ANALYTICAL SCIENCES OCTOBER 2018, VOL. 34

Highlights

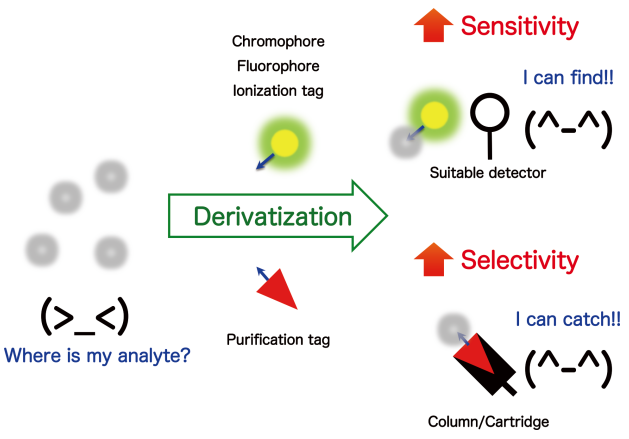
Derivatization Techniques for Chromatographic Analysis

Naoya KISHIKAWA

Course of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852–8521, Japan

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.[1]

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 determination method for phytanic acid, long-chain fatty acid, using 2-nitrophenylhydrazine as a derivatization reagent.[2] The derivatization reaction was carried out at room temperature for 20 min in the presence of a condensation reagent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). Although phytanic acid has no UV absorbance, its derivatized molecule could be detected with its absorbance at 228 nm. Shrestha *et al.* also used 2-nitrophenylhydrazine 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*-chloroformylmethyl-*N*-methylamino)-7-nitro-2,1,3-benzoxadiazole (NBD-COCl) 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-bromoacetylpyrene 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.



Derivatization technique can improve the detection sensitivity and control the chromatographic/extraction selectivity.

E-mail: kishika@nagasaki-u.ac.jp

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 fluorous derivatization reagent for carboxylic acid. The fluorous derivative could be retained selectively on a fluorous 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 Petasis 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. Zounr *et al.* have developed a gas chromatographic method for guanidino compounds using isovaleroylacetone and ethyl chloroformate as derivatization reagents.[15] The derivatization technique is also useful to develop an electrophoretic analysis. A traditional chromogenic derivatization reagent for a thiol group, 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

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

1. M. Yamaguchi and J. Ishida, "*Modern Derivatization Methods for Separation Sciences*", ed. T. Toyo'oka, **1999**, John Wiley and Sons, New York, Chichester, Brisbane, Toronto.
2. A. Miyamoto, T. Aoyama, M. Okamura, N. Fukuda, T. Ueno, M. Abe, and Y. Matsumoto, *Anal. Sci.*, **2017**, *33*, 365.
3. R. Shrestha, Y. Miura, K. Hirano, Z. Chen, H. Okabe, H. Chiba, and S.-P. Hui, *Anal. Sci.*, **2018**, *34*, 575.
4. A. Kawasaki, M. Yasuda, K. Mawatari, T. Fukuuchi, N. Yamaoka, K. Kaneko, R. Iijima, S Yui, M. Satoh, and K. Nakagomi, *Anal. Sci.*, **2018**, *34*, 841.
5. N. Inoue, *Anal. Sci.*, **2017**, *33*, 1375.
6. Y. Meng, J.-F. Liu, S.-Z. Yang, R.-Q. Ye, and B.-Z. Mu, *Anal. Sci.*, **2015**, *31*, 377.
7. Y. Meng, H.-Z. Gang, S.-Z. Yang, R.-Q. Ye, and B.-Z. Mu, *Anal. Sci.*, **2018**, *34*, 541.
8. T. Toyo'oka, *Anal. Sci.*, **2017**, *33*, 555.
9. S. Dong, Z. Yan, and H. Yang, *Anal. Sci.*, **2016**, *32*, 1333.
10. J. Pei, Y. Wang, and K. Yu, *Anal. Sci.*, **2018**, *34*, 335.
11. X.-L. Shan, X.-T. Liu, C. Gong, and X. Xu, *Anal. Sci.*, **2018**, *34*, 283.
12. Y. Sakaguchi, H. Yoshida, T. Hayama, M. Yoshitake. M. Itoyama, K. Todoroki, M. Yamaguchi, and H. Nohta, *J. Pharm. Biomed. Anal.*, **2011**, *55*, 176.
13. M. El-Maghrabey, M. Mine, N. Kishikawa, K. Ohyama, and N. Kuroda, *Talanta*, **2018**, *180*, 323.
14. Y. Suzuki, A. Okano, K. Kabayama, A. Nishina, M. Tanigawa, K. Nishimura, and Y. Kushi, *Anal. Sci.*, **2016**, *32*, 487.
15. R. A. Zounr, M. Y. Khuhawar, T. M. Jahangir, and M. Alamgir, *Anal. Sci.*, **2016**, *32*, 141.
16. A. V. Ivanov, K. A. Nikiforova, P. O. Bulgakova, E. D. Virus, and A. A. Kubatiev, *Anal. Sci.*, **2018**, *34*, 505.
17. N. J. Oborny, E. E. M. Costa, L. Suntornsuk, F. C. Abreu, and S. M. Lunte, *Anal. Sci.*, **2016**, *32*, 35.