



Assessment of nicotine metabolites and specific cigarette smoke constituents in human urine

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Summary

Introduction

Biomarker

- The most often recommended method to assess the exposure of smokers to smoke constituents.
- The knowledge about representative smoke exposure data is important for the assessment of potential reduced exposure products.

• Biomarkers of Exposure

- It is a constituent or metabolite that is measured in a biological fluid or tissue
- Nicotine and its metabolites (nicotine+5) is a good biomarker for overall smoke exposure.

• Biomarkers of Effect

- Biomarkers that may act as surrogate markers of the relative risk of developing smoking-related diseases.

Introduction

Biomarkers of tobacco smoke exposure recommended for routine use

Biomarker	Precursor	Biological matrix	Comments/selected reference
Nicotine and at least five major nicotine metabolite	Nicotine	Urine	Recommended for inclusion in all exposure studies of PREP(2001, Balint; 1992, Byrd; 2005, Roedig)
CO/COHb	CO	Exhaled breath; blood	(2002, Cunnington et, al; 2004, Hughes; 1998, Smith et, al ; 1981, Wald)
Urine mutagenicity	Mutagens, carcinogen	Urine	(2002, Bowman; 2005, Roedig)
1-Hydroxypyrene	Pyrene	Urine	Surrogate for PAH(2004, Hatsukami ; 2004, Murphy)
3- Hydroxypropyl-mercapturic acid	Acrolein	Urine	(2001, Mascher et, al)
Trans, trans-muconic acid(tt-MA)	Benzene	Urine	(1998, Scherer)
S-phenyl-mercapturic acid(S-PMA)	Benzene	Urine	(1995, Boogaard et, al)
Monohydroxy-butenyl -mercapturic acid (MHBMA)	1,3-Butadiene	Urine	(2003,Urban; 2000, Van Sittert et, al)
NNAL + NNAL-glucuronide	TSNAs	Urine	(2003, Breland et, al ; 2004, Hughes)
4- Aminobiphenyl hemoglobin adducts	4-Aminobiphenyl	Urine	(1990, Bartsch et, al)
N-(2-cyanoethyl)valine hemoglobin adducts	Acrylonitrile	Blood	(2000, Fennell; 1999, Perez; 2002,Schettgen et, al)

Introduction

Urinary Biomarkers of Exposure

Smoke Component	Urinary Biomarker
Nicotine	nicotine, nicotine-glucuronide cotinine, cotinine-glucuronide trans-3'-hydroxycotinine), trans-3'-hydroxycotinine-glucuronide
Acrolein	3-Hydroxypropyl mercapturic acid (3-HPMA)
1,3-Butadiene	Monohydroxybutenyl-mercapturic acids(MHBMA)
Mutagen	Urine Mutagenicity

Objective

- Measure the levels of selected biomarkers of cigarette smoke exposure in urine samples from Korean healthy smoker and non-smoker participants.
- Analyze relationship between nicotine metabolites and specific cigarette smoke constituents.



Study design

- **Characteristic of participants**

- Number of participants : smokers(n=7, S1~S7), non-smoker(n=1, NS)
- Gender : Male

	S1	S2	S3	S4	S5	S6	S7	NS
Age	34	55	55	51	40	34	44	35
Number of cigarette smoked	30	20	20	20	15	10	5	0
Level of Tar	5~6	1~6	3~5	1~3	3~5	5~6	3~5	0

- **Urine collection**

- 24hr Urine samples from participants were obtained and stored at -70°C.

- **Sample preparation**

- Centrifugation(3000rpm,15min) → Solid phase extraction with a internal standard.
→ Elution, reconstitution and injection into LC-MS/MS.

- **Instrument**

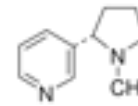
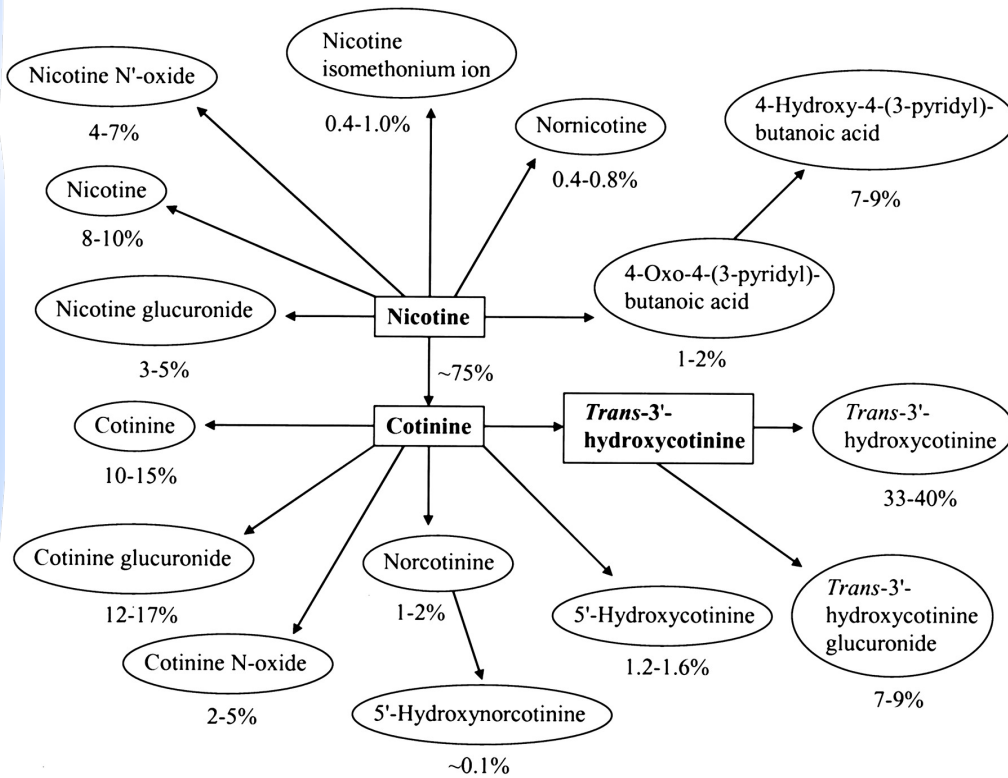
- LC : HPLC 1100 series
- MS/MS : AB science API 4000



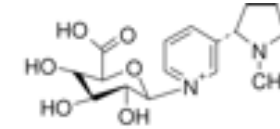
Biomarker♪

Nicotine & nicotine metabolites

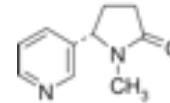
Analytes



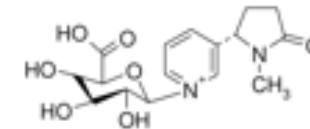
Nicotine



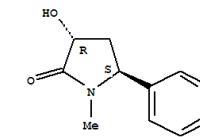
Nicotine *N*-glucuronide



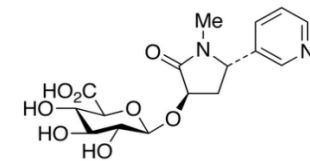
Cotinine



Cotinine *N*-glucuronide



trans-3'-hydroxycotinine

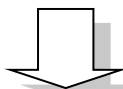


trans-3'-hydroxycotinine glucuronide

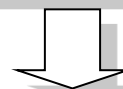
Analytical Procedure

**24hr urine centrifugation
(12000rpm, 15min)**

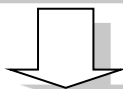
Upper phase



**0.45um filtration
(Captiva 96well filtration kit)**



100ul urine spiked ISTD mix



**Autosampler vial
LC-MS-MS**

Instrumental Condition

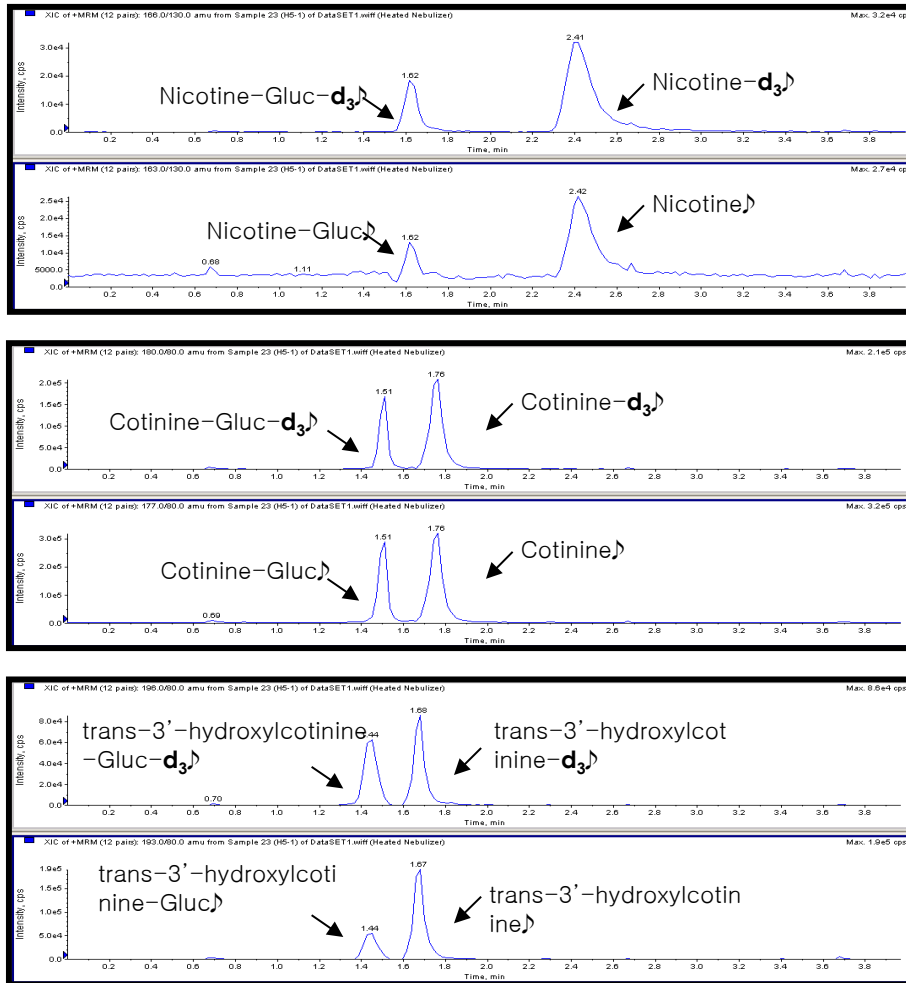
HPLC	
Column	Synergi MAX RP80(150x4.6mm, 4 μ m, 80Å)
Mobile phase	A : 10mM Ammonium acetate buffer B : Methanol Isocratic 20% A and 80% B
Flow rate / injection	1ml/min , 1ul
LC-MS/MS	
Ion source	APCI
Mode of analysis	Positive
Acquisition mode	MRM
Ion transition (m/z)	methyl nicotine-d₃ : 166 → 130 nicotine : 166 → 130 nicotine glucuronide : 166 → 130 methyl cotinine-d₃ : 180 → 80 cotinine : 180 → 80 cotinine glucuronide : 180 → 80 Hydroxylcotinine-d₃ : 196 → 80 3-hydroxylcotinine : 177 → 80 3-hydroxylcotinine-glucuronide : 177 → 80

Results(I)

Chromatogram of nicotine and its metabolites

ISTD

Analyte



- Calibration range 100~5000ng/ml
- R²>0.99

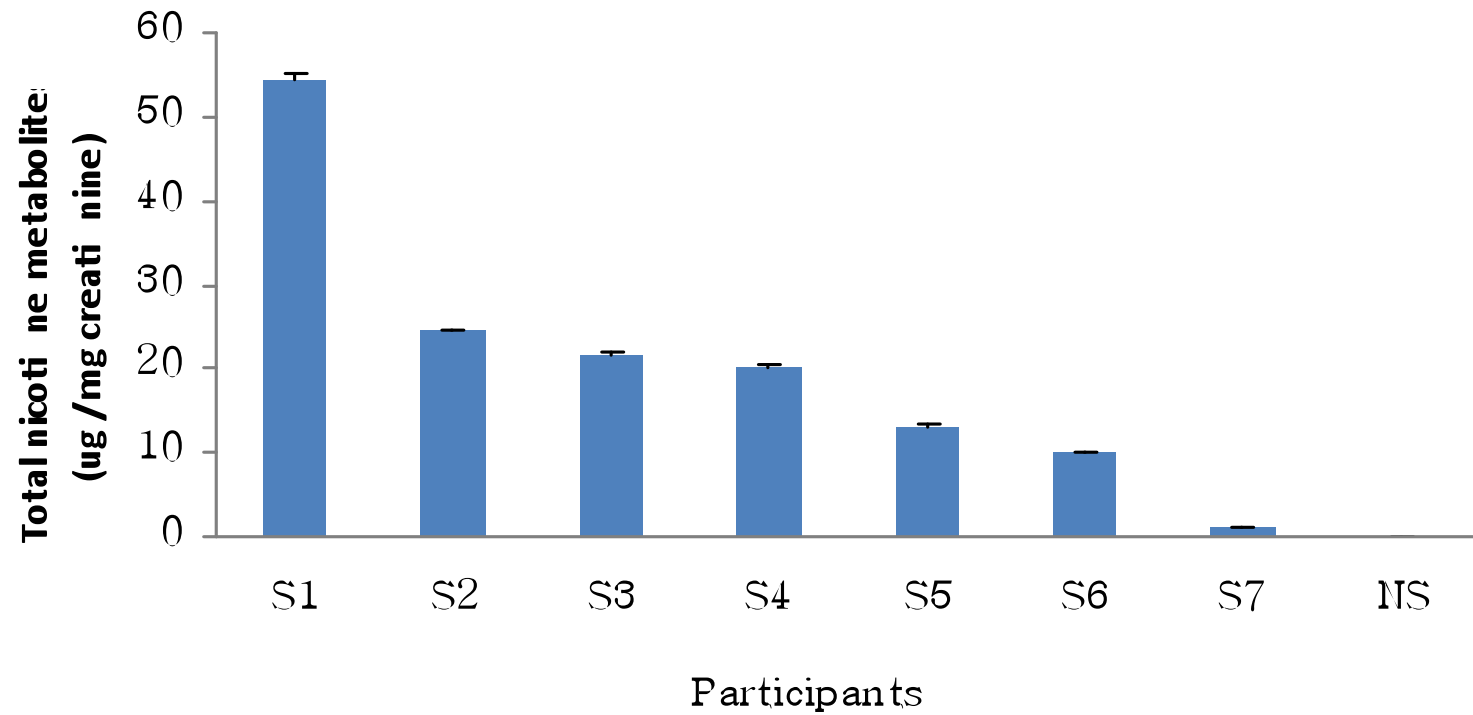
Results(2)

Concentration of nicotine and five metabolites in urine of participants

Participant	Nicotine	Cotinine	THC	Nic-gluc.	Cot-gluc.	THC-gluc.	SUM
S1	7.34	8.14	14.04	3.77	15.81	5.48	54.58
S2	0.63	2.56	10.48	0.78	5.56	4.47	24.48
S3	3.16	4.80	5.10	1.57	5.20	1.82	21.65
S4	1.27	3.43	6.48	1.42	5.32	2.19	20.12
S5	0.50	2.23	5.16	0.61	2.67	2.01	13.18
S6	1.43	0.99	2.44	0.94	3.30	0.96	10.06
S7	0.00	0.08	0.13	0.00	0.20	0.07	0.48
N	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Results(3)♪

Total quantity of nicotine and nicotine metabolites ♪

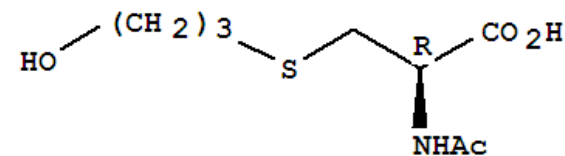
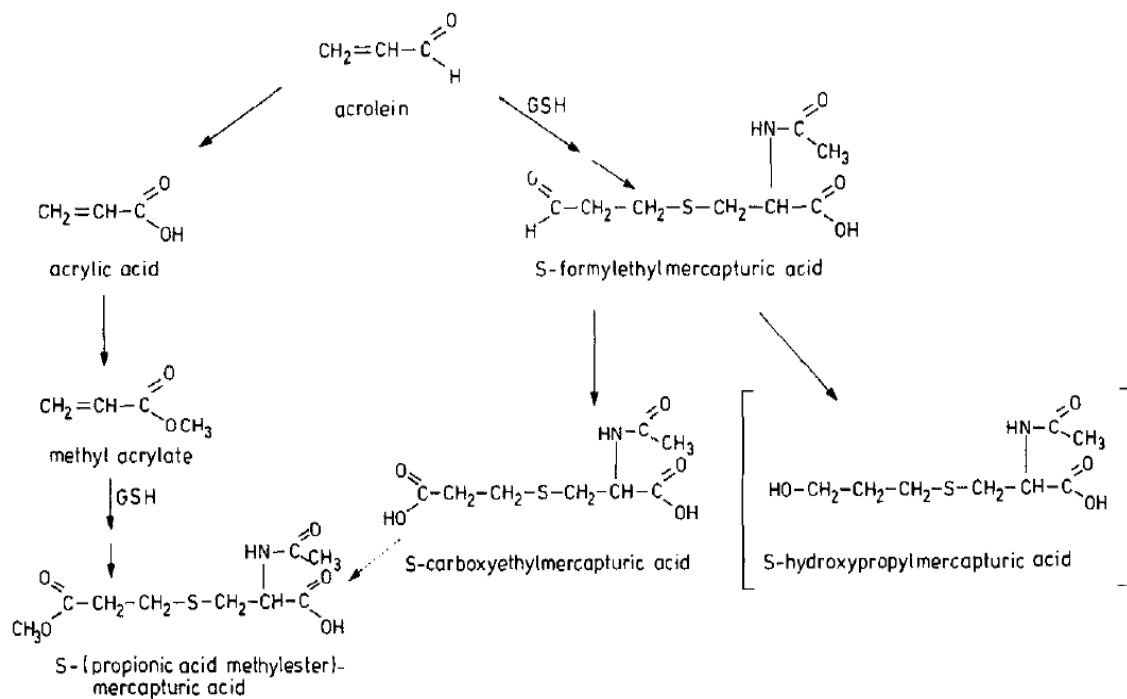


Biomarker

3-HPMA

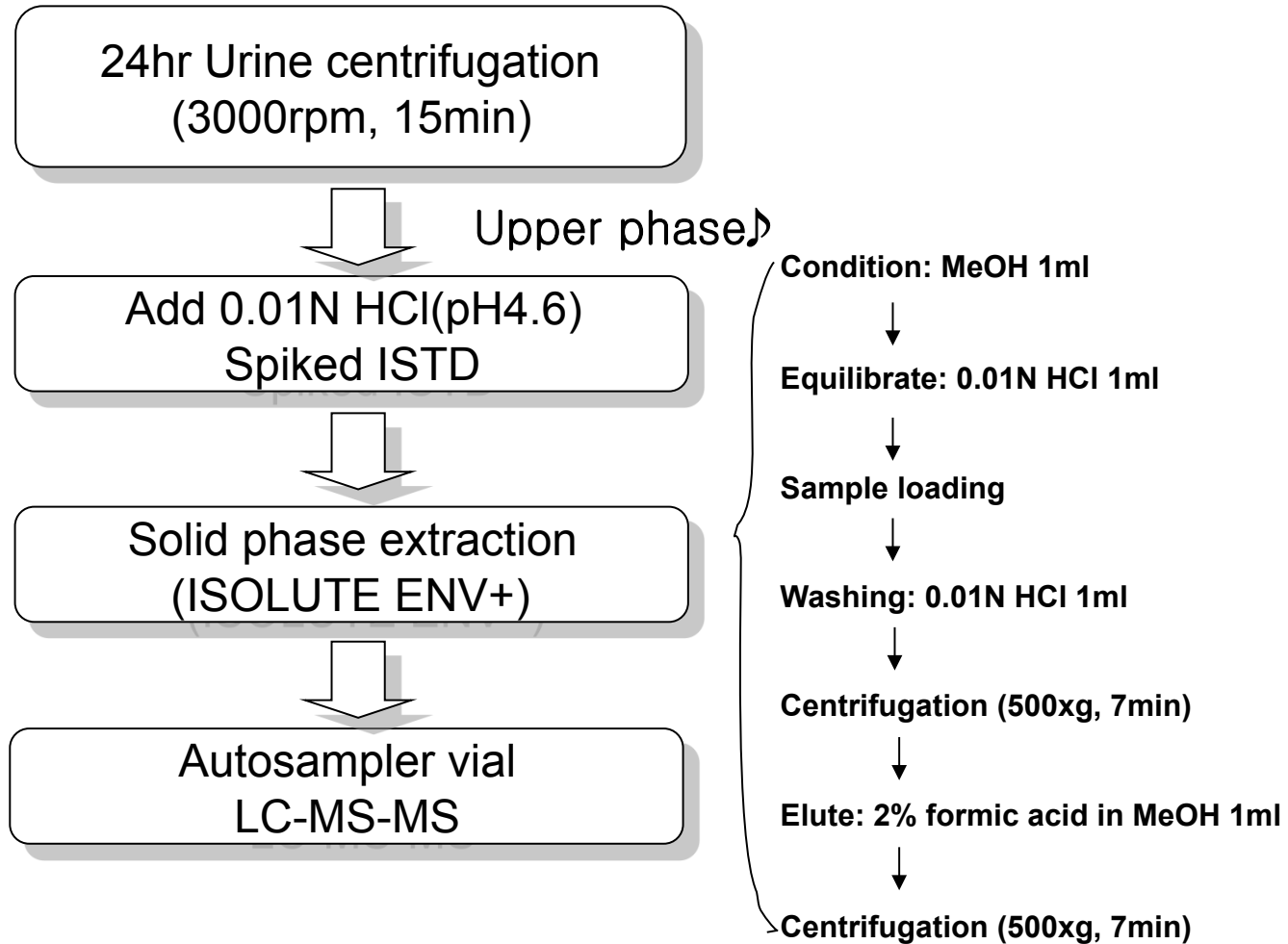


Analyte



3-HPMA 

Analytical Procedure



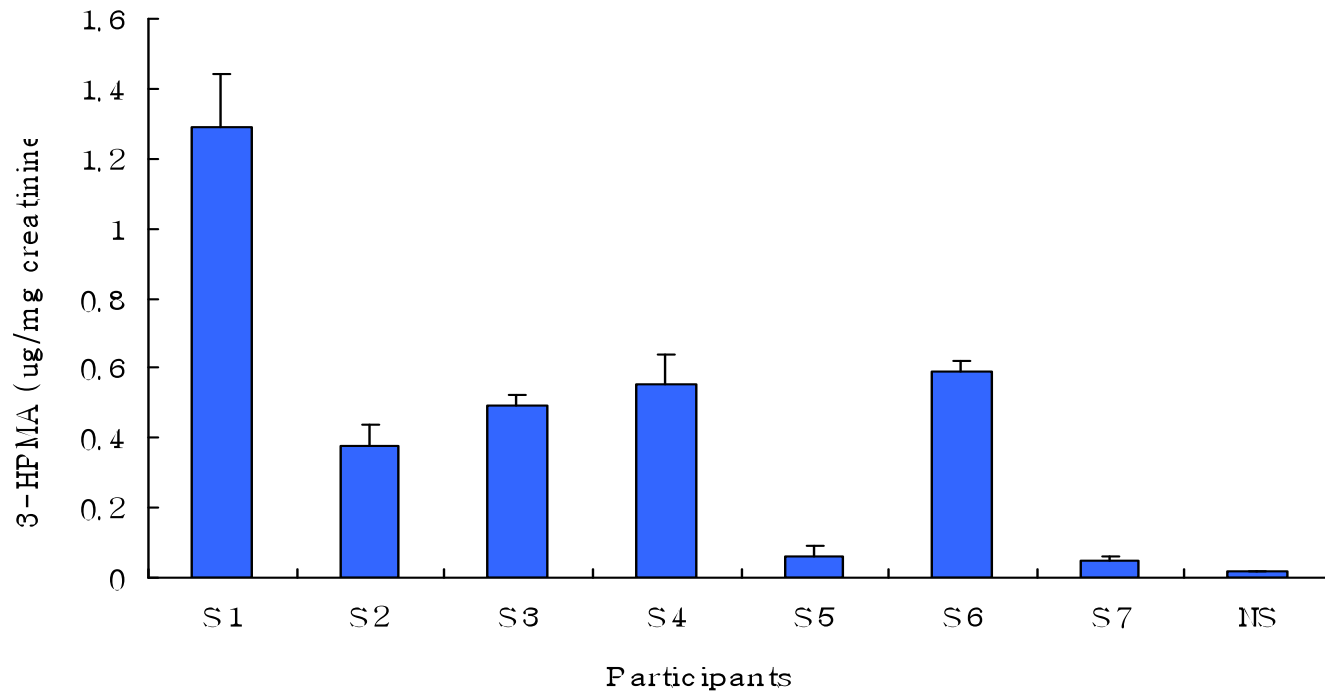
Instrumental Condition

HPLC	
Column	Synergi MAX RP80(150x4.6mm, 4 μ m, 80Å)
Mobile phase	A : 10mM Ammonium acetate buffer B : Methanol Isocratic 50% A and 50% B
Flow rate / Injection	0.8ml/min, 10 μ l
LC-MS/MS	
Ion source	APCI
Mode of analysis	Positive
Acquisition mode	MRM
Ion transition (m/z)	HPMA-d₃ : 225→133 HPMA(quantifier) : 222→117 HPMA(quantifier) : 222→91

- Calibration range : 50~5000ng/nl
- R²=0.9981

Results(I)

Total quantity of 3-HPMA

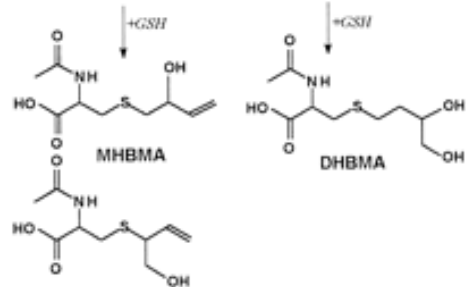
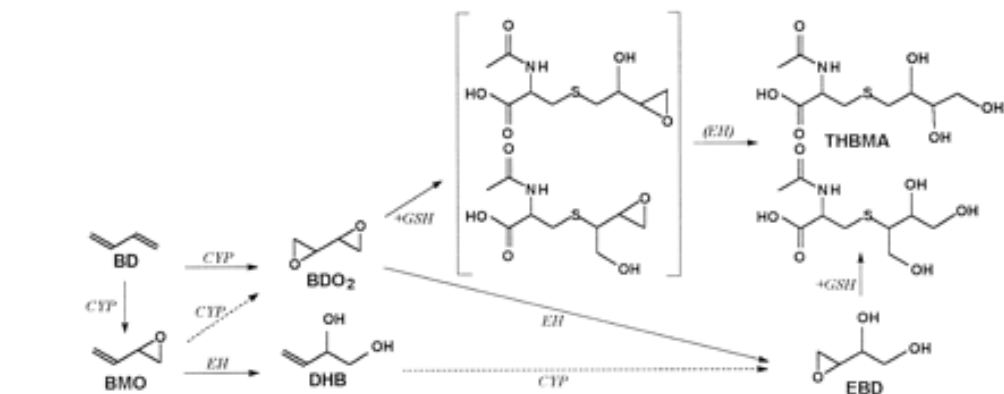


Biomarker

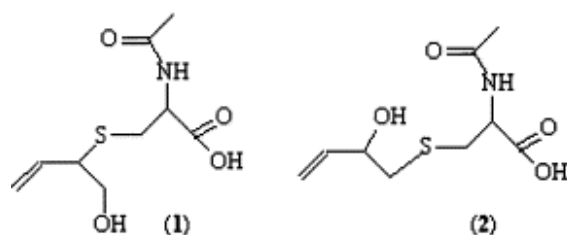
MHBMA



Analyte



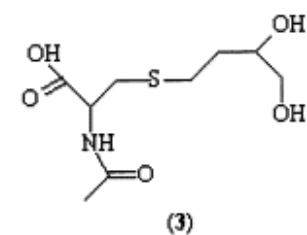
MHBMA:



R,S-1-Hydroxy-2-
(*N*-acetylcysteinyl)-3-butene

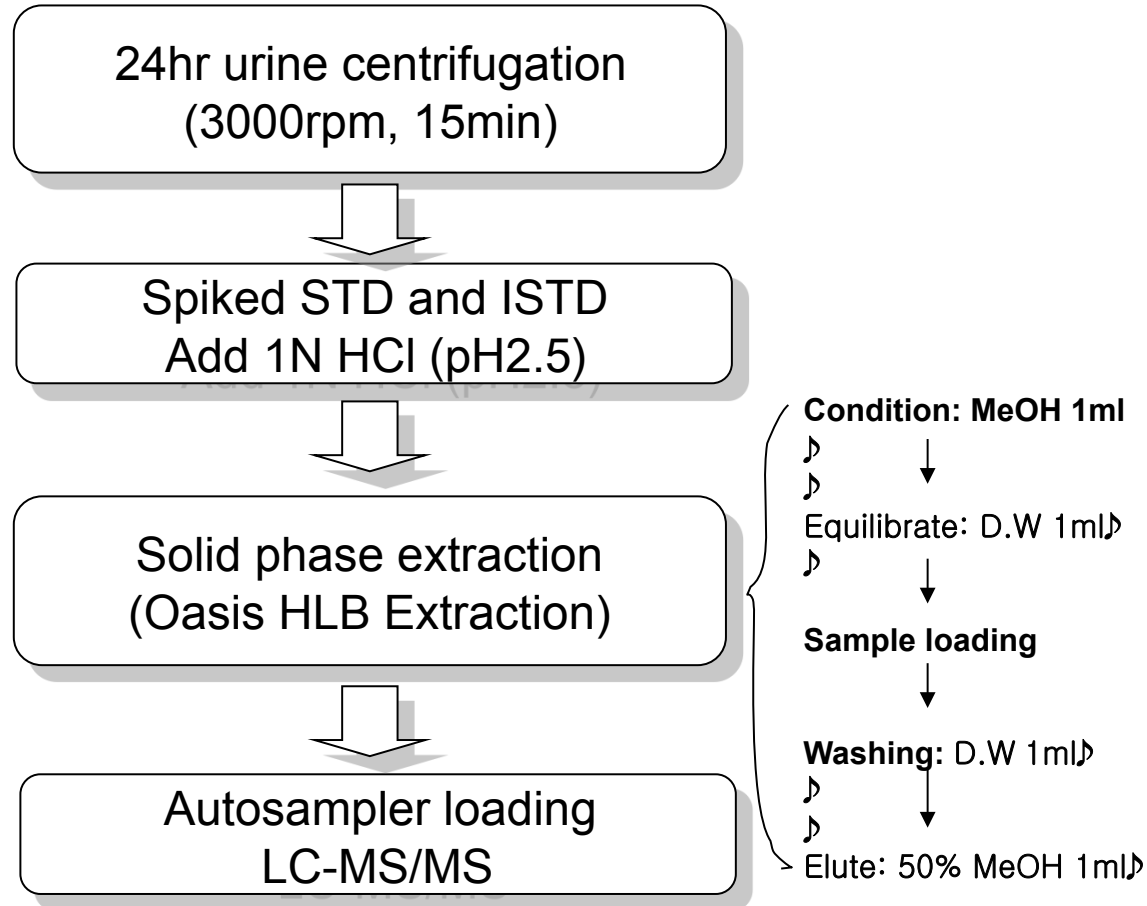
R,S-2-Hydroxy-1-
(*N*-acetylcysteinyl)-3-butene

DHBMA:



R,S-1,2-Dihydroxy-4-
(*N*-acetylcysteinyl)-butane

Analytical Procedure



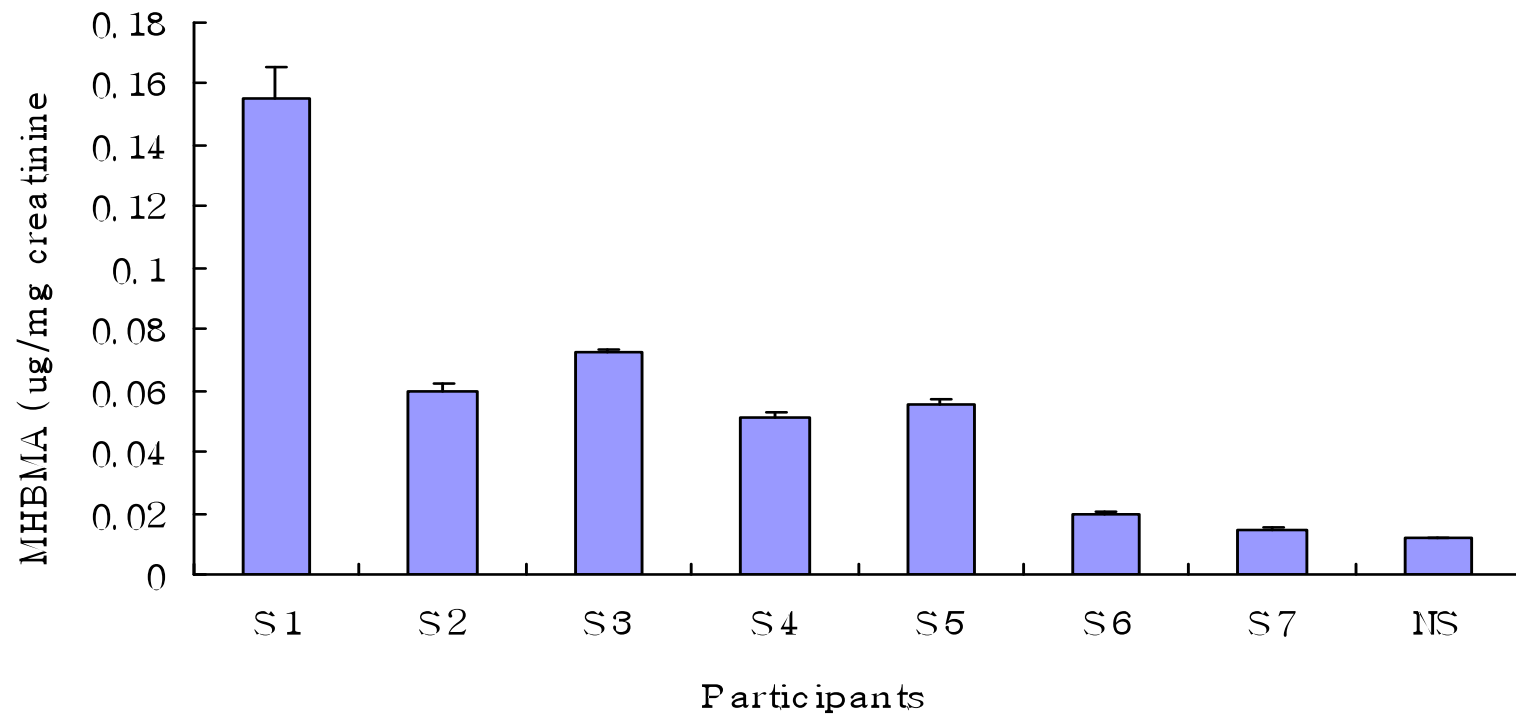
Instrumental Condition

HPLC	
Column	RP-8 anion exchange(100x2.1mm, 7μm)
Mobile phase	5mM Ammoniumformate buffer in 70% methanol
Flow rate / Injection	300ml/min, 10ul
LC-MS/MS	
Ion source	ESI
Mode of analysis	Negative
Acquisition mode	MRM
Ion transition (m/z)	MHBMA : 232.1→103.0 MHBMA-d₆ : 238.1→109.0m/z DHBMA : 250.1→121.0 DHBMA-d₇ : 257.2→128.1

- Calibration range : 0~1000ng/nl
- R²=0.9995

Results(I)

Total quantity of MHBMA

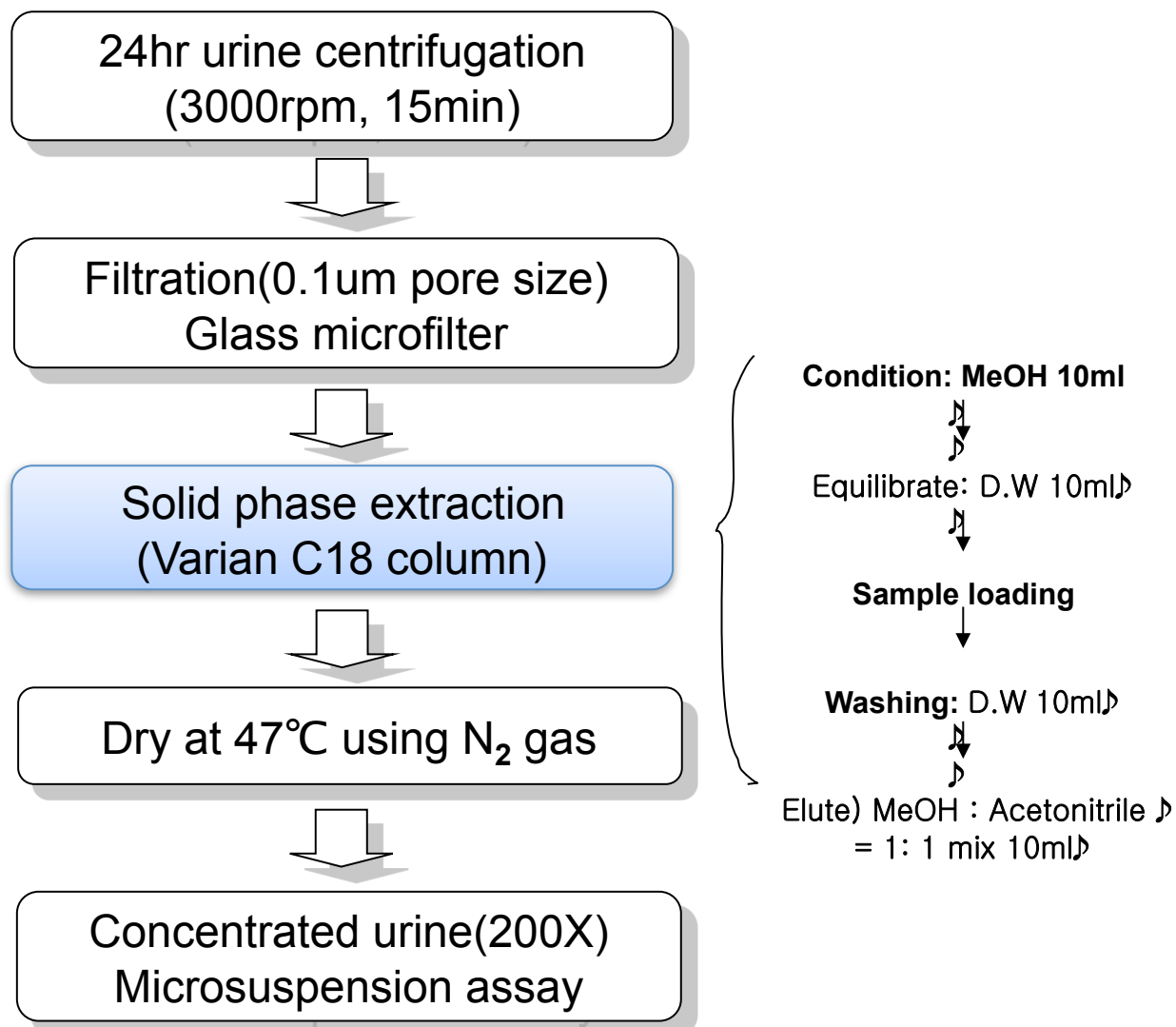




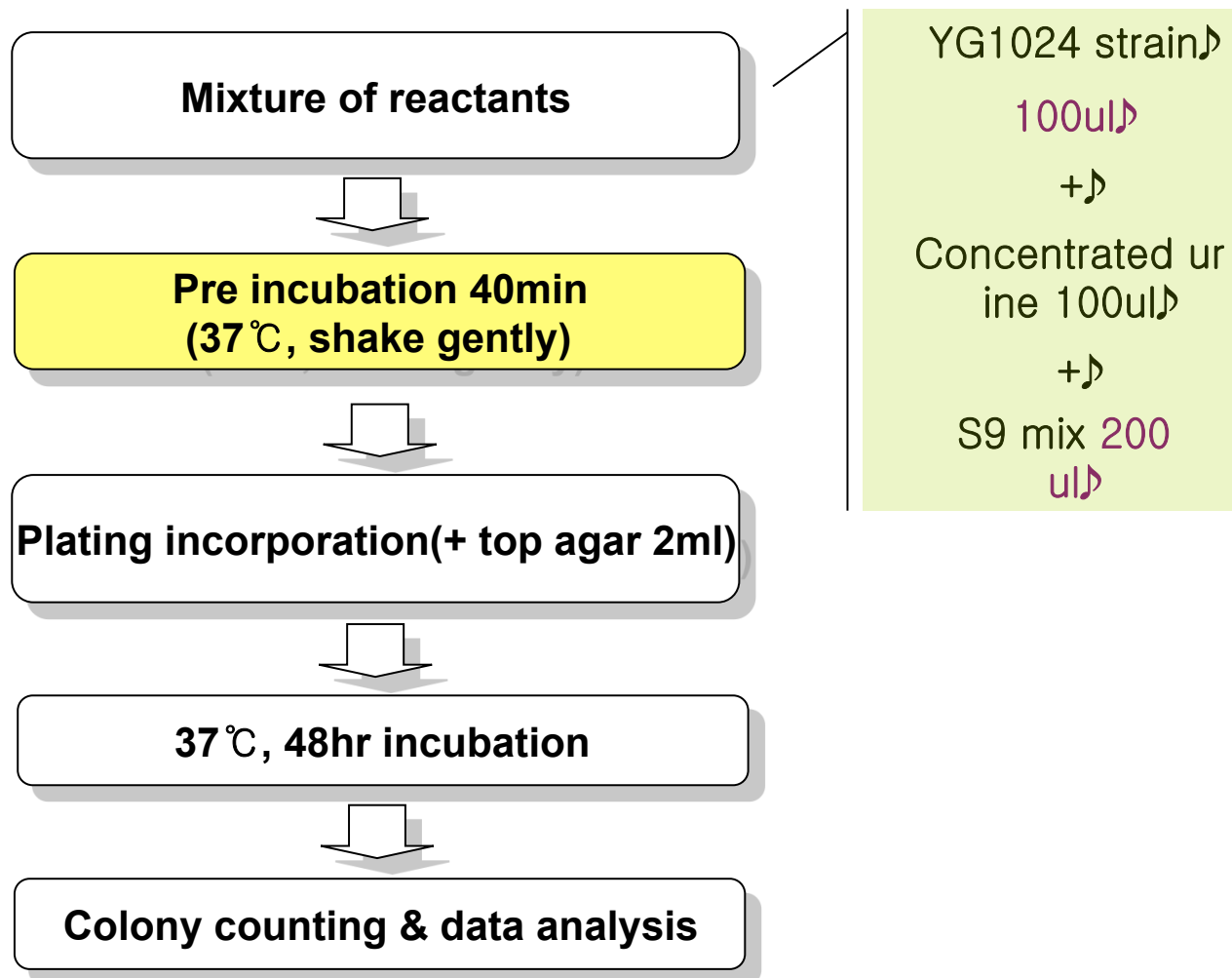
Biomarker♪

Urine Mutagenicity

Procedure of sample preparation

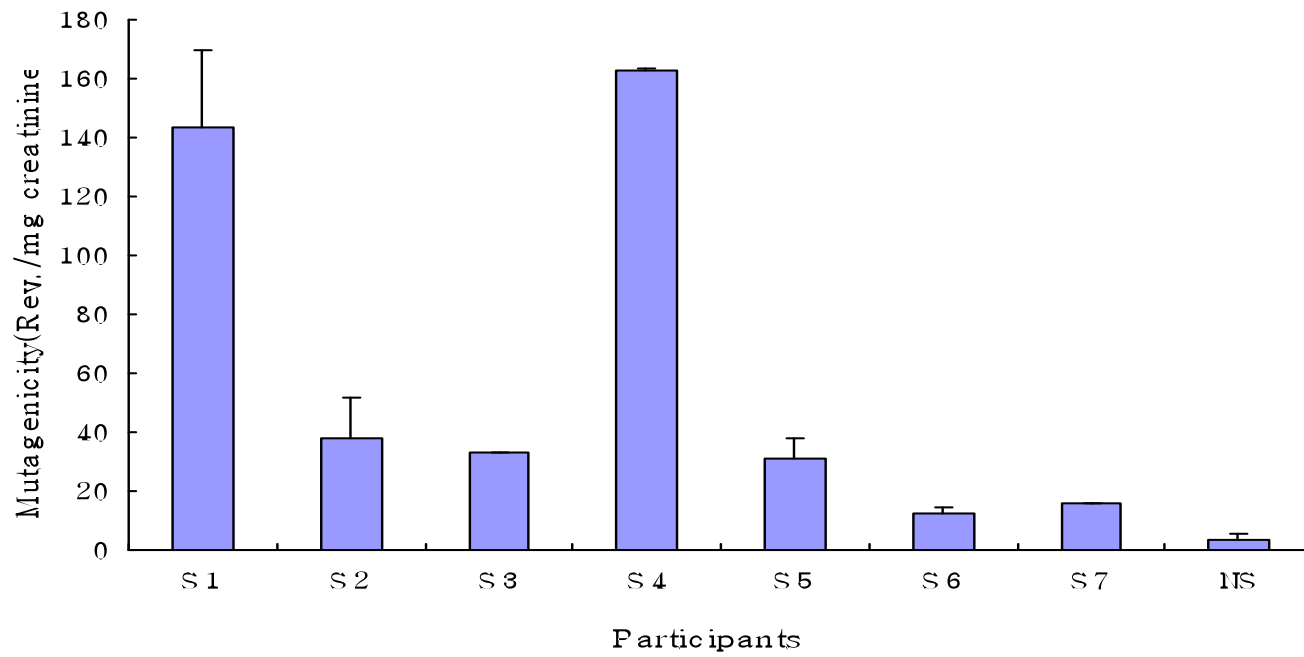


Procedure of Microsuspension assay

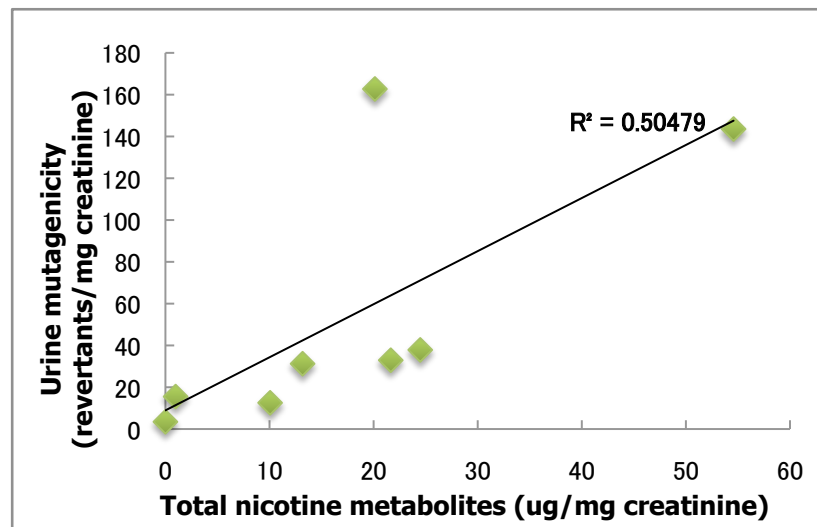
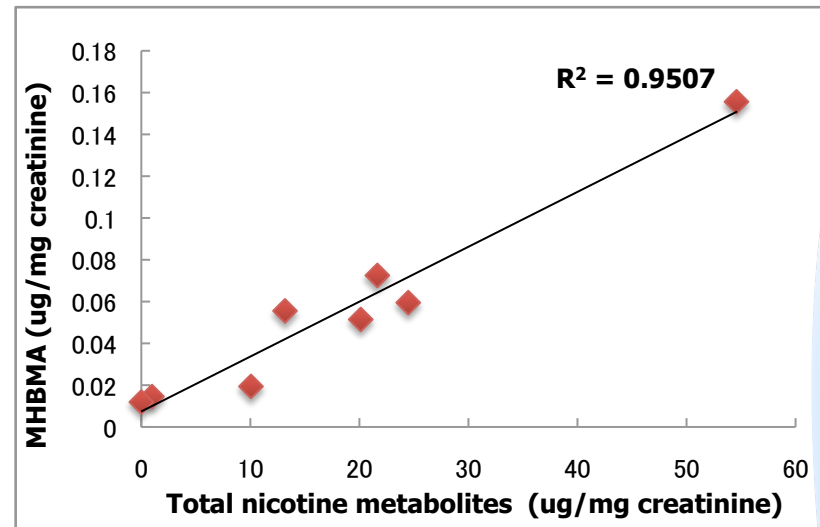
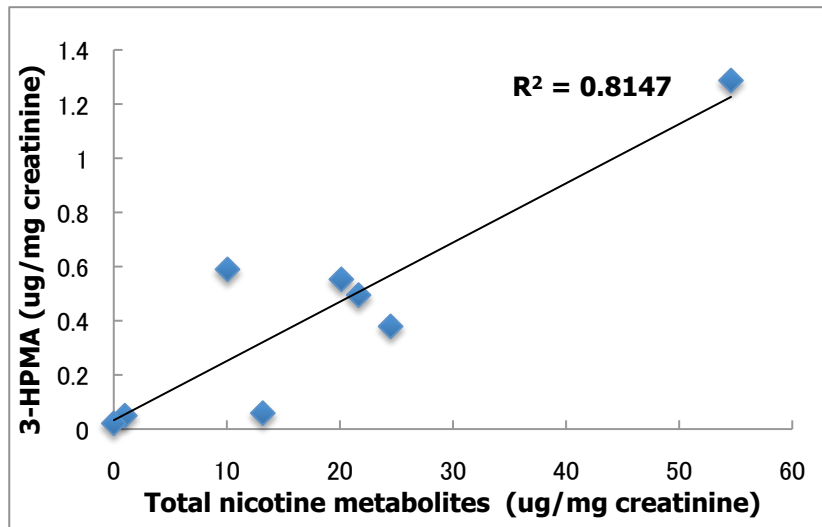


Results(I)

Urine mutagenicity in Participants



The relation between nicotine metabolites and specific cigarette smoke constituents



Summary

- In this study, we measured the levels of selected biomarkers of cigarette smoke exposure in urine samples from participants.
- We assessed the nicotine and 5 kinds of nicotine metabolites level.
- Also we measured the specific cigarette smoke constituents biomarker for acrolein, 1,3-butadiene and urine mutagen.
- We analyzed relationship between nicotine metabolites and specific cigarette smoke constituents.
- As a result, selected biomarkers such as acrolein, 1,3-butadiene and urine mutagenicity have a correlation with nicotine and nicotine metabolites.
- So, we're going to use nicotine and smoke constituents in human urine to evaluate the exposure of smoke in Korea

THANK YOU FOR ATTENTIONN