

The Heavy Metals Test for Volatile Oils*

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Among the more comprehensive problems undertaken in connection with the preparation of the Twelfth Revision of the Pharmacopœia, the complete restudy of the heavy metals test for chemicals may be mentioned. As a result of this study the test has been placed on a quantitative basis and therefore brought into accord with the present-day procedures as commonly applied to reagent chemicals.

As a consequence of the authors' contacts with this problem, the desirability of placing the heavy metals test for the volatile oils on a similar basis seemed to be of sufficient importance to warrant investigation. It is the purpose of this paper to discuss some of the work that has been done and to describe a method of procedure.

There are twelve volatile oils for which a heavy metals test is required. These oils are: anise, bitter almond, chenopodium, cinnamon, clove, coriander, eucalyptus, fennel, juniper, orange, sassafra and wintergreen. Commercial samples of these oils with U. S. P. labeling were purchased in the open market and used in the present study.

The test of the U. S. P. XI reads as follows: "Shake 10 cc. of the oil with an equal volume of distilled water to which a drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated: no darkening is produced in either the oil or the water (*lead or copper*)."

From the standpoint of simplicity there certainly can be no criticism of the test; but one might reasonably ask how accurately the presence or absence of darkening of the mixture can be determined particularly when no standard of comparison is provided. Such a standard might be obtained by passing an inert gas, such as nitrogen, through a similar oil and water mixture in the same manner as

the hydrogen sulfide is passed through the test mixture. In this case, however, it might well be expected that color differences quite independent of any heavy metals effect might occur owing to the marked difference in reactivity of hydrogen sulfide and of nitrogen.

EXPERIMENTAL

In our search for an improved method we have tried several procedures bearing in mind the desirability of having a standardized method which could be applied to each of the oils. An obvious method depending upon the ashing of the oil sample has doubtlessly been extensively examined. Our experience with this method has led to unsatisfactory results. The ashing process is long and tedious and requires considerable skill and experience. Perhaps with a rather elaborate temperature control satisfactory and consistent results can be obtained. The method of wet ashing also received consideration including the conventional sulfuric and nitric acid mixture and also a number of other oxidizing agents. In every case, we were faced with the serious difficulty of the necessity of adding considerably more heavy metals, already present in the reagents required, than might be reasonably expected to be present in the oil itself.

We have found one method which seems to offer considerable promise and it will be described in some detail. In this method a water mixture of the oil to be examined is subjected to steam distillation until the oil is practically completely removed. The residual aqueous solution is then tested for heavy metals.

For the steam distillation we have used a very simple arrangement as shown in Fig. 1. A 500-cc. Pyrex flask which serves as the steam generator is equipped with a two-holed rubber stopper through which are passed a safety tube and a steam delivery tube which leads almost to the bottom of a 25-x 150-mm. Pyrex glass tube.

In carrying out the test, a steady stream of steam is generated. Obviously there must be no bumping in the generator. One gram of the oil to be tested is weighed into the test tube and 5 cc. of distilled water is added. The tube is then placed in the position shown in the figure and the distillation is allowed to proceed for 15 min. during which time gentle heat is applied to the test tube so as to prevent excessive condensation therein. At the end of this period the total volume of the liquid in the test tube should be 10 to 15 cc. In the case of some of the oils this liquid was quite clear and water-white while in others it showed more or less milkiness suggesting emulsifi-

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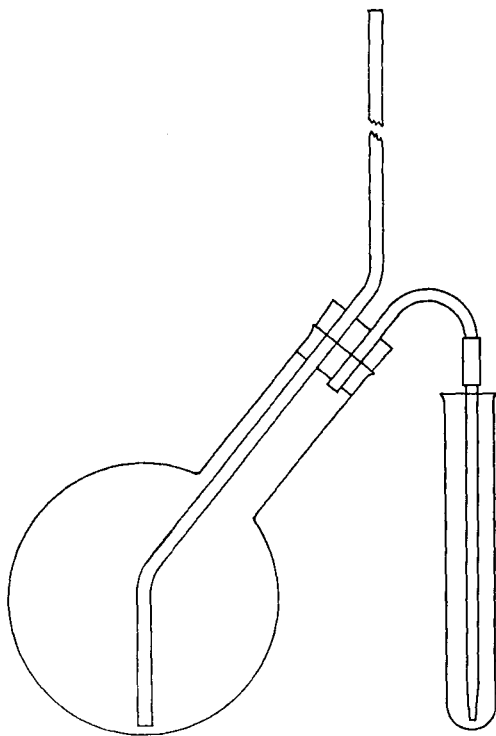


Fig. 1.

cation. Cloudy liquids are, of course, entirely unsuitable for the final step of the heavy metals test. In an effort to eliminate the formation of the cloudy residual solutions we have tried the addition of electrolytes to the water originally mixed with the oil sample. For example, 1 Gm. of sodium chloride was added to 5 cc. of the water before mixing with the oil. By this method no consistent improvement was obtained.

By the addition of one further step in the procedure we have been able to obtain clear and colorless residual solutions. The residual solution after the steam distillation is transferred to a separator, 25 cc. of ether is added and the mixture is extracted. The clear aqueous layer is separated and heated briefly on a steam bath until the ether is completely removed. The cooled solution is acidified by the addition of 1 cc. of 0.1 *N* hydrochloric acid and is then diluted to 25 cc. with distilled water.

This test solution is then treated with 10 cc. of hydrogen sulfide T. S. and after standing 10 min. its color is compared with that of 25 cc. of a standard aqueous solution containing 1 cc. of 0.1 *N* hydrochloric acid and the desired amount of lead as lead nitrate solution and to which has been added 10 cc. of hydrogen sulfide T. S. The two solutions are placed in similar comparator tubes and are observed by looking down through the tubes held above a white field. This portion of the test is identical with that of the heavy metals test for chemicals as adopted for the U. S. P. XII.

By means of this steam distillation method with subsequent ether extraction we have obtained consistently satisfactory results with ten of the twelve volatile oils previously listed. Even with numerous and extensive variations in the method we have been unable to adapt it to the oil of orange. Test solutions obtained from oil of sassafras, after the addition of the hydrogen sulfide, invariably became sufficiently tinted to obscure the final color comparison. Commercial U. S. P. samples of the other ten volatile oils were found to be below a ten parts per million heavy metals limit when examined by the proposed method. Some of the oils were found to be within a five parts per million limit. In each determination a comparison test was made in which ten parts per million of lead, as lead nitrate solution, was added to a 1-Gm. sample of the oil and then carried through the procedure outlined. An adequate recovery of this additional lead was consistently obtained, indicating that there is no appreciable loss in inorganic lead in the course of the procedure. Similar results were also obtained when copper, as copper sulfate solution, was employed as the added heavy metal.

We have experienced no difficulty in carrying out the test as proposed and recommend it as an improvement over the present test of the Pharmacopœia. With this recommendation, however, several points should be kept in mind. The heavy metal content of the volatile oils is to be attributed to one or both of two sources. Heavy metals may be present as a natural constituent of the original material and they may be introduced as contamination products during the processing of the oil. In either case, it is important to know if the heavy metals are present in organic combination and if such compounds are volatile with steam. Furthermore, if they are not appreciably volatile with steam they could be lost to the final test during the ether extraction step if sufficiently soluble in that solvent. The desirability of including oil of orange and oil of sassafras in any proposed method is fully recognized. Further work is being conducted along these lines.

SUMMARY

1. A procedure for the determination of heavy metals in volatile oils utilizing the principles of steam distillation has been described.

2. The method works quite satisfactorily for ten of the twelve volatile oils which require the test. Unsatisfactory results have been obtained with oil of orange and oil of sassafras.

3. All of the commercial U. S. P. oil samples examined in the course of the work gave a heavy metals test of less than ten parts per million.