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Note

Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) has been used for the determination of menadione and phytomenadione in a variety of matrices[1–9], but there are few data on the determination of the water-soluble form of menadione [menadione hydrogen sulphite (MHS)] by this technique[4–10] and prior to HPLC analysis MHS was converted into menadione itself. There are no reports of the direct determination of MHS present in some multivitamin formulations. Nicotinamide (NA), a component of many mixtures of vitamins, is usually determined spectrophotometrically or microbiologically according to the USP XX[11] or by HPLC with spectrophotometric detection[12–15].

We therefore decided to establish chromatographic conditions that would allow the direct and simple determination of both vitamins in the mixtures present in some pharmaceutical formulations.

EXPERIMENTAL

Apparatus

A Pye Unicam HPLC system consisting of an LC-XPD pump, an LC-XP gradient programmer, an LC-UV variable-wavelength detector and a PM 82-51 single-pen recorder was used. It was equipped with a Rheodyne 7125 injector fitted with a 20-μl loop.

Materials

All reagents were of analytical-reagent grade. The eluents were filtered and degassed by sonication.

The trihydrate sodium salt of MHS (corresponding to 83.3% of sodium MHS), two multivitamin formulations, Polfasol B compositum (POB), which is a pre-mix for veterinary use (batches 1 and 2), and Falvit (FAV) coated tablets, and nicotinamide (NA) were obtained from Polfa, Poland. Hetrazeen (HTZ), containing a water-soluble MHS product, used as a raw material for POB, was obtained from Heterochemical Co. (U.K.).

Conditions for chromatographic separation and quantification

A Nucleosil 10 C_{18} column (250 × 4.6 mm I.D.) was used. Mixtures of meth-

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anol or acetonitrile with water, modified by addition of several substances such as acetic acid, triethylamine, cetyltrimethylammonium bromide or sodium dodecyl sulphate, were tested as mobile phases. A mobile phase consisting of methanol–1% acetic acid (25:75) was finally selected.

The flow-rate was 1 ml/min, the recorder chart speed was 0.5 cm/min and UV detection at 270 nm was applied.

Standard solutions for calibration graph

Amounts corresponding to 0.73, 1.17, 1.77, 2.59 and 3.39 mg of sodium MHS of 100% purity were weighed and transferred into 100-ml volumetric flasks, dissolved in water and diluted to volume. Amounts of NA of 1.074, 1.601, 2.466, 3.814 and 5.184 mg were weighed and transferred into 25-ml volumetric flasks, dissolved in water and diluted to volume.

Determination of menadione hydrogen sulphite and nicotinamide

Standard solutions. About 1 mg of sodium MHS and 10–16 mg of NA were

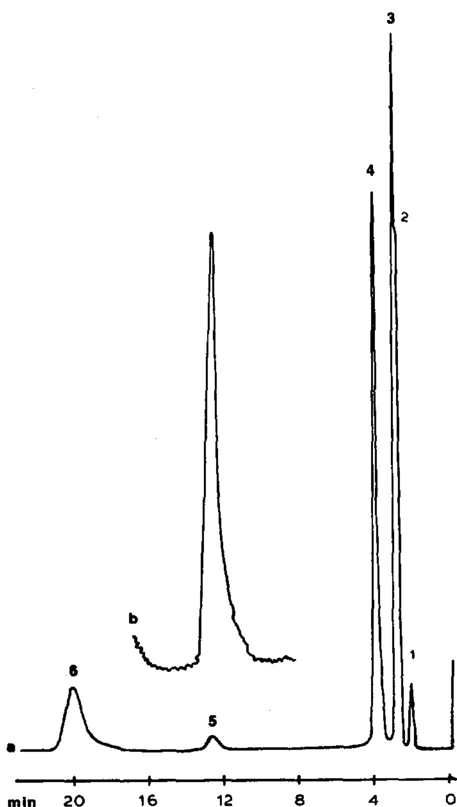

Fig. 1. Chromatogram of an aqueous extract of POB (about 0.5 g of pre-mix in 50 ml). Peaks: 1 = thiamine; 2,3 = pyridoxine + ascorbic acid; 4 = NA; 5 = MHS; 6 = riboflavin. Chromatographic conditions: Nucleosil 10 C_{18} column (250 × 4.6 mm I.D.); mobile phase, methanol–1% acetic acid (25:75); flow-rate, 1 ml/min; UV detection (270 nm); 0.64 a.u.f.s. (a) and 0.02 a.u.f.s. (b); recorder chart speed, 0.5 cm/min.

weighed accurately and transferred into 100-ml volumetric flasks, dissolved in water and diluted to volume.

Sample preparation. About 0.5 g of POB pre-mix or FAV ground tablet powder was weighed into a 50- or 100-ml volumetric flask, respectively, and extracted with water, then the mixtures were diluted to volume and filtered. About 2 mg of HTZ was weighed accurately and transferred into a 100-ml volumetric flask, extracted with water and diluted to volume. The concentrations of MHS were 4–10 μg/ml and those of NA 90–160 μg/ml.

Sample preparation for recovery experiments. Amounts of POB corresponding to about 0.5 mg of MHS (according to the mean result of previous determinations on that batch) were weighed into 50-ml volumetric flasks, then about 1 mg of sodium MHS was added to each, mixed, extracted and diluted with water as above.

RESULTS AND DISCUSSION

Optimization of the mobile phase

Isocratic elution with mobile phases such as those used for the analysis of other water-soluble vitamins mixtures that include NA in the absence of MHS [11–13] appeared to be unsuitable for this compound. Good results have been obtained with gradient elution. Several gradient elution profiles were employed, the fastest of them being provided by a linear increase in methanol concentration from 20 to 40% (v/v) in 1% acetic acid solution over 1 min, then kept fixed for 15 min. Nevertheless, the time required to accomplish the programme was not less than the duration of the isocratic elution with methanol–1% acetic acid (25:75) owing to the necessity for re-equilibration of the column with the starting mobile phase before injection of a subsequent sample. We also obtained satisfactory results using this mobile phase in isocratic elution and it was therefore finally selected for the quantitative determination.

Fig. 1 shows a chromatogram of an aqueous extract of POB multivitamin formulation chosen as a model mixture. The peak of NA is well resolved from the first eluted components and that of MHS is sufficiently distinct from it for necessary changes in the range of detector attenuation. The last peak (riboflavin) indicates the end of the elution and appears about 20 min after injection of the sample.

Calibration graph

The ratio of the concentrations of MHS and NA in the pharmaceutical prep-

TABLE I

RELATIONSHIP BETWEEN PEAK HEIGHT (h) AND CONCENTRATION OF THE NA SOLUTIONS (c)

c ($\mu g\ ml^{-1}$)	Detector range (a.u.f.s.)	h (mm)	h at 0.01 a.u.f.s./c
42.96	0.16	145	54.00
64.04	0.16	214	53.47
98.64	0.32	162	52.55
152.56	0.64	121	50.76
207.36	0.64	159	49.07

TABLE II

RESULTS OF THE DETERMINATION OF MHS AND NA

Formulation	*MHS**		*NA*		
	HPLC method (mg/g)	*R.S.D.*	*HPLC method (mg/g)*	*R.S.D.*	*Control method (mg/g)***
POB pre-mix containing vitamins K, PP, B_1, B_2, B_6, B_{12} and C and mineral salts	0.84 0.89	0.04 (n = 8) 0.04 (n = 4)	16.30 16.25	0.03 (n = 9) 0.01 (n = 4)	15.26 15.45
Hetrazeen containing amount of MDHS*** corresponding to 334 mg/g of MHS	351	0.02 (n = 2)	–	–	–
FAV coated tablets containing vitamins PP, A, B_1, B_2, B_6, B_{12}, D_3 and C and mineral salts	–	–	20.20	0.05 (n = 4)	22.09

* As $C_{11}H_{10}O_5S$.
** POB, microbiological; FAV, polarographic.
*** Menadione dimethylpyrimidinol hydrogen sulphite.

TABLE III

RECOVERY OF SODIUM MHS

Sodium MHS (mg)			*Recovery (%) (D)**
Calculated in sample (A)	*Added (B)*	*Determined (C)*	
0.484	0.974	1.443	98.45
0.500	0.853	1.333	97.65
0.489	0.778	1.261	99.23
0.490	0.813	1.288	98.15
			Mean: 98.4

* $D(\%) = \frac{100\,(C - A)}{B}$.

arations is about 1:10, so the concentrations of NA solutions were greater than those of MHS. The calibration graph obtained for MHS was linear in the chosen range; the mean ratio of the height of the peaks calculated at an attenuation of 0.01 a.u.f.s. to the concentration was 15.45 mm/μg $\cdot$ ml^{-1} (R.S.D. = 0.02, n = 5). This ratio for NA decreased from 54.0 to 49.1 mm/μg $\cdot$ ml^{-1} in the concentration range studied (Table I). Therefore, for the quantitative determination of NA the concentrations of the sample and standard solutions must be approximately equal.

Quantitative determination

In addition to POB, we determined NA and MHS in preparations that contained only one of these vitamins, *viz.*, NA in the FAV multivitamin coated tablets and MHS in HTZ used for the production of POB pre-mix. We compared our results of the NA determination with those obtained in the control analysis by microbiological or polarographic methods and, as can be seen (Table II), they agreed satisfactorily. It was essential to wash the column daily with methanol for at least 1 h in order to remove the components that had not been washed out with the mobile phase.

Our method was checked on MHS by its recovery from POB samples with standard additions of MHS (Table III). The amounts of MHS in these samples were calculated from the mean result of the determination of MHS in samples from the same batch. The mean recovery was 98.4%. The precision of the method was investigated by analysing one batch of FAV and HTZ and two batches of POB. Relative standard deviations of 0.01–0.05 were obtained for NA and 0.02 or 0.04 for MHS (Table II).

The method described here appears to be suitable for the direct determination of MHS in complex vitamin mixtures. It could be also used for the determination of riboflavin both in the studied formulations and in others of similar composition within a reasonable analysis time.

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