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Short Communication

High-performance liquid chromatography with electrochemical detection for the determination of thioctic acid and thioctic acid amide

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Introduction

Thioctic acid (1,2-dithiolane-3-valeric acid; TA) and thioctic acid amide (1,2-dithiolane-3-valeric amide; TAA) are used extensively for the treatment of liver disease. Many methods are currently available for the analysis of TA and TAA, including; thin-layer chromatography [1–4], polarography [5] and gas–liquid chromatography [6, 7]. However, none of these methods is altogether satisfactory due to their non-specificity, need for derivatization and lack of speed of analysis.

High-performance liquid chromatography (HPLC) is a highly sensitive, selective and relatively rapid technique for analysis of compounds of similar structure. However, very little work has been done on the determination of TA and TAA by HPLC. Howard *et al.* [8] separated TA and some of its analogues by reversed-phase LC on a C_{18} column using UV detection at 330 nm. As described the method appears to lack the sensitivity required for measuring these compounds at low levels.

In the present study TA and TAA are shown to undergo electrochemical oxidation which enables their detection at low concentrations by means of an amperometric detector. It is anticipated that this work will lead to further study of the analysis of TA and TAA by HPLC.

Experimental

Reagents and standards

Thioctic acid and thioctic acid amide were purchased from Sigma (St Louis, MO, USA). Both stock and standard solutions in methanol were prepared freshly before use. HPLC-grade acetonitrile, methanol, potassium dihydrogen phosphate (KH_2PO_4) and phosphoric acid were obtained from Wako (Osaka, Japan). All buffers were prepared in double distilled water.

Chromatographic apparatus and conditions

The HPLC apparatus consisted of a JASCO Model BIP-I pump (Japan Spectroscopic, Tokyo, Japan), a Rheodyne injector Model 7125 equipped with a 1-μl loop (Rheodyne, Berkeley, CA, USA), a JASCO Model 860-CO column oven, a Model VMD-101A electrochemical detector (Vanagimoto, Kyoto, Japan) and a Shimadzu chromatopac C-R3A digital integrator. The oxidation potential of the detector was set at +1.1 V versus a Ag/AgCl reference electrode. Separation was achieved with a prepacked Shim-pack CLC ODS column (Shimadzu, Kyoto, Japan) (150 × 6.0 mm i.d. 5 μm) at a column oven temperature of 40°C. The elution solvent was 50% acetonitrile in 0.05 M KH_2PO_4 adjusted to pH 2.0 with phosphoric acid. Before use it

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was filtered through a Millipore membrane filter (0.45 μm; Millipore, Bedford, MA, USA) followed by degassing using sonication under vacuum. The eluent was pumped at a flow rate of 1.0 ml min^{-1}.

Sample analysis

For formulations containing about 3% of TA or TAA 10-mg quantities were weighed accurately into 100-ml volumetric flasks and 90 ml of methanol was added. After sonication for 20 min, the flask was cooled and the solution made up to volume. An aliquot was centrifuged (2000 rpm) and 1 ml of the supernatant diluted to 50 ml with mobile phase. The final concentration was 3.0 μg ml^{-1} TA or TAA.

Results and Discussion

In view of the chemical characteristics of TA and TAA, and the requirement of a supporting electrolyte for amperometric detection, reversed-phase HPLC with a C_{18} stationary phase was selected along with a mobile phase consisting of acetonitrile and an aqueous solution of potassium dihydrogen phosphate (KH_2PO_4); conditions which led to the satisfactory separation of TA and TAA. The dependence of the separation on the mobile phase acetonitrile content, pH and on ionic strength of the mobile phase were studied. The capacity factors of TA and TAA are found to decrease with increasing acetonitrile content according to the relationship $\log k' = f[x]$ where [x] is the modifier concentration. For sufficient separation of TA and TAA, within a reasonable analysis time, an eluent containing about 50% acetonitrile was selected. The dependences of the capacity factors on the mobile phase pH and ionic strength were not almost shown.

Figure 1 illustrates the relationship between the oxidation potential applied and the relative response obtained after injection of a constant amount of TA and TAA (the highest reading obtained is taken as 100%). A potential of +1.1 V versus Ag/AgCl was selected for the electrochemical determination since it represented the point on the voltammograms where maximum sensitivity for each compound could be obtained.

The effect of the mobile phase pH and the concentration of KH_2PO_4 on the sensitivity then were examined. As can be seen from Figs 2 and 3, the sensitivity of the detector increased with decreasing mobile phase pH and with increasing concentration of KH_2PO_4 between 0.01–0.08 M. The sensitivity was almost constant in the range 0.05–0.08 M KH_2PO_4. Therefore, a 0.05 M KH_2PO_4 solution at pH 2.0 was selected as mobile phase.

The linearity of the detector response was tested by injecting 1-μl volumes of 0.5–5.0 μg

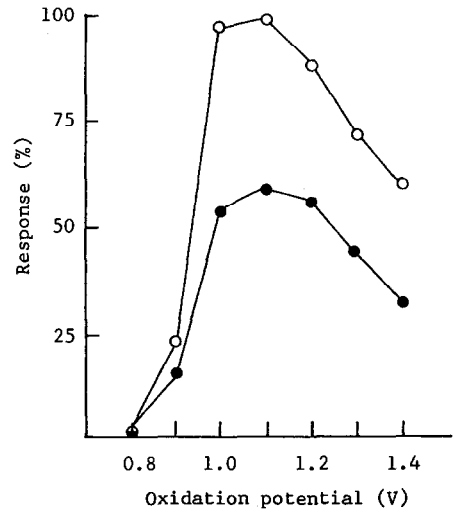

Figure 1
Dependence of electrochemical response upon applied oxidation potential. Mobile phase: 50% acetonitrile–0.05 M KH_2PO_4 (pH 2.0); flow rate, 1.0 ml min^{-1}; column temperature, 40°C. ●, Thioctic acid, ○, thioctic acid amide.

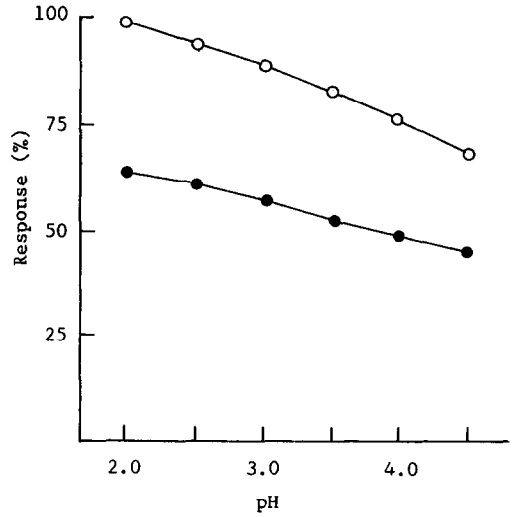

Figure 2
Dependence of the detector response upon the mobile phase pH. Mobile phase: 50% acetonitrile–0.05 M KH_2PO_4, adjusted to pH with H_3PO_4; flow rate, 1.0 ml min^{-1}; column temperature, 40°C; electrode potential, +1.1 V vs Ag/AgCl. ●, Thioctic acid; ○, thioctic acid amide.

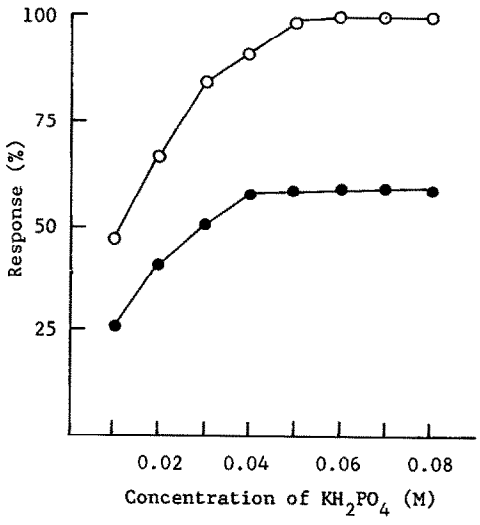

Figure 3
Dependence of the detector response upon mobile phase ionic strength. Mobile phase: various concentration of KH_2PO_4 (pH 2.0) with 50% acetonitrile; flow rate, 1.0 ml min^{-1}; column temperature, 40°C; electrode potential, +1.1 V vs Ag/AgCl. ●, thioctic acid; ○, thioctic acid amide.

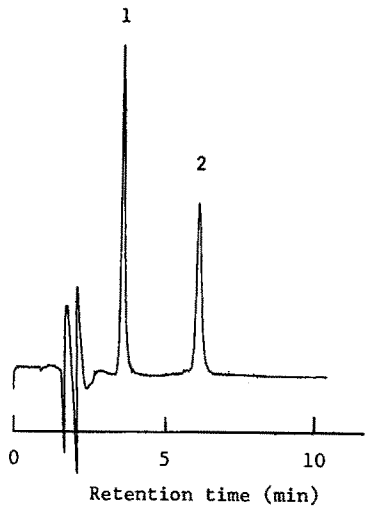

Figure 4
Chromatogram of a standard solution of thioctic acid (3 μg ml^{-1}), thioctic acid amide (3 μg ml^{-1}). Conditions as in the text.

Table 1
Recovery of thioctic acid (TA) and thioctic acid amide (TAA) from a spiked placebo

Sample no.	Amount added (mg g^{-1})	Recovery (%) TA	TAA
1	30	102.76	100.65
2	30	99.23	100.37
3	30	98.63	98.82
4	30	100.38	101.03
5	30	101.47	99.61
6	15	98.52	98.81
7	15	99.28	98.73
8	15	101.43	99.86
9	15	99.17	100.72
10	15	98.31	98.57
Mean		99.92	99.72
Range		98.31–102.76	98.57–101.03
% RSD		1.515	0.944

ml^{-1} solutions of TA and TAA. The relationships between the peak-height and the amount of TA and TAA were linear over the range 0.5–5.0 ng, with correlation coefficients of 0.997 and 0.998, respectively. A typical chromatogram of a mixture of TA and TAA is shown in Fig. 4. Under the present conditions the electrochemical detector was found to be more sensitive than the UV detector at 330 nm [8] by at least a factor of one hundred. The limits of detection for TA and TAA at 64 nAFS (nanoampere full scale) were 0.05 and 0.1 ng, respectively, at a signal to noise ratio of two.

The accuracy of the procedure was determined by spiking a placebo formulation with known concentrations (Table 1). Recoveries of TA and TAA averaged 99.92 and 99.72% with relative standard deviation (RSD) of 1.52 and 0.94%, respectively.

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