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Short Communication

Rapid determination of malonic acid by ion-pair reversed-phase high-performance liquid chromatography

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Introduction

Malonic acid (methanedicarboxylic acid, propanedioic acid) is often a starting material or an intermediate in many syntheses. It is the parent compound of diethyl malonate, which is widely used in the pharmaceutical industry. One of the most striking properties of the double charged malonate ion is its ability to form complexes with many metals [1, 2]. Recently, it has been shown that coordination compounds of malonate with platinum are very effective against tumors [3–5]. Biochemical interest arises from the fact that malonate is an intermediate in fatty acid metabolism, produced by a malonate-CoA-transferase catalysed reaction [6].

In order to extend biomedical studies of platinum coordination complexes with malonate, synthesis of C-14 and C-11-labelled malonic acid had to be optimized. Hence a rapid, simple and specific determination of malonic acid is needed.

Analytical methods described up till now are either not specific, e.g. gravimetric determination and titrations [7], or require time-consuming derivatization, e.g. gas chromatography [8–10] and thin-layer chromatography [11]. High-performance liquid chromatography of organic acids uses either ion-suppression or ion-pair techniques [12–15].

A disadvantage of the ion-suppression technique is the very low pH of the mobile phase needed for chromatography of the acids, causing corrosion of metal parts and

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deterioration of column performance. So we developed an ion-pair reversed-phase high-performance liquid chromatographic method for the rapid determination of malonic acid in the presence of acetic acid, which is the most likely impurity.

Experimental

Chemicals

Malonic acid (99%) was purchased from Janssen Chimica (Beerse, Belgium). Acetic acid p.a. (99–100%) was obtained from U.C.B. (Leuven, Belgium).

All other reagents were of analytical grade. Water was deionized and double-distilled.

Apparatus and conditions

Chromatography was performed using a Waters Pump M-45. A Vari-Chrom liquid chromatography detector (Varian) was used at 220 nm. Full scale deflection was set at 0.1 a.u.f.s.

The mobile phase consisted of a solution containing 0.5% K_2HPO_4 and 0.001 M tetrabutylammonium hydrogen sulphate (TBA); this solution was buffered at pH 6.25 with H_3PO_4. The eluent was degassed in the ultrasonic bath and filtered through a 5 μm filter. A LiChrosorb RP C_{18} 10 μm column (250 × 4.6 mm) (Chrompack) was used. It was protected with a Direct Connect Guard Column, dry-packed with pellicular 30 μm RP C_{18} packing material (Alltech).

All determinations were performed at room temperature. The flow rate was 2 ml min^{-1}. The injection volume was 20 μl in all cases.

Quantitation

The calibration curve was constructed by manual injection of six aqueous solutions with different concentrations, ranging from 150 to 1750 μg ml^{-1} malonic acid. Peak heights were measured manually. The six values obtained were subjected to linear regression analysis and the slope and intercept were calculated.

Results and Discussion

Figure 1 shows the separation of acetic acid (0.9 mg ml^{-1}) and malonic acid (1.3 mg ml^{-1}). With a lower concentration of TBA as ion-pairing reagent (0.0001 M), no retention of malonic acid was seen. By increasing the concentration of TBA to 0.001 M, malonic acid was increasingly retained on the column and yet separated from acetic acid, the retention of which did not increase to the same extent. As a consequence of the pKa values of malonic acid ($pKa_1 = 2.83$, $pKa_2 = 5.69$), the pH of the mobile phase had to be raised above 5.69. At pH = 6.25, malonic acid is twice dissociated, resulting in retention, a good peak shape and separation from acetic acid (pKa = 4.75). Under these conditions, citric, fumaric and succinic acid were also separated.

The repeatability of the measurements was investigated by repeating chromatography ten times with the same solution of malonic acid (1312 μg ml^{-1}). A relative standard deviation of 0.546% was obtained.

A typical standard curve for malonic acid (concentration range 150–1750 μg ml^{-1}) resulted in the following linear least-squares regression equation: $y = 88.0x + 1.80$ ($r^2 = 0.9995$). The minimum amount detectable was 1.5 μg (signal to noise ratio of 5) at the wavelength used (220 nm).

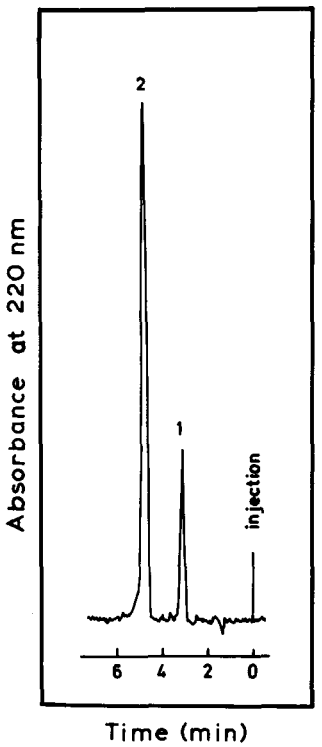

Figure 1
Chromatogram of a synthetic mixture containing acetic acid [1] and malonic acid [2].

The method was checked by standard addition recovery experiments on artificial mixtures, using the regression data. A relative recovery of 100.1% (conc. 1312 $\mu g\ ml^{-1}$) was obtained.

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