The Behavior of Fish Oils With Uranium Nitrate and Pyrogallic Acid

Developing a Possible Method for Detection of Adulteration in Cod Liver Oil.

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THERE has been considerable desire on the part of importers and dealers in Cod Liver Oil for the development of a means of determining the purity of that commodity which would be, (while reasonably dependable), more rapid than the present method prescribed by the U. S. Pharmacopoeia, which requires the determination of unsaponifiable matter, saponification and iodine numbers.

The following data, which was assembled while the writer was conducting experiments with a view to obtaining a chemical test for the identification of vitamins, seems to offer an avenue for research, which may, upon further investigation, develop a rapid means of confirming the purity of Cod Liver Oil, within reasonable limits.

Reagents Selected

Uranium Nitrate and Pyrogallic Acid were used for the following experiments, because, first of all, uranium nitrate being a radio-active chemical, was considered a likely substance for the development of a chromogenetic reaction. According to the U. S. Dispensatory, it is incompatible with the alkaloids, chloral hydrate, salts of arsenous and phosphoric acids; and changes the color of cochineal and its derivatives. The oxide is frequently used in photography. Pyrogallic acid was chosen because of its reaction with trichloracetic acid and kerosene in the detection of vitamins and also because of its use in photography as a developer.

Variations of Vitamin Tests

Knowing that pyrogallic acid when used with trichloracetic acid, kerosene and hydrogen peroxide, will develop a pink coloration with the vitamins of Cod Liver Oil,[1] I substituted powdered uranium nitrate for the trichloracetic acid, but the results were negative. When, after experimenting for some time, a mixture of equal parts of the uranium nitrate and pyrogallic acid were powdered together in a mortar, one-tenth of a gram of this mixture placed in 5 cc of oil in a test tube and shaken without heat, no reaction resulted; but on heating the test tubes over a Bunsen flame a color developed much more quickly with the other oils than with Cod Liver Oil. This appeared to be a good lead for a cod liver oil purity test and each chemical used was tested separately for its solubility in the various fish and marine animal oils worked with. Both the uranium nitrate and pyrogallic acid were soluble in all the oils tested, which included cod liver oil, menhaden oil, pilchard oil, whale oil, sperm oil, sardine oil and herring oil.

Experiments on Characteristic Reactions

It was this stage of the experiment that led to more encouraging

[1] Am. Jour. of Phar., July, 1926, p. 422.

results. In the first series of tests 0.1 gr. of pyrogallic acid was added to 5 cc of each of the oils, followed by a trace of powdered uranium nitrate. This mixture was placed in the steam bath for a few minutes and occasionally shaken. With all the oils, other than cod liver oil, a precipitate was formed and the color of the mixtures became a very dark red. With the cod liver oil, the mixture assumed a medium red color but no precipitate appeared. In the next experiment 2 grs. of pyrogallic acid were dissolved in 100 cc of ether; 5 cc of this solution were added to 5 cc of each oil in a test tube and the tubes placed in a water bath at 45°C. to evaporate the ether. After the ether was evaporated, 10 milligrams of powdered uranium nitrate were added to each oil, and the mixtures placed in the steam bath for thirty minutes, after which they were removed and the colors compared before an electric lamp. All the oils had turned a dark reddish or a brown color and were opaque, except cod liver oil, which although of a light red color was translucent. The ether solution of the pyrogallic acid was found to be an undesirable reagent, as it turned red on standing.

Another experiment was conducted by making a 1 percent alcoholic pyrogallic acid solution, using 95% alcohol. It was necessary to use 95% alcohol, as pyrogallic acid in weaker alcoholic solution turns yellow on standing. In this experiment a reversal of the procedure in the experiment above was adopted.

Ten (10) milligrams of the powdered uranium nitrate were placed in a test tube with 5 cc of the oil. The tube was placed in the steam bath for about two minutes to dissolve the uranium salt. Then 5 cc of the alcoholic pyrogallic acid solution was added and the mixture heated until most of the alcohol had evaporated. The tube was then removed from the bath to cool. The following colorations in the tubes resulted:

Oil	*Primary coloration*	*Treated with alcohol and sulphuric acid*
Cod Liver Oil (U.S.P.)	Orange Red	Decolorized
Harden Fish Oil	Orange Red	Decolorized
Sperm Oil	Orange Red	Decolorized
Sardine Oil	Yellowish Brown	Yellow
Whale Oil	Yellowish Brown	Yellow
Menhaden Oil	Yellow	Canary Yellow
Pilchard Oil	Yellow	Yellow
Newfoundland Cod Oil	Orange Red	Bright Orange
Herring Oil	Light Orange Red	Yellow
Blank Determination	Red	Decolorized

Cod Liver Oil (U.S.P.) orange red
Hardened Fish Oil orange red
Sperm Oil orange red
Sardine Oil yellowish brown
Whale Oil yellowish brown
Menhaden Oil yellow
Pilchard Oil yellow
Newfoundland Cod Oil orange red
Herring Oil light orange red
Blank Determination red

Next 10 drops of a 1-4 mixture of alcohol and sulphuric acid were

added to each tube, and the contents well shaken, with the results in table on page 327.

A further variation of the experiment was conducted as follows: Ten milligrams of the powdered uranium nitrate were placed in a test tube with 5 cc of the oil. The tube was placed in the steam bath to dissolve the uranium nitrate. One-tenth of a gram of pyrogallic acid was added and the mixture again heated in the steam bath for two minutes. All the oils except cod liver oil gave a precipitate and a color. They were allowed to stand overnight and in the morning examined to observe the appearance of the precipitate under the microscope. By this time the cod liver oil had precipitated white crystals which had the appearance of branches of trees each with a number of small twigs extending from the branch. Whale, menhaden and sardine oils gave a different form of crystal. In these three oils the crystals were more of a disc-mould or jelly-fish shape with many fine hairy legs and at the end of each leg a small ball. These objects were different in appearance from the fatty acid crystals which could be seen throughout the slides. Herring Oil gave a needle-shaped crystal, while Pilchard Oil gave crystals which looked more like staphylococcus bacilli. The original oils were tested to see if moulds were present but none was found.

Proposed Test for Cod Liver Oil

The following simple experiment can, I believe, be used to differentiate between cod liver oil (U.S.P.) and contaminated cod liver oil. Directions: Place in a test tube 10 milligrams of powdered uranium nitrate and 3 cc of the fish oil to be tested, then place the tube in the steam bath for 20 minutes, shaking occasionally, remove and observe the color, which should be as follows:

Cod Liver Oil (U.S.P.)—Amber color, showing a greenish cast with transmitted light

Norwegian Sperm Oil—Light amber color, no difference with transmitted light

Menhaden Oil—Crimson red

Pilchard Oil—Light red

Whale Oil—Light brownish red

Herring Oil—Blood red

Sardine Oil—Blood red

Newfoundland Cod Oil—Blood red

Samples of cod liver oil that were thought to be pure, answering all the U. S. P. requirements, except that they were high in unsaponifiable matter, gave a red color within six minutes. These samples were later declared contaminated.