Analysis of phenolic antioxidants and erucamide slip additives in polypropylene formulations

Excellent performance of the Agilent 1120 Compact LC and Agilent Method Translator to obtain ASTM-equivalent methods

Application Note

Food

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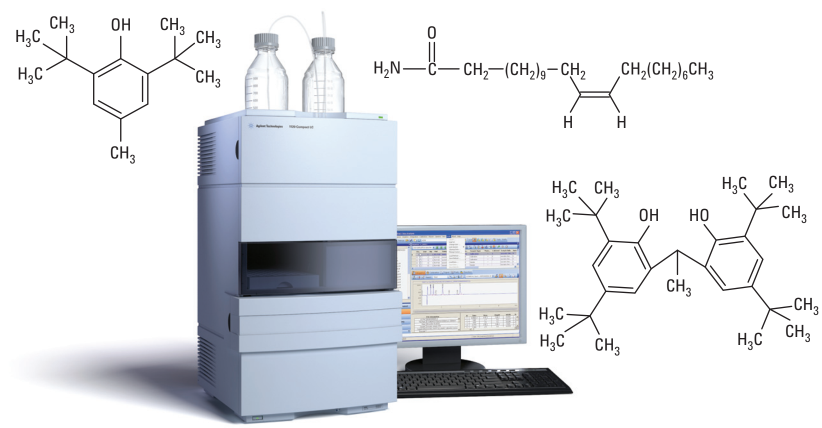


Abstract

Phenolic antioxidants, such as vitamin E, and erucamide slip additives in polypropylene formulations were separated and detected using an Agilent 1120 Compact LC with UV/VIS detection. The method was executed under the guidelines of ASTM Method D6042-09 and adapted to various column dimensions with the help of Agilent Method Translator software (Version 2.0). Agilent ZORBAX Eclipse Plus columns were used for the separation. Linearity of the standards using the Agilent 1120 Compact LC was demonstrated. Tinuvin P was used as the internal standard for the experiment. The effects of reducing injection volume by changing column dimensions on peak shape and sensitivity are also discussed.

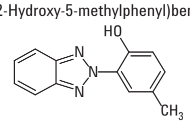
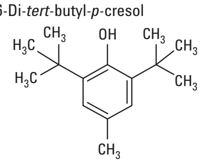
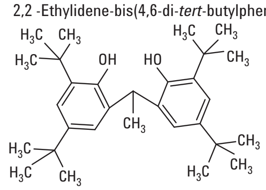
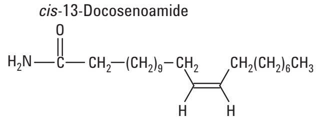
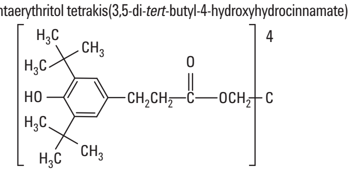
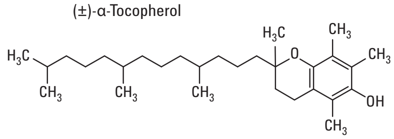
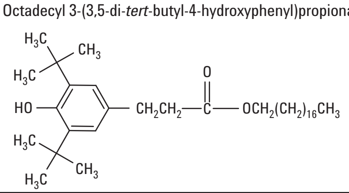
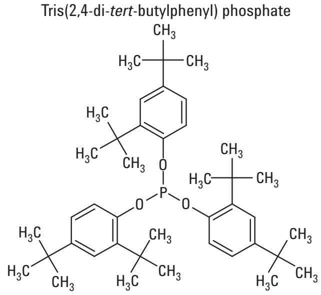
Name	Formula	Molecular weight	CAS No	Chemical name	Structure
Tinuvin P	$C_{13}H_{11}N_3O$	225.25	2440-22-4	2-(2-Hydroxy-5-methylphenyl)benzotriazole	
BHT	$C_{15}H_{24}O$	220.35	128-37-0	2,6-Di-*tert*-butyl-*p*-cresol	
Isonox 129	$C_{30}H_{46}O_2$	438.69	35958-30-6	2,2 -Ethylidene-bis(4,6-di-*tert*-butylphenol)	
Erucamide	$C_{22}H_{43}NO$	337.58	112-84-5	*cis*-13-Docosenoamide	
Irganox 1010	$C_{73}H_{108}O_{12}$	1117.63	6683-19-8	Pentaerythritol tetrakis(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)	
Vitamin E	$C_{29}H_{50}O_2$	430.71	10191-41-0	(±)-α-Tocopherol	
Irganox 1076	$C_{35}H_{62}O_3$	530.86	2082-79-3	Octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate	
Irgafos 168	$C_{42}H_{63}O_3P$	646.92	31570-04-4	Tris(2,4-di-*tert*-butylphenyl) phosphate	

Table 1

Chemical details of antioxidants and Tinuvin P

Introduction

Polymers are susceptible to degradation by ultraviolet light, heat and oxygen. To control this degradation process, various additives are blended into the polymeric material. These additives are also used to modify some of the physical properties of polymer formulations. Erucamide, Irgafos 168, Irganox 1010, Vitamin E (tocopherol), Irganox 1076, BHT, and Irgafos 168 are often used as antioxidants to prevent the degradation of polypropylene polymer formulations. HPLC methods are available for the quantitation of an additive or mix of additives in a polymeric material. ASTM D 6042-09[1] describes a conventional HPLC method for this purpose.

Accuracy of analysis is the most important requirement for ensuring that the additives and levels are suitable for the intended use. A liquid chromatography system with robust gradient pump, precise autosampler, and linear UV detector are required to execute this method. This Application Note evaluates the ability of the Agilent 1120 Compact LC to execute the ASTM D-6042 method for quantitation of the above mentioned additives. The ultraviolet (UV) absorbance for the compounds are measured at 200 nm and Tinuvin P is used as an internal standard according to the ASTM method. The samples for the analysis are prepared by an ultrasonic bath extraction procedure using a solvent mixture of 75:25 (v/v) dichloromethane:cyclohexane[1]. Chemical details of antioxidants and Tinuvin P are tabulated in Table 1[2,3].

Experimental

Chemicals

All antioxidant standards and Tinuvin P were purchased from Sigma-Aldrich. HPLC grade water was freshly taken from a MilliQ water purification system. Super gradient grade acetonitrile (ACN), isopropyl alcohol (IPA) and dichloromethane (DCM) were purchased from Labscan (Bangkok Thailand). Cyclohexane for sample extraction was purchased from SD Fine Chemicals (India).

Sample/Solution preparation

- Individual stock solutions are prepared in IPA to 1000 µg/mL.
- Standard mixtures of BHT, Isonox 129, Erucamide, Irganox 1010, Vitamin E, Irganox 1076, Irgafos 168, all 125 µg/mL in IPA, were prepared by diluting individual standard stock solutions.

- Dichloromethane and cyclohexane were mixed in the ratio 75:25. A 7.5 mg amount of Tinuvin P was added to 150 mL of the above solvent mixture to prepare an extraction solvent mixture with an internal standard (Tinuvin P) concentration of 50 µg/mL.
- Samples: Three polypropylene formulations (self-prepared from polypropylene sample tubes, from poly propylene syringes, and packaged drinking water bottles) were extracted by ultrasonication according to Method ASTM-D6042-09. All three polypropylene formulation extracts were spiked with 25 µg/mL standard mix.
- Linearity levels of standard mixtures were prepared at the following concentrations: 4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, 100 µg/mL. A concentration of approximately 50 µg/mL Tinuvin P was maintained in all linearity levels as an internal standard.

Columns

Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm (p/n 959993-902)

Agilent ZORBAX Eclipse Plus C18, 150 × 3.0 mm, 3.5 µm (p/n 959963-302)

Agilent ZORBAX Eclipse Plus C18, 100 × 3.0 mm, 3.5 µm (p/n 959961-302)

Agilent ZORBAX Eclipse Plus C18, 50 × 4.6 mm, 3.5 µm (p/n 959943-902)

LC system

An Agilent 1120 Compact LC, consisting of a gradient pump with integrated degasser, autosampler with vial tray, column oven, variable wavelength detector, and Agilent EZChrom Elite Compact software was used.

LC Parameters

The LC method used was ASTM-D6042-09. The run time was 30 min, with no post run time. The method details and the gradient used are tabulated in Table 2.

Parameter	Details
Column	Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm (p/n 959993-902)
Column Oven	50 °C
Mobile phase A	Water
Mobile phase B	Acetonitrile
Flow rate	1.5 mL/min
Needle wash	Using 100% acetonitrile
Injection volume	Variable, 10 µL and 5 µL
Detection	200/4 nm; no reference
Peak width	> 0.125 s
Data acquisition rate	20 Hz

Time	%B
0	75
5	100
25	100
25.1	75
30	75

Table 2
LC method details (ASTM method).

Procedure

A blank injection was performed in all trials to check the chromatographic interference in the resolution. Standard spike mix, extracted samples, and spiked extracted samples were also injected. The retention times of extraction solvents and individual standards were confirmed by individual standard injections.

Results and Discussion

Effect of injection volume on peak shape

According to method ASTM D 6042-09, DCM and cyclohexane in the ratio 75:25 are used for the extraction of antioxidants from polypropylene formulations. Both DCM and cyclohexane are immiscible with the acetonitrile and water mobile phases. Upon injection of the extract, a peak splitting is observed with an injection volume of 10 µL. When the injection volume was reduced from 10 µL to 5 µL, the peak shape improved (Figure 1). The effect of injection volume was not prominent with standards dissolved in IPA.

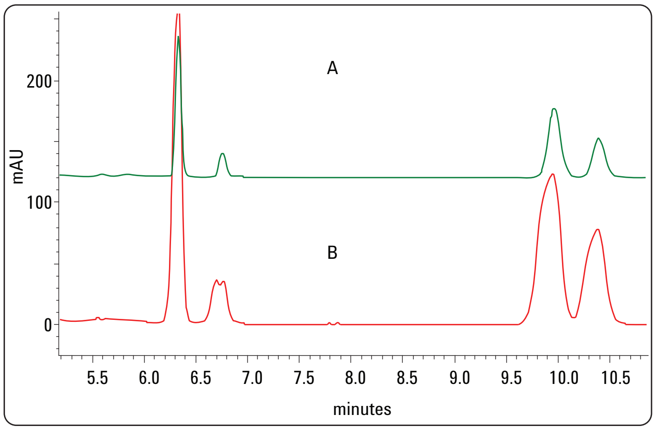


Figure 1
Effect of injection volume on peak shape. The lower trace (A) is with 10 µL injection volume and the upper trace (B) is with 5 µL.

Based on the observed injection volume effect, a 5-µm injection volume was maintained throughout this study. According to the ASTM method, the lowest level of phenolic antioxidant detectable under optimum conditions is approximately 2 µg/mL and the suggested injection volume is 10 µL. To maintain the same on-column concentration while checking the limit of detection, a 4 µg/mL standard solution was prepared and 5 µL were injected. The observed signal-to-noise (S/N) values for all individual peaks are included in Table 4.

Chromatographic representation of ASTM method

A standard spike mix of all additives at a concentration of 125 µg/mL in IPA was prepared and injected. The representative chromatogram of the standard spike solution as per ASTM method using an Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm column is shown in Figure 2.

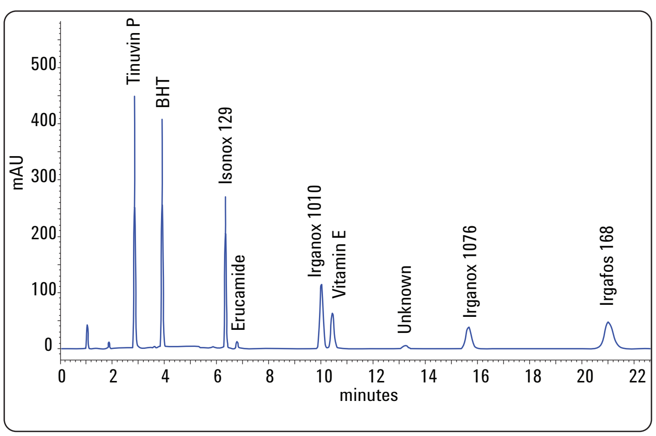


Figure 2
Representative chromatogram of a standard mix as per ASTM method (all at 125 µg/mL in IPA). (Column used: Agilent ZORBAX Eclipse Plus C18, 150 × 4.6 mm, 5 µm).

An unknown peak was observed at approximately 13.2 minutes, which is an impurity from Irgafos 168.

The chromatograms of the three extracted samples overlaid with spiked standard mix is shown in Figure 3.

Erucamide was present in all three extracted samples. In Sample I, the unknown peak was very prominent along with trace amounts of Irganox 1010, Irgafos 168 and Irganox 1076. Sample II contained Irganox 1010, an unknown peak and Irgafos 168, while Isonox 129 and Irganox 1076 were present in trace levels. Sample III, contained Irganox 1010, an unknown impurity, Irganox 1076 and Irgafos 168 in trace levels only.

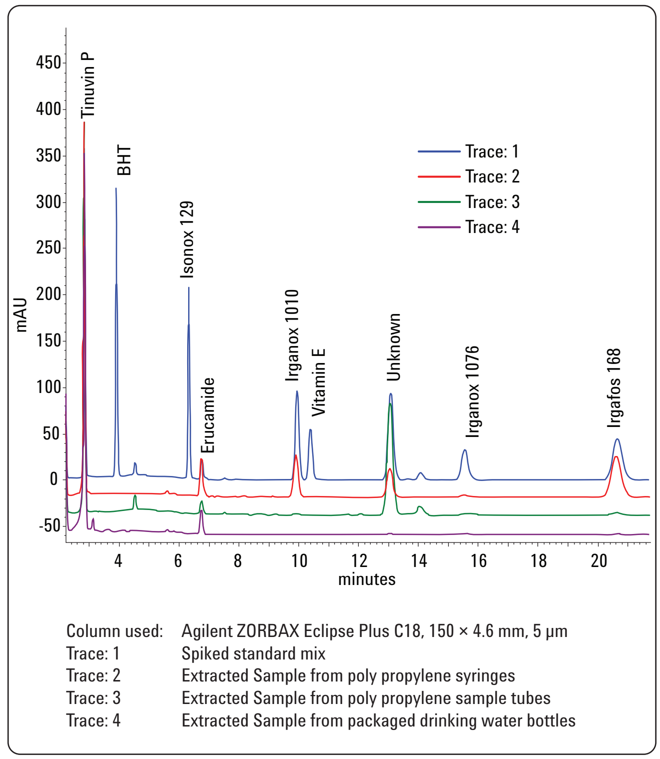


Figure 3
Chromatographic overlay of three extracted samples with spiked standard mixture.

Linearity and precision

Linearity tests were performed in the concentration range of 4 µg/mL to 100 µg/mL. The linearity levels were 4 ppm, 8 ppm, 16 ppm, 32 ppm, 64 ppm and 100 ppm. To demonstrate the precision of area and retention time, five replicate samples for all linearity levels were injected and relative standard deviation (RSD) for retention time (RT) and area were calculated. The graphical representation of RSD for RT and area can be shown (Figure 4 a and b). The observed RSD values throughout the linearity levels for retention time (<0.18%) and for area (<0.92%) are well within the acceptance limit of 1.0% which confirms the excellent precision in area and retention time.

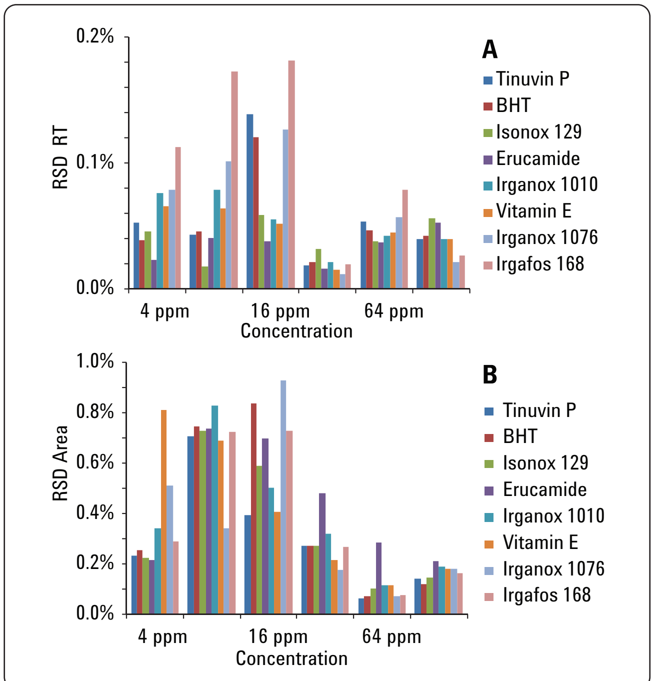


Figure 4
Graphical representation of RSD for (A) RT and (B) area.

A calibration graph was constructed by plotting the peak area of each standard against nominal concentrations (4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, and 100 µg/mL). The linearity of the relationship between peak area and concentration is demonstrated by the correlation coefficients obtained above r^2> 0.999. The overlaid linearity curves for all standards are shown in Figure 5. ASTM signal-to-noise ratios for all standards at 20 ng on-column (4 ppm solution, 5 µL injection) concentration along with r^2 values are tabulated in Table 3. The RT RSD across the linearity levels is also included in Table 3.

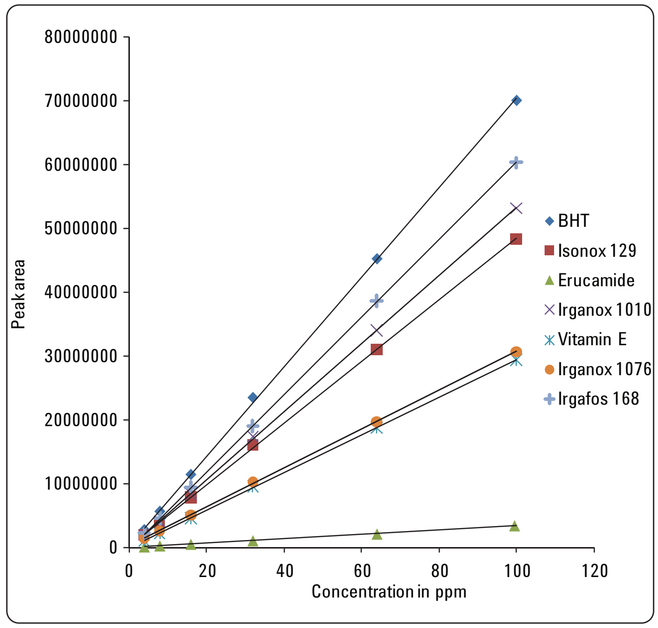


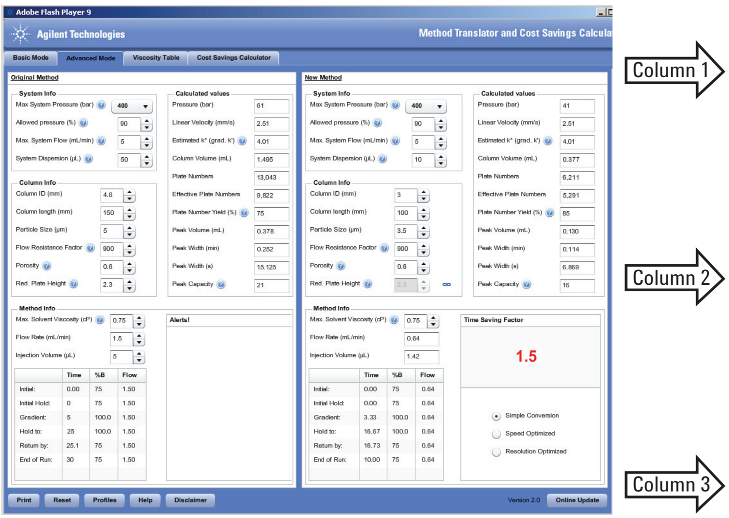
Figure 5
Linearity curves for all standards.

Compound	r^2 Values	S/N(ASTM) value at 4 ppm level, 5 µL	RT RSD across the linearity levels (6 levels)%
BHT	0.9998	650	0.19
Isonox 129	0.9999	234	0.05
Erucamide	0.9999	15	0.02
Irganox 1010	0.9999	245	0.12
Vitamin E	0.9999	131	0.10
Irganox 1076	0.9999	263	0.24
Irgafos 168	0.9999	244	0.38

Table 3
r^2 values and S/N for each standard at 20 ng on-column concentration and RT RSD for each standard across linearity levels.

Trials with reducing column dimension and particle size

In order to demonstrate the improved sensitivity and reduced analysis time by changing the column dimensions and particle size, the initial ASTM method was recalculated using Agilent Method Translator software for three other column dimensions. Agilent ZORBAX Eclipse Plus C18 columns were used for this study. The screenshots of Method Translator are shown in Figure 6, and the corresponding chromatograms are shown in Figure 7.



	Time	%B	Flow
Initial:	0.00	75	0.64
Initial Hold:	0.00	75	0.64
Gradient:	5.00	100.0	0.64
Hold to:	25.00	100.0	0.64
Return by:	25.10	75	0.64
End of Run:	30.00	75	0.64

Simple Conversion
Speed Optimized
Resolution Optimized

	Time	%B	Flow
Initial:	0.00	75	0.64
Initial Hold:	0.00	75	0.64
Gradient:	5.00	100.0	0.64
Hold to:	25.00	100.0	0.64
Return by:	25.10	75	0.64
End of Run:	30.00	75	0.64

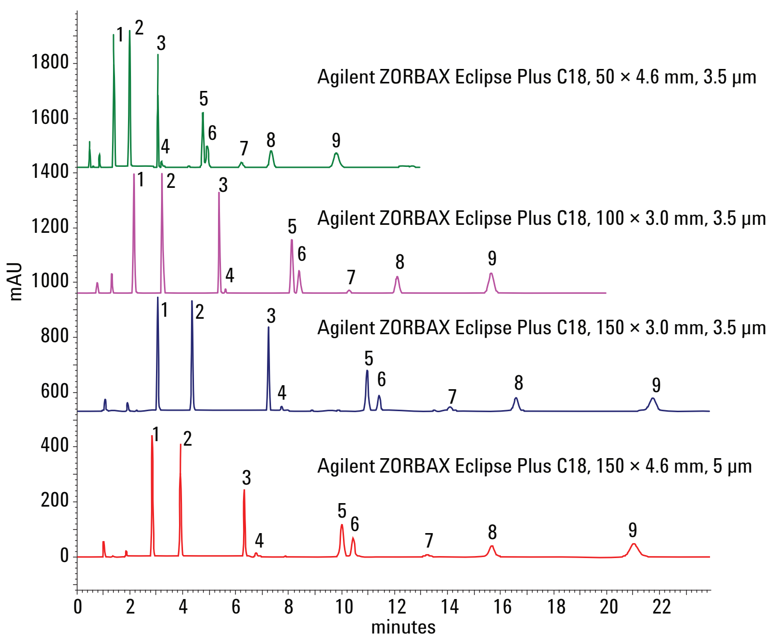
Simple Conversion
Speed Optimized
Resolution Optimized

	Time	%B	Flow
Initial:	0.00	75	1.13
Initial Hold:	0.00	75	1.13
Gradient:	1.67	100.0	1.13
Hold to:	11.00	100.0	1.13
Return by:	8.37	75	1.13
End of Run:	10.00	75	1.13

Simple Conversion
Speed Optimized
Resolution Optimized

Column 1: Agilent ZORBAX Eclipse Plus C18, 150 × 3.0 mm, 3.5 µm
Column 2: Agilent ZORBAX Eclipse Plus C18, 100 × 3.0 mm, 3.5 µm
Column 3: Agilent ZORBAX Eclipse Plus C18, 50 × 4.6 mm, 3.5 µm

Figure 6
Agilent Method Translator screenshot.



1. TinuvinP
2. BHT
3. Isonox 129
4. Erucamide
5. Irganox 1010
6. Vitamin E
7. Unknown (Impurity from Irgafos 168)
8. Irganox 1076
9. Irgafos 168

Figure 7
Chromatographic separation of additives using various column dimensions.

Resolution values for two critical pairs (Isonox 129 and Erucamide and Irganox 1010 and Vitamin E) were evaluated to illustrate a comparison in chromatographic performance. (Table 4). The S/N value for Erucamide (20 ng on-column) is included in the same table. With 5 cm columns, the first solvent peak starts eluting at 0.4 minutes, affecting the ASTM S/N calculation. Therefore, S/N values were calculated manually by selecting 0 to 0.35 minutes as the noise region (ASTM uses a default noise region of 0 to 0.6 minutes for the calculation).

Critical pairs	150 × 4.6 mm 5 µm Original method	150 × 3.0 mm 3.5 µm	100 × 3.0 mm 3.5 µm	50 × 4.6 mm 3.5 µm
Isonox 129 Erucamide	4.25	5.71	3.17	2.47
Irganox 1010 Vitamin E	1.86	2.56	1.83	1.31
S/N value for 20 ng Erucamide on-column	48	168	225	108

Table 4
The resolution values for critical pairs along with observed S/N value for 20 ng on-column concentration of Erucamide using Agilent ZORBAX Eclipse Plus columns.

The Agilent ZORBAX Eclipse Plus 100 × 3.0 mm, 3.5 µm column gives a better peak height compared to other column dimensions used in this study. This improves S/N values above the conventional ASTM column dimension of 150 × 4.6 mm, 5 µm. The lowest resolution value observed with this column was 1.83, (between Irganox 1010 and Vitamin E) and is well within the Standard Pharmacopeia specification limit. Resolution provided by ZORBAX Eclipse Plus 50 × 4.6 mm, 3.5 µm column between Irganox 1010 and Vitamin E is usually not acceptable by Pharmacopeia (1.31), but included here to demonstrate the effect of resolution and sensitivity by reducing the column length.

Conclusions

Phenolic antioxidants, Vitamin E and Erucamide slip additives in polypropylene formulations are well resolved and detected using Agilent's 1120 Compact LC with variable wavelength detection at 200 nm UV/VIS detection. The excellent performance of Agilent 1120 Compact LC pump is established by demonstrating high precision for retention times and areas as well as excellent signal-to-noise ratios. Exceptional linearity of the Agilent 1120 Compact LC detector is demonstrated. The observed r^2 values for all standards are >0.999. This Application Note also demonstrates the applicability of Agilent Method Translator software to obtain an equivalent method from ASTM D6042-09 when using different column dimensions. Improvement in peak sensitivity and lowest detection limits are shown by reducing the column diameter and particle size.

References

1. ASTM D6042-09, "Standard Test Method for Determination of Phenolic Antioxidants and Erucamie Slip Additives in Polypropylene Formulations Using Liquid Chromatography (LC)". Volume 08.03 Plastics (III): July 2009

2. Wei Luan, Chunxiao Wang (Shanghai-China) and Michael Woodman (Wilmington USA) "Fast Analysis of Tocopherol, Phenolic Antioxidants and Erucamide Slip Additives in Polypropylene Homopolymer Formulations Using 1200 Rapid Resolution Liquid Chromatography (RRLC) with Rapid Resolution High Throughput (RRHT) Columns and Method Translator", Agilent Technologies Publication 5989-5849EN, 2009 .

3. Chun-Xiao Wang, Wei Luan (Shanghai-China) and Michael Woodman (Wilmington USA) "Analysis of Phenolic Antioxidant and Erucamide Slip Additives in Polymer by Rapid-Resolution LC", Agilent Technologies Publication 5989-5850EN 2009.

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