Derivatization Techniques for Chromatographic Analysis

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High-performance liquid chromatography (HPLC) is a useful analytical tool to determine trace analytes in complicated samples due to its high sensitivity and selectivity. In order to further increase the sensitivity and selectivity, derivatization techniques have frequently been applied to a chromatographic analysis. Derivatization with some chromophore is a traditional derivatization technique that allows for UV detection of non-UV absorption compounds. Miyamoto et al. have recently developed a derivatization technique that allows for UV detection of non-UV absorption compounds.2

In this method, microwave-assisted heating was used to develop an HPLC-UV method for fatty acids.3 In this method, microwave-assisted heating was employed to accelerate the reaction. The derivatization reaction was completed at only 10 s under microwave irradiation in the presence of EDC. In addition to shorten the reaction time, the formation of any by-products could be reduced. Derivatization with fluorophore is widely used to develop a more sensitive HPLC-fluorescence (FL) method than HPLC-UV. Until now, various fluorophores have been adopted to convert a non-fluorescent compound to a highly fluorescent derivative. Among them, benzoxadiazole-type reagents have frequently been utilized because of their excellent fluorescent properties. For example, 4-(N,N-dimethylaminosulfonyl)-7-piperazino-2,1,3-benzoxadiazole (DBD-PZ) and 4-(N-chloroformaldehyde-N-methylamino)-7-nitro-2,1,3-benzoxadiazole (NBD-COCI) were used to develop an HPLC-FL method for sialic acid and aromatic diamines, respectively.4,5 Besides two small molecules, Meng et al. developed an HPLC-FL method for cyclic lipopeptide, surfactin using 1-bromooctylpyrene as a fluorescent reagent.6,7 Recently, derivatization with a reagent having a high ionization efficiency is also increasing for adaptation of the LC-MS method. As the derivatization reagent for LC-MS, conventional fluorescence derivatization reagents, such as dansyl chloride and 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F), can be used in the same manner as specially designed reagents for LC-MS,8,9 because these fluorescent reagents also have highly ionizable moiety. Additionally, the application of methanol as the derivatization reagent for the LC-MS/MS analysis of quinones was reported,10 in which the introduction of a methoxy group by the reaction of methanol to quinone was used to increase the ionization efficiency.

On the other hand, derivatization techniques with a purification tag have been reported in order to increase the selectivity. After derivatization, the derivative can be selectively separated from other interfering components by a treatment with a LC column or SPE cartridge, which can interact with the purification tag. Shan et al. used bromine as a derivatization reagent for triacylglycerols to control the retention behavior on the reversed-phase LC column.11 Sakaguchi et al. used perfluoroalkylamine as a fluoruous derivatization reagent for carboxylic acid. The fluoruous derivative could be retained selectively on a fluoruous phase LC column to separate it from the plasma components.12 El-Maghrabey et al. reported on a dual derivatization with both fluorophore and purification tags to develop a selective HPLC method for glyoxylic acid.13 In this method, glyoxylic acid was derivatized with 1-pyreneboronic acid (fluorophore) and 2-aminoethanesulfonic acid (purification tag) based on the Petas reaction. The derivative could be retained on a strong anion exchange SPE cartridge, whereas the un-reacted excess fluorophore could not. Although the large blank peaks derived from an excess reagent often become an issue in derivatization techniques,14 this approach could reduce such blank peaks on the chromatogram.

In the case of gas-chromatography, derivatization is usually employed to increase the volatility of a non-volatile analyte. Zour et al. have developed a gas chromatographic method for guanidino compounds using isovalerylacetone and ethyl chlorofomate as derivatization reagents.15 The derivatization technique is also useful to develop an electrophoretic analysis. A traditional chromogenic derivatization reagent for a thiogroup, 5,5′-dithiobis(2-nitrobenzoic acid), could be applied to develop a capillary electrophoresis with a UV detection method for aminothiols.16 Oborny et al. developed a portable microchip

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electrophoresis of neuroactive amines after the fluorescence derivatization with naphthalene-2,3-dicarboxaldehyde and sodium cyanide.17

Although the derivatization techniques described above can improve the sensitivity and selectivity of chromatographic analysis, the derivatization procedure is sometimes laborious and time consuming. In order to overcome such issues, further efforts will be needed to develop more sophisticated derivatization techniques.

**Keywords** Chromatography, derivatization

**References**