Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Agarose Hydrogel-enhanced Paper Spray Ionization Mass Spectrometry for Metabolites Detection in Raw Urine

Liujuan Zhan,[a] Zhuanghao Hou [a] and Guangming Huang*[a, b]

a. Department of Chemistry, School of Chemistry and Materials Science, University of Science and Technology of China , Hefei Anhui, 230026, People's Republic of China.

b. National Synchrotron Radiation Laboratory, University of Science and Technology of China, Hefei, Anhui 230029, P.R. China.

*Corresponding Author:

Email: gmhuang@ustc.edu.cn ; Fax: +86-551-63600706; Tel: +86-551-63600706

Table of Contents

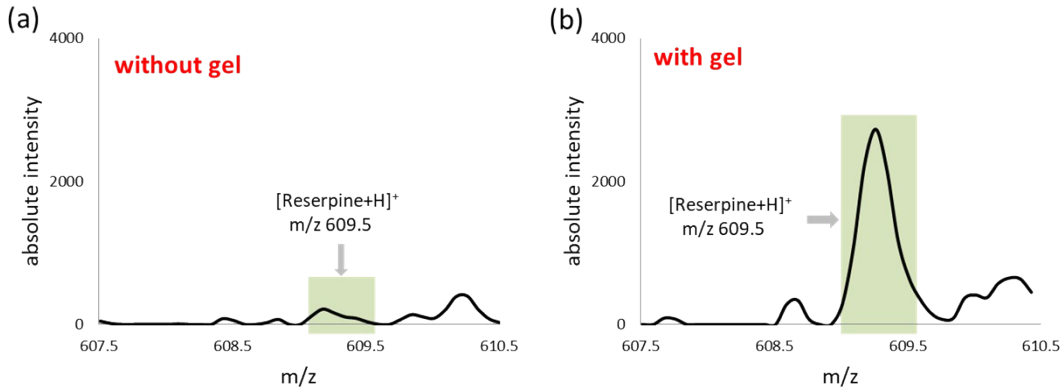

Fig. S1 Mass spectra (with background subtraction) of raw urine spiked with 1 mg/L reserpine (a) without and (b) with hydrogel conditioning.

In order to prove applicability of agarose hydrogel conditioning coupled with PSI MS, raw urine samples spiked with 1 mg/L reserpine were tested. Other than fragment ions produced by collision induced dissociation, the improvement on protonated ion of reserpine was also observed. As shown in Fig. S1, the absolute intensity of reserpine using the agarose hydrogel conditioning was ~15 folds higher than that obtained by direct paper spray analysis. The result suggested that agarose hydrogel enhanced-PSI MS could improve ion signal intensity of reserpine spiked in raw urine.

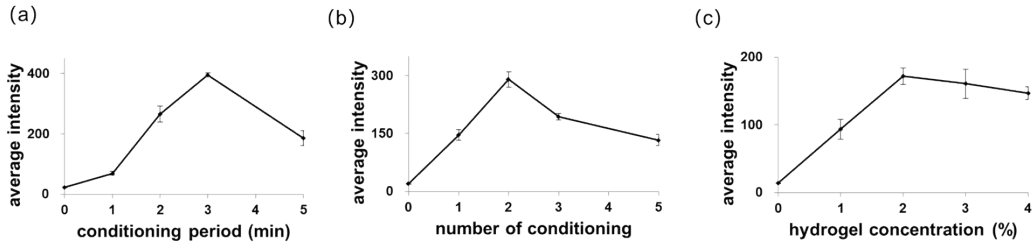

Fig. S2 Influence of (a) conditioning period, (b) number of conditioning, and (c) hydrogel concentration for the hydrogel enhanced-paper spray of signal improvement.

To maximize the improvement of the analytical performances, several key experimental parameters involve conditioning periods, number of conditioning and agarose concentration were optimized. Raw urine samples spiked with 1 mg/L reserpine were applied to optimize the experiments.

First, different conditioning period (0, 1, 2, 3, 5 mins) were tested. 1 μL sample was preloaded on the paper, 2% hydrogel block was gently placed on the paper with 1, 2, 3, 5 mins conditioning periods. Then, the hydrogel block was removed from the surface of paper, 15 μL spray solvent (CH3OH:H2O = 8:2, v/v) was applied for analysis. As shown in Fig. S2a, the signal of reserpine raise gradually as the conditioning period increased until 3 min, and reserpine signal dropped when conditioning period was longer than 3 min. It seems that prolonged contact between the hydrogel and the analytes on the surface of the paper causes more analytes to adhere to the bottom of the hydrogel, resulting in signal decrease. The conditioning period of 3 mins may have reached the exchange equilibrium between the paper surface and the layer of hydrogel with minimal loss of analytes. Thus, 3 mins was selected as the conditioning period.

Second, number of conditioning (0, 1, 2, 3, 5 times) was investigated. 1 μL sample was preloaded on the paper, 2% hydrogel block was gently placed on the paper with 1, 2, 3, 5 times conditioning. The results suggested that twice conditioning process gave the best signal of reserpine. Hence, the number of conditioning was set to 2 to achieve the best cleanup effect (Fig. S2b).

Third, the effect of agarose concentration (1%, 2%, 3%, 4%) was also studied. Different concentration hydrogel block was gently placed on the paper with 3 mins conditioning periods and repeat twice. When agarose concentration was 2%, the curve reached the max point, and after that, curve trended to equilibrium with no significant difference. Therefore, 2% agarose concentration was set to achieve desirable signal intensity (Fig. S2c).

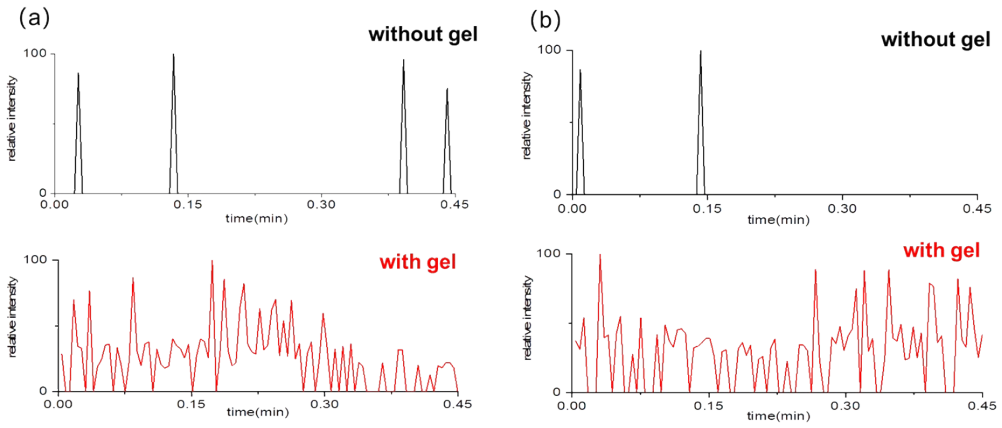

Fig. S3 Extracted ion chronograms (EIC) of endogenous metabolites with (red) and without (black) hydrogel conditioning: (a) guanine and (b) phosphocreatine in raw urine, show an increasing frequency of detection with hydrogel conditioning.

To evaluate the analytical performance of the developed method for endogenous metabolites, raw urine was investigated here. 1 µL raw urine sample was preloaded on the paper, then hydrogel block was gently placed on the paper substrate for 3 mins and the conditioning was repeated twice. After that, the hydrogel block was removed from the surface of paper, 15 µL spray solvent (CH3OH:H2O = 8:2, v/v) and 4.0 kV voltages was applied for the following PSI MS analysis. The extracted ion chronograms (EIC) of guanine and phosphocreatine in raw urine were shown in Fig. S3, respectively. With agarose hydrogel conditioning-PSI MS, guanine and phosphocreatine show an increase of detection frequency. The average signal intensity increased ~72 folds for guanine (*m/z* 152.0577) and ~86 folds for phosphocreatine (*m/z* 212.0461) compare with direct PSI MS. It should be noted that due to the discontinuity of the signal, the average signal intensities of endogenous metabolites are taken from 0-0.1 mins of the spectrum. These results demonstrated the feasibility of agarose hydrogel conditioning could enhance the signal intensity of some metabolites in raw urine of PSI MS.

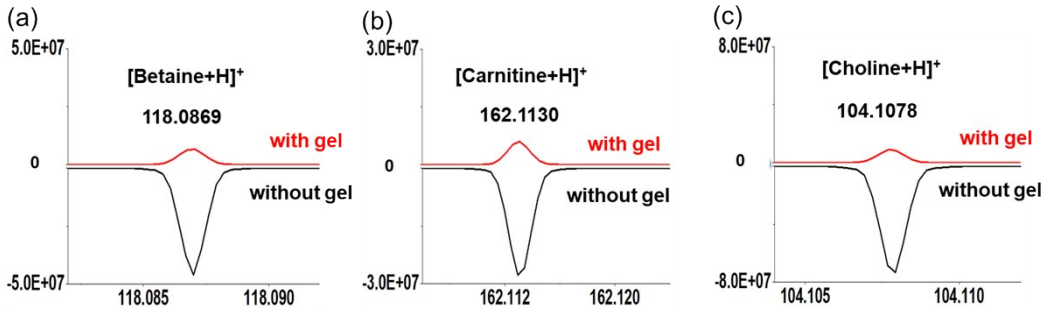

Fig. S4 Mass spectra of reduced signal of endogenous metabolites with (red) and without (black) hydrogel conditioning: (a) betaine, (b) carnitine, and (c) choline in raw urine.

The possible reasons for the signal intensity enhancement were also initially investigated here. For endogenous metabolites analysis in raw urine, it was found that the signal intensities of some endogenous metabolites decreased with agarose hydrogel conditioning-PSI MS. As shown in Fig. S4, the signal intensities decreased ~7 folds for betaine (*m/z* 118.0869), ~5 folds for carnitine (*m/z* 162.1130), ~10 folds for choline (*m/z* 104.1078) compare with direct PSI MS. The presence of these quaternary ammonium salts could also cause ion suppression of target compounds. Therefore, the result indicated that the improvement of therapeutic drugs and endogenous metabolites ion intensity might be attributed to the reduction of quaternary ammonium salts.

Table S1. Summary of the parameters for tandem mass spectra of each therapeutic drug.

Analytes	Parent ion	Product ion	Collision energy (eV)
Reserpine	609	397	40
Adriamycin	544	397	50
Propranolol	260	183	35
Norfloxacin	320	276	35
Tioconazole	387	131	35
Curcumin	369	177	35

Table S2. Improvement of characteristic fragment ions intensity of other therapeutic drugs after hydrogel conditioning.

Analytes	Concentration	PS	Gel-PS	Fold
Reserpine	1 mg/L	21.9	309	14.12
Curcumin	50 mg/L	74.1	786	10.61
Tioconazole	200 μg/L	13.1	116	8.85
Norfloxacin	2 mg/L	1110	6440	5.80
Propranolol	1 mg/L	506	2780	5.49
Adriamycin	2 mg/L	28.2	145	5.14

Table S3. Relative standard deviation (RSD) for reserpine, tioconazole, and adriamycin in different concentrations.

Analytes	Concentration	RSD (n=3)	
		PS	Gel-PS
Reserpine	50 μg/L	7.1%	9.7%
	500 μg/L	8.4%	3.2%
	1000 μg/L	5.9%	6.5%
Tioconazole	50 μg/L	4.0%	2.4%
	500 μg/L	7.0%	5.3%
	1000 μg/L	8.5%	6.8%
Adriamycin	200 μg/L	5.5%	9.8%
	1000 μg/L	6.8%	7.4%
	5000 μg/L	9.7%	2.6%

Table S4. Change folds of partial endogenous metabolites signal in raw urine after hydrogel conditioning.

Name	Formula	Ion type	Theoretical *m/z*	Measured *m/z*	Relative error(ppm)	Gel-PS	PS	Fold
Niacinamide	$C_6H_6N_2O$	$[M+H]^+$	123.0553	123.0560	5.69	1.20E6	1.74E5	6.9
Nicotinic acid	$C_6H_5NO_2$	$[M+H]^+$	124.0393	124.0401	6.45	2.86E5	7.73E3	36.9
Ornithine	$C_5H_{12}N_2O_2$	$[M+H]^+$	133.0972	133.0977	3.75	9.84E4	3.67E3	26.8
Niacinamide	$C_6H_6N_2O$	$[M+Na]^+$	145.0372	145.0378	4.14	2.11E6	1.79E5	11.7
Glutamine	$C_5H_{10}N_2O_3$	$[M+H]^+$	147.0764	147.0760	-2.72	2.98E6	1.78E6	1.7
Guanine	$C_5H_5N_5O$	$[M+H]^+$	152.0567	152.0577	6.57	3.30E5	4.60E3	71.7
Histidine	$C_6H_9N_3O_2$	$[M+H]^+$	156.0768	156.0774	3.84	3.86E7	1.17E7	3.3
Imidazolelactic acid	$C_6H_8N_2O_3$	$[M+H]^+$	157.0608	157.0614	3.82	1.62E5	6.12E4	2.6
Indolecarboxylic acid	$C_9H_7NO_2$	$[M+Na]^+$	162.0550	162.0533	-1.85	4.39E6	3.58E5	12.3
2-Furoylglycine	$C_7H_7NO_4$	$[M+H]^+$	170.0448	170.0454	3.53	1.02E6	6.00E4	17.0
Nicotinuric acid Picolinoylglycine	$C_8H_8N_2O_3$	$[M+H]^+$	181.0608	181.0608	0	3.25E5	3.88E3	83.7
Tyrosine	$C_9H_{11}NO_3$	$[M+H]^+$	182.0812	182.0811	-0.55	7.68E5	2.44E5	3.2
Acetyldopamine	$C_{10}H_{13}NO_3$	$[M+H]^+$	196.0968	196.0951	-9.68	1.36E6	4.70E4	28.9
Phosphocreatine	$C_4H_{10}N_3O_5P$	$[M+H]^+$	212.0431	212.0452	9.90	3.98E5	4.63E3	85.9
octenoylglycine	$C_{10}H_{17}NO_3$	$[M+Na]^+$	222.1101	222.1106	2.25	6.82E4	1.70E4	3.8
Valproylglycine	$C_{10}H_{19}NO_3$	$[M+Na]^+$	224.1257	224.1264	3.12	1.36E6	9.25E4	14.7
Acetyldopamine Acetylaminooctanoic acid	$C_{10}H_{13}NO_3$	$[M+K]^+$	234.0527	234.0527	0	2.68E5	3.64E4	7.4
Oleamide	$C_{18}H_{35}NO$	$[M+H]^+$	282.2791	282.2800	3.18	1.46E8	8.52E6	17.1
Sphinganine	$C_{18}H_{39}NO_2$	$[M+H]^+$	302.3054	302.3064	3.31	4.93E6	4.76E5	10.7
Sphingosine	$C_{18}H_{37}NO_2$	$[M+Na]^+$	322.2716	322.2725	2.79	2.21E6	5.22E4	42.3
Unknown	\	\	\	111.0466	\	1.05E8	1.64E7	6.4
	\	\	\	133.0464	\	3.07E7	3.28E6	9.4
	\	\	\	149.0239	\	5.18E7	8.15E6	6.4
	\	\	\	190.6335	\	2.00E7	3.43E5	58.4
	\	\	\	220.6497	\	3.72E7	1.38E6	27.0
	\	\	\	233.0738	\	2.25E7	4.81E6	4.7
Choline	$C_5H_{14}NO$	$[M+H]^+$	104.1075	104.1078	2.88	7.68E6	7.58E7	0.10
Trimethylaminoacetone	$C_6H_{14}NO$	$[M+H]^+$	116.1075	116.1077	1.72	1.77E7	8.50E7	0.21
Betaine	$C_5H_{11}NO_2$	$[M+H]^+$	118.0863	118.0869	5.08	5.59E6	3.93E7	0.14
Homoserine Allothreonine Threonine	$C_4H_9NO_3$	$[M+H]^+$	120.0655	120.0662	5.83	4.99E5	7.54E6	0.066
Creatine	$C_4H_9N_3O_2$	$[M+K]^+$	170.0326	170.0333	4.12	0	3.32E4	0

Leucine	$C_6H_{13}NO_2$	$[M+H]^+$	132.1019	132.1024	3.78	1.02E6	7.10E6	0.14
Proline betaine	$C_7H_{13}NO_2$	$[M+H]^+$	144.1019	144.1029	3.47	2.67E8	5.48E7	0.048
4-Trimethylammonibutanoic acid	$C_7H_{16}NO_2$	$[M+H]^+$	146.1181	146.1181	0	9.51E4	4.51E6	0.02
1-Nitroheptane								
Carnitine	$C_7H_{15}NO_3$	$[M+H]^+$	162.1125	162.1130	3.08	5.81E6	2.55E7	0.22
Acetylcarnitine	$C_9H_{17}NO_4$	$[M+H]^+$	204.123	204.1239	4.41	2.31E6	2.37E7	0.097
Leucylproline	$C_{11}H_{20}N_2O_3$	$[M+H]^+$	229.1547	229.1553	2.62	2.27E7	3.25E8	0.069
Butyrylcarnitine	$C_{11}H_{21}NO_4$	$[M+H]^+$	232.1543	232.1552	3.87	8.84E6	3.34E7	0.26
Tiglylcarnitine	$C_{12}H_{21}NO_4$	$[M+H]^+$	244.1543	244.1551	3.28	1.99E5	3.38E6	0.059
Unknown	\	\	\	169.1342	\	6.62E6	3.44E7	0.19
	\	\	\	180.8951	\	4.89E6	2.61E7	0.17
	\	\	\	227.1259	\	7.67E7	4.63E8	0.16
	\	\	\	228.1293	\	6.57E6	4.04E7	0.16
	\	\	\	230.1587	\	1.70E6	3.62E7	0.047
	\	\	\	257.1617	\	3.52E5	3.15E7	0.011

Compounds that cannot be found in the database (http://www.hmdb.ca/) were marked as “unknown”.