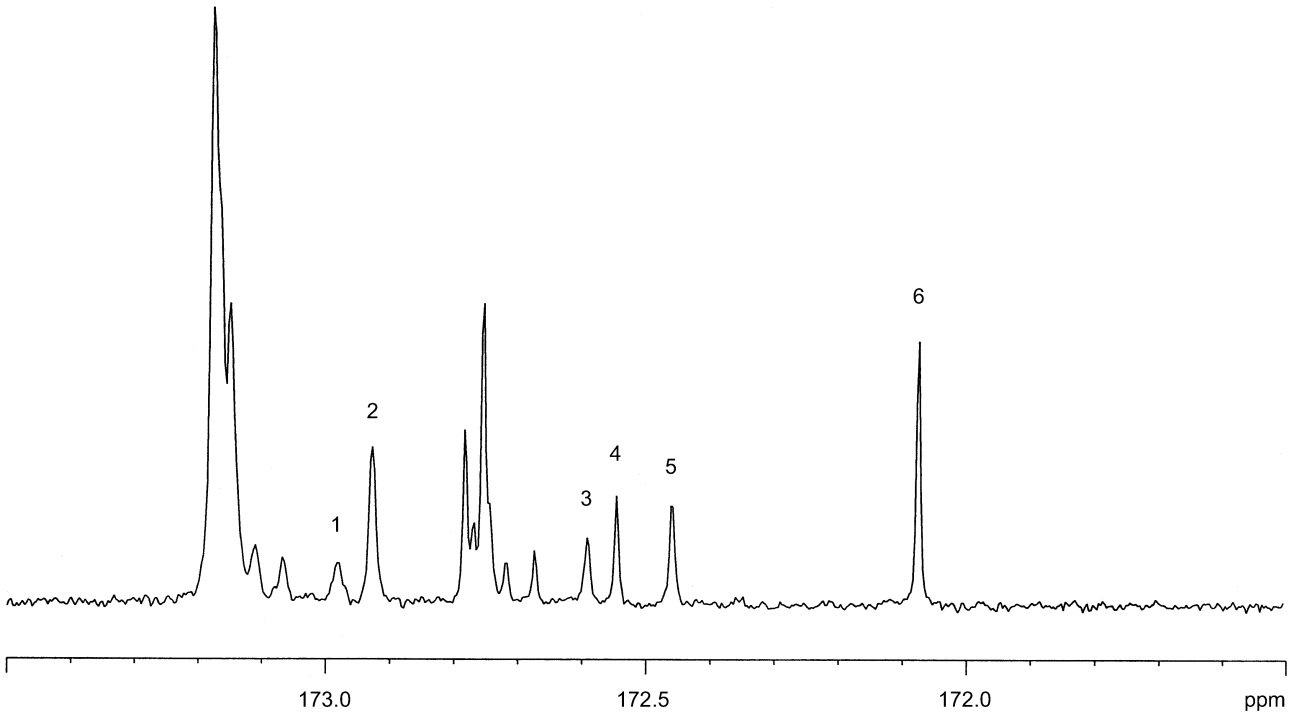

1. α C18:4
2. α EPA
3. β C18:4
4. β EPA
5. α DHA
6. β DHA

Figure 1910.-2. – *^{13}C NMR spectrum carbonyl region of farmed salmon oil*

01/2008:1848
corrected 6.0

SAW PALMETTO FRUIT

Sabalis serrulatae fructus

DEFINITION

Dried ripe fruit of *Serenoa repens* (Bartram) Small. (*Sabal serrulata* (Michaux) Nichols).

Content: minimum 11.0 per cent of total fatty acids (dried drug).

CHARACTERS

Characteristic, strong, unpleasant but not rancid odour.

IDENTIFICATION

First identification: A, B, D.

Second identification: A, B, C.

A. The fruit is an ovoid to subspherical drupe, with a dark brown to blackish, roughly wrinkled surface and more or less coppery sheen, up to 2.5 cm long and 1.5 cm in diameter. The apex sometimes bears the remains of the style and tubular calyx, with 3 teeth, and the base bears a small depression with the scar of the stalk. The epicarp and underlying mesocarp form a thin fragile layer, which partially peels off, revealing the thin, hard, pale brown endocarp, which is fibrous and easily separable. The seed is irregularly spherical to ovoid, up to 12 mm long and 8 mm in diameter, with a hard, smooth or finely pitted surface which is reddish-brown with a paler, raised and membranous area over the raphe and micropyle; cut transversely, the seed has a thin testa, narrow perisperm and a large area of dense, horny, greyish-white endosperm, with the embryo positioned to one side.

B. Reduce to a powder (710) (*2.9.12*). The powder is reddish or blackish-brown and oily. Examine under a microscope using *chloral hydrate solution R*. The powder shows fragments of epicarp composed of several layers of thin-walled, reddish-brown, pigmented, polyhedral cells (10 μm to 40 μm) which are strongly cuticularised; those of the outer layers are much smaller than those of the inner layers. Parenchyma cells of the mesocarp may be large and filled with oil droplets, or smaller and containing nodules of silica. Groups of xylem tissue of the mesocarp show small lignified, annular or spirally thickened vessels. Stone cells of the mesocarp (20 μm to 200 μm) may be found scattered, usually singly but sometimes in small groups, the walls are moderately thickened, distinctly striated and finely pitted. Fragments of endocarp contain groups of elongated sclereids about 300 μm long, with strongly thickened walls and numerous pits. The seed testa consists of small, thin-walled cells with brownish contents and underlying sclereids; albumen cells are thick-walled with large conspicuous pits and contain aleurone grains and fixed oil.

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 1.5 g of the powdered drug (710) (*2.9.12*), add 20 ml of *alcohol R* and stir for 15 min. Filter.

Reference solution. Dissolve 4 mg of *β-amyrin R* and 10 mg of *β-sitosterol R* in 10 ml of *alcohol R*.

Plate: *TLC silica gel plate R* (2-10 μm).

Mobile phase: *acetic acid R, ethyl acetate R, toluene R* (1:30:70 *V/V/V*).

Application: 8 μl of the test solution and 2 μl of the reference solution, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *anisaldehyde solution R*; dry the plate at 100-105 °C for 5-10 min; examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones are present, especially in the lower third, in the chromatogram obtained with the test solution.

Top of the plate	
	A strong blue zone
———	———
	A faint blue zone
β-Amyrin: a blue zone	A faint blue zone
	A strong bluish-violet zone
β-Sitosterol: a blue zone	
	A faint blue zone
———	———
	A faint blue zone
Reference solution	**Test solution**

D. Examine the chromatograms obtained in the assay of total fatty acids.

Results: the characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to the characteristic peaks in the chromatogram obtained with the reference solution. The principal peak is due to lauric acid.

TESTS

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 5.0 per cent.

ASSAY

Total fatty acids. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 0.47 g of *methyl pelargonate R* and 0.47 g of *methyl margarate R* in 20.0 ml of *dimethylformamide R* and dilute to 100.0 ml with the same solvent.

Test solution. Reduce 50 g of the drug to a powder (200). Place 4.0 g of the powdered drug in a 100 ml volumetric flask. Add 60.0 ml of *dimethylformamide R*. Mix using sonication for 15 min and then shake for 30 min. Dilute to 100.0 ml with *dimethylformamide R*. Allow to stand for a few minutes and filter. To 20.0 ml of this solution add 4.0 ml of the internal standard solution and dilute to 25.0 ml with *dimethylformamide R*. To 0.4 ml of this solution add 0.6 ml of an 18.84 g/l solution of *trimethylsulphonium hydroxide R* in *methanol R* and mix.

Reference solution. Dissolve 32.0 mg of *caproic acid R*, 62.0 mg of *caprylic acid R*, 68.0 mg of *capric acid R*, 0.699 g of *lauric acid R*, 0.267 g of *myristic acid R*, 10.0 mg of *palmitoleic acid R*, 0.217 g of *palmitic acid R*, 0.115 g of *linoleic acid R*, 18.0 mg of *linolenic acid R*, 0.870 g of *oleic acid R* and 49.0 mg of *stearic acid R* in *dimethylformamide R* and dilute to 10.0 ml with the same solvent. To 1.0 ml of the solution add 4.0 ml of the internal standard solution and dilute to 25.0 ml with *dimethylformamide R*. To 0.4 ml of this solution add 0.6 ml of an 18.84 g/l solution of *trimethylsulphonium hydroxide R* in *methanol R* and mix.

Column:

– *material*: fused silica,

– *size*: *l* = 25 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.20-0.53 mm,

– *stationary phase*: *poly(dimethyl)siloxane R*.

Carrier gas: *helium for chromatography R*.

Flow rate: 0.5 ml/min.

Split ratio: 1:40.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	150
	2 - 7	150 → 190
	7 - 22	190 → 220
Injection port		300
Detector		300

Detection: flame ionisation.

Injection: 1.0 µl.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution.

Use the expression below to determine the percentage content of the different fatty acids. Determine the content of caproic acid, caprylic acid, capric acid and lauric acid using methyl pelargonate as the internal standard. Determine the content of myristic acid, palmitoleic acid, palmitic acid, linoleic acid, linolenic acid, oleic acid and stearic acid using methyl margarate as the internal standard. The peak area of lauric acid is not less than 20 per cent of the total area of the peaks.

$$\frac{A_1}{A_3} \times \frac{A_2}{A_4} \times \frac{m_2}{m_1} \times p \times 0.5$$

A_1 = area of the peak due to the considered derivatised fatty acid in the chromatogram obtained with the test solution,

A_2 = area of the peak due to methyl pelargonate or methyl margarate in the chromatogram obtained with the reference solution,

A_3 = area of the peak due to methyl pelargonate or methyl margarate in the chromatogram obtained with the test solution,

A_4 = area of the peak due to the considered derivatised fatty acid in the chromatogram obtained with the reference solution,

m_1 = mass of the test sample, in grams,

m_2 = mass of the considered fatty acid in the reference solution, in grams,

p = percentage purity of the considered fatty acid in the reference solution.