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Determination of tris(2-ethylhexyl)trimellitate released from PVC tube by LC–MS/MS

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ABSTRACT

Tris(2-ethylhexyl)trimellitate (TOTM) is used as an alternative plasticizer of polyvinyl chloride (PVC) medical devices. A method for the determination of TOTM released from PVC medical devices into intravenous preparations was developed, which uses liquid chromatography-tandem mass spectrometry (LC–MS/MS). A PVC tube was filled with an intravenous preparation and extraction was carried out by shaking for 1 h at room temperature. LC was performed with an Inertsil®-C8 (50 mm × 2.1 mm, 5 μm) column. The isocratic mobile phase was acetonitrile:purified water (90:10, v/v) at a flow rate of 0.2 ml/min. MS detection was accomplished with an MS/MS detector equipped with a turbo ionspray ionization source in the positive ion mode. The limit of detection and the limit of quantification for the standard solution of TOTM was 0.5 ng/ml (S/N = 3) and 1.0 ng/ml (S/N ≥ 10), respectively. When Prograf® (tacrolimus) was used, the average recovery of TOTM was 101.1% (R.S.D. = 4.72%; n = 3). When our method was applied to the determination of TOTM released from unsterilized and gamma-ray-sterilized PVC tubes, we found that a higher concentration of TOTM was released from the unsterilized PVC tube than from the gamma-ray-sterilized one.

1. Introduction

Polyvinyl chloride (PVC) is used in a wide range of medical devices because of its versatility. Di(2-ethylhexyl)phthalate (DEHP) is the most commonly used plasticizer for PVC medical devices. However, such devices easily release DEHP when used with lipophilic substances (Tickner et al., 2001; Hanawa et al., 2005; Kambia et al., 2001a,b; Nakamura et al., 2004; Muramatsu et al., 2000; Ito et al., 2005a). It has been reported that some phthalate esters including DEHP exhibit reproductive, developmental, and testicular toxicities (Poon et al., 1997; Parks et al., 2000; Li et al., 2000; Kang et al., 2006). While the reproductive risk seems minimal with general levels of intake, specific uses, such as long-term medical treatment, have presented potential problems (McKee et al., 2004). The US FDA has reported that some individuals undergoing medical treatment may be exposed to high doses of DEHP (FDA, 2001). The Ministry of Health, Labour and Welfare of Japan has recommended that alternatives be used to reduce DEHP exposure, especially for high-risk patients (The Ministry of Health, Labour and Welfare of Japan, 2002). Polybutadiene, polyethylene, and polyurethane are known alternatives of PVCs, and adipate esters, citrate esters, and trimellitate esters are known alternative plasticizers.

Tris(2-ethylhexyl)trimellitate (TOTM), a trimellitate ester, is commonly used as an alternative plasticizer for medical devices in Japan (Japan Medical Devices Manufacturers Association, 2006). In 2006, the Japan Medical Devices Manufacturers Association reported a list of PVC-free and DEHP-free medical devices available in Japan (Japan Medical Devices Manufacturers Association, 2006). In that list, notable was that the number of medical devices using PVC/TOTM as an alternative to PVC/DEHP was increased compared to previous years. It has been reported that TOTM shows weaker hepatotoxicity than DEHP (Rathinam et al., 1990; Kambia et al., 2004) and exerts estrogenic activity on estrogen receptors alpha and beta (Ter Veld et al., 2006). TOTM migration from hemodialysis tube into blood has been reported (Christensson et al., 1991; Kambia et al., 2001a,b; Flaminio et al., 1988). TOTM analyses were performed with liquid chromatography–ultraviolet

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Table 1
Intravenous preparations used in this study

Product name of intravenous preparations	Principal drug	Concentration for medical use (mg/ml)	Additive
5% glucose	Glucose	–	None
Prograf®	Tacrolimus hydrate	0.01	Polyoxyethylated hydrogenated caster oil, dehydrated ethanol
Florid®-F	Miconazole	1	Polyoxyethylated hydrogenated castor oil
Sandimmun®	Cyclosporine	0.5	Polyoxyethylated castor oil, ethanol

detection (LC–UV) (Christensson et al., 1991; Kambia et al., 2001a,b; Senshu et al., 2004) or gas chromatography–mass spectrometry (GC–MS) (Flaminio et al., 1988). In the case of GC–MS, the derivatization of TOTM was required prior to analysis. On the other hand, TOTM might be difficult to detect with LC–UV since it is released at very low levels into 5% glucose solution. The aim of this study is to develop the analytical method by liquid chromatography-tandem mass spectrometry (LC–MS/MS) to determine the amount of TOTM released from PVC medical devices. In addition, we previously found that the concentration of DEHP released from gamma-ray-sterilized PVC sheet was low compared with that from the unsterilized one (Ito et al., 2005a,b). Therefore, a PVC tube was irradiated with gamma rays to simulate conventional sterilization process and the amounts of TOTM released from gamma-ray-sterilized and unsterilized PVC tubes were compared.

2. Materials and methods

2.1. Chemicals and materials

TOTM (99%) was purchased from Sigma–Aldrich, Inc., and environmental analytical grade DEHP-d_4 as internal standard was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Acetonitrile for HPLC and analytical grade acetone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The water purification system was a Milli-Q gradient A 10 equipped with an EDS polisher (Millipore, Bedford, MA, USA). All glassware used in this study were previously rinsed with acetone and heated at 240 °C for more than 2 h.

PVC tube (inside diameter: 2.1 mm, thickness: 0.65 mm) as test material was kindly supplied by the manufacturer. As sterilization process, the PVC tube was irradiated with gamma rays (^{60}Co; 25 kGy), courtesy of the Japan Radioisotope Association. The sterilization conditions were set with reference to commercially available medical devices. The control sample was not exposed to gamma rays.

The intravenous preparations used for the TOTM migration test were 5% glucose solution for injection (Otsuka Pharmaceuticals Co., Tokyo, Japan), Sandimmun® (Novartis Pharma K.K., Tokyo, Japan), Prograf® (Astellas Pharma, Inc., Tokyo, Japan), and Florid®-F (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) (Table 1).

2.2. Preparation of standard solution and samples

A stock solution of TOTM was prepared in acetonitrile at 1000 μg/ml in glass tubes. Working solutions for sample spiking and the calibration curve of TOTM were diluted with acetonitrile to concentrations ranging from 1 to 100 ng/ml. DEHP-d_4 was used as the internal standard. The structures of TOTM and DEHP-d_4 are shown in Fig. 1. The intravenous preparations used in this study were diluted with 5% glucose solution for injection to the desired concentration based on the package inserts.

2.3. Instrumentation and LC–MS/MS conditions

A 1100 series liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000™ (Applied Biosystems Japan, Tokyo, Japan) (triple quadrupole) equipped with a Turbo Ionspray™ ionization source. Mass spectrometric data were processed with Analyst® 1.3.2 software. An Inertsil®-C8 column (50 mm × 2.1 mm, 5 μm particle size) from GL Sciences was used for separation.

After 5 μl of the sample was injected with an auto sampler, it was loaded onto the analytical column by introducing the mobile phase at the flow rate of 0.2 ml/min. Separation was carried out with the isocratic mobile phase of acetonitrile/purified water = 90/10 (v/v). Run time was 15 min and column oven temperature was maintained at 40 °C.

TOTM was detected with the turbo ionspray™ ionization source in the positive ion mode and was monitored in the multiple reaction monitoring (MRM) mode. The optimum parameters for MS/MS were as follows: curtain gas (N_2) pressure, 0.138 MPa (20 psi); nebulizer gas (N_2) pressure, 0.207 MPa (30 psi); turbo ionspray™ gas (N_2) pressure, 0.414 MPa (60 psi). The ion source temperature was maintained at 400 °C and the turbo ionspray™ voltage was 5500 V. TOTM and DEHP-d_4 were detected in the positive ion mode. The mass spectra of TOTM and DEHP-d_4 obtained with the LC–MS/MS system are shown in Fig. 2. The combinations of precursor and product ions were as follows: TOTM (precursor ion → product ion, m/z 547 → 305), and DEHP-d_4 (m/z 395 → 153). The collision gas (N_2) pressure was set at 5 units.

2.4. Method validation

After the conditions for LC–MS/MS were optimized, the method was validated using TOTM standard solution. The linearity of response of the proposed system was examined with a calibration curve obtained from the standard solution containing a certain amount of the internal standard, DEHP-d_4. Linear regression was

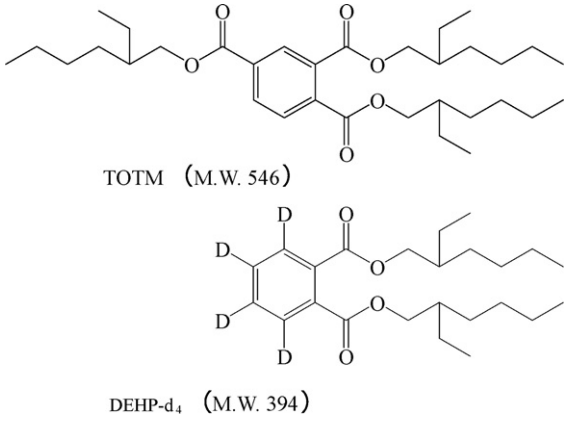

Fig. 1. Chemical structures of TOTM and DEHP-d_4.

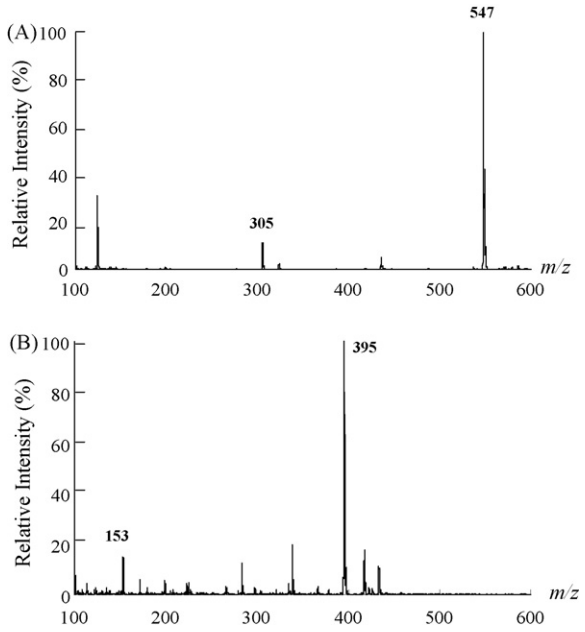

Fig. 2. Mass spectra of (A) TOTM and (B) DEHP-d_4. These mass spectra were obtained by injecting 1 μg/ml solution.

performed using the ratio of TOTM peak area/DEHP-d_4 (internal standard) peak area plotted against the concentration. The method was applied to 5% glucose solution spiked with 100, 500, and 1000 ng/ml TOTM and certain amounts of DEHP-d_4 for the recovery test. In addition, Prograf®, Sandimmun®, and Florid®-F spiked with 1, 5, and 10 μg/ml TOTM and certain amounts of DEHP-d_4 were examined. Each intravenous preparation was diluted with 5% glucose solution according to the instructions on the package insert before TOTM solution was spiked. All the samples of recovery test were appropriately diluted prior to LC–MS/MS analysis. Recovery was determined from five replicate analyses ($n = 5$). In addition, to assess the accuracy and precision of this method, 5% glucose solution spiked with the lower limit of quantification (LLOQ; 1 ng/ml) of TOTM was determined by using calibration curve with matrices. Intraday precision and accuracy were determined by replicate analysis of 5% glucose solution spiked with LLOQ level of TOTM in 1 day ($n = 6$), and interday precision and accuracy were determined over a span of 5 days.

2.5. Sample analysis

The PVC tube was cut to 10 cm length and filled with the sample to 8 cm tube length. Then, extraction was carried out by shaking the PVC tube at room temperature for 1 h. The extract was pipetted into another test tube and an aliquot was pipetted into vials containing a certain amount of DEHP-d_4. Then, the sample solution was appropriately diluted prior to LC–MS/MS analysis.

3. Results

3.1. Optimization of LC–MS/MS

When a conventional ODS column (150 mm × 4.6 mm) was used, the run time was relatively long because of the lipophilicity (log P = 10.49 calculated from ChemAxon) of TOTM [18]. Therefore, in this study, a short C8 column (50 mm × 2.1 mm) was used for analysis with acetonitrile/purified water (90/10, v/v) as the mobile phase, and the retention time of TOTM was approximately 7.5 min.

Table 2
Validation of LC–MS/MS for analysis of TOTM

Product	RSDRT (%)	LOD (ng/ml)	Linear range (ng/ml)	Correlation coefficient (r)
5% glucose	0.36	0.5	1–100	0.999
Prograf®	0.57	0.5	1–100	0.999
Florid®-F	0.63	0.5	1–100	0.999
Sandimmun®	0.70	0.5	1–100	0.999

$n = 3$; 5 μl of each solution was repeatedly injected. RT: retention time, LOD: limit of detection (S/N = 3).

There was no peak interference from other compounds present in the extraction solvents.

Deuterium-labeled TOTM or ^{13}C-labeled TOTM is not commercially available. The chemical structure of TOTM is similar to that of DEHP, and both are used as plasticizers of PVC medical devices. When we used DEHP-d_4 as the internal standard for TOTM determination in the intravenous preparations, the corrected recoveries were improved. Precursor spectral investigation by MS/MS in the scan mode was achieved: when TOTM and DEHP-d_4 standard solutions were subjected to MS/MS, ions at m/z 547 and 395, which were assigned to $[M+H]^+$ (Fig. 2), were observed. Moreover, in the product ion spectral investigation, the MRM ions of TOTM and DEHP-d_4 were detected at m/z 547 → 305 and m/z 395 → 153, respectively. When the turbo ionspray voltage was optimized at 5500 V, maximum peak intensity was obtained. Data acquisition and analysis were performed with Analyst® 1.3.2 software on a PC-based data system.

3.2. Method validation

In the proposed method, the limit of detection (LOD) and the limit of quantification (LOQ) of TOTM were 0.5 ng/ml (signal-to-noise ratio (S/N) = 3) and 1.0 ng/ml (S/N ≥ 10), respectively. For TOTM measurement, the calibration curve was obtained by plotting TOTM/DEHP-d_4 peak area ratio versus TOTM concentration. However, as the intravenous preparations had different matrices, standard solution of a known concentration was added to them, and a calibration curve was obtained for each (Table 2). When Sandimmun®, Prograf®, and Florid®-F were used, the linear range was 1–100 ng/ml ($r = 0.999$). The chromatogram of Prograf® spiked with TOTM is shown in Fig. 3. Then, we examined the recovery using each intravenous preparation. The average recoveries of TOTM in 5% glucose solution, Sandimmun®, Prograf®, and Florid®-F were 100.4–102.5% (relative standard deviation; R.S.D. = 1.58–3.57%), 84.7–115.0% (R.S.D. = 4.39–5.02%), 99.8–108.6% (R.S.D. = 1.92–3.73%), and 82.3–102.3% (R.S.D. = 2.90–5.15%), respectively (Table 3). The precision was expressed as R.S.D., which was calculated by measuring 5% glucose solution spiked with LLOQ level of TOTM (1 ng/ml) at six times ($n = 6$) in 1 day. The accuracy was expressed as standard error (S.E.), which was calculated as follows: (mean measured concentration − nominal concentration)/nominal concentration × 100 (%). The acceptable criterion was 15% or better. As Table 4 shows, all the values of intra- and inter-day precision and accuracy were less than 10% in the proposed method.

3.3. Determination of TOTM released from PVC tube

The proposed method was applied to the determination of TOTM released from PVC tube. TOTM at 0.16–5.6 μg/ml was released from both gamma-ray-sterilized and unsterilized PVC tubes (Fig. 4). Sandimmun®, Prograf®, and Florid®-F were diluted with 5% glucose solution for injection prior to analysis. The amount of TOTM released was higher when Prograf®, Sandimmun®, and Florid®-F were used as extraction solvent, than when 5% glucose solution was

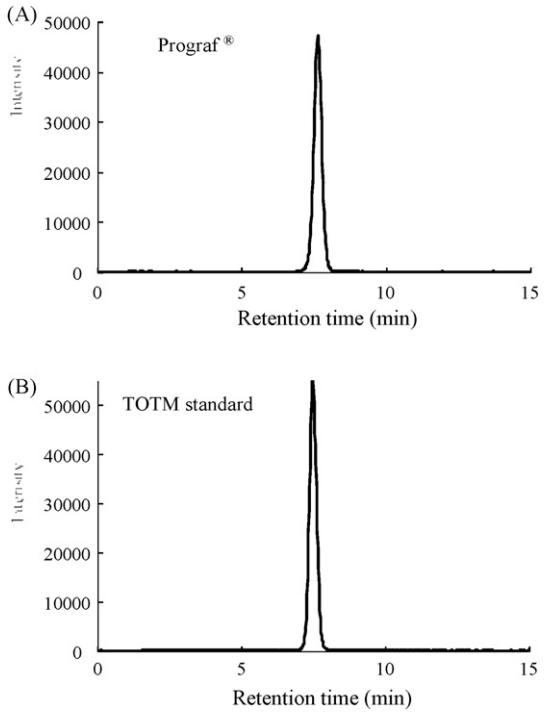

Fig. 3. Typical chromatograms of TOTM. MRM chromatogram of Prograf® spiked with 100 ng/ml TOTM. MRM chromatogram of 100 ng/ml TOTM standard solution. These chromatograms were obtained by monitoring m/z 574 → 305. The retention time of TOTM is 7.5 min.

Table 3
Recoveries of TOTM from each intravenous preparation

Product	Spiked amount (μg/ml)	Average recovery (%)	R.S.D. (%)
5% glucose	0.1	102.5	3.57
	0.5	100.4	2.87
	1	101.3	1.58
Prograf®	1	103.0	2.70
	5	108.6	1.92
	10	99.8	3.73
Florid®-F	1	99.5	3.41
	5	102.3	5.15
	10	82.3	2.90
Sandimmun®	1	84.7	5.02
	5	115.0	4.78
	10	94.7	4.39

$n = 5$; Each intravenous preparations was diluted by adding 5% glucose solution based on instructions on the package insert.

Table 4
Precision and accuracy for the analysis of TOTM in 5% glucose solution

	Detected concentration ±S.D. (ng/ml)	Precision (R.S.D., %)	Accuracy (S.E., %)
Intraday assay			
Day 1	1.01 ± 0.04	4.07	1.35
Day 2	1.01 ± 0.05	4.44	1.47
Day 3	0.98 ± 0.04	4.11	−2.50
Day 4	1.01 ± 0.06	5.66	1.47
Day 5	0.95 ± 0.07	7.71	−5.48
Interday assay			
	0.99 ± 0.03	3.17	−0.74

Intraday precision and accuracy were examined replicate analyses ($n = 6$). LLOQ (1 ng/ml) level of TOTM spiked in 5% glucose solution was determined.

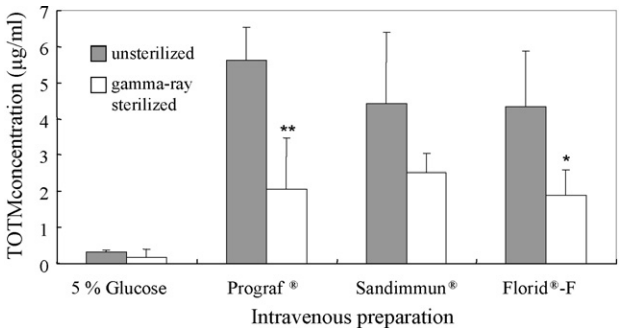

Fig. 4. Amount of TOTM migrating from PVC tube into intravenous preparations. Each column represents the amount of TOTM obtained from triplicate analysis ($n = 3$). Error bar represents standard deviation (S.D.). *Significantly different from unsterilized control ($p < 0.01$); t-test. **Significantly different from unsterilized control ($p < 0.05$); t-test.

used. The amount of TOTM released from gamma-ray-sterilized PVC tube into 5% glucose solution was 0.16 μg/ml, while that released into Prograf® was 2.1 μg/ml, which was 10 times higher. It has been reported that DEHP migration from PVC medical devices is related to the concentration of surfactant added to pharmaceuticals as solubilizer (Hanawa et al., 2003; Muramatsu et al., 2000). Except for 5% glucose solution, all the three intravenous preparations contained polyoxyethylated hydrogenated castor oil or polyoxyethylated castor oil as solubilizer. Therefore, the large amount of TOTM released into lipophilic intravenous preparations, such as Prograf®, is expected, similar to the case of DEHP.

The amount of TOTM released was compared with previously reported values (Ito et al., 2005b). TOTM release from PVC tube was performed under the same extraction conditions as those for DEHP release from PVC tube. In the case of Florid®-F, the amounts of DEHP and TOTM released per unit area were 307 and 24.4 ng/cm^2, respectively. It was confirmed that the amount of TOTM released was approximately 10 times smaller than that of DEHP.

3.4. Comparative study of TOTM release from sterilized and unsterilized PVC tubes

The amount of TOTM released from gamma-ray-sterilized PVC tube was approximately half that of TOTM released from the unsterilized one (Fig. 4). In the case of Prograf® and Florid®-F, there is the significant difference between the concentration of TOTM released from the gamma-ray-sterilized PVC/TOTM tube and that from unsterilized control. It is known that the surface structure of PVC was changed by gamma-ray irradiation. In fact, it was found that the amount of DEHP released from gamma-ray-sterilized PVC tube was small compared with the unsterilized control. On the other hand, a large amount of mono(2-ethylhexyl)phthalate (MEHP) migrated from gamma-ray-sterilized PVC tube compared with the unsterilized control (Ito et al., 2006). In that study, it was surmised that MEHP was a breakdown product of DEHP. A similar case is surmised for TOTM. Therefore, it is possible that the breakdown product of TOTM migrated from the gamma-ray-sterilized PVC tube as well. However, when the 5% glucose solution and Prograf® were used for migration test from gamma-ray-sterilized PVC tube, no other peak was observed both negative and positive in the total ion chromatogram (scan mode) except that of TOTM.

4. Discussion

In this study, we developed an analytical method for the determination of TOTM released from PVC medical devices, which uses LC–MS/MS. Our results demonstrate that TOTM is a superior alter-

native to DEHP for use in medical devices because of its lower leachability. In addition, it is a first paper that examined TOTM released from gamma-ray-sterilized PVC medical device. Even if the amount of TOTM released from the gamma-ray-sterilized PVC tube is small, it is necessary to evaluate the risk of TOTM released from PVC medical devices. In addition, we should examine the possibility of adsorption of drug into PVC/TOTM tubes, since it has been reported that adsorption of drug into PVC (Yano et al., 2001).

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