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

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Reviewer 4

Most importantly, the results & discussion part has a significant introduction/materials and methods part and first experimental results appear at section 3.3. A thorough restructuring is therefore needed.

Following your recommendations, we have completely reorganized the manuscript in two distinct parts (2. Results and discussion and 3. Materials and methods) for more clarity. So we have started the Results and discussion section with the API quantification results (Part 2.1, line 99).

Figure 2 orthography: dependent variables, elimination of artefacts, comparison of NMR spectra.

Now Figure 1. Correction have been done in the text

Figure 3 + S1: What does significance of the results mean? Why does HCl/NaOH treatment not have significance?

Now Figure 2. For reconstitute mixtures (M1, M2, M3), we have theoretical values for each product (API and DPs). It was not the case for the real mixtures (HCl and NaOH), that’s why we have not significance. Moreover, for the 5 replicates (●, ◊, ○, +, △) we have indicated the mean value by ●.

You use HRMS and 900 MHz NMR, please elaborate the structure of DP5 in more depth. Remove the list of possible compounds from Figure 4 f) it will not be readable. Rather propose a structure, which (in combination with NMR data) explains the fragments.

We have removed the list of possible compounds on Figure 4 (now Figure 3). These hypothetic candidates were described in the text (section 2.2). The complete NMR characterization of DP5 (with quaternary carbons assignment) was described in Supplementary Materials Figure S4. For HRMS, the fragments are described Figure 5. The NMR data correlate with MS/MS data.

Figure 6: explain the considerable deviations of experimental vs predicted shifts!

Now Figure 5. Chemical shift in NMR depends of: pH, ionic strength and solvent. Generally, the prediction software didn’t take these parameters into account. These explain the deviation between experimental vs predicted shifts. Software helps us in the interpretation, but it’s necessary to check.

Line by line comments: 21: ...industry, where a number..., 26: replace tolerances by "deviations" or similar, 30: DP (singular) levels, 39: API means active PHARMACEUTICAL ingredient, 40 leading to the identification, 44 mass spectrometric detector, 49 you also constructed calibration curves by diluting your mixes - please reformulate, 58 amino acid (singular), drug stability (sing.), 59 offer a global tool ... to pharmaceutical industry, 61 determination of the remaining api, 68 multivariate tools were(?) considered.

We have corrected in the text.
Materials and Methods:

*If you did not use a certified reference material to construct calibration curves please at least indicate to purity / quality of your API and DP1-4.*

Purity of API and DP1, DP3, DP4 (DP2 not available) were added in section ‘Chemicals’ (3.2). These purities were established by UV.

**Briefly also state how you defined LOD and LOQ (not only in the supplement).**

We have added a brief description in our text (lines 419-422).

**115 & 120 what does qs mean - please state.**

qs means quantum sufficit.

**158 as follows, , 215 indapamide DP (sing.) structures, 248 data, which , 257 DP (sing.) structures, 258 the contribution of multivariate analysis was evaluated.**

Done in the text.

**259 which makes the use of a calibration curve unnecessary - I do not clearly understand how - you used a cal. curve...**

Calibration curves were used to measure the percentage of remaining API. In the absence of standard, it was not possible to quantify our DPs (ex: DP5) with this method, that’s why we have used the qNMR technique.

**275 APIs? pharmaceutical drugs?**

API means active pharmaceutical ingredient

Results and Discussion

**3.3: R² is not a means to determine linearity it only describes deviations from a proposed function. Hence, either reformulate or perform a fitting test such as Mandel’s!**

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.

**Renumber 2.4 to 3.4!**

Done.

**Discuss the results of drift time / no drift time filtering not only in a figure caption.**

Indeed, UNIFI software seeks the ions with the same drift time, eliminates the others (e.g. [M+Na]− adduct at m/z 388.0493 and interference at m/z 419.9834) to obtain a clarified fragmented MS spectrum without the need of a specific MS/MS experiment. (Line 150 to152).
We have also added complementary information in the part MS and NMR analysis (3.4, line 392 to 405).

381 - 391 You have all experimental data at hand. Be more concise on what you think the structure is - probably figure 4f/figure 6 need to be updated.

Done on figure 4f (now 3f) and on figure 6 (now 5).

413 eliminated, 426 This compound was not directly obtained, 438 targeted search, 471 loadings plot.

Done.

Conclusion

Many of your claims are not to be found in the paper / not discussed thoroughly enough reduce to 3 or max. 5

Figure 1 (workflow) summarizes very well the different step of our methodology. So we have reduced our conclusion to gain clarity and to highlight the main results of our work.

494 of the API, 500 and validated an accurate assay.

Done.