Rapid Simultaneous Analysis of Vitamin A as Retinyl Palmitate and Beta Carotene Using the Agilent 1290 Infinity LC and STM Columns

Application Note

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Introduction

Advancements in LC instrumentation and column design have drastically increased the potential efficiency of many LC analyses. The instrumental quantification of beta carotene and retinyl palmitate can be completed 8x faster than the current Schwabe North America (SNA) in-house instrument method using a STM (sub-2 µm) column, the Agilent 1290 Infinity LC System, and an alternative instrument method developed at SNA; it also reduces solvent consumption significantly.

Agilent Technologies

Beta carotene and retinyl palmitate

Beta carotene is a form of vitamin A produced in plants. Fruits and vegetables high in beta carotene typically have a deep reddish-orange color. Technically, beta carotene is a vitamin A precursor, and as such, does not have the known toxic effects of high doses of preformed vitamin A. In animals, it is converted as needed into usable vitamin A in the body. Absorption of beta carotene in the gut is enhanced by higher fat intake, as beta carotene is a fat-soluble compound.

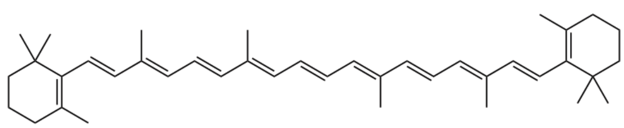
Vitamin A palmitate (also known as retinyl palmitate) is a synthetic alternative for vitamin A acetate in vitamin supplements. Because it is an active vitamin A form, there is toxicity risk in high doses.

Extraction procedure

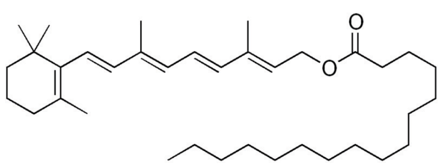
USP Method II: A Liquid/Liquid shake-out into *iso*-octane using DMSO to break down any gelatin encapsulants. The *iso*-octane is diluted into methanol for analysis.

Results and Discussion

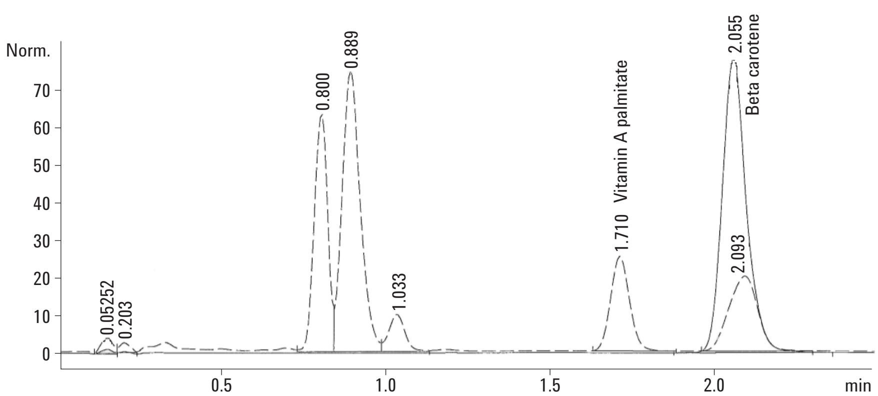
The new method and instrumentation can separate the two forms of vitamin A for quantification in less than 3 minutes (Figure 1). This is drastically faster than the SNA analysis, which takes approximately 19 minutes (Figure 2). The SNA analysis uses a non-Agilent 4.6 × 150 mm, 3 µm C-18 column, while the new method uses an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column.



Beta carotene



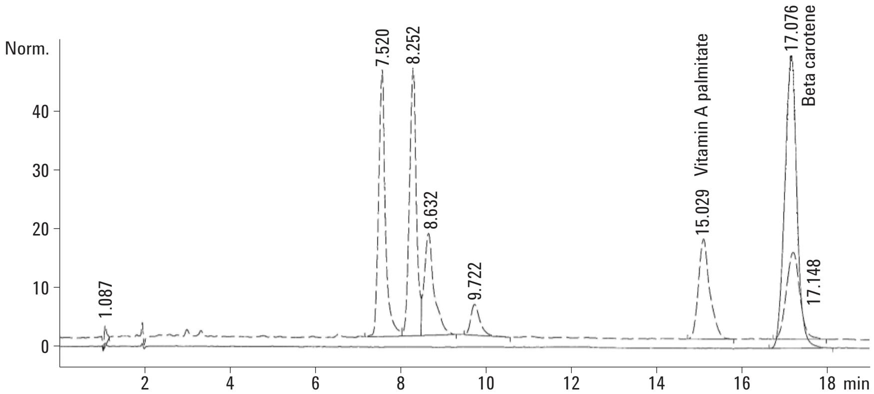
Retinyl palmitate



Instrument parameters

Temperature	40 °C
Injection amount	5 µL
Detection	UV (DAD), 325 nm for retinyl palmitate, 460 nm for beta carotene
Flow rate	1.0 mL/minute
Mobile phase	70/30 methanol with 3.2 g/L ammonium acetate/acetonitrile (isocratic, premixed)

Figure 1. Retinyl palmitate and beta carotene in a multivitamin matrix. Agilent 1290 Infinity LC System with an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm.



Instrument parameters

Temperature	40 °C
Injection amount	20 µL
Detection	UV (DAD), 325 nm for retinyl palmitate, 460 nm for beta carotene
Flow rate	1.5 mL/minute
Mobile phase	70/30 methanol with 3.2 g/L ammonium acetate/acetonitrile (isocratic, premixed)

Figure 2. Retinyl palmitate and beta carotene in a multivitamin matrix. SNA method using a non-Agilent C18, 4.6 × 150 mm, 3.0 µm column.

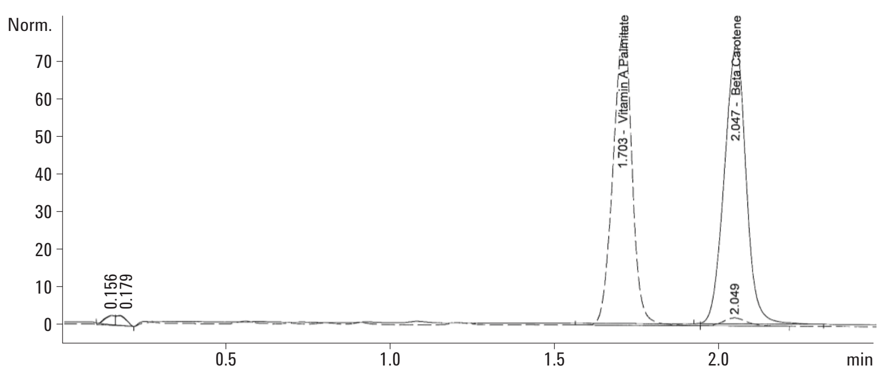


Figure 3. Reference standards.

Retinyl palmitate	*Spectrum #V1159 – Lot#1BI0736, calibration range = 0.534–13.35 µg/mL, R^2 = 0.99995.*
Beta carotene	*Sigma #C9750 – Lot#091M1417V, calibration range = 0.17–4.34 µg/mL, R^2 = 0.99994.*

Conclusion

In analyzing complex multivitamins, the Agilent 1290 Infinity LC coupled with STM columns can speed up analysis time, thus saving cost in labor and solvent use. This is done without any loss in resolution.

References

1. United States Pharmacopeia, Fat Soluble Vitamins, Method 2, USP36/NF31 p. 1632.

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