Response to Reviewer 3 Comments

Point 1: define MDPV.

Response 1: Thank you for your comment. We defined it.

Point 2: Introduction: The authors should compare the present method with other similar published methods, indicate any limitation of those methodologies, and what is novel about the one presented.

Response 2: Thank you for your comment. We compared our method in "results and discussion" section in terms of chromatographic separation for isomeric pears and sample treatment.

Point 3: The authors may also discus which are the most prevalent NPS in Italy (ex. literature).

Response 3: Thank you, we added it.

Point 4: Sample pre-treatment/validation: Blood origin: How many different type/sources of blood were investigated in the matrix effect experiment and authentic samples? Is it always whole blood or do you analyse also plasma with the present method?

Response 4: Thank you for your comment. The method was validated for whole blood. Selectivity and specificity were evaluated on 10 different blank whole blood samples, with no significative variations due to the donor. In matrix effect evaluation, three replicates for the same whole blood were analysed.

Point 5: Validation/results: The authors should compare the concentrations (LLOQ and ranges) validated with the ones reported in previous recent publications. The LLOQ should be also validated in terms of variation (RSD%) and not only S/N.

Response 5: Thank you for your comment. We compared our LLOQ values with the ones from the recent literature as we stated in the manuscript. However, we decided to not fully described the comparison as it would be very confusing for the reader for the high number of published methods and for the different classes/substances included in each method. We are aware about it, and for this reason we evaluated LOD and LOQ as "the lower concentrations which met the identification, precision and accuracy criteria at signal-to-noise ratios (SNR) $\geqslant$ 3 and $\geqslant$ 10, respectively"

Point 6: How the quality control (QC) were prepared? Did you check the quantification (at least for the classic drugs) with external QCs or proficiency test with the present method?

Response 6: Thank you for your comment. As we stated: "quality control (QC) samples were prepared by spiking blank blood…". We did not test yet the method with external QCs or

proficiency test. However, as usual in our laboratory, QC concentrations were tested with our previous method, providing highly comparable results.

Point 7: The fact that some analogues drugs did not fulfil the criteria should be discussed more in depth (ex. chromatographic baseline separation for those which present almost the same retention time and transitions, include specific figures chromatograms for those compounds, solutions). If they include these compounds also in the same calibration curves, the quantification would not be neither OK (as there would be overlapping).

Response 7: Thank you for your comment. In figures 1-3, chromatograms are available also for isomeric pairs that are not chromatographically separated, i.e. 3-MMC/mephedrone (figure 1, #25 and #26), 5/6-APB (figure 1, #7 and #8) and 5/6-MAPB (figure 1, #9 and #10). For these molecules, we used the same colour and a single peak to highlight that separation can not be achieved. For the other isomeric pairs, identification was effective, in terms of chromatographic separation or MS as you can see in figure 1. However, we added the reference to figure 1 in text.
In order to avoid overlapping, calibrators did not contain isomers affected by an ineffective chromatographic separation.

Point 8: Table 2: Include the QC concentrations evaluated (in the legend or in the table).

Response 8: Thank you for your comment. We added them.