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Determination of Tinuvin 292 in Acrylic Resins by Reversed-phase High-performance Liquid Chromatography–Electrospray Ionization–Ion Trap Mass Spectrometry

Insook Park,[1] Kyung-Hwan Yoon,[2] and Hyun Namgoong*[1]
[1]*Analysis and Evaluation Research Institute, Kolon Central Research Park, Yongin 446-797, Korea*
[2]*Department of Chemistry, Seoul National University, Seoul 151-747, Korea*

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Due to recent reports on toxicity of hindered-amine light stabilizers (HALS) in polymer materials, demand for the regulation of the dosage of HALS is increasing. Therefore development of a quantitation method for low concentration of HALS is required either for toxicity study of HALS in water or saliva extracts from polymeric materials or for accurate determination of reduced amounts of HALS in polymeric materials. Here we present a novel quantitation method for Tinuvin 292, a worldwide used HALS, in acrylic resins using high-performance liquid chromatography–electrospray ionization–ion trap mass spectrometry at a broad concentration ranging from 10 ppb to 50 ppm. Collision-induced dissociation was performed using helium gas, and the multiple reaction monitoring parameters were optimized for detection of parent/daughter ions of Tinuvin 292. Sample preparation employed a very simple extraction of Tinuvin 292 from acrylic resins with considerable recovery. The method was successfully applied to quantitate Tinuvin 292 in various acrylic polymers.

During the photooxidative degradation of polydienes, the resulting singlet oxygen ($^1O_2$) was found to attack carbon double bonds to form allylic hydroperoxide groups.[1–3] Hindered piperidines act as a singlet oxygen quencher by reacting with $^1O_2$ to produce nitroxyl radicals.[4–7] Light stabilizers containing hindered piperidines are called hindered-amine light stabilizers (HALS), which are used very commonly in the polymer industry. Since the introduction of the first HALS by Sankyo in 1967,[8] various HALS were developed and their applications extended to a variety of polymers from polydienes to polypropylene, polyethylene, polyesters, polyacrylate, polystyrene, and polyurethane.[9–15]

The concentration of HALS has not been regulated until recently. Manufacturers tend to use as much HALS as possible, considering the depletion of HALS by thermal evaporation during manufacturing processes.[16] According to a manufacturer's guide, the recommended concentration of Tinuvin 292, a widely used liquid HALS composed of bis(1,2,2,6,6-pentamethylpiperidin-4-yl) sebacate (**T1**) and 1-methyl-8-(1,2,2,6,6-pentamethylpiperidin-4-yl) sebacate (**T2**) at a mixing ratio of 75:25–85:15 (Figure 1), is 0.5–1.5 wt %. However, Shi et al. found that Tinuvin 292 effectively worked at a concentration as low as 0.15 wt %.[12] When combined with a suitable antioxidant, even a small amount (0.01–0.1 wt %) can achieve satisfactory stabilization effects.[17] Although we could not find a toxicity report on Tinuvin 292, Sótonyi et al. reported Tinuvin 770, one of the HALS used worldwide, caused severe damage to cardiac myocytes at a low concentration of 25 nmol[18] due to its action as an L-type Ca^{2+}-channel blocker.[19] Recently it was also reported that considerable amount of Tinuvin 770 could migrate from commercial products to saliva,[20] which suggests potential risks to children with a habit of sucking and biting plastics. Therefore, a regulation on the concentration of HALS should be introduced as soon as possible, and a quantitative analysis method for detection of low amounts of HALS is required either for toxicity study in water or saliva extracts from polymeric materials or for determination of the reduced amount of HALS in polymeric materials.

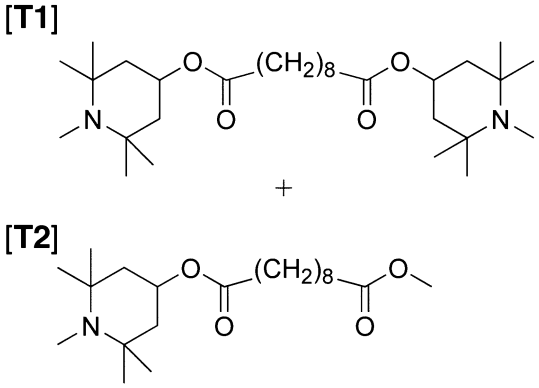

Figure 1. Chemical structures of Tinuvin 292.

Various analysis methods have been performed to determine the concentration of HALS, including electron spin resonance spectroscopy,[21] gas chromatography,[22–25] high-performance liquid chromatography (HPLC) equipped with evaporative light scattering detection,[24] UV detection,[24,26,27] and mass spectrometry (MS).[28,29] Most methods were designed for detection of relatively high amounts (≈1%) of HALS, and include laborious sample preparation processes. Recently more powerful methods using tandem MS/MS have appeared due to high selectivity and low limit of detection. Lowe et al. succeeded in identification of various HALS in coil coatings using LC-MS/MS,[30] and Gill et al. reported successful quantitation of Tinuvin 770 in aqueous extracts of plastic materials at a very low concentration level (1–200 ng mL^{-1}).[31] Here we introduce a very simple sample preparation process and the subsequent quantitation method of HALS at a very broad concentration range using LC-MS/MS. This novel method can be employed to determine either the concentration of HALS in polymeric materials (100–10000 ppm range) or the concentration of HALS in water or saliva extracts (10–1000 ppb range). Tinuvin 292 in acrylic resins was chosen as a representative study, since its toxicity and quantitation method were rarely reported in spite of its active usage.

A standard Tinuvin 292 was dissolved in acetonitrile at a concentration of 0.1 wt %. The solution was introduced to a Varian LC 500 Ion trap mass spectrometer via a syringe pump,

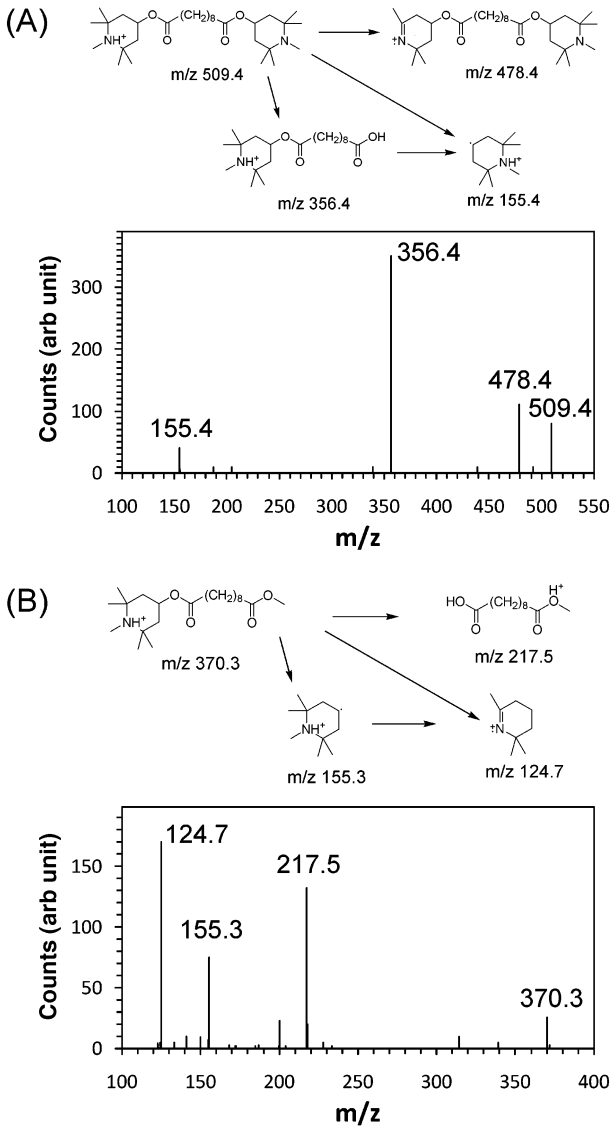

Figure 2. MS/MS results of Tinuvin 292.

and the parameters at the strongest intensity were determined as follows: positive electrospray, +600 V of spray shield voltage, 60 psig of nebulizer pressure, 40 psig of drying nitrogen pressure, 300 °C of drying gas temperature, and 5500 V of needle voltage. Under these parameters, m/z values of **T1**, **T2**, and TEA were found to be 509.4, 370.3, and 150.2, respectively. Helium gas was applied to induce the collision-induced dissociation (CID) of the selected m/z ions. CID results of **T1** and **T2** are shown in Figure 2.

T1 (m/z 509.4) was dissociated into m/z 478.4, 356.4, and 155.4, whereas **T2** (m/z 370.3) was dissociated into m/z 217.5, 155.3, and 124.7. For MS/MS quantitation, MRM parameters were set to the parent/daughter ions with the highest peak intensity: m/z 509.4 → 356.5 for **T1**, m/z 370.3 → 217.4 for **T2**, and m/z 150.2 → 133.4 for TEA as the internal standard.

A gradient HPLC method was developed for simultaneous detection of **T1**, **T2**, and TEA. Distilled water containing 0.01% trifluoroacetic acid (Solvent A) and acetonitrile (Solvent B)

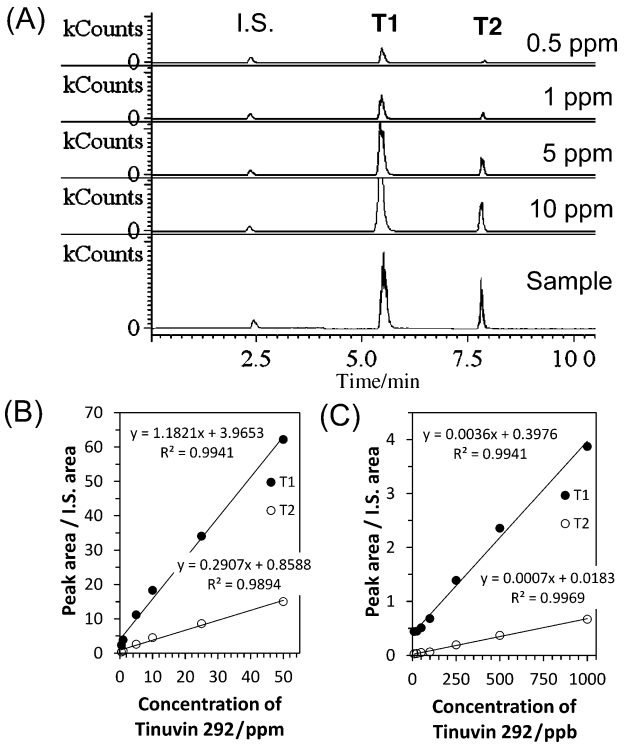

Figure 3. Representative LC-MS/MS chromatograms (A) and standard calibration curves of Tinuvin 292 at ppm range (B) and ppb range (C).

were eluted through Optimapak C18 column (5 μm, 4.6 × 250 mm) at a flow rate of 1 mL min^{-1}. The solvent gradient was achieved as follows: A/B (v/v) = 80/20 (at 0 min) → 5/95 (at 10 min, hold for 5 min) → 80/20 (at 16 min, hold for 4 min). Column temperature was maintained at 35 °C, and the injection volume was 20 μL.

A typical solvent gradient chromatogram is shown in Figure 3A, where TEA elutes first, followed by **T1** and **T2** subsequently. To draw the standard calibration curve in ppm range, 0.5, 1, 5, 10, 25, and 50 μg g^{-1} (ppm) concentrations of Tinuvin 292 were prepared in acetonitrile containing a fixed amount of the TEA (5 ppm). For the standards calibration curve in ppb range, 10, 25, 50, 100, 250, 500, and 1000 ng g^{-1} (ppb) concentrations of Tinuvin 292 were prepared in the same manner. The peak areas of **T1** and **T2** were divided by that of TEA, and plotted against the initial concentrations of Tinuvin 292. The average area ratio of **T1** between **T1** and **T2** was 81.2%, which is somewhat consistent with the component ratio of **T1** (75–85%), although such direct comparison is not meaningful due to different behaviors of different ion species during ionization and CID. At all concentration points, five injections were made to obtain the average relative standard deviations of 5.7% (**T1**) and 7.3% (**T2**). Low limit of quantitation was found to be 10 ppb with the relative standard deviations of 17.1% (**T1**) and 19.3% (**T2**). In ppm range (Figure 3B), both species resulted in good linear regression with R^2 of 0.9941 and 0.9894, respectively, and in ppb range (Figure 3C), R^2 values were 0.9941 and 0.9969, respectively. All standard calibration curves gave reasonable calculated concentration values.

Table 1. Quantitation results of Tinuvin 292 from five different acrylic resins

Polymer	Concentration of Tinuvin 292/ppm[a]	Recovery/%
A	0	0
B	620 ± 71	76.5
C	764 ± 126	94.3
D	761 ± 53	94.0
E	464 ± 53	57.3

[a]The calculated values from the calibration curve were multiplied by ≈100 (the sample dilution ratio).

Five different acrylic resin samples were prepared as follows. A commercial acrylic solution was polymerized by UV radiation without Tinuvin 292 (Polymer A) and with 810 µg of Tinuvin 292 per 1 g of sample (810 ppm) (Polymer B). Polymer B was aged either at room temperature for 120 h in a vacuum chamber (Polymer C) or at 80 °C for 120 h under ambient atmosphere (Polymer D). Polymer E was prepared by aging polymer B at 80 °C for 120 h in a vacuum chamber. Thickness of the polymer films were 30 ± 3 µm. Instead of laborious dissolution–precipitation or time-consuming soxhlet extraction, we simply placed 1.0 g of the samples (chopped in pieces of less than 2 mm in length) in 100 mL of the internal standard solution (5 ppm in acetonitrile), and vortically mixed the mixture for 1 h at 60 °C under atmospheric pressure in order to fully extract the Tinuvin 292. The resulting solutions were directly injected into LC-MS/MS.

LC-MS/MS chromatograms obtained from the acetonitrile extracts of acrylic resins showed good sensitivity for **T1** and **T2** (See Figure 3A, Sample polymer D). However, the average peak area ratio of **T1** between **T1** and **T2** was found to be 75.2%, indicating insufficient extraction of **T1** compared with **T2**. Assuming **T2** was extracted more ideally than **T1**, we calculated the concentration of Tinuvin 292 using the calibration curve of **T2** after compensation of the dilution ratio (100:1). The results are summarized in Table 1.

No Tinuvin 292 was detected in Tinuvin-free polymer A. In freshly polymerized polymer B, only 76.5% of Tinuvin 292 was recovered, whereas in aged polymer C and D, 94.3% and 94.0% were recovered, respectively. This result suggests possible structural changes such as microcavities or microcracks in aged polymer films, which facilitated migration of inner Tinuvin 292 toward the surface.[15] For satisfactory recovery of Tinuvin 292 from a freshly made polymer (i.e., over 90%), more extraction time was required. The recovery in polymer E was only 57.3% due to the accelerated evaporation of Tinuvin 292 caused by the synergetic effect of heat and vacuum.[16] This analysis method was also successfully applied to 50–150 ppb-level determination of Tinuvin 292 control samples with accuracy within 10%, implying its potential usage in study of trace amount of Tinuvin 292 in water or saliva extracts.

In conclusion, we developed a simple extraction and quantitation method of Tinuvin 292 using LC-MS/MS at a concentration range 10 ppb to 50 ppm. The method was successfully applied to determine hundreds to thousands ppm concentration of Tinuvin 292 in acrylic resins with recovery higher than 90%. This method is also adoptable for determination of trace amounts of Tinuvin 292 at ppb-level, which is a suitable range for toxicity study in water or saliva extracts from polymeric materials.

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