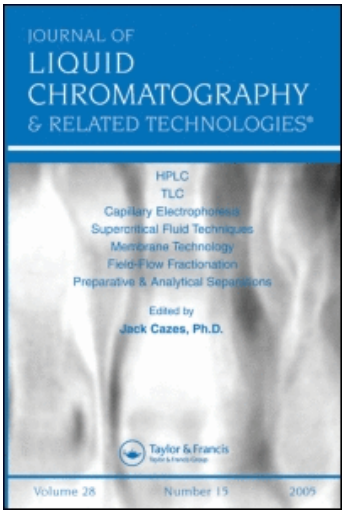

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THIN-LAYER CHROMATOGRAPHY OF ALIPHATIC THIOLS AFTER FLUORESCENT LABELLING WITH METHYL 4-(6-METHOXYNAPHTHALEN-2-YL)-4-OXO-2-BUTENOATE

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ABSTRACT

Aliphatic thiols of biopharmaceutical (cysteine,N-acetylcysteine,homocysteine,captopril,glutathione,mercaptop ropionylglycine) and cosmetic (thioglycolic acid, monothioglycerol,ammonium thiolactate) interest react under mild reaction conditions (10 min at room temperature) with methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate to give fluorescent adducts which can be separated on TLC silica gel plates. The fluorescent spots are visualized on irradiation at 254 and 366 nm.

INTRODUCTION

The analysis of aliphatic thiols of biopharmaceutical (Glutathione, cysteine, N-acetylcysteine etc.) and cosmetic (thioglycolic acid, thioglicerol, etc.) importance presents some difficulty, primarily owing to the weak detectability and high reactivity (oxidation to disulfide) of these compounds.

In the past few years, there has been a considerable interest in the development of fluorogenic reagents capable of yielding stable and highly fluorescent thiol derivatives suitable for subsequent chromatographic (HPLC, TLC) analyses (1-4). In particular, bromobimanes (5) and halogenobenzofurazans (6,7) have been applied successfully in thin-layer or high-performance thin layer chromatography of thiols with fluorimetric detection. Recently, naphthoylacrylic compounds also have been shown to be useful fluorogenic reagents for fluorimetric (8) and HPLC - Fluorescence (9,10) determinations of bioactive aliphatic thiols, providing good selectivity for the thiol function under mild reaction conditions. As an extension of this studies, the present comunication deals with the use of the methyl ester of 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butanoic acid as prechromatographic derivatization reagent for TLC identification of aliphatic thiols of pharmaceutical and cosmetic interest.

EXPERIMENTAL

Materials

Reduced glutathione, cysteine, N-acetylcysteine, N-(mercaptopropionyl)glycine (Thiola) and 2-mercaptoetanol were purchased from Fluka (Buchs,Switzerland) ;monothioglycerol was from Sigma (St. Louis,MO,USA) and 70% thioglycolic acid was obtained from Farmitalia C.Erba (Milan,Italy); Captopril was supplied by Squibb (Rome,

Italy) and ammonium thiolactate was supplied by Intercosmo (Bologna,Italy).

All other chemical and solvents were obtained from Farmitalia C.Erba (Milan, Italy).

Pre-coated flexible TLC silica gel sheets (Baker-flex IB2 20X20 cm; 200 μm thickness) were used.

Reagent and solutions

The reagent, methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate, was prepared and purified as previously described (10). The reagent solution was prepared in tetrahydrofuran(THF)(2 mg/ml) and the thiol solutions (5 mg/ml) were made in deionized doubly distilled water.

Derivatization procedure

In a 5 ml glass tube, 0.1 ml of aqueous thiol solution and 0.1 ml of 5% sodium acetate solution were added to 0.5 ml of the reagent solution. After 10 minutes at room temperature the reaction mixture was subjected to the TLC analysis. A similar procedure was followed with the reagent blank.

Thin-layer chromatography

TLC separations were carried out using the followimg mobile phases: (a),chloroform-methanol-acetic acid 8.5:1.5:1 (v/v/v),and (b),chloroform-methanol-acetic acid 9:1:0.2 (v/v/v).

A 3 μl aliquot of the derivatization reaction mixture was spotted at about 1 cm from the bottom of the TLC plate. The spotted plate was rapidly air dried and then developed,using the above indicated mobile phases into a standard TLC container, until the solvent front reached a height of about 10 cm. The spots were visualized through their intense fluorescence on UV irradiation at 254 and 366 nm.

RESULTS AND DISCUSSION

The reagent, methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate, is a pale yellow compound devoid of significant native fluorescence;the addition of thiols at the side chain double bond results in fluorescent thiol adducts.

The experimental conditions for the thiol derivatization were conveniently modified with respect to those used for reversed -phase HPLC analysis (10); in particular, a higher content of organic solvent(THF) in the reaction mixture was used in order to meet the requirements for a practical sample application and sodium acetate solution was used to obtain the adequate pH for the derivatization . Under the described conditions the reaction was found to be essentially complete after 10 min at room temperature

Aliphatic thiols of biopharmaceutical (cysteine,L-acetylcysteine,mercaptopropionilglycine,captopril,homocyste

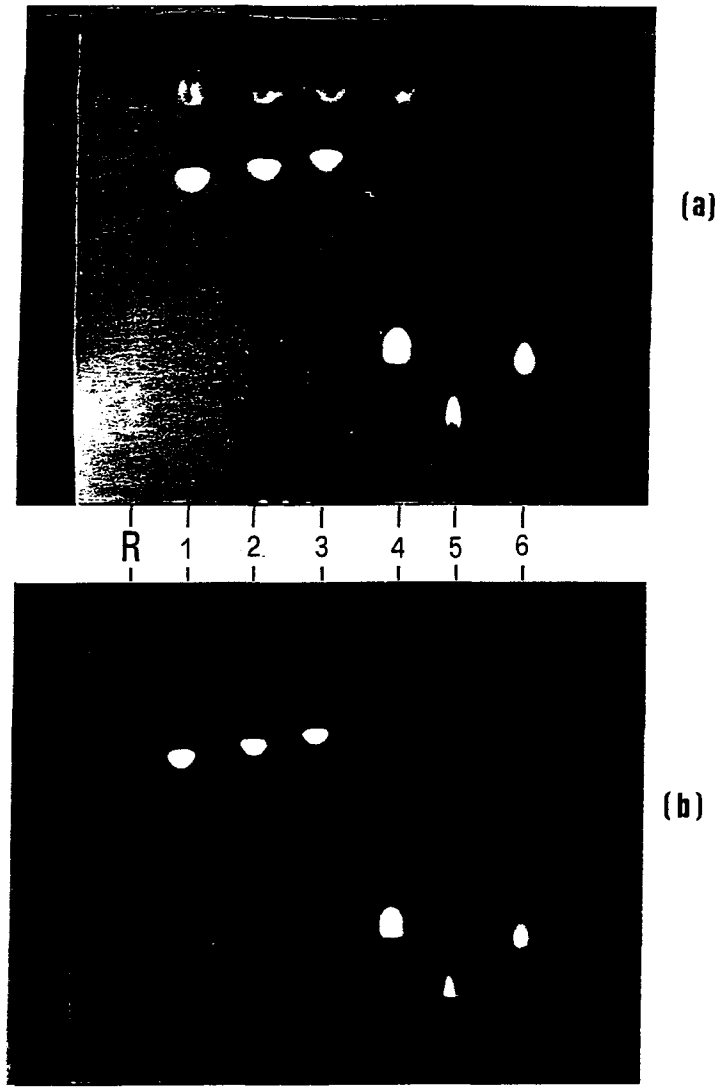

Figure 1

Typical TLC (silica gel) separation of thiol adducts from: 1:acetylcysteine, 2:mercaptopropionylglycine, 3:captopril, 4:L-cysteine, 5:glutathione, 6:homocysteine; R: reagent submitted to the reaction conditions. UV detection at 254 nm (a) and 366 nm (b). Mobile phase: chloroform-methanol-acetic acid 8.5:1.5:1.

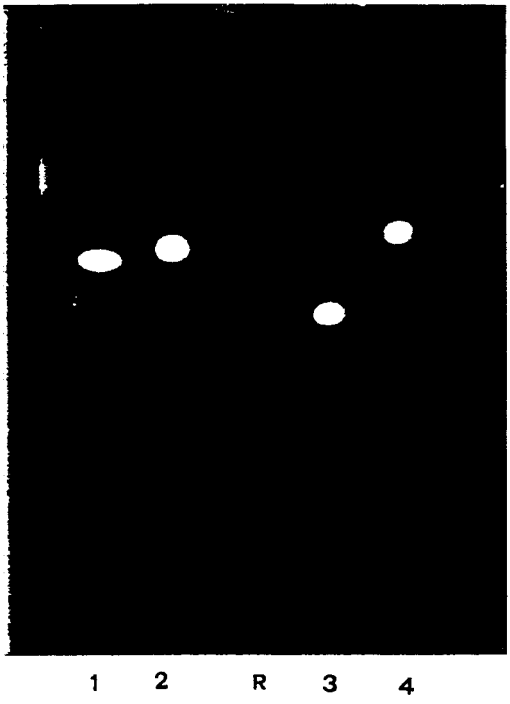

Figure 2

Typical TLC (silica gel) separation of thiol adducts from: 1:thioglycolic acid, 2:ammonium thiolactate, 3:monothioglycerol, 4:mercaptoethanol; R:as in Fig. 1. UV Detection at 366 nm. Mobile phase: chloroform-methanol-acetic acid 9:1:0.2.

ine , reduced glutathione) and cosmetic (thioglycolic acid, monotioglycerol, ammonium thiolactate) importance were subjected to the derivatization with methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate and the reaction mixture was directly chromatographed. Representative TLC separations are illustrated in Figs 1 and 2. As can be seen, the prechromatographic derivatization converts the aliphatic thiols in fluorescent derivatives which can be separated on TLC plates as fluorescent spots of satisfactory shape.The excess reagent migrates close to the solvent front and does not exhibits fluorescence Moreover, the derivatization reaction did not provide interfering reagent spots and side reaction products. Concerning the mobile phase composition, acetic acid was found to be useful to obtain compact spots. The Rf values obtained for the examined thiols were satisfactorily reproducible (RSD%:1.5-2.1). The detection limit by direct eye visualization was about 0.2 mg/ml for the initial thiol solution.

The described conventional TLC method can constitute a useful, selective identity test for aliphatic thiols, as the amino- and hydrxygroups are substantially unreactive under the reported reaction conditions. Moreover,the proposed reagent can offer the opportunity for the development of HPTLC-densitometric analyses (fluorimetric detection) of various aliphatic thiols in complex matrices.

Acknowledgements

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