FLUIDEXTRACT OF SQUILL.*

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Some time ago we called attention to the fact that the official dose of Squill preparations was not in proportion to the toxicity required for them by the biological standards of the U. S. P. IX, and we further pointed out that the commercial fluidextracts vary considerably in toxicity. We have also noticed since that time that commercial preparations vary considerably in the amount of total solids (10–30 Gm. per 100 mils) and the percentage alcohol (37–55%) which they contain.

We have deemed it advisable, therefore, to ascertain the cause for these variations and to devise a proper method for making fluidextract of squill. The U. S. P. IX process for making fluidextract of squill prescribes that the drug in *No. 20 powder* be macerated and percolated with *two parts alcohol and one water.* After a *definite volume* of the percolate has been received, it is directed that the alcohol be distilled and the liquid concentrated to a *definite volume.* After allowing the residue to cool, the gum, etc., are precipitated with alcohol and the residue washed with a diluted alcohol (4 parts alcohol and 1 part water). The combined alcoholic liquids are *distilled to a definite volume* and finally enough dilute alcohol is added to make a fluid, each part of which corresponds to one part of drug.

In any process for making fluid squill, the object in view is to obtain a product, of which 1 mil fully represents the active and therapeutic principles of 1 gramme of the drug. The process, which accomplishes this purpose in the simplest, most direct way is of course the best. In our opinion, few persons would say that the U. S. P. IX process is not complicated, but it might be assumed by some that if the directions were carried out properly a uniform product could be obtained.

The difficulties which are encountered in applying the U. S. P. process are: (1) the use of No. 20 powder, which becomes so gelatinous that percolation cannot be carried out; (2) the regulation of the definite volumes in the first and second stages of the operation. Upon this control of volume depend the qualities, chemical and physiological, of the finished product.

Our first experiments were made to find out if squill coarser than No. 20 powder could be used to obtain a satisfactory product. Fluidextracts were therefore made in accordance with the following specifications:

I. Squill No. 20 powder was exhausted according to the U. S. P. IX process, but the drug became so gelatinous that percolation could be perfected only by mixing purified sawdust with the drug.

II. Squill, very coarse powder, U. S. P. IX process. Percolation was satisfactorily carried out.

III. Whole commercial squill U. S. P. IX percolation process, which was satisfactory.

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The three preparations were examined chemically for total solids and alcoholic contents, and biologically for toxicity by both the one-hour and twelve-hour frog assay methods. The one-hour frog test was made strictly according to the method of the U. S. P. IX. The preparations were also examined by the Houghton twelve-hour frog method, except that in calculating the H. T. U. per mil, 200,000 rather than 100,000 H. T. U. per Gm. of Kombé Strophanthin was used. The M. L. D. and H. T. U. per mil for a standard F. E. Squill were assumed to be 0.0012 mil per Gm. frog and 80 H. T. U. per mil, respectively.

The chemical and biological tests on preparations I, II, and III are given in the following table:

TABLE I.

Fluidextract No.	Solid matter in 100 mils.	Alcohol % by volume.	Percentage of toxicity. 1-Hour frog.	12-Hour frog.	Ratio between 1-hr. and 12-hr. frog.
I	7.2	62.5	192.0	250.0	1:1.302
II	5.7	72.5	138.4	175.0	1:1.265
III	11.0	60.7	204.0	286.0	1:1.402

No. 20 powder causes considerable trouble during percolation and the data in Table I indicates that a coarse powder or even the whole commercial squill will give equally good products and at the same time facilitate the carrying out of the percolation process.

IV. This experiment was made to find out if it was necessary to have the finished product as strongly alcoholic as the menstruum which is employed to extract the drug, and which at the same time would retain the original toxicity. For this purpose, the alcohol from 100 mils of preparation No. III was reduced from 60.7 percent to 37 percent. This yielded a cloudy solution which was filtered. This preparation is designated as No. IV, and had 9.4 percent total solids, 37 percent alcohol and toxicities of 140.0 percent and 187.5 percent by the one-hour and 12-hour frog methods, respectively. It will be seen by comparison of assays on fluidextracts III and IV that only 1.4 percent total solids and 31 percent toxicity were lost, when calculated on the assumption that fluidextract No. III contained 100 percent total solids and toxicity, respectively. The lower toxicity may be due to the effect of the heat applied in reducing the alcoholic content.

Our next experiments were made in order to determine the proper solvent for extracting the drug. The following fluidextracts were prepared, using in each case very coarse squill:

V. 100 Gm. of squill was exhausted with hot water, the solution concentrated to about 60 mils and then sufficient alcohol was added to obtain 100 mils.

VI. Fluidextract was made according to the U. S. P. VII process. A product was obtained which separated into two layers (30 parts top layer and 70 parts bottom layer).

VII. The drug was extracted with 90 percent alcohol, the alcoholic solution was evaporated and the residue taken up with 50 percent alcohol. The fluidextracts thus prepared were examined as to the amount of solid matter and the percentage of alcohol which they contained, and they were also tested physiologically, both by the one-hour frog method and the twelve-hour frog method.

The following preparations, namely, VIII, IX, and X, were each made in triplicate, and are designated as (*a*), (*b*) and (*c*). Preparations (*a*) in each case were made from one lot of drug, and preparations (*b*) and (*c*) in each case, from a second lot of drug.

VIII. (*a*) (*b*) (*c*) Squill extracted according to the U. S. P. IX method.

IX. (*a*) (*b*) (*c*) 100 Gm. of squill was digested with two portions of hot water (750 mils and 250 mils). The mixture was strained through cheesecloth. The bulk of the strained solution was then evaporated to a syrupy consistence, 200 mils of alcohol were added, the mixture stirred well and the alcoholic liquid decanted. The combined alcoholic liquids were then concentrated and the residue dissolved in sufficient dilute alcohol to make 100 mils.

X. (*a*) (*b*) (*c*) Drug was extracted with 80 percent alcohol and then proceeded as under VII.

These preparations were examined chemically and biologically with results as recorded in Tables II and III.

TABLE II.

Fluidextract No.	Solid matter in 100 mils.	Alcohol % by volume.	Percentage of toxicity. 1-Hour frog.	12-Hour frog.	Ratio between 1-hr. and 12-hr. frog.
IV	9.4	37.0	140.0	187.5	1:1.340
V	36.0	38.0	78.5	97.8	1:1.245
VI	53.6	42.9	213.0	220.0	1:1.041
VII	3.4	50.0	120.0	132.2	1:1.110

TABLE III.

Fluidextract No.	Solid matter in 100 mils.	Alcohol % by volume.	Percentage of toxicity. 1-Hour frog.	12-Hour frog.	Ratio between 1-hr. and 12-hr. frog.
VIII.					
a	9.0	57.3	120.0	173.0	1:1.440
b	12.4	63.0	132.0	152.8	1:1.158
c	20.8	52.8	101.5	172.0	1:1.694
Averages	14.1	57.7	117.8	165.9	1:1.407
IX.					
a	29.2	39.6	83.5	133.4	1:1.596
b	12.4	38.0	126.6	116.0	1:0.917
c	20.9	37.0	95.0	101.4	1:1.066
Averages	20.8	38.2	101.7	116.9	1:1.149
X.					
a	16.2	50.0	128.0	153.8	1:1.202
b	13.1	51.6	165.0	105.6	1:0.640
c	14.8	49.5	97.8	114.4	1:1.170
Averages	14.7	50.4	130.3	124.5	1:0.957

DISCUSSION OF THE RESULTS.

The results given in Tables II and III show that water extracts the toxic principles of squill together with the gums and sugars which must finally be eliminated by precipitation with alcohol. By using 64 percent alcohol (U. S. P. IX solvent), we obtained the toxic principles, also the gums and sugars which must be removed by precipitation. Nothing was gained by the use of a 70 percent alcohol (U. S. P. VII 1890 solvent). Our experimental data show further that an 80 percent alcoholic menstruum gives a product of high toxicity and one in which the gums and a greater part of the sugars remain in the marc. The use of this solvent decidedly simplifies the unwieldy U. S. P. IX process and at the same time produces a fluidextract of squill containing a maximum of the therapeutic principles of the drug. By employing a 90 percent alcoholic menstruum, we obtained a product which was nearly as toxic and one which did not contain much

extractive matter. While the presence of gum, etc., does not greatly affect the toxicity of the preparation, they might as well be eliminated.

The U. S. P. IX process is capable of yielding a satisfactory product, and may be fundamentally correct. The process, however, is very unwieldy and evidently its practical application to large scale manufacture was not taken into consideration when it was designed. In the U. S. P. IX process, the volume of the original residue is difficult to control, hence by this process it is almost always impossible to obtain fluidextracts which are uniform in alcoholic strength and percentage of extractive matter.

While it is not within the scope of this paper to discuss the methods of biologic estimation of drugs in detail, we wish, however, to take this opportunity to say a few words regarding the relative merits of the two frog assay methods employed in this investigation.

A satisfactory biologic assay should give results which are comparable to chemical analyses in respect to accuracy, absolute as well as relative. The factor, which enters into all frog assay methods and which tends more than any other to prevent consistent and reliable results, is the rate of absorption of the drug. This variation in absorption may be due either to an idiosyncrasy of the test animal, temperature of medication (which should of course be kept constant) during the assay, or finally the nature and concentration of the drug itself. The 12-hour method practically eliminates this variability, as it allows sufficient time for complete absorption of the drug, regardless of the conditions which may be delaying absorption. As a result of this, the end-point in the 12-hour frog tests is never in doubt, that is, the animal is either dead or alive. On the other hand, the one-hour frog method of the U. S. P. IX leaves the interpretation of the end-point entirely to the experience and judgment of the operator. The degree and rate of absorption of the drug varies greatly in the one-hour frog tests and is the principal cause of the unreliableness of this method. While the 12-hour method takes longer to complete, the cost per assay is only half as much as for the one-hour method, viz.: ($0.60 as compared with $1.25).

It has already been stated in this paper, that in the 12-hour frog tests the percentage toxicities of the squill preparations were calculated on a basis of 200,000 H. T. U. per Gm. of Kombé Strophanthin. Repeated tests on samples of Kombé Strophanthin from the largest manufacturers have always shown that in the 12-hour frog test, the M. L. D. for K. Strophanthin is approximately 0.000,0005 Gm. per Gm. frog rather than the average M. L. D. of 0.000,0010 Gm. per Gm. frog given by some authorities.

It is, therefore, the greater consistency, reliability and accuracy of the 12-hour assay, when made from season to season and with different lots of frogs, that makes it preferable to the official one-hour frog test for biologic evaluations of heart tonic drugs, and it is the method which furnishes the basis for discussing the relative toxicities of our squill preparations.

SUMMARY.

1. A satisfactory fluidextract of squill can be made with a powdered squill coarser than No. 20.

2. When either a 64 percent alcoholic menstruum (U. S. P. IX process), water, or a 70 percent alcoholic menstruum (U. S. P. VII process), is employed, the toxic principles of squill are extracted together with the gums and sugars which must later be eliminated by precipitating with a stronger alcohol.

3. The most satisfactory menstruum for extracting squill was found to be an 80 percent alcohol. This solvent removes a maximum of the therapeutic principles and at the same time leaves the gums and the sugars in the marc. The use of 80 percent alcohol simplifies the preparation of fluidextract squill and the product which is obtained is more uniform and the cost of production is greatly reduced.

4. The 12-hour frog method is more satisfactory and accurate than the official U. S. P. IX one-hour frog method for the biologic estimation of the relative toxicities of fluidextract squill or similar preparations.

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