

**Supplementary Figure1.** High resolution ion chromatogram obtained for the m/z value (± 10 ppm) of 4-HPR and its main metabolites in a tumor sample.



**Supplementary Figure2**. MS/MS spectra of 4-HPR and its identified metabolites, highlighting some of the observed ion fragments.

**Supplementary Table1:** Comparison of the characteristics of the current method with others present in the literature

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| --- | --- | --- | --- | --- | --- | --- |
|  | **Matrices**  **analyzed** | **Analytes**  **analyzed** | **Common features** | **Differences** | **LOQ in plasma** | **Improvements** |
| **F. Formelli et al., 1993** [12] | Plasma  Breast tissue | 4-HPR  4-MPR | * Handling procedures. * Chromatographic characteristics:   reverse column, gradient features and run time below 10 min. | * Extractive solvent: CH3CN * HPLC-UV (λ= 340) * 3 points calibration plasma range: 5-2500 ng/mL. | 5 ng/ml | * Wider range of linearity applied reduces the need for dilutions. * The simultaneous measurement of the analyte and the metabolites allowed a more thorough analysis of the pharmacokinetic characteristics and limited the amount of matrix needed. |
| **J. Vratilova et al., 2004** [13] | Tumour | 4-HPR  4-MPR | * Extractive solvent: CH3CN * HPLC-UV (λ= 340). * 6 points calibration tumor range: 0.5-20 µg/mL. | 0.5 µg/mL. |
| **J. I. Lee et al., 2008** [10] | Plasma | 4-HPR  4-MPR | * Extractive solvent: CH3CN * HPLC-APCI-MS/MS. MRM + * 8 points calibration plasma range: 0.5-100 ng/mL. * Target ions m/z 392.4/283.3 for 4-HPR and m/z 406.3/283.2 for 4-MPR. | 0.5 ng/ml |
| **H.E. Cho et al., 2017** [14] | Plasma | 4-HPR  4-MPR  4-oxo-4-HPR | * Extractive solvent: CH3CH2OH * HPLC-ESI-QTRAP. MRM + * 8 points calibration plasma range: 0.2-50.0 ng/mL. * Target ions m/z 392.3/283.3 for 4-HPR, m/z 406.3/283.2 for 4-MPR and 406.3/297.2 for 4-oxo-4-HPR. | 0.2 ng/ml |
| **Present method** | Plasma  Tumour | 4-HPR  4-MPR  4-oxo-4-HPR  DH-4-HPR | * Extractive solvent: CH3CN * HPLC-APCI-MS/MS. MRM + * 8 points calibration plasma range: 1-500 ng/mL. * 6 points calibration tumour range: 0.35-14.0 µg/g. * Target ions m/z 392.4/283.3 for 4-HPR, m/z 406.3/283.2 for 4-MPR and m/z 390.2/281.1 for DH-4-HPR. | 1.0 ng/ml |