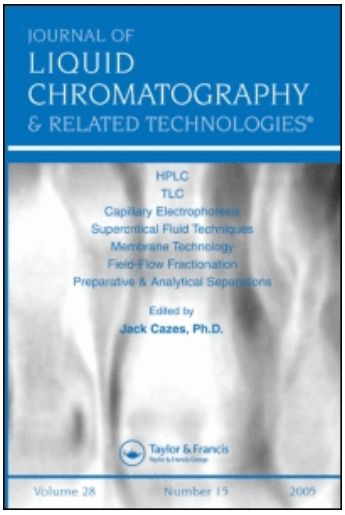

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Simultaneous Determination of Di(2-ethylhexyl)phthalate, Mono(2-ethylhexyl)phthalate, and Phthalic Acid Migrating from Gamma-Ray Irradiated Polyvinyl Chloride Sheet by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract: The aim of the present study was to establish a liquid chromatography-tandem mass spectrometric (LC-MS/MS) method for the simultaneous determination of di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl)phthalate (MEHP), and phthalic acid (PA). In the proposed method, the limits of detection of DEHP, MEHP, and PA were 5, 0.5, and 1 ng/mL, respectively, and the limits of quantification with standard solutions were 20, 2, and 5 ng/mL, respectively. Intra- and interday assays showed good accuracy and repeatability. The recoveries of DEHP, MEHP, and PA from respective extraction solvents ranged from 98.9 to 104.2% (relative standard deviation was below 10.3%). DEHP and its breakdown products migrating from gamma-ray irradiated polyvinyl chloride (PVC) sheets were determined simultaneously since DEHP is easily eluted from PVC medical devices. DEHP migration was noted from

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both gamma-ray irradiated and control PVC sheets. Compared with the migration from the control PVC sheet, MEHP showed significant migration from the gamma-ray irradiated PVC sheet. In contrast, PA migration was noted only from the gamma-ray irradiated PVC sheet.

Keywords: LC-MS/MS, Di(2-ethylhexyl)phthalate, Mono(2-ethylhexyl)phthalate, Phthalic acid, Gamma-ray sterilization

INTRODUCTION

Phthalate esters, particularly di(2-ethylhexyl)phthalate (DEHP), are extensively used as plasticizers to increase the flexibility of polyvinyl chloride (PVC) products. PVC is one of the most widely used plastic polymers in such medical products as blood containers, blood tubing, and catheters. However, it has been reported that DEHP was easily eluted from PVC products into food, drugs, and body fluids.[1–4] DEHP is considered to exhibit reproductive and developmental toxicity,[5,6] carcinogenicity, and testicular toxicity.[7–9] It was also found to affect the reproductive organs and fertility.[10] It has been reported that DEHP is hydrolyzed enzymatically into mono(2-ethylhexyl)phthalate (MEHP),[11–13] and that MEHP may be even more toxic than the parent compound. In vitro studies have shown that MEHP inhibits FSH stimulated cAMP accumulation in cultured Sertoli cells,[14–18] in addition to reducing 17β-estradiol production and aromatase mRNA expression.[19,20] DEHP was determined to be the common plasticizer migrating from PVC medical devices into the blood.[2,3] MEHP was also determined to be the metabolite of DEHP. PA was not determined because it is not as toxic as DEHP or MEHP and the amount of PA in blood is negligible, although it is also produced by the enzymatic hydrolysis of DEHP.[21]

In our previous studies, we observed that not only DEHP but also MEHP migrated from PVC medical devices into simulated pharmaceuticals even without enzymatic hydrolysis.[4,22] In addition, we found that hydrolysis may occur during the sterilization process, particularly gamma-ray sterilization.[22] Therefore, a method for the simultaneous analysis of DEHP, MEHP, and PA was developed to confirm that MEHP and PA are produced from DEHP even without enzymatic hydrolysis. In addition, the method was used to determine DEHP, MEHP, and PA migrating from gamma-ray irradiated PVC sheets into purified water, 5% glucose solution, and polyoxyethylated hydrogenated castor oil 60 (HCO-60). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was proven to be a suitable method for the determination of DEHP, MEHP, and PA with high sensitivity, precision, and selectivity.

EXPERIMENTAL

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). PA and PA-d_4 were purchased from CDN Isotope Central Chemicals Co., Inc. (Tokyo, Japan). Phthalic acid esters, analytical grade acetonitrile, and acetone were used in the experiments. Analytical grade formic acid was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 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 sheets subjected to gamma-ray (^{60}Co; 24.2 kGy). The gamma-ray doses were set with reference to sterilization conditions used by commercial medical devices. The control sample was not irradiated gamma-ray. These PVC sheets were 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.

Instrumentation and Chromatographic Conditions

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000^{TM} (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo Ionspray^{TM} ionization source. Mass spectrometry data were processed with Analyst 1.3.2 software. An Inertsil-Ph3 column (50 mm × 2.1 mm, 5 μm particle size) from GL Sciences was used for the separation.

After 5 μL of the sample was injected with an autosampler, it was loaded onto the analytical column by flowing mobile phase at the flow rate of 0.2 mL/min. Acetonitrile (mobile phase A) and 0.05% formic acid in water (mobile phase B) were used. Separation was carried out with the following profile: mobile phase A/B was 15/85 (0–4 min) → 90/10 (4.01–15 min for elution) → 15/85 (15.01–25 min for equilibration) (v/v). The column oven was maintained at 40°C for LC.

MS/MS Conditions

The working parameters for turbo ion spray ionization MS/MS were as follows: curtain gas flow rates, 10 psi (DEHP and DEHP-d_4 for positive ion mode) and

20 psi (MEHP, PA, and their internal standards for negative ion mode); nebulizer gas (N_2) pressure, 20 psi for positive ion mode and 30 psi for negative ion mode; and turbo ion spray gas (N_2) pressure, 10 psi for positive ion mode and 80 psi for negative ion mode. The ion source temperature was maintained at 650°C and the turbo ion spray voltages for positive ion mode (DEHP, DEHP-d_4) and negative ion mode (MEHP, PA, and their internal standards) were 3500 and −4500 V, respectively. DEHP and DEHP-d_4 were detected in the positive ion mode, whereas MEHP, PA, and their internal standards were detected in the negative ion mode. The product ion mass spectra of DEHP, MEHP, and PA obtained by the LC-MS/MS system are shown in Figure 1. 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), MEHP-d_4 (m/z 281 → 138), PA (m/z 165 → 121), and PA-d_4 (m/z 169 → 125). The collision gas (N_2) pressures were set at 5 units (positive ion mode) and 4 units (negative ion mode).

Method Validation

After selection of the optimum conditions for sample preparation and LC-MS/MS, the method was thoroughly evaluated using DEHP, MEHP, and PA standard solutions. The linearity of the response of this system was examined with a calibration curve obtained at six different concentrations of the standard solution containing the certain amount of internal standard. Linear regression was performed using the ratio of DEHP peak area/DEHP-d_4 (internal standard) peak area plotted against the concentration. The calibration curves for MEHP and PA were also obtained in the same way. To assess the accuracy and precision of this method, low and high quality control samples were determined by replicate analysis. Intraday precision and accuracy were determined by replicate analysis of standard solutions in one day ($n = 3$), and interday precision and accuracy were determined over a span of three days.

The method was applied to 5% glucose solution and HCO-60 (0.02 mg/mL) samples that were spiked with 100 ng/mL DEHP, MEHP, and PA standards and certain amounts of internal standards. Each recovery was obtained from three replicates.

Migration Test

The migration of DEHP, MEHP, and PA from PVC sheets (1 × 3 cm) into 5 mL of each extraction solvent was examined. Five percent glucose solution, HCO-60, and purified water were used as extraction solvent, and served as simulated pharmaceuticals. HCO-60 is a surfactant that is

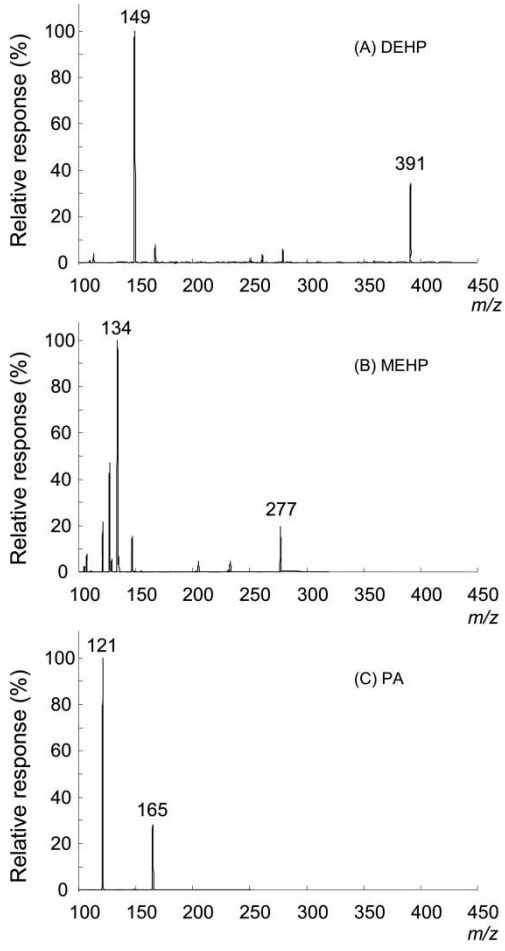

Figure 1. Product ion mass spectra of DEHP, MEHP and PA standard solutions. (A) 1 μg/mL DEHP standard solution, (B) 1 μg/mL MEHP standard solution, (C) 1 μg/mL PA standard solution. Each standard solution was infused directly into the MS system.

involved in the migration of DEHP into drugs such as Prograf®. The extent of DEHP migration was dependent on the concentration of HCO-60;[23] however, the injection of DEHP at high concentrations contaminated the MS system. Therefore, in this study, 0.02 mg/mL HCO-60 was prepared for the migration test. The samples were kept in test tubes and extraction was carried out by shaking at room temperature for 1 hr. A 1 mL aliquot of the extract was pipetted into another test tube, and DEHP-d_4, MEHP-d_4, and PA-d_4 were added. Then, the sample solution was appropriately diluted prior to LC-MS/MS analysis.

RESULTS AND DISCUSSION

Optimizing the LC-MS/MS Method

In the scan mode, DEHP, MEHP, and PA were monitored at m/z 391, 277, and 165 which were assigned to $[M + H]^+$, $[M\text{-}H]^-$, and $[M\text{-}H]^-$, respectively. Moreover, in the product ion MS/MS measurement, the selective reaction monitoring ions (SRM) of DEHP, DEHP-d_4, MEHP, MEHP-d_4, PA, and PA-d_4 were set depending upon their precursor ions. For the separation and the MS ionization, formic acid was added to purified water as the mobile phase. The optimum concentration of formic acid in purified water was 0.05% (Figure 2). In addition, the sample solution was acidified (1%) to improve separation. No interference from peaks of other compounds present in the extraction solvents was noted. The SRM chromatograms of DEHP, MEHP, and PA spiked into HCO-60 were shown in Figure 3.

Validation of the Method

In the proposed method, the limits of detection (LODs; signal-to-noise ratio = 3) of DEHP, MEHP, and PA were 5, 0.5, and 1 ng/mL, respectively. The limits of quantification (LOQs) (signal-to-noise ratio > 10) of DEHP, MEHP, and PA were 20, 2, and 5 ng/mL, 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 20 to 1000 ng/mL ($r = 0.999$). 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 2 to 1000 ng/mL ($r = 0.999$). For PA measurement, a calibration curve was obtained by plotting the peak area ratio (PA/PA-d_4) versus PA concentration, and was linear over the range of 2 to 1000 ng/mL ($r = 0.999$).

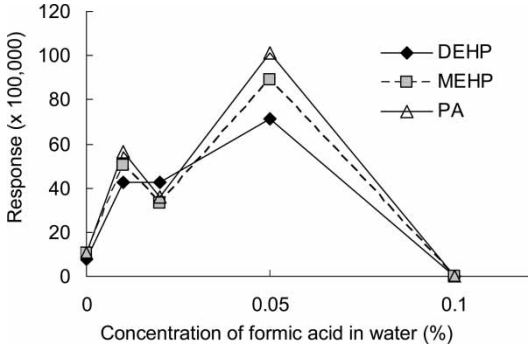

Figure 2. Effect of concentration of formic acid in purified water on response.

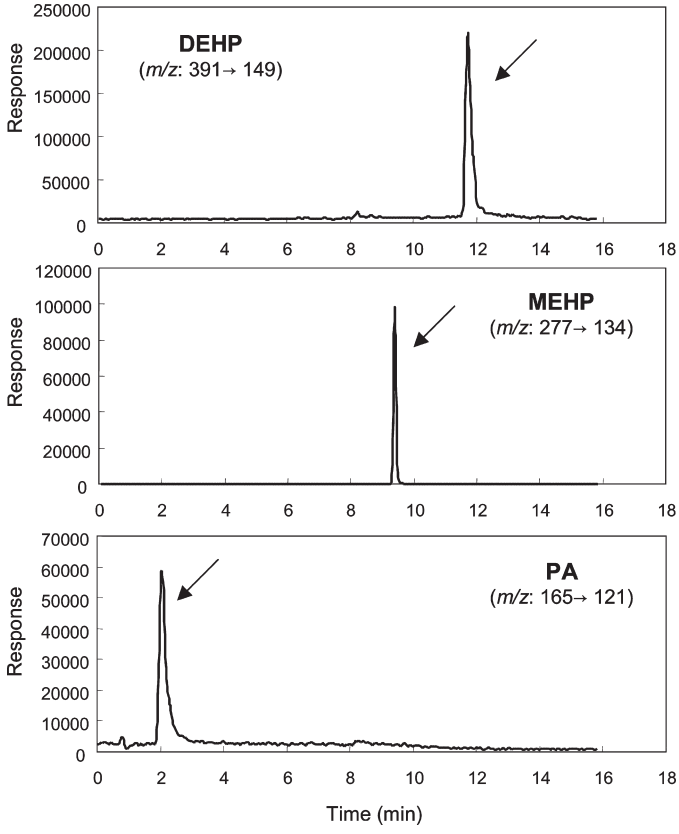

Figure 3. Chromatograms of DEHP, MEHP, PA spiked into HCO-60. 100 ng/mL of DEHP, MEHP, and PA were spiked into HCO-60 solution. SRM chromatograms were monitored as follows; DEHP (m/z 391 → 149), MEHP (m/z 277 → 134), and PA (m/z 165 → 121).

Intraday precision was expressed as relative standard deviation (RSD), which was calculated by measuring low (50 ng/mL) and high (500 ng/mL) concentrations of the standard solution three times (n = 3) in one day. Interday precision and accuracy were calculated using values measured at two concentrations (50 and 500 ng/mL) of the standard solutions over a span of three days. As Table 1 shows, all values of intra- and interday precision were less than 10%.

We also examined recovery using 5% glucose solution and HCO-60 as extraction solvents. For 5% glucose solution that was spiked with 100 ng/mL DEHP, MEHP, and PA, the average recoveries ranged from 100.4 to 104.2% (RSD <9.5%; Table 2). For HCO-60 that was spiked with 100 ng/mL DEHP, MEHP, and PA, the average recoveries ranged from 98.9 to 102.9% (RSD <10.3%; Table 2).

Table 1. Results of intra- and interday assays to validate proposed LC-MS/MS method

		Intraday			Interday		
Analyte	Concentration (ng/mL)	Detected average (ng/mL)	RSD (%)	Accuracy (%)	Detected average (ng/mL)	RSD (%)	Accuracy (%)
DEHP	50	54.4	4.0	106.8	50.3	8.9	100.6
	500	508.6	0.8	101.7	503.6	1.4	100.7
MEHP	50	48.9	3.5	97.8	48.6	1.1	97.1
	500	500.9	1.6	100.2	503.0	1.3	100.6
PA	50	49.9	4.8	99.9	49.4	1.6	98.9
	500	501.5	1.7	100.1	503.0	0.3	100.6

(n = 3).

Table 2. Recoveries of DEHP, MEHP and PA

Compound	Spiked amount (ng/mL)	Average recovery (%)		
		Water	5% Glucose solution	0.02 mg/mL HCO-60
DEHP	100	106.9 ± 6.9	104.2 ± 5.9	100.6 ± 10.3
MEHP	100	104.2 ± 2.4	103.4 ± 9.5	102.9 ± 5.8
PA	100	105.6 ± 7.6	100.4 ± 1.8	98.9 ± 8.2

(Mean ± SD, n = 3).

DEHP, MEHP, and PA Migration from Gamma-Ray Irradiated PVC Sheet

The proposed method was applied to the determination of DEHP, MEHP, and PA migration from the gamma-ray irradiated PVC sheet. DEHP migrated from both irradiated and unirradiated PVC sheets. The concentrations of DEHP that migrated from gamma-ray irradiated and unirradiated PVC sheets into purified water, or 5% glucose solution, were almost the same level (53.0–69.1 ng/mL). In contrast, the concentrations of DEHP that migrated from irradiated and unirradiated PVC sheets into HCO-60 were both high level (average concentration: 88.9 ng/mL) compared with the other solution. These concentrations were similar to those reported previously.[22] In our previous study, we noted that temperature and optical irradiation had an influence on DEHP release from the PVC sheet.[24] Therefore, DEHP release from the examined PVC sheet might have been influenced by temperature and/or optical irradiation, although the PVC sheet was stored in the dark at room temperature. The concentrations of MEHP that migrated from gamma-ray irradiated and unirradiated PVC sheets were also similar to those reported previously.[22] Gamma-ray irradiated PVC sheets released a high concentration of MEHP (Figure 4). In contrast, PA migration from unirradiated PVC sheets into any of the extraction solvents was not detected, whereas the gamma-ray irradiated PVC sheets released detectable levels of PA (Figure 4).

In our previous study, not only DEHP but also MEHP migrated from PVC medical devices into simulated pharmaceuticals. MEHP migration from PVC sheets was detected even though MEHP was not used as a plasticizer. In addition, MEHP was detected in gamma-ray irradiated PVC sheets but was not detected in PVC sheets sterilized by autoclaving or exposure to ethylene oxide gas.[22] The concentration of MEHP migrating from gamma-ray irradiated PVC sheets was significantly high compared with that from the unirradiated ones. Moreover, the concentration of PA migrating from gamma-ray irradiated PVC sheets was increased compared with unirradiated ones. MEHP and PA migrated from gamma-ray irradiated PVC sheets into

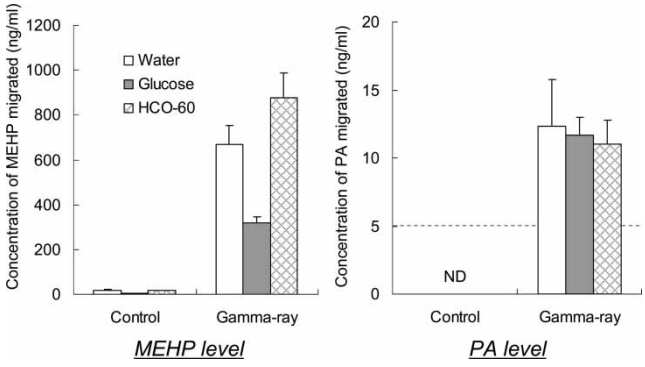

Figure 4. Concentrations of MEHP and PA migrating into various solutions from gamma-ray irradiated PVC sheets. Control is a PVC sample without gamma-ray irradiation. Each column is the mean of triplicate analysis (n = 3). Error bar represents standard deviation (S.D.). ND means "not detected." The dotted line at 5 ng/mL PA represents the limit of quantification.

both HCO-60 and purified water. We have already shown that MEHP was produced from DEHP as a breakdown product.[22] MEHP was produced by cleavage of one of two ester bonds in DEHP (Figure 5). We surmise that if MEHP was produced from DEHP by gamma-ray irradiation, PA would be produced by the same mechanism.

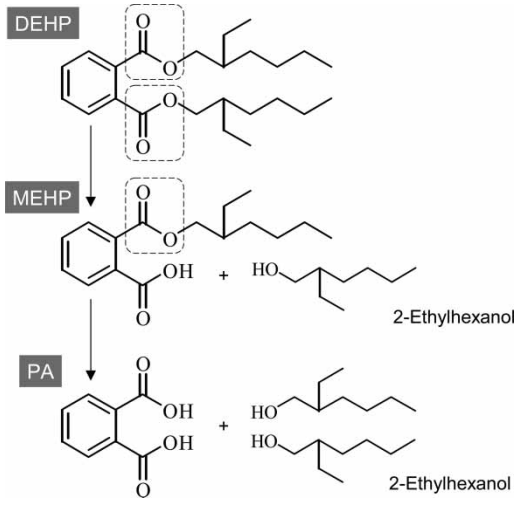

Figure 5. Chemical structures of DEHP, MEHP, and PA. The dotted circle represents the ester bond. MEHP was produced by cleavage of one of two ester bonds in DEHP. PA was produced by cleavage of two ester bonds in DEHP, and by cleavage of an ester bond in MEHP.

CONCLUSIONS

In this study, a method for the simultaneous determination of DEHP, MEHP, and PA was developed. The method had sufficient precision and accuracy to determine the concentrations of DEHP and its breakdown products migrating from PVC medical devices. Using the developed method, not only MEHP but also PA was found to be the breakdown product of DEHP. MEHP is thought to be more toxic than DEHP. The assessment of DEHP exposure in high risk patients is necessary to determine exposure to MEHP and PA, although PA is not as toxic as MEHP.

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REFERENCES

1. Earls, A.O.; Axford, I.P.; Braybrook, J.H. J. Chromatogr. A **2003**, *983*, 237–246.
2. Inoue, K.; Kawaguchi, M.; Okada, F.; Yoshimura, Y.; Nakazawa, H. Anal. Bioanal. Chem. **2003**, *375*, 527–533.
3. Takatori, S.; Kitagawa, Y.; Kitagawa, M.; Nakazawa, H.; Hori, S. J. Chromatogr. B **2004**, *804*, 397–401.
4. Ito, R.; Seshimo, F.; Miura, N.; Kawaguchi, M.; Saito, K.; Nakazawa, H. J. Pharm. Biomed. Anal. **2005**, *39*, 1036–1041.
5. Koizumi, M.; Ema, M.; Hirose, A.; Hasegawa, R. Jpn. J. Food Chem. **2000**, *7*, 65–73.
6. Koizumi, M.; Ema, M.; Hirose, A.; Kurokawa, Y.; Hasegawa, R. Jpn. J. Food Chem. **2001**, *8*, 1–10.
7. Tickner, J.A.; Schettler, T.; Guidotti, T.; McCally, M.; Rossi, M. Am. J. Ind. Med. **2001**, *39*, 100–111.
8. Yakubovich, M.; Vienken, J. Med. Device Technol. **2000**, *11*, 18–21.
9. Hill, S.; Shaw, B.; Wu, A. Clin. Chim. Acta **2001**, *304*, 1–8.
10. Thomas, J.A.; Northup, S.J. Toxicol. Environ. Health **1982**, *9*, 141–152.
11. Lake, B.G.; Phillips, J.C.; Linnel, J.C.; Gangolli, S.D. Toxicol. Appl. Pharmacol. **1977**, *39*, 239–248.
12. Albro, P.W.; Thomas, R.O. Biochim. Biophys. Acta **1973**, *306*, 380–390.
13. Albro, P.W.; Lavenhar, S.R. Drug Metab. Rev. **1989**, *21*, 13–34.
14. Heindel, J.J.; Chapin, R.E. Toxicol. Appl. Pharmacol. **1989**, *97*, 377–385.
15. Grasso, P.; Heindel, J.J.; Powell, C.J.; Reichert, L.E. Biol. Reprod. **1993**, *48*, 454–459.
16. Richburg, J.H.; Boekelheide, K. Toxicol. Appl. Pharmacol. **1996**, *137*, 42–50.

17. Lee, J.; Richburg, J.H.; Shipp, E.B.; Meistrich, M.L.; Boekelheide, K. Endocrinology **1999**, *140*, 852–858.
18. Richburg, J.H.; Nanex, A.; Williams, L.R.; Embree, M.E.; Boekelheide, K. Endocrinology **2000**, *141*, 787–793.
19. Davis, B.J.; Maronpot, R.R.; Heindel, J.J. Toxicol. Appl. Pharmacol. **1994**, *128*, 216–223.
20. Lovekamp, T.N.; Davis, B.J. Toxicol. Appl. Pharmacol. **2001**, *172*, 217–224.
21. Shintani, H. Chromatographia **2000**, *52*, 721–726.
22. Ito, R.; Seshimo, F.; Miura, N.; Kawaguchi, M.; Saito, K.; Nakazawa, H. J. Pharm. Biomed. Anal. **2006**, *41*, 455–460.
23. Haishima, Y.; Seshimo, F.; Higuchi, T.; Yamazaki, H.; Hasegawa, C.; Izumi, S.; Makino, T.; Nakahashi, K.; Ito, R.; Inoue, K.; Yoshimura, Y.; Saito, K.; Yagami, T.; Tsuchiya, T.; Nakazawa, H. Int. J. Pharm. **2005**, *298*, 126–142.
24. Ito, R.; Seshimo, F.; Haishima, Y.; Hasegawa, C.; Isama, K.; Yagami, T.; Nakahashi, K.; Yamazaki, H.; Inoue, K.; Yoshimura, Y.; Saito, K.; Tsuchiya, T.; Nakazawa, H. Int. J. Pharm. **2005**, *303*, 104–112.

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