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Note

Gas chromatographic determination of the purity of vitamin K_3 (menadione)

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Menadione (2-methyl-1,4-naphthoquinone) or vitamin K_3, is a simple synthetic analogue with the physiological properties of vitamin K (refs. 1 and 2) which can be synthesized by oxidation of 2-methylnaphthalene with chromic acid–acetic acid or $K_2Cr_2O_7$–sulphuric acid[3]. In industrial processes yields are *ca.* 50–70%. The commercial product thus obtained contains small amounts of 2-methylnaphthalene and traces of tervalent chromium.

Methods for the determination of the purity of menadione are based on reductive titration with titanium(II) chloride[4], on reduction by potassium iodide and titration of the iodine generated[5], and on treatment with an excess of bromine and titration of unchanged bromine[6]. Official sources[6–9] suggest reduction to the hydroquinone form by $Zn–H_2SO_4$, followed by oxidative titration with cerium(IV) sulphate in the presence of *o*-phenanthroline–iron(II) sulphate as indicator. This gives correct results with purified menadione (purity $\geqslant 99.5\%$), but values which are too high when applied to commercial products. This is confirmed by the yield, lower than expected, obtained in the synthesis of water-soluble vitamin K by formation of its sodium bisulphite adduct[10,11].

Therefore, one or more unidentified products can be presumed to be present in variable amounts in commercial menadione, which are titrated by the cerium(IV) sulphate method but do not undergo the adduct synthesis.

Previously suggested methods for gas chromatographic (GC) analysis of menadione in pharmaceutical products[12–15] used SE-30, XE-60, NPGS and NPGA columns, but did not find any product which could be responsible for the observed effects. The isolation and identification of the unknown components have therefore been thoroughly investigated.

SEPARATION OF IMPURITIES

GC analysis of samples of commercial menadione on columns having the length, diameter and concentration of liquid phase suggested by Sheppard and Hubbard[13–15] did not show any extraneous peaks, except small amounts of methylnaphthalene and traces of 1,4-naphthoquinones. The use of columns filled with more

XE-60 than previously reported (5% on Chromosorb G AW DMCS, 80–100 mesh) having a length of 2 or 3 m and an I.D. of 2.2 mm, allowed the separation of a compound, present in low concentration, which was eluted very close to the menadione peak (see Fig. 1a). This unknown product (X) can be concentrated by crystallization of pure menadione from saturated solution in hot aliphatic alcohols. Repetition of this procedure gives menadione containing 20–30% of X. A chromatogram of the enriched mixture is shown in Fig. 1b.

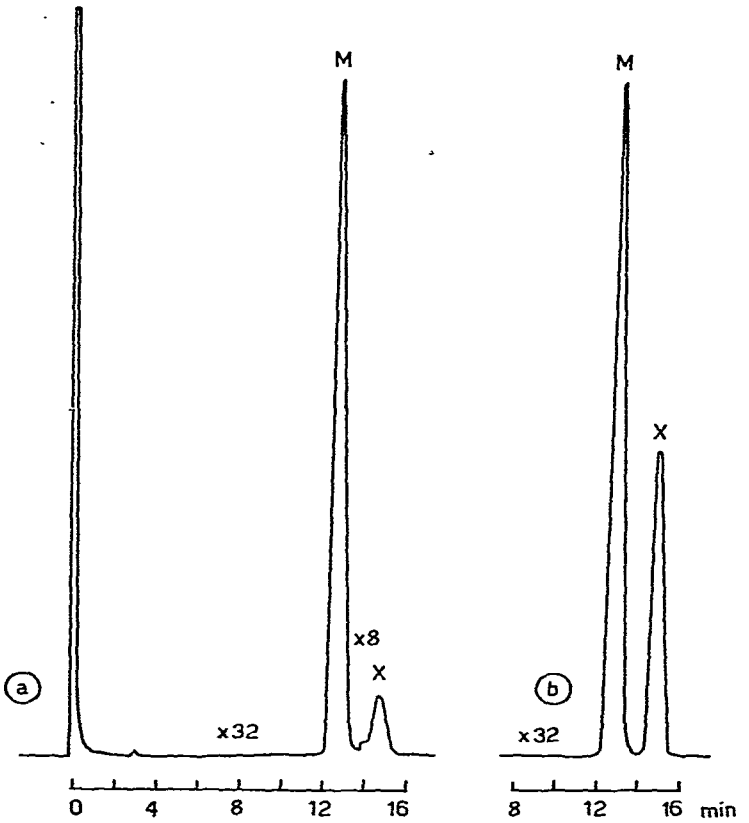

Fig. 1. Analysis of (a) commercial menadione (M) and (b) X-enriched mixture on 5% XE-60; 3m × 1/8 in. I.D. glass column, 190°, 30 ml/min carrier gas.

Other liquid phases previously suggested by various authors[12–15] [SE-30 2.5%; NPGA 1%; CRAIG polyester (BDS) 20%] were tested for separation of menadione and X by analysis of the enriched mixture. SE-30 2.5% did not separate the two components and a more enhanced tail of the menadione peak was only observed with mixtures containing about 20% of X. NPGA 1% and CRAIG polyester 20% gave the results shown in Fig. 2. BDS columns (or other polyester liquid phases) yielded a satisfactory difference in retention times but the peak tailing is high and therefore the determination of small amounts of X is difficult.

On the contrary, as shown in Fig. 1, the peaks on XE-60 5% are fairly sym-

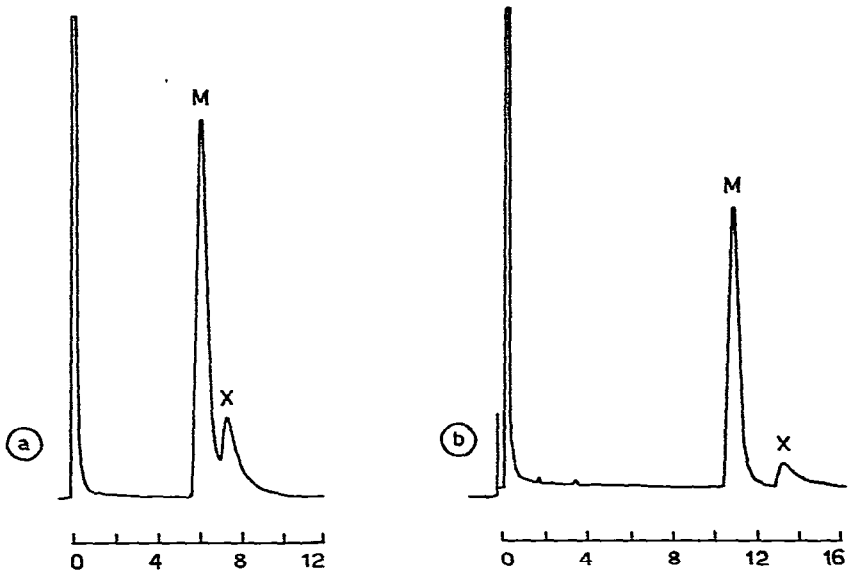

Fig. 2. Analysis of X-enriched mixture on (a) NPGA 1%, 190° and (b) CRAIG polyester 20%, 210°.

metrical and do not show appreciable tailing. Tailing factors of 98 and 94% were obtained for menadione and X peaks, respectively, 100% being the value for a perfectly symmetrical peak.

It is therefore possible that the tail shown by the menadione peak with lower concentrations of liquid phase, as reported previously[12], is due to incomplete separation of traces of X.

IDENTIFICATION OF THE UNKNOWN COMPONENT

The enriched mixture, containing *ca.* 30% of X, was further purified by preparative GC on an Aerograph Autoprep A 700, column 3 m × 1/4 in. I.D., containing the same stationary phase as used for analysis (XE-60 5%) at 180° and with helium as carrier gas. Samples of *ca.* 200 μl of concentrated solution in acetone were injected. The separation was initially very satisfactory, but after repeated injections column efficiency decreased. The previous resolution was completely restored by overnight conditioning at 220°.

The melting point of the X collected fraction, measured by a Mettler FP 52 melting point apparatus, mounted on a Reichert microscope, was 89–90°. GC–mass spectrometric (MS) data revealed that X is an isomer of menadione, with the methyl group substituted in the benzenoid ring. This is shown by the presence of a fragment with *m/e* 118, derived from the molecular ion (*m/e* 172) by loss of C_3H_2O, whereas the menadione spectra shows the typical peak at *m/e* 104 (refs. 16 and 17). On the basis of MS data, the *p*-quinone form is indicated by the similar intensity of the peak at *m/e* 144 (loss of CO from the molecular ion) in both menadione and X spectra. *o*-Quinones generally show more pronounced CO loss. Bar-graph MS spectra are shown in Fig. 3, and IR spectra in Fig. 4.

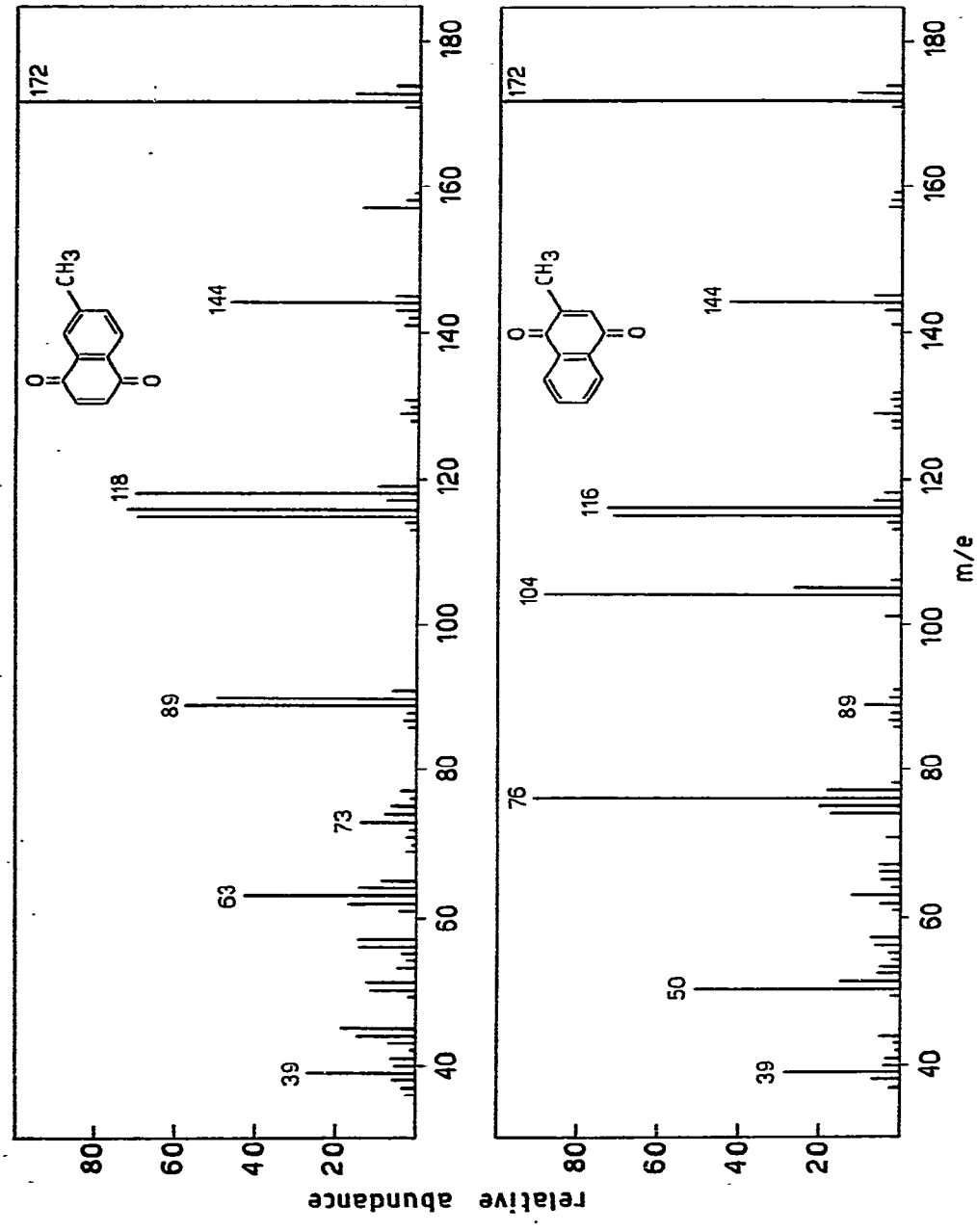

Fig. 3. MS spectra of X (6-methyl-1,4-naphthoquinone (a) and of menadione (2-methyl-1,4-naphthoquinone) (b).

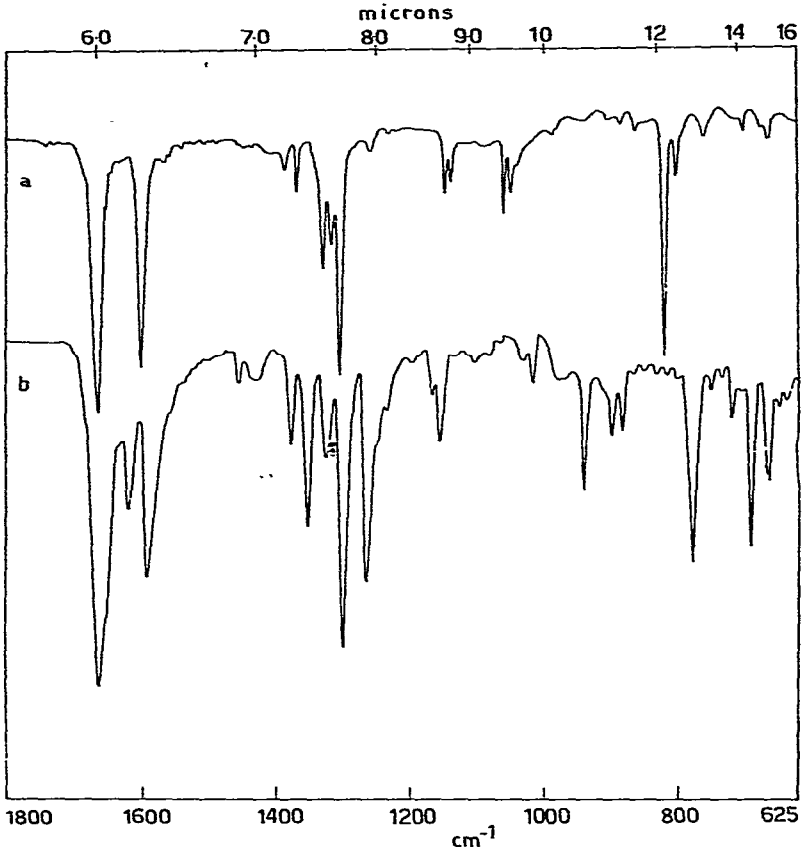

Fig. 4. IR spectra of (a) X, 6-methyl-1,4-naphthoquinone, and (b) menadione (2-methyl-1,4-naphthoquinone).

From the above results it can be suggested that the most probable identity of compound X is 6-methyl-1,4-naphthoquinone. This was confirmed by the preparation of authentic samples of 6-methyl-1,4-naphthoquinone, by modified diene synthesis of 1,4-naphthoquinones starting from isoprene and benzoquinone[18,19]. IR spectra, melting point and MS spectra of the synthesized standard were identical with those of X. The retention times on XE-60 and CRAIG columns of the synthesized compound perfectly correspond to those of X. Analysis of mixtures of menadione and 6-methyl-1,4-naphthoquinone on the XE-60 column previously described yielded fairly separated peaks (resolution of 1.5) with *ca.* 2400–2700 theoretical plates.

These results show that oxidation of 2-methylnaphthalene by $K_2Cr_2O_7$–H_2SO_4, yielding 50–70% of menadione, also produces variable amounts of 6-methyl-1,4-naphthoquinone (0.5–15% depending on reaction conditions). Generally speaking, menadione batches that give a low yield in the synthesis of water-soluble vitamin K, notwithstanding their high titration content, may have a high content of 6-methyl-1,4-naphthoquinone.

QUANTITATIVE ANALYSIS

The true content of commercial menadione can be determined using a fully GC technique, with internal standard (IS) method. The choice of the compound to be used as IS depends on the liquid phase used. With XE-60 5% the use of diethyl phthalate allows the IS peak to be eluted close to the 6-methyl-1,4-naphthoquinone (Fig. 5a), with a peak shape suitable for easy quantitation. The flame ionization detector (FID) correction factor of diethyl phthalate with respect to menadione was *ca.* 1.28. The FID correction factor for 6-methyl-1,4-naphthoquinone/menadione was 1 ($\pm$ 0.0015), as expected on the basis of the chemical nature of the two compounds.

Normal eicosane (C_{20}) was also used as IS, but it elutes far from menadione (Fig. 5b) and therefore the correction factor may depend on the analysis conditions.

Another suitable IS is 1,4-naphthoquinone (see Fig. 5c). Its correction factor

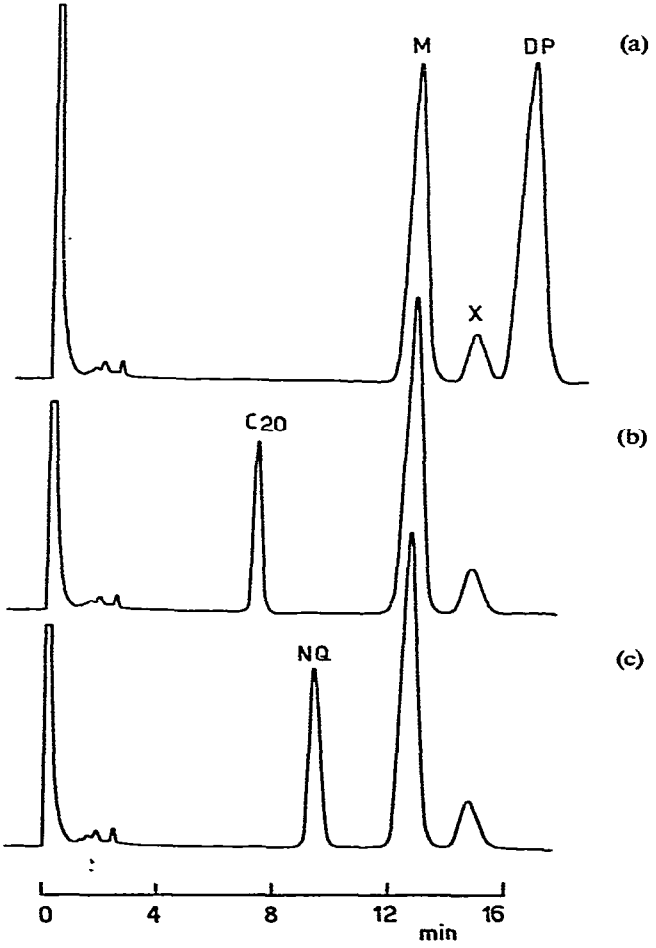

Fig. 5. Use of various internal standards in the quantitative determination of menadione (M) and 6-methyl-1,4-naphthoquinone (X). DP, diethylphthalate; C_{20}, *n*-eicosane; NQ, 1,4-naphthoquinone.

with respect to menadione, under the analysis conditions used, was 0.946. Two additional points have to be considered when using this compound as IS. First, it may be present in trace amounts in commercial menadione, as the 2-methylnaphthalene contains trace amounts of naphthalene, and this fact must be corrected for. Secondly, the concentration of 1,4-naphthoquinone standard solutions tends to change with time and on exposure to light, and therefore a fresh standard must be used for every series of analyses.

The standard official methods for determining the content of commercial menadione are based on titration techniques[8,9] which do not permit the determination of 6-methyl-1,4-naphthoquinone, as it is quantitatively titrated with its isomer and therefore gives a incorrect content of menadione. One can correct for this using GC analysis. As reported above, analysis of calibrated mixtures of pure menadione and of the synthesized and carefully purified 6-methyl-1,4-naphthoquinone showed that the relative responses to the FID of these two compounds are the same. Therefore, the ratio of the peak areas gives the relative amounts of the two components. No standard sample is required and time-consuming dilutions and calibrations are avoided. The presence of other reducing agents in the sample would influence the results of the titration. This is a limitation inherent in the official method, but can be generally excluded because menadione has been prepared by oxidation.

1,4-Naphthoquinone, titrated by the official methods, can be detected and measured, if present, by the GC technique (see Fig. 5c).

The values of accuracy (relative error) and precision (relative standard deviation)[20] of a series of 10 analyses of a known mixture of menadione and 6-methyl-1,4-naphthoquinone accomplished by a purely GC (IS 1,4-naphthoquinone) and by a titration–GC method are in Table I.

TABLE I

ACCURACY AND PRECISION OF THE TWO ANALYTICAL METHODS SUGGESTED FOR THE DETERMINATION OF MENADIONE

Method	*Relative error (%)*	*Relative standard deviation (%)*
Internal standard (1,4-naphthoquinone)	0.74	1.28
Cerium titration + GC	0.49	0.13

When small amounts of menadione have to be measured in pharmaceutical preparations the GC–IS method is the method of choice, using any of the standards listed above, depending on the retention time of other components eluted by the XE-60 column, in order to avoid interferences.

It is important to note that alkaline hydrolysis of water-soluble vitamin K (sodium bisulphite adduct) yields pure menadione, because 6-methyl-1,4-naphthoquinone does not follow the menadione reaction during the synthesis of vitamin K. This fact can be demonstrated only by GC analysis, because official titration methods do not discriminate between menadione and its isomer.

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