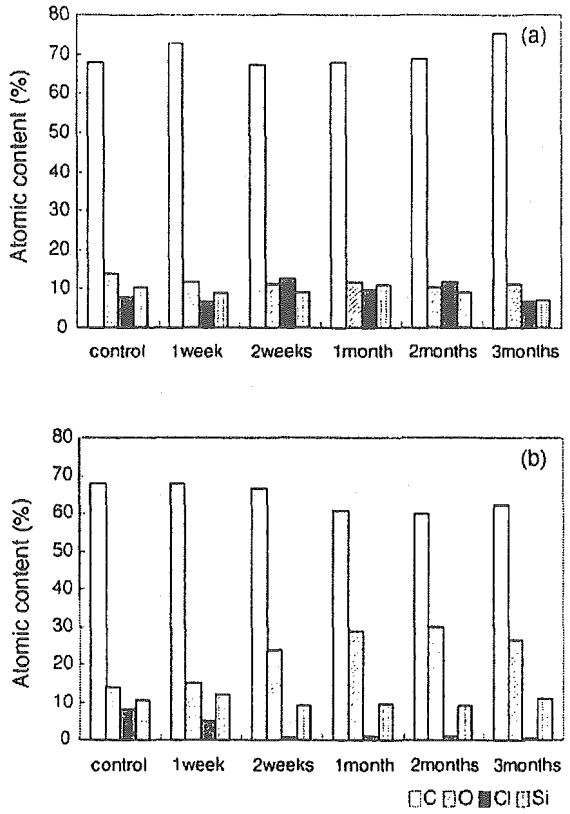

Fig. 4. Elemental analysis of PVC sheets by ESCA: (a) typical bar graph of PVC sheet samples subjected to heat treatment or visible light irradiation, and negative control and (b) bar graph of PVC sheet sample subjected to UV irradiation.

sheets, respectively. We hypothesize that the difference in the contact angle is due to differences in the embossed processing of the outer and inner surfaces (Fig. 5c (control) and Fig. 5b (control)). As shown in Fig. 5, the static angle of contact of the PVC sheets using the Sandimmun® injection as the wetting agent was not affected by either heat treatment or visible light irradiation (Fig. 5a). On the other hand, the static angle of contact of the inner surface of the UV-irradiated PVC sheets was decreased with time (Fig. 5b). In addition, the static angle of contact of the inner surface of the UV-irradiated positive control PVC sheets was almost the same as that of the inner surface of the PVC sheets UV-irradiated for 3 months. On the other hand, the static angle of contact of the outer surface of the UV-irradiated PVC sheets was increased markedly from the control to 3 months (Fig. 5c).

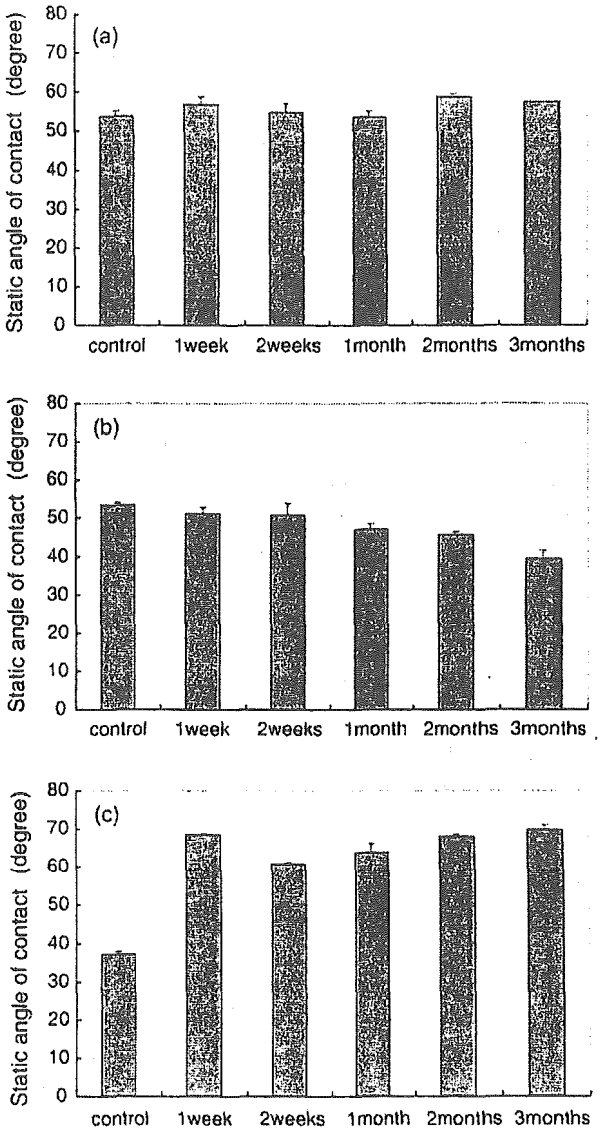

Fig. 5. Static angle of contact of PVC sheet samples Sandimmun® injection was used to determine the static angle of contact of PVC sheets subjected to heat treatment or optical irraditation. Typical bar graph of PVC sheet samples subjected to heat treatment or visible light irradiation. Bar graph of PVC sheet samples subjected to UV irradiation. The static angle of contact obtained by the inner surface of PVC sheet. Bar graph of PVC sheet samples subjected to UV irradiation. The static angle of contact obtained by the outer surface of PVC sheet.

Therefore, surface structure of the heat-treated PVC sheets and visible light irradiated PVC sheets did not change with time. On the other hand, surface structure of the UV-irradiated PVC sheets was markedly changed.

Table 2
Maximum force to break the PVC sheets for tensile test

	4 °C	37 °C	60 °C	Visible light	UV light
1 week	37.03 ± 1.68	36.54 ± 1.15	37.64 ± 2.01	36.63 ± 1.32	37.59 ± 0.86
2 weeks	36.12 ± 1.34	36.92 ± 0.52	36.20 ± 0.80	36.97 ± 1.12	36.28 ± 0.67
1 month	36.12 ± 1.07	36.86 ± 2.13	36.46 ± 1.39	36.70 ± 1.69	35.73 ± 0.76
2 months	37.84 ± 1.93	36.43 ± 2.14	36.08 ± 1.56	37.03 ± 0.39	36.43 ± 0.52
3 months	36.76 ± 1.48	36.28 ± 2.04	36.52 ± 0.81	36.86 ± 1.77	36.76 ± 1.05

(n = 4) Negative control samples: 36.37 ± 0.78 N; positive control samples subjected to heat treatment: 37.28 ± 0.92 N; positive control samples irradiated with visible light: 37.11 ± 1.33 N; positive control samples irradiated with UV: 33.07 ± 2.88 N.

3.4. Tensile test

Flexibility and stability are some of the reasons why PVC products are used widely. In order to examine the deterioration of PVC products subjected to heat treatment or optical irradiation, the tensile strength and elasticity were measured. The maximum force to break the PVC sheets ranged from 33.1 ± 2.9 to 37.8 ± 1.9 N regardless of treatment (Table 2).

Therefore, there were no notable changes in the tensile strength and elasticity of the PVC sheets when heat treatment or optical irradiation was applied.

4. Conclusions

The DEHP content and the surface structure of the PVC products, and the levels of DEHP migrating from them were measured in order to determine the influence of external factors on PVC products during storage. In addition, a tensile test was carried out to determine the tensile strength and elasticity of the PVC products. It was hypothesized that UV irradiation led to changes in the surface structure, and that change was responsible for the decreased levels of DEHP migrating from the PVC products using a drug solvent diluted according to the package insert.

In order to clarify the change in the surface structure, we examined the surface by ESCA. In UV-irradiated PVC sheets, we observed that the hydrogen chloride level was decreased and oxidation proceeded with time. Similarly, in the FT-IR spectra, we observed that the absorption band characteristic of C–Cl stretching vibration from PVC and the C–H band from the aromatic compound were decreased with time. In addition, the absorption bands in the FT-IR spectra were found to broaden with time. PVC oxidation and crosslinking were surmised to explain these events. Based on these results, the UV irradiation of PVC products induced a decrease in the levels of DEHP migration, and the PVC products maintained their features, such as flexibility and stability. Some studies have reported the decreased DEHP release from PVC products by modifying the surface structure of the products under various conditions such as UV-irradiation with sodium azide as an enhancer for absorbing UV-energy, gamma-ray irradiation, in an aqueous solution containing water-soluble compounds such as methacrylic acid, and gas plasma treatment under reduced pressure (Jayakrishnan et al., 1995; Krishnan et al., 1991). In comparison with these techniques, the simple UV-irradiation method described in this study seems to have a great advantage, because it can be performed easily under atmosphere conditions without reagents or special instruments.

Today, the medical device industry is searching for a substitute for DEHP as a plasticizer. Our results suggest that the levels of DEHP migrating from a PVC product can be reduced by easy surface treatment without changing the type of plasticizer. This could be useful method to develop novel PVC products, if other safety aspects are confirmed. Possible biological changes should not be ignored, since increased oxygen content on the surface could have an important impact on the activation of the clotting system and complements. A detailed investigation is in progress in our laboratory to develop novel PVC products.

Acknowledgement

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References

Arcadi, R.A., Costa, C.E., Imperatore, C., Marchese, A., Rapisarda, A., Salemi, M., Trimarchi, G., Costa, G., 1998. Oral toxicity of DEHP during pregnancy and suckling in the long-Evans rat. Food Chem. Toxicol. 36, 963–970.

Center for Devices and Radiological Health, US Food and Drug Administration, 2001. (Web site at http://www.fda.gov/cdrh/nespg.html) September.

Faouzi, M.E.A., Dine, T., Luyckx, M., Brunet, C., Mallevais, M.-L., Goudaliez, Gressier, B., Cazin, M., Kablan, J., Cazin, J.C., 1995. Stability, compatibility and plasticizer extraction of miconazole injection added to infusion solutions and stored in PVC containers. J. Pharm. Biomed. Anal. 13, 1363–1372.

Gray, E., Wolf, C., Lambright, C., Mann, P., Price, M., Cooper, R., Ostby, J., 1999. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, *p,p'*-DDE and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate PCB 169 and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the rat. Toxicol. Ind. Health 14, 94–118.

Haishima, Y., Hayashi, Y., Yagami, T., Nakamura, A., 2001. Elution of bisphenol-A from hemodialyzers consisting of polycarbonate and polysulfone resins. J. Biomed. Mater. Res. (Appl. Biomater.) 58, 209–215.

Hanawa, T., Muramatsu, E., Asakawa, K., Suzuki, M., Tanaka, M., Kawano, K., Seki, T., Juni, K., Nakajima, S., 2000. Investigation of the release behavior of diethylhexyl phthalate from the polyvinyl-chloride tubing for intravenous administration. Int. J. Pharm. 210, 109–115.

Hayashi, Y., Matsuda, R., 1994. Deductive prediction of measurement precision from signal and noise in liquid chromatography. Anal. Chem. 66, 2874–2881.

Hayashi, Y., Matsuda, R., Haishima, Y., Yagami, T., Nakamura, A., 2002. Validation of HPLC and GC–MS systems for bisphenol-A leached from hemodialyzers on the basis of FUMI theory. J. Pharm. Biomed. Anal. 28, 421–429.

Hayashi, Y., Matsuda, R., Poe, R.B., 1996. Probabilistic approach to confidence intervals of linear calibration. Analyst 121, 591–599.

Health Canada Expert Advisory Panel on DEHP in Medical Devices, 2002. (Web site at http://www.hc-sc.gc.ca/hpb-dgps/therapeut/hemleng/whatsnew.html) January.

Hill, S.S., Shaw, B.R., Wu, A.H.B., 2001. The clinical effects of plasticizers, antioxidants, and other contaminants in medical polyvinylchloride tubing during respiratory and non-respiratory exposure. Clin. Chim. Acta. 304, 1–8.

Huber, W., Grasl-Kraupp, B., Schulte-Hermann, R., 1996. Hepatocarcinogenic potential of DEHP in rodents and its implications on human risk. Crit. Rev. Toxicol. 26, 365–481.

Jayakrishnan, A., Sunny, M.C., Rajan, M.N., 1995. Photocrosslinking of azidated poly(vinyl chloride) coated onto plasticized PVC surface—route to containing plasticizer migration. J. Appl. Poly. Sci. 56, 1187–1195.

Jenke, D.R., 2001. Evaluation of model solvent systems for assessing the accumulation of container extractables in drug formulations. Int. J. Pharm. 224, 51–60.

Krishnan, V.K., Jayakrishnan, A., Francis, J.D., 1991. Radiation grafting of hydrophilic monomers on to plasticized poly (vinyl chloride) sheets II. Migration behaviour of the plasticizer from *N*-vinyl pyrrolidone grafted sheets. Biomaterials 12, 489–492.

Ljunggren, L., 1984. Plasticizer migration from blood lines in hemodialysis. Artif. Organs. 8, 99–102.

Rock, G., Labow, R., Tocchi, M., 1986. Distribution of di (2-ethylhexyl)phthalate and products in blood and blood components. Environ. Health Perspect. 65, 309–316.

Takeishi, M., Okawara, M., 1970. Reaction of poly (vinyl chloride) containing azide groups. J. Polym. Sci. Polymlett. 8, 829–833.

The Ministry of Health, Labour and Welfare, 2000. (Web site at http://www1.mhlw.go.jp/shingi/s0006/txt/s0614-1_13.txt).

Tickner, J., Schettler, T., Guidotti, T., McCally, M., Rossi, M., 2001. Health risks posed by use of di-2-ethylhexyl phthalate (DEHP) in PVC medical devices: a critical review. Am. J. Ind. Med. 39, 100–111.

Yakubovich, M., Vienken, J., 2000. Is there a need for plasticizer-free biomaterials in dialysis therapy? Med. Device Technol. 11, 18–21.

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Effect of sterilization process on the formation of mono(2-ethylhexyl)phthalate from di(2-ethylhexyl)phthalate

Rie Ito, Fumie Seshimo, Naoko Miura, Migaku Kawaguchi, Koichi Saito, Hiroyuki Nakazawa *

Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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Abstract

The risk assessment of di(2-ethylhexyl)phthalate (DEHP) migrating from polyvinyl chloride (PVC) medical devices is an important issue. Many studies have been conducted to determine the level of DEHP migration. A recent report has indicated that DEHP in blood bags is hydrolyzed by esterase into mono(2-ethylhexyl)phthalate (MEHP). However, MEHP is thought to be even more toxic than the parent compound. Therefore, a method for the simultaneous determination of DEHP and MEHP was developed. The limits of quantification (LOQs) of DEHP and MEHP were 2.5 and 0.75 ng/ml, respectively. In this study, the effect of sterilization process on the levels of DEHP and MEHP migration was investigated. The level of migration of DEHP from gamma(γ)-ray sterilized PVC sheet was low compared with that of the unsterilized control. By contrast, the level of MEHP migration from the γ-ray sterilized PVC sheet was high compared with that of the unsterilized control. In addition, a high content of MEHP was found in the γ-ray sterilized PVC sheet.

Keywords: Sterilization; Di(2-ethylhexyl)phthalate; Mono(2-ethylhexyl)phthalate; γ-Ray

1. Introduction

Polyvinyl chloride (PVC) is one of the most widely used polymer materials in the medical field. Flexible PVC is used in the manufacture of blood storage bags, blood tubing, and so on. Plasticizers are incorporated into PVC medical devices to increase their flexibility. The esters of phthalic acid, particularly di(2-ethylhexyl)phthalate (DEHP), are the most preferred plasticizers for medical grade PVC. However, the migration of DEHP from PVC medical devices has been reported [1–5]. DEHP in PVC products easily migrates into foods, drugs and body fluids [6–8]. The toxicity of DEHP has been evaluated [5,9–12], and a risk assessment study has suggested that it is relatively safe for humans.

However, it has been reported that DEHP is hydrolyzed enzymatically into mono(2-ethylhexyl)phthalate (MEHP) [13–15]. In vitro studies have indicated that MEHP inhibits FSH-stimulated cAMP accumulation in cultured Sertoli cells [16–20], in addition to reducing 17β-estradiol production and aromatase mRNA expression [21,22]. These results suggest that MEHP is an active metabolite of DEHP, and that any toxic effects of orally ingested DEHP are more likely to be due to the properties of the corresponding monoester rather than the intact DEHP, and that MEHP may be even more toxic than the parent compound. Medical devices are sterilized because they directly contact or are inserted into the human body. It has been reported that DEHP is hydrolyzed by such enzymes as lipases into MEHP in blood bags [13–15]. Some reports have indicated that the hydrolysis may have occurred during sterilization by autoclaving [23,24]. For medical devices, such sterilization processes as autoclaving, gamma(γ)-ray irradiation, and exposure of ethylene oxide gas (EOG), are usually performed.

Therefore, we investigated the effect of the sterilization process on the levels of migration of DEHP and MEHP from PVC medical devices. The PVC sheets that were subjected to various sterilization processes were extracted with purified water, 5% glucose solution or polyoxyethylated hydrogenated castor oil 60 (HCO-60). Moreover, the contents of DEHP and MEHP in the PVC sheets were examined. We have developed the column-

* Corresponding author. Tel.: +81 3 5498 5763; fax: +81 3 5498 5765.
E-mail address: nakazawa@hoshi.ac.jp (H. Nakazawa).

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switching (CS) liquid chromatography–tandem mass spectrometry (LC–MS/MS) as the method for determining DEHP and MEHP with high sensitivity and selectivity.

2. Experimental (materials and methods)

2.1. Chemicals and materials

Environmental analytical grade DEHP and DEHP-d_4 were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). MEHP and MEHP-d_4 were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). The structures of DEHP, MEHP and their surrogate standards are shown in Fig. 1. Phthalic acid esters, analytical grade acetonitrile and acetone were used in the experiments. The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

The test material was PVC sheet that was subjected to γ-ray irradiation (^{60}Co: 24.2 kGy), autoclaving (115 °C × 40 min) or EOG (50 °C × 8 h) as the sterilization process. In addition, commercially available PVC tubing on which γ-ray sterilization (20–25 kGy) was performed was used. None of the sterilization processes were performed on the control sample that was kindly supplied by the manufacturer.

The extraction solvents were 5% glucose solution for injection (Otsuka Pharmaceuticals Co., Tokyo, Japan), polyoxyethylated hydrogenated castor oil 60 (HCO-60) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and purified water.

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000™ (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo Ionspray™ ionization source. Mass spectrometry data were processed with Analyst 1.3.2 software. A Shimadzu (Kyoto, Japan) LC-10 AS pump was used for providing flow through the extraction column to load and wash the sample and to equilibrate the extraction column. A Mightysil® RP-18 GP column (5 mm × 2.0 mm, 5 μm particle size) from Kanto Chemical was used for the separation. An Oasis® HLB extraction column (20 mm × 2.1 mm, 25 μm particle size) from Waters was used for the extraction and clean-up.

2.3. Chromatographic and extraction conditions

The column-switching system was used for sample injection [25]. After 10 μl of the sample was injected with an autosampler, it was loaded onto the extraction column by flowing pure water at the rate of 1 ml/min using the LC-10 AS pump for 3 min. The matrices in the sample were eluted whereas DEHP and MEHP were retained on the extraction column. After the 3 min period, the switching valve was changed to configuration B (Fig. 2). The column oven was maintained at 40 °C. Separation was carried out with a mobile phase of acetonitrile/water (90/10, v/v) at a flow rate of 0.2 ml/min. The eluate from the analytical column was directed to the electrospray MS. After elution for 8 min, the switching valve was returned to the original position (configuration A in Fig. 2).

2.4. MS/MS conditions

The working parameters for turbo ionspray ionization MS/MS were as follows: declustering potentials, 81 V (DEHP and DEHP-d_4) and −60 V (MEHP and MEHP-d_4); curtain gas flow rates, 20 psi (DEHP and DEHP-d_4) and 30 psi (MEHP and MEHP-d_4); nebulizer gas (N_2) pressure, 30 psi; and turbo ionspray gas (N_2) pressure, 0 psi. The ion source temperature was maintained at 650 °C and the turbo ionspray voltages for DEHP (DEHP-d_4) and MEHP (MEHP-d_4) were 5500 and −4500 V, respectively. DEHP and DEHP-d_4 were detected in the positive mode, whereas MEHP and MEHP-d_4 were detected in the negative mode. The product ion mass spectra of DEHP, DEHP-d_4, MEHP and MEHP-d_4 obtained by the LC–MS/MS system

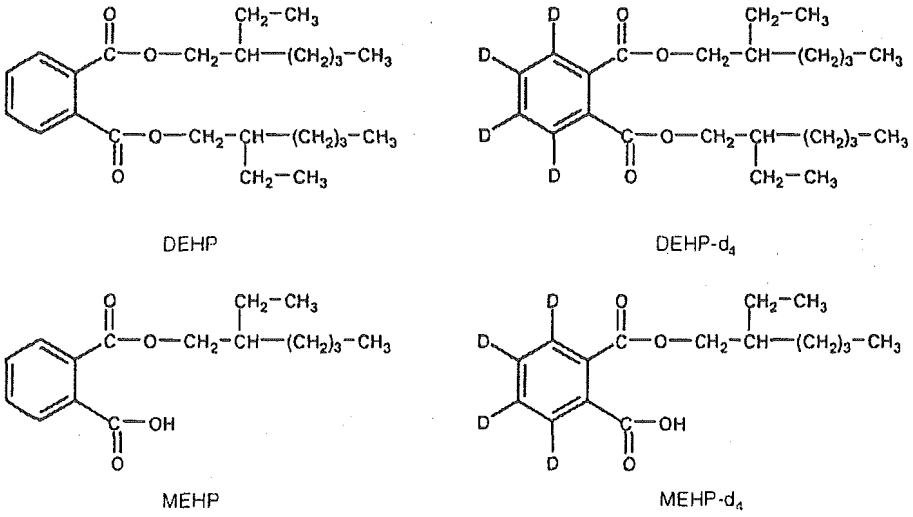

Fig. 1. Chemical structures of DEHP, MEHP and their internal standards.

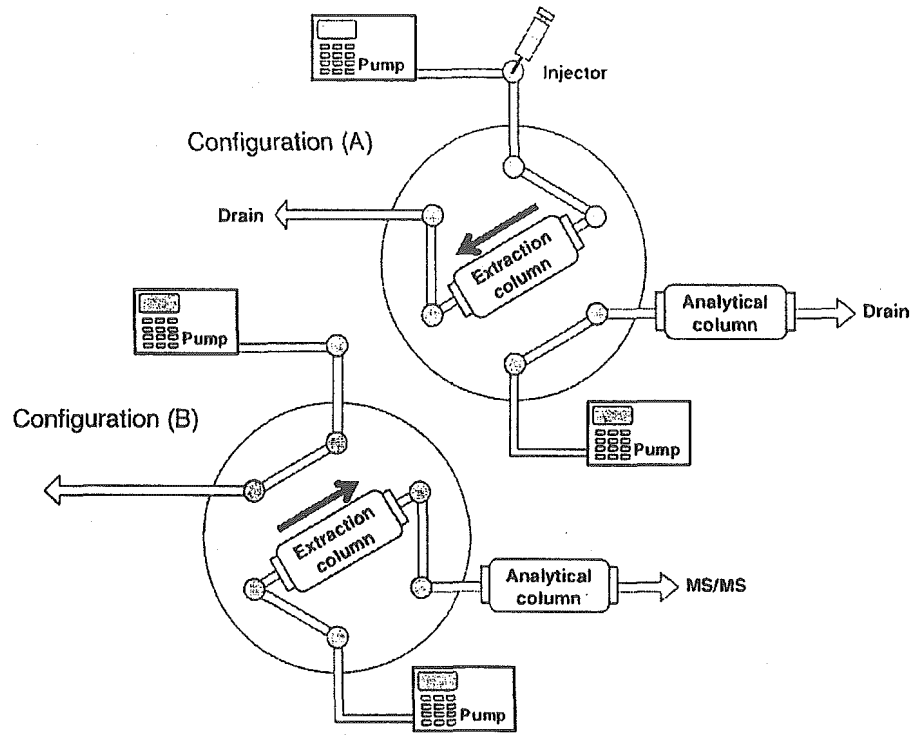

Fig. 2. Schematic representation of the column-switching LC–MS/MS system. (A) Configuration for sample loading and washing; (B) configuration for sample elution.

are shown in Fig. 3. The combinations of precursor ion and product ions were as follows: DEHP (precursor ion → product ion, *m/z* 391 → 149), DEHP-d_4 (*m/z* 395 → 153), MEHP (*m/z* 277 → 134), and MEHP-d_4 (*m/z* 281 → 138). The collision gas (N_2) pressures were set at 2 units (DEHP and DEHP-d_4) and 1 unit (MEHP and MEHP-d_4).

2.5. Migration test

The migration of DEHP and MEHP from the PVC sheet (1 cm × 3 cm) was examined in 5 ml of each solvent. HCO-60 is a surfactant that is used in the formulation of such drugs as Prograf® and is involved in the migration of DEHP. In addition,

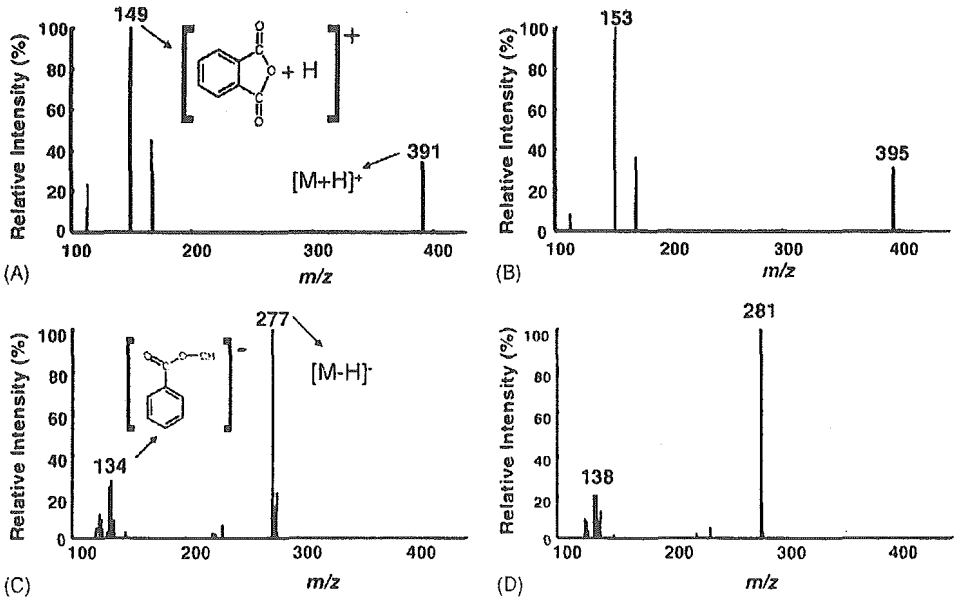

Fig. 3. Product ion mass spectra of DEHP, MEHP and their internal standards. (A) DEHP; (B) DEHP-d_4 (internal standard for DEHP); (C) MEHP; (D) MEHP-d_4 (internal standard for MEHP).

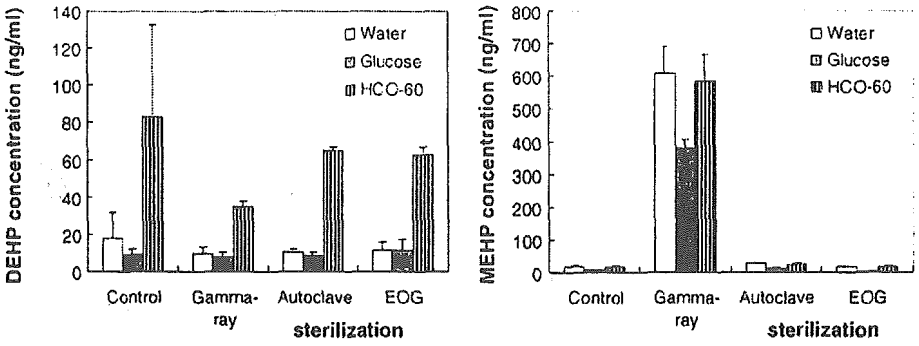

Fig. 4. Levels of DEHP and MEHP migration into various solutions from PVC sheet samples. Each plotted column is the mean the levels of DEHP or MEHP with triplicate analysis ($n = 3$). The error bar represents the standard deviation (S.D.).

it has reported that the level of DEHP migration was dependent on the concentration of HCO-60 [25]. We prepared 0.02 mg/ml HCO-60 for the migration test [26]. The samples were kept in test tubes and extraction was carried out with shaking at 37 °C for 1 h. A 1 ml aliquot of the extract was pipetted into another test tube, and DEHP-d_4 and MEHP-d_4 were added. Then, the sample solution was appropriate diluted prior to LC–MS/MS analysis.

The PVC tubing was cut to 10 cm length and filled with the solvents (tube length, 8 cm). The tubing was subjected to extraction with shaking at 37 °C for 1 h. The extracts were pipetted into another test tube containing DEHP-d_4 and MEHP-d_4. Then, all the samples were appropriate diluted prior to LC–MS/MS analysis.

2.6. Contents of DEHP and MEHP in PVC

A PVC sample (5 mg) was completely dissolved in 5 ml of THF. The solution was appropriate diluted with acetonitrile. Then, the internal standard was added prior to the analysis.

3. Results and discussion

3.1. Analysis of DEHP and MEHP by on-line SPE–LC–MS/MS

In the proposed method, the limits of quantification (LOQs) (signal-to-noise ratio >10) of DEHP and MEHP were 2.5 and 0.75 ng/ml with the standard solutions, respectively. For DEHP measurement, a calibration curve was obtained by plotting the peak-area ratio (DEHP/DEHP-d_4) versus DEHP concentration, and was linear over the range of 2.5–500 ng/ml ($r = 0.998$). For MEHP measurement, a calibration curve was obtained by plotting the peak-area ratio (MEHP/MEHP-d_4) versus MEHP concentration, and was linear over the range of 0.75–500 ng/ml ($r = 0.997$). We also examined the recovery using 5% glucose solution. For the glucose solution that was spiked with 50 ng/ml DEHP and MEHP, the average recoveries of DEHP and MEHP were 99.2% (R.S.D. = 3.2%, $n = 6$) and 109.0% (R.S.D. = 3.4%, $n = 6$), respectively.

3.2. Determination of DEHP and MEHP migration from PVC sheet and tubing

The developed method was applied to the determination of DEHP and MEHP migration from the PVC sheets that were subjected to various sterilization processes (Fig. 4). The migration of DEHP and MEHP from all the PVC sheets was observed. The level of DEHP migration had the following order: HCO-60 > water ≥ 5% glucose solution, similar to the report of Hanawa et al. [25]. Furthermore, when the PVC sheets were extracted with purified water and HCO-60, the levels of DEHP migration from all the PVC sheets that were subjected to the sterilization processes, particularly γ-ray sterilization, were low compared with the unsterilized control. On the other hand, the levels of MEHP migration from the unsterilized control, autoclaved and EOG sterilized PVC sheets were not different, whereas the γ-ray sterilized PVC sheet released a large amount of MEHP.

Then, the levels of DEHP and MEHP migration from the commercially available PVC tubing sterilized by γ-ray were compared with those of the unsterilized one (Fig. 5). As expected, DEHP more easily migrated in HCO-60 than in water or glucose solution. In HCO-60, the level of DEHP migration from the γ-ray sterilized PVC tubing was low compared with that of the unsterilized one. Moreover, the unsterilized PVC tubing released little MEHP whereas the γ-ray sterilized PVC tubing released approximately 30–40 times more MEHP compared with the unsterilized one. In addition, MEHP was also released from the γ-ray sterilized PVC tubing that was extracted with water or glucose solution.

3.3. Contents of DEHP and MEHP in PVC sheet and tubing

The developed method was also applied to determine the contents of DEHP and MEHP in the PVC sheets (Table 1) and the PVC tubing (Table 2). We thought that the DEHP contents were almost the same in the various sterilized sheets because the DEHP contents were 26.8–27.8% (w/w) in the sterilized PVC sheets. By contrast, MEHP was detected in only the γ-ray sterilized PVC sheet. Therefore, PVC material might inherently contain MEHP, and the MEHP migrated directly into the solvent.

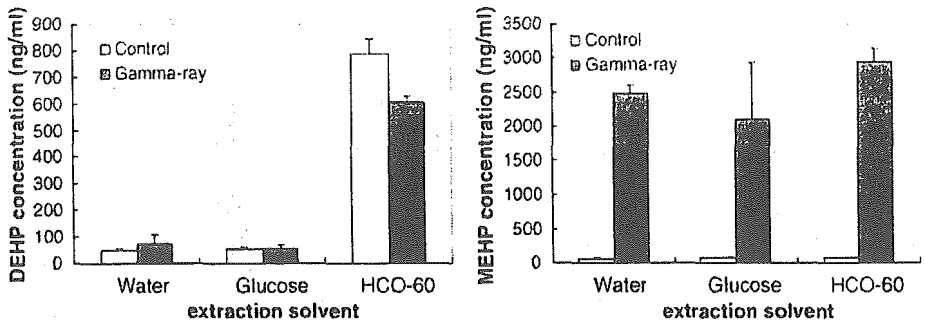

Fig. 5. Level of DEHP and MEHP migration into various solutions from commercially available PVC tubing. Each plotted column is the mean the levels of DEHP or MEHP with triplicate analysis ($n = 3$). The error bar represents the standard deviation (S.D.).

Table 1
Contents of DEHP and MEHP in PVC sheet samples treated with various sterilization

	Control	Gamma-ray	Autoclave	EOG
DEHP (%, w/w)	32.1 ± 5.7	27.8 ± 0.8	26.8 ± 1.6	26.8 ± 0.8
MEHP (mg/g)	<0.25	0.38 ± 0.05	<0.25	<0.25

Mean ± S.D., $n = 3$.

The commercially available PVC tubing which was sterilized by γ-ray was confirmed to contain MEHP, although the level was below the LOQ. A high dilution ratio was required because of the difference in level between DEHP and MEHP. Therefore, MEHP could not be determined in some of the sterilized PVC sheets.

In this study, the following phenomena were observed: (1) the level of DEHP migration from the γ-ray sterilized PVC sheet was low compared with that of the unsterilized sheet. Surface processing, an example of which is plasma treatment, is known to suppress DEHP migration [27,28]. We speculated that a similar surface processing occurred with γ-ray irradiation; (2) the level of MEHP migration from the γ-ray sterilized PVC sheet was significantly high compared with that of the unsterilized control. In addition, MEHP was released from the γ-ray sterilized PVC sheet regardless of the solvent used. We hypothesized that MEHP was inherently contained in the PVC sheet, and then directly migrated from it. To confirm this hypothesis, we determine the DEHP and MEHP contents in the PVC sheet; (3) MEHP was detected in the γ-ray sterilized PVC sheet. Although MEHP was also found to migrate from the PVC sheets sterilized by EOG or autoclaving, MEHP was not detected in them. We speculated that the MEHP contents in the other sterilized sheets were very low compared with that in the γ-ray sterilized PVC sheet. In addition, the most plausible reason for not detecting MEHP in the PVC sheets was the high dilution ratio of the samples; (4) although MEHP was also detected in PVC tubing, its amount could not be determined.

Table 2
Contents of DEHP and MEHP in commercially available PVC tubing

	Control	Gamma-ray
DEHP (%, w/w)	44.7 ± 2.9	53.9 ± 2.5
MEHP (mg/g)	<0.25	<0.25 (0.23)

Mean ± S.D., $n = 3$.

Taking these into consideration, we surmised that MEHP was inherently present in PVC and migrated directly from it, although MEHP was thought to be hydrolyzed by enzymes as lipases or by autoclave sterilization until now. In addition, we speculated that the sterilization by γ-ray was sufficient to decompose DEHP into MEHP in PVC materials. We conducted a risk assessment of MEHP migration. The level of MEHP migration was calculated as follows: we found that when γ-ray sterilized PVC sheet (1 cm × 3 cm) was extracted with 5 ml of solvent, approximately 600 ng/ml MEHP migrated from it. Therefore, the amount of MEHP migration was 3.0 μg (=600 ng/ml × 5 ml). As the superficial area involved in the migration was 6 cm^2, the level of MEHP migration per unit area was 0.5 μg/cm^2. The superficial area of the commercially available infusion set was 101.3 cm^2 at the maximum. Therefore, the amount of MEHP migration from the infusion set was 50.65 μg (=101.3 cm^2 × 0.5 μg/cm^2). When this infusion set was used for 2 days in a patient with 50 kg body weight, the patient was exposed to 0.51 μg/kg/day of MEHP. In 2001, the Center for Devices and Radiological Health of the U.S. Food and Drug Administration reported DEHP assessment as "Safety assessment of DEHP released from PVC medical devices." In Annex C of the report, "Aggregate safety assessment for coexposure to DEHP and MEHP," the relative potency of MEHP/DEHP was calculated to be 10. Therefore, an MEHP exposure of 0.51 μg/kg/day meant a DEHP exposure of 5.1 μg/kg/day. In addition, in this report, the tolerable intake (TI) of DEHP was 40 μg/kg/day by oral administration and 600 μg/kg/day by parenteral route. Although the level of MEHP that migrated from the γ-ray sterilized PVC did not exceed the TI, we must investigate the effect of formation of MEHP from DEHP by γ-ray sterilization for high risk patients such as infant and pregnant women.

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References

[1] T. Hanawa, E. Muramatsu, K. Asakawa, M. Suzuki, M. Tanaka, K. Kawano, T. Seki, K. Juni, S. Nakajima, Int. J. Pharm. 210 (2000) 109–115.
[2] K. Inoue, T. Higuchi, F. Okada, H. Iguchi, Y. Yoshimura, A. Sato, H. Nakazawa, J. Pharm. Biomed. Anal. 31 (2003) 1145–1152.
[3] M.A. Fauzi, F. Khalfi, T. Dine, M. Luyckx, C. Brunet, B. Gressier, F. Goudalez, M. Cazin, J. Kablan, A. Belabed, J.C. Cazin, J. Pharm. Biomed. Anal. 21 (1999) 923–930.
[4] M.C. Allwood, Int. J. Pharm. 29 (1986) 233–236.
[5] J.A. Tickner, T. Schettler, T. Guidotti, M. McCally, M. Rossi, Am. J. Ind. Med. 39 (2001) 100–111.
[6] A.O. Earls, I.P. Axford, J.H. Braybrook, J. Chromatogr. A 983 (2003) 237–246.
[7] K. Inoue, M. Kawaguchi, F. Okada, Y. Yoshimura, H. Nakazawa, Anal. Bioanal. Chem. 375 (2003) 527–533.
[8] S. Takatori, Y. Kitagawa, M. Kitagawa, H. Nakazawa, S. Hori, J. Chromatogr. B 804 (2004) 397–401.
[9] M. Koizumi, M. Ema, A. Hirose, R. Hasegawa, Jpn. J. Food Chem. 7 (2000) 65–73.
[10] M. Koizumi, M. Ema, A. Hirose, Y. Kurokawa, R. Hasegawa, Jpn. J. Food Chem. 8 (2001) 1–10.
[11] M. Yakubovich, J. Vienken, Med. Device Technol. 11 (2000) 18–21.
[12] S. Hill, B. Shaw, A. Wu, Clin. Chim. Acta 304 (2001) 1–8.
[13] B.G. Lake, J.C. Phillips, J.C. Linnel, S.D. Gangolli, Toxicol. Appl. Pharmacol. 39 (1977) 239–248.
[14] P.W. Albro, R.O. Thomas, Biochim. Biophys. Acta 306 (1973) 380–390.
[15] P.W. Albro, S.R. Lavenhar, Drug Metab. Rev. 21 (1989) 13–34.
[16] J.J. Heindel, R.E. Chapin, Toxicol. Appl. Pharmacol. 97 (1989) 377–385.
[17] P. Grasso, J.J. Heindel, C.J. Powell, L.E. Reichert, Biol. Reprod. 48 (1993) 454–459.
[18] J.H. Richburg, K. Boekelheide, Toxicol. Appl. Pharmacol. 137 (1996) 42–50.
[19] J. Lee, J.H. Richburg, E.B. Shipp, M.L. Meistrich, K. Boekelheide, Endocrinology 140 (1999) 852–858.
[20] J.H. Richburg, A. Nanex, L.R. Williams, M.E. Embree, K. Boekelheide, Endocrinology 141 (2000) 787–793.
[21] B.J. Davis, R.R. Maronpot, J.J. Heindel, Toxicol. Appl. Pharmacol. 128 (1994) 216–223.
[22] T.N. Lovekamp, B.J. Davis, Toxicol. Appl. Pharmacol. 172 (2001) 217–224.
[23] A. Arbin, J. Östelius, K. Callmer, J. Sroka, K. Hänninen, S. Axelsson, Acta Pharm. Suec. 20 (1983) 20–33.
[24] G. Smistad, T. Waaler, P.O. Roksvaag, M. Midtsem, Acta Pharm. Nord. 1 (1989) 321–326.
[25] T. Hanawa, N. Endoh, F. Kazuno, M. Suzuki, D. Kobayashi, M. Tanaka, K. Kawamo, Y. Morimoto, S. Nakajima, T. Oguchi, Int. J. Pharm. 267 (2003) 141–149.
[26] R. Ito, F. Seshimo, N. Miura, M. Kawaguchi, K. Saito, H. Nakazawa, J. Pharm. Biomed. Anal. 39 (2005) 1036–1041.
[27] M. Asai, Jpn. J. Artif. Organs 8 (1979) 389–390.
[28] R. Terada, J. Suzuki, Y. Mori, S. Nagaoka, T. Kikuchi, S. Nishiumi, K. Hatada, H. Kobayashi, Jpn. J. Artif. Organs 11 (1982) 1187–1190.