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DETERMINATION OF VITAMIN U AND ITS DEGRADATION PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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SUMMARY

A high-performance liquid chromatographic method has been developed for the determination of vitamin U in tablets and capsules. Threonine was employed as the internal standard throughout the assay. The *o*-phthalaldehyde derivatives were prepared and then chromatographed isocratically on a reversed-phase C_{18} column. The optimum reaction time for both vitamin U and threonine at pH 10.5 is 5 min. Vitamin U and its major degradation product in the dosage forms, *viz.*, methionine sulphoxide, were separated and quantified with a relative standard deviation of about 1%, using a fluorescence detector with excitation and emission wavelengths at 340 and 450 nm respectively. A linear relationship has been established between the peak area ratio of vitamin U/threonine and the concentration of vitamin U over the range of 2.5–50 μg/ml.

INTRODUCTION

Vitamin U (DL-methionine-S-methylsulphonium chloride) is an anti-ulcer vitamin extracted from cabbage leaves and other green vegetables[1]. The extracts and the synthetic compound have been widely used in Asia for the treatment of gastric disorder in conjunction with antacids and anticholinergic agents. There are more than ten over-the-counter formulations containing vitamin U available locally in Hong Kong. Most are in the form of plain tablets, capsules and sugar-coated tablets, in which the vitamin U is mixed with other active ingredients, the amounts present in each tablet or capsule being in the range of 4 to 50 mg. In one preparation, vitamin U is embedded centrally in a shell of antacids in a composite tablet. There are only a few reports in the literature on the determination of vitamin U. One involves the assay of dosage forms by amperometric titration[2] and the other the study of vitamin U metabolism in human and animals by gas and column liquid chromatography[3]. The former method is not selective as it cannot differentiate vitamin U from its degradation products in dosage forms. The latter method is quite complicated to perform, requires special apparatus for the generation of dimethyl sulphide from vitamin U and again lacks selectivity for the assay of dosage forms.

Vitamin U is very hygroscopic and is hydrolysed rapidly in alkaline media. When

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compounded in tablets or capsules with other basic ingredients such as aluminium hydroxide and magnesium hydroxide, decomposition may occur upon absorption of moisture from the atmosphere. Specific methods for the separation of vitamin U from its degradation products are therefore required for the analysis of vitamin U preparations.

When applying the high-performance liquid chromatographic (HPLC) method for the determination of vitamin U, one major problem encountered is that vitamin U has no UV or visible absorption characteristics. Reversed-phase chromatographic systems with short wavelength UV detection (210 nm) have been tested without success. *o*-Phthalaldehyde (OPA) has been demonstrated to be very useful for the precolumn and postcolumn fluorigenic detection of amines and amino acids[4–9]. Making use of the primary amine functional group of vitamin U, derivatization with OPA yields the corresponding fluorescent isoindole which can be detected by a fluorescence detector.

The OPA derivatives of methionine and its sulphone have been chromatographed by reversed-phase HPLC[9]. However, there is no report on the HPLC separation of vitamin U and its degradation products. This paper reports the development of a reversed-phase HPLC method for the quantitation of vitamin U and its major degradation product by derivatization with OPA and fluorescence detection.

EXPERIMENTAL

Instruments

An ISCO Model 2350 HPLC pump (Isco, Lincoln, NE, U.S.A.) was used at a flow-rate of 1.7 ml/min. Analysis was performed using a 250 mm × 4.6 mm I.D. stainless-steel column packed with 7-μm ODS particles (Cat. No. CP-10-5; Chemco Scientific, Osaka, Japan) using a Chemco slurry packing apparatus Model 124AA. The sample was injected into the column using a Rheodyne 7125 injector (Rheodyne, Cotati, CA, U.S.A.) with a 20-μl loop. The column effluents were monitored with a Beckman 157 fluorescence detector (Beckman Instruments, Berkeley, CA, U.S.A.) equipped with a coloured glass excitation filter (305–395 nm) and a wide band interference emission filter (430–470 nm). The integrator used was a Shimadzu Chromatopac C-R3A (Shimadzu, Kyoto, Japan) operated by the 100-mV output from the detector.

Reagents

Methanol and acetonitrile were HPLC grade, obtained from Ajax (Auburn, Australia). OPA was obtained from Sigma (St. Louis, MO, U.S.A.). Analytical reagent grade anhydrous disodium hydrogenphosphate and sodium dihydrogenphosphate dihydrate (Riedel-de Haen, Hannover, F.R.G.) were used to prepare a buffer solution. 2-Mercaptoethanol was from E. Merck (Darmstadt, F.R.G.). The internal standard, DL-threonine, and DL-methionine sulphoxide were obtained from BDH Chemicals (Poole, U.K.) and TCI (Tokyo Kasei Kogyo, Tokyo, Japan) respectively.

Mobile phase

A stock phosphate buffer solution was prepared by dissolving 5.8 g of sodium

dihydrogenphosphate dihydrate and 15.4 g of anhydrous disodium hydrogenphosphate in 500 ml of water.

In the preparation of the mobile phase, 80 ml of acetonitrile were first placed in a 500-ml stoppered measuring cylinder, followed by 25 ml of the stock phosphate buffer solution and water was then added to make up the volume. The mobile phase was degassed with an ultrasonic bath prior to use. The volume of acetonitrile may have to be slightly modified in order to obtain the optimum chromatographic performance.

Derivatization reagents

Borate buffer. 0.5 *M* Sodium borate (31 g in 1 l of water) was adjusted to pH 10.5 with potassium hydroxide pellets.

OPA solution. About 50 mg of OPA were dissolved in 5 ml of methanol. This solution was freshly prepared daily.

2-Mercaptoethanol solution. A 5-μl volume of 2-mercaptoethanol was added to 5 ml of methanol. This solution was also freshly prepared daily.

Vitamin U stock standard solution

About 10 mg of vitamin U were accurately weighed and dissolved in 200 ml of water.

Vitamin U working standard solutions

To four 100-ml volumetric flasks were added respectively 5, 10, 25 and 50 ml of the stock standard solution and made up to the mark with water. These working standard solutions contained respectively 2.5, 5, 12.5 and 25 μg/ml of vitamin U.

Internal standard solution

About 3 mg of DL-threonine were weighed and dissolved in 100 ml of water.

DL-Methionine sulphoxide standard solution

About 3 mg of DL-methionine sulphoxide were accurately weighed and dissolved in 100 ml of water.

Sample preparation

Twenty capsules or tablets were weighed and powdered. An accurately weighed quantity of the powder equivalent to about 2 mg of vitamin U was transferred to a 100-ml volumetric flask and about 90 ml of water were added. The contents of the flask were sonicated for 30 min and then made up to the mark with water. The mixture was filtered through Whatman No. 542 filter-paper. The first 10-ml portion of the filtrate was discarded and the remainder collected in a glass-stoppered flask.

Derivatization procedure

Volumes of 100 μl of each of the vitamin U working standard solutions and sample solutions were quantitatively transferred to 5-ml reaction vials. A 50-μl volume of internal standard solution was added, followed by 50 μl of borate buffer, and the solution was mixed thoroughly. Volumes 100 μl each of OPA and 2-mercaptoethanol solution were then added and mixed. A 4-ml volume of methanol was added to stop the reaction after 5 min. The resulting solutions were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

A study was carried out at room temperature to select the time for completion of the derivatization reaction of vitamin U and the internal standard (threonine) with OPA. The reaction conditions described above were used with various reaction times. As shown in Figs. 1 and 2, the optimum reaction time for completion of derivatization of both vitamin U and the internal standard is 5–10 min. The results for D-methionine sulphoxide which is the major degradation product of vitamin U (*vide infra*) were similar. Thus room temperature for 5 min was chosen as the derivatization condition throughout the determination. The OPA derivatives were found to be stable for more than 1 h.

Under the experimental conditions established, a linear relationship between the peak area ratio (vitamin U/threonine) and concentration of vitamin U was demonstrated over the range of 2.5–50 μg/ml and the correlation coefficient, r, was found to be 0.9990. The peak area ratio (DL-methionine sulphoxide/threonine) was also linearly related to the concentration of DL-methionine sulphoxide over the range of 5–50 μg/ml and r was 0.9999. Typical chromatograms of DL-methionine sulphoxide and vitamin U are shown in Fig. 3, the amounts injected being 14 and 12 ng respectively.

Since more than ten over-the-counter formulations containing vitamin U are available in Hong Kong, four typical ones were selected on the basis of their differences

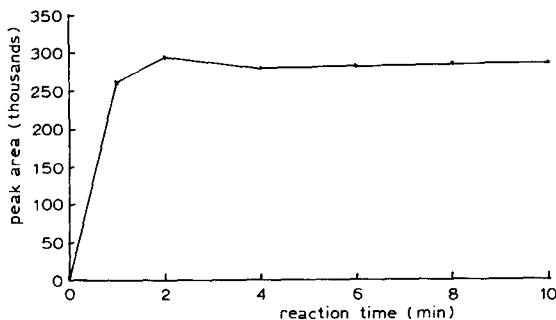

Fig. 1. Variation of the peak area of vitamin U with reaction time.

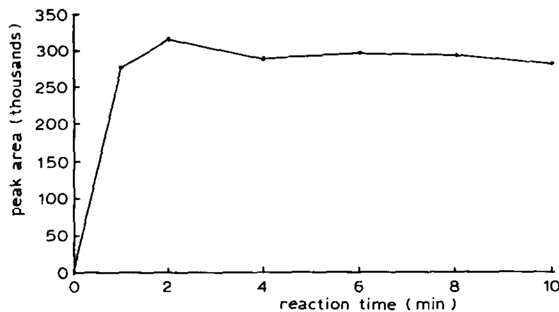

Fig. 2. Variation of the peak area of DL-threonine (the internal standard) with reaction time.

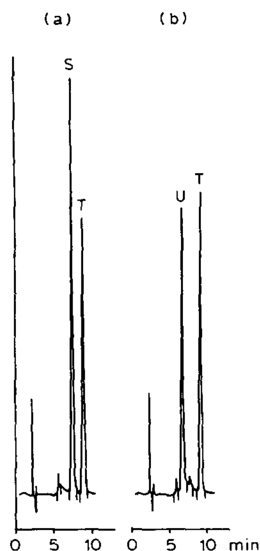

Fig. 3. Chromatograms of (a) DL-methionine sulphoxide (14 ng) and (b) vitamin U (12 ng). Peaks: U = vitamin U; S = DL-methionine sulphoxide; T = DL-threonine.

in dosage forms, origin and the presence of other active ingredients. The results of analysis of these four commercial samples using the HPLC method proposed are shown in Table I. The intra-assay coefficients of variation were between 0.3 and 1.4%, while the inter-assay coefficients of variation were between 0.4 and 2.9% in the range of 2.5–50 μg/ml of vitamin U.

Antacids and anticholinergic agents such as aluminium hydroxide, magnesium trisilicate, atropine, scopolamine and hyoscyamine were found not to interfere with the derivatization and HPLC separation when present at the analysis concentrations. A recovery experiment was carried out on a freshly prepared synthetic mixture containing a known amount of vitamin U and with appropriate quantities of aluminium hydroxide and magnesium trisilicate. The recovery was found to be 99.1 $\pm$ 0.6%.

Vitamin U is stable when dry, but not after absorbing moisture and in the presence of antacids which are usually basic in nature. Based on its structure, there are several possible degradation products, *viz.*, methionine sulphoxide, methionine sulphone, methionine and homoserine. However, it is noteworthy that the reference standard solutions did not show any significant loss in vitamin U content after standing at room temperature for 75 days, showing that the degradation might be the result of the presence of the alkaline medium.

Chromatograms of the four samples examined are shown in Fig. 4. Compared with the chromatogram of standard vitamin U, it was observed that for the first three samples the vitamin U peak was smaller than expected and there was a relatively large second peak which was present in very small quantity in the standard. Subsequent analysis showed that this peak corresponded to methionine sulphoxide which should be the major degradation product of vitamin U in the first three samples in which vitamin U was in direct contact with the antacids. Absorption of moisture might be the

TABLE I

DETERMINATION OF VITAMIN U AND METHIONINE SULPHOXIDE IN PHARMACEUTICAL PREPARATIONS

Preparation	*Origin*	*Labelled contents*	*Percentage of labelled content found*[a]	
			Vitamin U	*Methionine sulphoxide*[b]
Plain tablet	Japan	Vitamin U 4.17 mg Aluminium magnesiumsilicate 67 mg Scopolia extract 1.7 mg Carboxymethylcellulose calcium 10.5 mg	24.5 ± 0.3	46.6 ± 0.3
Capsule	Hong Kong	Vitamin U 30 mg Belladonna dry extract 15 mg Dried aluminium hydroxide gel 150 mg Magnesium hydroxide 150 mg	8.7 ± 0.1	49.2 ± 0.8
Sugar-coated tablet	China	Vitamin U 50 mg Dried aluminium hydroxide gel 123 mg Magnesium trisilicate 50 mg	35.3 ± 0.5	34.4 ± 0.6
Composite tablet	Japan	Inner tablet: vitamin U 25 mg diastase 60 mg Outer tablet: sodium glycyrrhizinate 33 mg glucuronic acid 17 mg dried aluminium hydroxide gel 160 mg magnesium trisilicate 145 mg	98.1 ± 0.3	0.7[c]

[a] Mean of five determinations, with standard deviation.
[b] Calculated as the equivalent of vitamin U.
[c] This is an approximation as the peak area lies outside the calibration range.

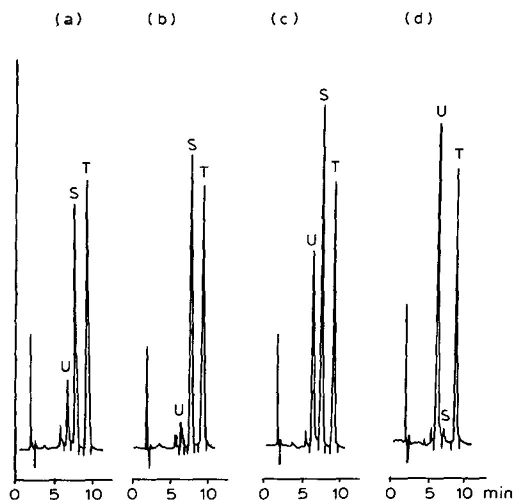

Fig. 4. Chromatograms of (a) plain tablet, (b) capsule, (c) sugar-coated tablet, (d) composite tablet; peaks as in Fig. 3.

cause of degradation. Quite surprisingly, methionine, methionine sulphone and homoserine were not detected.

For the last sample of a composite tablet, little decomposition of vitamin U was observed. It should therefore be the most stable dosage form as the vitamin U is separated from the antacids and is protected from moisture by the outer shell. As expected, the sugar-coated tablet was relatively more stable than the plain tablet and capsule because of better protection from atmospheric moisture.

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REFERENCES

1 *The Merck Index*, Merck & Co., Rahway, NJ, 10th ed. 1983, p. 1439.
2 M. I. Lebeveva, B. I. Isaeva, A. N. Dubovitskaya and M. V. Terekhina, *Farmatsiya (Moscow)*, 33 (1984) 79, *Anal. Abstr.*, 46 (1984) 8E22.
3 A. A. Bezzubov and N. N. Gessler, *J. Chromatogr.*, 273 (1983) 192.
4 M. Roth, *Anal. Chem.*, 43 (1971) 880.
5 M. Roth and A. Hampaï, *J. Chromatogr.*, 83 (1973) 353.
6 T. P. Davis, C. W. Gehrke, C. W. Gehrke, Jr., T. D. Cunningham, D. Thomas, K. C. Kuo, K. O. Gerhardt, H. D. Johnson and C. H. Williams, *Clin. Chem.*, 24 (1978) 1317.
7 L. D. Mell, *J. Liq. Chromatogr.*, 1 (1978) 261.
8 R. E. Subden, *J. Chromatogr.*, 166 (1978) 310.
9 P. Lindroth and K. Mopper, *Anal. Chem.*, 51 (1979) 1667.