Current Topics

Applications of Fluorescence Detection in Current Pharmaceutical Research

Foreword

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The high sensitivity and selectivity of fluorescence (FL)-based methods has meant that they have become among the most widely used tools for detecting trace amounts of bioactive compounds or medications in complex matrices such as biological samples. Determination of trace amounts of bioactive compounds exhibiting enzyme activity can provide conclusive evidence in the diagnosis of diseases. In addition, analysis of medications is necessary to promote their rational use. Indeed, the application of FL-based methods has become essential in the areas of quality control, pharmacokinetics, pharmacodynamics, pharmacology and toxicology of medicines. Viewed in this context, FL detection methods are likely to play an increasingly important role in pharmaceutical research. Currently, ultra-sensitive analyses require that samples be pretreated using methods such as derivatization, and a variety of purification strategies have been developed in conjunction with FL detection. This edition of Current Topics in *Chem. Pharm. Bull.* contains four original articles on recent advances in FL that have been contributed by researchers who are leaders in the field.

The first regular article entitled, "Liquid Chromatographic Determination of *o*-Phosphoethanolamine in Human Plasma Using Fluorescent Derivatization" by Dr. Tomita, Prof. Hayama (Fukuoka University) and colleagues describes an HPLC-FL detection method for *o*-phosphoetanolamine (PEA), which is a potential biomarker in human plasma for use in the diagnosis of a major depressive disorder. Prior to HPLC analysis, PEA was derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and then purified with a titanium dioxide-modified monolithic silica spin column. The separation of this PEA derivative without interfering components in plasma that is stable over a wide pH range in the mobile phase was achieved by using an amide-type hydrophilic interaction chromatography (HILIC) column. The highly sensitive and selective detection method was applied to the identification of PEA in plasma samples from patients with mental illness, and its applicability was successfully demonstrated.

The second regular article by Prof. Kabashima (Nagasaki International University) *et al.* was entitled, "A Facile Method to Determine Prolidase Activity Using a Peptide-Specific Fluorometric Reaction." They developed a new method to determine prolidase activity, which plays an important role in wound healing, inflammation, vascularization, cell proliferation, and protein synthesis. The method is based on a selective FL reaction between 3,4-dihydroxyphenylacetic acid and the substrate of prolidase, *N*-terminal glycine-containing peptide. Enzyme activity is measured by estimating the decrease in FL intensity. The advantage of the method lies in its simplicity because it does not require the pre-incubation or deproteinization procedures that are typically employed in conventional ninhydrin-based assays. Moreover, measurement of prolidase activity in fibroblasts and HeLa cells was successfully demonstrated.

The third regular article entitled, "Extremely Simple and Rapid HPLC Analysis of Tocilizumab in Human Serum with Selective Precipitation Using Alkylamine" by our research group (Sanyo-Onoda City University) describes an HPLC-FL assay for detecting tocilizumab (TCZ) in human serum. The rapid measurement of TCZ concentrations in biological samples would be highly advantageous in clinical settings, because conventional measurement methods require pretreatment steps that are time-consuming to perform. Specifically, TCZ in serum is selectively recovered by pretreating samples with acetonitrile containing alkylamine without the need for any time-consuming procedures. The TCZ is then separated by high-temperature reversed-phase LC and detected by its native FL. The established method was well-validated, and suggested the utility of monitoring the therapeutic efficacy of treating cytokine-release syndrome associated with the 2019 coronavirus disease.

The final regular article by Prof. Saito (Hoshi University) *et al.* was entitled, "Analysis of Methamphetamine in Urine by HPLC with Solid-Phase Dispersive Extraction and Solid-Phase Fluorescence Derivatization." Methamphetamine (MA) is one of the most frequently abused amphetamine derivatives in Japan, and its recreational use is a serious global problem. Therefore, the requirement for a simple and rapid analytical method for MA in the field of forensic science has become apparent. In this study, a new HPLC-FL detection method for MA determination in urine was established. The pretreatment of urine was completed by solid-phase dispersive extraction with reversed-phase polymeric solid-phase gel and fluorescence derivatization with 9-fluorenylmethyl chloroformate (FMOC) in the solid phase. The higher stability of FMOC-MA was compared to that of conventional liquid-phase methods. In addition, different reaction rate constants were calculated for different temperature conditions, and physicochemical parameters, including activation energy and activation entropy involved in the degradation reaction, were obtained from an Arrhenius plot and analyzed thermodynamically.

The organizer believes that the articles presented in this edition of Current Topics will provide useful information to researchers who need to determine trace levels of bioactive compounds or medications in biological samples. Finally, I would like to extend my gratitude to all of the contributing authors for their valuable contributions.

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