AL04 Development of Highly Selective Detection and High-Performance Separation Methods for Pharmaceuticals and Their Applications

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It is very important to develop highly selective detection and high-performance separation methods for drugs and their metabolites in biological fluids. Highly selective detection of penicillin antibiotics (PCAs) and β-lactamase inhibitors (BLIs) could not be attained by reaction with derivatization reagents because of their degradation reactions such as opening of a β-lactam ring. We found that PCAs and BLIs, respectively, reacted with 1,2,4-triazole (or MeOH - NaOH) and $HgCl_2$, and 1,2,4-triazole (or MeOH - NaOH) to yield penicillenic acid mercury (II) mercaptide or methyl penamaldate, and enamine amide or enamine ester. These reactions were applied to highly sensitive and selective analysis of PCAs and BLIs in biological fluids by pre- or post-column reactions in LC.

Regarding high-performance separation methods for pharmaceuticals and their applications, we developed three LC packing materials, which include restricted access media (RAM), protein-based chiral stationary phases (CSPs) and molecularly imprinted polymers (MIPs). RAM, which exclude proteins and retain a low molecular weight compound, could be used for direct serum injection assays of a drug and its metabolite(s). Mixed functional phase (MFP) materials, which have hydrophobic and hydrophilic phases in both internal and outer surfaces of silica gels, were developed. The MFP materials had octyl, phenyl and butyl groups as hydrophobic phases and glycerylpropyl groups as hydrophilic phases. Furthermore, β-cyclodextrin was introduced as hydrophobic phases for chiral separation of drugs in biological fluids. The MFP materials are now commercially available.

Protein-based CSPs could be used for enantioseparation of drugs. The third domain of ovomucoid from turkey (OMTKY3), which has the chiral recognition ability for some class of compounds, was isolated. The interaction of U-80413, which is one of profen analogs, with OMTKY3 was examined using ^{1}H-NMR and computational chemistry, and the chiral recognition mechanism of U-80413 on OMTKY3 could be elucidated. Furthermore, a new glycoprotein from chicken egg whites was isolated, and its amino acid sequences and sites of sugar chains and S-S bridges were clarified. The new glycoprotein was termed chicken α_1-acid glycoprotein (cAGP). It was found that its chiral recognition site(s) was present in a protein part and Trp26 was responsible for chiral recognition of drugs tested. cAGP- and pepsin-based CSPs were commercialized.

MIPs could be prepared for drugs, biologically important compounds and environmental pollutants using multi-step swelling and polymerization, which gives spherical and mono-disperse beads. The MIPs could be used for a target- and group-specific recognition in LC. Furthermore, RAM-MIPs, which have both RAM and MIP functions, were developed and applied to direct serum injection assays of a drug and its metabolite(s). It is well known that the leakage of a template molecule from the MIP affects on accuracy and precision of the assay in the case of ultra-trace analysis. The leakage problem has been overcome by imprinting a structurally related analog (pseudo-template molecule) and combining with chromatographic separation. The MIP and RAM-MIP for a isotope-substituted compound were used for the selective solid-phase extraction of the target compound in environmental samples or biological fluids combined with LC-MS or LC-MS/MS. Furthermore, MIPs for hydrophilic compounds such as creatinine, chlorogenic acid, arbutin and rutin, which are first dissolved in a hydrophilic solvent, were prepared by modified precipitation polymerization.

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