

SUPPLEMENTARY MATERIAL TO
**Dispersive liquid–liquid microextraction for determining
urinary muconic acid as benzene biological indicator**

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Table S-I. Matrix of of designed runs and related normalized peak areas

	Dispersive (μL)	Extraction (μL)	pH	Salt% (w/v%)	Centrifuge time (min)	Peak Area
1	100	150	6	4	3	7.12
2	200	100	8	6	4	64.45
3	300	250	6	4	3	90.27
4	300	150	2	4	3	5.66
5	400	100	8	2	4	1.30
6	300	150	6	4	3	44.54
7	400	200	4	6	2	1.44
8	300	150	6	8	3	66.48
9	300	50	6	4	3	5.70
10	300	150	6	4	1	55.23
11	300	150	6	4	3	36.59
12	400	200	4	2	4	4.93
13	200	200	8	6	2	49.01
14	500	150	6	4	3	72.80
15	400	100	8	6	2	61.78
16	300	150	6	4	3	51.41
17	200	200	4	6	4	69.83
18	400	200	8	2	2	35.02
19	300	150	6	0	3	35.60
20	200	100	4	2	2	29.65
21	300	150	10	4	3	100.00
22	300	150	6	4	3	52.49

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23	300	150	6	4	5	37.91
24	200	200	8	2	4	97.97
25	400	100	4	6	4	46.64
26	300	150	6	4	3	47.94

Table S-II. Analysis of variance (ANOVA), summary statistics of the quadratic model, in the current microextraction study

Source of Variation	Sum of square	Df ^a	Mean Square	F-Value ^b	p-value (Prob > F)	Status
Model	20471.48	11	1861.043	41.17	< 0.0001	significant
A-Dispersive Solvent	3095.271	1	3095.271	68.48	< 0.0001	
B-Extraction Solvent	4642.807	1	4642.807	102.72	< 0.0001	
C-pH	7707.412	1	7707.412	170.52	< 0.0001	
D-Salt	907.399	1	907.399	20.08	0.0005	
E-Centrifuge Time	373.4016	1	373.401	8.26	0.0122	
AB	3626.333	1	3626.333	80.23	< 0.0001	
AC	1863.888	1	1863.888	41.24	< 0.0001	
BE	4386.713	1	4386.713	97.05	< 0.0001	
CD	589.010	1	589.010	13.03	0.0028	
CE	2688.925	1	2688.925	59.49	< 0.0001	
DE	8302.758	1	8302.758	183.69	< 0.0001	
Residual	632.787	14	45.199			
Lack of Fit	468.827	10	46.883	1.144	0.4875	not significant
Pure Error	163.960	4	40.990			
Cor Total	21104.27	25				

^a: Degree of freedom. ^b: Test to comparing model variance with residual variance or significance of included factors.

Table S-III. Result of simplex optimization for the best recovery of tt-MA

Variables	Optimum Value
Extractant solvent volume (μL)	300
Disperser solvent volume (μL)	300
Salt amount (w/v,%)	3.4
Centrifuge time (min)	4
pH	8

Table S-IV. Comparison between the proposed DLLME method and other preconcentration methods using HPLC for the extraction of *t,t*-MA from urine matrix

Ref.	Method (analytical technique)	Extraction solvents/method	Additional solvents	Sample volume	Extraction time	LDR ($\mu\text{g/mL}$)	LOD ($\mu\text{g/L}$)
1	SLM ^a (HPLC-PDA)	m-xylene; 6-undecanone, and di-n-hexyl ether, tri-n-octylphosphine oxide	Acetonitrile	3 ml	13 min	0-0.2	10
2	LLE (HPLC-UV)	Ethyl acetate	-	8 ml	-	0.25-5	16.7
3	SPE (HPLC-DAD)	Oasis [®] MAX sorbents	HCl, Ethyl acetate, Formic acid	50 μl	10 min>	30 min>	-
4	SPE (HPLC-UV)	SAX sorbent	10% (v/v) Acetic acid	50 ml	-	-	100
5	SPE (HPLC-UV)	Elut-SAX cartridges	Acetonitrile, Acetic acid	1 ml	13 min	0.05-2	10.8
6	MIP ^b (HPLC-UV)	Ionic exchange extraction	Phosphate buffer, Methanol	2 ml	20 min>	0.1-10	10
7	HFLPME ^c (HPLC-UV)	Trioctylphosphine oxide(TOPO)	-	11 ml	120 min	0.005-1.2	1
8	MEPS ^d (HPLC-UV)	SAX sorbent	10%(v/v) Acetic acid	0.5 ml	4 min	0.1-2	32
9	MIMEPS ^e (HPLC-UV)	MIP in packed sorbent	Ethanol, Acetic acid	500 μl	5 min	0.015-2	15
10	Ion-pair HFLPME (HPLC-UV)	1-Octanol containing 10% w/v of Aliquat-336	-	10 ml	60 min	0.001-0.9	0.1
11	PDLLME (HPLC-UV)	Chloroform	Tetrahydrofuran, Methanol	5 ml	15 min>	0.1-10	0.11
12	SPME (HPLC-UV)	zirconium-based metal-organic frameworks	Acetonitrile, Acetic acid	-	-	0.01-50	10
13	In syringe IL-DLLME (HPLC-UV)	([P14,6,6,6]Cl (ionic liquid, 40 μL))	-	6.5 ml	10 min >	0.032-10	11
14	DLLME-SFOD ^f (HPLC-UV)	1-undecanol	Methanol	10 ml	10 min >	0.02-5	6
This work	DLLME (HPLC-UV)	Chloroform	Acetonitrile	2 ml	8 min	0.008-5	2.4

^aSupported Liquid Membrane/^bmolecularly imprinted polymer/^cHollow-fiber liquid-phase microextraction/^d microextraction by packed sorbent/^ecombination of MIP and MEPS/^fSolidified loading organic droplet

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