Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Data

A novel microextraction technique aided by air agitation using a natural hydrophobic deep eutectic solvent for the extraction of fluvastatin and empagliflozin from plasma samples: Application to pharmacokinetic and drug-drug interaction study

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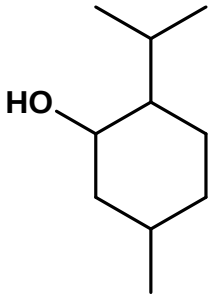
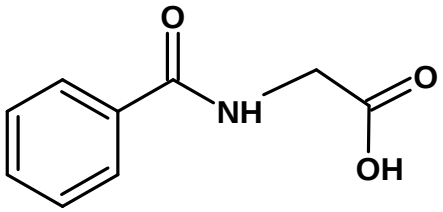


Menthol



Hippuric acid

4 : 1

Fig. S1. Chemical structure of NDES

Table S1. Preparation of hydrophobic DESs.

HBD	HBA	Mole ratio (HBD: HBA)	Physical state	Extraction recovery (%)	
				FLV	EMP
Hippuric acid	Menthol	1:1	Tubid	-	-
Hippuric acid	Menthol	1:3	Tubid	-	-
Hippuric acid	Menthol	1:4	Clear	96.0	92.0
Hippuric acid	Thymol	1:1	Tubid	-	-
Hippuric acid	Thymol	1:3	Tubid	-	-
Hippuric acid	Thymol	1:4	Tubid	-	-
Butyric acid	Menthol	1:1	Clear	62.0	79.0
Butyric acid	Menthol	1:3	Clear	56.0	84.0
Butyric acid	Menthol	1:4	Clear	51.0	87.0

Table S2. The experimental levels of the factors used in the central composite design.

Factor	Levels of factors				
	Low axial (-α)	Low factorial (-1)	Center (0)	High factorial (+1)	High axial (+α)
Sample pH	3	4	5	6	7
DES volume	50	100	200	250	250
Centrifugation time	5	10	15	20	25
Number of air-agitation cycles	2	4	6	8	10

Table S3. CCD matrix and the obtained extraction recoveries.

Run	A: pH	B: NDES volume (µL)	C: Centrifugation time (min.)	D: Air-agitation cycles	Recovery FLV (%)	Recovery EMP (%)
1	4	200	20	4	96	88
2	5	150	15	6	91	86
3	5	50	15	6	70	75
4	6	100	20	8	72	80
5	6	200	20	4	87	87
6	4	100	20	4	87	84
7	6	100	20	4	72	86
8	6	100	10	4	71	77
9	5	250	15	6	95	92
10	6	200	20	8	86	90
11	5	150	15	2	80	82
12	6	100	10	8	69	71
13	4	100	10	8	84	71
14	6	200	10	4	86	86
15	3	150	15	6	95	85
16	6	200	10	8	83	81
17	4	200	10	4	96	83
18	7	150	15	6	75	85
19	4	100	10	4	85	74
20	5	150	25	6	86	86
21	5	150	15	6	85	85
22	4	200	20	8	95	90
23	5	150	15	6	86	84
24	4	100	20	8	86	80
25	5	150	15	6	86	86
26	4	200	10	8	93	80
27	5	150	15	6	86	83
28	5	150	15	10	78	74
29	5	150	5	6	80	77
30	5	150	15	6	86	86

Table S4. Analysis of the variance for the fitted quadratic polynomial model for the extraction of FLV and EMP.

Parameter	FLV	EMP
R^2	0.9725	0.9531
Adjusted R^2	0.9468	0.9093
Predicted R^2	0.8957	0.7684
Adeq Precision	22.2695	18.0473

Table S5. Quantitative parameters of the developed method for the analytes.

Parameter	**FLV**	**EMP**
LR (ng mL^{-1})	20.0 - 380.0	5.0 - 300.0
R^2	0.9986	0.9966
LOD (ng mL^{-1})	6.3	1.5
LOQ (ng mL^{-1})	19.2	4.6
EF	48	42
ER (%)	96	92
Precision, RSD (%)	Intra-day: 1.4–2.7 Inter-day: 1.1–2.2	Intra-day: 2.1–3.2 Inter-day: 1.7–2.8

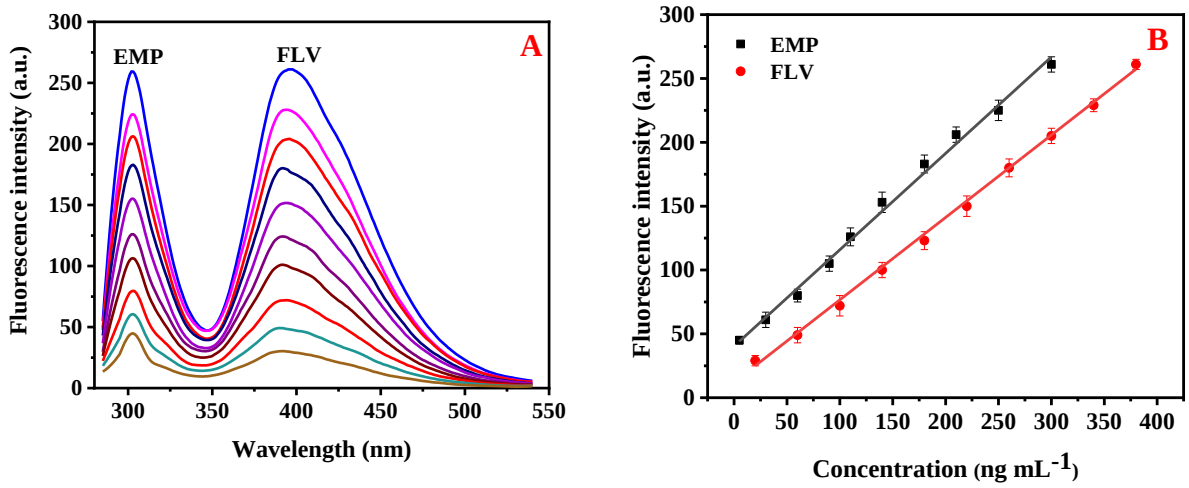


Fig. S2. (A) Fluorescence spectra for different concentrations of FLV and EMP in spiked water samples after NDES-DLLME, (B) the linear calibration curve.

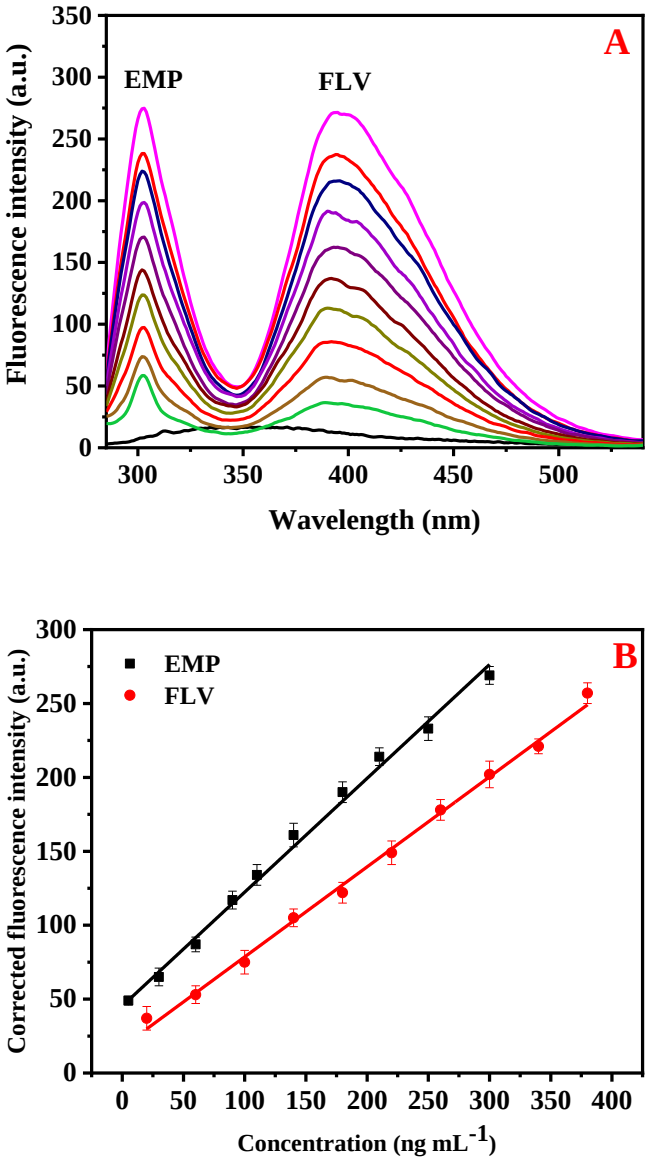


Fig. S3. (A) Fluorescence spectra for different concentrations of FLV and EMP in spiked plasma samples after NDES-DLLME, **Black line** represents blank plasma (B) the linear calibration curves.

Table S6. Relative recoveries (%) from analysis of FLV and EMP in plasma samples at optimum conditions (n=3).

Drug	Amount added (ng mL^{-1})	Amount found (ng mL^{-1})	Relative recovery (RR%)	RSD%
FLV	60.0	67.3	95.17	2.12
	180.0	184.2	96.67	1.54
	300.0	299.5	96.43	3.21
EMP	30.0	39.5	90.00	2.70
	110.0	109.9	88.54	1.75
	210.0	205.2	91.76	2.81

Amount measured in the real sample (Blank) (at zero concentration of the studied drugs) = 10.2 ng mL^{-1} for EMP and 12.5 ng mL^{-1} for FLV.

Table S7. Selectivity of the proposed method for 100 ng mL^{-1} of FLV and EMP in the presence of different ions and molecules (n = 3).

Ions and molecules	FLV	**EMP**
	Tolerable limit (ng mL^{-1})	**Tolerable limit (ng mL^{-1})**
Ca^{2+}	3500	4000
K^{+}	5000	3000
SO_4^{2-}	6000	5000
CO_3^{2-}	7000	6000
Mg^{2+}	3000	4000
F^{-}	5000	3000
Cl^{-}	5000	6000
Lactose	1400	1800
Glucose	1800	1900
Zn^{2+}	2500	2600
Fe^{3+}	3500	4500
Tryptophan	700	600
Tyrosine	800	900
Phenylalanine	900	800
Vitamin A	900	700
Riboflavin	600	800
Creatinine	800	900
Pyridoxine	500	1000

Table S8. Pharmacokinetic parameters of FLV after a single oral administration of EMP alone and after oral coadministration of EMP in the studied rabbits (mean ± SD, n = 3)

Parameters	**FLV alone**	**FLV co-administered with EMP**	**P value***
C_{max} **(ng mL^{-1})**	250.2 ± 22.58	352.2 ± 20.11	0.0057
T_{max} **(hr)**	1.5 ± 0.0	1.5 ± 0.0	
K_e **(hr^{-1})**	0.16 ± 0.024	0.090 ± 0.071	0.0081
$t_{0.5}$ **(hr)**	4.27 ± 0.78	7.73 ± 0.96	0.0045
K_a **(hr^{-1})**	2.24 ± 0.42	2.52 ± 0.42	0.058
$t_{0.5a}$ **(hr)**	0.31 ± 0.14	0.28 ± 0.42	0.079
AUC_{0-24} **(ng hr mL^{-1})**	1343.0 ± 452.3	2576.25 ± 245.2	0.0012
$AUC_{0-\infty}$ **(ng hr mL^{-1})**	1367.08 ± 143.5	2896.39 ± 189.25	0.0040
MRT (hr)	6.99 ± 0.89	7.66 ± 0.77	0.059

*A difference was considered significant at $P < 0.05$