# Chromai Online Solid Phase Extraction Two Dimensional Liquid Chromatography Assisted Analysis of Vitamin A, D, and E

**Chromai Online SPE-2DLC**
**Vitamin ADE Analysis System**

**CHROMAI** PRODUCTS

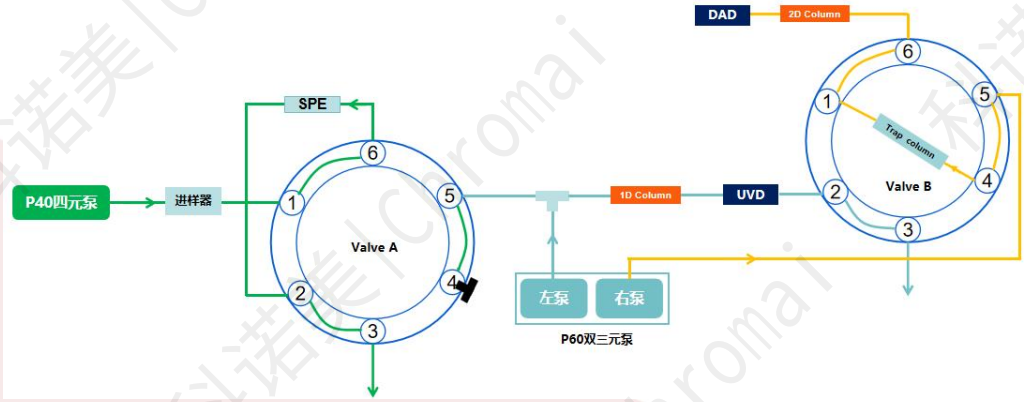
Fat soluble vitamins A, D, and E are essential nutrients for maintaining normal metabolism and function in the human body. Fortified foods and special medical formula foods are important ways to supplement vitamins. Accurately measuring the vitamin content in it is of great significance for scientific dietary guidance and ensuring

food safety. However, in the actual analysis process, due to the complexity of such matrices and the significant interference of conventional chromatographic analysis, a tedious sample pretreatment process is required.

The national food safety standard GB5009.296-2023 "Determination of Vitamin D in Food" includes online column switching reverse phase liquid chromatography as a standard method for the first time, providing a better reference for the determination of vitamin D. On this basis, Chromai Technology has introduced Online Solid Phase Extraction (Online SPE) technology into two-dimensional liquid chromatography, achieving a technological upgrade in the sample pretreatment process and bringing a qualitative improvement in analytical efficiency to customer laboratories.

## Online SPE technology simplifies pre-processing flow

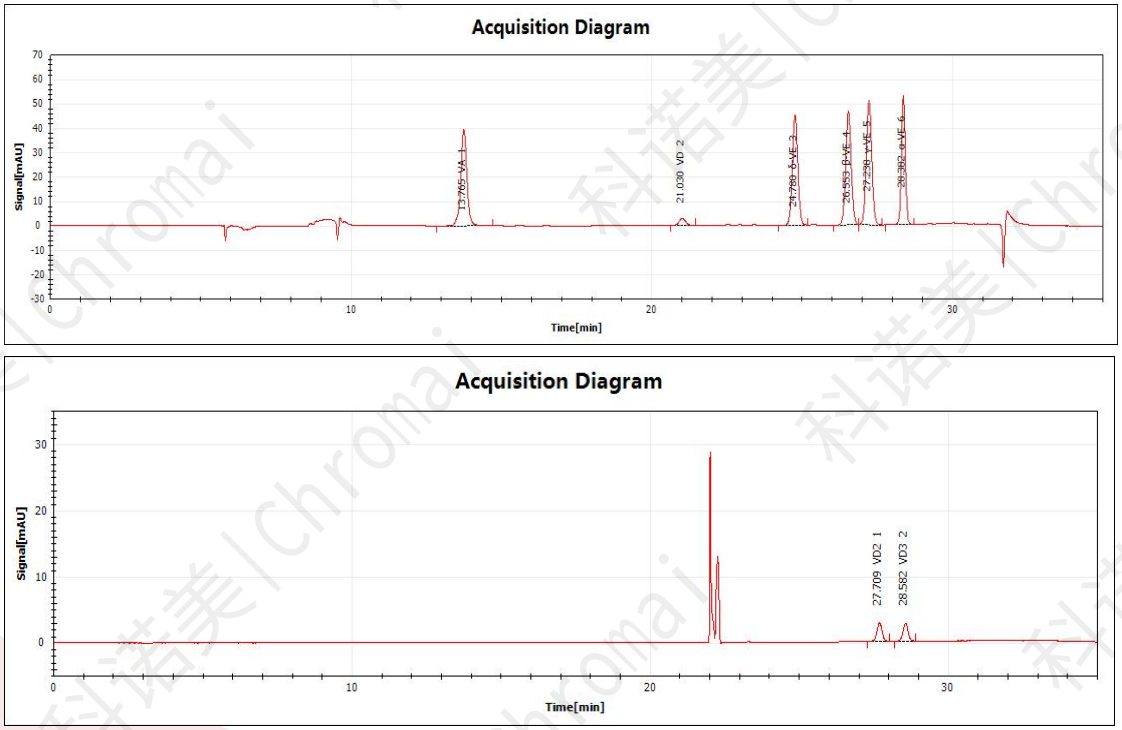

## Domestic good instrument: dual ternary gradient pump+high-sensitivity total reflection diode array detector


**System Schematic**

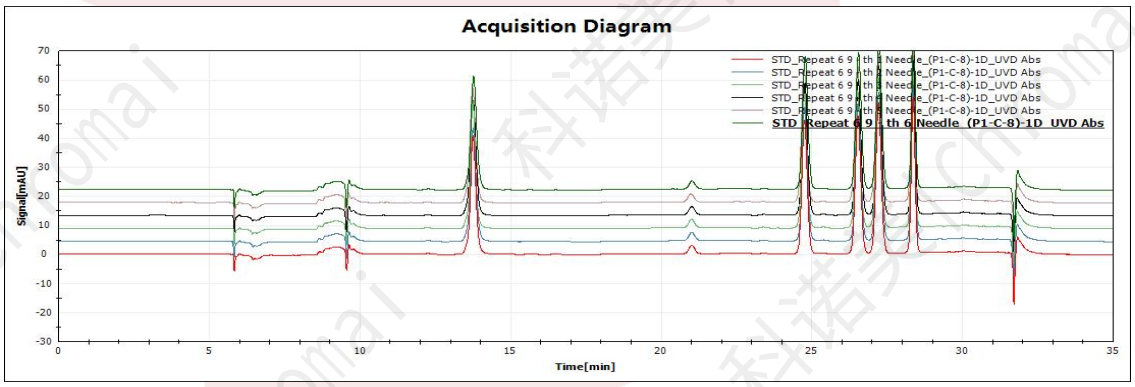
## Perfect separation of vitamin ADE and its isomers

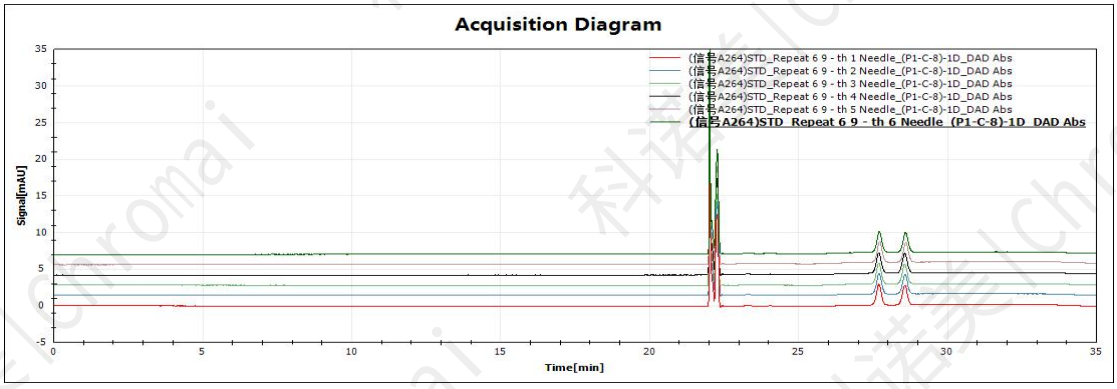
A single injection of VA, VD2, VD3, α - VE, β - VE, γ - VE, and δ - VE can achieve perfect separation. The separation degree of β - VE and γ - VE in the first dimension chromatography is 2.0, and the separation degree of VD2 and VD3 in the second dimension chromatography is 2.8, fully meeting the requirements of the method determination.




## Reproducitivity verification

Take a mixed standard solution and inject 6 consecutive injections. The overlapping spectra are shown below. The results indicate that the retention time RSD of all compounds is less than 0.1%, and the RSD of peak area is less than 0.5%.






## linear relationship

| 化合物 Chemical compound | 保留时间 Retenthion（min） | 浓度 Density (μg/mL) | R2 |
|---|---|---|---|
| VA | 13.765 | 0.02~1 | 0.9999 |
| α-VE | 28.382 | 0.4~20 | 0.9999 |
| β-VE | 26.553 | 0.4~20 | 0.9999 |
| γ-VE | 27.238 | 0.4~20 | 0.9999 |
| δ-VE | 24.780 | 0.4~20 | 0.9999 |
| D2 | 27.709 | 0.002~0.1 | 0.9999 |
| D3 | 28.582 | 0.002~0.1 | 0.9999 |

## Recovery

Take formula milk powder samples and add standard solutions of target substances (VA, VD2/VD3, α - VE) at high, medium, and low levels respectively. Prepare the test solution according to the sample pretreatment method, and calculate the recovery rate of each

target substance according to the following formula. The results are shown below. The recovery rates of all compounds are between 91.7% and 109.1%, indicating that this method has high accuracy.

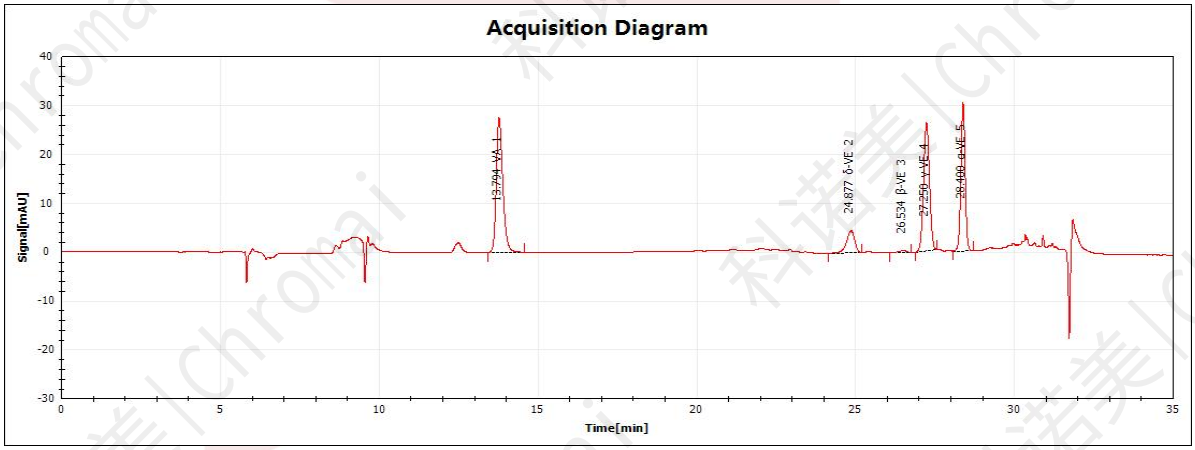
| 化合物 compound | 加标浓度 Density | 样品峰面积 Sample peak area | 标品峰面积 Reference peak area | 回收率 Recovery（%） |
|---|---|---|---|---|
| VA | 0 | 374.66 | - | - |
| | 0.1 μg/ml | 569.37 | 191.28 | 101.8 |
| | 0.2 μg/ml | 783.68 | 380.39 | 107.5 |
| | 0.5 μg/ml | 1387.85 | 941.20 | 107.6 |
| α-VE | 0 | 267.21 | - | - |
| | 2.5 μg/ml | 410.48 | 135.79 | 105.5 |
| | 5.0 μg/ml | 568.30 | 276.06 | 109.1 |
| | 12.5 μg/ml | 1002.29 | 679.77 | 108.1 |
| VD2 | 0 | N.D. | - | - |
| | 2 ng/ml | 7.02 | 7.68 | 91.7 |
| | 4 ng/ml | 14.16 | 14.7 | 96.4 |
| | 10 ng/ml | 35.4 | 37.32 | 94.8 |
| VD3 | 0 | 14.64 | - | - |
| | 2 ng/ml | 21.78 | 7.68 | 92.5 |
| | 4 ng/ml | 31.32 | 16.56 | 100.6 |
| | 10 ng/ml | 55.68 | 41.46 | 98.9 |

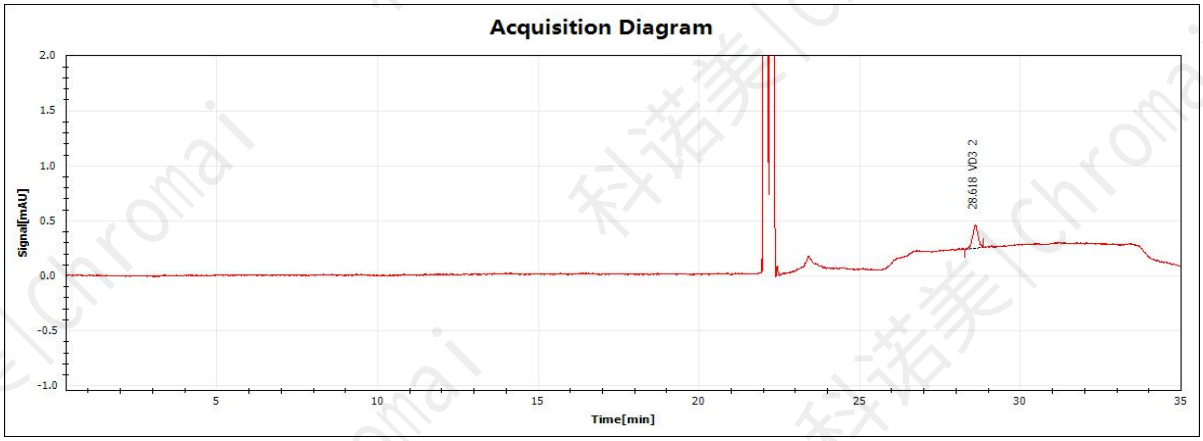
## Sample analysis

Take five commercially available formula milk powder samples, prepare sample solutions according to the sample pretreatment method, and analyze them on the machine under the determined chromatographic analysis conditions. The typical sample separation spectrum is shown in the following figure, and each target peak is well separated, meeting the separation requirements of the target analyte. The content of each compound is shown in the table below, all of which comply with relevant national food

safety standards.

| 样品 Sample | VA (μg/100g) | α-VE (mg/100g) | β-VE (mg/100g) | γ-VE (mg/100g) | δ-VE (mg/100g) | VD2 (μg/100g) | VD3 (μg/100g) |
|---|---|---|---|---|---|---|---|
| 全脂牛乳粉 Whole milk powder | 248 | 1.15 | N.D. | 0.23 | 0.10 | N.D. | 0.00 |
| 儿童配方奶粉 Children’s formula milk powder No.1 | 676 | 13.71 | 0.45 | 7.11 | 3.14 | N.D. | 13.43 |
| 儿童配方奶粉 Children’s formula milk powder No. 2 | 784 | 15.65 | 0.15 | 6.35 | 3.66 | N.D. | 15.74 |
| 儿童配方奶粉 Children’s formula milk powder No. 3 | 692 | 11.25 | 0.28 | 10.09 | 2.69 | N.D. | 7.94 |
| 儿童配方奶粉 Children’s formula milk powder No. 4 | 725 | 9.61 | 0.27 | 2.21 | 1.18 | N.D. | 12.70 |






Typical chromatogram of formula milk powder

## Conclusio

This scheme refers to the T/SHFCA 002-2024 group standard and establishes an analysis method for the content of vitamins A, D, and E in formula milk powder using Konomi online solid-phase extraction center cut two-dimensional liquid chromatography.

This scheme uses Chromai vitamin ADE analysis dedicated chromatographic column, which can achieve good separation of four isomers of vitamin A and vitamin E, as well as vitamin D2 and D3; The use of online solid-phase extraction technology can greatly simplify the sample pretreatment process, thereby improving detection efficiency and reducing human errors; Choosing the Konomi high-sensitivity total reflection diode array detector can accurately quantify low levels of vitamin D. Finally, this method has demonstrated good reproducibility and stability in durability testing, and can be widely applied as an efficient and stable method for analyzing VA, VD, and VE content in children's formula milk powder.