AN ADSORPTION METHOD FOR THE MEASUREMENT OF VITAMIN A STABILITY

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A number of methods are at present available for the determination of the stability of oils containing vitamin A. One of the earliest, the "Swift" method[1] subsequently modified[2,3,4], depended on passing air or oxygen through vitamin A oils at an elevated temperature, the undecomposed vitamin A being determined after varying periods of time; in the petri dish method[5] the degradation of the oil was produced by placing the oil in a petri dish or similar receptacle and exposing it to air at elevated temperatures in a thermostatically controlled oven. The oxygen absorption method[6,7,8] involved the determination of the amount of oxygen absorbed by an oil during oxidation either directly or by calculation from the pressure change.

It was felt that all these methods had certain disadvantages. Modifications based on the Swift method although reliable, are somewhat tedious and are more suitable for use in laboratories concerned with the stability of vitamin A oils as an everyday routine. The petri dish method is more convenient in operation but has been found, especially with some oils, to give less reliable and less reproducible results. Better results can be obtained if small vessels are used and the complete contents of a vessel are used for each assay, small glass or porcelain rings or cups being used as containers[9]. The direct oxygen absorption method suffers from the disadvantage that actual measurements of vitamin A potencies are not made, the results depending on measurements of oxygen absorption. In the last two methods oxidation takes place mainly at the surface and any skin formation will interfere with the rate of oxidation.

Stability experiments by all 3 methods are generally performed between 37° C., and 100° C., in order to shorten the time involved, although the use of higher temperatures introduces the possibility of thermal decomposition which is unlikely to occur below 25° C. In addition the artificial conditions necessarily adopted may not reflect accurately the stability of the oil in a particular pharmaceutical preparation.

A method was therefore evolved which is simple in operation and specially suitable for the determination of the stability of vitamin A oils when present in tablets or when adsorbed on solids. The vitamin A oil or concentrate was adsorbed on a powder, the mixture being placed in a number of small wide-mouth screw cap bottles which could be closed and stored at 25° C. or 37° C. At suitable intervals the contents of the bottles were weighed, extracted and assayed either spectrophotometrically at 328 mμ or by the antimony trichloride reaction. The method gave a much accelerated rate of decomposition without rise in temperature and was found to be particularly applicable to the determination of the

stabilities of vitamin A oils intended for use in tablets and in certain animal feeding supplements where the vitamin A is adsorbed on a solid medium.

Calcium phosphate, kieselguhr, barium sulphate, and silica were tried as adsorbents. It was difficult to obtain an even distribution of vitamin A oils on available barium sulphate powders; kieselguhr was also unsatisfactory since absorption on this medium caused decomposition of the vitamin A which was too rapid for convenient measurements of stability. Silica and calcium phosphate were both found to be suitable although some difficulty was found in quantitative extraction of low potency oils from silica. It was finally decided after many trials to use calcium phosphate B.P. (primary calcium phosphate has been reported to react as a synergist presumably due to its acidity[10]). The ratio of the weight of vitamin A oil to adsorbent was kept constant at 1:50.

Experimental

Mix 1 g. of oil with 49 g. of calcium phosphate B.P. by grinding together with a glass pestle and mortar; add the calcium phosphate at first in 1, 2, 4 and 8 g. quantities. The whole mixing must be done very rapidly. The efficiency of this stage determines the accuracy of the experiment. Distribute the mixture into 10 or 15 small, wide-mouthed screw cap bottles which are then stored at the chosen temperature. After appropriate time intervals weigh the whole contents of one container into a conical flask and extract the vitamin A with portions of chloroform or *cyclo*hexane by decantation. Make up to volume and filter if necessary. Determine the vitamin A spectrophotometrically or by the antimony trichloride reaction. Table I shows typical results obtained at 25° C. with some high potency vitamin A concentrates.

TABLE I

	High potency (1,500,000 I.U./g.) vitamin A alcohol unstabilised — per cent. of original potency	Synthetic vitamin A acetate 1,000,000 I.U./g. — per cent. of original potency	High potency (370,000 I.U./g.) natural concentrate — per cent. of original potency	Synthetic vitamin A palmitate (1,000 000 I.U./g.) stabilised — per cent. of original potency
1 day	2	98	100	100
2 days	—	92	100	100
3 days	—	79	100	100
4 days	—	55	100	100
6 days	—	10	95	98
8 days	—	—	86	94
10 days	—	—	76	89
12 days	—	—	64	82
14 days	—	—	54	74
16 days	—	—	44	66
18 days	—	—	29	48

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