

Quantitative Analysis of an Antioxidant Additive in Insoluble Plastics by Surface-Assisted Laser Desorption/Ionization Mass Spectrometry (SALDI-MS) Using TiO₂ Nanoparticles

Issey OSAKA,¹ Koji OKUMURA,² Naoki MIYAKE,² Takehiro WATANABE,²
Kazuyoshi NOZAKI,² Hideya KAWASAKI,² and Ryuichi ARAKAWA^{2*}

¹ *Center for Nano Materials and Technology, Japan Advanced Institute of Science and Technology, Nomi, ISHIKAWA, JAPAN*

² *Faculty of Chemistry, Materials and Bioengineering, Kansai University, Suita, OSAKA, JAPAN*

The applicability of matrix-free surface-assisted laser desorption/ionization mass spectrometry using porous silicon (DIOS-MS) and TiO₂ nanoparticles (TiO₂-SALDI-MS) was examined by analyzing an antioxidant added to insoluble polypropylene (PP) materials. The optimized solvent extraction, which involved freezing and crushing the PP, results in a good recovery, 89±4 and 97±8% for 0.5 wt% of the added antioxidant, tetrakis[methylene3-(3,5-di-*t*-butyl-4-hydroxyphenyl)propionate]methane (commercial name; Irganox1010) in laboratory-produced and commercial PP composites, respectively. The positive ion measurements in DIOS and TiO₂-SALDI-MS yielded sodium adduct ions without any interference from other signals. Using an internal standard with a similar structure, a good calibration curve linearity was established in the Irganox1010 concentration range of 0.01–2.0 mg/mL in PP, which was sufficient for monitoring the plastic additives. However, TiO₂-SALDI-MS was superior to DIOS-MS in terms of mass resolution and easiness of sample preparation. A solvent extraction method followed by TiO₂-SALDI-MS appears to be a anticipated method for the analysis of additives in insoluble plastics.

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1. Introduction

A number of additives to plastic materials, including antioxidants, light stabilizers and ultraviolet stabilizers are required to prevent the deterioration of quality of the material. Adding the stabilizers has an aging effect on polymeric materials, resulting in tarnishing and degradation of the materials. These stabilizers, which are present in trace amounts in various polymer materials, are typically analyzed by gas chromatography (GC) or liquid chromatography (LC) after their extraction from the substrate. Quantitative analyses of tetrakis[methylene3-(3,5-di-*t*-butyl-4-hydroxyphenyl)propionate]methane, an antioxidant that is marketed under the name Irganox1010, along with Irganox1076, 1035, 259 and related additives, have utilized pyrolysis-GC.¹⁾ However, it is often difficult to quantitatively analyze these antioxidants and light stabilizers, especially with GC-MS and liquid chromatography (LC)-MS, because many are nonvolatile, nonpolar compounds and their quantitative recovery is thus quite low. Therefore, rapid, simple methods for quantitatively analyzing such additives in plastic materials are needed.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been widely used for

the analysis of biomolecules such as proteins and peptides,^{2)–4)} because it can effectively ionize thermally-unstable, high molecular-weight compounds. Recently, it has also been used for the characterization of synthetic polymers, such as polyethyleneglycol and polystyrene.^{5)–8)} In MALDI, the chemical matrix yields intense matrix-molecule ions in the low mass region, which occasionally interfere with the characterization of low-mass target components. Furthermore, the selection and combination of chemical matrixes must be done with caution in that cationizing agents and solvents must be chosen carefully, to obtain reliable MALDI mass spectra. Since MALDI spectra are particularly dependent on the crystal condition of an analyte and matrix, in which solubility plays a role in miscibility when the solvent is removed, optimization of the concentration ratio of reagents is required. It is generally difficult to quantify the species observed in the MALDI mass spectra, because the absolute intensities of the observed peaks are strongly dependent on the laser power used and the conditions for sample preparation.⁹⁾ An oligomeric hindered amine light stabilizer (HALS) in polypropylene (PP) was directly determined by solid sampling MALDI-MS.^{10), 11)} The matrix and the HALS-containing PP were co-ground in liquid nitrogen using a freezer mill and the powdered mixture was directly spotted onto the sample plate for MALDI-MS.

Siuzdak's group recently reported on a matrix-free ionization method and referred to it as laser desorption/ionization on porous silicon (DIOS).^{12), 13)} Porous

* Correspondence to: Ryuichi ARAKAWA, *Faculty of Chemistry, Materials and Bioengineering, Kansai University, 3-3-35 Yamatecho, Suita, Osaka 564-8680, JAPAN*, e-mail: arak@ipcku.kansai-u.ac.jp

silicon is a UV-absorbing semiconductor with a large surface area and is produced through electrochemical anodization or the chemical etching of crystalline silicon. In DIOS measurements, analytes are deposited on a DIOS chip without a matrix. The DIOS mass spectra are free from cluster ions derived from the matrix, such that the resulting scant background signals facilitate the detection of ions of small molecules. As a result, DIOS-MS has been widely used for the analysis of a variety of compounds, including peptides,¹²⁾ low molecular weight organic molecules¹⁴⁾ and synthetic, hydrophilic polymers¹⁵⁾ such as surfactants and polyethylene glycol. DIOS-MS without the use of matrix can be regarded as a more quantitative method than MALDI-MS. DIOS-MS has also been used in the qualitative analysis of polymer additives¹⁶⁾ and the quantitative analysis¹⁷⁾ of salicylic acid in serum.

More recently, surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS), in which nanomaterials are used, has received considerable attention because the method does not require the use of organic compounds as a matrix. Nanomaterials such as titania sol-gel thin films,^{18), 19)} silicon nanoparticles,²⁰⁾ and gold nanoparticles²¹⁾⁻²⁵⁾ have been used for SALDI-MS. For these inorganic matrixes, with high surface areas, the sample-preparation can be simplified, and flexibility can be achieved for sample deposition under different conditions.

In this study, MALDI-, DIOS-, and SALDI-MS with TiO₂ nanoparticles (TiO₂-SALDI-MS) were examined as a possible rapid and simple method for analyzing the antioxidant Irganox1010 in insoluble PP materials. We particularly assessed the quantitative method in which TiO₂-SALDI-MS is used. The capability of this method for quantitative analysis is discussed herein.

2. Experimental

2.1 Sample preparation

Standard solutions containing 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/mL of Irganox1010 (Ciba Specialty Chemicals) in tetrahydrofuran (THF) were prepared to obtain a calibration curve. A PP sample containing 0.5 wt% Irganox1010 was laboratory-produced by kneading hot-melt PP (200°C) and Irganox 1010. Commercial PP samples containing Irganox1010 (0.5 wt%), calcium stearate (0.05 wt%) and Irganox 1076 (octadecyl-3-(3',5'-di-*tert*-butyl-4'-hydroxyphenyl) propionate, 0.011 wt%) were obtained. For extraction of the additives, the PP polymers, residue from an electric drill, were ground using a ball mill MM200 (Retsch, Haan, Germany) cooled with liquid nitrogen for 40 min. Irganox1010 was extracted from 100 mg of

the PP powder with 1 mL THF. Irganox1098 (Ciba Specialty Chemicals) as an internal standard (IS) was dissolved in THF and added to the standard solutions and the extracted solutions. The chemical structures of Irganox1010 (1176.8 Da) and Irganox1098 (636.5 Da) are shown in Fig. 1.

2.2 Instruments

2.2.1 Liquid chromatography atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS)

When LC-MS was applied to the THF solution that was used to extract Irganox1010, no Irganox1010 was detected because it had precipitated upon mixing with water in the mobile phase (H₂O/acetnitrile (MeCN)=1/9). Therefore, to avoid the precipitation of Irganox 1010 in the mobile phase, the THF extraction solution was first dried and the residue was then completely dissolved in a solvent mixture consisting of H₂O/MeCN=3/7 (v/v) using a vibrating stirrer.

In the APCI experiments, the 150 μL solution with the addition of 30 μL of the internal standard (IS) solution (Irganox1098) was measured using LC-MS. The IS concentration was 0.1 mg/mL. An HP1100 (Agilent, Palo Alto, CA) instrument with a column of Cosmosil 5C8-AR-300 (4.6 × 150 mm, Nacalai Tesque Inc., Kyoto, Japan) was used for the HPLC measurements. The mobile phase was a mixed solution of H₂O/MeCN=1/9 containing ammonium acetate (5 mM) and the flow rate was 0.5 mL/min. A 5 μL sample solution was injected.

APCI mass spectra were recorded using a TSQ triple-stage quadrupole mass spectrometer (ThermoElectron, San Jose, CA). The data were acquired using Xcalibur software (version 1.2). Parameters for APCI negative ion analysis were as follows: APCI desolvation temperature, 500°C; corona current, 6.5 μA; heated capillary, 250°C; and cone voltage, 5–20 V. Nitrogen was used as the desolvation and nebulization gas. Deprotonated ions of Irganox1010 and 1098 (IS) were recorded using the selected ion monitoring (SIM) mode.

2.2.2 MALDI-MS

For the MALDI analysis, 2,5-dihydroxybenzoic acid (DHB) as a matrix and NaI as a cationizing agent were used. The sample solution was obtained by adding 50 μL of the IS solution to a 100 μL solution of the extracted additive to give an IS concentration of 0.05 mg/mL. A mixture of sample/matrix (13 mg/mL)/cationizing solution (1 mg/mL)=5/15/1 (v/v/v) was prepared, 0.7 μL was spotted onto the sample target, followed by drying at room temperature.

Mass spectra were acquired using an AXIMA-CFR time-of-flight mass spectrometer (Shimadzu/Kratos,

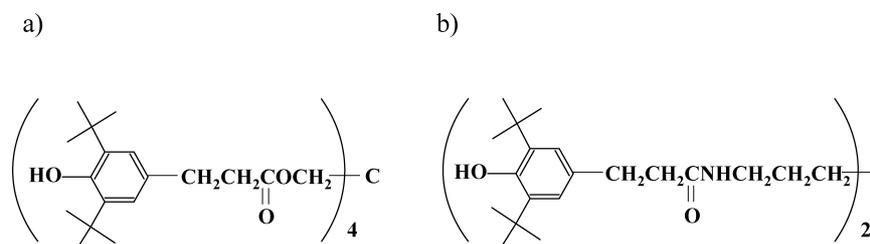


Fig. 1. Structures of a) Irganox1010 (1176.8 Da) and b) Irganox1098 (636.5 Da, internal standard, IS).

Manchester, UK) with a pulsed nitrogen laser (3 ns pulse width, 337 nm) in the linear positive ion mode. Protonated ions of DHB, angiotensin I and insulin were used for mass calibration. All mass spectra were acquired by averaging 100 individual laser shots with the threshold laser power (LP=90) that was required to detect 0.01 mg/mL analyte of Irganox1010 with $S/N > 3$. The threshold laser powers for DIOS- and TiO₂-SALDI-MS were also determined in the same manner and were found to be LP=65 and 75, respectively. The ion intensities were obtained from the peak height by smoothing ten points. The data were obtained under the same experimental conditions from more than six different sample spots. The measurements were carried out over a period of three days.

2.2.3 DIOS-MS

DIOS chips were prepared as described previously.^{16,17} Briefly, n-type silicon (100) wafers with resistivity $\rho = 0.008\text{--}0.02 \Omega\text{cm}$ were obtained from Sumitomo Mitsubishi Silicon Corporation (Tokyo, Japan). They were anodically etched in a 1:1 (v/v) solution of ethanol (EtOH)-HF (46%) under exposure to white light using a 100 W tungsten filament bulb at a distance of 20 cm. After etching with a current of 5 mA/cm² for 1 min, the DIOS chips were exhaustively washed with EtOH and then stored in EtOH. The DIOS sample preparation was carried out under the same conditions as for MALDI and reproducibility was evaluated in the same manner, although the matrix was not used.

2.2.4 TiO₂-SALDI-MS

Amorphous TiO₂ nanoparticles with an average size of < 50 nm (Wako Pure Chemical Industry, Ltd.) were suspended in 2-propanol (special grade, Wako Pure

Chemical Industry, Ltd.) at a concentration of 0.33 wt%. The solution was treated with an ultrasonic processor for 5 min and a 0.6 μL aliquot of the suspension was spotted on the sample target and dried in the atmosphere. The solution of the additives extracted from the PP sample was mixed with those of the internal standard and cationizing agent. The mixed solution was then dropped onto the TiO₂ nanoparticle spot and dried in the atmosphere.

3. Results and Discussion

3.1 Determination of sample recovery by LC-APCI-MS

We were successful in detecting the extracted Irganox1010. Since hydrophobic Irganox1010 precipitates upon being mixed with even a small amount of water, the sample must be dissolved in a solvent with a higher water-component than the mobile phase solvent before injection into the LC-APCI-MS. This inhibits the precipitation of Irganox1010 in the mobile phase.

The Irganox1010 recovery factors for the laboratory-produced and commercial PP were determined using LC-APCI-MS. The measurements were carried out four times on the same day for three consecutive days. Since PP is insoluble, the Irganox1010 that adhered to the surface of the milled PP powder could be obtained by extraction with THF. Since Irganox1010 is not ionized by ESI because of its low polarity, LC-APCI-MS was used to determine the recoveries. The values for the recoveries were 89 ± 4 and $97 \pm 8\%$ for the laboratory-produced and commercial PP, respectively.

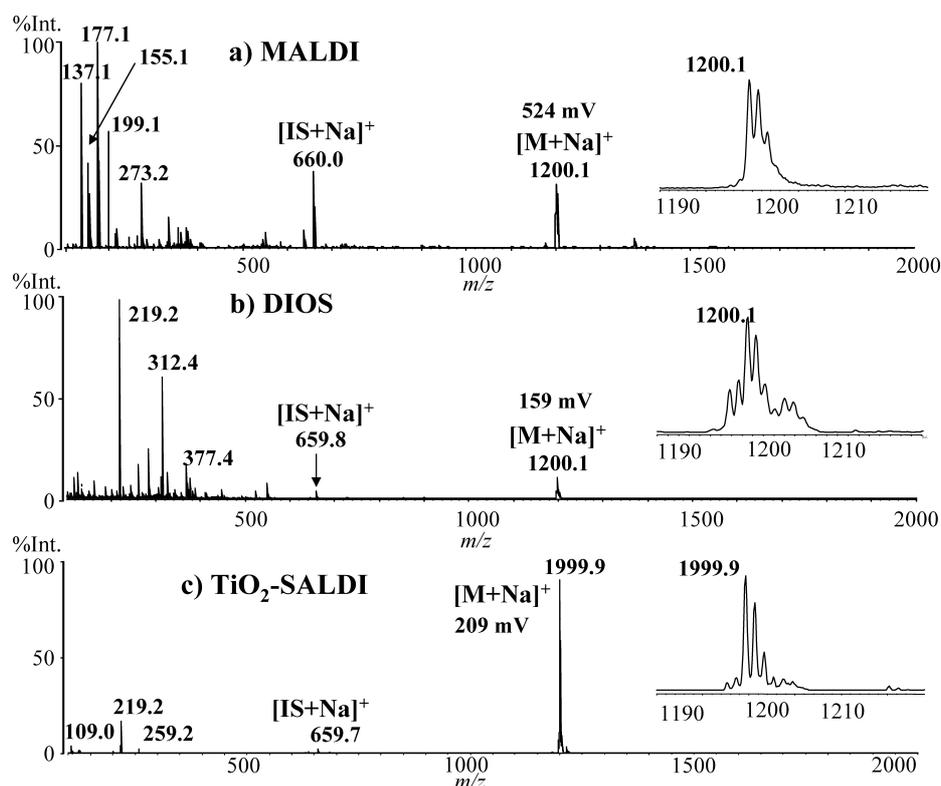


Fig. 2. Positive ion mass spectra of Irganox1010 (M) and Irganox1098 (IS) by MALDI, DIOS-, and TiO₂-SALDI-MS.

3.2 Comparison of MALDI, DIOS, and TiO₂-SALDI mass spectra

A comparison of the MALDI, DIOS, and TiO₂-SALDI mass spectra for the standard solution (2.0 mg/mL) at the threshold laser power is shown in Fig. 2. The ions at m/z 660.0 and 1200.1 are [IS+Na]⁺ and [M+Na]⁺ where IS (internal standard) and M represent molecules of Irganox1098 and Irganox1010, respectively. In the MALDI spectrum, ions at m/z 155.1, 177.1, 199.1, 375.2, and 551.2 are assigned to matrix clusters [DHB+H]⁺,

[DHB+Na]⁺, [DHB-H+2Na]⁺, [2DHB-2H+3Na]⁺, and [3DHB-3H+4Na]⁺, respectively. The other ions are unidentified. In the DIOS and TiO₂-SALDI spectra, ions at m/z 219.2, 259.2, 312.4, and 377.4 are also unidentified. The ion intensities of Irganox1010 for the MALDI, DIOS, and TiO₂-SALDI spectra were 524, 159, and 209 mV as indicated on an instrumental scale (Fig. 2), respectively. The background noise below m/z 500.0 was higher for DIOS than for TiO₂-SALDI. The DIOS performance related to background noise and mass

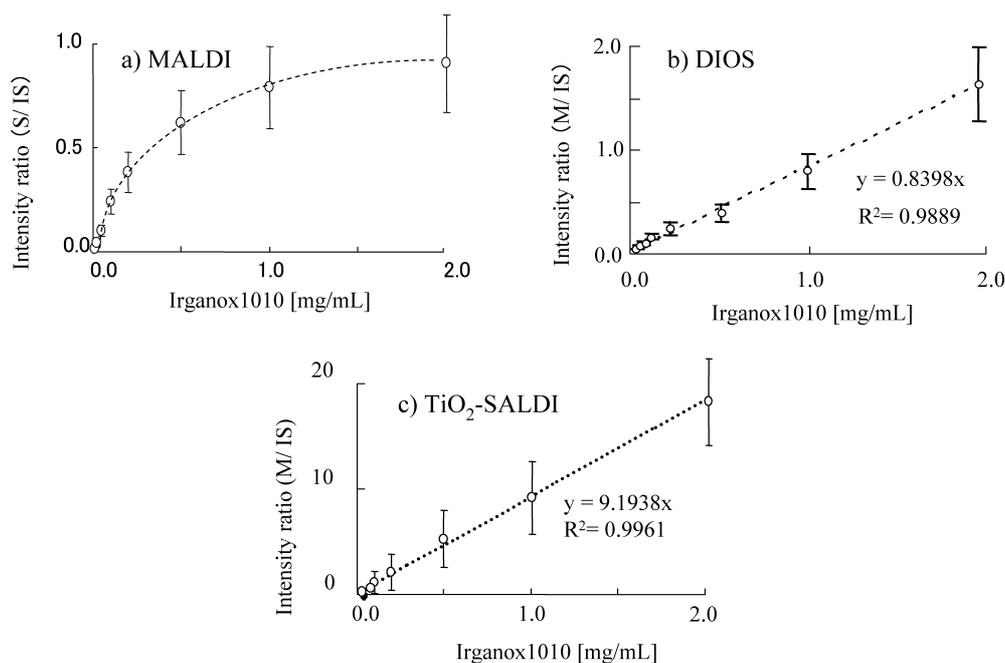
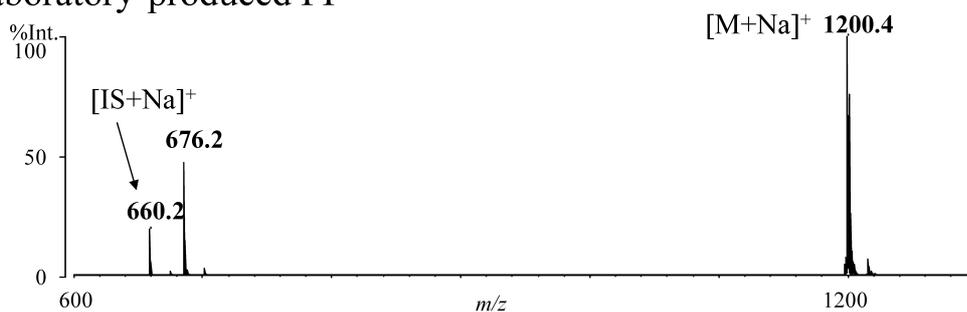


Fig. 3. The relationship between the Irganox1010 content and the intensity ratio of Irganox1010 (M) to Irganox1098 (IS) by MALDI-, DIOS-, and TiO₂-SALDI-MS.

a) laboratory-produced PP



b) commercial PP

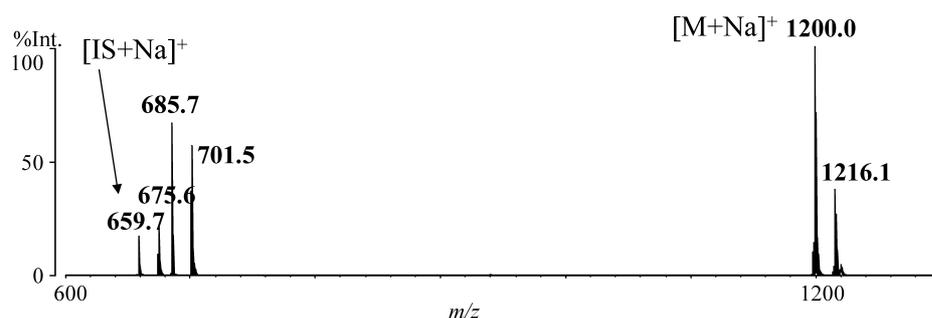


Fig. 4. Positive ion mass spectra of extracted Irganox1010 from the PP plastics by TiO₂-SALDI-MS.

resolution appears to be inferior to TiO₂-SALDI. The DIOS noise is attributable to ambient contamination of the surface, because the noise increases with the time. The DIOS mass resolution for the measurement of a 2.0 mg/mL solution of Irganox1010 was improved when the laser power was decreased below LP=65 and was determined at a concentration of 0.01 mg/mL. The ion intensity of Irganox1098 for TiO₂-SALDI-MS was much less sensitive than that of Irganox1010. In addition, the side peaks (m/z 1998.1, 1999.2, 1204.4 or 1205.4) of the Irganox1010 ion were observed in the DIOS and TiO₂-SALDI spectra. The reason why the side peaks were detected is presently unclear.

3.3 Quantitation by MALDI, DIOS, and TiO₂-SALDI-MS

The relationship between the Irganox1010 content and the ratio of the ion intensities of Irganox1010 relative to IS was obtained from MALDI, DIOS, and TiO₂-SALDI-MS (Fig. 3). For MALDI-MS, a good linear relationship was obtained only at a low analyte concentration. At higher concentrations, however, the relationship leveled off (Fig. 3a). This indicates that the ionization efficiency for MALDI decreases as the analyte concentration relative to the matrix increases, while the IS concentration remains constant. In other words, the ionization efficiency for MALDI is dependent on the ratio of the analyte and matrix concentration. Therefore, when a matrix is used, MALDI-MS cannot be recommended to use in quantitative analysis of these types of materials.

Good linear relationships were obtained from DIOS and TiO₂-SALDI mass spectra with coefficients of determination $R^2=0.9889$ and 0.9961 , respectively (Fig. 3b, c). The relative standard deviations (RSD) of the ion intensities for MALDI, DIOS, and TiO₂-SALDI-MS for 20 repeated runs were approximately 20% for each concentration. The calibration curves and the RSD value are reasonably useful for a quantitative analysis using matrix-free LDI-MS.

TiO₂-SALDI-MS was evaluated for the quantitative analysis of antioxidants in an actual PP material. Fig. 4 shows the TiO₂-SALDI mass spectra for Irganox1010 extracted from the laboratory-produced and commercial PP, and the percent of Irganox1010 were determined to be 0.51 wt% and 0.48 wt%, which are consistent with the actual content of 0.5 wt%. Irganox1076 (0.011 mg/g) and calcium stearate added to the commercial PP was not detected in the spectra. As a result, TiO₂-SALDI-MS was found to be applicable to the quantitative analysis of antioxidants in the concentration range of 0.01–2.00 wt% in PP.

4. Conclusion

A quantitative method for determining antioxidant levels using solvent extraction followed by MALDI, DIOS and TiO₂-SALDI-MS is described. Optimized solvent extraction resulted in a high recovery of Irganox 1010 from the insoluble PP plastics. The Na⁺ adducts of analytes in the DIOS and TiO₂-SALDI spectra yielded good linearity in the analyte concentration range of 0.01–2.00 mg/mL. TiO₂-SALDI-MS was applied to the quantitative analysis of Irganox1010 in an actual PP material and the results were found to be

consistent with those of LC-APCI-MS. TiO₂-SALDI-MS appears to be a viable method, in that it allows a simpler and more rapid measurement of plastic additive levels than DIOS-MS. The major reason for this is that the preparation of DIOS chips is a time-consuming task.

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