Focusing Review

Development of novel fluorescence derivatization systems enabling highly sensitive and selective detection of biological compounds

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Abstract

A fluorescence derivatization-liquid chromatography (LC) method is a powerful tool for the analysis of bioactive compounds with high sensitivity and selectivity. In this paper, the author reviews the development of the following three types of novel fluorescence derivatization-LC analytical systems: (1) simultaneous LC analysis of melatonin and its related compounds through post-column electrochemical demethylation and fluorescence derivatization, (2) LC analysis of 5-hydroxyindoles based on fluorescence derivatization by online pre-column photocatalytic oxidation with benzylamine, and (3) reagent peak-free LC analysis for aliphatic amines and amino acids using F-trap pyrene as a fluorous tag-bound F-trap fluorescence derivatization reagent. The authors have also successfully applied these systems to biological and pharmaceutical analyses.

Key Words: Fluorescence derivatization, Pinpoint targeting reagent, Electrochemical oxidation, Photocatalytic oxidation, Fluorous separation, F-trap fluorescence derivatization

1. Introduction

A fluorescence derivatization-LC method, which enables the femtomole-level detection of analytes, is a powerful tool for the analysis of biological compounds with high sensitivity and selectivity. Thus far, various fluorescent derivatization reagents for varieties of functional groups have been reported. For example, dansyl chloride, fluorescein isothiocyanate, FMOC-Cl, DMEQ-COCl, and DIB-Cl for amines are commercially available and widely used. However, a major drawback associated with the use of this method for most commercially available fluorescence derivatization reagents is that large peaks appear in the chromatograms due to unreacted reagents. Because it is often difficult to distinguish these peaks from the target analyte peaks, they interfere with the quantification of the analytes. Therefore, there is a demand for comprehensive pretreatment processes (liquid-liquid extraction and solid-phase extraction processes) or a sophisticated LC separation technique that involves the column-switching method.

On the other hand, we have previously used reagent peak-free fluorescence derivatization reagents, which are pinpoint targeting (PPT) reagents that do not recognize functional groups such as amines and thiols, but rather recognize specific chemical structures. Among the PPT reagents, benzylamine reacts with serotonin-related compounds, such as 5-hydroxyindoles (5-HIs), in the presence of an oxidizing agent to produce highly fluorescent benzoxazole derivatives [1, 2] (Fig. 1). The reagent is selective for 5-HIs, and is then applied to post-column fluorescence derivatization LC for the determination of 5-HIs in human plasma [3] and in microdialysis samples obtained from rats [4, 5] and mice [6]. This method is thus very selective for 5-HIs; however, it presents the following problems: (1) it is does not show any response to melatonin-related 5-methoxyindoles (5-MIs) and (2) it requires a harmful and unstable chemical oxidizing agent, such as potassium

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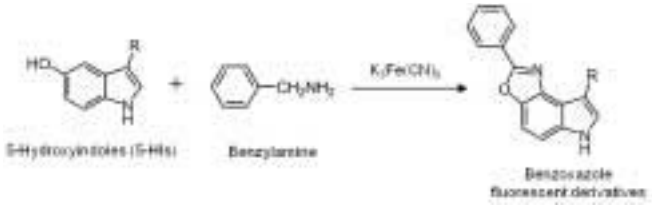

Fig. 1 Fluorescence derivatization reaction of 5-HIs with benzylamine.

hexacyanoferrate (III), during the PPT reaction. To solve these problems, we have developed the following two novel fluorescence derivatization-LC analytical systems: (1) simultaneous LC analysis of melatonin and its related compounds through post-column electrochemical demethylation and fluorescence derivatization and (2) LC analysis of 5-hydroxyindoles based on fluorescence derivatization by online pre-column photocatalytic oxidation with benzylamine. In addition, we have developed a reagent peak-free LC analysis method for aliphatic amines and amino acids using F-trap pyrene as a fluorous tag-bound F-trap fluorescence derivatization reagent. We have also successfully applied these systems to biological and pharmaceutical analyses.

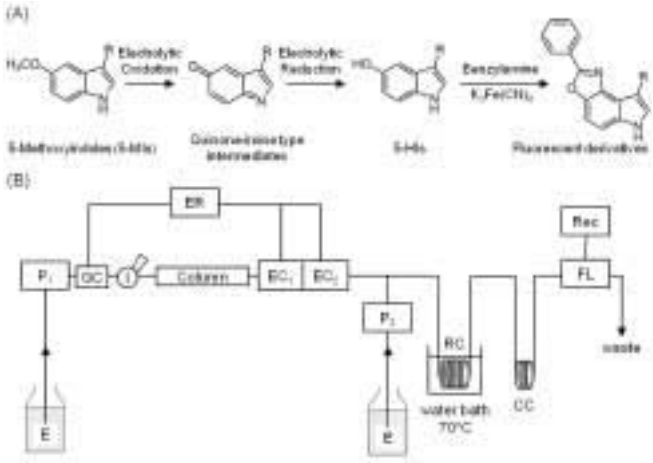

Fig. 2 (A) Fluorescence derivatization of 5-MIs through electrochemical demethylation followed by PPT reaction with benzylamine, and (B) Schematic diagram of the post-column LC-fluorescence derivatization system for the analysis of 5-MIs. P_1 and P_2, LC pumps; I, injection valve (20 μL); GC, guard cell; EC_1 and EC_2, electrolytic cells; M, mixing device; RC, reaction coil; CC, cooling coil; ER, electrolytic regulator (coulometer); FD, fluorescence detector; Rec, integrator; E, eluent; R, reagent solution.

2. Simultaneous determination of 5-MIs based on post-column electrochemical demethylation and fluorescence derivatization [7]

Melatonin (Mel), one of the 5-MIs, is an indole-based hormone synthesized from L-tryptophan and secreted by the pineal gland. In various species, Mel has been linked to the functional regulation of circadian and seasonal rhythms, the immune function, and tumor inhibition, and most recently, it has been found to behave as a free radical scavenger and as an antioxidant. Various methods have been described for the quantification of Mel in several tissues and body fluids; these methods include radioimmunoassays, gas chromatography-mass spectrometry, enzyme-linked immunosolvent assays, capillary electrophoresis, and LC with either electrochemical detection or fluorescence detection. Although the above-mentioned methods allow Mel to be determined, not all of them are useful when seeking to determine the other 5-MIs, including Mel precursors and metabolites such as 5-methoxytryptamine (5-MT), 5-methoxytryptophol (5-MTOL), and 5-methoxyindole-3-acetic acid (5-MIAA). The LC-electrochemical detection method permits the simultaneous determination of these 5-MIs. However, because its operation potential is relatively high, most electroactive compounds present during this process can become co-oxidized, which causes interfering peaks to appear, as well as an intense level of background noise. The fluorescence derivatization method is also unsuitable for the simultaneous analysis of the 5-MIs because there is no reactive functional group common to all the 5-MIs in their molecule. Therefore, a convenient, robust, highly selective, and sensitive method for the simultaneous analysis of 5-MIs remains elusive.

We have successfully applied the highly selective and sensitive PPT reaction of 5-HIs with benzylamine to the post-column derivatization of 5-MIs, including melatonin, by introducing an electrochemical demethylation step.

Figure 2 shows the fluorescence derivatization of 5-MIs and a schematic diagram of the LC system. We separated the 5-MIs through reversed-phase LC using isocratic elution, and then we demethylated them electrochemically—using a coulometric technique—to obtain their corresponding 5-HIs. We optimized the conditions of the LC separation, electrochemical demethylation, and fluorescence derivatization for the sensitive and selective determination of 5-MIs.

Figure 3 shows a typical chromatogram obtained using a standard mixture of the four 5-MIs. We achieved a good separation of the 5-MI derivatives within 30 min on the ODS column when using isocratic elution with 250 mM acetate buffer–acetonitrile–methanol [pH 6.5; 8:1:1 (v/v)] as the mobile phase; the retention times were 5.3 (5-MIAA), 7.6 (5-MT), 21.8 (5-MTOL), and 24.7 min (Mel). In this analytical system, we expected that the 5-MIs would become demethylated electrochemically to form the 5-HIs, which would then react with benzylamine in the presence of potassium hexacyanoferrate (III) to produce fluorescence. To confirm this hypothesis, we subjected the eluates for Mel and NAS, which

is a demethylated derivative of Mel, to this process in order to compare their fluorescence spectra and mobilities in the reversed-phase LC system used for the separation of the pre-labeled derivatized 5-HIs. The fluorescence spectra of the eluates were almost identical in terms of both the shape and wavelength of their excitation and emission maxima (345 and 470 nm, respectively). The retention time of the post-labeled derivative of Mel was in good agreement with the retention times obtained for the pre- and post-labeled derivatives of NAS.

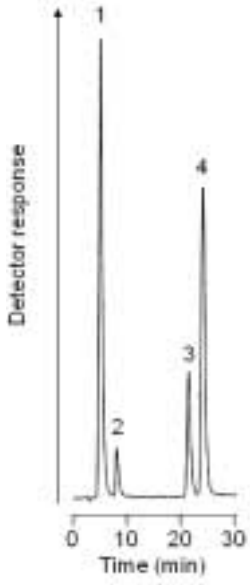

Fig. 3 Chromatogram obtained using a standard mixture of 5-MIs. A portion (20 μL) of a standard solution (20 pmol each on column) was applied to the LC system. Peaks: 1, 5-MIAA; 2, 5-MT; 3, 5-MTOL; and 4, Mel.

Furthermore, we used LC-MS with an ESI interface to analyze the structures of Mel and NAS derivatives (data not shown). Solutions of the post-labeled derivatives of both Mel and NAS and of the pre-labeled derivative of NAS were subjected to LC-MS analyses in the positive-ionization mode. Under an ion monitoring mode selected in a scan range of 319.7−320.7, each peak of the three derivatives was detected at the same retention time. Mass spectra for the peaks also provided the corresponding quasi-molecular ion ($[M + H]^+$) of the expected derivative (319.2 Da) as a base peak. All these observations strongly support the notion that the fluorescence derivatization reactions of the 5-MIs occur as shown in Fig. 2.

The relationships between the peak areas and the amounts of the individual 5-MIs were linear (correlation coefficients > 0.995; n =7) over a concentration range of 2−200 pmol per 20 μL injection volume for all the substances tested. We established the intra-day precision of this process by performing repeated determinations (n =7) using a standard mixture of the 5-MIs (1 μM). The relative standard deviations were 2.9 (5-MIAA), 5.6 (5-MT), 4.6 (5-MTOL), and 1.9% (Mel). The detection limits (signal-to-noise ratio =3) were 12 (5-MIAA), 93 (5-MT), 4.6 (5-MTOL), and 21 fmol (Mel) per 20 μL injection, i.e., the sensitivity of this present method is similar for Mel and 5-MIAA, but slightly lower for 5-

Table 1 Detection limits (DL) and retention times (tR) for 5-MIs, 5-HIs, other indoles, CAs and CA metabolites.

5-MIs	**DL[a] (fmol)**	**tR (min)**
5-Methoxyindole-3-acetic acid	12	5.3
5-Methoxytryptamine	93	7.6
5-Methoxytryptophol	36	21.8
5-Methoxytryptophan	46	5.5
Melatonin	21	24.7
6-Hydroxymelatonin	2,200	8.0
5-HIs		
5-Hydoroxyindole-3-acetic acid (5-HIAA)	14	3.4
Serotonin (5-HT)	135	3.5
5-Hydroxytryptophol (5-HTOL)	37	5.6
5-Hydroxytryptophan (5-HTP)	90	3.3
N-Acetylserotonin	56	6.3
Other indoles		
Indole-3-acetic acid	230	5.5
Tryptamine	170	8.3
Tryptophol	660	23.2
Tryptophan	160	5.0
4-Hydroxyindole	370	7.7

Catecholamines	**DL[a] (fmol)**	**tR (min)**
Dopamine	1,500	3.4
Epinephrine	4,700	3.1
Norepinephrine	2,700	3.2
3,4-Dihydroxyphenylalanine	3,200	4.4
Catecholamine metabolites		
Metanephrine	4,500	3.2
Normetanephrine	1,300	3.3
3-Methoxytyramine	740	3.7
3,4-Dihydroxymandelic acid	6,000	2.9
3,4-Dihydroxyphenylacetic acid	170	3.0
3,4-Dihydroxyphenylglycol	190	3.2
4-Hydroxy-3-methoxyphenylglycol	240	3.9
4-Hydroxy-3-methoxymandelic acid	5,300	3.1
4-Hydroxy-3-methoxyphenylacetic acid	320	3.8

a) Defined as the amount per injection volume (20 μL) giving a signal-to-noise ratio of 3.

MT and 5-MTOL, as compared to the results obtained when using the LC-ED method. We also studied the reactivity of this system toward 5-HIs, catechols, and methoxyphenols. Each compound we tested provided a single peak in the chromatogram. Table 1 lists the retention times for the benzylamine derivatives and their detection limits. All the 5-HIs, the four catecholamine metabolites (DOPAC, DOPEG, MOPEG, and HVA) produced relatively large peaks in the chromatogram. Four other indoles (IAA, TA, TOL, and Trp) also produced relatively large peaks in the chromatogram.

We successfully applied this system to the analysis of the small amount of impurities in commercial melatonin supplements. Despite vendors' claims that these supplements are chemically pure, trace amounts of tryptamine (23 nmol per tablet), tryptophol (116 nmol per tablet), and *N*-acetylserotonin (1.16 nmol per tablet) were detected.

3. Online photocatalytic fluorescence derivatization of 5-hydroxyindoles with benzylamine [8]

The PPT reaction is so selective for 5-HIs that it can be applied to the LC analyses of biological samples [2-6, 9, 10]. Unfortunately, the potassium hexacyanoferrate (III) solution employed as a chemical oxidizing agent is harmful and requires preparation immediately prior to its use because of its instability. In addition, it can be absorbed into the inner walls of the flow-lines and flow-cells of the fluorescence detector; such accumulation greatly increases the background noise, which decreases the sensitivity of the detection of the 5-HIs. In order to retain high sensitivity during these analyses, frequent washing of the flow-line and flow-cell thus becomes necessary. As part of our research to discover a more valuable oxidation method, in this study, we have focused on carrying out a photocatalytic reaction using titanium dioxide (TiO_2). This photocatalytic oxidation approach has received much attention in recent years due to its ability to significantly degrade organic pollutants in water and air. The main mechanism of this oxidation process is the photogeneration of electrons and holes in the photocatalyst. The excitation transfer of an electron from the valence band to the conduction band creates an oxidizing site (hole) and a reducing site (electron). The photogenerated hole has the potential to oxidize a variety of substrates by means of electron transfer. Many reports have described flow-injection analyses following the online photocatalytic oxidation of organic contaminants in water. The drawbacks of these systems, however, are that they lack the barotolerance and endurance needed for their direct connection to a conventional analytical LC system. We have successfully applied photocatalytic oxidation using titanium dioxide (TiO_2) to the online pre-column fluorescence derivatization of 5-HIs with benzylamine. For this method, we prepared a photocatalytic column comprising tefzel tubing packed with TiO_2-coated glass beads, which served as a pre-column reactor.

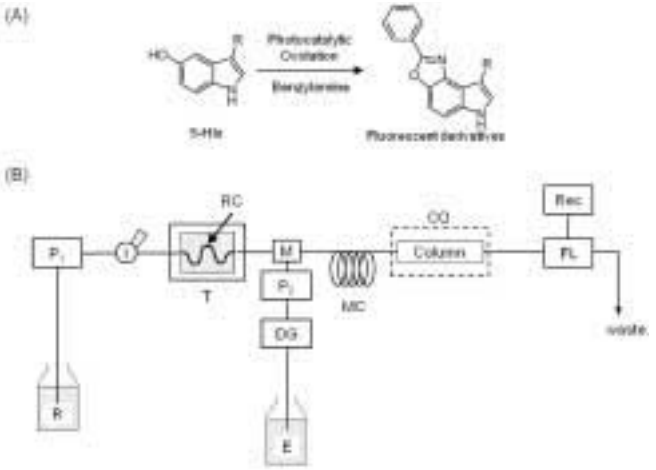

Fig. 4 (A) Fluorescence derivatization of 5-HIs, followed by their photocatalytic oxidation with benzylamine, and (B) Schematic flow diagram of the online pre-column LC-fluorescence detection system for the analysis of 5-HIs. P_1 and P_2, LC pumps; DG, degasser; I, injection valve (5 μL); T, transilluminator (365 nm, 25 W × 4); R, reagent solution; RC, reaction column; M, mixing tee; MC, mixing coil; E, eluent; CO, column oven; FL, fluorescence detector; Rec, integrator.

Figure 4 shows the fluorescence derivatization of 5-HIs and a schematic diagram of the LC system. We derivatized the 5-HIs with benzylamine—using photocatalytic oxidation—to obtain their corresponding derivatives, and then we separated them by ion-pair reversed-phase LC. We optimized the conditions of the photocatalytic oxidation, LC separation, and fluorescence derivatization for the sensitive and selective determination of 5-HIs. As shown in Fig. 4, fluorescence derivatization proceeded during the passage of the sample through the reaction column while under near-UV irradiation. The derivatives were separated continuously through a reversed-phase LC system under isocratic elution and were then detected fluorometrically. Figure 5 (A) shows a typical chromatogram that we obtained using a standard mixture of 5-HIs. We achieved a good separation of the 5-HI derivatives within 50 min on the ODS column when eluting isocratically using a mobile phase comprising 100 mM acetate buffer (pH 4.6)-acetonitrile (72: 28, v/v) containing 3 mM sodium 1-octanesulfonate; the retention times were 22.5 (5-HT), 26.3 (5-HIAA), and 44.8 min (NAS). In contrast, we observed no peaks in the chromatogram in the absence of near-UV irradiation (Fig. 5 (B)). For comparison, we also injected offline-derivatized 5-HI samples, which we had treated under chemical oxidation conditions [4], into the analytical system (Fig. 5 (C)). In this case, the peak heights of the derivatives were ca. twice as intense as those obtained in Fig. 5 (A); however, their

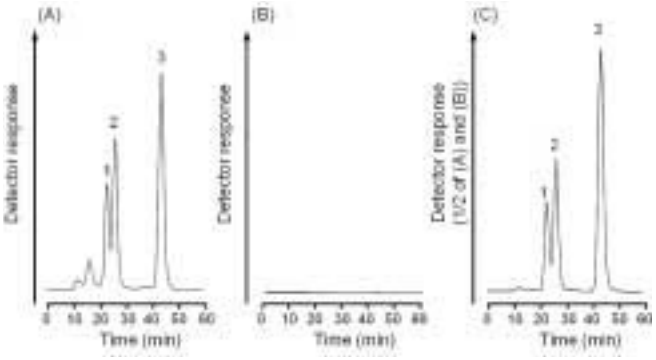

Fig. 5 Chromatograms obtained using a standard mixture of 5-HIs (100 pmol each on column). (A) Under near-UV irradiation, (B) without near-UV irradiation, (C) pre-derivatized samples of 5-HIs under near-UV irradiation. Peaks: (1) 5-HT, (2) 5-HIAA, and (3) NAS.

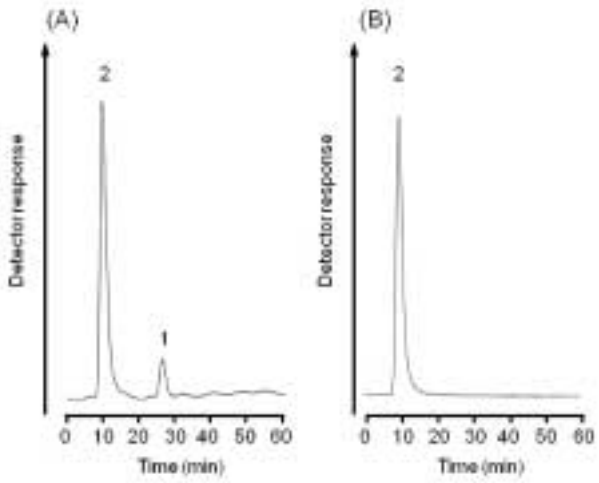

Fig. 6 Chromatograms of a urine sample obtained from a healthy human subject: (A) under near-UV irradiation, (B) without near-UV irradiation. Peaks: (1) 5-HIAA (25.2 μM), (2) endogenous fluorescent compounds.

retention times were in good agreement. The fluorescence spectra of the eluates were almost identical with respect to their shapes and the wavelengths of their excitation and emission maxima (350 and 465 nm, respectively). All these observations strongly support the notion that the fluorescence derivatization reactions of the 5-HIs occur through online photocatalytic oxidation with benzylamine, as shown in Fig. 4. The relationships between the peak heights and the amounts of the individual 5-HIs were linear (correlation coefficients >0.999; n=4) over a concentration range of 2.5 pmol−1 nmol (for 5-HT and 5-HIAA) and of 1−500 pmol (for NAS) per 5 μL injection. We established the intra-day precision of this process by performing repeated determinations (n=5) using a standard mixture of the 5-HIs (20 μM). The relative standard deviations of the peak heights were 12.5 (5-HT), 11.6 (5-HIAA), and 11.2 % (NAS). The detection limits (signal-to-noise ratio =3) were 360 (5-HT), 300 (5-HIAA), and 160 fmol (NAS) per 5 μL injection, i.e., the sensitivity of this present method for the analysis of 5-HIs is similar to that obtained when using the post-column chemical oxidation/derivatization method [2]. In a further step, we applied this method to the determination of the major metabolite of 5-HT in urine, 5-HIAA, which is important for the diagnosis of carcinoid tumors and for monitoring their therapeutic treatment. Thus, we applied our method to the determination of urinary 5-HIAA. Figure 6 (A) shows a typical chromatogram that we obtained when our method was applied to urine from a healthy human. We identified the peak for 5-HIAA on the basis of its retention time relative to that of the standard compound, and through its co-elution. We observed peak 2 in the chromatogram, even when we carried out the reaction in the absence of benzylamine and/or near-UV irradiation (Fig. 6 (B)). These results suggest that it is the endogenous fluorescent compounds in human urine that are responsible for the presence of peak 2. Using our present method, we measured the concentration of 5-HIAA in the urine of healthy human volunteers (n=6) and obtained values of 11.4−46.8 μM, which are similar to those previously obtained by other researchers (2−50 μM).

4. Reagent peak-free LC analysis for aliphatic amines and amino acids using F-trap pyrene [11] as a fluorous tag-bound F-trap fluorescence derivatization reagent

As mentioned in the introduction, a major drawback associated with the use of most commercial fluorescence derivatization reagents is that large peaks appear in the chromatograms due to unreacted reagents. In contrast, fluorogenic derivatization reagents, which are themselves non-fluorescent but which react with amines to generate fluorescence, such as *o*-phthalaldehyde with 2-mercaptoethanol, NBD-F, DBD-F, and AccQ-Tag, are commercially supplied and widely used for the highly sensitive analysis of amino biomolecules such as amino acids and peptides. However, the fluorescence properties (excitation and emission wavelengths, Stoke's shift), fluorescence quantum yields, and chemical stabilities of these reagents do not always satisfy the user's purposes. Although excess unreacted reagent will not fluoresce, it can influence the LC separation of the analytes. In addition, these reagents control the fluorescence quantum yields by means of their unique emission mechanism; therefore, other fluorophores (for example, pyrene, coumarin, dansyl, fluorescein, and rhodamine) cannot be applied for this purpose. We have developed a novel pre-column fluorescence derivatization reagent, F-trap pyrene, for use in the LC analysis of biological amines, and the use of this reagent does not result in the above-mentioned large peaks due to unreacted reagents (Fig.7). This reagent is comprises a fluorophore, amine-

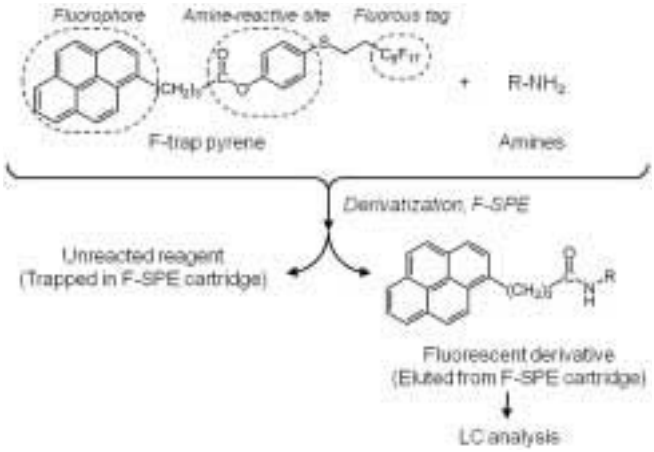

Fig. 7 Fluorescence derivatization of aliphatic amines using F-trap pyrene.

Table 2 Removal rate of reagent and average recovery of the amine derivatives by F-SPE with different fluorophobic elutions.

Eluent		Removal rate[a] (%)	Average recovery[b] (%)
Methanol	70%	＞99.9	36.4
	80%	＞99.7	84.2
	90%	＞98.2	92.6
Acetonitrile	60%	＞99.9	48.9
	70%	＞99.8	87.1
	80%	＞99.7	99.6
DMF	80%	＞99.9	60.9
	90%	＞99.8	70.1
DMSO	100%	＞99.9	72.2

reactive phenylthio ester (Marshall linker), and a fluorophilic perfluoroalkyl group (the so-called fluorous tag). Amines are fluorescently labeled with this reagent by being heated in the presence of pyridine concurrent with the elimination of 4-(3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 9, 9, 10, 10, 10-heptadecafluorodecylthio)phenol during the reaction. This compound is retained, along with excess reagent, by the fluorous solid-phase extraction (F-SPE) step prior to LC analysis. Only amine derivatives, then, are sensitively and selectively analyzed by this LC-fluorescence technique. The F-trap pyrene was synthesized by a one-step reaction of 1-pyrene-4-butyric acid and FluoMar in the presence of *N, N'*-diisopropylcarbodiimide as a condensation agent and *N, N*-dimethylaminopyridine as a catalyst. This compound was stable for at least one month at room temperature under N_2 atmosphere. The derivatization reaction for amines proceeded successively in the presence of the reagent and pyridine with heating at 90℃ within 60 min. A portion of the derivatization reaction solution was loaded onto a commercial FluoroFlash SPE cartridge, which was packed

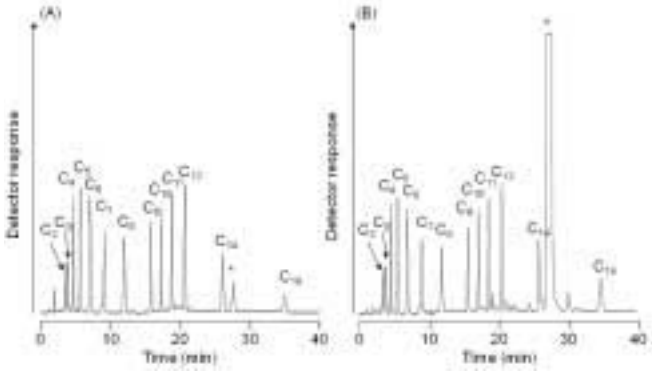

Fig. 8 Chromatograms of the pyrene derivatives of 13 aliphatic amines (A) with and (B) without F-SPE treatment (50 pmol each on column). Peaks: C_2, ethylamine; C_3, *n*-propylamine; C_4, *n*-butylamine; C_5, *n*-pentylamine; C_6, *n*-hexylamine; C_7, *n*-heptylamine; C_8, *n*-octylamine; C_9, *n*-nonylamine; C_{10}, *n*-decylamine; C_{11}, *n*-undecylamine; C_{12}, *n*-dodecylamine; C_{14}, *n*-tetradecylamine; C_{16}, *n*-hexadecylamine; *, F-trap pyrene.

with 2 g of perfluorooctyl-modified silica gel. The cartridge was fluorophobically eluted with 4 mL of aqueous 80% (v/v) aqueous acetonitrile. A portion of the resulting eluate was subjected to the LC system.

Figure 8 (A) presents a typical chromatogram that we obtained using a standard mixture of 13 types of aliphatic (C_2−C_{16}) amines. We achieved a good separation of the pyrene-aliphatic amine derivatives within 40 min on an ODS column by means of stepwise gradient elution with 80% (v/v) acetonitrile and acetonitrile−water−tetrahydrofuran (8:1:1, v/v) as the mobile phases. In this chromatogram, we observed a small peak of the unreacted F-trap pyrene at around 28 min; however, this peak did not interfere with the separation and the quantification of all the amines. For comparative study, a diluent of the reaction mixture without F-SPE treatment was injected into the same LC system (Fig. 8 (B)). In contrast to the results shown in Fig. 8 (A), an unreacted reagent peak that was significant disturbed the analysis of *n*-tetradecylamine (C_{14}). Although F-trap pyrene exhibited similar elution behavior to those of the C_{14} and C_{16} derivatives in reversed-phase LC, it was selectively removed by F-SPE treatment before LC analysis. From these results, it was found that the criteria for removal of the unreacted reagent are determined not by hydrophobicity, but rather by fluorophobicity. The removal rate of the reagent and the average recovery of the amine derivatives by F-SPE were calculated from both the chromatograms in Fig. 8. Using the F-SPE with 80% (v/v) aqueous acetonitrile elution, over 99.7% of the unreacted F-trap pyrene was removed. The average recovery (average of peak-height ratios of 13 amine derivatives with and without F-SPE treatment) was calculated as 99.6%. The relationships between

the peak heights and the amounts of the 13 aliphatic amines were all linear (correlation coefficients > 0.998; n=5) over a concentration range of 50 fmol−50 pmol (except for *n*-hexadecylamine) and 100 fmol−50 pmol (*n*-hexadecylamine) per 20 μL injection. The precision of these results was established by repeated determinations (n=5) using a standard mixture of amines (100 μM each in a sample solution, 5 pmol each per 20 μL injection volume). The intra- and inter-day precisions of the peak heights (n=5, 5 pmol per injection each) were less than or equal to 5.8% and 7.0%, respectively. The detection limits (signal-to-noise ratio=3) of the amines were in the range of 3.6−25 fmol per 20 μL injection. We applied the proposed derivatization method to the analysis of amino acids. Figure 9 shows typical chromatograms that we obtained using a standard mixture of 16 amino acids with and without F-SPE treatment. We observed all the fluorescent peaks of the corresponding pyrene derivatives within 30 min. Although a peak of 1-pyrene-4-butyric acid, which is a hydrolysate of the reagent, was observed, no peaks of the unreacted F-trap pyrene were detected in the chromatograms after F-SPE treatment.

As distinct from other fluorogenic derivatization reagents in the F-trap fluorescence derivatization method, the criteria for the removal of the unreacted reagent are determined not by its hydrophobicity, but rather by its fluorophobicity. Therefore, a variety of F-trap fluorescence derivatization reagents with different fluorophores could be easily designed. In addition, because there are no perfluoroalkyl compounds in biological components, no attention needs to be paid to the decrease in the removal rates of analytes during F-SPE, in contrast to other solid-phase (normal-phase, reversed-phase and ion-exchange) extractions or liquid-liquid extraction.

For these reasons, we believe that F-trap pyrene could become a powerful tool for amine analysis that demands high sensitivity and comprehensive detection in proteomics and metabolomics research.

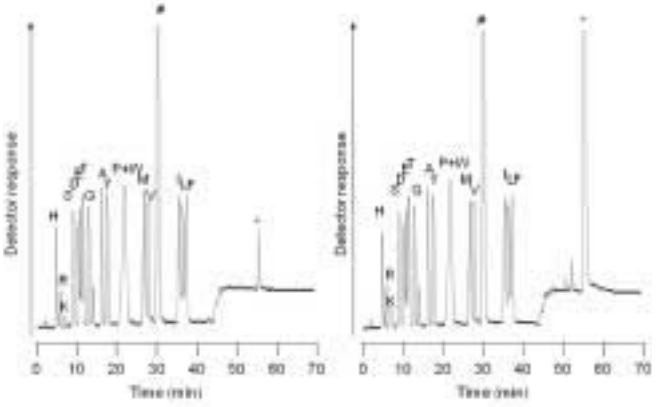

Fig. 9 Chromatogram of pyrene derivatives of 16 amino acids (A) with and (B) without F-SPE treatment (5 pmol each on column) using F-trap pyrene. Peaks: *, F-trap pyrene; #, Hydrolysate of the reagent.

5. Conclusion

We have developed the following three types of novel fluorescence derivatization-LC analysis systems: (1) simultaneous LC analysis of melatonin and its related compounds through post-column electrochemical demethylation and fluorescence derivatization, (2) LC analysis of 5-HIs based on fluorescence derivatization by online pre-column photocatalytic oxidation with benzylamine, and (3) reagent peak-free LC analysis for aliphatic amines and amino acids using F-trap pyrene as a fluorous tag-bound F-trap fluorescence derivatization reagent. All these methods allow reagent peak-free LC analysis that exhibits high sensitivity and selectivity toward its analytes, i.e., 5-MIs, 5-HIs, and aliphatic amines. We have also successfully applied these developed systems to biological and pharmaceutical analyses. We believe that these systems and reagents could become a powerful tool for the analysis of various pharmaceutical and biological samples.

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