Journal of Chromatography, 273 (1983) 192—196
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1529

Note

Gas—liquid and column liquid chromatography for studying vitamin U metabolism in humans and animals

A.A. BEZZUBOV* and N.N. GESSLER

Bakh Institute of Biochemistry, U.S.S.R. Academy of Sciences, Leninskii Prospect 33, Moscow 117071 (U.S.S.R.)

In recent years the mortality rate from gastrointestinal diseases has increased all over the world, evidently due to greater environmental contamination. Normally, peptic and duodenal ulcers as well as gastritis are treated with the synthetic vitamin U preparation (D,L-S-methylmethionine sulfonium chloride) [1]. Vitamin U was first isolated from cabbage juice [2] and parsley leaves [3]. Later it was detected in various natural sources, mainly of plant origin [4—6]. This allowed a wide use of food products with a high content of vitamin U (different varieties of cabbage, beets, parsley, celery, etc.) for the prophylaxis of gastrointestinal diseases.

In view of the important biological role of vitamin U, great attention is paid to the methods of studying its metabolism in the human and animal body. Existing microbiological and chemical methods are of low specificity and are laborious and time-consuming [7—9]. Emphasis should be given to chromatographic techniques, particularly to liquid and gas—liquid chromatography.

The present paper describes the study of vitamin U assimilation in animals and humans by means of chromatographic methods.

EXPERIMENTAL

Apparatus

The experiments were carried out using a gas chromatograph Chrom-41 (Laboratory Equipment, Prague, Czechoslovakia) with a flame ionization detector. It was supplied with a glass column (150 × 0.3 cm) packed with Polychrome-1 (Olaine Chemical Producing Plant, Riga, U.S.S.R.) having 0.25—0.5 mm grains impregnated with 10% Carbowax 20M. The temperature of the column was 70°C and that of the evaporator and detector 130°C. The carrier gas was helium flowing at a rate of 30 ml/min. Hydrogen and air flow-rates were 25 and 250 ml/min, respectively.

0378-4347/83/0000—0000/$03.00

The spectropolarimeter used was a Jasco Model J 40-AS (sensitivity 20 × 10^{-4} degrees/cm, time constant 16 sec and chart speed 5 × 10^{-1} cm/min).

Reagents

The reagents used were: D,L-S-methylmethionine sulfonium chloride (MMSCI or MMS for free base) of 96,3% purity (Ufa Vitamin-Producing Plant, Ufa, U.S.S.R.); toluene, carbon tetrachloride and dimethylsulfide were analytical reagent grade.

Animals

The experiments were carried out on Wistar white rats weighing 200 g. MMSCI in the form of a 2% aqueous solution was administered per os at a dose of 10 mg per 100 g body weight. The rats were decapitated at different time intervals after drug administration. Immediately after decapitation blood and viscera were removed and placed on ice. The amount of MMS excreted was measured in 24-h urine samples collected from five rats.

Gas—liquid chromatography

A batch of the tissue (liver, kidneys) was homogenized in cooled borate buffer pH 9.8—10 at a ratio of 1:2 (w/v). The suspension was transferred to 10-ml vials tightly stoppered with rubber caps. Blood and urine (3—5 ml) were placed into vials and the solution was made up to 10 ml with borate buffer. The vials were quickly heated on a boiling water bath and then autoclaved for 30 min at 120°C and 0.2 MPa. After cooling 40—100 μl of *n*-octanol (to prevent foaming) were injected through the rubber stopper with the aid of a microsyringe, and the resultant dimethyl sulfide was distilled in the apparatus shown in Fig. 1. The temperature of the water bath was 80°C, the nitrogen flow-rate was 45 ml/min, and the distillation time was 30 min. Dimethyl sulfide was trapped in a toluene-containing trap cooled with a mixture of dry ice and acetone; 1 μl of toluene solution was analyzed in the gas chromatograph.

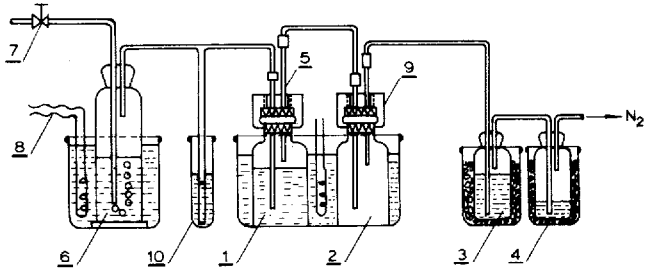

Fig. 1. Schematic diagram of the apparatus for dimethyl sulfide isolation from samples after MMS degradation. 1 = Vial with a sample homogenate, 2 = intermediate vial, 3 = trap with 40% $NaHSO_3$ cooled with ice, 4 = trap with toluene cooled with dry ice in acetone, 5 = syringe needle, 6 = Drexel bottle for nitrogen heating, 7 = fine-adjustment valve, 8 = heater, 9 = clamp, 10 = mercury cut-off seal.

Liquid chromatography

Cooled liver and kidneys were homogenized with two volumes of distilled water and supplemented with trichloroacetic acid to adjust the final concentra-

tion to 10%. The homogenates were centrifuged and the clear solution was extracted three times with sulfuric ether to remove the trichloroacetic acid. Aqueous solutions were evaporated in vacuo on a rotor evaporator at max. 40°C. The residue was extracted with 80% methanol and the precipitate discarded. The water—methanol solution was evaporated to bring the volume to 0.5—1.0 ml. The cooled urine was filtered and the filtrate evaporated in vacuo. The dry residue obtained in this way was dissolved in 5—10 ml of 80% methanol and the precipitate was discarded. The solution was concentrated to bring the final volume to 0.5—1.0 ml.

The resultant concentrates containing MMS were placed on a column packed with 15 g of silica gel (Woelm, Eschwege, G.F.R.) in 90% methanol. The column was washed with 90% methanol to eliminate completely the yellow zone. The MMS was eluted in the solvent system methanol—formic acid—water (4:1:4). Fractions of 1 ml were collected and assayed by thin-layer chromatography on silica gel plates (Silufol, Prague, Czechoslovakia) in the solvent system *sec.*-amyl alcohol—formic acid—water (4:4:2) (detection with ninhydrin). The vitamin U-containing fractions were combined and evaporated in vacuo. The quantity of isolated vitamin was measured by gas—liquid chromatography.

Circular dichroism

Circular dichroic spectra of the isolated vitamin U preparation were recorded after treatment with *o*-phthalaldehyde in the presence of mercaptoethanol [10].

RESULTS AND DISCUSSION

The gas chromatographic assay of vitamin U was performed with respect to dimethyl sulfide, its decomposition product which is formed in equimolecular quantities as a result of S-methylmethionine degradation in alkaline medium following the reaction

$$OH^- + (CH_3)_2S^+\text{-}CH_2CH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOO^- \rightarrow (CH_3)_2S + HOCH_2CH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOO^-$$

The yield of dimethyl sulfide is 91% (heating at 120°C and pH 9.7—10 for 30 min).

Carbon tetrachloride was used as internal standard. The calibration curve was established and calculations were made as described previously [11]. Gas chromatograms are given in Fig. 2.

In order to determine the sensitivity and error of the method used, MMSCI was added to homogenates of different rat organs. The data obtained indicate a direct correlation between the amount of vitamin U administered and measured in the concentration range 0.02—1.0 mg/g. The lowest detectable quantity of vitamin U in the sample was 20 μg, the relative error ± 3.0%.

The gas chromatographic data of the vitamin U concentration in rat liver, kidneys and blood at different time intervals after its oral administration are

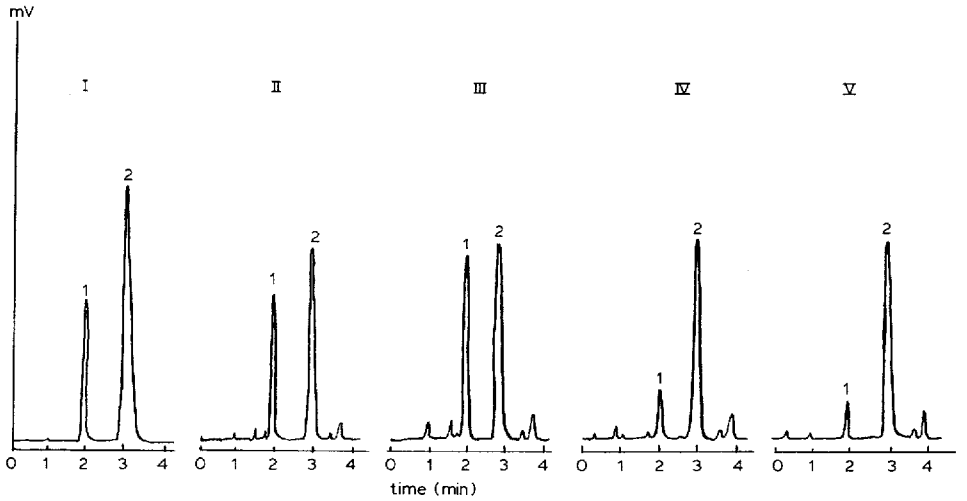

Fig. 2. Gas—liquid chromatograms of volatile substances obtained by heating homogenates of rat organs and urine. 1 = Dimethyl sulfide, 2 = carbon tetrachloride; toluene peak is not shown. I = MMSCI, II = kidneys, III = liver, IV = blood, V = urine.

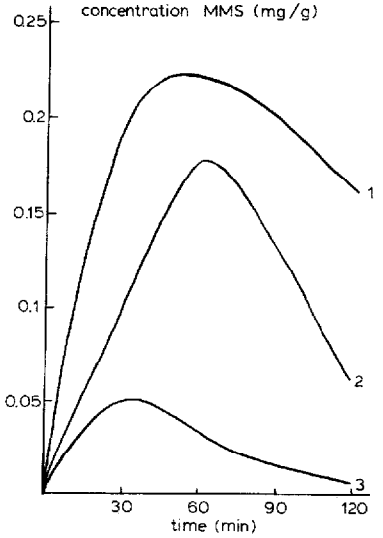

Fig. 3. The variation of MMS concentration in rat organs with time elapsed after drug administration. 1 = Kidneys, 2 = liver, 3 = blood.

presented in Fig. 3. It can be seen that the vitamin U concentration reaches a maximum in blood at 30 min and in liver and kidneys at 60 min after administration, and then decreases rapidly. On the second day after administration, only traces of the preparation can be found in blood and organs. We failed to detect vitamin U in the blood or organs of control rats fed with a standard vivarium diet. This suggests that vitamin U is actively involved in metabolic processes occurring in the animal body.

In order to verify the gas chromatographic data, vitamin U was isolated from rat organs using silica gel column partition chromatography. First, pro-

TABLE I

COMPARISON OF GAS CHROMATOGRAPHIC ANALYSIS OF VITAMIN U IN RAT ORGANS BEFORE AND AFTER PURIFICATION USING COLUMN LIQUID CHROMATOGRAPHY

Rat organ	Detected before sample purification (mg)	Detected after sample purification (mg)	Recovery after purification (%)
Liver (57 g)	2.85	2.0	70
Kidneys (13 g)	1.95	1.6	82
Vitamin preparation (1 mg)	1.0	0.8	80

teins from liver and kidney homogenates were precipitated with trichloroacetic acid. The vitamin-containing fraction was decomposed to yield dimethyl sulfide, which was determined by gas chromatography. As follows from the data obtained (Table I), the gas chromatographic technique can be used to measure vitamin U in animal tissues without preliminary preparation of the sample.

Measurements of vitamin U in the urine of rats and men given the drug at a dose of 100 mg/day showed its renal excretion at a rate of 5—6% of the dose administered. Using circular dichroism, it was demonstrated that the vitamin excreted in the urine was the D-isomer.

REFERENCES

1 V.N. Bukin and V.E. Anisimov (Editors), Vitamin U (S-methylmethionine), Nature, Properties, Application, Nauka, Moscow, 1973 (in Russian).
2 R.A. McRorie, G.L. Sutherland, M.S. Lewis, A.D. Barton, M.R. Glazener and W. Shive, J. Amer. Chem. Soc., 76 (1954) 115.
3 T. Challenger and B.J. Hayward, Chem. Ind., (1954) 729.
4 F.I. Skodak, F.F. Wong and L.M. White, Anal. Biochem., 13 (1965) 568.
5 E.V. Kovatscheva and I.A. Popova, Nahrung, 21 (1977) 465.
6 A.A. Bezzubov and N.N. Gessler, Gig. Sanit., 6 (1981) 85.
7 E. Hiraoka and T. Kishi, Vitamins, 28 (1965) 387.
8 X. Seno and D. Nagai, Vitamins, 31 (1965) 214.
9 S. Ogawa, M. Morita, A. Koshimoto and K. Dome, J. Vitaminol., 12 (1966) 162.
10 V.A. Voskova, V.V. Romanov, N.V. Sumbatyan, G.A. Korshunova and Yu.I. Shvachkin, Bioorg. Khim., 6 (1980) 731.
11 A.A. Bezzubov and N.N. Gessler, Prikl. Biochim. Microbiol., 13 (1977) 301.