

A Rapid Method of Separation and Estimation of Squalene from Fish Liver Oils Using Iatroscan Analyser

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Squalene is found present only in *Chiloscyllium griseum* liver oil among the four shark liver oils studied. The unsaponifiable matter (80% of oil) contained 71% squalene. The separation of squalene by column chromatography is discussed. For the quantitative recovery of 1 g of squalene on 10 g alumina column 30 ml petroleum ether is required. The detection of squalene is found to be higher using Iatroscan compared to column chromatography using alumina.

The occurrence of Squalene in small quantities in vegetable oils like olive oil and in high concentration in fish oils was first reported by Tsujimoto (1906). Liver oils of some sharks, especially the deep sea sharks, contain about 80% unsaponifiable matter of which 90% is squalene. 7-45% of Basking shark (*Cetorhinus maximus*) liver oil is composed of squalene and another hydrocarbon zamene (Lovern, 1962). Squalene is an intermediate in the biosynthesis of cholesterol. It has got bactericidal properties. It is widely used in the manufacture of pharmaceuticals, aromatics, surface active agents, rubber chemicals and also as additive in cosmetics. It is a good lubricant and is used in making dyestuffs, artificial silk, etc. (Bailey, 1952). A health food called squalene powder is popular in Japan.

Considering the various important industrial applications and uses of squalene a rapid method for the detection and quantitative estimation of it using Iatroscan Analyser was worked out. The details of the method and the results of the analysis compared to other methods are discussed in this paper.

Materials and Methods

Liver oils of four species of sharks

namely, *Scoliodon sp.*, *Sphyrna zygaena*, *Carcharhinus limbatus* and *Chiloscyllium griseum* (Dog shark) were analysed for unsaponifiable matter as per the methods of AOAC (1975). Specific gravity was determined at ambient temperature (29±1°C). Refractive index was measured using Abbe's Refractometer. Oil samples were collected from different areas. The data given represent the average values of the determinations.

In a glass column (300x10mm) a slurry of freshly heated chromatographic grade activated alumina was filled to a height of 10 cms. This was filled with petroleum ether (40-60°C) just to cover the alumina. The unsaponifiable matter separated from the oil was dissolved in petroleum ether and applied on the alumina column. It was eluted with petroleum ether and eluant collected in a beaker. The solvent was vapourised off and the beaker weighed to constant weight. The process was repeated until maximum hydrocarbon was recovered. From this mixture of hydrocarbon, squalene was determined by the pyridine sulphate-bromide reaction (AOAC, 1975).

Squalene was identified by thin layer chromatography technique (Bernad & Sherma, 1982). Thin layer plates of thickness 4µm were prepared using silica gel G. The hydrocarbon

mixture containing squalene, collected through alumina column was subjected to TLC in a solvent system of n-butanol : acetic acid: water (3:2:2). The plates were exposed to iodine vapour and squalene was identified. Experiments were carried out by adding a known quantity of squalene to oil which did not contain it and the recovery was estimated using alumina column and Iatroscan.

The Iatroscan TH-10 TLC/Analyser, MarkIII (Iatron Labs. Inc. Tokyo, Japan) used was equipped with a flame ionisation detector and an external electronic integrator/print-out system. The FID was operated under a hydrogen pressure of 0.7 kg/cm² and flow rate of 160 ml/min and air flow rate of 2000ml/min. The scanning speed was 0.4 cm/sec. A thermal printer plotter chromatocorder II (Systems Instruments Co.Ltd., Tokyo, Japan) was used for recording.

Squalene was estimated using Iatroscan analyser. Unsaponifiable matter from shark liver oil was dissolved in a suitable solvent and spotted (1 μ l) using a Drummond micro-dispenser 2 cms above the base of chromorod type-s. It was developed in a glass chamber (150x30x150 mm) containing solvent system n-hexane: diethyl ether (9:1) for 30 min. Chromarods were taken out and dried using a drier and heated to 100°C for 5 min in an air oven. These dried chromarods

were scanned in Iatroscan. Similarly the standards were also scanned. From the chart peak and retention time (RT) of the standard and experimental samples, the squalene content was estimated.

Results and Discussion

Table I gives the characteristics of liver oils from four species of shark. *Chiloscyllium griseum* liver oil has 80% unsaponifiable matter of which 71% is squalene. The liver oils of other species contain around 1.3% unsaponifiable matter and no squalene. The specific gravity of the *C.griseum* liver oils is 0.8577 which contains high amount of squalene. All other samples have specific gravity above 0.9 and contain no squalene. It indicates that when specific gravity is above 0.9 the oil samples may not contain squalene.

During the elution of the nonunsaponifiables through alumina column, it is observed that the solvent required was 30 ml on an average for recovery of one gram squalene in the above column. Addition of more solvent did not enhance further separation (Table 2,3). The results show that using 10 g of alumina and 30ml petroleum ether maximum recovery of squalene can be effected from 1 g sample (Table 2). It is also observed that 10cm column of alumina can absorb a maximum of 1.5 - 2.0 g of squalene and

Table 1. Specific gravity and percentage of unsaponifiable matter and squalene in four species of shark liver oils

Species	Specific gravity	Unsaponifiable matter %	Squalene % of Unsaponifiable matter
<i>Caroharhinus limbatus</i>	0.9107	1.385	Nil
<i>Scoliodon sp.</i>	0.9175	1.319	Nil
<i>Sphyrna zygaena</i>	0.9201	1.223	Nil
<i>Chiloscyllium griseum</i>	0.8577	80.000	71.

Table 2. *Percentage recovery of squalene from alumina column*

Weight of liver oils	Squalene added	Volume of solvent	Recovery of squalene	Volume of solvent	Recovery of squalene
g	g	ml	%	ml	%
0.851	0.109	30	98.16	40	98.16
0.843	0.157	30	98.08	40	98.09
0.636	0.304	30	98.35	40	98.40
0.490	0.510	30	98.01	40	98.03
0.931	1.059	30	97.50	40	97.50
9.019*	1.033	30	90.86	40	90.90
8.028*	1.996	30	95.75	40	95.80

*Commercial Samples.

Table 3. *Recovery of squalene from shark liver oil*

Volume of solvent	Recovery % (Average)
10	20.28
20	64.66
30	98.21
40	98.16
50	98.13
60	98.17

afterwards the absorption capacity decreased (Table 4).

Table 5 gives the relation of retention time and recovery of squalene. In Table 6, the percentage detection of squalene by using alumina column and that by scanning is given. Complete (100%) detection is possible using

Table 4. *Absorption capacity of alumina column*

Squalene added mg	Recovery of squalene %
106	98.16
236	97.95
469	98.01
740	97.89
1030	97.34
1189	97.21
1496	97.04
1732	98.84
2065	94.31
2512	92.11
2788	91.40
3051	88.78

Table 5. *Recovery of Squalene by Iatroscan analysis*

	Retention time (sec)	Recovery
Squalene	0.104	85.2695
Oil I	0.098	80.0276
Oil II	0.096	81.6044
Oil III	0.088	80.1740

Stationery phase : Chromarod - S

Mobile phase : n- hexane: diethyl:
ether: 9:1

Hydrogen flow - 160 ml/min;

Air flow - 20000 ml/min

Recorder sensitivity 10 mV ;

Scanning speed 30 sec/rod scan

Iatroscan from pure squalene while it is only 96.21% in alumina column. When squalene is added to different oils the percentage detection is 88 - 96 by Iatroscan and only 80 - 81 using alumina. The Iatroscan method is rapid and simple. Hence Iatroscan is an efficient analytical tool for the estimation of squalene.

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Table 6. *Percentage detection of squalene in alumina column and Iatroscan*

	By alumina column	By Iatroscan
	%	%
Squalene	96.2100	100.00
Oil I	80.0395	95.9924
Oil II	81.4025	88.1760
Oil III	80.1028	91.5663

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