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## **Supplementary information**

Enhanced solid phase extraction synergistically assisted by cloud point strategy prior to the determination of a recently FDA approved anti-hepatitis C drug velpatasvir: Application in Biological Fluids

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## Figures

Fig.1S: The chemical structure of VELP.

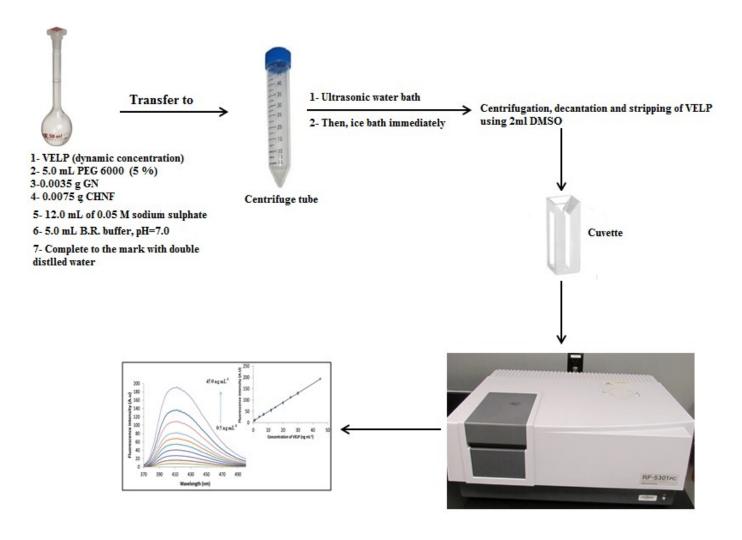
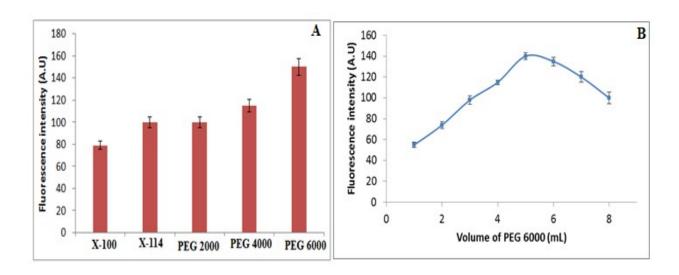
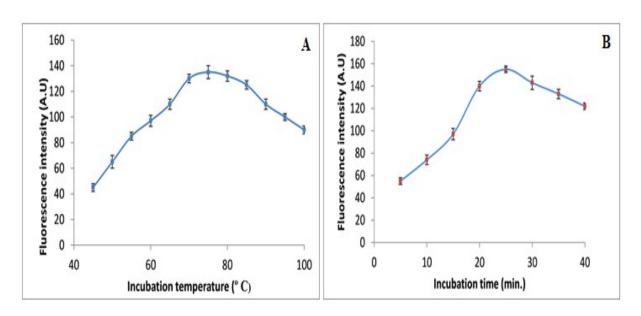


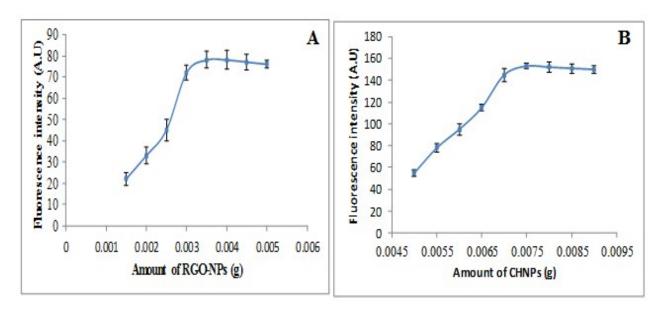
Fig.2S: Graphical representation of the proposed analytical procedure.



**Fig. 3S:** (A) The influence of surfactant type and (B) Influence of different volumes of PGE 6000 on fluorescence intensity of 30 ng/ml of VELP.



**Fig. 4S:** (A) The influence of incubation temperature and (B) Influence of incubation time on fluorescence intensity of 30 ng/ml of VELP.



**Fig. 5S:** (A) The influence of RGO-NPs amount using 0.0075 g CHNPs while (B) The influence of CHNPs amount using 0.0035 g of RGO-NPs on fluorescence intensity of 30 ng/ml of VELP.

## Tables

**Table 1S.** The precision of the proposed method for determination of VELP.

| Concentration of VELP | Inter-day precision |       | Intra-day precision |       |
|-----------------------|---------------------|-------|---------------------|-------|
| $(ng mL^{-1})$        | % Recovery± SD*     | % RSD | % Recovery±         | % RSD |
|                       |                     |       | SD*                 |       |
| 10                    | 99.5±1.8            | 1.81  | 100.9±2.6           | 2.57  |
| 20                    | 100.3±2.5           | 2.49  | 97.8± 1.7           | 1.73  |
| 30                    | 98.8±1.9            | 1.92  | 102.3±2.0           | 1.96  |

<sup>\*</sup> Average of five replicates.

Table 2S. Recovery study for determination of VELP in tablets by standard addition method.

| Amount taken   | Amount of      | Amount found   | % Recovery ± SD* | % RSD |
|----------------|----------------|----------------|------------------|-------|
| $(ng ml^{-1})$ | standard added | $(ng mL^{-1})$ |                  |       |
|                | $(ng mL^{-1})$ |                |                  |       |
|                | 2              | 1.98           | 99.0± 1.7        | 1.72  |
|                | 5              | 4.98           | 99.6±2.2         | 2.20  |
| 5              | 10             | 10.15          | $101.5 \pm 1.8$  | 1.77  |
|                | 15             | 15.08          | $100.5 \pm 2.4$  | 2.38  |
|                | 20             | 19.88          | $99.4 \pm 1.5$   | 1.51  |
|                | 25             | 24.55          | 98.2± 1.8        | 1.83  |

<sup>\*</sup> Average of five replicates.

| <b>able 3S.</b> The robustness of the proposed m | recovery (±SD*) | RSD  |
|--|-----------------|------|
| No variation                                     | 99.7± 2.1       | 2.11 |
| рН   |                 |      |
| 6.0  | $98.2 \pm 2.0$  | 2.04 |
| 8.0  | $98.7 \pm 2.3$  | 2.33 |
| Volume of surfactant (mL)                        |                 |      |
| 4.5  | $99.7 \pm 2.1$  | 2.11 |
| 5.5  | 99.5± 1.8       | 1.81 |
| Amount of RGO-NPs (g)                            |                 |      |
| 0.003  | 100.2±2.3       | 2.29 |
| 0.004  | 99.8±1.7        | 1.70 |
| Amount of CHNPs (g)                              |                 |      |
| 0.007  | 97.6±2.5        | 2.56 |
| 0.008  | 97.2±1.9        | 1.95 |
| Incubation temperature (°C)                      |                 |      |
| 70   | $100.8 \pm 2.4$ | 2.38 |
| 80   | 97.5±2.7        | 2.77 |
| Incubation time (min.)                           |                 |      |

99.7±2.6

101.3±2.0

2.61

1.97

23

27

<sup>\*</sup> Average of six replicates.