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The analysis of the plasma vitamin D of the pre-school children

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ABSTRACT

The analysis of the plasma vitamin D of the pre-school children in paediatric department, in The University Hospital (CHU) of Tlémcen (Algerian willaya is located in the North West), is carried out by reversed phase high performance liquid chromatography with UV detection. This method is characterized by direct injection of the supernatant obtained by treatment of 100 µL of plasma with the mixture solvent $C_2H_5OH/CH_3COOC_2H_5$ (1: 1). The quantification of the concentrations is carried out by using Cholecalciferol as an external standard; elution is made ingredient mode by using mixture CH_3OH/CH_3CN (70: 30%) and 1.5 mL/mn flow rate. The percentage of deficiency found in this study is 2.63 %.

Keywords: Vitamin D, Chromatography, Plasma, Deficiency

INTRODUCTION

Vitamin D has long been regarded as an essential element for the skeletal system. The recent evidence asserts that it also defends against cancer, heart disease, fractures and falls, type 2 diabetes, and depression. In addition, it plays a major role in regulating the immune system [1]. Vitamin D_3 (Cholecalciferol) is produced in our skin from the 7-dehydro-cholesterol by a non-enzymatic process which is catalyzed by UV light energy. Vitamin D is stored in plasma [2].

Vitamin D is substantial in many biochemical functions such as the maintenance of plasma calcium homeostasis, in conjunction with parathyroid hormone and bone metabolism.

Vitamin D is produced in two forms: Vitamin D_2 and Vitamin D_3, which differ by the presence of a double bond and methyl group on the aliphatic side chain. The issues involved in assessing Vitamin D status arise from the complexities of the metabolic pathways leading to a number of active forms. The complex metabolic pathway for Vitamin D_3 is summarized in Figure 1 [3].

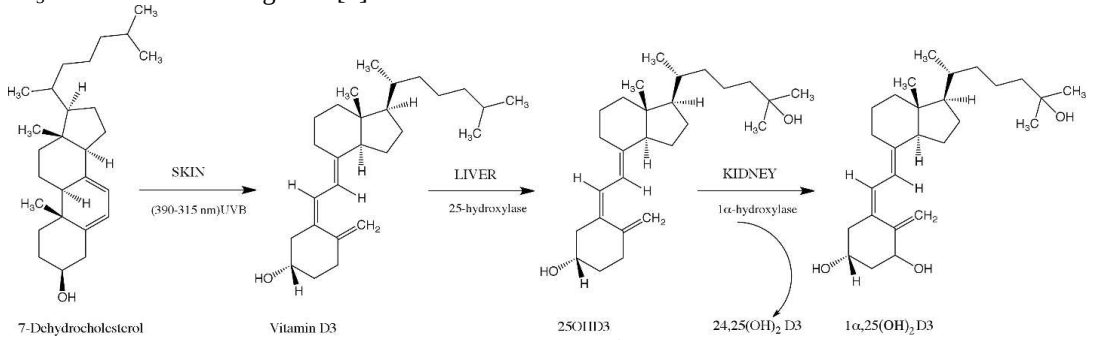


Figure 1 Metabolic pathway for vitamin D_3

In this work, a fast, automated and sensitive high-performance liquid chromatography method using 1 ml serum for determination of vitamin D_3.

MATERIALS AND METHODS

Sample nature

Samples are plasmas of preschool children (12-59 months). Their samples are obtained by the pediatric Department of Hospital University of Tlémcen (CHU) from three different areas of Tlémcen (Algerian willaya is located in the Northern West): coastal Northern area, the central mountainous area, and the southern steppe area. Each sample was conducted a questionnaire. Volume 2 ml of blood for each child was collected in Lithium heparin tube protected from light by a foil pouch. These blood samples are transported to the laboratory in a cooler where they were centrifuged at 5000 tr/ min [5]. Plasma obtained in an amount of 1 ml was pipetted into black and placed in tubes 2 aliquots (a and b) 0.5 ml for each. The tube "a" was reserved for the determination of Cholecalciferol and tube "b" used double as a backup or for further study on other micronutrients. The foil-covered tubes were frozen at -25 ° C.

HPLC apparatus

All analytical separations were performed with a reversed phase high performance liquid chromatography with UV detection (Model Shimadzu) equipped with a variable wavelength UV detector, injection was by means injection valve with 100 µl fixed loop.

Design of the initial extraction procedure

Serum sample (50 µl) were denatured with 200 µl of a 1:1 (v/v) ethanol/ ethyl acetate mixture, vortexes, and then centrifuged at 11000 rpm for 4 min. Then, 100 µl of the denatured sample was injected onto the chromatographic system [4].

Chromatographic Profile and Peak Identification

The chromatographic profile obtained with injection of the supernatant obtained by treatment of 100 µL of plasma.

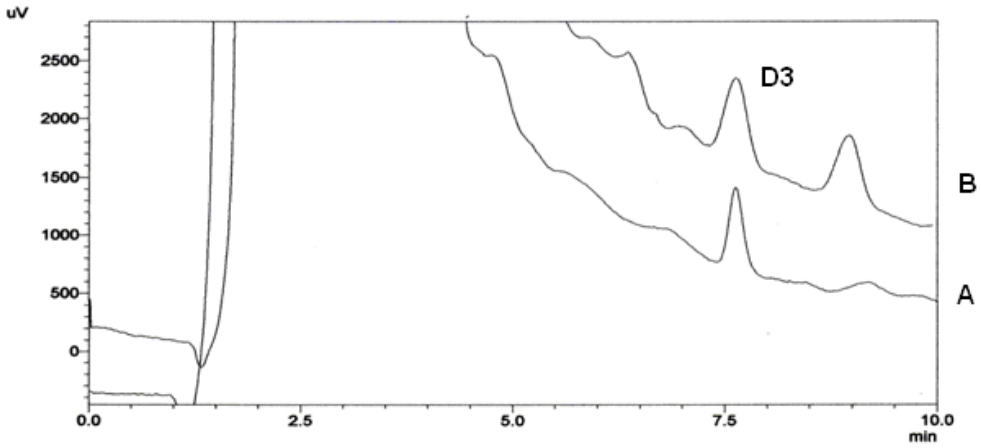


Figure 2 Identification of the Peak of Cholecalciferol in Plasma Sample

A: pure standard; B: Sample of treated plasma.
(Elution mode: linear gradient; Mobile phase: (70% CH_3OH; 30% CH_3CN); Flow rate: 1.5 ml/min; Detection: λ= 265 nm attenuation 0.005 AUFS.)

Calibration Graph:

The dilute vitamin standard solution used to obtain calibration graphs (injecting different concentrations from 80 nmol/l to 400 nmol/l).

Table 1 shows the values of the parameters *a* and *b* of the equation $y = a + bx$, where y is the peak area and *x* is the amount of analyte injected in nanomoles per liter.

Table.1

Level	Concentration of Cholecalciferol in nmol/l	Peak Area	Peak height
Level-1	80.327	8983	321
Level-2	160.65	18064	651
Level-3	240.98	26982	958
Level-4	321.31	36243	1291
Level-5	401.63	44931	1598

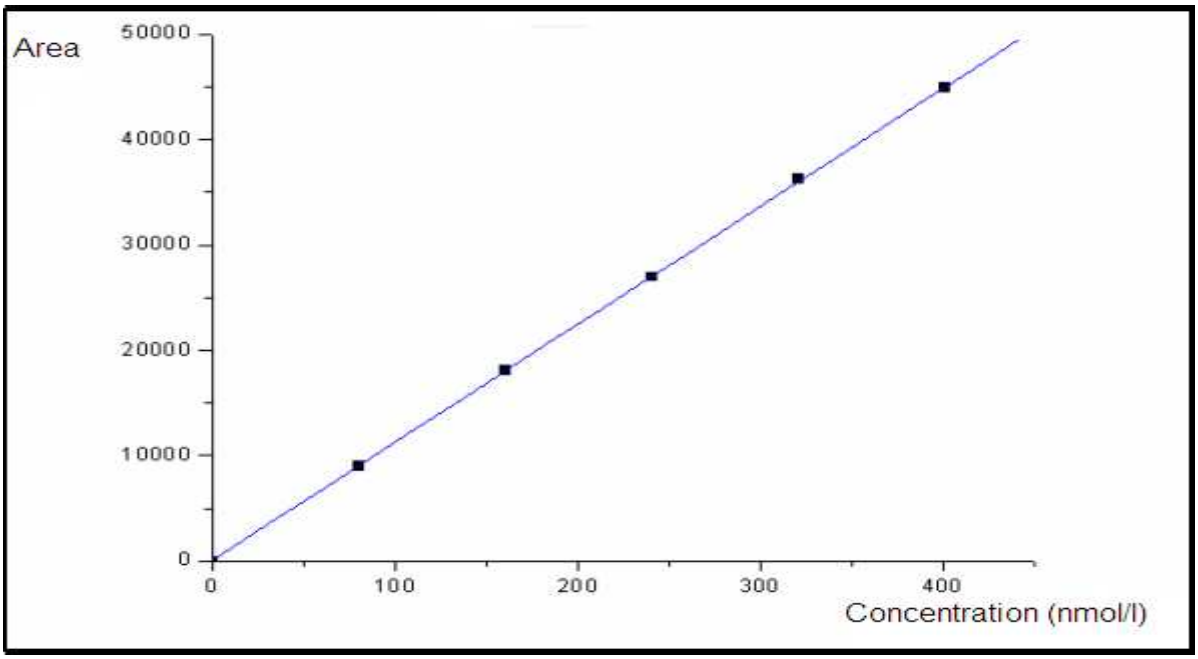


Table.2 Parameters of Calibration Graph

Equation of calibration	Coefficients	
	r	r^2
y = 112.16 x + 8.63	0.9999	0.9998

RESULTS AND DISCUSSION

Linearity:
For the study of linearity, calibration curves were run covering the concentration range of the analyses usual in serum samples, Regression analysis was performed using the analyses area/internal standard area ratio versus concentration of each analysis. The linearity equation:

y = 112.16 x + 8.63 and the correlation coefficient (r^2) are 0.9998.

Accuracy:
The recoveries at three different concentrations (70, 100 and 130 % of Cholecalciferol), were found to be within the range of 82 % to 122 %. Results are given in Table 3.

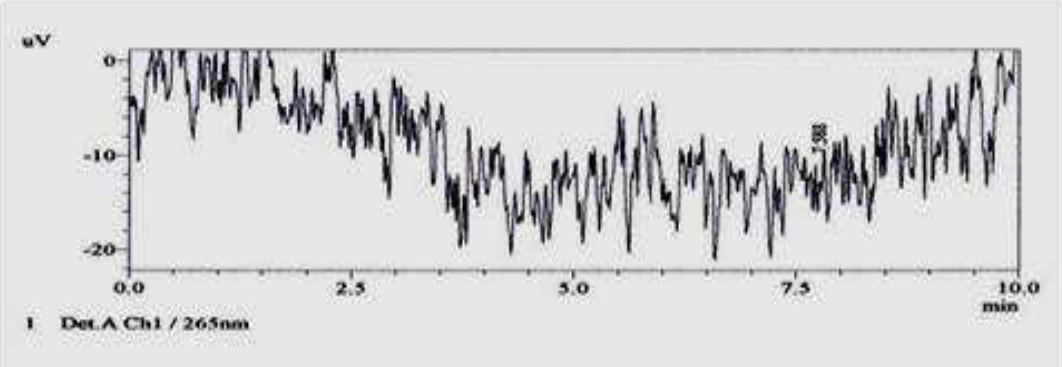
Table.3

Level	Recovery
70 % level	**82 %**
100 % level	**108 %**
130 % level	**122 %**

Detection and quantification limits:

Table.4

Noise (Ns)	LOD = 3 Ns	LOQ = 10 Ns
3	0.150	0.5



Analysis results

Sample	*Retention time*	*Area*	*Height*	*Concentration*
Sample 1	7,695	4496	210	40,00579488
Sample 2	7,586	747	77	6,582715213
Sample 3	7,567	2717	99	24,14565652
RS	7,715	6124	367	54,51973825
Sample 5	7,714	1054	96	9,319681192
Sample 6	7,708	6891	489	61,3576956
Sample 7	7,552	3348	191	29,77114685
Sample 8	7,643	1882	126	16,70146566
Sample 9	7,652	1122	92	9,925914699
Sample 10	7,662	983	112	8,68670209
Sample 11	7,645	2418	168	21,48001212
RS	7,677	6071	385	54,04723272
Sample 13	7,643	3236	202	28,7726446
Sample 14	7,559	961	73	8,49056772
Sample 15	7,644	1499	189	13,28694458
Sample 16	7,623	2221	92	19,72371799
Sample 17	7,686	3019	169	26,8380465
Sample 18	7,697	2142	128	19,0194173
RS	7,704	5988	344	53,30727124
Sample 20	7,704	3061	215	27,21248484
Sample 21	7,68	3332	223	29,62850367
Sample 22	7,657	219	56	1,875490336
Sample 23	7,66	2181	148	19,36711005
Sample 24	7,683	2995	216	26,62408173
Sample 25	7,646	2560	159	22,74597033
Sample 26	7,671	1887	135	16,74604165
Sample 27	7,598	104	38	0,850242493
Sample 28	7,655	2643	174	23,48593182
Sample 29	7,682	516	82	4,523304329
Sample 30	7,657	3714	269	33,03410955
RS	7,636	5981	321	53,24486485
Sample 32	7,67	2228	158	19,78612438
Sample 33	7,669	2903	207	25,80388346
Sample 34	7,555	4451	271	39,60461094
RS	7,544	5948	405	52,95066329
Sample 36	7,591	3567	211	31,72357535
Sample 37	7,508	1573	126	13,94666928
Sample 38	7,45	1948	115	17,28986877
Sample 39	7,525	1656	81	14,68663077
Sample 40	7,587	5984	348	53,27161044
Sample 41	7,644	2335	132	20,74005064
Sample 42	7,575	7977	495	71,03960131
Sample 43	7,588	2816	221	25,02826118
Sample 44	7,633	3067	209	27,26597604
RS	7,63	5955	312	53,01306968
Sample 46	7,664	1137	87	10,05964268
Sample 47	7,584	1480	136	13,11755581
Sample 48	7,599	2518	165	22,37153199
Sample 49	7,725	3280	220	29,16491334
Sample 50	7,691	3672	240	32,65967121
Sample 51	7,683	3739	246	33,25698952
RS	7,696	5980	279	53,23594965

Sample 53	7,688	3446	264	30,64483632
Sample 54	7,718	2075	111	18,42209899
Sample 55	7,648	3103	264	27,58692319
Sample 56	7,634	4015	248	35,71758434
Sample 57	7,64	4074	258	36,24358106
Sample 58	7,64	4888	311	43,50055274
Sample 59	7,567	5262	294	46,83483703
RS	7,676	5905	289	52,56730975
Sample 61	7,663	2222	163	19,73263319
Sample 62	7,661	7335	449	65,31604379
Sample 63	7,621	6229	359	55,45583411
Sample 64	7,6	4096	227	36,43971543
Sample 65	7,6	4176	256	37,15293132
RS	7,564	5972	237	53,16462806
Sample 67	7,567	2068	165	18,3596926
Sample 68	7,577	3174	223	28,21990229
Sample 69	7,59	2855	215	25,37595393
Sample 70	7,56	8899	600	79,25941445
Sample 71	7,654	1447	108	12,82335425
Sample 72	7,579	2994	238	26,61516654
Sample 73	7,591	3686	195	32,78448399
RS	7,565	5966	260	53,11113687
Sample 75	7,556	3603	238	32,0445225
Sample 76	7,567	3369	208	29,95836602
Sample 77	7,691	3299	219	29,33430212
Sample 78	7,699	1535	125	13,60789173
Sample 79	7,662	7462	475	66,44827402
RS	7,681	5784	201	51,48857072
Sample 81	7,651	1297	101	11,48607446
Sample 82	7,646	1813	135	16,08631695
Sample 83	7,595	1387	111	12,28844234
Sample 84	7,657	146	48	1,224680836
Sample 85	7,639	8235	393	73,33972256
Sample 86	7,609	2891	192	25,69690108
RS	7,606	5860	261	52,16612581
Sample 88	7,62	2305	162	20,47259468

RS: Reference sample.

CONCLUSION

The results of the study indicate that the proposed HPLC method is simple, precise, accurate, sensitive, economic and less time consuming. Therefore, this method can be applied for the routine analysis of Cholecalciferol in plasma.

The analysis of 88 samples of the plasma vitamin D of the pre-school children with a reversed phase high performance liquid chromatography RP-HPLC has led us to the percentage of deficiency of 2.63 %.

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