

Determination of Tocopherols in Human Serum Using Gas Chromatography

- Ion Trap Mass Spectrometry

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Introduction

The positive effects of vitamin E (alpha-tocopherol and to some extent beta-, gamma-, and delta-) as an antioxidant have been extensively studied.^{1,2} It appears to be the balance of alpha-, beta-, delta-, and gamma-tocopherols in the serum that is responsible for the positive effects.^{1,2} Some of the benefits include inhibition of atherosclerotic plaque formation, decreased platelet aggregation and thrombus formation.^{1,2} In one study the use of vitamin E as a dietary supplement has been shown to reduce the number of ischemic cardiac events in patients with documented coronary artery disease.² Although the use of vitamin E drugs has been popular for quite some time the actual consumption rates and serum levels are not well known.¹ The same study has shown that the use of alpha-tocopherol containing drugs has resulted in the reduction of the serum levels of beta- and gamma-tocopherols.¹ It is important to determine the relative serum concentrations of the tocopherols because it is the balance of the relative amounts of these that results in the benefit. This application note demonstrates the applicability of using an ion trap in single ion monitoring mode for the determination of the trimethylsilyl derivatives of the delta-, gamma-, and alpha-tocopherols.

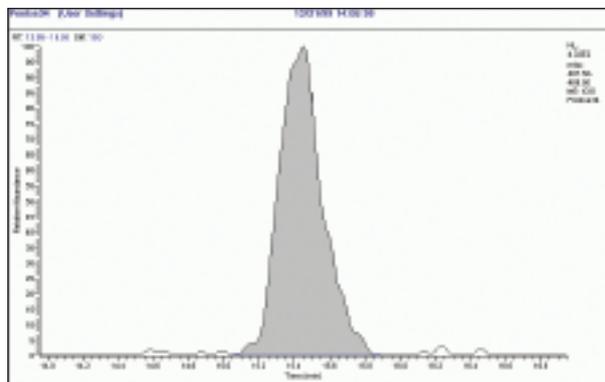


Figure 1: Mass chromatogram of gamma-Tocopherol-TMS in human serum detected at 0.96 ng

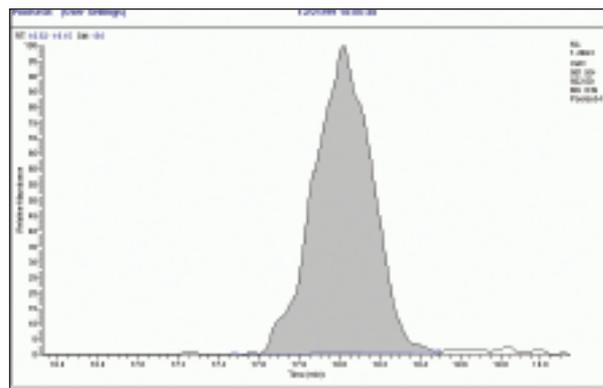


Figure 2: Mass chromatogram of alpha-Tocopherol-TMS in human serum detected at 5.98 ng

Results and Discussion

Previous analyses of these compounds have been performed using high performance liquid chromatography and fluorescence detection and gas chromatography-mass spectrometry of the trimethylsilyl derivatives.^{1,2} This report discusses the use of a gas chromatograph coupled to a bench top ion trap mass spectrometer running in single ion monitoring mode. Figures 1 and 2 on page 1 show the results of gamma- and alpha-tocopherol in a pooled serum sample that are at “low baseline” levels (i.e. from individuals not taking any alpha-tocopherol containing drugs). After some baseline determinations have occurred the serum could be monitored for changes in the relative amounts of tocopherols. Any individual who has been taking alpha-tocopherol containing drugs would show a diminished concentration of the gamma-tocopherol from the baseline. Figures 3, 4 and 5 shown at right depict the linearity of the three tocopherols from 1.35 ng to 5.5 ng except for alpha-tocopherol which was determined from 4.2 to 16.8 ng. Each point was analyzed in duplicate. Each figure also shows the structure of the native component. Mass 488, 474, and 502 were the ions monitored for trimethylsilyl derivative of gamma-, delta-, and alpha-tocopherol respectively. On this particular column the beta-, and gamma-tocopherol co-elute so a more polar column should be used when it is necessary to quantitate beta- and gamma-tocopherol at very low levels.

Key Words

- GC/MS
- Ion Trap
- Vitamin E
- Human Serum
- Tocopherols

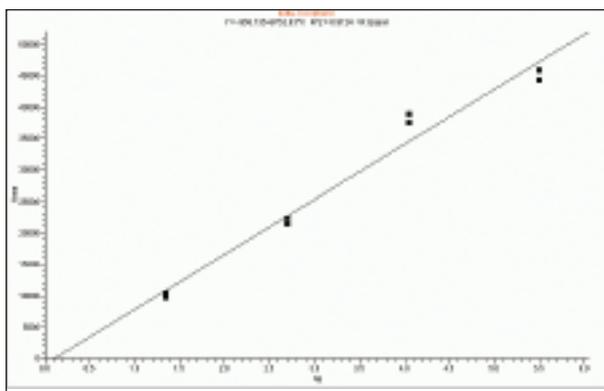


Figure 3: Calibration curve for delta-Tocopherol from 1.35 ng to 5.50 ng.

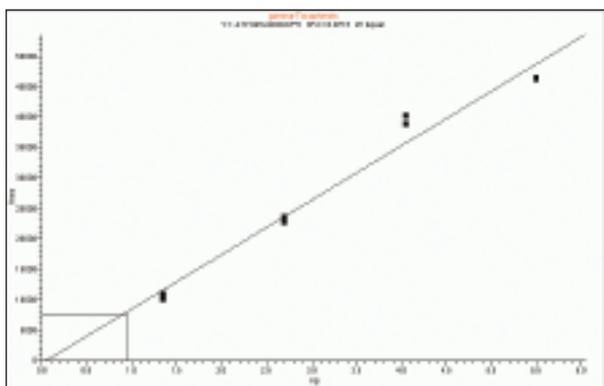


Figure 4: Calibration curve for gamma-Tocopherol from 1.35 ng to 5.50 ng.

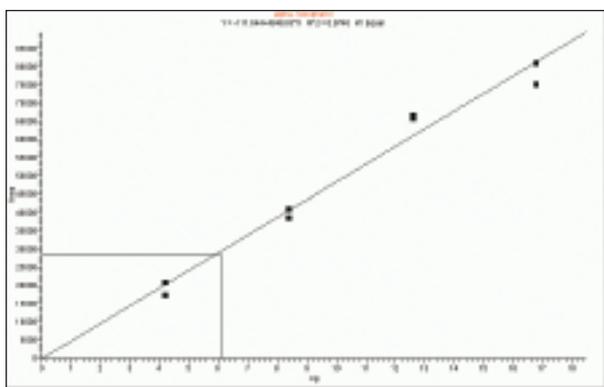


Figure 5: Calibration curve for alpha-Tocopherol from 4.20 ng to 16.8 ng

Instrument Conditions

AUTOSAMPLER: A200S

Before Injection:	
Solvent washes:	3
Sample cleans:	3
Sample pullups:	3
Hold time:	1.0 seconds
Sample:	
Sample volume:	1.0 µL
Air volume:	1.0 µL
After Injection:	
Pull-out delay:	3.0 seconds
Solvent washes:	5

GC: FINNIGAN Q-GC

Initial value:	220°C
Initial time:	1.00 minutes
5.00 °C/min:	285°C 5.00 min
Injector temp.:	275°C
Splitless time:	1.00 minutes
Column:	Restek Rtx®-5ms, 30M, 0.25mm ID, 0.25 µm df
Constant Velocity:	30.00 cm/sec Helium

MS: GCQ/POLARIS

Scan Mode:	SIM
Ion Source:	170°C
Transfer Line:	280°C
Scan Events:	
Start Time	Mass (width)
delta-tocopherol	
12.00 min.	474 (4)
gamma-tocopherol	
14.50 min.	488 (4)
alpha-tocopherol	
16.50 min.	502 (4)

Sample Preparation

500 µl human pool-serum were used for extraction and derivatization. The final volume of the sample was 200 µL (100 µL MSTFA + 100 µL iso-octane). The final sample is 2.5 times concentrated compared with the original volume of 500 µL serum.

Conclusion

The GCQ/Polaris is an excellent instrument for the detection of all tocopherols in human serum. The values shown in Figures 1 and 2 depict pooled serum samples from baseline individuals. This data proves that the instrument is sensitive and selective enough to detect the changes in concentrations in the different tocopherols that would occur from alpha-tocopherol containing drugs taken as a medicine or dietary supplement.

References

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