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Ethyl-propiolate as a novel and promising analytical reagent for the derivatization of thiols: Study of the reaction under flow conditions

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ABSTRACT

The present investigation demonstrates the potentials of ethyl-propiolate (EP), a novel derivatizing reagent for thiols. To the best of our knowledge this is the first systematic study of EP in analytical chemistry. The reaction was investigated under flow conditions using sequential injection (SI) analysis and UV detection at 285 nm. The reaction kinetics was affected by parameters such as the pH, the concentration of EP and the temperature and was thoroughly examined exploiting stopped-flow experiments. Cysteine (CYS) and captopril (CAP) were selected as model thiolic compounds in terms of chemical structures. Finally, the applicability of EP as a derivatization reagent for analytical purposes was demonstrated by the development and validation of a novel automated assay for the determination of CAP in pharmaceuticals.

1. Introduction

Thiols play an important role in the biological systems since they are related to a large number of biological phenomena. For instance, one of the naturally occurring thiols with simple structure, CYS, is a significant element in cellular metabolism process and protein structure [1]. On the other hand, thiol-containing drugs (e.g. CAP, tiopronine, penicillamine, etc.) are widely used for the treatment of a broad range of diseases. On this basis, there is an interest in developing analytical methodologies capable to determine both naturally occurring thiols and thiol-containing drugs in a variety of sample matrixes.

Derivatization is one of the most widely applied sample pretreatment protocols in analytical chemistry. The targets of derivatization reactions vary from improving the detectability of the analytes by tagging suitable chromo- or fluorophores, to modifying their chemical properties and increase their compatibility to the selected analytical techniques (e.g. improve the volatility for GC analysis, of fragmentation pattern for MS detection) [2]. In the case of separation techniques such as HPLC, derivatization can be carried out either pre-column (in batch or automated modes) or post-column. In capillary electrophoresis, derivatization is usually performed in batch, in-capillary or post-capillary modes. An ideal analytical derivatization reagent should fulfil most of the following demands: (i) rapid reaction under mild conditions, (ii) stability of the derivatives under the analysis conditions, (iii) minimum side-reactions, (iv) low cost and commercial availability, (v) high sensitivity and (vi) compatibility to modern analytical techniques.

The majority of the naturally occurring thiols such as CYS and its degradation product cysteamine, homocysteine, N-acetyl cysteine, reduced glutathione, the tripeptide glutathione or some drugs (CAP, penicillamine and 2-mercaptoethane sulfonate (MESNA)) lack of chromo- or fluorophore groups in their molecule. Consequently, their detectability is low and therefore their determination at low concentrations in complex matrixes may be problematic. A typical and widely accepted way to overcome this analytical problem is the derivatization of this group of compounds prior to the final measurement using suitable reagents.

The reagents used for the derivatization of thiols typically include tagging labels introducing UV–vis, fluorescent or electrochemical properties to the analytes. The analytical derivatization of thiols has previously been reviewed in detail [3–5]. Table 1 summarizes information about the most commonly used derivatizing reagents including data on the detection mode, the reaction conditions and the cost of the reagents. Fluorescent reagents such as monobromobimane (MBB) and 4-fluoro-7-sulfobenzofurazan (SBD-F) are not attractive due to the requirement of elevated temperatures and long reaction times [6,7]. Ellman's, 4,4′-dithiodipyridine (DTDP) and Thioglo®-3 (9-acetoxy-2-(4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)phenyl)-

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Table 1
Most popular derivatization reagents for thiolic compounds.

Reagent	Analyte	Detection mode	Reaction time (min)	Reaction conditions	Cost	Ref.
Monobromobimane (MBB)	Monothiols, polythiols	FL[a]	30	45 °C	110 €/25 mg[b]	[6]
7-Fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F)	Glutathione, cysteine, n-acetyl-cysteine, mercaptoacetic acid, γ-glutamylcysteine	FL	60	60 °C	60.5 €/5 mg[c]	[7]
Ellman's reagent	Total thiols	Vis	10	RT[d]	16.3 €/g[b]	[8]
4,4′-Dithiodipyridine (DTDP)	Mercaptans	UV	5	RT	29.7 €/g[b]	[9]
Methyl 9-maleinimido-8-methoxy-6,7-benzocumarin-3-carboxylate, (Thioglo®-5)	WR-1065	FL	10	RT	397 €/mg[b]	[10]
o-Phthaldialdehyde (OPA)	Organic thiols	FL	2	RT	47.3 €/250 mg[b]	[11]
Ethyl-propiolate	Cysteine glutathione homocysteine mercaptoethanol coenzyme A	UV	0.5	RT	36.8 €/5 g[b]	[12]

[a] Fluorescence.
[b] According to Sigma–Aldrich reagents' catalogue (2009).
[c] According to Fluka reagents' catalogue (2009).
[d] Room temperature.

3-oxo-3H-naphtho[2,1-b]pyran) react faster but the first two suffer from undesirable side-reactions while the high cost of Thioglo® – 3 limits its applicability to routine analyses [8–10]. o-Phthaldialdehyde (OPA) that is a common derivatizing reagent for primary amines, can also be used for the indirect fluorimetric or spectrophotometric determination of thiols [11]. Although OPA offer fast and sensitive assays, the main disadvantage of this reagent is the instability of the derivatives. Based on the previously-mentioned drawbacks of the already existing reagents for the derivatization of thiols and the biological significance of this group of compounds there is a continuous interest in proposing new alternative analytical approaches.

The "chemistry" and properties of propiolate esters are generally well-known mainly to organic chemists [12]. Ethyl-propiolate (EP) has recently been employed in a one-pot, four-components synthesis of benzene-1,2,3,5-tetracarboxylates promoted by triphenylphosphine [13], while its halogenated derivatives were used as substrates for direct, asymmetric alkynylation of cyclic β-ketoesters using chiral phase-transfer catalysts [14]. However, the feasibility and advantages of this group of compounds for analytical purposes has been roughly investigated only very recently by Owen [15]. In this study, the author presented data on the kinetics of the reaction, structure activity correlations, stereochemistry of the derivatives and preliminary results on the reaction of EP with proteins. The aim of this work was to carry out a systematic study of this promising derivatization reaction under flow conditions. The resulting data could be useful for more extensive applications of the reagent to separation science. For this purpose, cysteine (CYS) and captopril

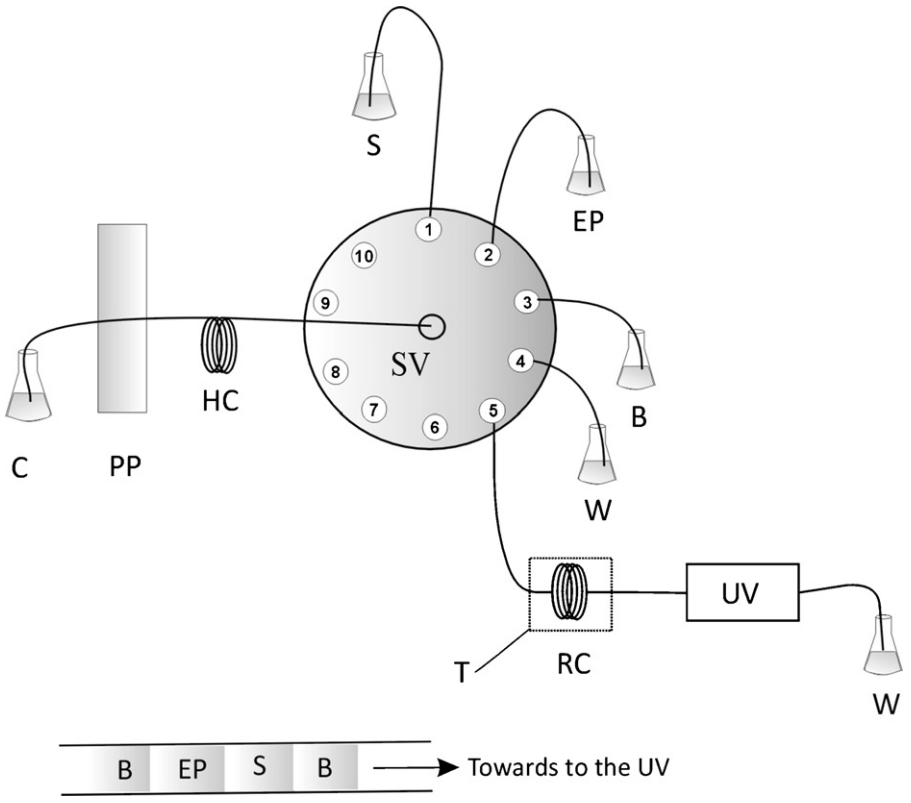

Fig. 1. SI manifold: C, carrier (water); PP, peristaltic pump; HC, holding coil (300 cm/0.5 mm i.d.); SV, selection valve; UV, detector (λ_{max} = 285 nm); S, sample; EP, ethyl-propiolate reagent; B, Britton–Robinson buffer; RC, reaction coil; T, thermostat; W, waste.