molecules on its surface. J. Chromatogr. A 2010; 1217, 7249–7254.
25. **Turiel E., Tadeo J. L., Cormack P. A. G., Martin-Esteban A.:** HPLC imprinted-stationary phase prepared by precipitation polymerization for the determination of thiabendazole in fruit. The Analyst 2005; 130, 1601–1607.
26. **Hoa K., Yeh W., Tung T., Liao J.:** Amperometric detection of morphine based on poly(3,4-ethylenedioxythiophene) immobilized molecularly imprinted polymer particles prepared by precipitation polymerization. Anal. Chim. Acta 2005; 542, 90–96.
27. **Tamayo F. G., Casillas J. L., Martin-Esteban A.:** Highly selective fenuron-imprinted polymer with a homogeneous binding site distribution prepared by precipitation polymerization and its application to the clean-up of fenuron in plant samples. Anal. Chim. Acta 2003; 482, 165–173.
28. **Pérez-Moral N., Mayes A. G.:** Comparative study of imprinted polymer particles prepared by different polymerisation methods. Anal. Chim. Acta 2004; 504, 15–21.
29. **Jin Y., Jiang M., Shi Y., Lin Y., Peng Y., Dai K., Lu B.:** Narrowly dispersed molecularly imprinted microspheres prepared by a modified precipitation polymerization method. Anal. Chim. Acta 2008; 612, 105–113.
30. **Hosoya K., Yoshizako K., Shirasu Y., Kimata K., Araki T., Tanaka N., Haginaka J.:** Molecularly imprinted uniform-size polymer-based stationary phase for high-performance liquid chromatography. Structural contribution of cross-linked polymer network on specific molecular recognition. J. Chromatogr. A 1996; 728, 139–147.
31. **Haginaka, J., Sanbe, H.:** Uniform-sized molecularly imprinted polymers for 2-arylpropionic acid derivatives selectively modified with hydrophilic external layer and their applications to direct serum injection analysis. Anal. Chem. 2000; 72, 5206–5210.
32. **Liua Y., Liub Z., Gaoa J., Daia J., Hana J., Wanga Y., Xiea J., Yan Y.:** Selective adsorption behavior of Pb(II) by mesoporous silica SBA-15-upported Pb(II)-imprinted polymer based on surface molecularly imprinting technique. J. Hazard. Mater. 2011; 186, 197–205.
33. **Djozan D., Ebrahimi B., Mahkam M., Farajzadeh M. A.:** Evaluation of a new method for chemical coating of aluminum wire with molecularly imprinted polymer layer. Application for the fabrication of triazines selective solid-phase microextraction fiber. Anal. Chim. Acta 2010; 674, 40–48.
34. **Čižmárik J., Lachová M., Lehotay J., Skačáni I.:** Extraction of 1-methyl-2-piperidinoethylesters of alkoxyphenylcarbamic acids from human plasma by imprinted polymers and its comparison with classic solid phase extraction. Čes. slov. Farm. 2009; 58, 78–82.
35. **Lachová M., Lehotay J., Skačáni I., Čižmárik J.:** Influence of polymerization mixture composition on molecularly imprinted polymers properties prepared with 1-methyl-2-

-piperidinoethylester of decyloxyphenylcarbamic acid as a template. Modern Analytical Chemistry. Prague 2008; 49–52.
36. **Jafari M.T., Rezaei B., Javaheri M.:** A new method based on electrospray ionisation ion mobility spectrometry (ESI-IMS) for simultaneous determination of caffeine and theophylline, Food Chem. 2011; 126, 1964–1970.
37. **Shih-Kai Ch., Mei-Jywan S.:** Via zinc(II) protoporphyrin to the synthesis of poly(ZnPP-MAA-EGDMA) for the imprinting and selective binding of bilirubin. Biomaterials 2009; 30, 1255–1262.
38. **Meng Z., Wang J., Zhou L., Wang Q., Zhu D.:** High performance cocktail functional monomer for making molecule imprinting polymer. Anal. Sci. 1999; 15, 141–144.
39. **Yoshimi Y., Arai R., Nakayama S.:** Influence of the solvent on nature of gate effect in molecularly imprinted membrane. Anal. Chim. Acta 2010; 682, 110–116.
40. **Moreira F. T. C., Kamel A. H., Guerreiro R. L., Azevedo V., Sales M. G. F.:** New potentiometric sensors based on two competitive recognition sites for determining tetracycline residues using flow-through system. Procedia Engineering 2010; 5, 1200–1203.
41. **Gavrilovic I., Mitchell K., Brailsford A. D., Cowan D. A., Kicman A.T., Ansell R. J.:** A molecularly imprinted receptor for separation of testosterone and epitestosterone, based on a steroidal cross-linker, Steroids 2011; 76, 478–483.
42. **Joshi V. P., Kulkarni M. G., Mashelkar R. A.:** Enhancing adsorptive separations by molecularly imprinted polymers: Role of imprinting techniques and system parameters. Chem. Eng. Sci. 2000; 55, 1509–1522.
43. **Zhiong Ch., Yuanzong L.:** The role of molecular recognition in regulating the catalytic activity of peroxidase-like polymers imprinted by a reductant substrate. J. Mol. Catal. A: Chem. 2006; 256, 9–15.
44. **Lachová M., Lehotay J., Skačáni I., Čižmárik J.:** Possibility of using of molecular imprinted polymers on sorption selectivity of some phenyl carbamic derivatives. Farm. Obzor 2008; 77, 37–40.
45. **Saifuddin, N., Nur, Y. A. A., Abdullah, S. F.:** Microwave enhanced synthesis of chitosan-graft-polyacrylamide molecular imprinting polymer for selective removal of 17β-estradiol at trace concentration. Asian J. Biochem. 2011; 6, 38-54.
46. **Zhou J., He X., Li Y.:** An acrylamide-based molecularly imprinted polymer for the efficient recognition of optical amino acid hydantoins. Anal. Commun. 1999; 36, 243–246.
47. **Thazhathuparambil E. M., Krishnapillai P. P., Talasila P. R.:** Synthesis of surface imprinted nanospheres for selective removal of uranium from simulants of Sambhar salt lake and ground water. J. Hazard. Mater. 2011; 188, 384–390.
48. **Luliński P., Maciejewska D.:** Impact of functional monomers, cross-linkers and porogens on morphology and recognition properties of 2-(3,4-dimethoxyphenyl)ethylamine imprinted polymers. Mater. Sci. Eng., C 2011; 31, 281–289.
49. **Lämmerhofer M., Gargano A.:** Monoliths with chiral surface functionalization for enantioselective capillary electrochromatography. J. Pharm. Biomed. Anal. 2010; 53, 1091–1123.
50. **Luo X., Zhan Y., Huang Y., Yang L., Tu X., Luo S.:** Removal of water-soluble acid dyes from water environment using a novel magnetic molecularly imprinted polymer. J. Hazard. Mater. 2011; 187, 274–282.
51. **Surong M., Da W., Ming J., Bin L., Jae-Min L., Yi-Kai Z., Yong-Ill L.:** Determination of trace bisphenol A in complex samples using selective molecularly imprinted solid-phase extraction coupled with capillary electrophoresis. Microchem. J. 2011; 98, 150–155.
52. **Moring S. E., Wong O. S., Stobaugh J. F.:** Target specific sample preparation from aqueous extracts with molecular imprinted polymers. J. Pharm. Biomed. Anal. 2002; 27, 719–728.
53. **Augusto de Barros L., Martins I., Rath S.:** A selective molecularly imprinted polymer-solid phase extraction for the determination of fenitrothion in tomatoes. Anal. Bioanal.Chem. 2010; 397, 1355–1361.
54. **Zhang T., Liu F., Chen W., Wang J., Li K.:** Influence of intramolecular hydrogen bond of templates on molecular recognition of molecularly imprinted polymers. Anal. Chim. Acta 2001; 450, 53–61.
55. **Guan G., Liu B., Wang Z., Zhang Z.:** Imprinting of molecular recognition Sites on nanostructures and its applications in chemosensors. Review, Sensors 2008; 8, 8291–8320.
56. **Xu X., Zhu L., Chen L.:** Separation and screening of compounds of biological origin using molecularly imprinted polymers. J. Chromatogr. B 2004; 804, 61–69.
57. **Soares da Silva M., Väo E. R., Temtem M., Mafra L., Caldeira J., Aguiar-Ricardo A., Casimiro T.:** Clean synthesis of molecular recognition polymeric materials with chiral sensing capability using supercritical fluid technology. Application as HPLC stationary phases. Biosens. Bioelectron. 2010; 25, 1742–1747.
58. **Lachová M., Lehotay J., Čižmárik J.:** Applications of molecularly imprinted polymers in analytical and pharmaceutical chemistry. Čes. slov. Farm. 2007; 56, 159–164.
59. **Baggiani C., Anfossi L., Giovannoli C.:** Solid phase extraction of food contaminants using molecular imprinted polymers. Anal. Chim. Acta 2007; 591, 29–39.
60. **Turiel E., Martin-Esteban A.:** Molecularly imprinted polymers for solid-phase microextraction, Review. J. Sep. Sci. 2009; 32, 3278–3284.
61. **Bunte G., Hurttlen J., Pontius H., Hartlieb K., Krause H.:** Gas phase detection of explosives such as 2,4,6-trinitrotoluene by molecularly imprinted polymers. Anal. Chim. Acta 2007; 591, 49–56.
62. **Kirsch N., Hedin-Dahlström J., Henschel H., Whitcombe M. J., Wikman S., Nicholls I. A.:** Molecularly imprinted polymer catalysis of a Diels-Alder reaction. J. Mol. Catal. B: Enzym. 2009; 58, 110–117.
63. **Theodoridis G., Zacharis C. K., Tzanavaras P. D., Themelis D.G., Economou A.:** Automated sample preparation based on the sequential injection principle. Solid-phase extraction on a molecularly imprinted polymer coupled on-line to high-performance liquid chromatography. J. Chromatogr. A 2004; 1030, 69–76.
64. **Molinelli A., Weiss R., Mizaikoff B.:** Advanced solid phase extraction using molecularly imprinted polymers for the determination of quercetin in red wine. J. Agric. Food. Chem., 2002; 50, 1804–1808.
65. **Su S., Zhang M., Li B., Zhang H., Dong X.:** HPLC determination of sulfamethazine in milk using surface-imprinted silica synthesized with iniferter technique. Talanta 2008; 76, 1141–1146.
66. **Mhaka B., Cukrowska E., Bui B. T. S., Ramström O., Haupt K., Tutu H., Chimuka L.:** Selective extraction of triazine herbicides from food samples based on a combination of a liquid membrane and molecularly imprinted polymers. J. Chromatogr. A 2009; 1216, 6796–6801.
67. **Xu Z., Hu Y., Hu Y., Li G.:** Investigation of ractopamine molecularly imprinted stir bar sorptive extraction and its application for trace analysis of β-agonists in complex samples. J. Chromatogr. A 2010; 1217, 3612–3618.
68. **Brambilla G., Fiori M., Rizzo B., Crescenzi V., Masci G.:** Use of molecularly imprinted polymers in the solid-phase extraction of clenbuterol from animal feeds and biological matrices,. J. Chromatogr. B 2001; 759, 27–32.
69. **Carabias-Martinez R., Rodriguez-Gonzalo E., Herrero-Hernandez E.:** Determination of triazines and dealkylated and hydroxylated metabolites in river water using a propazine-imprinted polymer. J. Chromatogr. A 2005; 1085, 199–206.
70. **Hoshina K., Horiyama S., Matsunaga H., Haginaka J.:** Simultaneous determination of non-steroidal anti-inflammatory drugs in river water samples by liquid chromatography-tandem mass spectrometry using molecularly imprinted polymers as a pretreatment column, J. Pharm. Biomed. Anal. 2011; 55, 916–922.
71. **Teixeira Tarley C. R., Kubota L. T.:** Molecularly-imprinted solid phase extraction of catechol from aqueous effluents for its selective determination by differential pulse voltammetry. Anal. Chim. Acta 2005; 548, 11–19.
72. **Lai J.-P., Niessner R., Knopp D.:** Benzo[a]pyrene imprinted polymers: Synthesis, characterization and SPE application in water and coffee samples. Anal. Chim. Acta 2004; 522, 137–144.
73. **Gros M., Pizzolato T., Petrovi M., Lopez de Alda M.J., Barcelo D.:** Trace level determination of β-blockers in waste waters by highly selective molecularly imprinted polymers extraction followed by liquid chromatography-quadrupole-linear ion trap mass spectrometry. J. Chromatogr. A 2008; 1189, 374–384.
74. **Jiang M., Zhang J. H., Mei S. R., Shi Y., Zou L. J., Zhu Y. X.:** Direct enrichment and high performance liquid chromatography analysis of ultra-trace bisphenol A in water samples with

narrowly dispersible bisphenol A imprinted polymeric microspheres column. J. Chromatogr. A 2006; 1110, 27–34.
75. **Watabe Y., Kondo T., Morita M., Tanaka N., Haginaka J., Hosoya K.:** Determination of bisphenol A in environmental water at ultra-low level by high-performance liquid chromatography with an effective on-line pretreatment device. J. Chromatogr. A 2004; 1032, 45–49.
76. **San Vicente B., Villoslada F. N., Moreno-Bondi M. C.:** Continuous solid-phase extraction and preconcentration of bisphenol A in aqueous samples using molecularly imprinted columns. Anal. Chim. Acta 2004; 380, 115–22.
77. **Qu S., Wang X., Tong Ch., Wu J.:** Metal ion mediated molecularly imprinted polymer for selective capturing antibiotics containing beta-diketone structure. J. Chromatogr. A 2010; 1217, 8205–8211.
78. **Bjarnason B., Chimuka L., Ramstrom O.:** On-line solid-phase extraction of triazine herbicides using a molecularly imprinted polymer for selective sample enrichment. Anal. Chem. 1999; 71, 2152–2156.
79. **Caro E., Marce R. M., Cormack P. A. G., Sherrington D. C., Borrull F.:** Molecularly imprinted solid-phase extraction of naphthalene sulfonates from water. J. Chromatogr. A 2004; 1047, 175–180.
80. **Caro E., Marce R. M., Cormack P. A. G., Sherrington D. C., Borrull F.:** On-line solid-phase extraction with molecularly imprinted polymers to selectively extract substituted 4-chlorophenols and 4-nitrophenol from water. Short communication. J. Chromatogr. A 2003; 995, 233–238.
81. **Le Moullec S., Begos A., Pichon V., Bellier B.:** Selective extraction of organophosphorus nerve agent degradation products by molecularly imprinted solid-phase extraction. J. Chromatogr. A 2006; 1108, 7–13.
82. **Zhu X., Yang J., Su Q., Cai J., Gao Y.:** Selective solid-phase extraction using molecularly imprinted polymer for the analysis of polar organophosphorus pesticides in water and soil samples. J. Chromatogr. A 2005; 1092, 161–169.
83. **Yang L., Zhao X., Zhou J.:** Selective enrichment and determination of nicosulfuron in water and soil by a stir bar based on molecularly imprinted polymer coatings. Anal. Chim. Acta 2010; 670, 72–77.
84. **Kootstra P. R., Kuijpers C. J. P. F., Wubs K. L., van Doorn D., Sterk S. S., van Ginkel L. A., Stephany R. W.:** The analysis of β-agonists in bovine muscle using molecular imprinted polymers with ion trap LCMS screening. Anal. Chim. Acta 2005; 529, 75–81.
85. **Crescenzi C., Bayoudh S., Cormack P. A. G., Klein T., Ensing K.:** Determination of clenbuterol in bovine liver by combining matrix solid phase dispersion and molecularly imprinted solid phase extraction followed by liquid chromatography/electrospray ion trap multiple stage mass spectrometry. Anal. Chem. 2001; 73, 2171–2177.
86. **Widstrand Ch., Larsson F., Fiori M., Civitareale C., Mirante S., Brambilla G.:** Evaluation of MISPE for the multi-residue extraction of β-agonists from calves urine. J. Chromatogr. B 2004; 804, 85–91.
87. **Hu Y., Li Y., Liu R., Tan W., Li G.:** Magnetic molecularly imprinted polymer beads prepared by microwave heating for selective enrichment of β-agonists in pork and pig liver samples. Talanta 2011; 84, 462–470.
88. **Martin P. D., Jones G. R., Stringer F., Wilson I. D.:** Comparison of extraction of a β-blocker from plasma onto a molecularly imprinted polymer with liquid-liquid extraction and solid phase extraction methods. J. Pharm. Biomed. Anal. 2004, 35, 1231–1239.
89. **Huanga H. Ch., Lin Ch. I., Joseph A. K., Lee Y. D.:** Photolithographically impregnated and molecularly imprinted polymer thin film for biosensor applications, J. Chromatogr. A 2004; 1027, 263–268.
90. **Zaidi S. A., Lee S. M., Cheong W. J.:** Open tubular capillary columns with basic templates made by the generalized preparation protocol in capillary electrochromatography chiral separation and template structural effects on chiral separation capability. J. Chromatogr. A 2011; 1218, 1291–1299.
91. **Andac M., Say R., Denizli A.:** Molecular recognition based cadmium removal from human plasma. J. Chromatogr. B 2004; 811, 119–126.
92. **Yavuz H., Say R., Denizli A;** Iron removal from human plasma based on molecular recognition using imprinted beads. Mater. Sci. Eng., C 2005; 25, 521–528.
93. **Hu S. G., Li L., He X. W.:** Comparison of trimethoprim molecularly imprinted polymers in bulk and in sphere as the sorbent for solid-phase extraction and extraction of trimethoprim from human urine and pharmaceutical tablet and their determination by high-performance liquid chromatography. Anal. Chim. Acta 2005; 537, 215–222.
94. **Bereczki A., Tolokan A., Horvai G., Horvath V., Lanza F., Hall A. J.:** Determination of phenytoin in plasma by molecularly imprinted solid-phase extraction. J. Chromatogr. A 2001; 930, 31–38.
95. **Andersson L. I., Hardenborg E., Sandberg-Ställ M., Möller K., Henriksson J., Bramsby-Sjöström I., Olsson L., Abdel-Rehim M.:** Development of a molecularly imprinted polymer based solid-phase extraction of local anaesthetics from human plasma. Anal. Chim. Acta 2004; 526, 147–154.
96. **Lachová M., Lehotay J., Skačáni I., Čižmárik J.:** Isolation of some derivatives of phenylcarbamic acid from human plasma using molecularly imprinted polymers. J. Liq. Chromatogr. Related Technol. 2009; 32, 167–181.
97. **Liu X., Liu J., Huang Y., Zhao R., Liu G., Chen Y.:** Determination of methotrexate in human serum by high-performance liquid chromatography combined with pseudo template molecularly imprinted polymer. J. Chromatogr. A 2009; 1216, 7533–7538.
98. **Blomgrena A., Berggrena Ch., Holmberga A., Larssona F., Sellergren B., Ensing K.:** Extraction of clenbuterol from calf urine using a molecularly imprinted polymer followed by quantitation by high-performance liquid chromatography with UV detection. J. Chromatogr. A 2002; 975, 157–164.
99. **Khorrami A.R., Rashidpur A.:** Design of a new cartridge for selective solid phase extraction using molecularly imprinted polymers: Selective extraction of theophylline from human serum samples. Biosens. Bioelectron. 2009; 25, 647–651.
100. **Javanbakht M., Attaranb A. M., Namjumanesh M. H., Esfandyari-Manesha M., Akbari-Adergani B.:** Solid-phase extraction of tramadol from plasma and urine samples using a novel water-compatible molecularly imprinted polymer. J. Chromatogr. B 2010; 878, 1700–1706.
101. **Boos K. S., Fleischer C. T.:** Multidimensional on-line solid-phase extraction (SPE) using restricted access materials (RAM) in combination with molecular imprinted polymers (MIP), Fresenius J. Anal. Chem. 2001; 371, 16–20.
102. **Yang J., Hu Y., Cai J., Zhu X. L., Su Q. D.:** A new molecularly imprinted polymer for selective extraction of cotinine from urine samples by solid-phase extraction. Anal. Bioanal.Chem. 2006; 384, 761–768.
103. **Caro E., Marce R. M., Cormack P. A. G., Sherrington D. C., Borrull F.:** A new molecularly imprinted polymer for the selective extraction of naproxen from urine samples by solid-phase extraction. J. Chromatogr. B 2004; 813, 137–143.
104. **Kriz D., Mosbach K.:** Competitive amperometric morphine sensor based on an agarose immobilised molecularly imprinted polymer. Anal. Chim. Acta 1995; 300, 71–75.
105. **Xie J., Chen L., Li Ch., Xu X.:** Selective extraction of functional components derived from herb in plasma by using a molecularly imprinted polymer based on 2,2-bis (hydroxymethyl)butanol trimethacrylate. J. Chromatogr. B 2003; 788, 233–242.
106. **Mullett W. M., Walles M., Levsen K., Borlak J., Pawliszyn J.:** Multidimensional on-line sample preparation of verapamil and its metabolites by a molecularly imprinted polymer coupled to liquid chromatography-mass spectrometry. J. Chromatogr. B 2004; 801, 297–306.
107. **B. Sellergren:** Direct drug determination by selective sample enrichment on an imprinted polymer. Anal. Chem. 1994; 66, 1678–1582.
108. **Lachová M., Lehotay J., Karasová G., Skačáni I., Armstrong D. W.:** Isolation of L-theanine from plant material using a molecularly imprinted polymer. J. Liq. Chromatogr. Related Technol. 2007; 30, 2045–2058.
109. **De Smeta D., Kodeckb V., Dubruelb P., Van Peteghema C. E. Schachtb, De Saeger S.:** Design of an imprinted clean-up method for mycophenolic acid in maize. J. Chromatogr. A 2011; 1218, 1122–1130.
110. **Lachová M., Lehotay J., Skačáni I., Čižmárik J.:** Selective solid-phase extraction of phenylcarbamic acid derivatives from rat serum by molecularly imprinted polymer. Acta Chim. Slovakia 2008; 1, 175–179.

111. **Lachová M., Lehotay J., Skačáni I., Čižmárik J.:** Molecularly imprinted solid-phase extraction of 1-methyl-2-piperidinoethylesters of alkoxyphenylcarbamic acid from human plasma, comparison with classical solid-phase extraction. J. Liq. Chromatogr. Related Technol. 2009; 32, 2293–2306.
112. **Möller K., Nilsson U., Crescenzi C.:** Investigation of matrix effects of urine on a molecularly imprinted solid-phase extraction. J. Chromatogr. B 2004; 811, 171–176.
113. **Claude B., Morin P., Bayoudh S., de Ceaurriz J.:** Interest of molecularly imprinted polymers in the fight against doping Extraction of tamoxifen and its main metabolite from urine followed by high-performance liquid chromatography with UV detection. J. Chromatogr. A 2008; 1196–1197, 81–88.
114. **Theodoridis G., Kantifes A., Manesiotis P., Raikos N., Tsoukali-Papadopoulou H.:** Preparation of a molecularly imprinted polymer for the solid-phase extraction of scopolamine with hyoscyamine as a dummy template molecule. J. Chromatogr. A 2003; 987, 103–109.
115. **Nakamura M., Ono M., Nakajima T., Ito Y., Aketo T., Haginaka J.:** Uniformly sized molecularly imprinted polymer for atropine and its application to the determination of atropine and scopolamine in pharmaceutical preparations containing scopolia extract. J. Pharm. Biomed. Anal. 2005; 37, 231–237.
116. **Hu Y., Wang Y., Chen X., Hu Y., Li G.:** A novel molecularly imprinted solid-phase microextraction fiber coupled with high performance liquid chromatography for analysis of trace estrogens in fishery samples. Talanta 80 2010; 2099–2105.
117. **Hu S. G., Wang S. W., He X. W.:** An amobarbital molecularly imprinted microsphere for selective solid-phase extraction of phenobarbital from human urine and medicines and their determination by high-performance liquid chromatography. Analyst 2003; 128, 1485–1489.
118. **Yin J., Yang G., Chen Y.:** Rapid and efficient chiral separation of nateglinide and its L-enantiomer on monolithic molecularly imprinted polymers. J. Chromatogr. A 2005; 1090, 68–75.
119. **Caro E., Marce R.M., Cormack P.A.G., Sherrington D.C., Borrull F.:** Novel enrofloxacin imprinted polymer applied to the solid-phase extraction of fluorinated quinolones from urine and tissue samples. Anal. Chim. Acta 2006; 562, 145–151.
120. **Caro E., Marce R. M., Cormack P. A. G., Sherrington D. C., Borrull F.:** Synthesis and application of an oxytetracycline imprinted polymer for the solid-phase extraction of tetracycline antibiotics. Anal. Chim. Acta 2005; 552, 81–86.
121. **Zhang M. L., Xie J. P., Zhou Q., Chen G. Q., Liu Z.:** On-line solid-phase extraction of ceramides from yeast with ceramide III imprinted monolith. J. Chromatogr. A 2003; 984, 173–183.
122. **Dong X. C., Wei W. A., Ma S. J., Sun H., Li Y., Guo J. Q.:** Molecularly imprinted solid-phase extraction of (-)-ephedrine from chinese ephedra. J. Chromatogr. A 2005; 1070, 125–130.
123. **Zhu L. L., Xu X. J.:** Selective separation of active inhibitors of epidermal growth factor receptor from Caragana jubata by molecularly imprinted solid-phase extraction. J. Chromatogr. A 2003; 991, 151–158.
124. **Mena M. L., Agui L., Martinez-Ruiz P., Yanez-Sedeno P., Reviejo A. J., Pingarron J. M.:** Molecularly imprinted polymers for on-line clean up and preconcentration of chloramphenicol prior to its voltammetric determination. Anal. Bioanal.Chem. 2003; 376, 18–25.
125. **Sambe H., Hoshina K., Moaddel R., Wainer I. W., Haginaka J.:** Uniformly-sized, molecularly imprinted polymers for nicotine by precipitation polymerization. J. Chromatogr. A 2006; 1134, 88–94.