Asian Journal of Chemistry Vol. 18, No. 2 (2006), 1520–1522

NOTE

Spectrophotometric Estimation of Vitamin A in the Sturgeon Fish Liver of the Caspian Sea

MOHAMMAD REZA HADJMOHAMMADI, MOHAMMAD JAVAD CHAICHI* and VALI TAGHIPOUR
Department of Chemistry, Mazandaran University, Babolsar, Iran
Fax: (98)(11252)42002; Tel: (98)(11252)42025
E-mail: jchaichi@yahoo.com

Vitamin A present in sturgeon fish liver of Caspian sea was determined by Carr-Price reaction. In this reaction Vitamin A produces blue color (λ_{max} = 620 nm) with $SbCl_3$ in $CHCl_3$ solution which is the basis for analytical measurement of this vitamin in visible region. The amount of this vitamin was in the range of 54080 ± 4234–76410 ± 5983 USP units (13524 ± 1059–22923 ± 1795 ppm)/g of fish liver oil for three different species of this fish.

Key Words: Vitamin A, Sturgeon fish, Fish liver oil, Carr-Price reaction, *Acipenser stellatus*, *Acipenser guldenstadtii* and *Acipenser percicus*.

Vitamin A is one of the fat soluble vitamins. It is only found in animal organisms. The most abundant source of this vitamin is fish liver oils[1, 2]. The methods of extraction of Vitamin A are: extraction with solvent[3], saponification and then crystallization[4]. Vitamin A produces blue colour (λ_{max} = 620 nm) with strong Lewis acid, $SbCl_3$ in $CHCl_3$ solution. The reaction of $SbCl_3$ with Vitamin A (Carr-Price reaction) is the basis for analytical measurement of this vitamin in the visible region[5, 6]. Other analytical methods include UV absorption[2, 7], fluorescence[8], HPLC[9–11] and TLC[12].

The international unit of Vitamin A (USP unit) is equal to 0.344 μg of Vitamin A acetate[2]. The fish liver oil is a very important source of Vitamin A. Since catching of sturgeon fish in Iran is about 20 ton/yr and its liver is not used commercially, here extraction and determination of Vitamin A in sturgeon fish liver is investigated.

All chemicals were obtained from Fluka Chemical Company (Büchs, Switzerland). The instruments used were: spectrophotometer Cecil 5000 (Cambridge, England), freeze dryer Eyela FD-1 (Tokyo, Japan) and rotary evaporator Buchi (Flawil, Switzerland).

Sampling

All fish specimens were caught from Caspian Sea near Babolsar, Iran in the

middle of July. The analytical data derived from analysis of three species of sturgeon fish are recorded in Table-1.

TABLE-1
THE AMOUNT OF VITAMIN A AND FAT IN THE STURGEON FISH LIVER

Kind of fish	Sex	Weight of liver (g)	Dry weight of liver (%)	Fat per dry weight of liver (%)	Vitamin A (USP/g fat)
Acipenser stellatus	male	265	27	52	73080 ± 5769
	female	250	27	54	76410 ± 5983
Acipenser guldenstadtii	male	270	26	52	72820 ± 5701
	female	265	28	53	73210 ± 5732
Acipenser percicus	male	244	27	44	54080 ± 4234
	female	235	28	48	60850 ± 4765

Alkaline hydrolysis and standard addition

First the samples of fish liver were dried by a freeze dryer. Then, seven 250 mL round bottom flasks were chosen and to each of them 10 g of dried liver sample was added. Standard solution of Vitamin A acetate (1000 USP/mL) with volumes 5, 10, 15, 20, 25, 30 and 35 mL were added to flasks 1–7, respectively. After addition of Vitamin A acetate solution, 20 mL of 50% KOH solution was added to each flask and refluxed for 45 min. The material of each flask was filtered and liquid filtrates transferred to a 250 mL volumetric flask and diluted to mark with the 25% solution of EtOH as a solvent.

Extraction of Vitamin A

15 mL of the solution of Vitamin A in EtOH was added to a separatory funnel and extracted by 20 mL of *n*-hexane. The aqueous layer was discarded and the *n*-hexane layer was evaporated by a rotary evaporator at 60–65°C. The residue was dissolved in 1 mL of $CHCl_3$ and used for analysis.

Spectrophotometric determination of Vitamin A

The zero absorbance of spectrophotometer at 620 nm was adjusted by a solution containing 1 mL of $CHCl_3$ and 9 mL of 20% $SbCl_3$ solution in $CHCl_3$. For determination of Vitamin A, 1 mL of extracted solution of Vitamin A was mixed with 9 mL of 20% $SbCl_3$ solution in $CHCl_3$ in a spectrophotometer cell. After the reaction was completed, blue colour was produced and the absorbances of the solutions were recorded at 620 nm for each samples separately.

Extraction of fat

For determination of fat content in fish liver, the dried liver samples were weighed and added to a thimble and their fats extracted by diethyl ether.

The weight of fish liver and analytical data are presented for the three species in Table-1. The percentage of Vitamin A is higher for bigger liver and higher fat content. For this reason, the percentage of Vitamin A in the liver of *Acipenser*

stellatus is more than those of *Acipenser guldenstadtii* and *Acipenser percicus* and their ranges are 54080 ± 4234–76410 ± 5983 USP units (13524 ± 1059–22923 ± 1795 ppm)/g of fish liver oil.

A number of factors other than species of fish affect the potency of the oils. These include age, size, sex, nutritional condition and spawning stage of fish as well as season and geographical source. The value of the oils is related to Vitamin A potency. If the processing is delayed for any reason, a number of problems will arise and their severity will be directly related to the amount of decomposition that has occurred in the livers. Processing livers in which decomposition has started is complicated by problems such as formation of emulsions and increased difficulties in extracting the oil. These result in a lower yield of vitamins and sometimes a lower potency oil. Ranges of potency of Vitamin A in some of the important commercial species are given in Table-2[13, 14]. With comparison of the data in Tables 1 and 2, we conclude that the sturgeon fish liver is suitable source of Vitamin A.

TABLE-2
THE AMOUNT OF VITAMIN A IN SOME FISH LIVER OIL[13, 14]

Kind of fish	Oil content (%)	Vitamin A USP units/g of oil
Gadus sp.	20–60	1000–30000
Germo alaunga	7–20	10000–60000
Asphyrnidae sp.	30–75	5000–150000
Sarda chiliensis	4–12	15000–60000

REFERENCES

1. A.L. Leninger, Biochemistry, Worth Publisher Inc., New York (1982).
2. Krik-Othmer, Encyclopedia of Chemical Technology, Wiley-Interscience, New York (1995).
3. T. Nakayama, K. Kami and K. Sato, *Sci. Papers Inst. Phys. Chem. Res.*, **3**, 81 (1925).
4. H.N. Holmes and R.E. Corbet, *J. Am. Chem. Soc.*, **59**, 2042 (1937).
5. F.H. Carr and E.H. Price, *Biochemical J.*, **20**, 497 (1926).
6. B.L. Oser, D. Melmic and M. Pader, *Ind. Eng. Chem. Anal. Ed.*, **15**, 724 (1943).
7. J.G. Baxter and C.D. Robeson, *J. Am. Chem. Soc.*, **64**, 2411 (1942).
8. D.P. Parrish, *CRC Crit. Rev. Food. Sci. Nutr.*, **9**, 375 (1977).
9. M.H. Bui and B. Blanic, *Experienta*, **36**, 374 (1980).
10. D.M. Drexler, A.P. Land and S.W. Baldwin, *LC-GC*, **17**, 854 (1999).
11. A.E. El-Bayoumi, *J. Pharm. Sci.*, **7**, 1 (1998).
12. T. White and E.D. Nudelman, PCT Int. Appl. wo 9950655, 1999.
13. G. Wald and R. Hubbard, in: R.A. Morton (Ed.), International Encyclopedia for Food and Nutrition, Pergamon Press, Oxford (1970).
14. H. Higashi, Fish as Food, Vol. 1, Academic Press, New York (1961).

(*Received*: 15 September 2004; *Accepted*: 31 December 2005) AJC-4604