A deeper investigation of drug degradation mixtures using a combination of MS and NMR data: application to indapamide

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Reviewer 3

It does not seem that you are acquiring the 1D NMR spectra under quantitative conditions. Your relaxation delay of 13 s seems a bit short for a molecule the size as it also seems (zgcppr) that you use a 90 degree pulse. At 600 MHz i would expect emoting like 30 s and a 30 degree pulse would do better (snd would not result in unfeasibly long experiment times), but you could be OK, and could prove it by running an inversion recovery T1-determination.

The T1 measurements were performed with an inversion recovery pulse sequence t1ir, in order to fix the relaxation delay (D1 = 13 s) of 1H experiment (5*T1), it was specified in the text (line 439-441).

DP1, DP2, DP3, and DP4 are to be expected if you look at the structure. But you haven’t addressed racemization at position 16, which is rather likely, but not directly detectable with your chosen methods. Given that you are examining a drug, you should at least discuss this.

Our API (Indapamide) is a racemic compound (RS). We are for this work at the identification step of degradation products (DPs), and the research of compound configuration at this stage is not essential. It will be done later for the toxicology studies of DPs > 1%. Moreover, on DP1, DP2 and DP3 the degradation reactions led to the loss of the chiral center initially located at position 16 of API. Only the DP4, not observed in our study, kept the chiral center in its structure at position 5. In the case of enantiomers analytical studies in chiral conditions could be interesting.

Also, it seems (line 325, page 9 of 18) that you expect a linear calibration curve from Electrospray ionization, which is not generally the case. You evaluate linearity using r2, which is not sensitive enough to nonlinearity, but requires analysis of residuals, and subsequent fitting of a linear and e.g. a quadratic calibration curve and analysis of the results.

We have clarified this point in the Supplementary Materials Table S1. The lack of fit of the linear model was tested using the R software and we find no lack of fit with the linear regression.

Use of PLS to identify the NMR and MS signals for the different species is OK, but actually makes the use of 2D spectra slightly overkill, but nice that you have them.

In our case, 1H NMR was sufficient to build the PLS model because we had few overlap signals. But it should be possible to use 2D spectra to build PLS. In our work the 2D NMR spectra were used for identification.

With proper buffering of the samples (AS YOU HAVE; NICE) you should be able to fit the "raw" NMR spectra directly.

With our buffer, we still have some shift so it was better to align our spectra for more accuracy and the procedure was easy with NMR ProcFlow.
I am not happy with the way You determine LOD’s and LOQ’s. The ICH Q2 guideline section 6.4 recommends that you make a sample at the estimated limit and confirms the limit. "In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit".

We agree with you but our percentage of degraded API was in the range of 5-25% (recommendation ICH), considerably above the limit of quantification. That’s why, in our case, it was not necessary to test a sample at the detection limit. The methods for determination of LOQ and LOD was clarified in Supplementary Materials Table S1.