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Determination of Vitamin D_3 in serum by solid phase extraction on the ep*Motion*® 5075

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Abstract

A team at the Isala Hospital in Zwolle, NL, has been successful in establishing solid phase extraction (SPE) with subsequent distribution of the purified samples for quantification of the vitamin D content in serum on the ep*Motion*.

Vitamin D plays a major role in our body's calcium and phosphate metabolism. Vitamin D is primarily involved in bone growth and regulation, as well as body growth and prevention of rickets and osteomalachia. The most important representatives of this group are Vitamin D_3 – also called cholecalciferol and vitamin D_2 – also known as ergocalciferol. The blood value traditionally measured is not the actual vitamin D but rather its storage form, 25-OH-vitamin-D (25-hydroxy vitamin D). Starting with solid phase extraction and subsequent distribution of the samples into HPLC vials, the entire process, with the exception of the actual HPLC measurement, is performed on the ep*Motion* 5075.

Figure 1: ep*Motion* 5075vt

Introduction

Our food, with the exception of enriched margarine (D_2) or fatty fish (D_3) contains only small amounts of vitamin D. The natural source of this vitamin is a photochemical process in our skin. Vitamin D is produced under the influence of sunlight (UV-B) as an intermediate product during cholesterol synthesis. Worldwide, approximately one billion people are estimated to be vitamin D deficient.

The vitamin D supply over the past months may be accurately determined by measuring its storage form, 25-OH-vitamin-D (25-hydroxyvitamin D).

To this end, many laboratories worldwide use patient serum as the sample material to be processed. However, the sample number is frequently too high for convenient manual processing. For this reason, the Isala Hospital in Zwolle has moved towards establishing the preparatory steps, such as solid phase extraction (SPE) and the distribution of samples for subsequent HPLC-based quantification, on the ep*Motion* 5075, an automated liquid handling work station.

Material and Methods

> Eppendorf ep*Motion* 5075 VAC/TMX (ep*Motion* 5075vt)
> Dispensing Tool TS_1000
> Reservoir Rack
> Racks for single tubes
> Reservoirs 30 mL/Reservoirs 100 mL
> Eppendorf Deepwell Plate 96/2000 µL
> Tipholder for reloadable tips

> Eppendorf Centrifuge 5810

> Strata-X™ 33µ 96 well plate 30 mg/well

> Agilent® HP1260/1200 liquid chromatograph

> MF membrane filter
> HPLC Quard columns

> Vit D_3 controls Level I and II, Chromsystems
> Vit D_3 Calibrator, Chromsystems

> Precipitation reagent
> Methanol

Reagent preparation (SPE)
The serum is first de-proteinized. The serum should be well protected against light. These samples, along with controls and standards, are placed into racks on the worktable. The reservoir rack is then equipped with the individual reservoirs, filled with the respective liquids and also placed on the worktable.

Position	Reservoir	Content
1	30 mL	Internal standard
2	100 mL	Precipitation reagent
3	100 mL	5 % methanol
4	100 mL	5 % methanol
5	100 mL	100 % methanol

Table 1: Distribution of reservoir racks with the liquids for solid phase extraction

Subsequently the following positions of the worktable are occupied:

Position	Labware	Comment
T0	VacLid	
A2	epT.I.P.S.® Motion 1000 µL filter	
A3	epT.I.P.S. Motion 1000 µL filter	
A4	Sample plate	Deepwell plate 96
B1	Reagent reservoirs: Internal standard Precipitating agent 5 % methanol 5 % methanol 100 % methanol	 30 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir
B2	Racks containing samples, standards and controls	
B3	Racks containing samples, standards and controls	
B4	Waste tub Vac frame SPE Strata-X 30 mg/well	400 mL Filter plate
C1	Collection plate	
C2	Racks containing samples, standards and controls	
C3	Racks containing samples, standards and controls	
C4	Vac frame holder	

Table 2: ep*Motion* worktable details for the Vitamin D_3 solid phase extraction

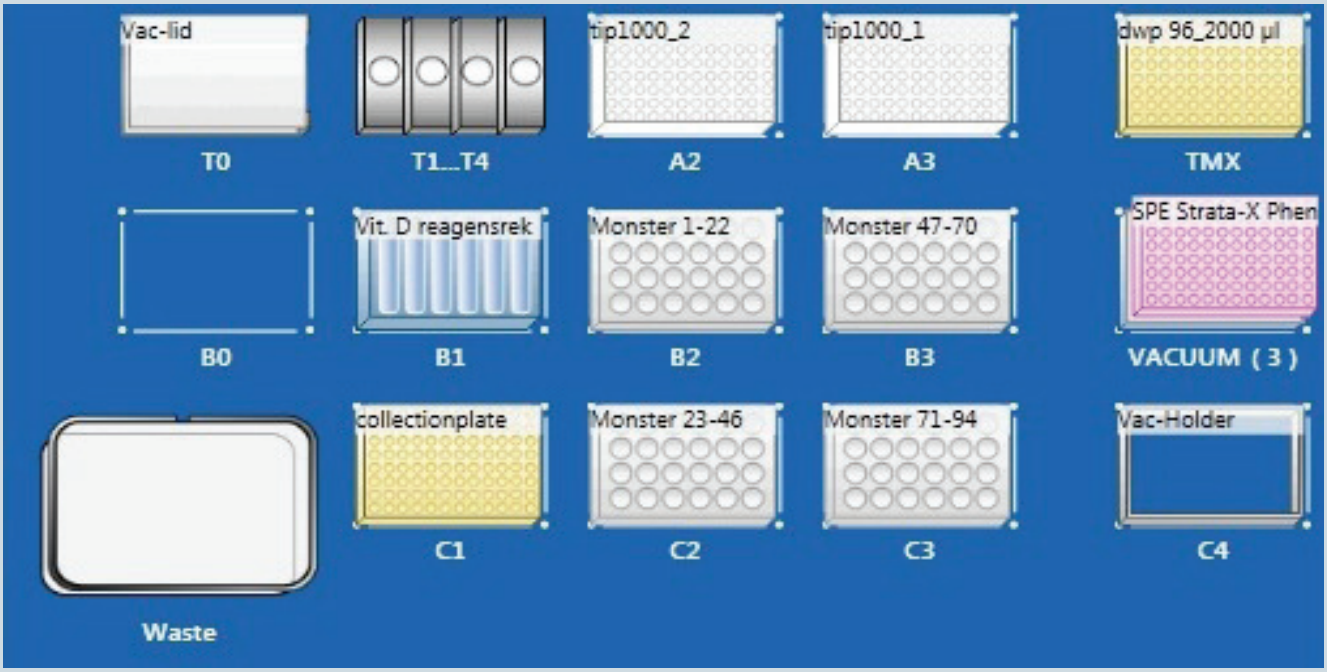

Figure 2: Screenshot of the worktable layout for solid phase extraction

In order to operate with as little negative pressure as possible, a small nick must be cut into the silicon seal of the Vac frame which will create a small leak. The negative pressure required is approximately -10 mbar.

Figure 3: Vac frame 2 with nicked silicon insert

Procedure

The de-proteinized serum samples, including the standard and control samples, are placed in racks on the worktable of the ep*Motion*. The individual samples are mixed with an internal standard and, following the mixing step, the precipitation reagent is added. After an external centrifugation step, the supernatant is then transferred to the Strata-X-Phen filter plate. Subsequently, two wash steps are performed.

Following two elution steps, the two phases are mixed well once again. The last step prior to HPLC measurement constitutes sample distribution from the collection plate into the individual HPLC vials. The samples are measured at 265 nm.

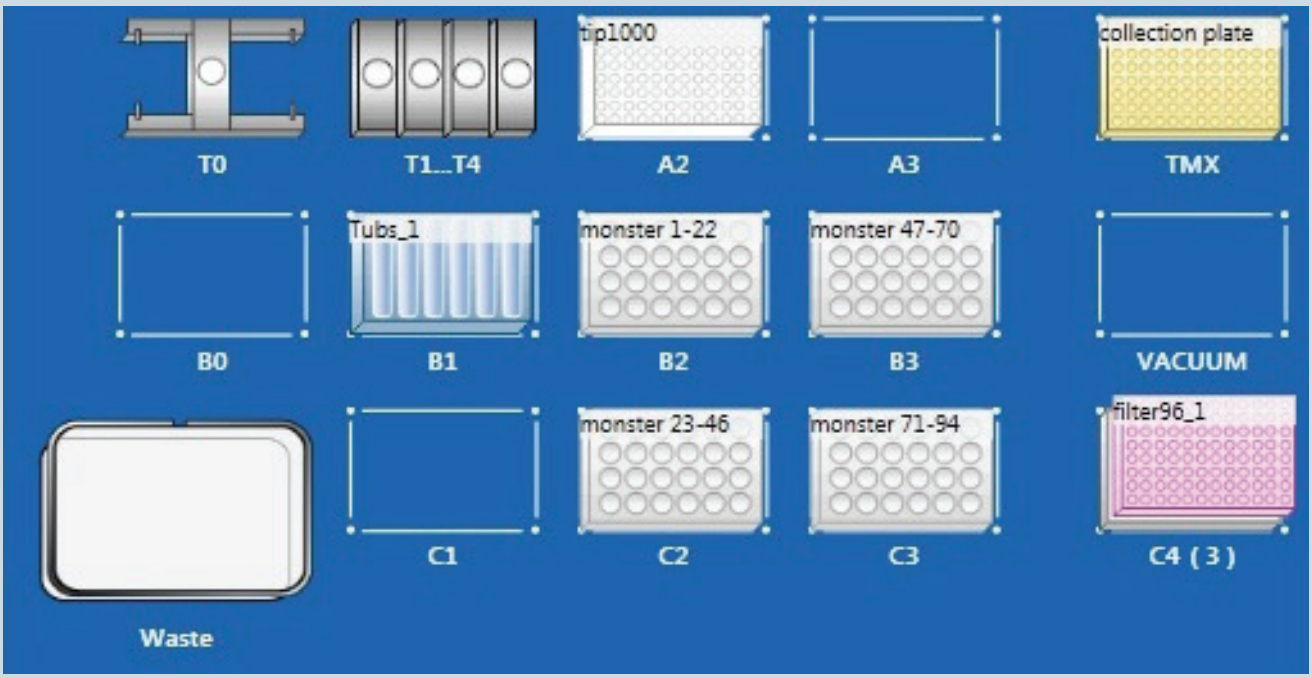

Figure 4: Layout of the worktable during distribution of purified samples into HPLC vials

Results

The quality controls 25-OH-Vit D_3 L 1 and 25-OH-Vit D_3 L2 by Chromosystems® were validated over a time period of three months. Standard deviation as well as coefficient of variance showed very good results.

	Between-run (n = 27)		
	x_{gem} nmol/L	SD nmol/L	% CV
25-OH-Vit D_3 control L 1 (lot 5012)	34.5	2.4	6.8
25-OH-Vit D_3 control L 2 (lot 1913)	127.6	5.26	4.12

Table 3: QC results from control reagents by Chromsystems obtained between 01 Oct and 31 Dec 2013.

A different approach comparing the results of the vitamin D_3 quantification obtained by either manual purification or by automated purification using the ep*Motion* demonstrates very good agreement between the two methods.

A comparison of the samples purified on the ep*Motion* with those manually prepared yields a linear correlation with the following values: n = 88, y = 0.9884 x -1.495 and R^2 = 0.9652. The almost entirely automated process on the ep*Motion* facilitates the otherwise fairly complex manual labor in the laboratory.

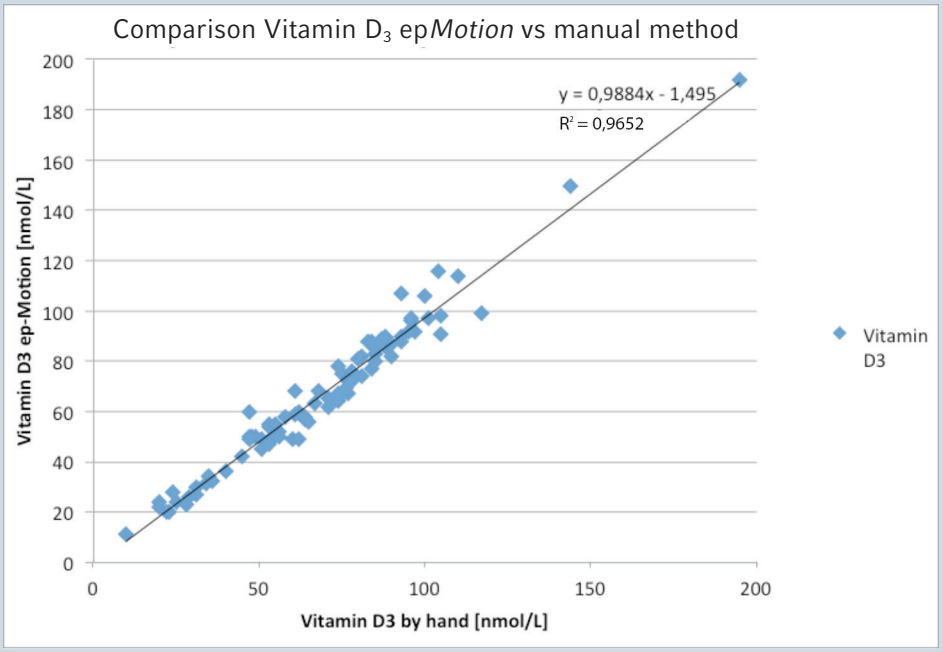

Table 4: Comparison of 88 manually and automatically purified vitamin D_3 values

Sample purification takes approximately 100 minutes, followed by distribution of the samples to the HPLC vials.

Conclusion

A team at the Isala Hospital in Zwolle, NL, has successfully transferred a solid phase extraction application for the purpose of purification of 25-OH-Vitamin D_3 to the ep*Motion*. All comparisons with the manual method were favorable, as well as control measurements using the standards. Establishment of this method on the ep*Motion* results in significant labor savings in every laboratory.

References

[1] Clin. Lab. 1999: 45:657–659, Evalution of the Bio-Rad 25 Hydroxyvitamin D_3 HPLC-assay Pekelharing 450-451 Vitamine D
[2] Operating manual for HP 1260 and HP 1200, Agilent
[3] Operating Manual for ep*Motion* 5075

Ordering information

Description	Order no. international	Order no. North America
ep*Motion*® 5075vt	5075 000.304	5075000304
Reservoir rack	5075 754.002	960002148
Reservoirs 100 mL	0030 126.513	960051017
Racks	5075 760.002	960002032
Tipholder for epT.I.P.S.®	5075 751.399	5075751399
Centrifuge 5810	5810 000.424	5810000424

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