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Note

High-pressure liquid chromatographic separation of 2-phenyl-1,3-indandione and its oxidation and rearrangement products

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2-Aryl-1,3-indandiones are still widely used as anti-coagulants[1–4]. Several methods have been reported for the analysis of 2-aryl-1,3-indandiones, but each has some disadvantage in specificity, sensitivity or convenience. The most commonly used analytical procedure involves solvent extraction followed by spectrophotometric determination in the UV region[5–7]. However, its specificity is questionable. 2-Aryl-1,3-indandiones have been reported to be unstable when exposed to air[8–15] and light[16,17]. The major oxidation products are 3-aryl-4-hydroxyisocoumarins (HIC) and 2,2'-diaryl-[2,2'-biindan]-1,1',3,3'-tetrones (dimers) and the light-induced rearrangement products are benzylidenephthalides (BZP). These compounds will interfere with the determination. In a previous paper we described a modification of the spectrophotometric method suitable for measuring 2-phenyl-1,3-indandione (PID) in the presence of its oxidation products[18]. The modification consists of calculation of the concentration by absorption at 461 nm, but unfortunately the sensitivity is low. The gas–liquid chromatographic (GLC) properties of PID have also been investigated[19]. GLC analysis after methylation of the acid function by the extractive alkylation technique has been reported to give satisfactory results. However, the GLC method has the disadvantage that it cannot be applied in studies on the stability of PID in air, because HIC will also be alkylated, and in addition, it is hydrolyzed in alkaline medium[12,13] (used in the extractive alkylation technique). Further, the dimer is not volatile enough to be analyzed by this technique and it is known that it can undergo base- and heat-catalyzed conversions[10,11]. Finally, a procedure has been described for the determination of 2-aryl-1,3-indandiones in which solvent extraction is followed by polarographic analysis[20], but this method has not been widely applied.

In attempting to devise a procedure by which the need for sensitivity and specificity in estimating PID in the presence of its oxidation and rearrangement products is satisfied, we tried high-pressure liquid chromatography (HPLC), which has been used successfully for the qualitative analysis of PID[21] and for the quantitation of the two structurally related 4-hydroxycoumarins, warfarin[22] and phenprocoumon[21].

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chro-

matograph model ALC/GPC 204, was used throughout this study. The system was equipped with a fixed-wavelength (254 nm) detector and a 12.5-μl flow cell. The flow-rate of the solvent mixture was 1.7 ml/min, with a column input pressure of 2000 p.s.i. Chromatograms were recorded on a Kipp model BD8 single-pen recorder with a 0–10 mV span.

Column

The chromatograph was fitted with a 30 cm long, 6.7 mm O.D., 4 mm I.D. reversed-phase μBondapak C_{18} column.

Solvents

All organic solvents were of analytical grade. The mobile phase consisted of a 3:1 mixture of methanol and distilled water.

Chemicals

PID was purchased from E. Merck (Darmstadt, G.F.R.). HIC, dimer and BZP came from the laboratory stock.

Sample preparation and calculation

Calibration curves were constructed using mixtures of equal amounts of the four compounds in a 1:1 mixture of methanol and chloroform, saturated with argon. The concentrations were 0.5, 1, 2, 5, 10, 20, 40, 60, 120 and 240 μg/ml. 25 μl of the methanol–chloroform solutions were injected. The extinctions (corresponding to the peak heights) were plotted against the amounts of compound added.

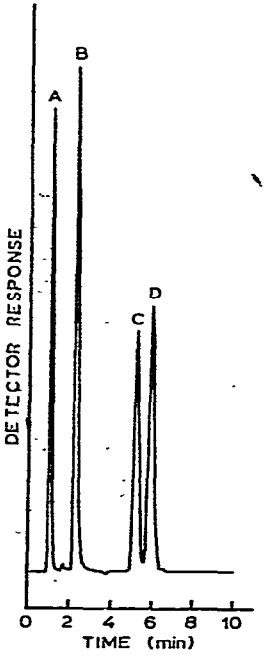

Fig. 1. High-pressure liquid chromatogram showing (A) PID; (B) HIC; (C) dimer; (D) BZP. The concentration of all four compounds was 20 μg/ml.

RESULTS AND DISCUSSION

A chromatogram of PID, HIC, dimer and BZP is shown in Fig. 1. The peaks corresponding to the four compounds have retention times of 1 min 3 sec, 2 min 16 sec, 5 min 12 sec and 5 min 57 sec, respectively. Since no peak overlap occurred, it may be concluded that HPLC is specific for all four compounds and, therefore, suitable for use in studies of the stability of PID. The following calibration curves were obtained:

PID: $E = 0.00023Q + 0.00061$ with $r = 0.998$ and $s = 0.0114$
where E is extinction and Q the quantity injected (in ng).
HIC: $E = 0.00028Q - 0.00406$ with $r = 0.999$ and $s = 0.0081$.
dimer: $E = 0.00015Q - 0.00233$ with $r = 1.000$ and $s = 0.0031$.
BZP: $E = 0.00014Q - 0.00231$ with $r = 1.000$ and $s = 0.0033$.
With a range of 25 ng–3 μg, the detector response was linear for each compound. It should be noted that depending on the injection volume the peak height can vary.

The method described here for the quantitative analysis of PID has a high sensitivity, the specificity is excellent, and its simplicity makes it useful for rapid analysis.

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