

Di(2-ethylhexyl)phthalate DEHP

Abstract

Validation of High Performance Liquid Chromatographic Method with UV Detector for the Determination of Di(2-ethylhexyl)Phthalate in Plasma in some Korean Male Workers

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Objectives: This study was conducted to validate a simple, rapid and sensitive reverse-phase high-performance liquid chromatographic method with UV detector (HPLC-UV) and present the plasma level of di(2-ethylhexyl)phthalate (DEHP) in some Korean male workers.

Methods: HPLC-UV for quantification of plasma DEHP was validated by the following guideline from the Center for Drug Evaluation and Research (CDER) - calibration/standard curve, precision, accuracy and recovery. Plasma DEHP from 255 healthy Korean male workers aged from 30 to 60 years was analyzed by validated HPLC-UV method.

Results: The calibration curve over the range 0~150 µg/ for the plasma DEHP standard solution showed linearity($r^2=0.999$). The limit of detection (LOD) and limit of quantification (LOQ) of plasma DEHP were 5.22 µg/ and 15.81 µg/ , respectively. The accuracy and precision for 2.5 µg/ of DEHP were acceptable in CDER guideline on the second and third day but not first day, and those for 50 µg/ and 150 µg/ of DEHP were acceptable on all three days(Ed-confirm this addition). The distribution of plasma DEHP level was skewed to the left and ranged from 0 to 18.9 µg/ . The plasma DEHP level was lower than 10 µg/ for 98 % of subjects and lower than 5 µg/ for 85 % . The geometric mean and standard deviation of plasma DEHP were 0.4 ± 1.5 µg/ .

Conclusions: The HPLC-UV method for quantification of plasma DEHP was acceptable by CDER guideline. The plasma DEHP of 255 Korean male workers ranged from 0 to 18.9 µg/ and the distribution was skewed to the left.

Key Words: Di-(2-ethylhexyl)phthalate, HPLC-UV, CDER guideline, Validity, Plasma, Korean male.

Di-(2-ethylhexyl)phthalate(DEHP)
 bis-(2-ethylhexyl)phthalate
 가
 40~50 %
 (Latini, 2000).
 (NTP, 2000).
 DEHP (IARC, 2000; Davis et al, 1994; Siddiqui & Srivastava, 1992)
 (Park et al, 2002; Gray et al, 1999; Poon et al, 1997).
 DEHP 1998
 DEHP 60 %
 (, 2000).
 DEHP
 (Kang et al, 2003).
 PVC 가
 가 phthalate 가
 (Lee et al, 2001; MAFF, 1994).
 (Page & Lacroix, 1995; Sharman et al, 1994).
 g DEHP (Faouzi et al, 1999)
 4~30 µg/kg (Doull et al, 1999).
 DEHP
 (Doull et al, 1999)
 가 가
 DEHP (Takatori et al, 2004; Inoue et al, 2003; ColOn et al, 2000),
 DEHP Koo (2003)
 DEHP ppb

(CDER, 1998).
 가
 DEHP
 (High Performance Liquid Chromatography, HPLC)
 (Saito et al, 2002a; Saito et al, 2002b; Kessler et al, 2001; Luks et al, 2001; Suna et al, 2001; Suzuki et al, 2001; Gray et al, 1999; Egestad et al, 1996; Albro et al, 1984).
 (Kambia et al, 2001),
 HPLC septum 가
 가 가 (Kessler et al, 2001; Pollack et al, 1985; Teirlynck & Rosseel, 1985).
 HPLC-Ultraviolet/Visible
 (UV) DEHP
 (Park et al, 2002; Suna et al, 2001) CDER
 (1998)
 DEHP
 가
 1.
 1)
 DEHP, Di-n-octyl phthalate(DNOP),
 Sigma-Aldrich (Saint-Quentin-Fallavier, France) 99.9%
 HPLC
 J.T. Baker(USA) Milli-Q system(Milli-Q, Millipore, Saint-Quentin Yvelines, France)
 Sigma-Aldrich(Saint-Quentin-Fallavier, France)
 2

2)
 DEHP DNOP
 6 mg/ml 5 mg/ml
 4
 DEHP 0, 10, 20, 40, 80, 120, 200, 300, 400,
 600 µg/ DNOP 100 µg/
 100 µl DEHP DNOP 50 µl

DEHP 0, 2.5, 5, 10, 20, 30, 50, 75,
 100 150 µg/ , DNOP 25 µg/
 DEHP
 DNOP (peak area ratio)
 DEHP

3)
 2003 2004
 5 30
 60 255

3000 rpm 10
 -20

4)
 Suna (2001)
 Kambia (2001)
 4 µl 100 µl DNOP
 50 µl , 1M 400 µl
 1.7 µl 20 20 µl
 1000 g 15
 , 1.6 ml 가
 , 100 µl

5) HPLC
 DEHP HPLC Suna
 (2001) Park (2002) HPLC(501
 pump, Waters, U.S.A) UV (484 waters,
 USA) 230 nm
 1% 85:15(v/v)
 0.8 ml/min, 30 µl
 XTerra RP18 5 µm (3.9 mm x 150 mm,
 Waters, U.S.A)
 0.45 µm

2.
 CDER(1998)
 1) (Calibration curve) (Linearity)
 DEHP 10
 가

2) (Limit of Detection, LOD)
 (Limit of Quantification, LOQ)
 DEHP 10가

가 3.3 , 10

(y) , S
 (ICH, 1996).

$$LOD = \frac{3.3}{S} \quad LOQ = \frac{10}{S}$$

3) (Precision)
 DEHP
 3가 3
 (mV/s)

3 (%)
 4) (Accuracy)
 DEHP 3 3

(, %) 가
 (Recovery, %) = ——— × 100

5)
 Duncan
 (SAS 8.01).

1.
 1) DEHP

(DEHP/DNOP) DEHP

(Fig. 1).

(Table 1) $y=0.043x+0.004$

(y: , x: ($\mu\text{g}/\text{L}$) y
 $0.043 \pm 0.004, 0.004$
 ± 0.063 (r) 0.999

50 150 $\mu\text{g}/\text{L}$

5.2 % 1.1%

35.8%

2.5 $\mu\text{g}/\text{L}$

가 50 150 $\mu\text{g}/\text{L}$
 7.8% 11.2%

15%

2.5 $\mu\text{g}/\text{L}$

28.1%

4.7%

2.5 $\mu\text{g}/\text{L}$

가

2)

DEHP

5.22 $\mu\text{g}/\text{L}$

15.81 $\mu\text{g}/\text{L}$

($p=0.037$), 50 150 $\mu\text{g}/\text{L}$

가

($p>0.05$)(Table 2).

3)

(Precision)

DEHP

Table 2

4)

(Accuracy)

DEHP

2.5, 50 150

DEHP 가 2.5, 50 150 $\mu\text{g}/\text{L}$ 가

$\mu\text{g}/\text{L}$

3

3

3

3

2.5 $\mu\text{g}/\text{L}$

30.5 %

9.7%, 2.3% 50 150 $\mu\text{g}/\text{L}$

가 가 7.0 % 3.1 % 가

가 4.7% 1.2%

2.5 $\mu\text{g}/\text{L}$ 16.9% 50 150 $\mu\text{g}/\text{L}$

3.9% 3.1%

3.

DEHP

가

(Fig. 2).

DEHP

(Fig. 3).

DEHP

255

DEHP 가 1 $\mu\text{g}/\text{L}$

63

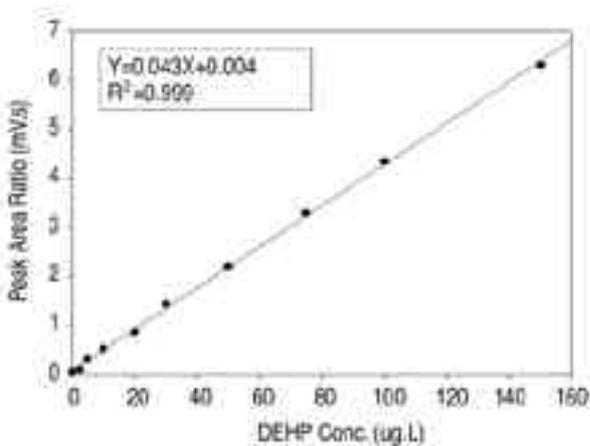


Fig. 1. A daily calibration curve for DEHP representing all analytes. The calibration curve was linear across the range from 0 to 150 $\mu\text{g}/\text{L}$ with concentration coefficient typically exceeding 0.999.

Table 1. Recovery and coefficient of variation(CV) of DEHP representing all analytes

Amount added ($\mu\text{g}/\text{L}$)	Amount found ($\mu\text{g}/\text{L}$)	Recovery (%)	CV (%)
0	-0.5 ± 0.8 (0.068*)		
2.5	2.1 ± 0.1	84.0	6.2
5	5.3 ± 0.6	106.3	10.4
10	11.0 ± 1.5	110.0	13.7
20	20.4 ± 0.3	102.0	1.5
30	30.5 ± 0.3	101.6	1.0
50	50.0 ± 0.8	99.9	1.6
75	75.4 ± 0.8	100.6	1.0
100	100.0 ± 2.8	100.0	2.8
150	146.5 ± 4.3	97.7	2.9

*: 0.068 is the standard deviation of response

25 % , 5 µg/ 217
 85 % . 5~10 µg/ 33
 , 10 µg/ 5 가
 DEHP 18.9 µg/ DEHP
 0.4±1.5 µg/

DEHP
 (r=-0.098, p=0.452).

(
), (
),
 6 ~ 8 가
 가 (CDER, 1998).
 DEHP Takatori (2004) 2.2~3.9 µg/
 , ColOn (2000) 70

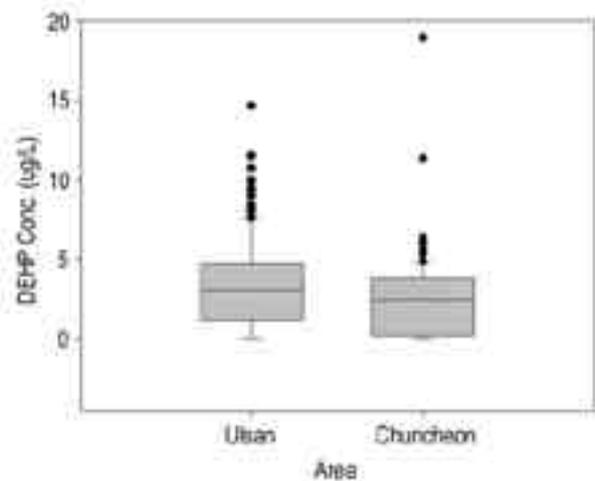


Fig. 2. Box plot of DEHP in plasma of workers in Ulsan and Chuncheon area.

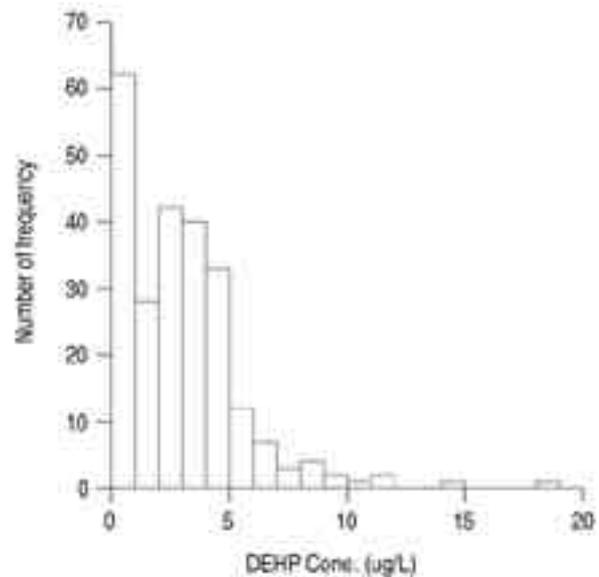


Fig. 3. Frequency distribution of DEHP in plasma in some Korean adult male.

Table 2. Analysis of variance of interday and intraday assay for precision at 2.5, 50 and 150 µg/ of DEHP

DEHP Conc.(µg/)	Peak area ratio			CV(%)	P-value
	1st day	2nd day	3rd day		
2.5	0.22	0.08	0.09	35.8	0.037
	0.15	0.12	0.10		
	0.13	0.09	0.09		
CV*(%)	28.1	22.1	4.7		
Duncan grouping	A	B	B		
50	1.99	1.81	1.91	5.2	0.204
	2.04	1.80	2.09		
	2.18	2.00	1.79		
CV(%)	4.7	6.0	7.8		
Duncan grouping	A	A	A		
150	6.58	6.52	6.92	1.1	0.948
	5.43	6.14	6.02		
	6.68	6.25	6.14		
CV(%)	11.2	3.1	7.7		
Duncan grouping	A	A	A		

* CV : coefficient of variation

DEHP HPLC

10 0~150 25

(r²) 99% 5.22 14.0 15.81

(CDER, 1998) 가 (aliquots)

(Thomsen et al, 2003). 5 37

2.5 84% 15% 20%

가 가 가 가

가 100%가

(CDER, 1998). HPLC-UV 가 (, ,) (100 %)

DEHP 50 150 PVC)

g/ (Aignasse et al, 1995). (CDER, 1998). 2.5, 50 150 37 3

ng/ml (Kambia et al, 2001), DEHP 20 2.5 37 3

(Koo et al, 2003). DEHP 10 2.5 가 가

Inoue (2003) col- umn-switching system-LC/MS 가 (p=0.037). 2.5

Table 3. Analysis of variance of inter- and intraday assay for accuracy at 2.5, 50 and 150 µg/ of DEHP

DEHP Conc. (µg/)	Amount found			Recovery (%)			CV (%)
	1st day	2nd day	3rd day	1st day	2nd day	3rd day	
2.5	5.0	4.7	4.0	199.4	186.3	159.3	16.9
	3.3	5.6	4.1	133.6	224.5	163.0	
	2.8	4.9	3.9	112.4	197.3	155.5	
CV (%)				30.5	9.7	2.3	
50	48.1	44.9	43.9	96.1	89.8	87.8	3.9
	49.2	44.7	47.7	98.4	89.4	95.4	
	52.6	49.3	50.5	105.2	98.6	101.1	
CV (%)				4.7	5.6	7.0	
150	159.8	154.6	153.8	106.5	103.0	102.6	3.1
	154.6	145.6	156.9	103.0	97.1	104.6	
	162.1	148.3	157.4	108.1	98.9	104.9	
CV (%)				2.4	3.1	1.2	

* CV: coefficient of variation

가
 , DEHP
 . 50 µg/ 150 µg/
 가 (p>0.05). 50,
 150 µg/ 87.8~ 105.2%, 97.1~108.1%
 2.5 µg/ 112.4~224.5 %
 2
 DEHP
 DEHP가
 가
 DEHP 3,700 µg/
 (Teirlynck & Rosseel, 1985) Pollack (1985)
 HPLC DEHP 50 µ
 g/
 . GC
 septum (Kessler et
 al, 2001). , (Inoue et al, 2003)
 (Mitani et al, 2003)
 HPLC
 DEHP
 .
 (Mahara et al, 1997).
 ,
 (Koo et al, 2003; Inoue et al, 2003;
 Kambia et al, 2001; Aignasse et al, 1995)
 ,
 CDER(1998)
 가
 CDER(1998) HPLC-
 UV 255 DEHP
 . DEHP
 19 µg/ ,
 DEHP 5 µg/ .
 DEHP
 .
 DEHP 41
 2,098 µg/ , 35 719 µg/
 가 (ColOn et al, 2000).
 2.2~3.9 µg/ (Takatori et
 al, 2004), DEHP 5.78 µg/
 (Koo et al, 2003). 255
 10 µg/ (98 %) , 5 µg/
 85 % .

DEHP
 ,
 EU(European Union) IARC(International
 Agency for Research on Cancer)
 DEHP 5~21 µg/kg (CSTEE,
 2004; IARC, 2000). DEHP
 mono(2-ethylhexyl)phthalate(
 MEHP) 가 (Takatori et al,
 2004; Pollack et al, 1985; Teirlynck & Rosseel,
 1985). DEHP가
 , DEHP
 10~ 18 MEHP 3~6
 (Doull et al,
 1999) . DEHP
 MEHP (MEHP/DEHP) 6-12:1
 (Pollack et al, 1985; Teirlynck & Rosseel, 1985),
 Takatori (2004)
 MEHP/DEHP 2.0~4.7:1 .
 (Lake
 et al, 1977). MEHP/DEHP
 DEHP가
 DEHP MEHP ,
 가 DEHP DEHP
 MEHP (Koch et
 al, 2004). DEHP 가
 DEHP MEHP
 . Koo (2002)
 DEHP , , ,

UV 0 ~ 150 µg/
 (r²=0.999). 5.22 µg/ , 15.81
 µg/ . 2.5 µg/ DEHP
 CDER
 . 50 µg/ 150 µg/ DEHP
 CDER 98 %
 10 µg/ , 85% 5 µg/
 , 18.9 µg/
 DEHP
 0.4±1.5 µg/
 : HPLC-UV
 DEHP CDER
 DEHP 0 ~ 18.9 µg/

. 2000.

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