Electronic Supplementary Material

Application of ultrasonic for facilitate extraction of hippuric acid and methyl hippuric acid in real samples by using Fe_3O_4@SiO_2@sodium dodecyl sulfate: Experimental design methodology

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2.1. Reagents and chemicals

The analytical standards of hippuric acid and 3- methyl hippuric acid with purity 98% were purchased from Sigma -Aldrich (St. Louis, MO, USA). Ferric chloride ($FeCl_3.6H_2O$), ferrous chloride ($FeCl_2.4H_2O$), ammounium hydroxide (NH_4OH), sodium hydroxide (NaOH), hydrochloride acid (HCl), acetonitrile (ACN), sodium chloride (NaCl), isopropanol, and ethanol were obtained from Merck (Darmstadt, Germany).Tetraethyl orthosilicate ($Si(OEt)_4$) was supplied from Sigma-Aldrich. Deionized water was obtained from a Milli-Q Water System (Millipore, MA, USA).

2.2. Apparatus

Samples analysis were carried out by a reversed-phase HPLC instrument (Knauer Company, Germany), consisted of a K-1001 series high-pressure pump, a K2006 ultra violet detector and a VS injection valve, equipped with a 20-µL loop. The chromatographic separation of the analytes were achieved on a knauer RP- C18 (250 mm × 4.6 mm × 5 µm) column. The mobile phase composition was a mix of acetonitrile: purified water (37.5:62.5) and 62.5 µL of acetic acid with a flow-rate of 1.0 mL min^{-1} in isocratic mode. The detector's wavelength was set at 254 nm.

2.3. Synthesis of Fe_3O_4@SiO_2 nanoparticles

The procedure previously described by Li was applied for synthesis of magnetic Fe_3O_4 nanoparticles [1]. Accordingly, 10.4 g of $FeCl_3$. 6 H_2O and 4 g of $FeCl_2$. 4 H_2O were weighed by a digital scale and then dissolved in a 200 mL of double distilled water. The mixture were purged with a stream of nitrogen and to heated and heated to 80 °C. Thereafter, 15 mL of ammonium hydroxide (NH_4OH 32%) was added dropwise to the mixture and stirred for 1 h at 500 rpm. After finishing the synthesis step, the prepared nanoparticles were separated from the solution by applying an external magnetic force. The obtained magnetic nanoparticles were washed with double distilled water to achieve pH of 7. The prepared nanoparticles were

then dried at 70 º C. In In a subsequent step, 1.5 g of Fe_3O_4 nanoparticles were weighed and dispersed in the mixture solution of 60 mL pure water, 240 mL absolute ethanol and 10 mL of NH_4OH. The mixture solution was dispersed through ultrasonic waves for 10 min. After mechanically stirring for 15 min at 40 º C, 2.1 mL of TEOS was added to the mixture solution. Finally, the reaction solution was mechanically stirred for 48 h. The acquired precipitation was collected by an external magnetic field and washed with double distilled water for several time and dried at 70 º C.

3.3. Effects of matrix

The effects of matrix were investigated via determining the percentage of signal suppression/enhancement acquired under the optimum conditions of extraction for the real urine samples. In this respect, the obtained peak area from the injection of spiked blank urine was compared with those to the corresponding spiked solvent (mobile phase) at the same concentration level. The obtained data indicated that the recoveries were 88-95% (n = 5). It was also shown that there was very slight matrix effects (in terms of signal suppression) when the blank were spiked with 50 μg L^{-1} of HA and MHA.

[1] Li, C., Chen, L., Determination of pyrethroid pesticides in environmental waters based on magnetic titanium dioxide nanoparticles extraction followed by HPLC analysis. *Chromatographia* 2013, 76, 409-417.

Table 1S: Variables and their levels for Box-Behnken design (BBD).

Variable	Level		
	Lower	Central	Upper
SDS concentration (mmol L^{-1})	4	5	6
sorbent amount (mg)	5	10	15
pH	1	2	3
Sonication time (min)	1	2.5	4

Table 2S. Comparison of the developed sample preparation method with recently published articles.

Determination method	Sample preparation technique	Metabolites	MDL [a]	RSD %	Ref.
GC-FID	µ-SPE	HA	16.5	1.6	[27]
GC-MS	LLE	HA and MHA	17-20	1- 4.2	[28]
HPLC-UV	MINPs	HA	0.15	<6.1	[29]
HPLC-UV	LPME	MHA	2-3	3.8-7.3	[30]
HPLC-UV	---	HA	700	3.2	[31]
HPLC-UV	LLE	HA and MHA	5000-6000	5.3-6.7	[32]
GC-MS	SPE	HA and MHA	1000-10000	2.5-7.4	[33]
HPLC-UV	SPE	MHA	4000	1.1-11.7	[34]
LC/MS/MS	SPE	HA	5	3-10	[35]
HPLC-UV	SA-d-SPE	HA and MHA	0.2	3.2-7.1	This study

a) Method detection limit (µg L^{-1})