Application Note 140

Analysis of Fat Soluble Vitamins from Tablets, Using SPME/HPLC

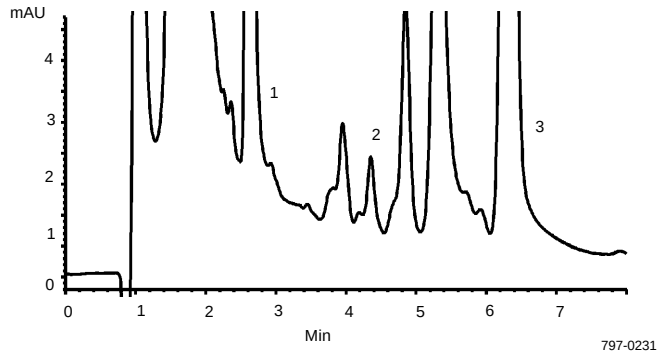
With the recent advent of the SPME/HPLC interface, solid phase microextraction (SPME)* presents a novel and simple approach to concentrating and extracting vitamins A, D_3, and E from high potency tablets, for HPLC analysis.

The best extraction for all three vitamins from a tablet was obtained with polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Figure A). We found that small adjustments to the solvent concentration shifted the equilibrium to such an extent that it considerably affected the fibers' extraction capabilities. Therefore, when determining your own sample extraction procedure, be sure to optimize it carefully.

Figure A. Fat Soluble Vitamins by SPME/HPLC

Sample:	2.5mL supernatant obtained from dissolving vitamin tablet in 1% acetic acid / 33% isopropanol
SPME Fiber:	**PDMS/DVB, 65µm**
Cat. No.:	**57311**
Extraction:	immersion (40 min), with stirring
Desorption:	static, 2 min in mobile phase; dynamic, valve open during run
Column:	**SUPELCOSIL™ LC-8, 25cm x 4.6mm ID, 5µm particles**
Cat. No.:	**5-8297**
Mobile Phase:	acetonitrile:methanol:water (63:33:4)
Flow Rate:	2mL/min
Temp.:	35°C

1. Vitamin A
2. Vitamin D_3
3. Vitamin E



The best procedure for sampling vitamins A, D_3, and E from a generic brand high potency tablet is as follows: We sampled tablets which contained these vitamins at levels of 5000IU, 400IU, and 30IU, respectively. To prepare the vitamin tablet sample, we added 4mL of 1% acetic acid to one tablet weight of ground tablet powder. A low actinic container was flushed with nitrogen and shaken occasionally, then heated for 2 minutes in a 55°C water bath. After the container cooled to room temperature, 2mL of isopropanol was added, for a final volume of 6mL (33% isopropanol). The sample was gently shaken for 2 minutes, then centrifuged. The vitamins were extracted from 2.5mL of the resulting supernatant using SPME, by immersing the exposed fiber for 40 minutes, with stirring.

The analytes are desorbed from the fiber in the SPME/HPLC interface. The fiber is exposed inside the desorption chamber for 2 minutes, for static desorption with mobile phase, before the valve is opened and dynamic desorption occurs during the run.

Ordering Information:

Description	Cat. No.
SPME Fibers for HPLC, pk. of 3	
50µm CWX/TPR▲	**57315**
65µm PDMS/DVB	**57317**
SPME Fibers Assortment Kit	
1 fiber each of 5-7315, 5-7317, 5-7301	**57323-U**
SPME Fiber Holder**	
HPLC holder	**57331**
SPME/HPLC Interface, includes 2 ferrules	
Valco® valve version	**57350-U**
Rheodyne® valve version	**57353**
SUPELCOSIL LC-8 Column	
25cm x 4.6mm ID, 5µm particles	**58297**
Fat Soluble Vitamins	
100mg, neat, unless otherwise noted. For additional vitamins refer to our catalog.	
Retinol acetate	**46958**
Retinol palmitate	**46959-U**
DL-α-Tocopherol	**47783**
rac-β-Tocopherol, 50mg/mL in 1mL hexane	**46401**
δ-Tocopherol	**47784**
γ-Tocopherol, 10mg	**47785**
DL-α-Tocopherol acetate	**47786**
Cholecalciferol (D_3)	**47763**
Ergocalciferol (D_2)	**47768**

*Solid phase microextraction technology licensed exclusively to Supelco. US patent pending; European patent #0523092.

**Initially you must order both holder and fiber assembly. Holder is reusable indefinitely.

▲Carbowax®/templated resin.

Trademarks
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Rheodyne – Rheodyne, Inc.
SUPELCOSIL – Sigma-Aldrich Co.
Valco – Valco Instruments Co., Inc.

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 800-359-3044 or 814-359-5468) for expert answers to your questions.

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