GLC Analysis of Menadione Bisulfite Addition Compounds by On-Column Pyrolysis to Menadione

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Abstract □ The GC determination of the on-column pyrolysis product of menadione bisulfite addition compounds as menadione is described. Differential thermal analysis, mass spectroscopy, and GC retention times conclusively prove the feasibility of on-column pyrolysis of menadione bisulfite addition compounds to menadione. Recovery values of menadione content of powdered and liquid injectable menadione bisufite compounds were the following: menadione sodium bisulfite (trihydrate) powder, 98.59 ± 3.4%, 5 mg./ml. liquid injectable, 97.04 ± 1.46%, and 10 mg./ml. liquid injectable, 100.40 ± 0.50%.

Keyphrases □ Menadione bisulfite addition products—GLC analysis, on-column pyrolysis to menadione, thermal decomposition confirmed by differential thermal analysis and GC–mass spectroscopy □ Pyrolysis, menadione bisulfite to menadione—GLC analysis of menadione bisulfite addition products, thermal decomposition confirmed by differential thermal analysis and GC–mass spectroscopy □ GLC—analysis, menadione bisulfite *via* pyrolysis to menadione □ Differential thermal analysis—determination, menadione bisulfite pyrolysis to menadione □ GC–mass spectroscopy—determination, menadione bisulfite pyrolysis to menadione

Menadione sodium bisulfite (sodium salt of 2-methyl-1,4-naphthoquinone-2-sulfonic acid) is a water-soluble derivative of menadione (2-methyl-1,4-naphthoquinone); menadione is the primary synthetic analog with the physiological properties of vitamin K. The NF XII method for analysis of menadione sodium bisulfite consists of extraction of menadione with chloroform (formed from an alkaline solution of menadione sodium bisulfite), followed by a reduction step and ceric sulfate titration (1). Menadione sodium bisulfite also is quantitated by comparing the UV spectra of extracted menadione with a solution of menadione reference standard in chloroform (2).

However, with chloroform extraction of menadione from an alkaline solution, decomposition of menadione subsequently results and often low recovery yields are obtained. To avoid the decomposition problem, Johnson (3) recently described a diatomaceous earth[1] chromatographic method which removes menadione from an aqueous alkaline solution nearly as quickly as it is formed. GC methods also have been used to analyze menadione and menadione sodium bisulfite solutions (4–6), but here again the chloroform extraction of alkaline solutions of menadione sodium bisulfite is employed. Without a doubt, direct analysis of menadione sodium bisulfite without alkaline treatment would be the method of choice. This report describes a direct GC analysis of menadione sodium bisulfite as the on-column desulfonated pyrolytic product, menadione. Results generated by differential thermal analysis and GC–mass spectroscopy establish that menadione is the major volatile product of the thermal decomposition of menadione sodium bisulfite.

[1] Celite.

EXPERIMENTAL

Materials—The following materials were used: (*a*) reference standard of menadione NF[2]; (*b*) menadione sodium bisulfite (trihydrate)[3]; (*c*) menadione sodium bisulfite complex (trihydrate)[2]; (*d*) 5- and 10-mg./ml. menadione sodium bisulfite injection solutions[4]; (*e*) diethyl phthalate for internal standard[5]; (*f*) A. W. Chromosorb W support, 80–100 mesh[6]; (*g*) Gas Chrom Q support, 100–120 mesh[7]; (*h*) OV-17 liquid phase[6] (silylated); (*i*) Dexsil 300 liquid phase[8]; and (*j*) methanol, analytical grade[9].

Pretreatment of Solid Samples—Methanolic solutions containing 0.5 mg./ml. diethyl phthalate as the internal standard and 0.0625, 0.10, 0.25, 0.50, 1.0, 1.5, and 2.0 mg./ml. menadione or 0.0625, 0.25, 0.50, and 1.0 mg./ml. menadione sodium bisulfite (trihydrate) were employed to prepare the standard curves. Methanolic solutions containing 0.5 mg./ml. diethyl phthalate and 1.0 mg./ml. menadione sodium bisulfite complex (trihydrate) also were prepared for analysis.

Pretreatment of Liquid Samples—Aliquots of 50 mcg. menadione sodium bisulfite (trihydrate) taken from 5- and 10-mg./ml. menadione sodium bisulfite injection solutions were placed in 2-ml. conical tubes (G. S.) and evaporated to dryness at 37° with a stream of nitrogen. The evaporated samples were redissolved in methanol to a final concentration of 0.883 mg./ml., allowed to stand for 2–3 hr., and centrifuged immediately before GC analysis.

Column Preparation—*2% OV-17 on A. W. Chromosorb W (Silylated)*—Prior to coating, 19.6 g. of A. W. Chromosorb W, 80–100 mesh, was added to 100 ml. of chloroform in a 1-l. round-bottom indented boiling flask[10]. This mixture was slurried for 2–3 min. To the slurry, 0.4 g. of OV-17 was added and mixed well for 2–3 min. This mixture was placed on a rotating evaporator with high vacuum applied. After the chloroform evaporated, the material was left on the rotating evaporator for 2 hr. to ensure that it was dry. The flask occasionally was tapped to prevent the material from adhering to the sides which ensured even distribution of the liquid phase coating. The support was packed into a 1.83-m. (6-ft.) glass column with 0.32-cm. (0.125-in.) i.d., and the column was conditioned by attaching it to the inlet port only. The oven temperature was set at 250°, and the carrier gas (nitrogen) flow rate was 25 ml./min. The column was left overnight under these conditions. After conditioning, the 2% OV-17 column was silylated by injecting (about five times) 2 μl. of *N,O*-bis(trimethyl)acetamide[11] + trimethylchlorosilane[11] (5 + 1 v/v) solution.

3% Dexsil 300 on Gas Chrom Q, 100–120 Mesh—The 3% Dexsil 300 column was prepared in a manner similar to that described for the 2% OV-17 column, but this column was not silylated.

Differential Thermal Analyses[12]—The samples studied were menadione, menadione sodium bisulfite (trihydrate), and menadione sodium bisulfite complex (trihydrate). All analyses were performed

[2] Abbott Laboratories, North Chicago, Ill.
[3] Nutritional Biochemicals Corp., Cleveland, Ohio.
[4] Hykinone, Abbott Laboratories, North Chicago, Ill.
[5] Aldrich Chemical Co., Milwaukee, Wis.
[6] Supelco, Inc., Bellefonte, Pa.
[7] Applied Science Labs., Inc., State College, Pa.
[8] Analabs, Inc., New Haven, Conn.
[9] Mallinckrodt Chemical Works, St. Louis, Mo.
[10] Morton G. S.
[11] Purchased from the Pierce Chemical Co., Rockford, Ill.
[12] Differential thermal analyses were carried out on a Dupont 9000 DTA unit.

under a nitrogen atmosphere. The settings used for each sample are described in Fig. 1.

GC–Mass Spectroscopy—*Mass Spectrum of Menadione Sodium Bisulfite Products*—A GC–mass spectroscopy instrument[13] operated at 70 ev. ionizing energy, 60 μamp. ion current, and 270° ion source temperature was used to obtain this spectrum. Sample introduction was by direct probe.

Mass Spectrum of Menadione and Volatile Menadione Sodium Bisulfite Pyrolysis Product Obtained from GC–Mass Spectroscopy—The spectrum was obtained from a GC–mass spectroscopy instrument[13] operated under the same conditions as already described. The molecular separator temperature was set at 250° and the sample was introduced through the GC inlet. A 2.4-m. (8-ft.) × 0.63-cm. (0.25-in.) i.d. glass column, packed with 100–120-mesh Gas Chrom Q with 3% Dexsil 300, was used for all the combination GC–mass spectral analyses. The chromatographic conditions were the same as those already described.

GC Analyses—A gas chromatograph[14] equipped with the silylated 1.83-m. (6-ft.) × 0.32-cm. (0.125-in.) i.d., 2% OV-17 on A. W. Chromosorb W 80–100-mesh column and a hydrogen flame-ionization detector was used for analysis of menadione and menadione sodium bisulfite compounds. The injector port temperature was set at 250°, the column oven at 140°, and the detector block at 235°. The nitrogen flow rate was 25 ml./min., the hydrogen flow rate was 25 ml./min., and the air flow rate was 300 ml./min. A recorder[15] with a chart speed of ½ division/min. and an integrator[16] with attenuation at 1, digital baseline corrector at 75% maximum, slope sensitivity of peak width at 30, and filtering of peak width at 10 were used. For each determination, a volume of sample solution (2 μl.) containing 0.5 mcg./μl. of internal standard (diethyl phthalate) was injected at an attenuation of 32×10^{-11} amp./mv. The (sample/internal standard) counts ratio was applied to a (menadione/internal standard) standard curve to obtain milligrams of menadione per milliliter of sample. This value was then multiplied by the appropriate factor to obtain the total content of the sample analyzed.

RESULTS AND DISCUSSION

Differential Thermal Analysis—Differential thermal analyses were run to determine the thermal stability of menadione and menadione sodium bisulfite compounds. The results are shown in Fig. 1.

The differential thermal analysis plot of menadione shows a strong endotherm at m.p. 105–107°. There are no additional endotherms or exotherms until one exceeds 200°. Therefore, one concludes that menadione is thermally stable up to 200°.

The differential thermal analysis plot of menadione sodium bisulfite (trihydrate) (I) shows several endotherms. One observes a strong endotherm at 115–120°. There are two more small endotherms at 142 and 161°. It is quite probable these are related to dehydration and phase transformations. Thermal gravimetric analysis would be helpful in further determining the chemical nature of these endotherms. The major endotherm that appears above 200° has been shown to be desulfonation of I to menadione.

The differential thermal analysis plot of menadione sodium bisulfite complex (trihydrate) (II) is nearly identical with that of I. Again, there are several endotherms which are believed to be related to dehydration and bisulfite complex bond breaking. The desulfonation endotherm of II appears a bit below 200°. From these data, it appears that it is feasible to pyrolyze menadione bisulfite compounds upon injection into a GC inlet port set above 200° and chromatograph the resulting menadione pyrolysis product through a column set at 140°.

GC–Mass Spectroscopy—Mass spectral analyses of the volatile menadione bisulfite thermal decomposition product were carried out in two ways. In the first the bisulfite compound was introduced directly into the ion source of the mass spectrometer in a small glass vial on the direct probe. The probe was heated until thermal decomposition occurred and a mass spectrum of the volatile products was taken. This technique results in the analysis of all volatiles produced in the decomposition. The second technique consisted of a combination GC–mass spectral analysis of the volatile product with

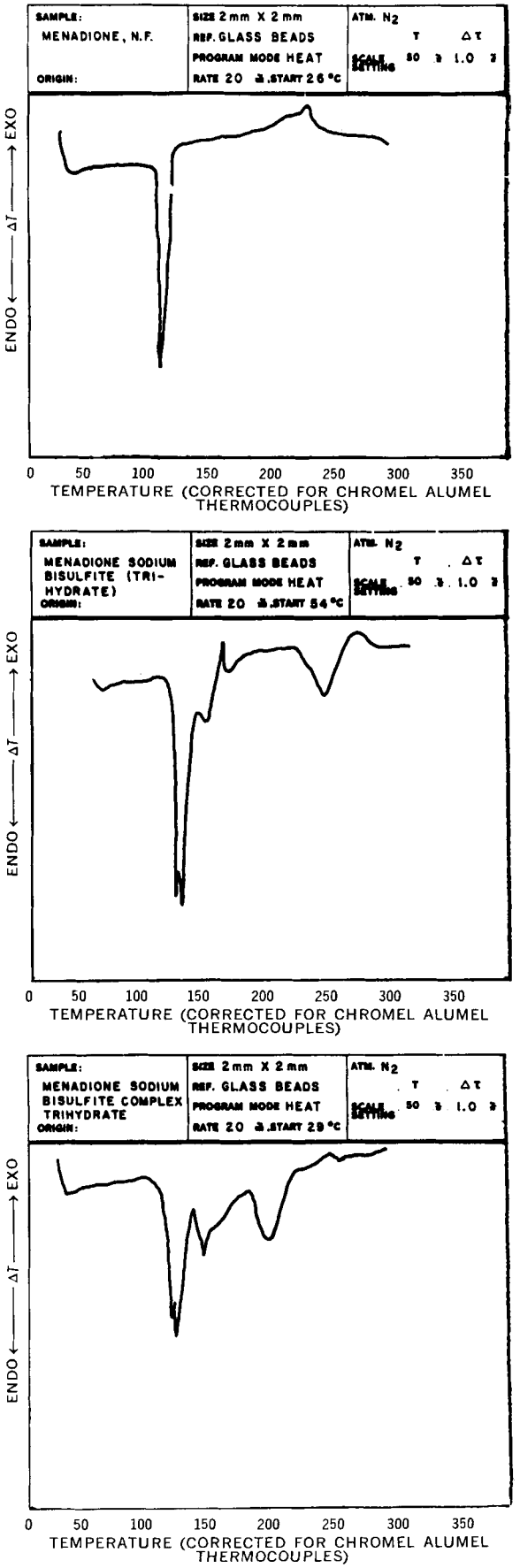


Figure 1—*Differential thermal analysis of: (top) menadione, (center) menadione sodium bisulfite (trihydrate), and (bottom) menadione sodium bisulfite complex (trihydrate).*

[13] LKB-9000.
[14] Varian model 2100.
[15] Varian model 20.
[16] Varian model 481.

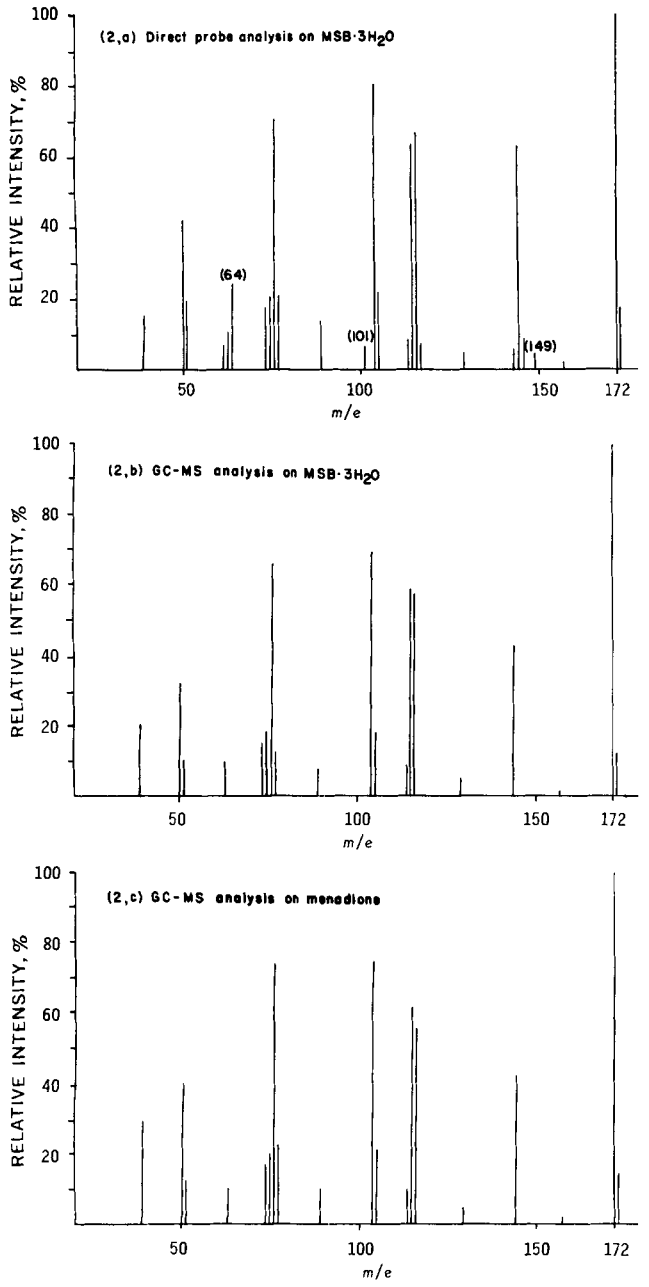
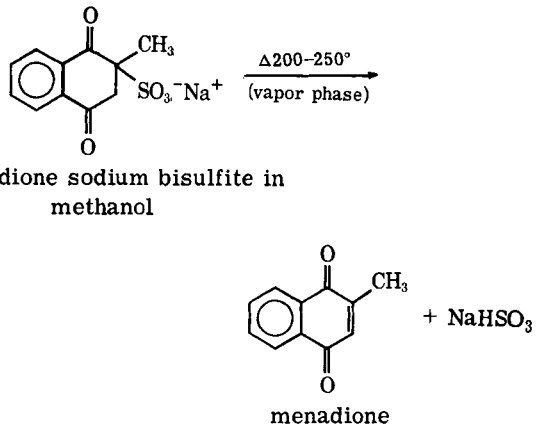


Figure 2—*Mass spectral analysis of:* (a) *menadione sodium bisulfite (trihydrate) direct probe volatile pyrolytic product(s),* (b) *menadione sodium bisulfite (trihydrate) GC volatile pyrolytic product, and* (c) *menadione GC volatile product. Conditions are given in text.*

GC properties identical to menadione. GC–mass spectral analysis of both the volatile bisulfite decomposition product and menadione showed that mass spectra of these compounds (Figs. 2*b* and 2*c*) are identical. The mass spectrum of volatile product(s) obtained by direct probe analysis (Fig. 2*a*) is also very similar to the other spectra but has additional fragment ions at *m/e* 64, 101, and 149. From these spectra, it may be concluded that the thermal decomposition of menadione bisulfite compounds yields menadione and at least one additional volatile product. This additional product(s) exhibits a prominent fragment ion at *m/e* 64 and apparently does not pass through the chromatograph for reasons of polarity or volatility. Thus, it may be concluded that menadione is a product of the thermal decomposition of the menadione bisulfite derivative. Although the pyrolysis equation (Scheme I) shows sodium bisulfite as a by-product for convenience, the authors are aware that there is insufficient evidence to determine the nature of the by-product(s), *i.e.*, products at *m/e* 64, 101, and 149.



Scheme I—Pyrolysis of Menadione Sodium Bisulfite

GC Analyses—Since it is now known that the various bisulfite addition products of menadione can be pyrolyzed to menadione, one is led directly into a GC analytical procedure for these derivatives. By adjusting the injection port temperature to 250° and the column temperature to 140°, desulfonation of menadione bisulfite occurs on initial injection and the resulting menadione pyrolysis product is chromatographed without being destroyed. There are no problems involved in the GC pyrolysis of bisulfite addition compounds, with the possible exception that the column is made acidic with the release of by-products as already mentioned. This is not a problem unless one attempts to use the same column for analyses of acid-labile compounds. The pyrolysis conditions remain constant for at least 2 months without further attention.

A typical chromatogram is shown in Fig. 3. By using diethyl phthalate as an internal standard, the concentration of the test bisulfite sample is determined by comparing the menadione/internal standard peak areas and multiplying the menadione content by the appropriate factors: 1.92 for I or 2.56 for II.

The standard curve for menadione shows linearity as measured by internal standard ratios of integrated counts and/or menadione integrated counts against micrograms of menadione injected (Fig. 4). When the bisulfite addition products of menadione are pyrolyzed and subjected to GC measurements under the same conditions, the retention times are the same and peak areas are in proportion to the theoretical menadione concentration. The plot of micrograms of menadione sodium bisulfite (trihydrate) *versus* micrograms of men-

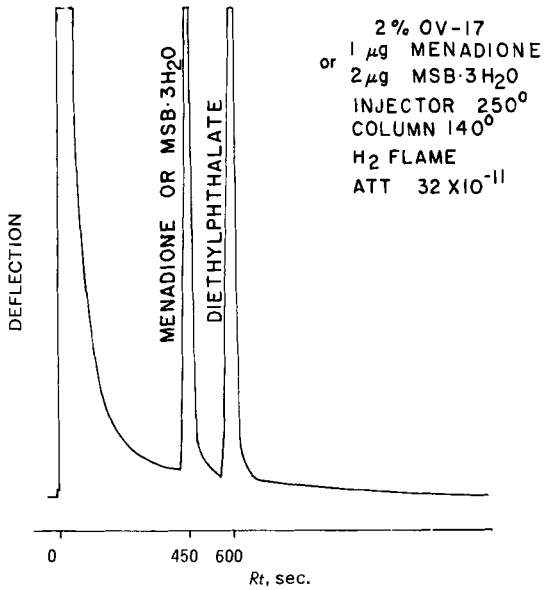


Figure 3—*Gas chromatogram of menadione and/or menadione sodium bisulfite (trihydrate) ($MSB \cdot 3H_2O$) with diethyl phthalate as internal standard. The 2% OV-17 on A. W. Chromosorb W, 80–100 mesh, column is silylated.*

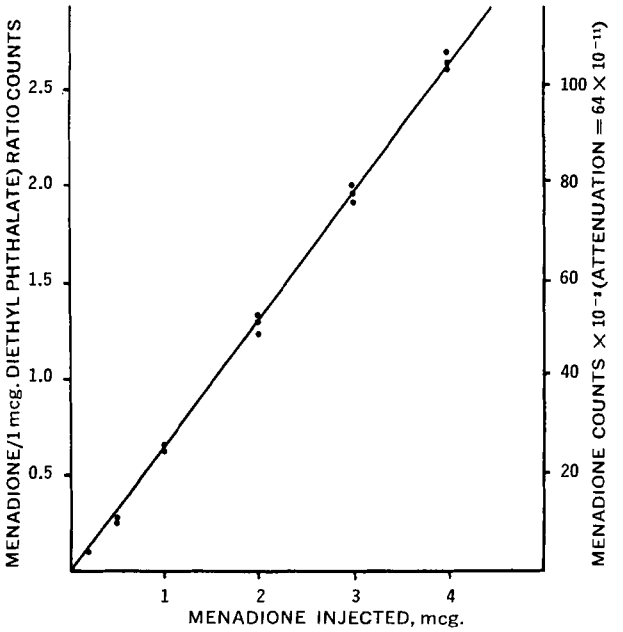


Figure 4—*Standard curve of menadione* versus *peak area integrated counts and/or (menadione/diethyl phthalate) ratio counts.*

adione shows menadione sodium bisulfite (trihydrate) to be about 52% menadione, in accordance with the molecular weight calculation (Fig. 5).

The accuracy and precision of the analytical method were tested on the following powdered and liquid samples: analytical grade (94+%) and liquid injectable (5 and 10 mg./ml.) menadione sodium bisulfite (trihydrate) and commercial grade (33+% menadione) menadione sodium bisulfite complex (trihydrate).

For the powdered sample of I, six different concentrations at 0.125, 0.25, 0.374, 0.50, 0.75, and 1.0 mg./ml. were assayed in triplicate, making a total of 18 different determinations. The final result showed 0.5127 mg. menadione/mg. bisulfite sample or 98.59 ± 3.4% *CV* of theory.

The results for six determinations on the bisulfite complex sample showed 0.331 ± 0.005 (*SD*) mg. menadione/mg. The commercial menadione sodium bisulfite complex usually contains about 33% menadione, and the GC value obtained was statistically equivalent to the menadione content determined by UV analysis.

The results for six determinations on the liquid injectable samples were as follows: 5 mg./ml. sample = 97.04 ± 1.46% *CV*, and 10 mg./ml. sample = 100.40 ± 0.50% *CV*. When six liquid injectable samples were spiked with an additional 0.3 mg. menadione sodium bisulfite (trihydrate)/ml., the final recovery value was 101.27 ± 2.6% *CV*.

CONCLUSION

The GC method described is accurate, sensitive, and specific for the determination of the menadione content in bisulfite addition compounds. The method is applicable to parenterals and solid and liquid dosage forms of menadione bisulfite compounds.

The principle of the assay is on-column desulfonation of the bisulfite upon injection at a temperature of 250° and subsequent chromatography of the menadione pyrolysis product at a temperature of 140°. Conclusive evidence of this process was obtained from differential thermal analysis, mass spectroscopy, and retention times of menadione and bisulfite addition compounds.

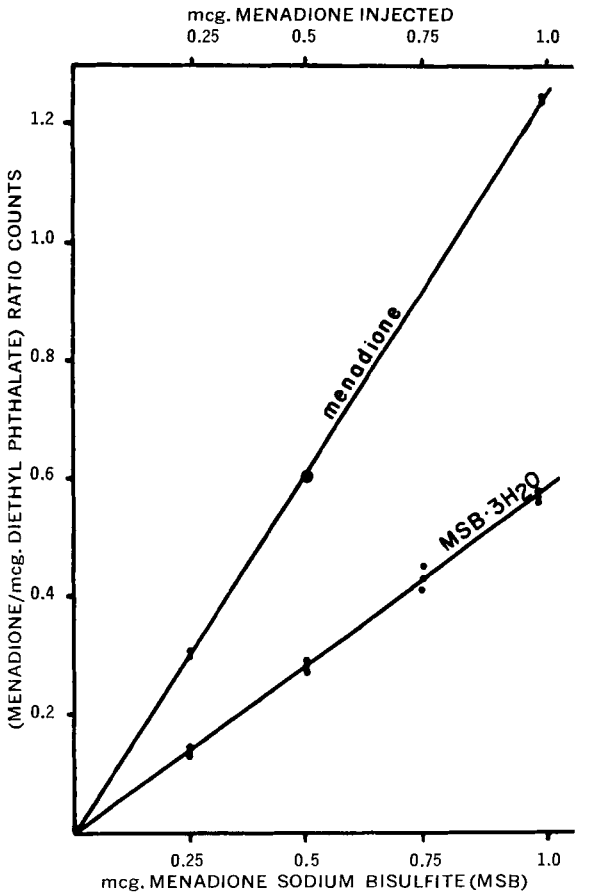


Figure 5—*Standard curves of menadione and menadione sodium bisulfite (trihydrate) (MSB·3H₂O)* versus *diethyl phthalate internal standard.*

REFERENCES

(1) "The National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, p. 230.

(2) K. Schoen, *J. Amer. Pharm. Ass., Sci. Ed.*, **34**, 247(1945).

(3) R. D. Johnson, *J. Ass. Offic. Anal. Chem.*, **54**, 593(1971).

(4) D. A. Libby and A. J. Sheppard, *J. Ass. Offic. Agr. Chem.*, **48**, 973(1965).

(5) W. D. Hubbard and A. J. Sheppard, *ibid.*, **51**, 15(1968).

(6) A. J. Sheppard and W. D. Hubbard, *ibid.*, **53**, 1093(1970).

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