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- Abstract -

The study on the pretreatments for the analysis of
benzidine metabolites in urine

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This study evaluates the pretreatment for analysis of benzidine metabolites in urine by measuring the recovery rates according to the temperature and periods of storage of the urine. By the solid phase extraction, the recovery rates of basic hydrolysis are benzidine 67.4 %, monoacetylbenzidine 105.1 %, and diacetylbenzidine 115.8 %, respectively. By the liquid extraction, the recovery rates of back-extraction into 0.1 M perchloric acid are benzidine 105.7 %, monoacetylbenzidine 94.2 %, diacetylbenzidine 72.8 %, respectively. The difference of the recovery rates between the back-extraction into 0.1 M HCl and 0.1 M perchloric acid after basic hydrolysis are 101 % and 98.8 %, respectively. When the recovery rates

of the urinary samples of pH 3, pH 7, pH 12 at 25 °C and -76 °C are compared for four weeks, there are no differences according to the temperature and the periods of storage.

The above results show that the solid phase extraction and back-extraction by 0.1 M perchloric acid after basic hydrolysis are suitable for the analysis of benzidine metabolites. There are no difference of the recovery rates of the urinary samples stored at 25 °C and -76 °C at pH 3, pH 7, pH 12, respectively for 28 days.

Key words : benzidine, monoacetylbenzidine, diacetylbenzidine, liquid phase extraction, solid phase extraction, metabolites

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I .

(1991)

(1995)

(, ,)
가

가가

, ,

‘ (skin)’

가

(ACGIH, 1995).

가

(free benzidine) 4~10 %,

7~16% 가 (Baselt, 1997).

DNA

, . 가

가

가

(Martin , 1982),

pH

(Babu , 1993). Lynn (1984)

[14C]benzidine

, N-

II.

, 3-

1.

pH 5.3

가 4-5

가

가

(Babu , 1992; 1993).

(High performance liquid

chromatography, HPLC)/

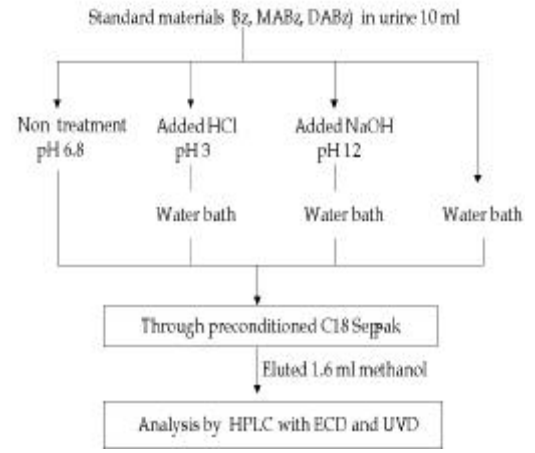
(ultra- violet

Charles

detector, UVD, Gilson 118/119)

(electrochemical detector, ECD, Gilson 102)
 C18 (5 μ m, 4.6mm \times 250mm,
 Higgins Analytical, USA)

가
 (Fig. 1).



Bz, benzidine; MABz, monoacetylbenzidine;
 DABz, diacetylbenzidine.

Fig 1. Pretreatment of benzidine metabolites in urine(solid phase extraction)

2.
 1)
 Birner Neumann (1988)
 (,)
 가 가
 (HP 5890/MSD 5972, USA)

2)
 HPLC
 1 L LiCl 2 g 22 78 ,
 1 L LiCl 2 g 37 63
 , 0.01 M
 45 55 , ECD
 , ECD
 가 UVD

4)
 , 가
 2 ml
 0.1 M (1 ml)
 (,
 1998; Baselt, 1997) Brown (1995)
 0.1
 M 1 ml
 2 ml
 NaOH 가 2 M 가
 2
 0.1 M 1 ml 0.1 M
 1 ml
 (Fig. 2).

3)
 (Sep-pak, Waters, USA)
 가 10 ml
 sep-pak 1.6 ml
 HPLC
 , 2
 , NaOH 가 pH가 12
 가 , 1 N pH가 3

5)

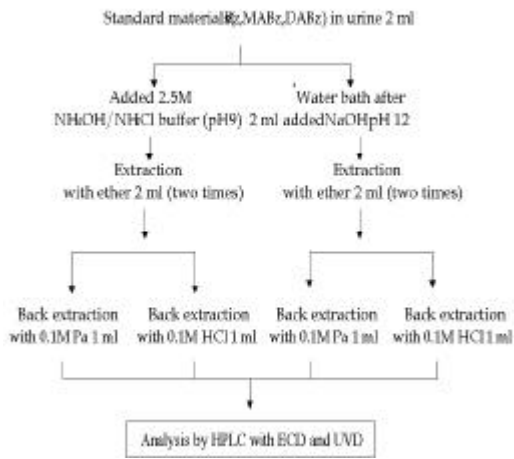
pH 3, pH 7, pH 12
7 28

2.

6)

SAS(Statistical Analysis System)

cal Analysis System)



Bz, benzidine; MABz, monoacetylbenzidine; DABz, diacetylbenzidine; Pa, perchloric acid.

Figure 2. Pretreatment of benzidine metabolites in urine (liquid phase extraction)

III.

1.

150ml 1g 200μl
가

Rf(relative flow rate)

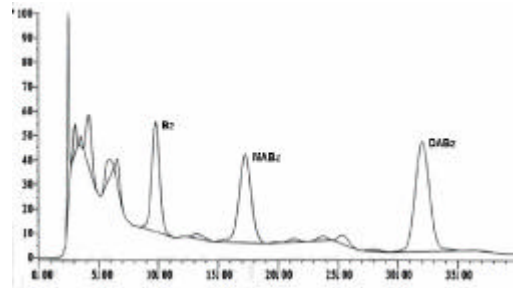
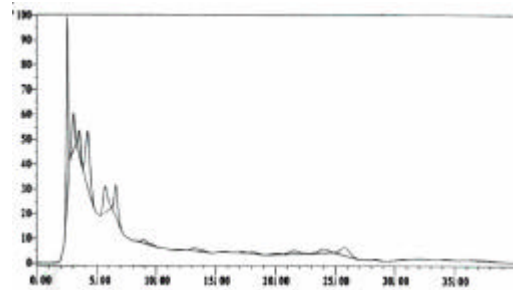
3가
1 l LiCl 2
1.2 ml/min
가 가

g

63 37

(Figure 3).

(ECD)
(UVD)
가 ECD
287nm
UVD
0.8
V



Upper : urine blank Lower : Bz, MABz, DABz in urine
Bz: benzidine(100 ng/ml), MABz:monoacetylbenzidine(100 ng/ml),
DABz: diacetylbenzidine(500 ng/ml).

Figure 3. Chromatograms of benzidine, monoacetylbenzidine and diacetylbenzidine in urine by high performance liquid chromatograph

3. 2)

1) , ,
 NaOH 가
 100 ng/ml, 500 ng/ml, 1000 ng/ml 2 ml 2 0.1 M
 가 , 0.1 M
 2 , (Table 2). 0.1 M
 NaOH 가 pH 12 가 91.5~119.8
 , 1 N pH 3 가 % , 92.1~102.8 % ,
 70.4 % 0.1 M HCl
 (Table 1), NaOH 가
 가 (P<0.001). 0.1 M
 가 가 0.1 M
 60.5~73.6 % , 93.3~117.6 가 (p>0.05).
 % , 91.4 ~ 140.2 % 가 .

Table 1. Recovery rates of benzidine metabolites by solid phase extraction

		Bz	MABz	DABz
A	a	30.0 ± 2.5*	14.6 ± 0.0	16.2 ± 0.0
	b	12.4 ± 0.4	3.4 ± 0.9	3.2 ± 0.0
	c	7.7 ± 0.1	3.2 ± 0.5	1.6 ± 0.0
B	a	58.7 ± 12.3	99.4 ± 28.9	132.4 ± 13.2
	b	35.1 ± 3.3	81.1 ± 8.3	88.9 ± 11.5
	c	49.4 ± 5.8	95.5 ± 4.7	102.3 ± 6.9
C	a	47.0 ± 3.9	102.6 ± 10.9	106.8 ± 5.2
	b	34.9 ± 4.6	84.0 ± 12.9	80.8 ± 12.5
	c	44.3 ± 7.1	88.7 ± 2.9	83.3 ± 2.9
D	a	73.6 ± 16.9	117.6 ± 15.5	140.2 ± 15.8
	b	60.5 ± 3.5	107.5 ± 4.4	119.9 ± 5.2
	c	62.4 ± 5.2	93.3 ± 5.2	91.4 ± 5.1

unit, %; * recovery, mean ± SD; A, no treatment; B, water bath; C, water bath after acid hydrolysis; D, water bath after base hydrolysis; a, 100 ng/ml; b, 500 ng/ml; c, 1000 ng/ml; Bz, benzidine; MABz, monoacetylbenzidine; DABz, diacetylbenzidine.

Table 2. Recovery rates of benzidine metabolites by liquid phase extraction

		Bz	MABz	DABz
A	a	119.8±19.2*	96.3± 3.8	70.6±5.0
	b	96.9±17.3	102.2± 7.8	70.4±3.4
	c	91.5± 9.7	92.1± 4.5	75.2±3.4
B	a	104.6± 4.1	88.0± 6.0	71.1±4.5
	b	92.7± 7.8	101.6±11.6	64.1±5.0
	c	105.5±17.4	88.2± 4.3	65.1±2.1
C	a	97.0± 3.0		
	b	105.0± 3.5		
	c	98.4± 5.0		
D	a	109.5± 9.8		
	b	90.1± 6.2		
	c	92.1± 5.7		

unit, %; * recovery, mean ± SD; A, back extraction by 0.1 M perchloric acid; B, back extraction by 0.1 M HCl; C, after basic hydrolysis back extraction by 0.1 M perchloric acid; D, after basic hydrolysis back extraction by 0.1 M HCl; a, 250 ng/mL; b, 500 ng/mL; c, 1000 ng/mL; Bz, benzidine; MABz, monoacetylbenzidine; DABz, diacetylbenzidine.

3) pH 가 69.6 % (Figure 5).
 가 (p>0.05).
 가 pH 3, pH 7, pH 12 (- 76 °C) 7 IV.
 28 가 .
 (p>0.05),
 (p>0.05).
 84.6~111.9 %, 84.8~
 114 % 52~70.4 %
 (Figure 4). 가
 (p> 0.05),
 (p>0.05).
 87.3 ~ 120.8 %, 80.9
 ~ 114 % 55.4 ~

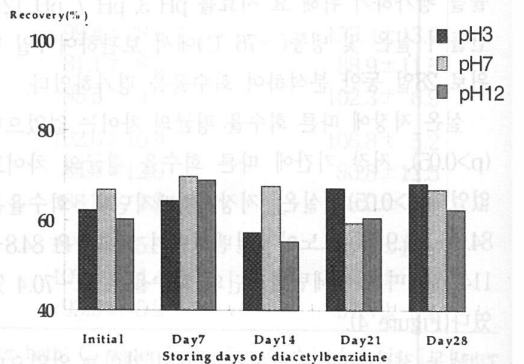
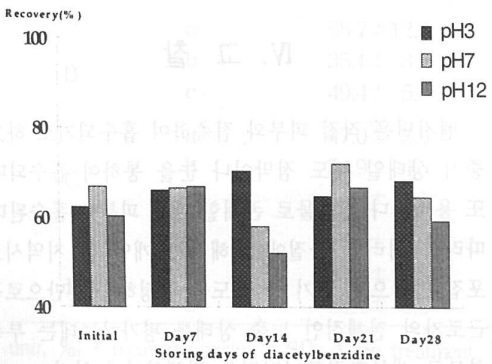
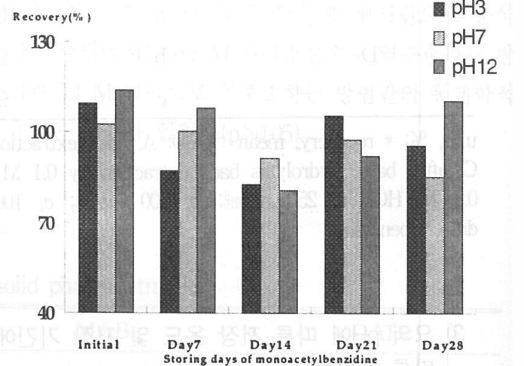
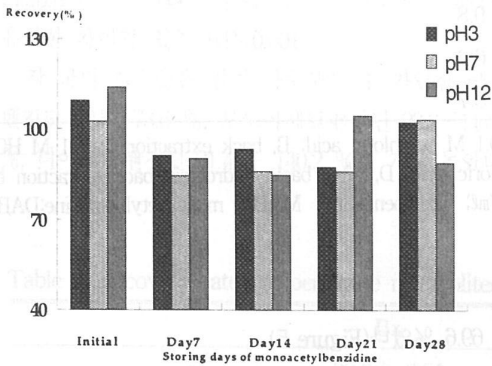
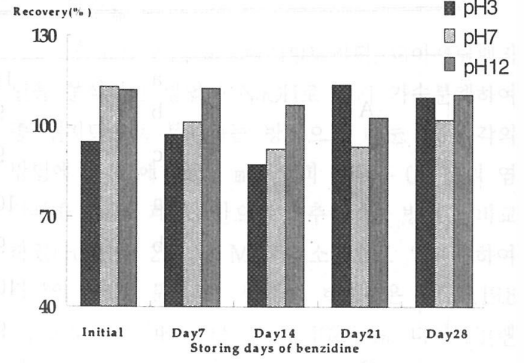
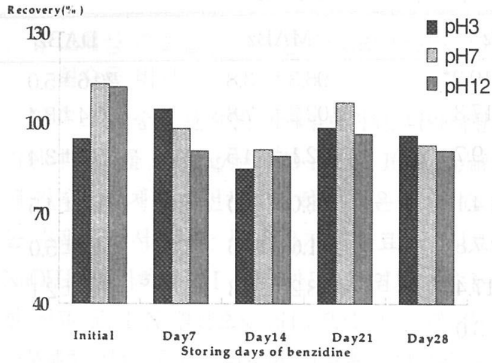


Fig. 4. Recovery rates according to storing period of benzidine metabolites at room temperature

Fig. 5. Recovery rates according to storing period of benzidine metabolites at -76°C

NaOH 가 4.7 M NaOH 가
 80 °C 2 가
 NaOH 2 M
 가 2

60.5~73.6%, 93. Brown (1995) 91 ~
 3~117.6 %, 91.4~140.2 % 100 % 가 .
 . Charles (1991) KOH pH 12 가

80 % NIOSH (1994)
 NaOH pH 12 가 가 (method 8304) - 20 °C
 NaOH 2 M 가 가 , GC/ECD
 가 100 % (method 8306) 가
 . Charles (1991) pH 3, pH 6, pH12
 가 28 pH
 pH 12 6 90 % 75 %
 2 M 가 가
 가 가 pH 3, pH 7, pH 12
 가 28 pH
 120.8 %, 80.9 ~ 114 %, 87.3 ~
 55.4 ~ 69.6 % 28
 가 28

0.1 M
 92.7 ~ , 가
 105.5 %, 88.2 ~ 101.6 %, 가
 64.1 ~ 71.1 % 0.1 M
 91.5 ~ 119.8
 %, 92.1 ~ 102.28 %, (Lynn , 1984; Babu
 70.4 ~ 75.2 % 가 , 1992; 1994),
 Baselt (1997) 가
 (103 %) 가

NaOH 가 0.1 M 가
 90.1 ~ 109.5 % 0.1 M 0.1 M
 97 ~ .
 105 % . Brown (1995) - NaOH 가 2 M
 (o- toluidine) (aniline) NaOH 가 2
 가 가

하는 것이 바람직하다고 하겠다.

V. 결 론

요중 벤지딘의 대사물질의 전처리 방법들을 평가하고 요의 pH에 따른 저장온도 및 저장기간에 따른 회수율을 평가하였다.

1. 고체상 추출법을 이용한 전처리 방법에 따른 회수율을 비교한 결과 염기 가수분해한 군의 회수율이 벤지딘 67.4 %, 모노아세틸벤지딘 105.1 %, 디아세틸벤지딘 115.8 %로 높았다.

2. 액상 추출법을 이용한 전처리 방법에 따른 회수율을 비교한 결과 0.1 M 과염소산으로 역추출하는 방법의 회수율이 벤지딘 105.7 %, 모노아세틸벤지딘 94.2 %, 디아세틸벤지딘 72.8 %로 높았다.

3. 액상 추출법을 이용한 전처리 방법에 따른 회수율 평가 중 시료의 NaOH 농도가 2 M한 후 끓는 수욕 중에서 2시간 동안 유지하여 가수분해하고 0.1 M 염산으로 역추출하는 방법과 0.1 M 과염소산으로 역추출하는 방법 간 회수율의 차이가 없었다.

4. 요의 pH에 따른 저장 온도 및 저장 기간에 따른 회수율 평가를 위해 요 시료를 pH 3, pH 7, pH 12로 하여 실온 및 냉동 보관하여 28일 동안의 회수율의 차이가 없었다.

본 실험 결과 요 중 벤지딘 대사물질 분석은 염기 가수분해 방법과 0.1 M 과염소산으로 역추출하는 방법이 적합하며, 요 시료는 28일 동안 저장 기간 및 저장 온도에 따른 회수율에 차이가 없었다.

REFERENCES

노재훈, 안연순, 김규상, 김치년, 김현수: 벤지딘계 염료제조 사업장 근로자의 벤지딘 폭로. 대한산업의학회지 1995; 7(2): 103-109.

염혜경, 송제석, 김치년, 원종욱, 노재훈: 벤지딘계

염료공장 근로자들의 피부노출평가. 대한산업의학회지 1998; 10(1): 83-93.

ACGIH: Threshold limit value for chemical substances and physical agents and biological exposure indices for 1995-1996. Cincinnati, OH 1995.

Babu SR, VM Lakshmi, FF Hsu, TV Zenser, and BB Davis: Role of N-glucuronidation in benzidine-induced bladder cancer in dog. Carcinogenesis 1992; 13: 1235-1239.

Babu SR, VM Lakshmi, FF Hsu, RE Kane, TV Zenser, and BB Davis: N-acetylbenzidine-N'-glucuronidation by human, dog and rat. Carcinogenesis 1993; 14(12): 2605-2611.

Babu SR, VM Lakshmi, TV Zenser, and BB Davis: Glucuronidation of N-acetylbenzidine by human liver. Drug Metab Dispos 1994; 22: 922-927.

Baselt RC: Biological monitoring methods for industrial chemical. Chemical Toxicology Institute 1997; 45-48.

Birner G, and HG Neumann: Biomonitoring of aromatic amines II: Hemoglobin binding of monocyclic aromatic amines. Arch Toxicol 1988; 62: 110-115.

Brown KK, AW Teass, S Simon, EM Ward: A biological monitoring method for o-toluidine and aniline in urine using highperformance liquid chromatography with electrochemical detector. Appl Occup Environ Hyg 1995; 10(6): 557-565.

Charles E: Analysis of urine to monitor exposures to benzidine, o-dianisidine, o-tolidine, and 4,4-methylenedianiline. Appl Occup Environ Hyg 1991; 6(11): 953-958.

Lynn RK, CT Garvie-Gould, DF Milam, KF Scott, CL Eastman, AM Ilias, and RM Rodgers: Disposition of the aromatic amine, benzidine, in the rat: characterization of mutagenic urinary and

biliary metabolites. *Toxicol Appl Pharmacol* 1984; 72: 1-14.

Martin CN, FA Beland, RW Roth, FF Kadlubar: Covalent binding of benzidine

and N-acetylbenzidine to DNA at the C-8 atom of deoxyguanosine in vivo and in vitro. *Cancer Research* 1982; 42: 2678-2696.