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# VITAMINS $D_2$ AND $D_3$ CONTENTS OF THE HEPATOPANCREAS OF *RAPANA VENOSA* (VALENCIENNES 1846)

## *RAPANA VENOSA* (VALENCIENNES 1846) HEPATOPANKREASININ VİTAMIN $D_2$ VE $D_3$ İÇERİĞİ

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*Vitamin $D_2$ and $D_3$ were identified in hepatopancreas of Rapana venosa. The extraction was made by dichloromethane from the hepatopancreas of R.venosa and analized by GC/MS.*

*Vitamin $D_2$ ve $D_3$ mevcudiyeti Rapana, venosa'nın hepatopankreasında tanımlandı. Ekstraksiyon R.venosanın hepatopankreasından diklormetan ile yapıldı ve GC/MS ile analize edildi.*

**Key words:** *Rapana venosa; Hepatopancreas; Vitamins $D_2$ and $D_3$; GC/MS analysis*

**Anahtar kelimeler:** *Rapana venosa; Hepatopankreas; Vitamin $D_2$ ve $D_3$; GC/MS analizi*

## Introduction

Shellfish, *Rapana venosa* (Valenciennes 1846) (Gastropoda, Prosobranchia) was introduced into the Black Sea in 1946 (1) and was detected in Turkish coasts in 1960 (2). Anotomy of *R.venosa* was investigated by Lupu (3). Later studies were carried out on its contents such as enzymes (4-8), heparinoid (9,10), fatty acids (11-13), sterols (12,14) and insulin (15,16).

Several provitamins D occur in nature. These are (3β) hydroxy $\Delta^{5-7}$ steroids that include α-calcidol, calcifediol, cholecalciferol, dihydrotachysterol and ergocalciferol. Vitamin D is a fat soluble vitamin available in several natural and synthetic forms. It is a three-ringed sterol compound that may be hydroxylated at specific points on the molecule. The vitamin $D_2$ of natural sources is cholecalciferol or Vit $D_3$ found particularly in certain fish oils. In animals, vitamin $D_2$ is produced in the skin by the action of ultraviolet light on its precursor 7-dehydrocholesterol. Synthetic forms of vitamin D are produced by the irradiation of plant sterols and are known as ergocalciferol (Vit $D_2$) and dihydrotachysterol. The other vitamin D derivates are listed in the table (17,18).

Vitamin D derivatives are found in various organs in fish, particularly in the liver (19-22).

Halibut-liver oil (Oleum hippoglossi, Oleum jecoris hippoglossi) is the liver of halibut species (genus: Hippoglossus, Pleuronectidae) which contains 3000 U vitamin $D_2$.

Cod-liver oil (Oleum morrhuae) obtained from fresh liver of *Gadus callarias* (*G.morrhua*) and other species of *Gadus* (Gadidae) contain 85 units/g of vitamin D (antirachitic activity).

Vitamins $D_2$ and $D_3$ contents of hepatopancreas of *R.venosa* are reported in the present paper.

### Materials

*Rapana venosa* (Valenciennes 1846) was collected from the Black Sea, close to the entrance of the Bosphorus.

Dichloromethane (HPLC grade, Lab-Scan).

Hexane (HPLC grade, Lab-Scan).

### Methods

*R.venosa* vas dissected, hepatopancreas was separated according to Lupu (1977) and stored at –30 $^0$C. The frozen organ was thawed, homogenized

* Correspondence

Table. Sources and provitamins of vitamins D.

| Vitamin | Provitamin | Source |
|---|---|---|
| $D_1$ | — | Vit $D_2$ and lumisterin are nof found in nature |
| $D_2$ | Irradiated ergosterol (Calsiferol) | Fungi, yeast |
| $D_3$ (natural Vit D) | 7-Dehydrocholestrin | Human and animals |
| $D_4$ | 22,23-Dehydroergosterol | Only synthetized |
| $D_5$ | 7-Dehydrositosterol | Only synthetized |
| $D_6$ | 7-Dehydrostigmasterol | Only synthetized |
| $D_7$ | 7-Dehydrocamposterol | Isomer of provitamin $D_4$ |

Vitamin $D_4$, $D_5$ and $D_6$ were vitamin D derivatives and only synthetized products. 7-Dehydrocholesterol is the provitamin of vitamine $D_3$ (Cholecalciferol). It was used in therapy as an antirachitic agent.

in cold and extracted with dichloromethane (DCM) in a Soxhlet apparatus for 4h. The extract was filtered and distilled at 40 $^0$C and then hydrolyzed with 5% KOH in methanol for 30 min under reflux in a water bath. After hydrolysis, two volumes of water were added and re-extracted with DCM. Organic phase was separated and distilled. The residue was dissolved in hexane and applied to mass spectrometer (GC/MS) for analysis.

*GC/MS analysis:* The analysis of sterol was run on an HP6890 capillary gas chromatograph connected to an HP MSD and controlled by an HP ChemStation. Capillary column; 50 m x 200 µm id, fused HP PONA (methylsiloxane). Column temperature programme was 110$^0$C - 290$^0$C at 6$^0$C min$^{-1}$; split injector temperature 250$^0$C; carrier gas helium, 44.7 psi.

Vit $D_2$ and $D_3$ were identified by comparing the spectrum of each peak with its corresponding spectrum from HP memory.

## Results

The GC/MS chromatogram of vitamins $D_2$ and $D_3$ are shown in figure1.

The spectra of vitamins $D_2$ and $D_3$ and those taken from HP memory are given in figures 2 and 3.

The characteristics in the fragment are:

For vitamin $D_2$: 396 (m/z), 363, 337, 253, 211, 197, 185, 171, 159, 157, 143, 128, 109, 93, 81, 69, 55.

For vitamin $D_3$: 384 (m/z), 369, 366, 351, 325, 211, 171, 145, 143, 119, 91, 69, 55.

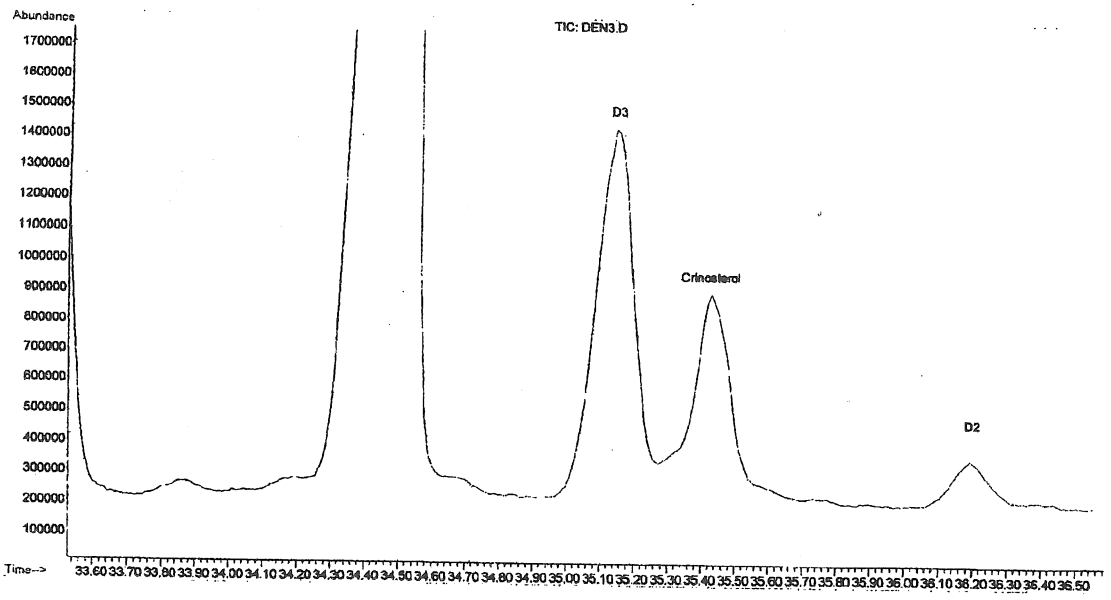


Fig. 1. GC/MS chromatogram of vitamin $D_2$ and $D_3$ extracted from *R.venosa.*

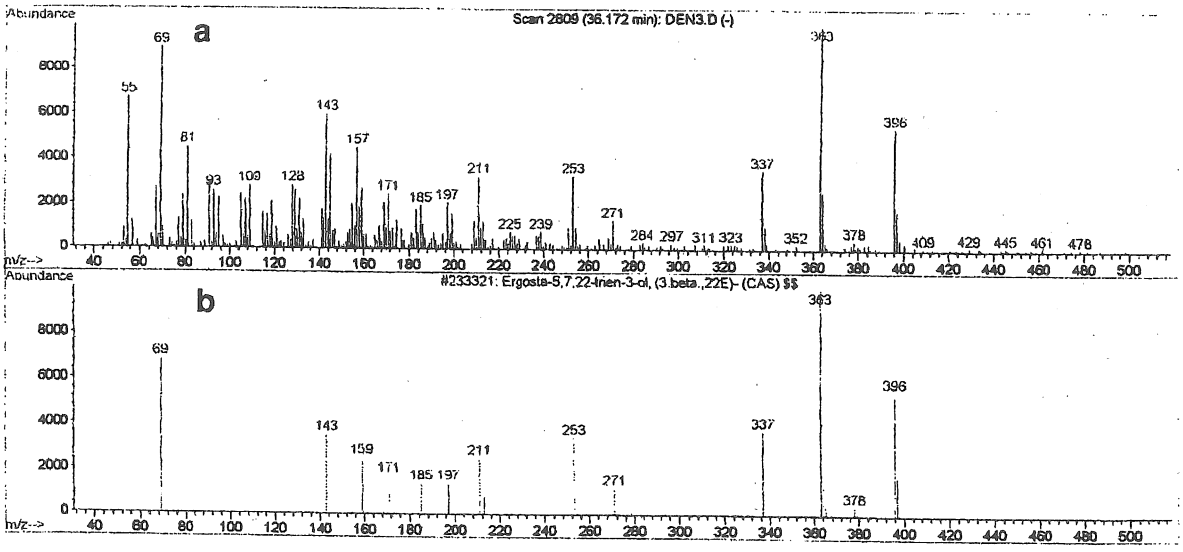


Fig. 2. a) Spectrum of vitamin $D_2$ extracted from *R.venosa*.
b) Spectrum of vitamin $D_2$ taken from HP memory.

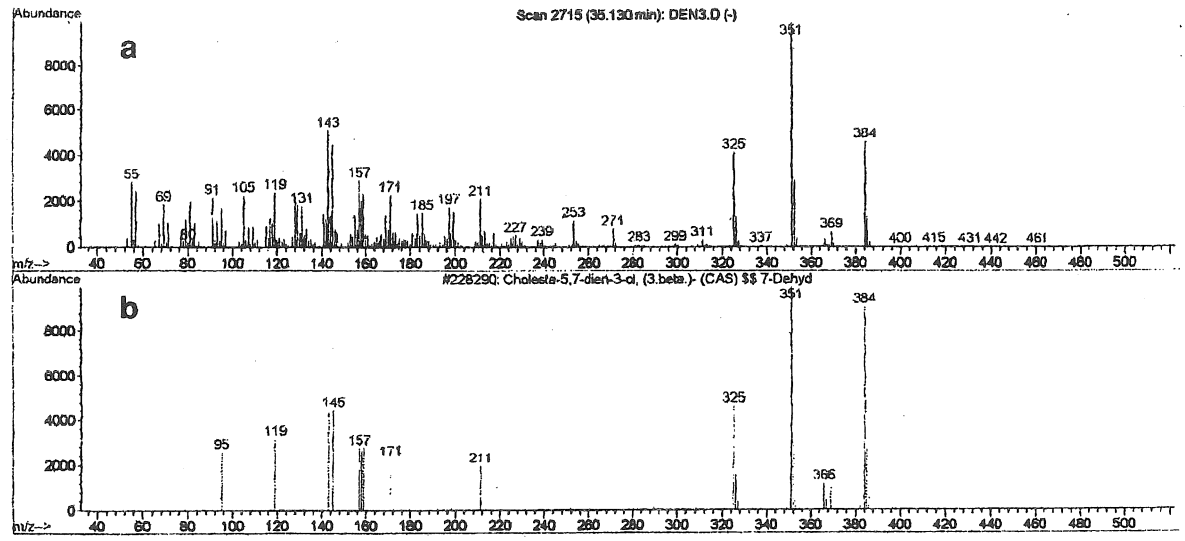


Fig. 3. a) Spectrum of vitamin $D_3$ extracted from *R.venosa*.
b) Spectrum of vitamin $D_3$ taken from HP memory.

The spectra of the vitamins D extracted from *R.venosa* were similar with that taken from GL/MS memory.

These findings proved that hepatopancreas of *R.venosa* contains vitamins $D_2$ and $D_3$.

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