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Short Communication

Improved high-performance liquid chromatographic separation for the analysis of oxalate in fungal culture media

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ABSTRACT

A rapid method to determine oxalate concentrations in liquid culture media of wood-rotting fungi has been developed using reversed-phase high-performance liquid chromatography. Separation was achieved on a Shandon Hypercarb column using 0.2 *M* orthophosphoric acid as the mobile phase, oxalate was detected at 210 nm. Oxalate was well resolved from other components in the culture medium, this enabled an accurate quantification of oxalate concentration in the range of 0.005–2 m*M*.

INTRODUCTION

The quantitative measurement of oxalate is required in many areas of biological research, such as medicine (urine and plasma analysis), the food and brewing industry and agriculture.

Many methods have been described for the detection of oxalate, the majority of which are based on enzyme assays, involving either oxalate oxidase [1] or oxalate decarboxylase [2]. These are highly specific reactions, but susceptible to interference in crude samples, therefore initial extraction and purification of oxalate is frequently required.

Gas–liquid chromatographic procedures have also been employed which require complex extraction and derivatisation of oxalate prior to analysis [3–6].

Of the numerous high-performance liquid chromatographic (HPLC) methods available, few are suitable for rapid analysis of a large number of biological samples. Many are time consuming, requiring pre-column removal of interfering substances, [7–9], in some cases with oxalate eluting close to the void peak preceding monocarboxylic acids such as formate and acetate. Many methods have detection problems due to sensitivity or selectivity, requiring derivatisation of oxalate prior to detection, such as precolumn derivatisation with *o*-phenylenediamine [10], or postcolumn derivatisation based on complexing with Fe^{3+} ions [11].

This paper describes a rapid, simple method to determine levels of oxalate in cultures of wood-rotting fungi by HPLC using a porous graphitic carbon column.

EXPERIMENTAL

Reagents

All reagents were prepared using HPLC-grade water supplied by Rathburn (Walkerburn, UK), HiPerSolv-grade orthophosphoric acid and Ana-

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laR-grade oxalic acid were purchased from BDH (Poole, UK).

Standards of oxalate were prepared in the range 0.005–2 m*M*.

Apparatus and procedure

A 20-μl aliquot of either sample or standard was injected onto a 10 cm × 4.6 mm I.D. graphitised carbon Hypercarb column (Shandon Scientific, Runcorn, UK), using a Merck–Hitachi LiChro-Graph HPLC System. Separation was achieved using 0.2 *M* orthophosphoric acid as the mobile phase.

The flow-rate of the mobile phase was maintained at 1 ml min^{-1} and separated compounds were detected at 210 nm.

Culture media preparation

Stationary cultures of the basidiomycete fungus, *Coniophora marmorata* 410 (Building Research Establishment, Department of the Environment) were grown in a defined liquid culture medium [12], using 10 g glucose as carbon source, for 35 days at 25°C.

Growth medium was collected and filtered to remove biomass. Aliquots (2 ml) were boiled for 5 min and subsequently centrifuged at 16 000 *g* for 10 min to remove denatured proteins from the media. The supernatant was filtered through a 0.45-μm membrane filter (Whatman, Maidstone, UK) prior to injection onto the column.

RESULTS AND DISCUSSION

Under the conditions described it was possible to determine oxalate levels in the culture medium. With 0.2 *M* orthophosphoric acid as eluent good resolution of oxalate from other components in the culture medium was achieved. At lower eluent concentrations (0.05 *M*) oxalate eluted as two peaks, which may be attributable to the differing pK_a values of the two carboxylic acid groups.

Linear regression analysis

Regression analysis was carried out on peak area against analyte concentration for oxalate. The correlation coefficient was 0.998 over the concentration range 0.005–2 m*M*. The detection limit was 0.1 nmoles on column in standard aqueous solutions and 1 nmole in culture medium.

Reproducibility

The variations in peak area were measured for an

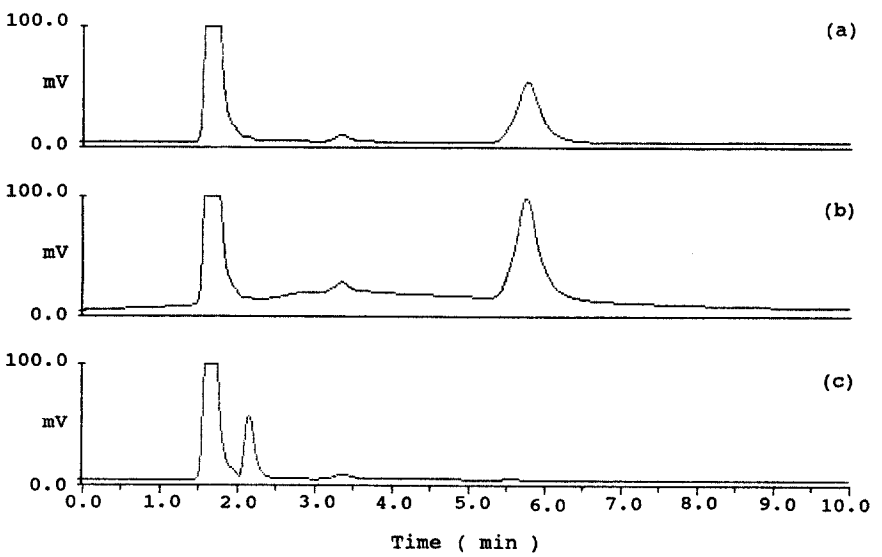

Fig. 1. Chromatogram of separation of oxalate in culture media: (a) after growth of *C. marmorata*, for 35 days at 25°C in a glucose–salts medium [12]; (b) spiked with 0.5 mmol of authentic oxalate; (c) degraded by the addition of 0.1 mg of oxalate decarboxylase in 0.2 ml of 0.2 *M* acetate buffer, pH 3.7, incubated at 37°C for 1 h. The peak at retention time 2.2 min corresponds to acetate from the acetate-buffered enzyme solution.

oxalate standard, and the cooefficient of variation between triplicate injections was found to be low (0.26%–1.28%), and between 10 different batches of eluent and oxalate standard was 4.84%. Standard oxalate solutions should be prepared fresh or kept at 4°C for a maximum of seven days to maintain the low coefficient of variation. Degradation of oxalate in solution has been reported [13].

Culture media analysis

The presence of oxalate in fungal culture media was identified by retention time and confirmed by spiking experiments with authentic oxalate and subsequent degradation with oxalate decarboxylase. Fig. 1 shows a representative chromatogram of the separation of oxalate in culture media after growth of *C. marmorata*, spiked with 0.5 mmol of oxalate, and its subsequent degradation by the addition of 0.1 mg of oxalate decarboxylase incubated at 37°C for 1 h. Some variation in retention time was apparent possibly as a result of other analytes present in the medium.

CONCLUSIONS

Oxalate has been satisfactorily resolved in fungal culture media without the need for isolation of oxalate and subsequent derivatisation, using reversed-phase HPLC on a Hypercarb column. Analysis was rapid and simple to perform thus facilitating analysis of a large number of samples.

As concentrated acids can be used as eluents on Hypercarb columns, separation is effected which would not be possible on acid-soluble silica columns. This method may therefore be useful for the determination of oxalate in other biological matrices.

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