

petroleum ether extract was allowed to stand over night. In the morning beautiful ruby red crystals were found to have separated in the flask. These were filtered on a pad of solid carbon dioxide and kept in a sealed tube filled with the gas. They melt at 192° , the melting point of β -carotene, and resemble β -carotene in solubility and crystalline form. Longer extraction with petroleum ether produced more of the red product and, finally, an orange-colored powdery precipitate, which is easily separated from the red crystals by washing with ether.

Work upon the woody portion of the root has scarcely begun. There is being obtained, however, a sterol-like substance and a white crystalline substance resembling those from the leaves and stem.

Fruit.—The outer husk was separated from the fruit and it and the berry were dried separately. The dried husks were ground in a mill. The berries, after drying, were rubbed on a coarse sieve with a large cork, and the arillus was thus separated from the seed. The coarse powder thus obtained was of a deep red color. It and the powdered husks were extracted separately with low boiling petroleum ether. From the deep red extracts no crystals separated upon standing. The extracts were then concentrated by distillation of the petroleum ether, and red fatty masses resulted, that from the arillus of the seed being the more intensely colored. This product was saponified by boiling with alcoholic potassium hydroxide. After the alcohol was evaporated water was added to the reaction mixture and it was shaken out with ether. Small deep red needle-shaped crystals separated upon evaporation of the ether. Only a small quantity of this material has been obtained. Neither it nor the other products of the hydrolysis have been investigated.

There have, therefore, been isolated from the plant, and partly identified:

One or more substances which respond to the tests for sterols.

One or more red crystalline substances which resemble carotene, probably β -carotene.

Two or more white crystalline substances, one of which yields upon hydrolysis a reducing sugar which forms an osazone with the melting point and crystalline form of galactosazone. The other is non-sugar in character, probably a sugar alcohol, possibly dulcitol.

All of these products, and others not so well characterized, are undergoing further examination and will be reported upon later.

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SOME OBSERVATIONS ON THE STABILITY OF QUININE SULPHATE DURING STORAGE.*

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Under certain conditions quinine sulphate may crystallize from water with 8 molecules of water of hydration. This product rapidly loses some of its water,

* Scientific Section, A. Ph. A., Washington meeting, 1934.

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even while drying during the process of manufacture, so that the more usual form contains moisture, equivalent to about 7 or $7\frac{1}{2}$ molecules of water. It has been known for many years that quinine sulphate containing more than 2 molecules of water of hydration is unstable. On standing at ordinary temperatures the salt loses water until the equivalent of only about two molecules remain. This change takes place very rapidly at 50° C. After the two-molecule stage has been reached the product remains stable unless subjected to temperatures appreciably higher than those usual in the laboratory or to other unusual treatment, such as storage in a desiccator.

The U. S. Pharmacopœia permits either 7 or 8 molecules of water of hydration. It prescribes a maximum limit for loss on drying (16.2 per cent) which is very nearly the value corresponding to 8 molecules of water of hydration (16.16 per cent). It prescribes no minimum limit for loss on drying. Concerning the instability of the fully hydrated salt the U. S. P. X makes the following statement:

"It effloresces rapidly when exposed to dry air or when heated to 50° C., losing all but two molecules of its water of crystallization and becoming lusterless."

Although there are several references in the literature to the instability of quinine sulphate during storage, exact information concerning the length of time required (at room temperature) for the crystallized salt to lose sufficient of its water to reach the stable form is inadequate.

In 1876 Cownley¹ observed that freshly prepared quinine sulphate contained $7\frac{1}{2}$ molecules of water of crystallization and that it lost $5\frac{1}{2}$ of these when freely exposed to the air for 28 hours.

In 1884 Parsons² reported that he had determined the water in 1015 samples of quinine sulphate by drying in a water oven. The average water content was 13.849% or slightly above $6\frac{1}{2}$ molecules (13.56 %). He suggested that the stable salt with 2 molecules of water of crystallization be introduced in the U. S. P. VII.

In 1886 Spalding³ reported that he had weighed the contents of two cans of quinine sulphate and repeated the weighings over a period of 12 months in one instance and 8 months in the other. The total loss in the first case amounted to between 8% and 9% and in the second to 11.39%. No information was given as to how long the containers remained open at the time of the weighings.

In 1892 Thompson⁴ reported that he had found an average water content of 11.74% in 183 samples of quinine sulphate on the market.

In 1924 Linnerseege⁵ called attention to this problem in its relation to public analysts in Great Britain. He says in part:

Owing to some samples of medicine containing an excess of quinine, seven samples of the sulphate were bought from pharmacies. The amount of moisture varied from 3.7 per cent to 12.0 per cent, with an average of 6.4 per cent, not one of them corresponding with the $7\frac{1}{2}$ molecules, or 15.3 per cent of water required by the B. P. description. The 1898 B. P. required the freshly

¹ "The Water of Crystallization in Quinine Sulphate," *Pharm. J. & Trans.* (3), 7 (1876), 189.

² "On the Water of Hydration in Quinine Sulphate," *Proc. A. Ph. A.*, 32 (1884), 457.

³ "Sulphate of Quinine—Loss in Weight when Packed in Cans," *Ibid.*, 34 (1886), 605.

⁴ "Quinine Sulphate U. S. P.," *Ibid.*, 40 (1892), 266.

⁵ "Quinine Sulphate," "Year Book of Pharmacy," 61 (1924), 756.

prepared salt to lose 15.2 per cent of moisture on heating. This test was omitted in the 1914 B. P., and nothing put in its place. Both Pharmacopœias remark under "Characters and Tests" that the crystals effloresce on exposure to dry air until two molecules of water remain. What, therefore, is the amount of moisture in the B. P. salt? Is it $7\frac{1}{2}$ molecules, as required by the description, or anything between that and two molecules, as is suggested by the latter statement? This is a difficult question for an analyst who receives samples of quinine sulphate or medicine containing it. The retail samples given above show how variable the salt is, and one wholesale sample contained 3.9 per cent of moisture.

Linerseege suggested that the next revision of the British Pharmacopœia should describe a salt containing but two molecules of water of crystallization. However, the new edition (1930) still describes a salt with $7\frac{1}{2}$ molecules of crystallization.

Two one-ounce bottles of quinine sulphate of the same brand were obtained by Sage¹ from the manufacturer. One was opened immediately and small portions were removed from time to time for a period of two years. The other was kept sealed beside the opened bottle. Water was then determined in each. The salt from the frequently opened bottle lost 3.76% on drying and the other 13.28%. The initial moisture content of the salt in the first opened bottle was not stated.

In 1933 Beal and Szalkowski² determined loss in weight at 100° on twenty-eight specimens of quinine sulphate, representing market material. A specimen containing 16.78% of H₂O and an anhydrous specimen were prepared. The market material lost from 4.39% to 12.29%, or calculated as H₂O, from 1.9 to 5.8 molecules. None of the market specimens conformed to the U. S. P. which limits the loss at 100° to 16.2%. Portions of both of the prepared specimens were exposed in desiccators 14 days to concentrated sulphuric acid, 3:1 sulphuric acid, 2:1 sulphuric acid, 1:1 sulphuric acid, saturated solution of potassium acetate (20% humidity), saturated solution of calcium chloride (30% humidity), saturated solution of potassium carbonate (40% humidity) and saturated solution of ammonium chloride (80% humidity). No marked changes took place after the ninth day. The dihydrate (4.60% H₂O) is the most stable form. The heptahydrate (14.43%), the octahydrate (16.16%) and the anhydrous salt tend to form the dihydrate. In changing from the heptahydrate to the dihydrate the salt loses its long, flaky crystalline form which results in short needles that have almost the appearance of fine powder. The salt also loses in bulk.

Wales³ has shown by vapor pressure measurements that two hydrates of quinine sulphate exist. The octahydrate is unstable under exposure to the air and is transformed into the dihydrate. His findings indicated that under ordinary conditions equilibrium with water vapor in the air would be reached somewhere on the sharp break in the vapor pressure curve and that the salt stored in an open container should contain slightly more than 2 moles of water.

Because of the insufficiency of the information in the literature, and because of the bearing which the facts would have on the administration of the food and drugs act it was decided to carry out some tests in the hope of determining how rapidly the salt would lose its water under such conditions as would ordinarily obtain on

¹ "Quinine Sulphate and Its Storage," *Pharm. J.*, 119 (1927), 264.

² "Notes on the Water of Crystallization of Quinine Sulphate," *JOUR. A. PH. A.*, 22 (1933), 1219-1225.

³ *Ibid.*, 23 (1934), 793.

the shelves of drug stores. Accordingly, in November 1932, seven packages of quinine sulphate were purchased directly from the manufacturer with the specifications that the material should all be of the same lot and that it should have been freshly made and packed. The material was contained in six glass bottles with screw caps, each containing 1 ounce, and in one 8-ounce tin with tightly fitting telescopic cover. The salt in the tin package was protected further by being placed in a sack of blue paper.

The following disposition was made of the material:

A 1-ounce package (A) was opened in the Washington Laboratory immediately after delivery and a small portion taken out for water determination,¹ the package being kept open for 15 minutes. This procedure was repeated once each week until the material no longer lost weight, care being taken to keep the package open for exactly 15 minutes each time a portion was taken out for analysis, and to take the sample from the surface of the contents without mixing the remainder. This was assumed to simulate conditions in prescription practice.

The 8-ounce package (B) was given the same treatment as the 1-ounce package above mentioned.

After an interval of 1 month another package (C), which had been kept sealed in the laboratory since purchasing, was opened and a sample taken for water determination, the container being kept open for 15 minutes. This procedure was repeated once each month until the material ceased to lose weight.

After an interval of 3 months a fourth package (D) (still sealed) was opened and a sample removed once each month for water determination, the package being left open for 15 minutes each time that a sample was removed. The process was repeated until the loss became constant.

After an interval of 4 months a fifth package (E) (still sealed) was opened and a sample removed for water determination once each month until the weight became approximately constant.

One of the 1-ounce packages (F) was sent unopened to the Minneapolis Station of the Food and Drug Administration, and another (G) to the Denver Station with the request that they be opened once each week for 8 weeks and kept uncovered for 15 minutes each time. The request was made also that the product be stored in such manner that direct sunlight would not fall on it. As soon as the specimens had been returned they were opened and the treatment accorded the third specimen given each.

Since all of the specimens were of the same lot it was assumed that the initial water content of all of the 1-ounce packages was the same as that of the two specimens which were first assayed.

At the expiration of one year from the time of purchase all of the specimens were reexamined for loss on drying. Also at the expiration of one year from the time of opening each specimen was again tested for loss on drying.

The results obtained with the specimens which were analyzed weekly are recorded in Table I; those obtained from the analyses on the monthly basis are given in Table II. The monthly results for Specimens A and B are included also in Table II, four weeks being considered as 1 month.

¹ Water was determined by drying at 90° C.

In Table III the principal results are condensed for ready reference.

TABLE I.—WATER CONTENT OF QUININE SULPHATE AFTER STORAGE FOR DIFFERENT PERIODS.

Sample.	Initial Water Content, Per Cent.	Water Content after 1 Week, Per Cent.	Water Content after 2 Weeks, Per cent.	Water Content after 3 Weeks, Per Cent.	Water Content after 4 Weeks, Per Cent.	Water Content after 5 Weeks, Per Cent.	Water Content after 6 Weeks, Per Cent.	Water Content after 7 Weeks, Per Cent.	Water Content after 8 Weeks, Per Cent.	Water Content after 9 Weeks, Per Cent.
A	12.91 12.91 13.18	12.97 12.91	12.58	11.63	8.93	7.77	9.18 8.35	7.94	6.98	6.65
B	13.48 13.44	12.75 12.44	11.27	10.25	9.56	7.86	6.92	5.81	6.40	6.97

Sample.	Initial Water Content, Per Cent.	Water Content after 10 Weeks, Per Cent.	Water Content after 11 Weeks, Per Cent.	Water Content after 12 Weeks, Per Cent.	Water Content after 13 Weeks, Per Cent.	Water Content after 14 Weeks, Per Cent.	Water Content after 15 Weeks, Per Cent.	Water Content after 16 Weeks, Per Cent.	Water Content after 7 Mos., Per Cent.
A	12.91 12.91 13.18	5.57	5.27	4.63	No assay	No assay	No assay	No assay	No assay
B	13.48 13.44	6.83	5.41	5.37	5.03	5.06	4.84	5.00	4.50

TABLE II.—WATER CONTENT OF QUININE SULPHATE AFTER STORAGE FOR DIFFERENT PERIODS.

Sample.	Initial Water Content, Per Cent.	Water Content after 1 Mo., Per Cent.	Water Content after 2 Mos., Per Cent.	Water Content after 3 Mos., Per Cent.	Water Content after 4 Mos., Per Cent.	Water Content after 5 Mos., Per Cent.	Water Content after 6 Mos., Per Cent.	Water Content after 7 Mos., Per Cent.	Water Content after 8 Mos., Per Cent.
A	12.91 12.91 13.18	8.93*	6.98*	4.63*	No assay	4.56	No assay	No assay	No assay
B	13.48 13.44	9.56*	6.40*	5.37*	5.00*	4.86	No assay	4.50	No assay
C		13.53** 13.00	12.65 11.84	8.70 8.45	6.95	4.56	4.68	4.62	No assay
D				12.09** 11.88	10.33	8.51	7.78	7.36	6.80
E					11.25** 11.21	9.78	8.95	8.56	7.4
F			12.10** 12.09	11.54 11.53	10.42	9.38	8.07	6.84	6.58
G			13.27** 11.25	9.13 9.06	8.78 8.20	9.51	7.94	6.99	No assay

Sample.	Initial Water Content, Per Cent.	Water Content after 9 Mos., Per Cent.	Water Content after 10 Mos., Per Cent.	Water Content after 11 Mos., Per Cent.	Water Content after 12 Mos., Per Cent.	Theory for $Q_5SO_4 \cdot 2H_2O$, Per Cent.
A	12.91 12.91 13.18	No assay	No assay	No assay	4.84	4.60
B	13.48 13.44	No assay	No assay	No assay	4.51	4.60
C		No assay	No assay	No assay	4.07	4.60
D		7.21	6.37	5.64	4.06	4.60
E		7.81	5.25	4.65	4.84	4.60
F		6.90	No assay	6.60	4.61	4.60
G		No assay	No assay	6.17	4.87	4.60

* Four weeks was considered as a month in these assays.

** Initial assay.

The results in Table I show that Specimen A became stable in 12 weeks and Specimen B in 7 months. The stable stage was reached when the product contained approximately 4.60 per cent of water, or two molecules. Specimen C contained 13.27 per cent of water after storage for one month unopened. It became stable in 4 months after opening, or 5 months after purchase. Specimen D contained 11.98 per cent of water after storage unopened for 3 months. It became stable in 9 months after opening, or 12 months after beginning storage. Specimen E contained 11.23 per cent of water on first opening 4 months after purchase. It became stable in 7 months after opening or 11 months after purchase. The specimens which were not opened until 1, 3 or 4 months after purchase contained nearly

as much water (initial analysis) as the specimens opened and assayed immediately after purchase. The specimen which had been opened weekly in Minneapolis for 8 weeks (but not analyzed there) (F) contained 12.1 per cent of water after its return to the Washington laboratory, or nearly 6 molecules. After monthly opening and sampling in Washington it became stable in 10 months after opening, or 1 year after purchase. Likewise, the specimen which had been opened in Denver (G) contained 12.26 per cent of water (or nearly 6 molecules) after its return to Washington. It became stable in 12 months after opening or 14 months after purchase.

TABLE III.—WATER CONTENT OF QUININE SULPHATE AFTER STORAGE FOR DIFFERENT PERIODS.

Sample.	Treatment before Initial Analysis, Per Cent.	Water Content Initial Analysis, Per Cent.	Water Content 1 Mo. after Opening, Per Cent.	Water Content 2 Mos. after Opening, Per Cent.	Water Content 4 Mos. after Opening, Per Cent.	Water Content 6 Mos. after Opening, Per Cent.	Water Content 8 Mos. after Opening, Per Cent.	Water Content 12 Mos. after Opening, Per Cent.
A	Opened immediately	12.91 12.91 13.18	8.93	6.98	No assay	No assay	No assay	4.84
B	Opened immediately	13.48 13.44	9.56	6.40	5.00	No assay	No assay	4.51
C	Stored 1 month after purchase	13.53 13.00	12.65 11.84	8.70 8.45	4.56	4.62	No assay	4.67
D	Stored 3 months after purchase	12.09 11.88	10.33	8.51	7.36	7.21	6.80	4.86
E	Stored 4 months after purchase	11.25 11.21	9.78	8.95	7.44	7.44	5.25	4.58
F	Opened weekly for 2 mos. but no samples taken, then opened monthly for analysis	12.10 12.09	11.54 11.53	10.42	8.07	6.58	No assay	4.74
G	Opened weekly for 2 mos. but no samples taken, then opened monthly for analysis	13.27 11.25	9.13 9.06	8.78 8.20	7.94	No assay	No assay	4.81

These experiments show that during storage in a climate comparable to Washington, D. C., crystallized quinine sulphate progressively loses water of crystallization until it contains about 2 molecules (4.60%) after which it remains practically stable. The more frequently the specimen is opened the more rapid is the loss. Specimens which are opened occasionally but which are not otherwise disturbed, such as the packages sent to Minneapolis and Denver, do not lose their water of crystallization very rapidly. The conditions under which Specimens A and B were kept probably represent those obtaining in drug store practice more nearly than is the case with the other specimens.

THE HYDRATION OF EMETINE HYDROCHLORIDE AND CODEINE PHOSPHATE.*

BY H. WALES.¹

I. *Emetine Hydrochloride*.—The statement in the tenth edition of the United States Pharmacopœia that emetine hydrochloride "contains variable amounts of water of crystallization" is the obvious conclusion to be drawn from the reports of those who have published data on this product.

Paul and Crownley (1) state that considerable difficulty was encountered in obtaining the salt in a state fit for analysis on account of the large quantity of

* Scientific Section, A. Ph. A., Washington meeting, 1934.

¹ Drug Control, Food and Drug Administration.