Electronic Supplementary Material (ESI) for Analyst

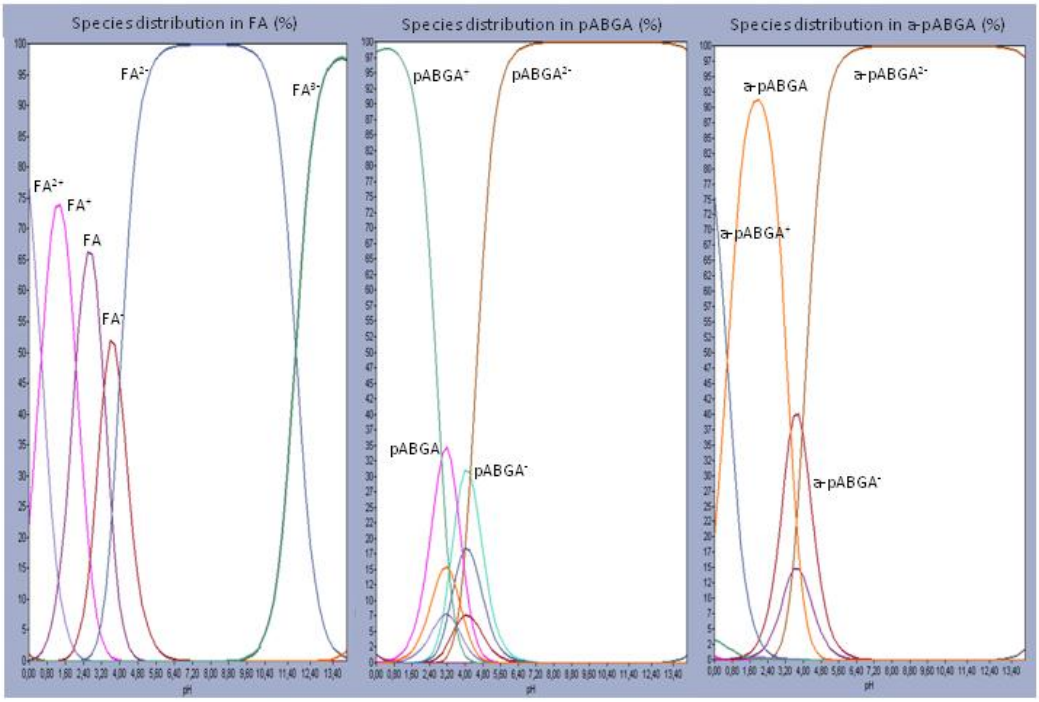
Approach for quantitative analysis of vitamins D and B_9 and their metabolites in human biofluids by on-line orthogonal sample preparation and sequential mass spectrometry detection

Carlos Ferreiro-Vera[a,b], Feliciano Priego-Capote*[a,b], María Dolores Luque de Castro[a,b]

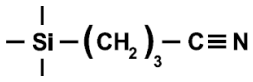
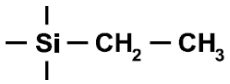
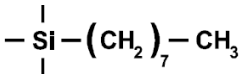
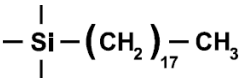
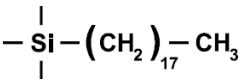
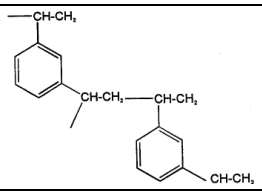
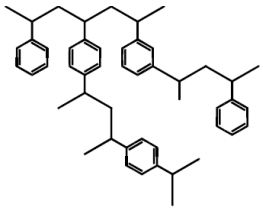
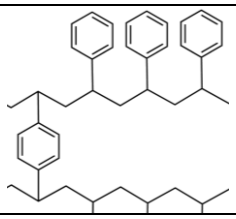
[a]Department of Analytical Chemistry, Annex C-3, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain.
[b]Institute of Biomedical Research Maimónides (IMIBIC), Reina Sofía Hospital, University of Córdoba, E-14071, Córdoba, Spain

Supplementary material

Supplementary Figure 1. pKa curves for polar target metabolites.



Supplementary Table 1. SPE sorbents evaluated to achieve the optimum retention of each vitamins and their metabolites.

Sorbent	Structure primary functional group	Description
CN (silica-based cyanopropyl phase)		A silica based cyanopropyl phase. Particle size 7 µm
C2 (silica-based ethyl phase)		A silica based cyanopropyl phase. Particle size 7 µm
C8 (EC) (end-capped silica-based octyl phase)		An endcapped silica based octyl phase. Particle size 10 µm
C18 (EC) (end-capped silica-based octadecyl phase)		An end-capped silica based octadecyl phase. Particle size is 7 µm
C18 (HD) (high-density silica-based octadecyl phase)		An end-capped, silica based phase with a high loading of octadecyl chains. The particle size is 7 µm, spherical shape. This phase is less polar then HySphere C18 (EC).
resin GP (polymeric polydivinylbenzene phase)		A polydivinyl-benzene. The particle size is 5-15 µm, spherical shape. This polymer phase can be used for the extraction of a wide variety of compounds
resin SH (strong-hydrophobic modified polystyrene-divinylbenzene)		A modified polystyrene-divinylbenzene phase, 20-50µm particles, irregular shape. This highly porous polymer based resin adsorbs compounds hydrophobic very strongly. It is often used for the extraction of phenols and polar analytes or for the extraction of a wide range of all different compounds.
MM anion (mixed-mode phase containing a strong anion exchange functional group)		A mixed mode anion exchanger with a polydivinyl-benzene backbone. The average particle size is 10 µm. This polymer phase can be used for the extraction of organic acids.

Supplementary Table 2. Optimization of the MS/MS step for qualitative and quantitative determination of D and B_9 vitamins and their metabolites.

Analyte	Precursor ion (*m/z*)	Voltage MS1 (V)	Product ion (*m/z*)	Collision energy (eV)	Quantification transition
24,25 $(OH)_2D_3$	399.3	100	295.1 159.0 121.1	30 30 30	121.1
1,25 $(OH)_2D_3$	399.3	100	161.1 147.1 107.0	30 30 30	107.0
25 OH D_2	395.3	100	377.0 199.0 133.1	30 35 35	133.1
25 OH D_3	383.3	100	365.3 173.1 159.1	30 30 30	159.1
D_2	397.3	100	379.2 159.1 107.1	35 35 35	107.1
D_3	385.3	100	259.2 147.1 107.1	30 35 35	107.1
FA	442.2	140	295.1 175.8	17 17	295.1
a-*p*ABGA	308.8	140	162.0	8	119.2
*p*ABGA	266.8	120	119.2	15	162.0

Supplementary Table 3. Characteristics of the method.

Analyte	Retention time (min)	Sample	Linear range[a]	Coefficient of regresion (R^2)	Limit of detection[a]	Limit of quantification[a]
24,25 $(OH)_2D_3$	3.27	Serum	0.10–80	0.9996	0.03	0.10
		Breast milk	0.09–60	0.9999	0.03	0.09
		Urine	0.50–80	0.9967	0.15	0.50
1,25 $(OH)_2D_3$	3.59	Serum	0.30–100	0.9986	0.09	0.30
		Breast milk	0.20–100	0.9996	0.06	0.20
		Urine	0.50–60	0.9930	0.15	0.50
25 OH D_2	5.54	Serum	0.70–100	0.9989	0.21	0.70
		Breast milk	0.50–80	0.9958	0.15	0.50
		Urine	0.50–80	0.9998	0.15	0.50
25 OH D_3	6.15	Serum	0.25–100	0.9984	0.07	0.25
		Breast milk	0.20–100	0.9936	0.06	0.20
		Urine	0.50–80	0.9980	0.15	0.50
D_2	11.27	Serum	0.25–80	0.9975	0.15	0.25
		Breast milk	0.20–80	0.9953	0.06	0.20
		Urine	0.10–60	0.9921	0.03	0.10
D_3	11.42	Serum	0.15–60	0.9985	0.04	0.15
		Breast milk	0.05–100	0.9965	0.02	0.05
		Urine	0.25–60	0.9978	0.08	0.25
FA	10.06	Serum	0.25–80	0.9999	0.08	0.25
		Breast milk	0.15–80	0.9996	0.04	0.15
		Urine	0.05–100	0.9999	0.02	0.05
a-*p*ABGA	5.09	Serum	0.30–60	0.9998	0.09	0.30
		Breast milk	0.10–100	0.9997	0.03	0.10
		Urine	0.07–100	0.9991	0.02	0.07
*p*ABGA	5.18	Serum	1–80	0.9999	0.30	1
		Breast milk	0.50–80	0.9973	0.15	0.50
		Urine	0.30–100	0.9987	0.09	0.30

[a]Expressed as ng mL^{-1}.

Supplementary Table 4. Repeatability (Sr), within-laboratory reproducibility (Swr), for each analyte.

	Sample	**Spiked (ng mL^{-1})**	**Analyte**								
			24,25(OH)$_2$D$_3$	1,25(OH)$_2$D$_3$	25 OH D$_2$	25 OH D$_3$	D$_2$	D$_3$	FA	a-*p*ABGA	*p*ABGA
Intra-day variability (%)	Serum	2.5	3.4	4.8	4.7	3.1	2.8	0.6	1.9	2.8	0.4
		5	4.6	4.5	2.6	2.2	2.2	0.6	1.9	3.5	3.8
		10	2.9	1.0	2.8	4.1	4.1	1.2	1.5	3.3	4.9
	Milk breast	2.5	0.8	0.9	2.0	1.5	0.8	2.3	3.3	1.1	3.8
		5	0.5	4.1	0.3	3.9	2.4	3.1	0.2	1.6	3.6
		10	0.3	1.8	1.9	0.4	0.3	2.6	2.6	0.9	1.5
	Urine	2.5	0.1	4.0	4.1	2.9	2.7	1.6	0.7	0.7	1.0
		5	2.3	3.4	1.0	2.3	1.7	0.4	0.7	0.2	0.8
		10	0.4	1.9	1.2	3.8	1.7	3.0	2.1	0.8	1.3
Inter-day variability (%)	Serum	2.5	4.9	6.7	6.6	4.3	4.0	0.8	2.6	3.6	5.5
		5	6.5	6.3	3.7	6.5	3.1	0.9	2.7	4.0	5.3
		10	4.1	1.4	3.9	0.4	5.8	1.7	1.6	4.6	6.1
	Milk breast	2.5	1.2	1.4	2.9	2.2	1.1	3.2	4.6	1.6	5.3
		5	0.7	5.8	0.5	5.5	3.4	4.7	0.3	2.3	5.1
		10	0.3	2.5	2.6	0.6	0.4	3.6	3.6	1.2	2.2
	Urine	2.5	0.2	5.6	5.8	4.1	3.8	2.2	1.0	0.9	1.4
		5	3.2	4.8	1.4	3.2	2.4	0.5	1.0	0.2	0.3
		10	0.6	2.7	1.6	5.4	2.4	4.2	3.0	1.2	1.9