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An easy-to-use excimer fluorescence derivatization reagent, 2-chloro-4-methoxy-6-(4-(pyren-4-yl)butoxy)-1,3,5-triazine, for use in the highly sensitive and selective liquid chromatography analysis of histamine in Japanese soy sauces

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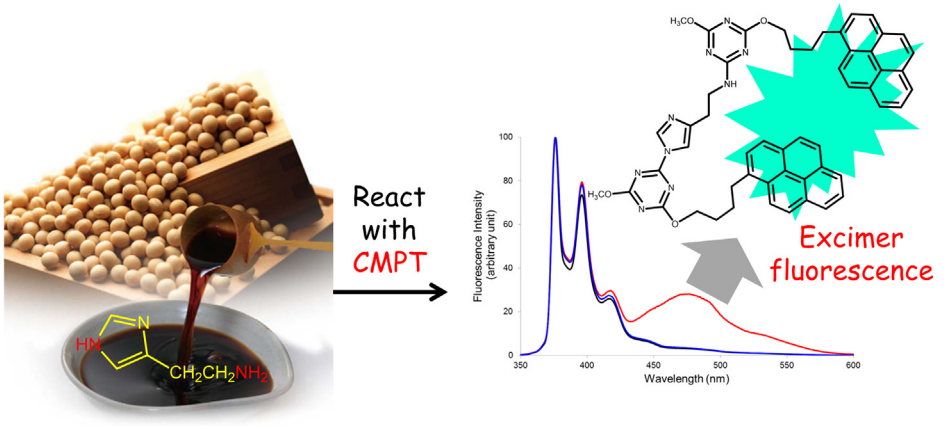
HIGHLIGHTS

- An easy-to-use excimer fluorescence derivatization reagent has been developed.
- Derivatives of histamine, tyramine and polyamines showed excimer fluorescence.
- The reagent is chemically stable and its reactivity is sustained for long period.
- The method was applied to analysis of histamine in commercial Japanese soy sauces.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a novel pre-column excimer fluorescence derivatization reagent, 2-chloro-4-methoxy-6-(4-(pyren-4-yl)butoxy)-1,3,5-triazine (CMPT), was developed for polyamines, specifically histamine. By CMPT derivatization, the polyamines, histamine and tyramine were converted to polypyrene derivatives, and emitted intra-molecular excimer fluorescence at 475 nm. This could clearly be distinguished from the normal fluorescence emitted from reagent blanks at 375 nm. Unlike conventional excimer fluorescence derivatization reagents, CMPT is chemically stable and its reactivity sustained over at least 36 days even in solution state. We successfully applied this reagent to the sensitive and selective analysis of histamine in different kinds of Japanese commercial soy sauces. The detection and quantification limits of histamine were 15 and 50 $\mu g L^{-1}$, respectively, equating to 1.35 pmol and 4.5 pmol for a 6 μL injection. This sensitivity helped the direct analysis of soy sauce samples only treated by one-step liquid–liquid

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Excimer fluorescence derivatization
Soy sauce

extraction without concentration. The histamine levels of commercial soy sauce samples (*koikuchi*, *usukuchi* and *saishikomi*) investigated were 1.24–768.5 mg L^{-1}.

1. Introduction

Histamine (Him) is well-known as a chemical mediator of allergy [1,2], gastric acid secretion [3,4], and neurotransmitter in the mammalian brain [5,6]. Because excessive intake of Him can cause allergy-like food poisoning, it is also known as a typical chemical marker of hazardous decomposition in food analysis [7,8]. Soy sauce is a traditional ingredient in East and Southeast Asian cuisines, where it is used in cooking and as a condiment. Soy sauce is produced from soybeans and wheat by fermentation due to microbes such as a lactic acid bacterium and yeast. During the fermentation process, it is possible that nonvolatile amines such as Him tyramine (Tym) and polyamines are synthesized. Similarly to soy sauce, fish sauces such as nuoc-mam and nam-pla may contain Him and Tym [9,10]. For export and import purposes, these sauces are globally treated according to the same safety regulations.

The maximum acceptable Him concentration in fish products is less than 200 mg L^{-1} in European Union (EU) countries [11], Switzerland and Norway [12]. Although Him levels in food products are not legally regulated in Japan, they will soon be regulated as in the EU. Therefore, a simple and sensitive monitoring method for Him during the production process of soy sauce is required. We recently established a simple, sensitive and reproducible method to analyze Him and Tym levels in Japanese soy sauce and its mash (called *moromi*). This method uses a hydrophilic interaction liquid chromatography with tandem mass spectrometry (HILIC-MS/MS). However, a simple and sensitive LC-based analytical method, which does not require large-scale MS, is desired by companies in the food manufacturing industry.

For this purpose, pre- and post-column fluorescence derivatization following LC analysis is an effective and practical approach. A number of reagents such as dansyl chloride [13], *o*-pthalaldehyde [14] and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate [15] have been used for Him derivatization. In these reagents, Him monoamines, amino acids, peptides and proteins are derivatized. As such, numerous peaks are detected in the chromatograms, which complicates the quantification of Him. In addition, an extraction procedure for Him using solid-phase extractions in cation-exchange or ion-pair mode is inevitable prior to the derivatization. The use of dansyl chloride further results in an excess of unreacted reagents, which are detected as large peaks in the chromatograms and interfere with Him quantification. Yoshitake et al. described a fluorometric LC method to determine Him concentrations in human urine, rat brain microdialysate and rat plasma and tissue, based on an intramolecular excimer-forming fluorescence derivatization with 4-(1-pyrene) butyric acid *N*-hydroxysuccinimide ester (PSE) [16–19]. The resulting polypyrene-labeled derivatives of Him provided intramolecular excimer fluorescence at a wavelength of 440–520 nm. These shifted markedly to the higher emission wavelengths compared to the wavelengths of non-derivatized pyrene monomers and monopyrene-labeled monoamines (360–420 nm). This chemistry facilitated the selective analysis of polyamines even in complex samples containing monoamino compounds. Further research led to the development of an excimer-forming fluorescence derivatization method using 4-(1-pyrene)butanoyl chloride (PBC) at mild conditions. This method can be used for derivatization of polyamines (putrescine, cadaverine, spermidine and spermine) [20], and the heterofunctional compounds tyrosine (Tyr) and Tym [21]. Both methods were successfully applied to analyze Him and polyamine concentrations in seafood [22] and soy sauces [23]. However, both PBC and PSE have some drawbacks: (1) they are moisture sensitive and hydrolyzed, (2) further purification is difficult. Therefore, the reactivity toward amines may differ between batches of commercial products. To eliminate these drawbacks, we developed the novel pre-column fluorescence derivatization reagent, 2-chloro-4-methoxy-6-(4-(pyren-4-yl) butoxy)-1,3,5-triazine (CMPT), for use in the highly sensitive and selective LC analysis of Him. Fig. 1 shows an intramolecular excimer-forming fluorescence derivatization reaction of Him with CMPT. This reagent has a highly fluorescent pyrene moiety and a chlorotriazinyl moiety which reacts to amines. By CMPT derivatization, Him and polyamines are converted to polypyrene derivatives and they emit intra-molecular excimer fluorescence. Since the intra-molecular excimer fluorescence derivatization enables highly selective detection of Him the sample pretreatment process was simplified to a one-step liquid–liquid extraction (LLE). The developed method was validated in terms of sensitivity, repeatability, linearity, accuracy and precision. We have successfully applied the method to a sensitive and selective analysis of Him in soy sauces. Quantification results were also compared with those reported using HILIC-MS/MS.

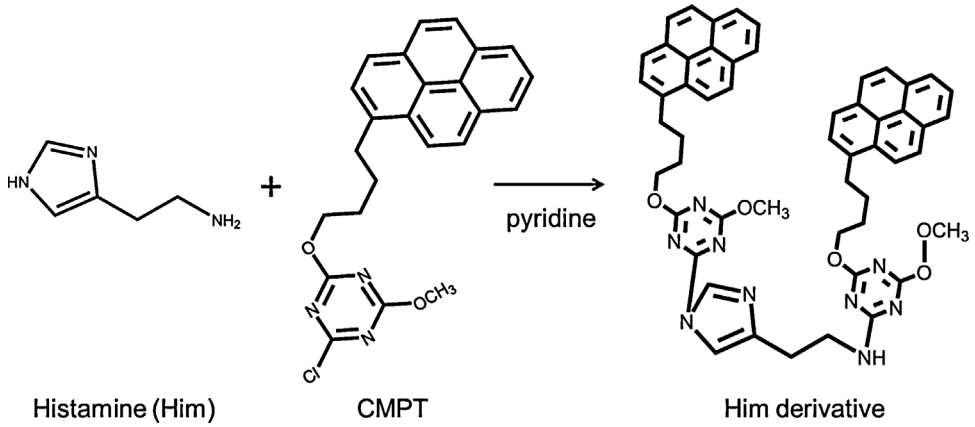

Fig. 1. Intramolecular excimer-forming fluorescence derivatization of Him with CMPT.