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Mechanochemical Degradation of Active Pharmaceutical Ingredients (APIs): A Simple Tool for the Prediction of Drug Stability

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Abstract: Knowledge of the potential degradation products of active pharmaceutical ingredients (APIs) is of major interest for the development and approval of new drugs. Therefore, methodologies for the time-efficient and precise prediction of degradation products and pathways are of great importance. Traditional degradation assessments typically involve solution-based forced degradations under acidic, basic, thermal, or photolytic conditions. However, such conditions often fail to accurately replicate degradation pathways relevant to solid-state formulations. A promising addition to the established solvent-based approaches are forced degradation processes in the solid-state using mechanochemistry. The newly developed methodologies enable a time-efficient and accurate simulation of degradation pathways under mild reaction conditions in the solid-state. Herein, the general principles of forced mechanochemical degradations will be discussed on the basis of published case studies involving marketed drugs.

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1. Introduction

1.1 *Regulatory Aspects and General Principles*

The quality of active pharmaceutical ingredients and drug products is a strictly regulated field. In order to harmonize the assessment of quality, efficacy, and safety of drug products in Europe, Japan, and the United States, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) was founded by international regulatory authorities. The ICH developed 14 guidelines (Q1-Q14) on the quality of drugs, which include guidance on stability studies, analytical procedures, and impurity testing.[1] Those are mirrored in the major pharmacopoeias, *i.e.* the European Pharmacopoeia,[2] the United States Pharmacopeia,[3] and the Japanese Pharmacopoeia.[4]

According to the ICH guideline Q3A (R2) impurities are any components of the API that are not the chemical entity defined as the API and can be classified into three categories: inorganic impurities, residual solvents, and organic impurities, including starting materials, by-products, intermediates, degradation products, reagents, ligands, and catalysts.[5]

Since the stability of an API is of decisive importance for its quality, safety, and efficacy, the ICH guideline Q1A (R2) advises testing the physical, chemical and (micro)biological attributes of an API and a drug product.[6] The formation of degradation products due to chemical instability can be caused by hydrolysis, oxidation, decarboxylation, and photolytic cleavage among other reactions as summarized by Prabu and Suriyaprakash.[7] Even though conditions for forced degradation being in acidic, basic, oxidative media are given, the guideline only provides general recommendations. In addition, such approaches can suffer from the inherent problem of requiring long-term measurements and solvent-related degradation products (*e.g.* solvolysis products).[8–10] Another approach is based on the treatment of APIs in the solid-state with varying levels of humidity and temperature for several days.[8,9] Other stress conditions can also be applied, namely mechanochemical degradation, not only for pure APIs but also for drug products, containing excipients such as polymers.

Because many of those conditions fail to accurately replicate degradation pathways relevant to solid-state formulations, which represent the majority of pharmaceutical products, there is an urgent need in the pharmaceutical industry for innovative, fast, and predictive methods to evaluate the stability of solid APIs and formulation mixtures at an early stage of development. To overcome the known limitations of solvent-based degradation studies, forced degradation processes using mechanochemistry are discussed as a modern and reliable approach.

1.2 *Mechanochemistry*

In recent years, mechanochemical techniques have increasingly been applied in organic synthesis.[11–20] In most cases, reactions under mechanochemical conditions are solvent-free. Besides this advantage, several other beneficial effects have been identified when compared to analogous solution-based transformations. They include, for example, that the desired reactions proceed faster. They might also not need additional heating, or even they can require less catalyst. Furthermore, applying mechanochemical techniques has led to altered product compositions.[21,22]

While the aforementioned strategies focus on bond-forming processes leading to molecularly enlarged organic entities (= synthesis), destructive mechanochemical pathways are also well-known. In this context, one of the first reports stems from Staudinger and Heuer, who, as early as 1934, documented the degradation of polystyrene in a ball mill.[23] By bond cleavage, a shortening of the polymer chains occurred. Such solid-state depolymerizations of nowadays ubiquitous synthetic polymers including polyolefins and polycondensates is attractive in the context of circular economy and several studies have highlighted the enormous potential of mechanochemistry.[24–30] Analogous destructive mechanochemical processes have been applied in degradations of lignin[31–33] and perfluorinated alkyl substances (PFAS).[34–36]

The previously mentioned transformations lead to modified products of polymeric or long-chain molecules. In contrast, untargeted mechanochemical degradation reactions of small molecules are less investigated. This is particularly true for APIs, which is surprising because drug substances are often subjected to mechanical stress during milling to control the particle size distribution.[37] Whereas in solution, accelerated degradation processes are well documented,[9] analogous solid-state conversions have remained mostly unstudied.[38]

2. Mechanochemical API Degradations

Solvent-free degradation of APIs under mechanochemical conditions have the potential to provide significant information on solid-state stability and degradation pathways. Both have high relevance for the pharmaceutical industry.

2.1 *Examples of Mechanochemical API Degradations*

To simulate the degradation of active pharmaceutical ingredients in the solid-state and to overcome the inherent solvent effects, which are associated with classical (solvent-based) degradation studies, Buschmann and Handler developed the basic concept for an operationally simple forced degradation process using

mechanochemistry.[38] As the reactions are carried out in the solid-state and no solvent is used, this approach is independent of solubility issues and solvent-dependent selectivities or reaction rates.

In their publicly funded proof-of-concept study, they investigated the forced mechanochemical degradation of the API Clopidogrel hydrogensulfate (**1**, Clp), a platelet aggregation inhibitor (Scheme 1).[39] Thus, Clp was milled in a commercially available ball mill with silica gel and other stressors (including organic and inorganic acids and bases as well as oxidizing agents). After milling, the obtained residue was dissolved in an organic solvent, passed through a filter and analyzed by GC-MS or HPLC-MS. Milling of Clp in the presence of sulfuric acid for 1 h led to the formation of the expected decomposition product, *i.e.* the respective carboxylic acid. Comparing these results with previously published studies[40] showed that the new approach allowed a qualitative and quantitative analysis within a much shorter period of time. Forced degradation tests applying $KMnO_4$ on aluminum oxide led to the formation of a dehydrogenated decomposition product, which was in accordance with previous studies,[40] and the formation of one unidentified compound. Interestingly, milling of Clp for a prolonged time under oxidizing conditions resulted in selective and increasing conversion of the API to the previously detected products.[38] No additional decomposition compounds were observed.

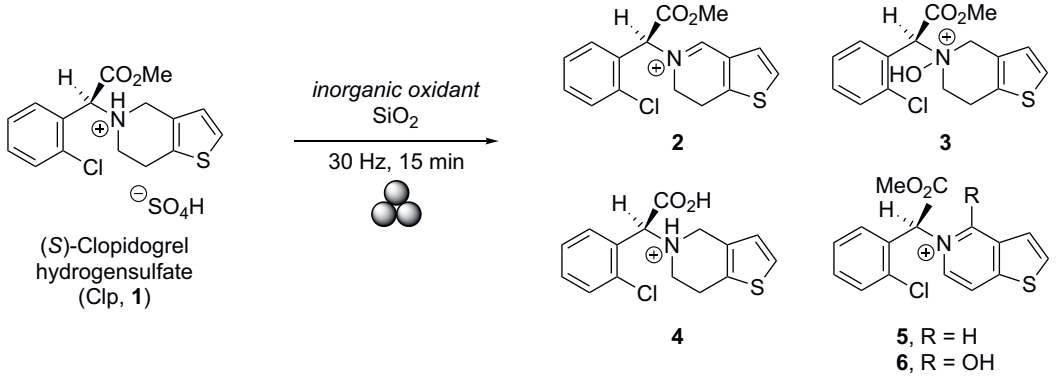
Besides allowing the simulation of degradation profiles in the solid state, this new approach also proved applicable in the investigation of the stability of polymorphs. Thus, Buschmann and Handler also showed that milling of Clp (polymorph II) without any additives at 10 Hz for 90 min led to only a minor change of the composition, whereas increasing the frequency to 25 Hz for the same time, resulted in amorphization. Finally, the authors also confirmed a high reproducibility of the data generated by the newly developed forced degradation method. The innovative character of the method was confirmed by an approved US patent[38] and further validated with industrial partners and academic groups under the supervision of the inventors Buschmann and Handler.

Motivated by the initial proof-of-concept study, Bolm, Holzgrabe, Beweries, and coworkers including Buschmann and Handler performed a more comprehensive, systematic study of the forced degradation of (*S*)-Clopidogrel under oxidative mechanochemical conditions.[41] In a typical experiment, Clp, silica gel, and an inorganic oxidant ($KMnO_4$, KNO_3 or the commercially available triple salt Oxone® consisting of $KHSO_5 \cdot 0.5KHSO_4 \cdot 0.5K_2SO_4$) were milled at 20–30 Hz for 1–15 min in a ZrO_2-Y jar (ZrO_2 stabilized with Yttria). While frequencies of below 20 Hz were not sufficient to induce degradation of Clp, milling at 30 Hz led to conversions of 30–40% and the formation of a characteristic degradation profile depending on the oxidant used (Scheme 1). For the analysis of the degradation products, HPLC-MS, NMR and IR spectroscopy were used.[41]

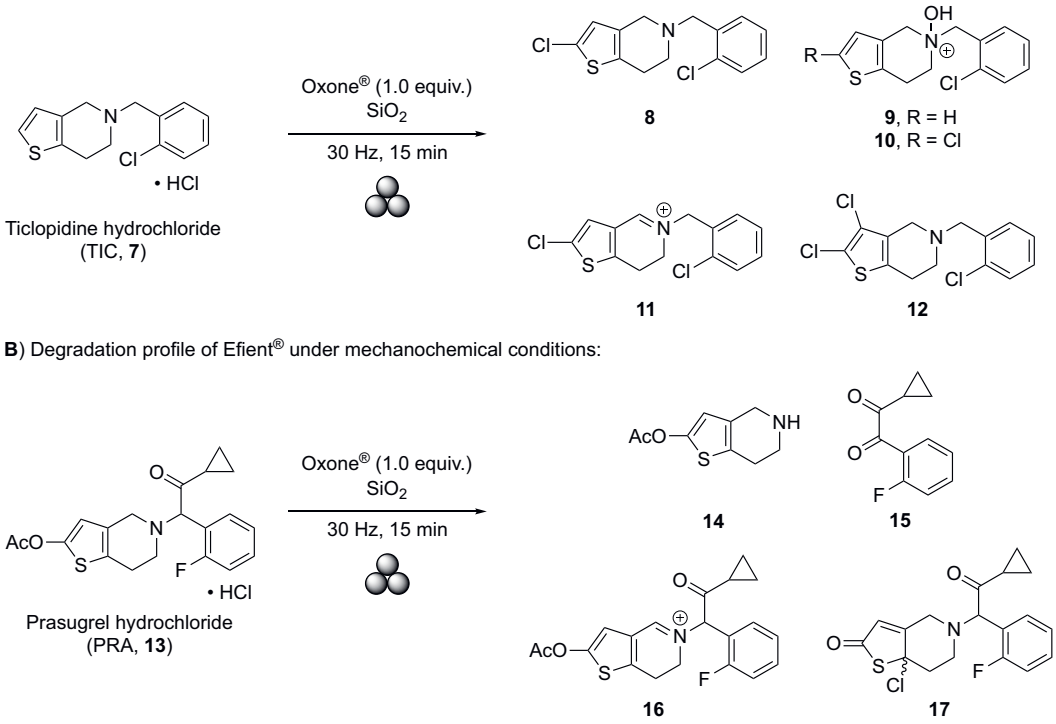
Applying $KMnO_4$ or KNO_3 as the oxidative stressor resulted in a dehydrogenation and the formation of compound **2** (often referred to as the *endo*-iminium product) as the main degradation product. While KNO_3 almost exclusively led to the formation of compound **2**, various other degradation products (compounds **2** and **4**–**6**) were observed in the case of $KMnO_4$, however, only in trace amounts.[41] In contrast, the application of Oxone® as the oxidative stressor gave *N*-oxide **3** as the main degradation product and only trace amounts of *endo*-iminium product **2**. Comparing the three used oxidants, Oxone® showed the most selective degradation profile. The authors further compared the obtained results with already published solvent-based studies (*e.g.* applying H_2O_2 as the oxidative stressor under harsh reaction conditions)[40,42–47] indicating similar degradation pathways, however with a higher selectivity and a significantly improved time efficiency of the mechanochemical approach.[41]

Since marketed drugs and finished products are usually complex mixtures of the API, several excipients, and additives, Beweries, Bolm, Holzgrabe, and coworkers next investigated the influence of these additives on the degradation profile of various thienopyridine-containing drugs under oxidative conditions (Scheme 2).[48] For the evaluation of the effect of various excipients and coatings, Clp was again chosen as the model compound. The additives examined included, among others, mannitol, cellulose, macrogol 6000, lactose monohydrate, carnauba wax, hydrogenated castor oil, Fe_2O_3 and TiO_2. In initial experiments, Clp, silica gel, Oxone®, and one additive at a time were milled in a ZrO_2-Y jar at 30 Hz for 15 min. None of the additives tested were found to have a significant influence on the degradation profile, and *N*-oxide **3** was obtained as the major degradation product. Only in the cases of cellulose, lactose monohydrate and mannitol were trace amounts of the hydrolyzed ester, acid **4**, observed. Consequently, the forced mechanochemical degradation method was further benchmarked by milling the Clp-containing marketed drug Plavix®. As expected, the degradation products remained the same as before (Scheme 1), and *N*-oxide **3** and *endo*-iminium product **2** were detected in 44% and 2% yields, respectively.[48]

After successfully demonstrating the applicability of the forced mechanochemical degradation on Plavix® further thienopyridine-containing drugs were investigated. Forced degradation of Ticlopidin-neuraxpharm® containing ticlopidine hydrochloride (**7**, TIC)[49] under oxidative solid-state conditions led to the formation of five degradation products as depicted in Scheme 2A. As the major decomposition product, 2-chloro thienopyridine **8** was observed as a result of an oxidative chlorination where the chlorine atom stems from the hydrochloride. Similar transformations of Clp in solution have been recently described by Krake and Baumann.[50] While the degradation products **8**–**10** have also been obtained under solution-based conditions,[51] compounds **11** and **12** were unknown in the literature.[48]



Scheme 1. Forced mechanochemical degradation of clopidogrel (**1**) under oxidative conditions.[38,41]



Scheme 2. Forced mechanochemical degradation of A) Ticlopidin-neuraxpharm® and B) Efient® under oxidative conditions.[48]

As another example, the oxidative degradation of Efient®, containing prasugrel hydrochloride (**13**, PRA),[52] was investigated under mechanochemical conditions. Although the chemical structure of PRA is closely related to that of Clp and TIC, a much more complex degradation pattern with several main products was observed (Scheme 2B). Among these structures were compounds **14** and **15** as the result of an oxidative cleavage of the expected *endo*-iminium compound **16**. While the aforementioned compounds were also described in related decomposition experiments in the literature before,[53–56] the formation of chlorinated compound **17** was unknown.[48]

In summary, the newly developed forced oxidative mechanochemical degradation method has proved to be highly efficient in the simulation of degradation profiles in the solid state for thienopyridines. Besides a faster prediction, a more realistic and reliable analysis of APIs has been achieved.

In a follow-up study Bolm, Holzgrabe, and coworkers explored the use of the developed solvent-free forced mechanochemical degradation method to investigate the degradation behavior of five widely used sartan-class antihypertensive agents: losartan potassium (**18**), irbesartan, valsartan, olmesartan medoxomil, and telmisartan, under oxidative conditions (Scheme 3).[57] Milling of the sartans was performed at 25 Hz for durations ranging from 15 to 90 min applying $KMnO_4$, KNO_3 or Oxone® as the oxidizing agents. Those oxidants were chosen in order to cover a wide range of mechanistic pathways leading to potential degradation products. The general aim was to simulate realistic, environmentally relevant stress conditions without the use of solvents, in line with green chemistry principles. The authors employed high-resolution liquid chromatography-mass spectrometry (LC-HRMS, see below in section 2.2) to identify the degradation products and impurities. Prior to degradation, untreated API samples were screened to distinguish native impurities from those newly generated during mechanochemical stress. Remarkably, even short milling times (15–60 min) led to substantial degradation in all five APIs, with many products aligning with known degradation pathways reported in the precedent monographs in the literature.[58–65] This consistency validates the mechanochemical approach as a viable predictive tool for solid-state degradation.

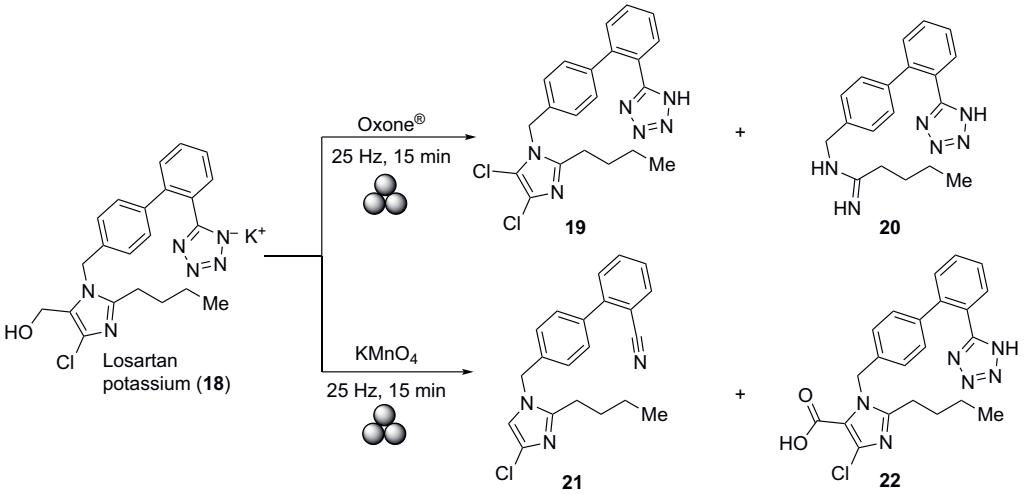
Each sartan displayed a distinct degradation behavior depending on the oxidant used, yet some patterns emerged. For instance, in the tetrazole-containing sartans (losartan, irbesartan, valsartan, and olmesartan), a common transformation involved the conversion of the tetrazole ring into a nitrile or formyl group. These changes were observed across multiple APIs and oxidants, indicating shared mechanistic features in the degradation process. Notably, losartan potassium (**18**) also showed evidence of chlorination and imidazole ring breakdown, while olmesartan medoxomil exhibited dioxolone ring degradation.

In contrast, telmisartan, which lacks the tetrazole group, followed different degradation pathways. Its degradation products included decarboxylated and hydroxylated forms, particularly when treated with $KMnO_4$ and Oxone®. No significant degradation was observed with KNO_3. Valsartan was unique in that it showed no detectable impurities prior to the addition of the oxidizing agent, yet under $KMnO_4$ stress, it produced multiple degradation products involving tetrazole ring transformation into a nitrile group and cleavage of the amide bond.

Regarding Valsartan, importantly, the oxidant type had a notable impact on the degradation outcome. $KMnO_4$ was the most effective at inducing degradation, producing four major products. In contrast, reactions with Oxone® or KNO_3 yielded only minor degradation, reinforcing that the oxidative strength and redox potential of the oxidant significantly influenced the degradation extent and profile. Another key finding from the study was the relatively minor influence of oxidant quantity on the degradation profile. Whether one or two equivalents of oxidant were used, the types and proportions of degradation products remained largely consistent. This suggests that the mechanochemical process is more dependent on reaction time and oxidative strength than on reagent excess.[57]

Overall, the authors demonstrated that mechanochemical oxidative stress testing can provide degradation profiles that closely mirror known pharmaceutical impurities,[58–65] while also identifying previously unreported products.

The autoxidation of drugs in the solid state was studied by Paudel and coworkers by co-milling amorphous mifepristone (MFP) and amorphous olanzapine (OLA) with polyvinylpyrrol-



Scheme 3. Forced mechanochemical degradation of a representative sartan.[57]

idone vinyl acetate (PVPVA), a polymeric excipient containing reactive free radicals (Scheme 4).[66] In these cases, milling led to the degradation of up to 5% of the drug in the solid state. The mechanism of autoxidation was evaluated in controlled experiments in solution using azobisisobutyronitrile (AIBN) as a stressing agent. The deconvolution of the effect of the milling frequency and the energy on the extent and kinetics of milling-induced autoxidation allowed for an extrapolation of mechano-activated degradation rates to zero milling frequencies. Furthermore, the autoxidation rates of drugs stored at high temperatures were found to follow an Arrhenius behavior, and good agreement was observed between model predictions obtained by mechano-activation to the reaction rates observed under accelerated temperatures.

In another study Moreno-Pirajan and coworkers have reported the mechanochemical degradation of diclofenac in the presence of SiO_2, Al, Al_2O_3, and Al-Al_2O_3 doped biochar using a planetary ball mill.[67] LC-MS analysis of the milling products suggested the formation of six subproducts through hydroxylation, demethylation, decarboxylation, oxidation reactions, and cleavage of the C–C and C–N bonds, likely by oxygen species generated from molecular oxygen in the presence of the dopants under high-energy mechanochemical conditions.

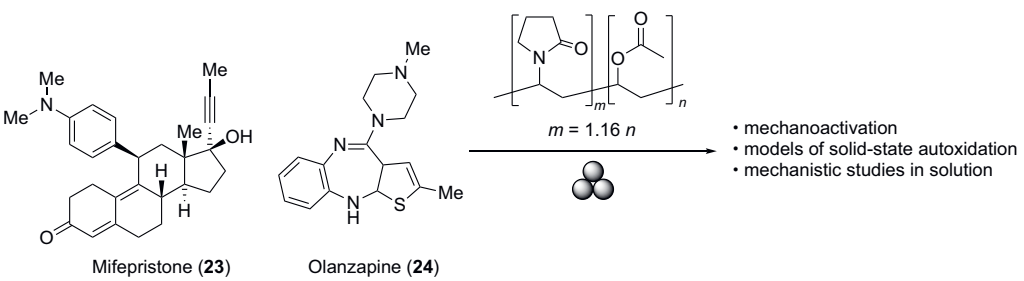
Holzgrabe and coworkers evaluated the configurational stability of (*S*)-Ibuprofen by ball milling.[68] A partial racemization was observed under basic conditions by applying long milling times and high milling frequencies. The products were analyzed using a chiral capillary electrophoresis (CE) method that was developed and validated according to the ICH guideline Q2(R1).

Racemization of the API Levetiracetam was demonstrated by Brandel, Cartigny, and coworkers using a mixer mill.[69] Of note, the kinetics of this racemization reaction were drastically improved compared to the solution-based approach. The authors addressed the importance of mixing efficiency regarding data reproducibility and showed that water contamination was detrimental for the reaction rate. The *in situ* X-ray diffraction analysis gave insights into the mechanisms of the mechanochemical racemization process.

In general, all of these approaches hold strong potential as predictive tools for impurity profiling during drug development and regulatory testing, especially for solid oral dosage forms. Additionally, their solvent-free nature and operational simplicity make them attractive alternatives to conventional solution-based stress testing methods. By presenting a time-efficient and environmentally sustainable protocol for generating relevant impurity data, these studies make meaningful contributions to pharmaceutical quality control. They open new avenues for applying mechanochemistry in stability testing and supports a broader movement toward greener, more realistic degradation methodologies in the pharmaceutical sciences.

2.2 *Structure Elucidation*

Commonly, the mechanochemical degradation experiments described above result in mixtures of degradation products of very different amounts, ranging from <0.1 percent up to a two-digit percentage. Ideally, those products could directly be identified in the solid product mixtures by modern techniques applied in mechanochemistry that do not require work-up, including solid-state NMR spectroscopy,[70] PXRD analysis,[71,72] or Raman spectroscopy.[73,74] Although these methods often allow significant organic species in complex reactions pathways to be identified,[75,76] their sensitivity is still not sufficient enough to generate unambiguous information of the diverse product portfolio obtained by the forced mechanochemical degradation discussed here. Hence, the product mixture needs to be worked-up to get rid of remaining reagents and the components of the crude mixture have to be separated by analytical or preparative high-performance liquid chromatography (HPLC). For a complete characterization of the degradation profile, the HPLC should be coupled to a high-resolution mass detector (HRMS). The struc-



Scheme 4. Autooxidative mechanochemical degradations of Mifepristone (**23**) and Olanzapine (**24**).[76]

tures derived from the mass data can then be confirmed by product isolation and analysis using NMR spectroscopy.

To distinguish between the genuinely present impurities stemming from the synthesis and the degradation products it is necessary to carefully analyze the non-treated API. For a known API, the impurities are typically reported, at least those present in amounts greater than 0.1 percent. The related-substance method given in a pharmacopoeia can be a starting point for HPLC analysis, although it might be necessary to adopt this method for a mass detection. In any case, in both a targeted and an untargeted approach a very close look into the impurity profile of the non-treated API by means of HPLC-HRMS is recommended in order to find impurities of very low concentration which might not be reported by the pharmacopoeias (*cf.* the losartan screening by Holzgrabe, Scherf-Clavel and coworkers[77]).

Subsequently, the structure of the unknown degradation product has to be elucidated by using a proteomics analogue approach as detailed by L. Backer.[78] For this purpose, it might be necessary to optimize the former HPLC-HRMS separation method. For detection, either triple quads (QqQ), Time-of-Flight (TOF) or a qTOF, being a hybrid mass spectrometer, are suitable not only for detection and quantification but also for structure elucidation. In the qTOF, two quadrupoles for selection and fragmentation of ions are combined with a TOF mass analyzer. Due to its high resolution, fast acquisition speed, high mass accuracy and sensitivity, a qTOF can be of great value for the identification of unknown compounds as shown by Ferrer and Thurmann.[79] The ESI positive and negative ionization mode should be chosen to avoid overlooking degradation products. For untargeted MS/MS acquisition methods such as MS^{All}, information-dependent acquisition (IDA) or sequential window acquisition of all theoretical fragment-ion spectra (SWATH) are available.[80] This procedure is well-known in the field of metabolomics and proteomics. Scherf-Clavel *et al.*,[81] Wohlfart *et al.*,[82] and Walther *et al*.[83] have successfully applied the so-called general unknown comparative screening (GUCS) to screen HRMS data of different drugs in search of unknown impurities. GUCS enables the comparison of a selected reference against one or multiple samples concerning acquired chromatographic and mass spectrometric data. Differences between the sample and the reference are displayed in a peak list and hence help to identify relevant features; along with this, it helps for structure elucidation as shown by Wohlfart *et al*.[82] and Marquet and coworkers.[84] The structures of the main degradation products should then be confirmed by NMR spectroscopy and synthesis.

Isolation of the degradation products from solid-state degradation experiments is often challenging as in many cases only traces of the respective compound are formed. However, classical techniques known from preparative organic chemistry such as liquid-liquid extraction, column chromatography and crystallization, followed by structure elucidation using standard techniques such as NMR spectroscopy or LC-MS can, in principle, be applied. The targeted synthesis of model compounds is helpful for the assignment of species from complex mixtures. For example, the selective synthesis of a halogenated product of prasugrel which is present during mechanochemical oxidation of this API with Oxone® (*vide infra*) was reported.[44] Furthermore, results of degradation studies in solution are helpful for the assignment of products of solid-state reactions. In this context, Baumann, Purohit, and others have presented valuable data for the oxidative degradation of thienopyridine-based APIs.[43,50,51,58,85,86] Similar studies are available for other classes of APIs, such as sartans.[57]

3. Outlook and Conclusions

Forced mechanochemical degradation offers a promising opportunity to simulate the degradation of active pharmaceutical ingredients in the solid-state. While established solvent-based approaches are generally time-consuming and can fail to accurately replicate degradation pathways relevant to solid-state formulations, the mechanochemical process is performed in the absence of a solvent. Compared to conventional solvent-based approaches, the newly developed methodology enables a time-efficient, selective and solvent-independent prediction of degradation-pathways under mild reaction conditions.

As previously discussed, mechanochemical degradation is not only applicable for the assessment of APIs but also to their complex formulations. Further confirmatory studies to expand the scope and applicability in the pharmaceutical industry are ongoing including quantum mechanical calculations to better understand the kinetics and degradation of solid drug substances and drug products.

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Author Contributions

All authors wrote and corrected the manuscript.

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