Journal of Chromatography, 606 (1992) 272–276
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 297

Short Communication

Determination of coenzyme Q by non-aqueous reversed-phase liquid chromatography

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(First received May 16th, 1991; revised manuscript received April 24th, 1992)

ABSTRACT

A non-aqueous reversed-phase liquid chromatographic method for determination of coenzyme Q_{10} in pharmaceutical formulations has been developed. The reversed-phase system provides better reproducibility and better selectivity for the separation of coenzyme Q_{10} analogues and degradation products than studied normal-phase systems. Furthermore, the non-aqueous mobile phase showed a very good solubility and provides a greater variety of work-up solvents for the lipophilic formulations than aqueous mobile phases. Validation studies showed detector response linearity over a concentration range of 0.2–100 μg/ml. The lower limit of detection was 2 ng on-column. The intra-assay precision (relative standard deviation) for a soy bean oil formulation was 2.0% (n = 11).

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) (see Fig. 1) is a lipid-soluble quinone which is the active component of various pharmaceutical preparations used in the treatment of cardiomyopathy and angina pectoris [1,2].

The determination of CoQ_{10} in formulations is commonly performed by straight-phase liquid chromatography [3]. This paper describes the usefulness of a non-aqueous reversed-phase liquid chromatographic (LC) method for analysis of the drug in various formulations. The non-aqueous mobile phase shows good solubility and provides a greater variety of work-up solvents for the lipophilic formulations than aqueous mobile phases. The method was applicable to the quantitative analysis of CoQ_{10} in samples as diverse as raw material, soy bean oil formulations and micelle-based preparations.

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EXPERIMENTAL

Chemicals

Methanol and *n*-hexane were of LiChrosolv or analytical grade (Merck, Darmstadt, Germany). Coenzyme Q_9 was from Sigma (St. Louis, MO, USA) and coenzyme Q_{10} from Dai-Nippon Chemicals (Osaka, Japan). Ubichromenol was synthesized in the laboratory by basic catalysis of CoQ_{10} with a tertiary amine by use of a method described elsewere [4].

Apparatus

The liquid chromatograph consisted of a Shimadzu LC pump, LC-6A (Shimadzu, Kyoto, Japan), with a Shimadzu SIL-6A autoinjector and an ABI S-1000 diode-array detector (Applied Biosys-

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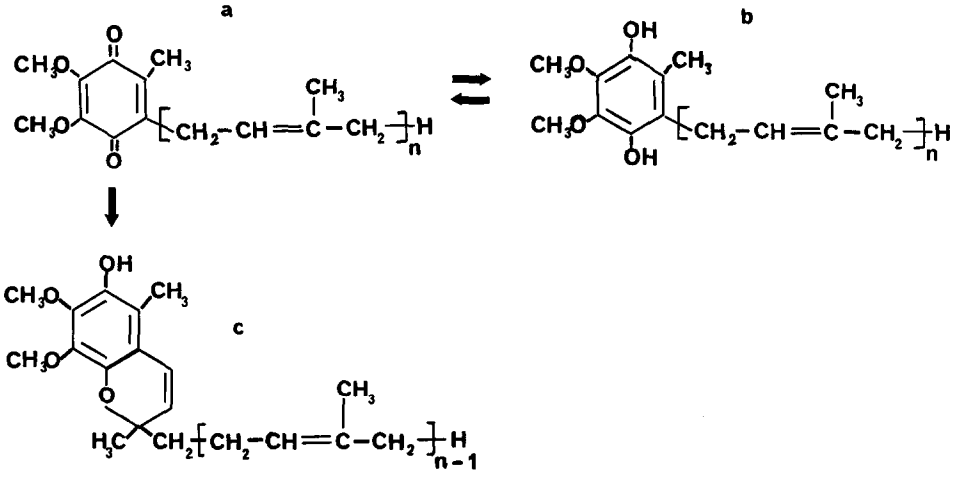

Fig. 1. (a) Chemical structure of CoQ_{10}, oxidized form, $n = 10$. (b) Chemical structure of CoQ_{10}, reduced form, $n = 10$. (c) Chemical structure of ubichromenol, $n = 10$.

tems, Foster City, CA, USA). For evaluation a Nelson 6000 chromatography data system (Perkin Elmer Nelson Systems, Cupertino, CA, USA) was used.

Sample preparation

(a) Soy bean capsules, 33 mg of CoQ_{10} (33.3 mg of CoQ_{10} suspended in soy bean oil). The capsule was opened with scalpel in a funnel over a 100-ml volumetric flask. The capsule was then transferred to the volumetric flask using forceps. The forceps, scalpel and the funnel were rinsed with hexane (the rinsing was performed by Pasteur pipette with 20 ml of hexane). The sample was ultrasonicated for 5 min and diluted to 100.0 ml with methanol and ultrasonicated again for another 2 min. A 2.00-ml aliquot of the sample was then diluted to 50.0 ml with methanol. A 20-μl aliquot was injected into the LC system.

(b) Mixed micelles, 4 mg/g CoQ_{10} (prepared from egg yolk lecithin/cholesterol and cholate). A 100-mg aliquot of the micelle preparation was dissolved in 100.0 ml of 20% *n*-hexane in methanol and ultrasonicated for 5 min. A 20-μl aliquot was injected into the LC system.

Chromatographic conditions

The analytical column (100 × 4.6 mm I.D.) contained Spherisorb ODS-2 material, 3-μm particles (Phase Separations, Queensferry, UK). The mobile phase, consisting of 10% *n*-hexane in methanol, was delivered at a flow-rate of 1.4 ml/min. The operative wavelength of the detector was 275 nm. The injected volume was 20 μl.

RESULTS AND DISCUSSION

The method uses an end-capped reversed-phase column with a mobile phase consisting of 10% *n*-hexane in methanol and UV detection at 275 nm. By using this non-aqueous LC method, problems associated with straight-phase liquid chromatography (*i.e.* non-reproducible retention times due to hydrogen bonding [5]) are avoided.

For the studied lipophilic formulations, which consisted of, for instance, triglycerides and phospholipids, this non-aqueous mobile phase showed a very good solubility compared with aqueous mobile phases, in which these components were poorly soluble.

Furthermore, this method provides better separation of CoQ analogues and major degradation products than straight-phase systems that have been studied. For instance, a heavily degraded CoQ_{10} solution gave very few, if any, extra peaks when it was analysed by the straight-phase method described by Palmer [5].

Fig. 2 shows the separation of oxidized and re-

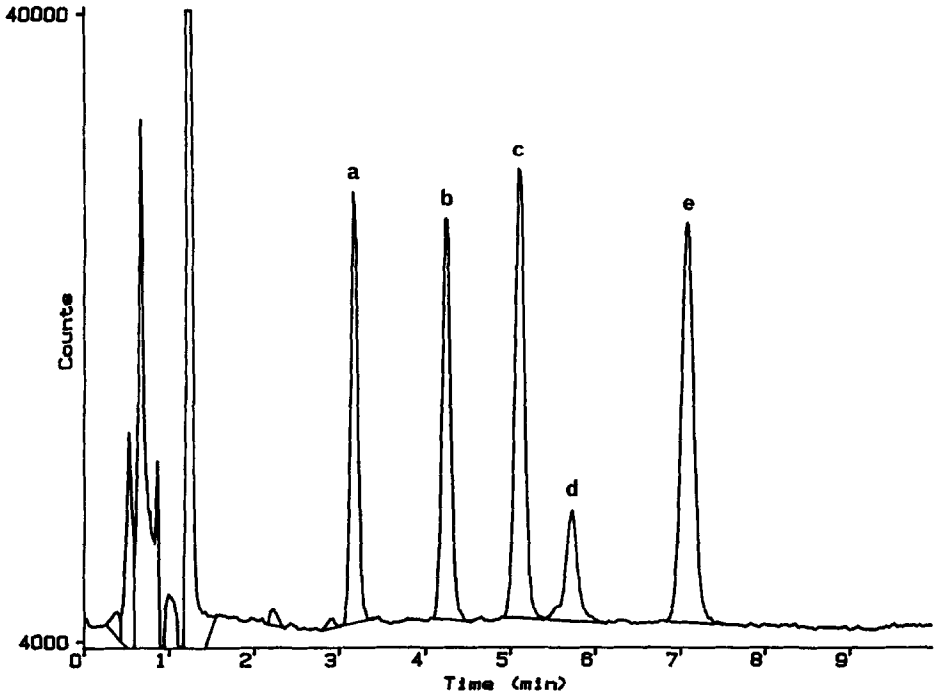

Fig. 2. HPLC separation of oxidized and reduced coenzyme Q_9, coenzyme Q_{10} and the major degradation compound ubichromenol. Peaks: a = CoQ_9, reduced form (100 μg/ml); b = CoQ_{10}, reduced form (100 μg/ml); c = CoQ_9, oxidized form (10 μg/ml); d = Ubichromenol (3.5 μg/ml); e = CoQ_{10}, oxidized form (10 μg/ml).

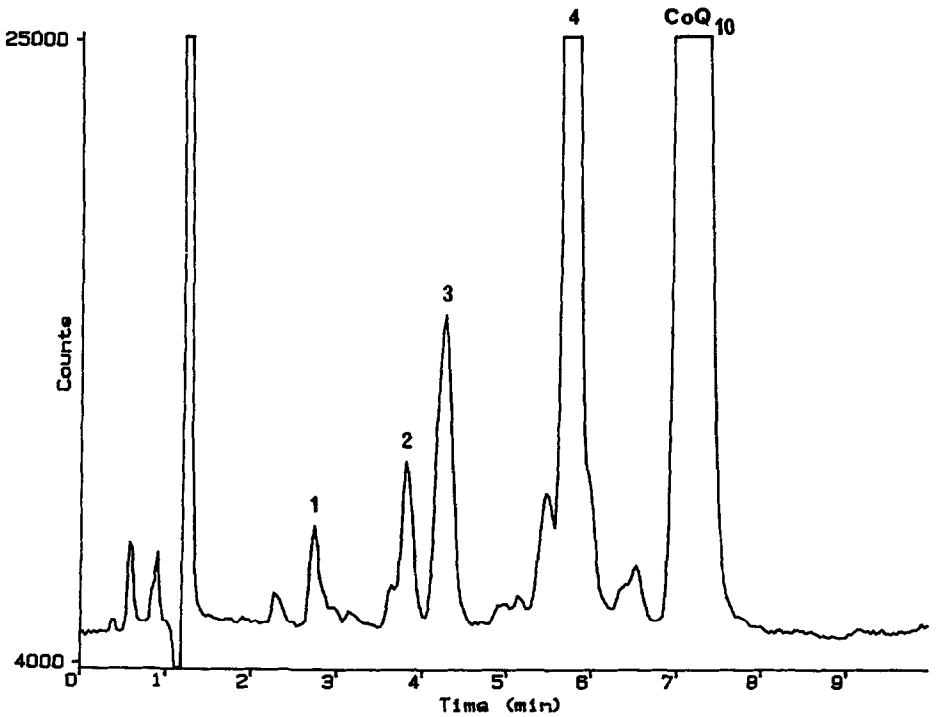

Fig. 3. Light-degraded raw material of CoQ_{10} after 20 h in daylight. Nominal content of CoQ_{10} was 500 μg/ml.

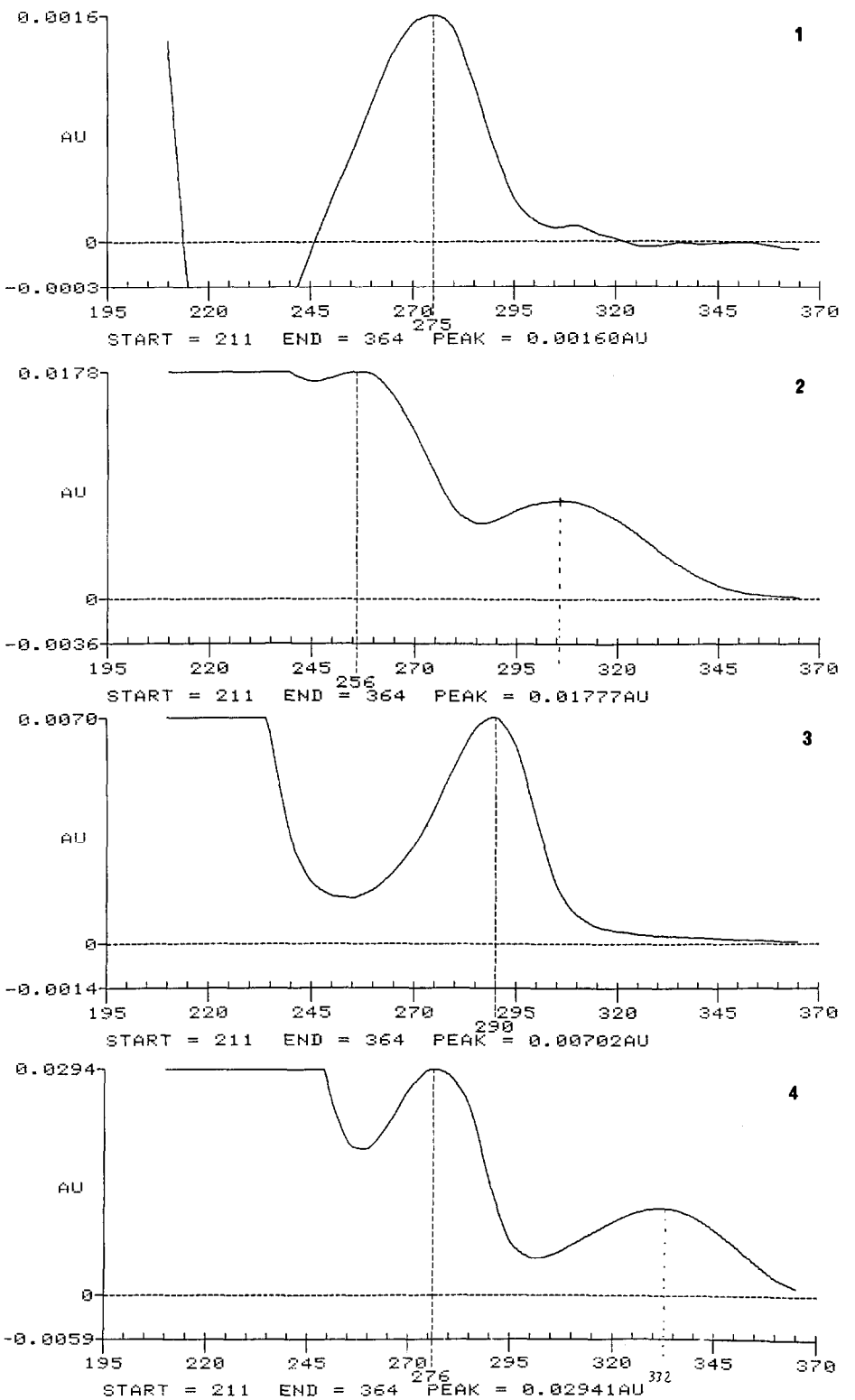

Fig. 4. UV spectra for some of the degradation products from the chromatogram in Fig. 3. 1 = Unknown; 2 = unknown; 3 = CoQ_{10}, reduced form; 4 = Ubichromenol.

duced CoQ_9, oxidized and reduced CoQ_{10} and the major degradation component ubichromenol (chemical structures in Fig. 1). These components could thus be easily quantitated within the same run.

In Fig. 3 a chromatogram from a light-degraded raw material is given. The identities of the unknown degradation products were checked using retention times and UV spectra. In Fig. 4, UV spectra for some of the unknown degradation products are shown.

For the studied formulations the determination of CoQ_{10} showed linear detector response in the concentration range 0.2–100 μg/ml. The lower limit of detection of CoQ_{10} at a signal-to-noise ratio of 3:1 (peak to peak) was 2 ng on-column. The intra-assay precision (relative standard deviation) of the method for soy bean capsules (5–20 μg/ml) was 2.0% (n = 11) and for micellar formulations (0.3–5.0 μg/ml) 2.6% (n = 6). The mean recovery of CoQ_{10} added to placebo capsules was 100.3 ± 1.9% (n = 6) and for CoQ_{10} added to a micelle vehicle was 99.7 ± 2.5% (n = 8).

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