Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 804 (2004) 397–401
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate in human serum using liquid chromatography-tandem mass spectrometry

S. Takatori [a,*], Y. Kitagawa [a], M. Kitagawa [a], H. Nakazawa [b], S. Hori [a]

[a] *Osaka Prefectural Institute of Public Health, 3-69, 1-chome, Nakamichi, Higashinari-ku, Osaka 537-0025, Japan*
[b] *Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, 2-chome, Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*

Received 1 October 2003; received in revised form 27 January 2004; accepted 27 January 2004

Abstract

Concentrations of mono(2-ethylhexyl)phthalate (MEHP), and di(2-ethylhexyl)phthalate (DEHP), in serum of healthy volunteers were determined by high performance liquid chromatography (HPLC) with tandem mass spectrometry (LC/MS/MS). The serum was extracted with acetone, followed by hexane extraction under acidic conditions, and then applied to the LC/MS/MS. Recoveries of 20 ng/ml of MEHP and DEHP were 101 ± 5.7 ($n = 6$) and 102 ± 6.5% ($n = 6$), respectively. The limits of quantification (LOQ) of MEHP and DEHP in the method were 5.0 and 14.0 ng/ml, respectively. The concentration of MEHP in the serum was at or less than the LOQ. The concentration of DEHP in the serum was less than the LOQ. Contaminations of MEHP and DEHP from experimental reagents, apparatus and air during the procedure were less than the LOQ and were estimated to be <1.0 and 2.2 ± 0.6 ng/ml, respectively. After subtraction of the contamination, the net concentrations of MEHP and DEHP in the serum were estimated at or <5 and <2 ng/ml, respectively. To decrease contamination by DEHP, the cleanup steps and the apparatus and solvent usage were minimized in the sample preparation procedures. The high selectivity of LC/MS/MS is the key for obtaining reliable experimental data from in the matrix-rich analytical samples and for maintaining a low level contamination of MEHP and DEHP in this experimental system. This method would be a useful tool for the detection of MEHP and DEHP in serum.

Keywords: Di(2-ethylhexyl)phthalate; Mono(2-ethylhexyl)phthalate

1. Introduction

Di(2-ethylhexyl)phthalate (DEHP) is a common plasticizer used to impart flexibility to polyvinylchloride (PVC). It leaches readily from PVC into the environment and transfers to other materials attached to the PVC or via the atmosphere. Patients undergoing medical procedures, such as intravenous therapy, nutritional support, blood transfusion, hemodialysis, cardiopulmonary bypass or extracorporeal membrane oxygenation (EMO) can be exposed to DEHP. Previous studies have shown detectable amounts of DEHP in blood products, in intravenous solutions, and in intravenous fat emulsions stored in PVC bags [1–5]. In animal studies, DEHP and/or MEHP are toxicants to the reproductive and developmental systems [6–10]. DEHP is hydrolyzed to MEHP in vivo and in blood products by esterase activities [11,12]. DEHP and MEHP have been detected in the blood of hemodialysed patients [13,14]. The FDA Center for Devices and Radiological Health (FDA/CDRH) has reviewed the potential health risks of DEHP leaching from medical devices [15]. Furthermore, the FDA/CDRH has recommended considering alternatives when high-risk procedures including transfusion, hemodialysis, total parenteral nutrition, EMO, or enteral nutrition are to be performed on male neonates, pregnant women who are carrying a male fetus, and peripubertal males [16].

To assess patient risk of DEHP and MEHP intake via medical procedures, the concentration of DEHP and MEHP in drugs, blood products and patients' serum should be determined accurately. However, the widespread usage and stability of DEHP in the experimental environment have led to DEHP being present as a ubiquitous contaminant. For this reason, the contamination of DEHP arising from

* Corresponding author. Tel.: +81-6-6972-1321; fax: +81-6-6972-2393.
E-mail address: takatori@iph.pref.osaka.jp (S. Takatori).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.01.056

the environment often injures the reliability of experimental data. There are documented cases of high levels of DEHP contamination in experimental environments and/or including reagents in DEHP measurements [17,18]. To decrease contamination by DEHP, it is reasonable to minimize the cleanup steps, and the apparatus and solvent usage. However, omission of the cleanup steps increases the potential for enough matrices remaining in the analytical samples to interfere with the accurate determination of analytes. To overcome this problem, we have adopted a high performance liquid chromatography with tandem mass spectrometry (LC/MS/MS) system for its high selectivity of the analytes. High performance liquid chromatography (HPLC) systems can measure MEHP without esterification at the carboxylic group of MEHP. Furthermore, elution performed in an isocratic mode is free from detection of MEHP and DEHP from in the LC systems including pump, lines, ferrules and eluents. These are advantages of HPLC systems over gas chromatography systems. Here, we describe a simple and sensitive method for determination of the concentrations of MEHP and DEHP in human serum by using LC/MS/MS.

2. Experimental

2.1. Materials

DEHP (99.6%), MEHP (99.3%), DEHP-d4 (99.0%) and MEHP-d4 (99.8%) were purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan). Environment analytical grade acetone, hexane and acetonitrile were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). HPLC grade acetonitrile and acetic acid were also obtained from Wako Pure Chemical Industries Ltd. The water for HPLC was purified by the Milli-Q system (Milli-Q, Millipore, Saint-Quentin Yvelines, France). The water for extraction was prepared by washing the Milli-Q water with hexane.

To eliminate contamination of DEHP and MEHP from glassware, the glassware was washed twice with acetone and hexane and then baked at 200 °C for 2 h in a clean oven.

2.2. Preparation of standard solutions and human serum

The stock solutions of DEHP, MEHP, and their isotopes were prepared in acetonitrile at 1.0 mg/ml using DEHP and MEHP-free glassware. They were mixed at the desired ratio and serially diluted for calibration curves. Human blood was obtained from four healthy volunteers with syringes made of glass through metal needles. To prepare serum samples, the blood was allowed to stand at 20 °C for 30 min in glass tubes with aluminum foil caps and then centrifuged at 3000 rpm for 10 min. The serum was stored at −40 °C until analysis. To avoid the contamination of DEHP and MEHP, all glassware and metal needles were washed and baked as mentioned above. The gender, age, body weight and nutrition of the volunteers are shown in Table 1.

Table 1
The gender, age, body weight and nutrition of four volunteers (A, B, C and D)

	Gender	Age (years)	Body weight (kg)	Meal[a]	Nutrition[b]
A	F	30	56	M1	*Bread* (40 g), *butter* (3 g), apple (40 g), coffee (400 ml)
				M2	*Rice* (80 g), grilled horse mackerel, deep-fried vegetables (pumpkin, onion, asparagus, eggplant; 20 g each), soup (miso 20 g, sweet potato, radish, 20 g each)
				M3	Pasta (200 g), source (200g; ground meat, tomato, onion, potato, cheese)
				M4	*Bread* (80 g), apple (40 g), coffee (200 ml), *yogurt* (50 g)
B	M	28	63	M1	Rice balls (200g)
				M2	Beef stew (250 g; beef, onion, carrot, potato, source), *deep-fried chicken* (100 g), beer (350 ml)
				M3	Pasta (100 g), mushrooms (30 g), thick white noodles made of wheat flour, salt and water (200 g)
				M4	Bread (90 g), coffee (180 ml)
C	M	29	62	M1	Cereal (30 g), milk (100 ml), coffee (200 ml), banana (90 g)
				M2	Rice (200g), boiled chicken (150 g), *lettuce* (100g), *spinach* (50 g), *soybean pulp* (50 g), soup (miso 20 g, potato, onion, 10 g each)
				M3	*Burger put deep-fried chicken* (200 g), french fries (50 g), *deep-fried chicken* (50 g), orange juice (200ml)
				M4	*Bread* (80 g), blueberry jam (10 g), milk (100 ml)
D	M	34	58	M1	Rice (80 g), *pancake* (200 g; wheat flour, pork, cabbage, egg), soup (miso 20 g, onion 20 g), omelet (20 g)
				M2	Rice (200 g), chinese-style dumpling (300 g; wheat flour, ground meat, chinese cabbage, onion, spring onion), boiled crab (50 g)
				M3	Doughnuts (150 g), *Corn snack foods* (75 g)
				M4	Rice balls wrapped with deep-fried soybean curds (250 g)

Blood sampling was performed at 10 a.m. (set at zero time). The nutrition of the volunteers taken prior to the blood sampling for 26 h was presented.
[a] M1, taken at 3–4 h; M2, taken at 13–16 h; M3, taken at 20–22 h; M4, 24–26 h.
[b] The weight of nutrition was at served. Italicized: nutrition served in a plastic dish or a package.

2.3. Sample preparation procedures

To a tube containing 0.50 g of serum, 10 ng of MEHP-d4 and DEHP-d4 was added at 4 °C. Then, 4.0 ml acetone was added and the sample was sonicated for 2 min and vigorously shaken for 5 min. The serum was centrifuged at 3×10^3 g and the supernatant was collected. To the precipitant, 1.0 ml acetone was added and extracted as described above. The supernatants were combined together and dried under an N_2 stream. To this tube 0.50 ml hexane-washed water and 4.0 μl acetic acid were added. After a 2 min sonication, MEHP and DEHP were extracted four times with 1.0 ml hexane. After drying under an N_2 stream, the extract was resolved in 0.50 ml acetonitrile. The analytical samples were placed in inactivated insert vials capped with aluminum foil and 5.0 μl of these samples were injected into an LC/MS/MS system.

2.4. LC/MS/MS conditions

LC/MS/MS analysis was performed on an API3000 (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization (ESI) interface and an Agilent 1100 series HPLC from Agilent Technologies (Waldbronn, Germany). The HPLC system consisted of a G1312A HPLC binary pump, a G1367A autosampler and a G1379A degasser. A reverse phase HPLC column (Wakosil3C18, 2.0 × 100 mm, 3 μm; Wako Pure Chemical Industries Ltd.) was used. The mobile phases consisted of 100% acetonitrile (A) and 0.05% aqueous acetic acid (B). Elution was performed using an isocratic mode (A/B: 15/85, v/v) at 0.2 ml/min. The ESI interface was controlled by the Analyst software (v.1.3.2). ESI-MS was operated in negative or positive ion mode. The heated capillary and voltage were maintained at 500 °C and ±4.0 kV (negative/positive mode), respectively. MEHP and MEHP-d4 were detected in the negative mode. DEHP and DEHP-d4 were detected in the positive mode. Daughter ion mass spectra of MEHP, MEHP-d4, DEHP and DEHP-d4 obtained by the LC/MS/MS system are shown in Fig. 1. The combinations of parent ions and daughter ions were as follows; MEHP (parent ion/daughter ion, 277/134), MEHP-d4 (281/138), DEHP (391/149), DEHP-d4 (395/153). The daughter ions were formed in the collision cell using N_2 gas as the collision gas. The optimum collision energies for MEHP (MEHP-d4) and DEHP (DEHP-d4) were −22.0 and 27.0 V, respectively.

3. Results

The retention times of MEHP, MEHP-d4, DEHP and DEHP-d4 were 3.0, 3.0, 25.6 and 25.3 min, respectively. The relative standard deviations of the retention times were <0.03%. The signal to noise ratios of the MRM (multiple reaction monitoring) of 1 ng/ml MEHP and DEHP were 4.0 and 3.5, respectively. For MEHP measurement, the calibration curve was obtained for the peak-area ratio (MEHP/MEHP-d4) versus the MEHP concentration. It was linear over the range of 2.0–500 ng/ml. The mean linear regression equations obtained from five replicates were $y = 0.0581x - 0.097$ ($r = 0.999$) with mean values for slope and intercept of 0.0581 ± 0.0012 (mean ± S.D.; S.D., standard deviation) and -0.097 ± 0.017, respectively (y, peak-area

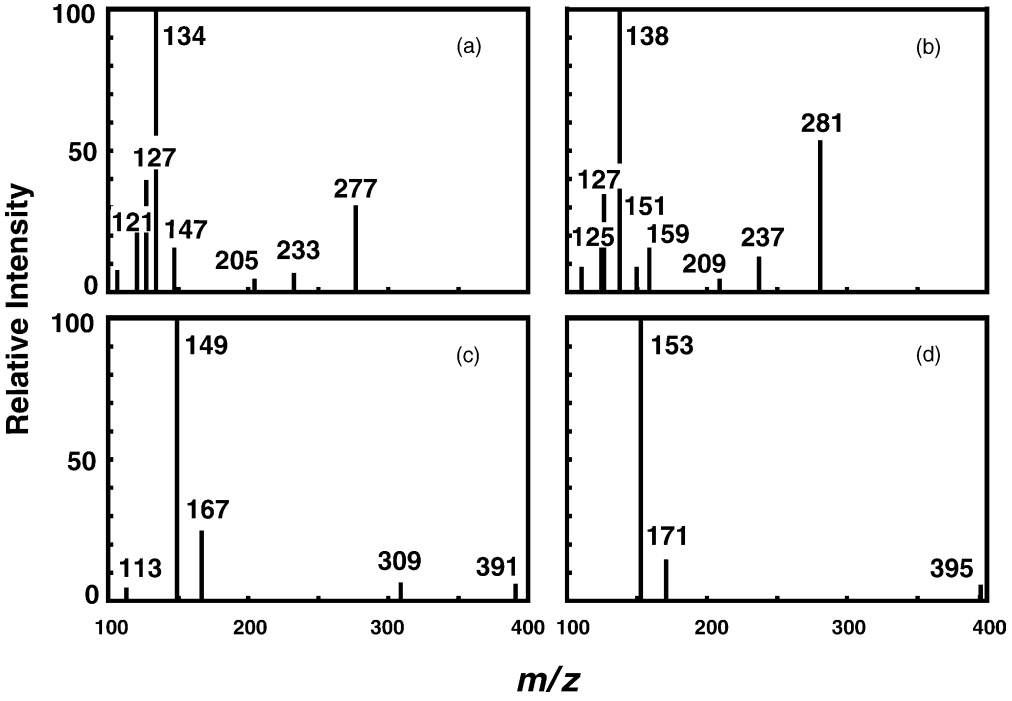

Fig. 1. Daughter ion spectra of MEHP (a), MEHP-d4 (b), DEHP (c), and DEHP-d4 (d).

Table 2
Concentrations of MEHP and DEHP in human sera

Serum from volunteers	Concentration (ng/ml)	
	MEHP[a]	DEHP[b]
A	5.7 ± 2.7	N.D. (3.8 ± 1.3)
B	N.D. (4.1 ± 1.5)	N.D. (3.7 ± 0.8)
C	N.D. (3.3 ± 0.6)	N.D. (2.9 ± 0.6)
D	N.D. (3.4 ± 0.6)	N.D. (3.9 ± 1.0)
Blank	N.D. (<1.0)	N.D. (2.2 ± 0.6)

The blank was the result of measurements of MEHP and DEHP in hexane washed water which contained >1 ng/ml MEHP and 1 ng/ml DEHP. Values in parentheses represent averages of the five independent measurements and SDs.

[a] N.D.; MEHP concentrations lower than 5 ng/ml.

[b] N.D.; DEHP concentrations lower than 14.0 ng/ml.

ratio; x, MEHP concentration ng/ml). For DEHP measurement, the calibration curve was obtained for the peak-area ratio (DEHP/DEHP-d4) versus DEHP concentration. It was linear over the range of 1.0–4000 ng/ml. The mean linear regression equations obtained from five replicates were $y = 0.0318x + 0.337$ ($r = 0.999$) with mean values for slope and intercept of 0.0318 ± 0.0012 and 0.337 ± 0.035, respectively (y, peak-area ratio; x, DEHP concentration ng/ml). The recovery tests were performed using MEHP-d4 and DEHP-d4 to avoid the effects of possible contamination by MEHP and DEHP. The recoveries of 20 ng/ml of MEHP-d4 and DEHP-d4 from human serum were 101 ± 5.7 ($n = 6$) and 102 ± 6.5% ($n = 6$), respectively. The recoveries of 100 ng/ml of MEHP-d4 and DEHP-d4 from human serum were 93.8 ± 6.8 ($n = 6$) and 102 ± 6.2% ($n = 6$), respectively.

To determine the contamination of DEHP and MEHP generated by this extraction method, a blank test was performed using hexane-washed water instead of human serum. The concentrations of MEHP and DEHP in sera of healthy volunteers and the blank are shown in Table 2. The typical MRM chromatogram of the human serum is shown in Fig. 2. The

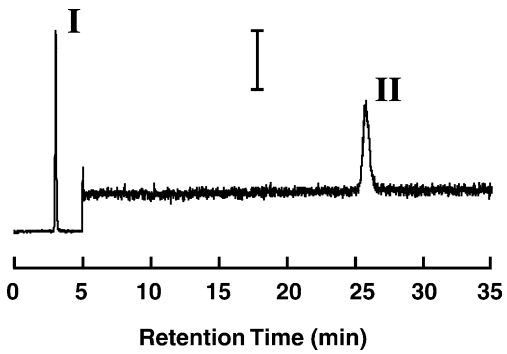

Fig. 2. The MRM chromatogram of human serum. From 0 to 5 min, monitoring of the daughter ion (m/z 134) of the parent ion (m/z 277), is in the negative mode. From 5 to 35 min, monitoring of the daughter ion (m/z 149) of the parent ion (m/z 391), is in the positive mode. The bar corresponds to 5.0×10^3 counts per second. The concentrations of MEHP (I) and DEHP (II) were estimated at 3.2 and 3.7 ng/ml, respectively.

blank of MEHP and DEHP were <1.0 and 2.2 ± 0.6 ng/ml, respectively. The limits of quantification (LOQ) of MEHP and DEHP in this method were determined by the formula, LOQ = 5 x (the blank + S.D.), and were 5 and 14.0 ng/ml, respectively. The concentrations of MEHP and DEHP in sera of healthy volunteers were at or below the LOQ. The concentrations of MEHP and DEHP under the LOQ are shown in parentheses. The concentrations include the blank levels of MEHP and DEHP. Thus, the net concentrations of MEHP and DEHP in the human serum were estimated at or <5 and <2 ng/ml, respectively.

4. Discussion

Severe contamination of MEHP and DEHP make it difficult to know the accurate concentrations of MEHP and DEHP in normal serum. In our trial to determine the MEHP in the serum by using gas chromatography with mass spectrometry, the contaminations of MEHP and DEHP derived from the esterification with 2,3,4,5,6-pentafluorobenzylbromide and subsequent clean up were 120 and 420 ng/ml, respectively (data not shown). By using an LC/MS/MS system, we developed a method to measure the concentrations of MEHP and DEHP with a low level contamination, and demonstrated that in serum of healthy volunteers these concentrations were at or less than the LOQ (5 and 14.0 ng/ml, respectively). Kessler et al. concluded that MEHP and DEHP in blood obtained from rats actually presented minute amounts, because there was no difference between the concentrations obtained from rat blood and water [17]. Inoue et al. have developed a method using LC/MS with column-switching systems for measurement of MEHP and DEHP in human blood samples and demonstrated that the concentrations of MEHP and DEHP in serum of healthy volunteers were <5 and <25 ng/ml, respectively [19]. Our results confirm their findings.

The direct injection methods using a column switching LC/MS system [19] and a solid-phase microextraction/HPLC [20] were effective in minimizing the contaminations of MEHP and DEHP during experimental procedures. However, these methods would have the potential for loading matrices into the LC/MS system or HPLC, which interfere with the accurate determination of analytes. The reliability of experimental data supported by MS/MS is one of the advantages of this method. Especially in the case of shortened cleanup steps, this advantage would be important. In our procedure, a large part of the contamination came from the solvents. Adopting the column switching system instead of the extraction steps in our procedures to decrease solvent usage would be possibly minimize the contamination and set the LOQ lower.

EU and IARC estimated that the human daily oral DEHP intake would be in the range of 5–21 μg/kg per day [21,22]. However, the concentrations of MEHP and DEHP in the serum of human that were orally administrated DEHP in

that range, have not yet been determined. After oral administration of DEHP, the concentration of DEHP in serum is lower than that of MEHP since a large part of orally administrated DEHP is absorbed after hydrolyzing to MEHP in the intestine [23,24]. The ratio of the concentration of MEHP and that of DEHP (MEHP/DEHP) in serum was 6–12:1 in rats [23,24]. In this study, MEHP/DEHP in human serum was calculated to be 2.0–4.7:1. There is difference in DEHP hydrolysis activities to MEHP among several species [12]. The DEHP hydrolysis activity of human intestine was conceived to be lower than that of the rat [12]. Assuming that the DEHP hydrolysis activity in the intestine reflect the MEHP/DEHP in serum, the large part of MEHP and DEHP detected in the serum should be sourced from the volunteers' nutrition. The concentrations of MEHP and DEHP in volunteers' nutrition did not determined in this study. There are few studies of human about relationship between the dose of orally administrated DEHP and the concentrations of MEHP and DEHP in serum [25]. To assess the daily exposure level of DEHP, determination of the concentrations of MEHP and DEHP in human serum would be informative.

The leaching of DEHP from medical devices into solutions was affected by the lipid content, the flow rate of solutions [26,27], and the concentration of the surface-active agent [28]. The exposure of DEHP to infants via TPN was estimated to be non-negligible from model studies [27]. To minimize the exposure of MEHP and DEHP to patients, improvement of medical devices using PVC, and determination of the checkpoints for handling of the medical devices would be important. Furthermore, model studies of the leaching of DEHP from medical devices, as well as investigations of the relationship between contamination and storage conditions of materials;, such as time, temperature and light would be informative to improve the medical devices. Changing DEHP in the medical device to an alternative would be effective in decreasing the exposure of MEHP and DEHP to patients. As a candidate of an alternative plasticizer for DEHP, trioctyltrimellitate (TOTM) is being used in medical devices for its minimal leaching and low hepatic toxicity [29–31]. For the safety of patients, more knowledge of the toxicities and application of TOTM in medical devices will be required. Thus, the risk assessment of medical usage of DEHP and the improvement of medical devices using DEHP should be continued. To achieve these goals, reliable methods for the measurement of MEHP and DEHP in blood is required. The method reported here would be applicable towards this end.

Acknowledgements

This research was supported by "Health Science Research Grants, 2003" from the Ministry of Health, Labor, and Welfare.

References

[1] T. Dine, M. Luyckx, M. Cazin, C. Brunet, J.C. Cazin, F. Goudaliez, Biomed. Chromatogr. 5 (1991) 94.
[2] T. Hanawa, E. Muramatsu, K. Asakawa, M. Suzuki, M. Tanaka, K. Kawanao, T. Seki, K. Juni, S. Nakajima, Int. J. Pharm. 210 (2000) 109.
[3] M.A. Faouzi, F. Khalfi, T. Dine, M. Luyckx, C. Brunet, B. Gressier, F. Goudaliez, M. Cazin, J. Kablan, A. Belabed, J.C. Cazin, J. Pharm. Biomed. Anal. 21 (1999) 923.
[4] W.N. Waugh, L.A. Trissel, V.J. Stella, Am. J. Hosp. Pharm. 48 (1991) 1520.
[5] H.I. Mazur, D.J. Stennet, P.K. Egging, J. Parent. Enter. Nutr. 13 (1989) 59.
[6] T.J.B. Gray, S.D. Gangolli, Environ. Health Perspect. 65 (1986) 229.
[7] P. Sjöberg, U. Bondesson, T.J.B. Gray, L. Plöen, Acta Pharmacol. Toxicol. 58 (1986) 225.
[8] E.J. Moss, M.W. Cook, L.V. Thomas, T.J.B. Gray, Toxicol. Lett. 40 (1988) 77.
[9] P.W. Albro, R.E. Chapin, J.T. Corbett, J. Schroeder, J.L. Phelps, Toxicol. Appl. Pharmacol. 100 (1989) 193.
[10] B.J. Davis, R. Weaver, L.J. Gaines, J.J. Heindel, Toxicol. Appl. Pharmacol. 128 (1994) 224.
[11] P.W. Albro, R.O. Thomas, Biochem. Biophys. Acta 360 (1973) 380.
[12] B.G. Lake, J.C. Phillips, J.C. Linnell, S.D. Gangolli, Toxicol. Appl. Pharmacol. 39 (1977) 239.
[13] L.M. Flaminio, R. Bergia, L.D. Angelis, M. Ferazza, M. Marinovich, G. Galli, C.L. Galli, Int. J. Artificial Organs 11 (1988) 428.
[14] G.M. Pollack, J.F. Buchanan, R.L. Slaughter, R.K. Kohli, D.D. Shen, Toxicol. Appl. Pharmacol. 79 (1985) 257.
[15] Center for Devices and Radiological Health, US Food and Drug Administration, 2001, http://www.fda.gov/cdrh/ost/dehp-pvc.pdf.
[16] Center for Devices and Radiological Health, US Food and Drug Administration, 2002, http://www.fda.gov/cdrh/safety/dehp.html.
[17] W. Kessler, W. Phokha, G.A. Csanády, J.G. Filser, Arch. Toxicol. 75 (2001) 62.
[18] T. Suzuki, K. Yaguchi, S. Suzuki, T. Suga, Environ. Sci. Technol. 35 (2001) 3757.
[19] K. Inoue, M. Kawaguchi, F. Okada, Y. Yoshimura, H. Nakazawa, Anal. Bioanal. Chem. 375 (2003) 527.
[20] K. Mitani, S. Narimatsu, F. Izushi, H. Kataoka, J. Pharm. Biomed. Anal. 32 (2003) 469.
[21] Scientific Committee for Toxicity, Ecotoxicity and the Environment (CASTEE), Opinion on the results of the risk assessment of: Bis(2-ethylhexyl)phthalate (DEHP)-opinion expressed at 29th CASTEE plenary meeting, Brussels, 9 January 2002, http://europa.eu.int/comm/food/fs/sc/sct/out141_en.pdf.
[22] International Agency for Research on Cancer (IARC), di(2-ethlhexyl)phthalate, IARC Monographs, vol. 77, Lyon, 2000, p. 41.
[23] G.M. Pollack, R.C.K. Li, J.C. Ermer, D.D. Shen, Toxicol. Appl. Pharmacol. 79 (1985) 246.
[24] O.A. Teirlynck, F. Belpaire, Arch. Toxicol. 57 (1985) 226.
[25] H.M. Koch, H.M. Bolt, J. Angerer, Arch. Toxicol., in press.
[26] K. Kambia, T. Dine, B. Gressier, A.F. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand, J. Chromatogr. B 755 (2001) 297.
[27] K. Kambia, T. Dine, B. Gressier, S. Bah, A.F. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand, Int. J. Pharm. 262 (2003) 83.
[28] T. Hanawa, N. Endoh, F. Kazuno, M. Suzuki, D. Kobayashi, M. Tanaka, K. Kawano, Y. Morimoto, S. Nakajima, T. Oguchi, Int. J. Pharm. 267 (2003) 141.
[29] L.M. Flaminio, L.D. Angelis, M. Ferazza, M. Marinovich, G. Galli, C.L. Galli, Int. J. Art. Org. 11 (1988) 435.
[30] K. Rathinam, S.P. Srivastava, P.K. Seth, J. Appl. Toxicol. 10 (1990) 39.
[31] K. Kambia, T. Dine, B. Gressier, M. Luyckx, C. Brunet, Int. J. Pharm. 229 (2001) 139.