1 **Development of magnetic solid phase extraction based on magnetic chitosan-graphene oxide**

2 **nanoparticles and deep eutectic solvents for the determination of flavonoids by high**

3 **performance liquid chromatography**

4 Zahra Jafari, Mohammad Reza Hadjmohammadi[*]

5 Department of Analytical Chemistry, Faculty of Chemistry, University of Mazandaran,

6 NirooHavayiiboulevard,47416-95447 Babolsar, Iran

7

8

9

10

11

12

13 [*]Corresponding author: Prof. M.R. Hadjmohammadi, Department of Analytical Chemistry,

14 Faculty of Chemistry, University of Mazandaran, NirooHavayiiboulevard,47416-95447

15 Babolsar, Iran

16 Tel.: +98 113532350; fax: +98 1135342350.

17 E-mail address: hadjmr@umz.ac.ir (M.R. Hadjmohammadi)

18
19
20

21 *Synthesis of GO*

22 Concentrated H_2SO_4 (23 mL) was affixed to a mix of graphite powder (1.0 g) and $NaNO_3$ (0.5 g)

23 in an ice bath and stirred. Then $KmnO_4$ (3.0 g) was added gradually to this mix for 20 min. After

24 5 h, the mix was removed from the ice-bath and extra 3 g of potassium permanganate was affixed

25 to the mix. After 12 h, 140 mL of water was poured into the mix. Then,1 mL of H_2O_2 (30% v/v))

26 was added dropwise to the mixture subsequently until the color of the mixture changed from

27 brownish into bright yellow. The mix was centrifuged. The obtained results were then washed with

28 30% HCl and water until the pH reached to neutral. The finally obtained product was vacuum-

29 dried at 50 °C.

30 *Calculation of analytical parameters*

31 Herein, to achieve the optimized efficiency of the MSPE method, extraction recovery (ER),

32 enrichment factor (EF), and spiking recovery (SR) was employed to investigate the extraction

33 efficiency under optimized conditions.

34 EF is defined as follows:

$$\%EF = \left(\frac{C_f}{C_i}\right) \times 100$$

35

36 That C_f and C_i are the concentrations of flavonoids in the desorption phase and the initial

37 concentration in the aqueous phase, respectively.

38 The extraction recovery of flavonoids is calculated as follows:

$$ER\% = \frac{n_a}{n_d} \times 100 = \frac{C_a V_a}{C_d V_d} \times 100 = EF \times \frac{V_a}{V_d} \times 100$$

39

40 where n_a the number of moles of analytes in the desorption phase and n_d number of moles of

41 analytes in the sample solution. Also, V_a and V_d are the volumes of desorption solvent and sample

42 solution, respectively.

43 The spiking recovery (SR) was applied in the analysis of real samples and is defined by the

44 following equation:

$$Spiking\ recovery\ (\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100$$

45

46 Here, C_{found} shows the concentrations of flavonoids after adding a specific amount of standard into

47 the real sample, C_{real} is the flavonoids initial concentration in the real samples, and C_{added} is the

48 concentration of the standard solution spiked into the real samples.

49 The ME% was investigated by the ratio of the slopes of the calibration curves in the desorption

50 solvent to slopes of the calibration curves in the matrix and determined as:

$$ME\% = \frac{(slope\ in\ matrix) - (slope\ in\ desorption\ solvent)}{(slope\ in\ desorption\ solvent)} \times 100$$

51

52

53

54

55

56

57

58 **Figure S1**. FT-IR spectra of the prepared DES, aloe vera gel, choline chloride, urea.

59 **Figure S2**. Pareto chart of the standardized effects obtained from a Plackett-Burman design.

60 **Figure S3.** Compare the desorption efficiency of DES with the conventional eluents such as

61 methanol, acetonitrile, ethanol and isopropanol.

62 **Figure S4**. Extraction efficiency of target analytes obtained by the GO, Fe_3O_4-GO and Fe_3O_4-CS-

63 GO

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87 **Table1S.** The experimental variable and levels of the Plackett-Burman design
88

Variables	Symbols	Low(-1)	High(1)
Sorbent amount	A	5	15
Extraction time (min)	B	2	15
Desorbtion time (min)	C	2	10
pH	D	3	7
elution solvent volume (µL)	E	100	500
Amount of salt 20 W/V%	F	0	20

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

Table S2. Analysis of variance table (ANOVA) of Plackett-Burman design

Source	df [a]	Sum of Squares	Mean Square	*F-Ratio*	*P-Value*
sorbent amount	1	1655645684	1655645684	29.88	0.003
extraction time	1	761183194	761183194	13.74	0.014
Desorb time	1	12724621	12724621	0.23	0.652
pH	1	118308920	118308920	2.14	0.204
solvent volume	1	3399357070	3399357070	61.36	0.001
Salt effect	1	713313780	713313780	12.87	0.016
Error	5	277022368	55404474		
Total	11	6937555637			

118 [a] degree of freedom

119

120

121

122

123

124

125

126

127

128

129

130

131

132

133

134

135

136 **Table S3**. The experimental design matrix and response of the Box-Bahnken design

Numbers	Extraction parameters				response surface
	A	B	C	D	
139 1	0	-1	0	1	153995
140 2	0	1	0	-1	150100
141 3	1	0	1	0	160347
142 4	0	0	0	0	143579
143 5	-1	0	-1	0	153745
144 6	-1	1	0	0	156817
145 7	0	0	-1	-1	141956
146 8	0	0	0	0	150135
147 9	0	1	-1	0	150566
148 10	0	0	-1	1	151011
149 11	1	0	0	-1	146446
150 12	0	-1	-1	0	152644
151 13	0	0	0	0	145728
152 14	-1	-1	0	0	161210
153 15	1	1	0	0	167071
154 16	-1	0	1	0	158617
155 17	0	1	1	0	168390
156 18	-1	0	0	1	159501
157 19	1	-1	0	0	145975
158 20	-1	0	0	-1	151528
159 21	0	-1	0	-1	153825
160 22	0	0	1	-1	152871
161 23	1	0	0	1	157687
162 24	1	0	-1	0	146011
163 25	0	-1	1	0	160108
164 26	0	0	1	1	164043
165 27	0	1	0	1	163904

166

Table S4. Analysis of Variance (ANOVA) table of Box –Behnken design

Source	DF	Sum of Squares	Mean Square	F-Value	P-Value
A	1	33915856	33915856	14.35	0.002
B	1	59857800	59857800	25.32	0.000
C	1	390336133	390336133	165.11	0.000
D	1	237763519	237763519	100.57	0.000
AA	1	151360195	151360195	64.02	0.000
BB	1	323080978	323080978	136.66	0.000
CC	1	106102409	106102409	44.88	0.000
DD	1	41208249	41208249	17.43	0.001
AB	1	192918210	192918210	81.60	0.000
AC	1	22406022	22406022	9.48	0.008
AD	1	26832400	26832400	11.35	0.005
BD	1	46471489	46471489	19.66	0.001
Error	14	33097994	2364142		
Lack-of-Fit	12	28515852	2376321	1.04	0.591
Pure Error	2	4582142	2291071		
Total	26	1424041226			

185

186

187

188

189

190

191 **Table S5.** Estimated determination coefficient of the BBD design

R^2	R^2 (pred)	R^2 (adj)
97.68%	89.41%	95.68%

192

193

194

195

196 **Table S6.** Optimized value of the factors obtained from BBD design (coded and un-coded values).

Factor	pH	Extraction time	Salt effect	Solvent volume
Coded value	-1	+ 1	+1	1
un-coded values	3	15	20% (w/v)	500

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217 **Table S7. Matrix effect (ME%) for real samples**

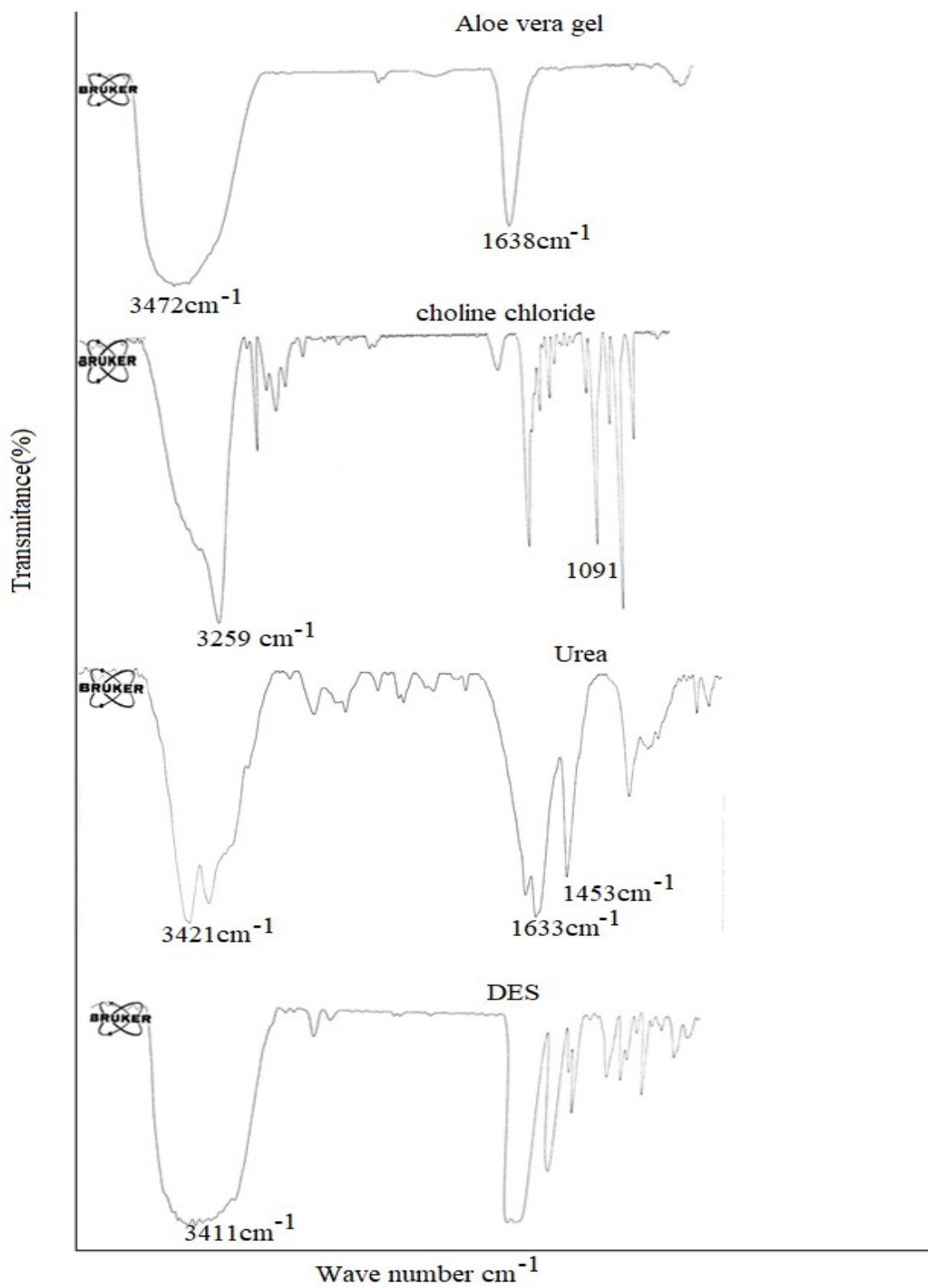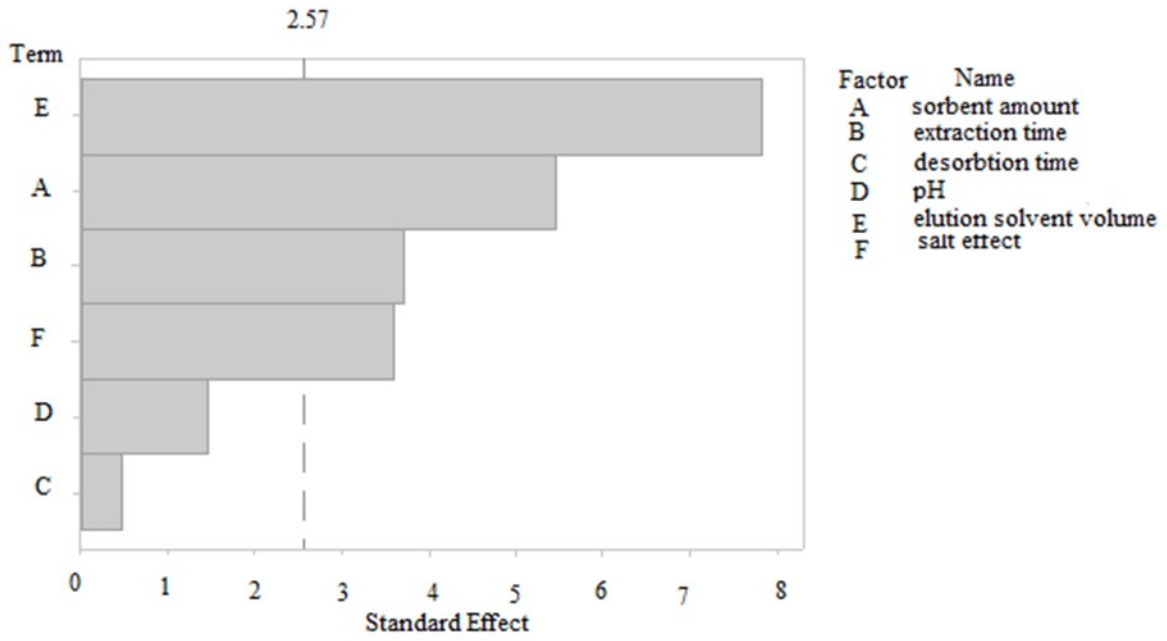| Sample	Matrix effect 100 μg L^{-1}
Natural orange juice	96
onion juice	93
Commercial apple juice	96
Natural apple juice	94
Green tea	90

218

219

220

221

222

Figure S1

225

226

227

228

229   Figure S2

230

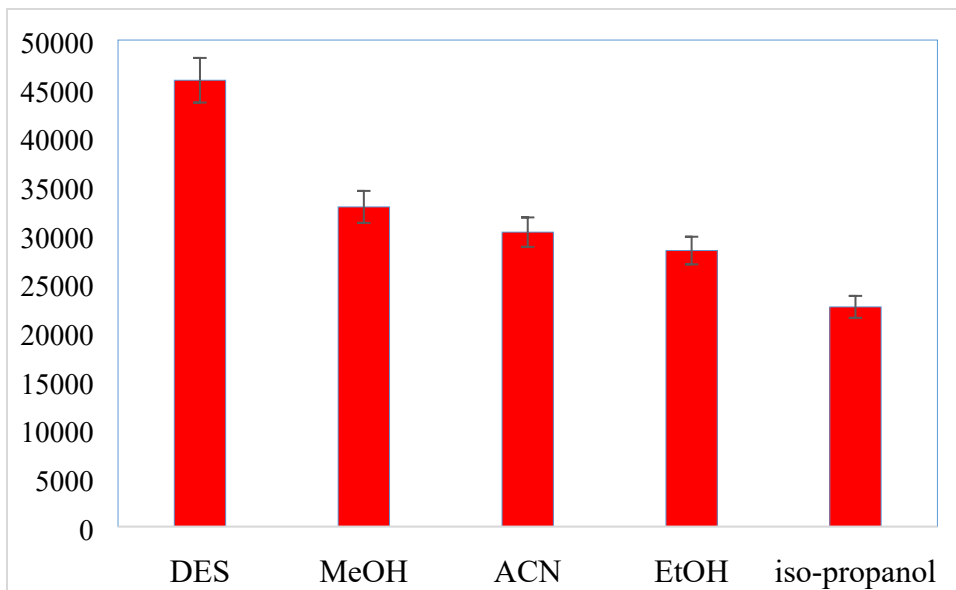231

232

233

234

235

236

237

238

239

240

241

242

243

Figure S3
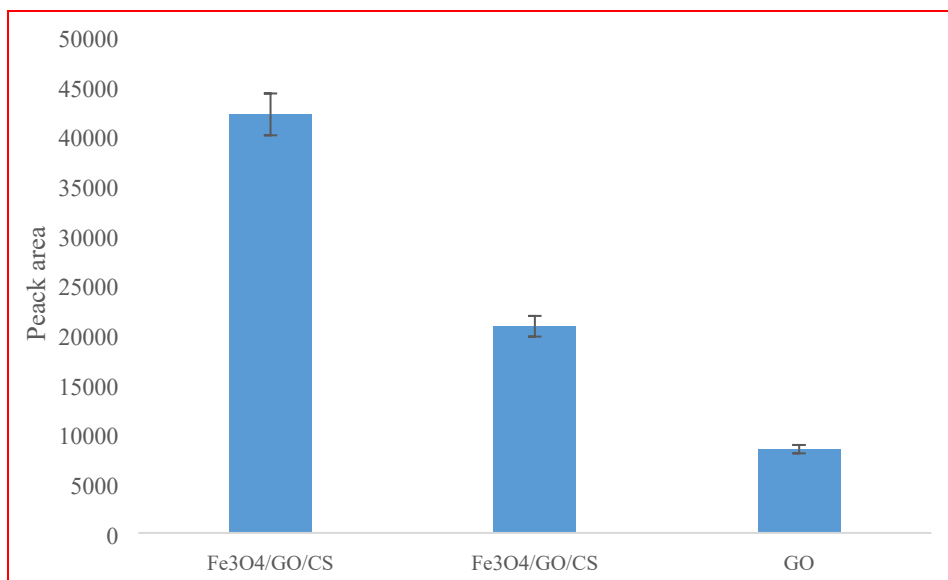
244
245
246
247
248
249
250
251
252
253
254
255
256

257

Figure S4

258

259