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Quantitative MALDI-TOF mass spectrometric analysis of biocidal polyhexamethylene guanidine (PHMG) oligomers in consumer products

Jung-eun Bae[a,1], Myungjun Park[a,1], Jung-min Lee[a], Insu Song[a], Yunha Ju[a], Choong Sik Lee[b], Jung-Hwan Kwon[c], Bongjin Moon[a,*,2], Han Bin Oh[a,*,2]

[a] Department of Chemistry, Sogang University, Seoul, Republic of Korea
[b] Department of Toxicology and Chemistry, Scientific Investigation Laboratory, Criminal Investigation Command, Ministry of National Defense, Seoul, Republic of Korea
[c] Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea

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ABSTRACT

In Korea, biocides, which were used for sterilizing household humidifiers, caused a large number of casualties. The biocides used were either guanidine-containing oligomers, such as polyhexamethylene guanidine (PHMG), or thiazolinone-based small molecules such as methylisothiazolinone/methylchloroisothiazolinone (MIT/CMIT). For the guanidine-containing oligomers, a quantitative analysis method has not yet been developed, as the quantification of the oligomers is very challenging due to their polydispersed molecular weight distribution. In this paper, we describe a new MALDI-TOF mass spectrometry (MS)-based quantitative analysis method for the PHMG oligomers contained in wet wipe products. Quantification was made using internal standard ^{13}C labeled PHMG oligomers that were custom synthesized. To get rid of matrix molecules, for example, polyethylene glycols (PEGs), included in the wet wipes, solid phase extraction (SPE) with an MCX SPE cartridge was carried out. In addition, an ionic liquid matrix (ILM) of 1-methylimadazolium α-cyano-4-hydroxycinnamate (1-MeIm-CHCA) was used to improve the reproducibility of the MALDI-TOF MS measurements. This new quantitative analysis method was validated, and good linearity was obtained in the calibration plot. This approach is expected to be equally applicable to other guanidine-containing oligomers, such as polyhexamethylene biguanide (PHMB) and oligo-(2-(2-ethoxy)-ethoxyethyl)-guanidinium-chloride (PGH) as well as to other consumer products.

1. Introduction

In 2011, the Korean government announced that dozens of people including children and women died due to the long-term inhalation exposure to biocides used for sterilizing household humidifiers [1–12]. Later, hundreds of more victims were identified through governmental search efforts. In this tragedy, a biocide was added to the water tank of the household humidifier to prevent bacterial growth, and it was vaporized in the form of an aqueous droplet. The lethal ingredients used in those biocide accidents were identified as oligomers containing guanidine functional groups, such as polyhexamethylene guanidine (PHMG), polyhexamethylene biguanide (PMHB), and oligo-(2-(2-ethoxy)-ethoxyethyl)-guanidinium-chloride (PGH) or thiazolinone-based small molecules, such as methylisothiazolinone (MIT) and methylchloroisothiazolinone (CMIT); these chemicals were used on purpose as active biocidal ingredients. Long-term inhalation of a mist of the guanidine-containing oligomers sprayed by humidifiers was reported to cause serious lung damage and even acute deaths [1,4,9]. Due to the increasing public concerns about the health and safety issues of guanidine-containing oligomers, governmental regulations for monitoring the use of the guanidine-containing oligomers in consumer products, particularly of the spray-type,

* Corresponding authors.
E-mail addresses: bjmoon@sogang.ac.kr (B. Moon), hanbinoh@sogang.ac.kr (H.B. Oh).
[1] These authors contributed equally to this work.
[2] These authors also contributed equally to this work.

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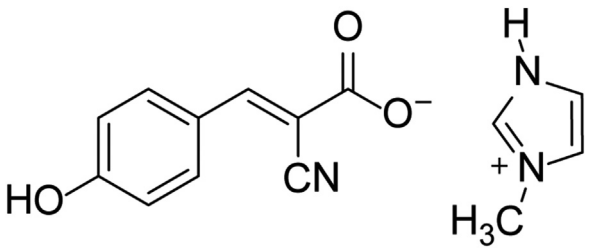


Scheme 1. The structure of 1-methylimadazolium α-cyano-4-hydroxycinnamate (1-MeIm-CHCA).

are being established and enforced in Korea. Therefore, a variety of academic efforts to reveal and eliminate the cause of the biocidal accidents have been made. One of the efforts is to develop an analytical method that allows one to identify and quantify guanidine-containing oligomers in a variety of consumer products [3,8].Initially, a spectrophotometric method was used for the analysis of the guanidine-containing oligomers [13–17]. In this method, EOSIN Y was complexed with the guanidine-containing oligomers in aqueous solution, and then by measuring the visible light absorbance at a specific wavelength, *i.e.*, 589 nm, the guanidine-containing oligomers could be quantified (the EOSIN Y method). However, the EOSIN Y method was found to be vulnerable to erroneous measurements if the sample under examination contains any molecule that absorbs light in the wavelength window used in the EOSIN Y method or any compound that leads to shift in the absorption spectrum of EOSIN Y.

To circumvent such an erroneous measurement, a method that utilizes matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) was suggested for the identification and characterization of the guanidine-containing oligomers [3,8,18–22]. MALDI-TOF MS has been well-known as a powerful tool for the characterization of oligomers and polymers, both synthetic and biological [23–28]. On the other hand, there have been numerous reports indicating that MALDI-TOF MS suffers from poor shot-to-shot, spot-to-spot, and sample-to-sample reproducibility, which makes MALDI-TOF MS an unsuitable tool for quantitative analysis [29]. Thus, a few methods were suggested to mitigate the poor reproducibility of the MALDI-TOF MS method [8,30–40]. For example, an ionic liquid matrix (ILM) has been reported to improve the reproducibility of MALDI-TOF MS measurements, which was clearly manifested in the pioneering works by the Armstrong group and the Gross group [30,31,33,34,38]. An ILM consists of an organic cation and an organic or inorganic anion with a melting point at or below 100 °C. A main cause for the poor reproducibility of the MALDI ionization method is known to the heterogeneous co-crystallization of the analytes and matrix molecules. In contrast, the ILM can form a homogeneous thin film of a mixture of analytes and ILM ingredients, thus greatly improving the reproducibility of MALDI-TOF MS measurements [31,34]. Thus far, the ILM has been successfully implemented for the measurements of peptides, lipids, proteins, and small molecules to date [35,38,41,42].

Recently, our group showed that an ILM, specifically, 1-methylimadazolium α-cyano-4-hydroxycinnamate (1-MeIm-CHCA, Scheme 1), could successfully improve the shot-to-shot, spot-to-spot, and sample-to-sample reproducibility of the MALDI-TOF MS measurements of PHMG oligomers [8]. It was also demonstrated that even the quantitative analysis of the PHMG oligomers could be made using the RRRRK peptide, which contains three guanine groups in the side chains of arginine, as an internal standard. A high linear regression coefficient of $R^2 = 0.975$ could be achieved with the use of the RRRRK internal standard. However, it was soon realized that in the quantitative analysis of PHMG oligomers contained in consumer products, the RRRRK standard could not be used as an internal standard. A sample preparation procedure, which often includes (either liquid-liquid or solid-phase) extraction, would not allow for identical recovery efficiencies of the PHMG oligomers and RRRRK internal standard. Therefore, it is necessary to introduce stable isotope-labeled PHMG oligomers as an internal standard, as this approach has been widely used in the quantitative analysis of omics research [43]. However, for the quantitative analysis of oligomers, special caution should be paid. Unlike small molecules or peptides with monodisperse characteristics, oligomers show a distribution of different numbers of monomer units [26]. Due to this reason, few studies that utilize isotope-labeled oligomers or polymers as an internal standard have been reported in quantitative MS analysis.

In the present study, we report the synthesis of ^{13}C isotope-labeled PHMG oligomers and the use of these oligomers for the quantification of PHMG oligomers in consumer products, particularly, wet wipes (the use of guanidine-containing oligomers/polymers in wet wipe products intended for use on babies is now banned in Korea). In the matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) analysis, an ILM was used for reliable and reproducible quantitative analysis. Furthermore, a SPE procedure utilizing mixed cation exchange (MCX) sorbent material was used to eliminate the matrix existing in wet wipes, *i.e.*, polyethylene glycol (PEG). Lastly, this quantitative method is validated following the conventional quantitative analysis guidelines.

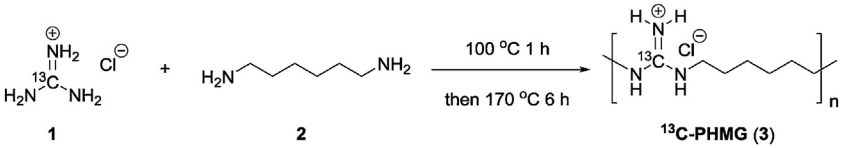
2. Materials and methods

2.1. Materials

PHMG chloride (CAS no. 57028-96-3) and ^{13}C labeled PHMG chloride (now commercially available from Futurechem (Seoul, Korea)) were synthesized and used without any further purification. Hexamethylene diamine and guanidine-^{13}C hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA) and American Custom Chemicals Corp. (San Diego, CA, USA), respectively. An MCX SPE cartridge, which was used for the purification and enrichment of PHMG oligomers, was available from Waters (1 cc, Milford, MA, USA). The other SPE cartridges, such as C_{18}, TiO_2, and porous graphite carbon (PGC), were purchased from Waters. Elution solvents, methanol (Fisher Scientific, Waltham, MA, USA) and water (Burdick & Jackson, Morristown, NJ, USA), were of HPLC grade. HCl was purchased from Daejung (36.0–38.0% w/w, Goryeong, Gyeongsanbukdo, Korea). α-Cyano-4-hydroxycinnamic acid (CAS no. 28166-41-8, Sigma, St. Louis, MO, USA) and 1-methyl-imidazole (CAS no. 616-47-7, Sigma, St. Louis, MO, USA) were used for making an ILM. Wet wipes were purchased from anonymous (domestic and international) shops.

2.2. Synthesis of ^{13}C labeled PHMG oligomers

In our previous studies, we used an arginine-containing peptide, RRRRK, as an internal standard for the quantitative analysis of PHMG oligomers [8]. However, for real sample quantitative analyses in which a SPE sample preparation procedure is included, isotope-labeled PHMG oligomers should be used as an internal standard. Otherwise, different recovery efficiencies between the analyte and internal standard would hinder quantitative analysis. As an internal standard, ^{13}C-labeled PMHG oligomers were synthesized. The synthetic procedure is as follows.



1,6-Hexamethylenediamine (**2**, 61 mg, 0.52 mmol) and ^{13}C-guanidine hydrochloride (**1**, 51 mg, 0.52 mmol) were mixed in a 5 mL conical vial equipped with a mechanical stirrer and vacuum system. The mixture was heated to 100 °C for 1 h and then at 170 °C for 5 h under atmospheric conditions. It was further heated to 170 °C for 40 min under vacuum conditions to remove the release, byproduct ammonia. The slightly yellow viscous liquid became solidified upon cooling to give ^{13}C-labeled PHMG (**3**) as a yellow amorphous solid (80 mg, 79%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34-6.79 (br m, 7 H), 3.29-3.10 (br m, 4 H), 1.50-1.35 (br m, 4 H), 1.35-1.19 (br m, 4 H); ^{13}C NMR (100 MHz, DMSO-d_6) 157.04, 155.83, 40.45. Syntheses were repeatedly made several times until the distribution of the synthesized oligomers became more or less similar to that of the analyte sample under examination.

2.3. Sample preparation (purification and enrichment of PHMG)

Wet wipes were squeezed out to obtain an extracted solution. Initially, a wet wipe was folded into a small piece and placed inside an empty 12 mL plastic syringe. The folded wet wipe was squeezed by pushing a plunger hard against the folded wet wipe. The volume of the extracted solution was measured. The ^{13}C labeled PHMG oligomer internal standard stock solution was prepared by dissolving a 1 mg of the ^{13}C labeled PHMG oligomers in water to make a total of 1 mL of aqueous solution. The analyte sample solution was made by mixing 100 μL of the ^{13}C labeled PHMG oligomer internal standard solution, 900 μL of the extracted solution, 3 μL of 0.1% HCl/methanol, and a 3 mL of methanol. The analyte sample solution was purified and enriched using a MCX SPE cartridge (or other SPE cartridges). Initially, the MCX cartridge was activated by eluting a 1 mL methanol three times, and then pre-wetted by elution with 1 mL of a 0.1% HCl/methanol solution (pH = 2–3) through the cartridge three times. The sample loading was made by eluting the 1 mL of the prepared analyte solution through the cartridge. Unbound material or matrix was washed away from the MCX beads by elution with 1 mL of 0.1% HCl/methanol solution three times. The PHMG bound onto the MCX beads was finally eluted by running 1 mL of a 2 M HCl/methanol (pH = 1∼2) solution through the cartridge. The eluted solution was dried in an Eppendorf tube using a SpeedVac (the inner part should be Teflon-coated so the rotor is not damaged). The water of HPLC grade was added into the tube to make a 1 mL final solution.

2.4. MALDI-TOF mass spectrometry

The analysis and characterization of the PHMG contents of wet wipes were performed using a MALDI-TOF MS (Bruker Daltonics, Autoflex Speed series, Bremen, Germany) and an ASTA (Tinkerbell LT 2.0, Suwon, Korea)), which are both equipped with a 355 nm (third harmonic of a Nd:YAG laser) light source. Before PMHG analyses, the *m/z* values of the MALDI-TOF mass spectrometers were carefully calibrated in order to minimize the mass measurement error.

To facilitate the quantitative analysis of PHMG, reproducible acquisitions of MALDI-TOF MS spectra were needed. The spot-to-spot, shot-to-shot, and sample-to-sample reproducibility could be improved by the use of 1-MeIm-CHCA ILM. The 1-MeIm-CHCA ILM preparation protocol was slightly modified from the previous one [8]. The 1-MeIm-CHCA ILM was prepared by dissolving 37.8 mg (0.2 mmol) of α-cyano-4-hydroxycinnamic acid and mixing 15.93 μL (0.2 mmol) of 1-methyl imidazole in methanol to make a 1 mL solution, which is then vortexed and sonicated for 10 min. The resulting solution is dried under nitrogen purging. The dried yellowish product was dissolved in 850 μL of methanol, and this solution was used as an ILM. A 5 μL aliquot of the extracted analyte sample solution, which included PHMG and ^{13}C labeled PHMG oligomer internal standard, was mixed with 5 μL of the ILM solution, which was then briefly vortexed. A 1 μL aliquot of the resulting solution was pipetted and deposited onto the MALDI plate.

The MALDI-TOF MS spectra were obtained in the positive ion mode at the repetition rate of 500 Hz by averaging 5000 spectra. Each of the five different points on the same sample spot were irradiated by the laser 1000 times. The mass spectra were acquired in the reflectron mode. The Nd:YAG laser power was carefully adjusted to minimize the photo-decomposition of the PHMG oligomers. The following parameters for the Bruker MALDI-TOF MS were used in data acquisition: ion-source 1 voltage, + 19.05 kV; ion-source 2 voltage, + 16.70 kV; laser power percentage, 48%; pulsed ion extraction, 140 ns; lens voltage, + 8.24 kV; reflectror voltage, + 20.99 kV; and reflector2 voltage, + 9.73 kV.

3. Results and discussion

3.1. MALDI-TOF MS analysis of untreated squeezed wet wipe extracts

In an effort to detect and identify PHMG oligomers from consumer products, such as wet wipes, MALDI-TOF MS spectra were acquired without any sample preparation for the solutions obtained by squeezing a wet wipe. Fig. 1 shows a prototypical MALDI-TOF mass spectrum obtained from a wet wipe that contains PHMG oligomers as a sanitizing agent. In this spectrum, a large number of polyethylene glycol (PEG) polymer peaks appeared in the *m/z* region of 400 to 1500, and no PHMG polymer peak appeared although a series of PHMG oligomer peaks were found to be present when analyzed after sample preparation. The peaks of PEG polymer, which is often used in various consumer products as a moisturizing agent, peaks interfered with the detection of other ingredients, particularly PHMG oligomer peaks, in the wet wipes. Due to the matrix effect observed when analyzing a real sample using MALDI-

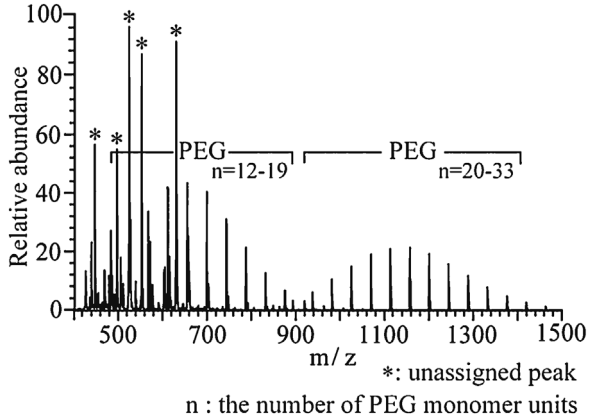


Fig. 1. A MALDI-TOF mass spectrum acquired for a solution squeezed from a wet wipe. The MALDI MS analysis was made without any sample preparation.

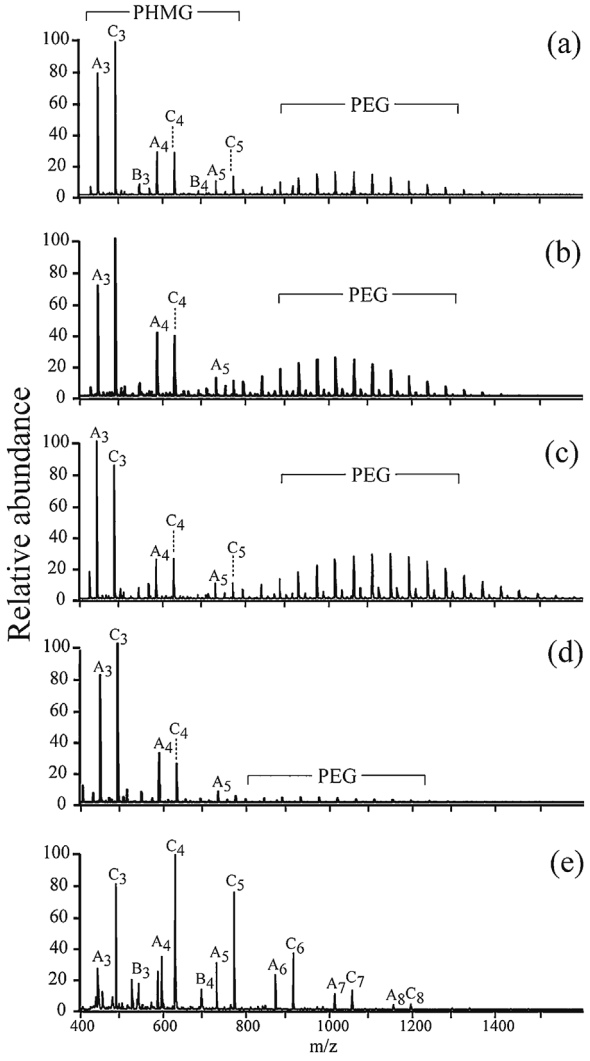


Fig. 2. MALDI-TOF MS spectra of the PHMG and PEG mixture obtained (a) without any purification and with purification using various SPE cartridge of (b) C_{18}, (c) PGC, (d) TiO2, and (e) MCX. Note that the labels, such as $A_{3\text{-}8}$, $B_{3\text{ or }4}$, and $C_{3\text{-}8}$, above the peaks refer to the PHMG oligomeric isomers (*vide infra*).

TOF MS, it is necessary to introduce a sample preparation procedure for removing the matrix, *e.g.,* PEGs.

3.2. Solid phase extraction (SPE)

Hence, an analytical procedure for the purification and simultaneous enrichment of the analytes is essential when analyzing a real sample. Among the various purification methods, we chose the solid phase extraction (SPE) method due to its simplicity and effectiveness.

For the development of an effective sample preparation method, a model solution was prepared by mixing PHMG and PEG oligomers. Sample preparation was performed using four SPE cartridges, *i.e.*, C_{18}, TiO_2, porous graphite carbon (PGC), and mixed cation exchange (MCX) cartridges. Fig. 2 shows MALDI-TOF spectra acquired (a) without any sample preparation (reference spectrum) and after (b) C_{18}, (c) TiO_2, (d) PGC, and (e) MCX cartridge extraction. As shown in Fig. 2, C_{18}, TiO_2, and PGC-based SPE exhibited only partial removal of PEG oligomers, although PGC cartridge purification showed relatively better selective purification of PHMG oligomers. In contrast, the extraction with the MCX cartridge, which consists of strong cation exchange beads and reversed phase beads, showed complete removal of PEG oligomers and only PHMG oligomer peaks appeared in the MALDI-TOF MS spectrum (see Fig. 2e). The PHMG oligomers that include multiple basic guanidine groups are likely to interact strongly with cation exchange beads, and thus, they are selectively enriched by the MCX SPE cartridge.

3.3. Qualitative MALDI-TOF spectra of PHMG, PHMB, and PGH

A number of wet wipe solutions were treated using the MCX-cartridge, and then the extracted solutions were subjected to MALDI-TOF MS analysis. Among these MALDI-TOF MS spectra, a few of them showed signature peaks of PHMG oligomers. Supplementary Fig. S1 shows a representative spectrum of a PHMG-containing product. In Supplementary Fig. S1, a variety of oligomer isomer peaks appeared, as shown in earlier studies. In general, PHMG oligomers are known to have several isomers depending on their end-groups; for example, A-, B-, C-, D-, and E-type isomers (see Scheme 2) [3,8,18–22]. These isomers were produced in the condensation reactions of 1,6-hexamethylenediamine and ^{13}C-guanidine hydrochloride. In Supplementary Fig. S1, A-, B-, C-, D and E-type PHMG isomers appear, and each isomer series exhibits a 141 Th difference between the adjacent oligomers. For example, the peaks appearing at the *m/z* values of 441.1, 582.5, 723.7, and 864.9 are A_3, A_4, A_5, and A_6, respectively. In general, only relatively small oligomers, instead of larger polymers, were observed in wet wipe solutions. The number average and weight average molecular masses, M_n and M_w, could be determined; for example, for the Supplementary Fig. S1 MALDI-TOF MS spectrum, M_n and M_w are determined to be 571.7 and 608.4, respectively, using the following Eqs. (1) and (2) [26].

$$M_n = \frac{\sum_i N_i M_i}{\sum_i N_i} \quad (1)$$

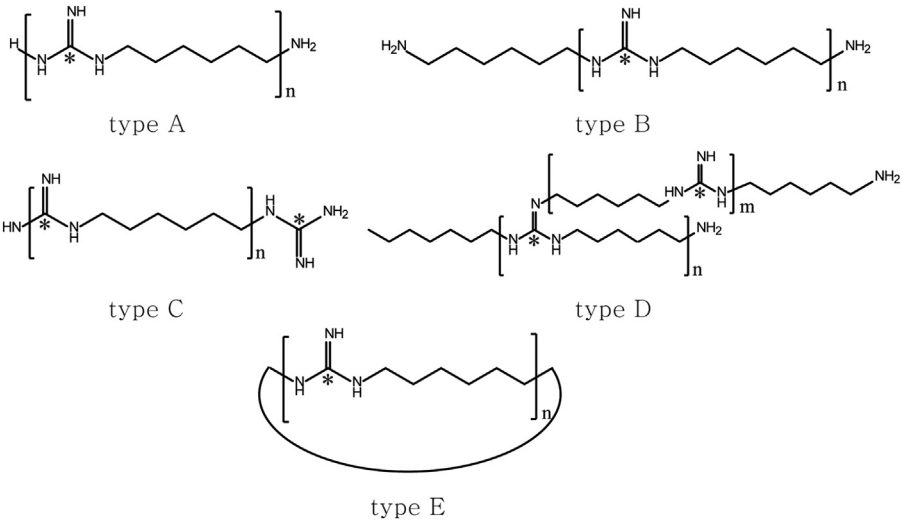
$$M_\omega = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i} \quad (2)$$

It is also notable that the isomeric oligomer peaks were observed only in a protonated form, *i.e.*, $[M+H]^+$, because metal cations were removed through the MCX SPE process.

3.4. Quantitative MALDI-TOF analysis

3.4.1. Ionic liquid matrix

Generally, MALDI-TOF MS is known to suffer from high shot-to-shot, spot-to-spot, and sample-to-sample variations, which hinders the use of MALDI-TOF MS as a quantitative analysis tool [31]. One of the main reasons for the poor reproducibility in MALDI-TOF MS measurements is thought to be heterogeneous matrix-analyte co-crystallization. To circumvent this, ILMs have been introduced for the quantitative analysis of small molecules, carbohydrates, lipids, and even synthetic oligomers [8,30–40]. ILMs were found to form a thin, homogeneous matrix-analyte film, thus providing low shot-to-shot, spot-to-spot, and sample-to-sample variations. In the recent studies, our group has shown that the introduction of an ionic liquid matrix, particularly, 1-methylimadazolium α-cyano-4-hydroxycinnamate (1-MeIm-CHCA, Scheme 1), could lower variations in the quantitative analysis of PHMG oligomers [8]. Indeed, it was found that the 1-MeIm-CHCA ILM produced very reproducible MALDI-TOF MS spectra for PHMG oligomeric contents in wet wipes. For the PHMG spectra, the relative standard devia-



Scheme 2. The structures of PHMG oligomer isomers. * indicates that the carbon is ^{13}C labeled in the internal standard.

tions (RSDs) of the shot-to-shot and sample-to-sample variations were measured to be less than 3%.

3.4.2. Isotope labeled internal standards

In the present study, PHMG oligomers containing a ^{13}C labeled monomer unit were synthesized as an internal standard (an MALDI-TOF MS spectrum for the ^{13}C labeled PHMG is shown in the Supplementary Fig. S2). Scheme 2 shows the structures of the ^{13}C labeled PHMG oligomers. In synthesizing the ^{13}C labelled PHMG oligomers, special care was taken to ensure that the oligomer distribution is similar to that of the analyte sample under examination as much as possible. If the distribution of the internal standard PHMG oligomers was different from that of the analyte PHMG oligomers, this would lead to an erroneous quantitative analysis result. Thus, in the quantitative analysis of oligomers, it is inevitable to have a certain error arising from the oligomer distribution discrepancy between the PHMG oligomers under examination and their counterpart ^{13}C labeled PHMG oligomer internal standard. For the ^{13}C labeled PHMG oligomers, it was found that the mass differences between the unlabeled and ^{13}C labeled PHMG oligomers became larger as the oligomers become longer. For example, the mass difference between A_2 and A_2^* was 2 Th, and it increased to 3 Th between A_3 and A_3^*.

3.4.3. Calibration plot

A calibration plot was constructed using the ^{13}C labeled PHMG oligomer internal standard. The concentration of the internal standard was set to 100 ppm. To ensure the reproducibility of the MALDI-TOF mass spectra, a 1-MeIm-CHCA ILM was used. For the construction of the calibration plot, working standard (unlabeled) PHMG solutions in the concentration range of 1 to 1000 ppm were prepared from a 1 mg/mL PHMG stock solution, each of which was mixed with the 100 ppm of ^{13}C labeled internal standard. The mixed solutions were then subjected to the MCX SPE sample preparation procedure. Fig. 3 shows the MALDI-TOF mass spectra acquired at the working standard (unlabeled) PHMG concentrations of 1, 5, 10, 50, 100, 300, 500, and 1000 ppm. It is easily noticeable that the relative abundances of the (unlabeled) PHMG oligomer peaks, *e.g.*, A_3, C_3, and A_4, decrease from the 1000 ppm MALDI spectrum to the 1 ppm spectrum. On the other hand, the relative abundances of the ^{13}C labeled internal standard peaks increase from the uppermost panel to the lowest panel. It is also notable that the *m/z* gaps between the unlabeled PHMG oligomer peaks and the counterpart ^{13}C labelled internal standard peaks become larger as the number of units of the oligomer increases because the unit mass of the ^{13}C labeled PHMG oligomer, *i.e.*, 142 Da, is 1 Da larger than that of the unlabeled PMHG oligomer (141 Da). In the *m/z* window between 400 and 1000, there was no overlap between the unlabeled and the ^{13}C labeled PHMG oligomer peaks. In terms of the limit of detection (LOD), it was determined to be a little lower than 1 ppm.

Fig. 4 shows a representative calibration plot that exhibits a linear relationship between the logarithmic values of the concentration of the (unlabeled) PHMG standard (x axis) and the logarithmic values of the relative quantities of the PHMG oligomers acquired through the MALDI-TOF MS spectral analyses (y axis); each y value was obtained by averaging five MALDI-TOF MS spectra. As shown in Fig. 4, the calibration plot shows good linearity ($R^2 = 0.9989$) over a wide concentration range (1–1000 ppm).

Here, careful caution should be exerted in determining the amounts of PHMG oligomers in real samples. The relative quantities of the PHMG oligomers can be easily over- or under-estimated if the overall oligomer distribution of the standard PHMG oligomers shows a significant discrepancy from that of the ^{13}C labeled PHMG oligomers. The distribution of the labeled PHMG oligomers should bear a similarity to that of the real sample of interest. Thus, in the quantitative analysis of PHMG oligomers, it is important to have a variety of synthetic ^{13}C labeled PHMG oligomer standards with different oligomer distributions. Then, a labeled PMHG oligomer standard with a similar oligomer distribution can be readily chosen for the reliable quantification of PHMG in consumer products. In the present study, the oligomer distributions of the several synthesized internal standards were visually compared with that of the analyte sample. In particular, we made sure that the oligomer isomer, *e.g.*, A_3, of the highest abundance in the real sample was also the highest abundance peak in the internal standard. As well known, it was almost impossible to make an internal standard that showed an oligomer distribution identical to that of the real sample under examination. However, the synthetic route to make PHMG oligomers is quite limited due to the cost of available starting materials and the synthesis yield, the discrepancy in the oligomer distribution is expected not to be too huge to be discarded as long as the oligomer isomer of the highest abundance is identical in two oligomers under comparison.

3.4.4. Precision, accuracy, and extraction efficiency

Intra- and inter-day assays were performed by analyzing the PHMG-containing solution at various concentrations in five separate analytical runs on three separate days, and the results are

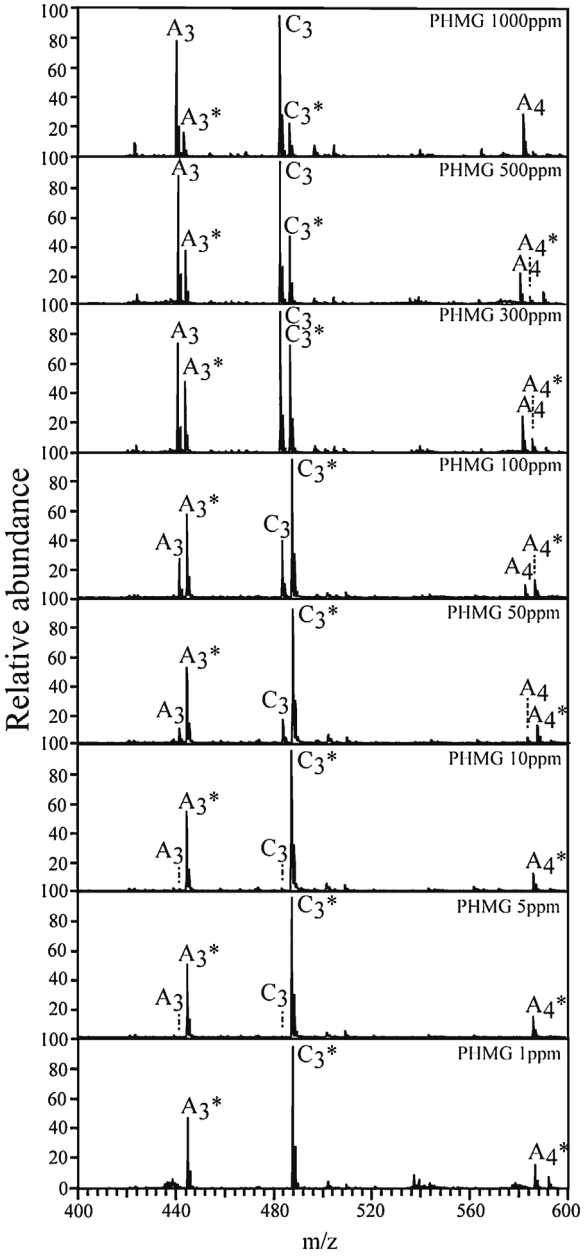


Fig. 3. MALDI-TOF spectra of various concentrations of PHMG with 100 ppm ^{13}C-labeled PHMG internal standard.

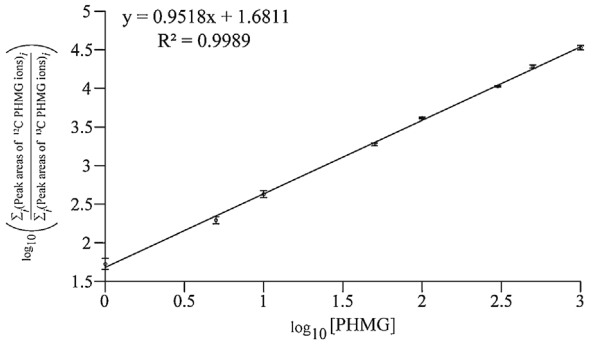


Fig. 4. A linear calibration plot for the quantification of PHMG using the ^{13}C labeled internal standard method.

shown in Table 1. The precisions were determined by calculating the coefficient of variation (CV, %) of replicates within runs on one day (intra-day) and between runs on three different days (inter-day). The CVs were well below 15% for a wide range of PHMG concentrations (1 to 1000 ppm), except for the 1 μg/mL concentration, in both intra- and inter-day assays. The accuracies could be obtained by calculating the percent deviation from the nominal concentration and were represented with relative errors (REs). The REs were determined to be mostly within 10% of the nominal values.

The recovery efficiency was determined by comparing the mean response of samples spiked with analyte and internal standard before extraction and that spiked with analyte and internal standard post-extraction. The recovery was determined to be 73%, indicating that some analytes were lost during the sample preparation. The MCX SPE column used in this analysis inevitably gave rise to some loss due to its dual loading materials, *i.e.*, C_{18} and strong cation exchange resins.

It is also notable that a good linearity was obtained in the linear least squares regression calibration plot of the response *versus* the PHMG concentration (see Table 1). In the inter-day assay, the mean R^2 was determined to be 0.9983.

3.5. Real sample analysis

The method described above was applied to the analysis of wet wipe products obtained in the foreign market. Supplementary Fig. S3 shows a representative mass spectrum in which a 10-fold diluted squeezed wet wipe solution was analyzed with a 100 ppm ^{13}C labeled PHMG oligomer internal standard. In Supplementary Fig. S3, a series of PHMG oligomer doublet peaks were observed. For example, the expanded insert clearly shows a 3 Th mass difference between the unlabeled and labeled A_3 peaks. It is also notable that the differences in the relative heights of the unlabeled and labeled oligomer peaks are more or less irregular, reflecting the differences in the abundance distributions of the PHMG oligomers existing in the wet wipes and the internal standard ^{13}C labeled PHMG. This difference eventually causes some error in the quantitative analysis, but unavoidable. Therefore, the selection of an internal standard ^{13}C labeled PHMG with an abundance distribution similar to that of the sample under examination is very important for minimizing measurement error.

Three repeated measurements were performed for each wet wipe and three different wet wipes from the same product were quantitatively analyzed. Three different wipe products were collected and analyzed to give a result. The final concentrations of PHMG oligomers from the wet wipes under examination were almost identical within an experimental error and the average concentration was determined to be 87 ppm using the calibration plot equation. Since the wet wipe solution under quantitative analysis was 10-fold diluted, the final result for the PHMG quantitative analysis is 870 ppm.

4. Conclusions

A new quantitative analysis method for the PHMG oligomers contained in wet wipe products was developed and validated. For the quantitative analysis, internal standard ^{13}C labeled PHMG oligomers were custom synthesized and added to the sample of interest at a 100 ppm concentration. Due to the matrices, *e.g.*, PEGs, in the wet wipes, MCX SPE sample preparation had to be applied to the squeezed wet wipe solutions with the added internal standards. With this method, calibration plots were obtained with a high linearity with $R^2 > 0.99$. Although it is not described in this paper, this method can be successfully applied to the quantitative analysis of

Table 1
Intra- and inter-day assay results. SD: standard deviation; CV: coefficient of variation; RE: relative error.

Intra- and inter-day assay precision and accuracy								
	1 μg/mL	5 μg/mL	10 μg/mL	50 μg/mL	100 μg/mL	300 μg/mL	500 μg/mL	1000 μg/mL
Day 1								
Mean	1.04	4.66	10.62	47.50	106.00	286.00	519.00	998.00
SD	0.17	0.54	1.21	2.15	2.40	6.51	25.70	69.45
CV(%)	16.09	11.66	11.40	4.52	2.27	2.27	4.95	6.96
RE(%)	3.04	7.31	5.68	5.15	5.93	4.53	3.76	0.46
N	5	5	5	5	5	5	5	5
Day 2								
Mean	0.91	4.99	10.43	56.17	107.17	307.17	467.17	921.51
SD	0.01	0.11	0.19	0.99	1.74	16.06	9.71	40.50
CV(%)	1.28	2.29	1.86	1.77	1.68	5.23	2.08	4.40
RE(%)	9.00	0.23	4.33	12.33	7.17	2.39	6.57	7.85
N	5	5	5	5	5	5	5	5
Day 3								
Mean	1.03	4.95	9.07	52.52	108.00	297.00	470.60	1020.40
SD	0.02	0.08	0.19	0.60	1.67	6.26	8.14	32.56
CV(%)	2.08	1.62	2.09	1.15	1.55	2.11	1.73	3.19
RE(%)	3.40	0.92	9.30	5.04	8.00	5.88	5.88	2.04
N	5	5	5	5	5	5	5	5

PHMGs in other consumer products such as contact lens cleaning solutions and cosmetic makeup removers.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijms.2018.10.001.

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