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Microwave-assisted forced degradation using high-throughput microtiter platforms

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ABSTRACT

Parallel microwave-assisted forced degradation in sealed HPLC/GC vials utilizing a high-throughput platform is described. The platform is made out of strongly microwave absorbing silicon carbide (SiC) plates providing 20 bore holes having the appropriate dimensions to be fitted with standard autosampler HPLC/GC vials serving as reaction vessels. Due to the possibility of heating up to four SiC platforms simultaneously (80 reactions) in a dedicated multimode microwave cavity with online temperature control, efficient parallel forced degradation studies can be performed at temperatures and pressures of up to 200 °C and 20 bar, respectively. Since degradation reactions and analyses are performed in the same vessel, the sample handling effort is reduced and errors caused by a required transfer step are avoided. As proof-of-concept, the platform was evaluated for the parallel testing of various stress conditions on the drug indomethacin. The obtained data provided a rapid overview over suitable stress conditions at high temperatures, implicating a significant reduction in time required for the forced degradation compared to conventional methods at room temperature. Applying acidic (0.01–0.1 M HCl, 1–15 M AcOH), basic (0.001–0.01 M NaOH, 0.001–0.01 M $NaHCO_3$) and oxidative (0.001–0.02% H_2O_2) stress conditions at 150 °C for 5 min resulted in similar indomethacin degradation levels requiring 0.5–20 h at lower temperatures (25–100 °C). In addition solvent stability tests exposing indomethacin to 20 different, mostly organic, solvents at 150 °C and 160 °C for 30 min and the exposure of the solid drug to various gases (N_2, Ar, O_2, NH_3, air), applying high temperatures are presented.

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1. Introduction

Forced degradation or stress studies of drug substances play an integral role in the development of pharmaceuticals [1–7]. Results from forced degradation studies reveal important data on the stability of a given drug molecule and on the generation of "pharmaceutical impurities" resulting from these degradation processes [1–7]. As these impurities may have pharmacological or toxicological relevance, the presence of these impurities must be carefully monitored. Long term storage tests performed to investigate the stability of a developed drug are expensive due to the time involved. Therefore, the method of forced degradation uses external stress conditions like acids or bases (typically ~1 M), oxidative stress (hydrogen peroxide up to 3%), temperature increase, and exposure to light, to enforce the degradation of a drug candidate [1–7]. Traditionally, forced degradation studies in solution are performed using reaction volumes between 10 and 100 ml (drug concentration 1–10 mg/ml), applying comparatively moderate temperatures ranging from room temperature up to ~100 °C (reflux conditions) which implicates long reaction times (from hours to days) [7–12], low sample throughput, and a time-consuming sample handling/analytical regime.

During the past ten years microwave-assisted chemistry has emerged as a very efficient and powerful technology to heat reaction mixtures in dedicated sealed reaction vessels/reactors. The ability to rapidly superheat solvents far above their boiling point up to 300 °C and 30 bar utilizing modern microwave instrumentation has been shown to dramatically reduce processing times compared to conventionally heated experiments under reflux conditions [13–15]. Somewhat surprisingly, the exploitation of microwave technology for forced degradation/stress studies has scarcely been reported in the literature, with the only two published protocols describing the use of domestic microwave ovens without temperature control [16,17]. As with all microwave-assisted chemistry, a proper and reliable control over the reaction parameters (temperature, pressure, stirring) is essential in order to obtain reproducible results that can be duplicated in other laboratories [15,18–21]. In particular, the monitoring of internal reaction temperature in microwave-assisted transformations is a non-trivial affair, and inaccuracies in temperature measurements have in the past frequently led to misinterpretations and erroneous conclusions as to

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the specific role of microwave irradiation in a particular process [18,19].

Herein, we present a microtiter platform made out of strongly microwave absorbing silicon carbide (SiC) plates providing bore holes with the appropriate dimensions to be fitted with 20 standard HPLC/GC vials to perform low-volume microwave-assisted forced degradations (0.5–1.5 ml). The HPLC/GC vials are sealed with aluminum crimp caps equipped with PTFE coated silicone septa and an additional sealing mechanism enables parallel high-temperature processing of 80 vials up to 200 °C and 20 bar [22]. Detailed measurements have demonstrated that microwave irradiation of the SiC reaction blocks leads to rapid and homogeneous heating of the entire plate, with minimal deviations in the temperature recorded at different positions of the plate or inside the glass vials [23]. Importantly, because of the strongly microwave-absorbing nature of the SiC ceramic, solvents with different microwave absorption characteristics can be heated in parallel in individual vials of the microtiter plate reaching the same temperature [23]. The SiC setup has been successfully applied as a high-throughput experimentation platform in organic synthesis [22,24], for GC derivatization protocols [25], the acid hydrolysis of proteins and peptides [26], the enzymatic hydrolysis of protein-bound selenium [27], and for microwave-assisted extraction protocols [28]. In this paper, microwave-assisted parallel forced degradation studies using indomethacin as a model drug substance, applying acidic, basic, and oxidative stress conditions under elevated temperature and pressure conditions are described. In addition, solvent stability tests as well as the exposure of solid compound to reactive gases in the same platform will be presented. Since degradation reactions and analyses are performed in the same vessel, the sample handling effort is reduced and errors caused by a required transfer step are avoided.

2. Experimental

2.1. Chemicals and reagents

Indomethacin was obtained from Sigma–Aldrich (Steinheim, Germany). All the other chemicals, solvents, and gases were purchased from commercial sources and used without further purification.

2.2. Heating equipment and temperature monitoring

Conventional reflux experiments were performed in an oil bath utilizing standard glassware. Single-mode microwave experiments were performed in a Monowave 300 microwave reactor equipped with a fiber optic probe for accurate internal temperature measurement (Anton Paar GmbH, Graz, Austria) [19] or an Emrys Initiator 8 EXP 2.0 (Biotage, Uppsala, Sweden) measuring the outside surface temperature of the microwave vial using an IR sensor. Preliminary optimization experiments were performed in conical microwave vials possessing a filling volume range from 0.5 to 2 ml in the Initiator instrument. For single-mode microwave experiments in the Monowave 300, 10 ml G10 Pyrex vessels (2–6 ml filling volume) or 10 ml silicon carbide vessels (2–6 ml filling volume, identical dimensions as the Pyrex vessel) were utilized [20]. Multimode microwave experiments were performed in a Synthos 3000 microwave instrument utilizing the 4 × 20 MGC rotor capable of holding up to four silicon carbide reaction blocks (80 HPLC/GC reaction vessels, Anton Paar GmbH, Graz, Austria) [22,23], containing 20 bore holes to be equipped with standard 1.5 ml HPLC/GC vials (32 mm × 11.6 mm, transparent, VWR International, Vienna, Austria) sealed with aluminum crimp caps in combination with PTFE coated silicone septa (1.3 mm thickness, sealing disks N 11 silicone/PTFE coated, Macherey-Nagel, Düren, Germany). Temperature controlled runs in the Synthos 3000 are based on IR temperature measurement where the IR sensor, located at the bottom of the instrument, is measuring the outside temperature of the SiC blocks. The pressure resistance of the heating setup is increased by fixing an additional aluminum top plate with six stainless steel bolts, allowing pressures up to 20 bar. Temperature measurement inside the HPLC/GC vials during the multimode microwave run was accomplished by using a multi-channel conditioner (TempSens signal conditioner, Opsens, Quebec, Canada) capable of using up to four OTG-F fiber optic temperature sensors simultaneously [23].

2.3. HPLC instrumentation

HPLC-UV analysis (Shimadzu LC 20 AD) was carried out on a C 18 reversed-phase analytical column (150 mm × 4.6 mm, particle size 5 μm) using mobile phases A (water/MeCN 90:10 (v/v) + 0.1% TFA) and B (MeCN + 0.1% TFA) at a flow rate of 1 ml/min. The following gradient was applied: linear increase from solution 30% B to 100% B in 5 min, hold at 100% solution B for 1 min. All compounds were analyzed at a wavelength of 230 nm.

HPLC–MS analysis (Shimadzu LC 20 AD) was carried out on a C 18 reversed-phase analytical column (150 mm × 4.6 mm, particle size 5 μm) using mobile phases A (water/MeCN 90:10 (v/v) + 0.1% HCOOH) and B (MeCN + 0.1% HCOOH) at a flow rate of 0.6 ml/min. The following gradient was applied: linear increase from solution 30% B to 100% B in 9 min, hold at 100% solution B for 6 min. The MS (Shimadzu LCMS 2020) conditions were as follows: positive electro spray ionization (ESI+) selecting a mass range between 50 and 500 *m*/*z*. Interface voltage was 4.5 kV and detector voltage was 1 kV.

2.4. Procedures

2.4.1. Conventional protocols

Room temperature experiments were performed in conical microwave vials, stirring 0.1 ml of a previously prepared indomethacin stock solution (5 mg/ml in MeCN) together with 0.9 ml 0.1 M HCl solution for 24 h and 72 h, respectively. After that the liquid was transferred to a HPLC/GC vial, diluted with 0.5 ml MeCN and analyzed by HPLC-UV. For experiments performed under reflux conditions a 10 ml round bottom flask was equipped with a stir bar, 0.5 ml of indomethacin stock solution was diluted with 4.5 ml of water or acidic (0.1 M HCl, 0.1 M H_2SO_4, 0.1 M AcOH), basic (0.1 M $NaHCO_3$, 0.1 M NaOH), and H_2O_2 (0.02%) solutions, respectively. Heating was maintained for 10, 30, 60, or 120 min (600 rpm stirring speed) and after cooling 1 ml of the solutions was diluted with 0.5 ml MeCN for subsequent HPLC-UV analysis (230 nm).

2.4.2. Single-mode microwave experiments

Preliminary single-mode microwave experiments utilized a temperature range from 100 to 160 °C and heating periods between 1 and 60 min using 0.1 ml of indomethacin stock solution (5 mg/ml) and 0.9 ml of the acidic, basic and peroxide solutions described in the previous section. Experiments were performed in conical 5 ml microwave vials utilizing the corresponding stir bars and 600 rpm stirring speed. After cooling with compressed air, the reaction mixtures were transferred to HPLC/GC vials, diluted with 0.5 ml MeCN and analyzed applying HPLC-UV analysis (230 nm). Comparison experiments utilizing 10 ml Pyrex or SiC vessels, immersing a ruby thermometer for accurate internal temperature measurement into the liquids during heating, were performed at 150 °C (5 min heating time and 600 rpm stirring speed) using 0.3 ml indomethacin stock solution and 2.7 ml HCl solution (0.1 M). After cooling the solutions with compressed air, 1 ml was transferred into HPLC/GC vials, diluted with 0.5 ml MeCN and analyzed by HPLC-UV (230 nm).

2.4.3. Multimode microwave experiments

Preliminary test runs focused on testing the temperature inside the HPLC/GC vials. Therefore a HPLC/GC vial was filled with 1 ml water, equipped with a stir bar and sealed with an aluminum crimp cap combined with PTFE coated silicone septa. An immersion tube was inserted into the vial to allow the introduction of OGT-F fiber optic probes into the vial [23] for accurate internal temperature recording. Initially, a screening testing various reagents at different concentrations (HCl 0.01–0.1 M, AcOH 1–15 M, $NaHCO_3$ and NaOH 0.001–0.01 M, H_2O_2 0.001–0.02%, and water) was performed, utilizing standard HPLC/GC vials as reaction vessels, equipped with proper stir bars (5 mm × 3 mm), 0.1 ml of indomethacin stock solution, 0.9 ml of reagent solution and the vials were sealed with aluminum crimp caps combined with PTFE coated silicone septa. The sealed HPLC/GC vessels were put in the SiC heating platform and by fixing an additional aluminum top plate with six stainless steel bolts processing at elevated temperature and pressure conditions was enabled. The reaction block was placed onto the corresponding 4 × 20 MGC rotor and the setup was heated for 5 min (hold time) selecting stirring level 3 which correlates to 600 rpm. A preset IR temperature of the Synthos 3000 of 144 °C was selected to maintain 150 °C reaction temperature inside the HPLC/GC vials (maximum output power 500 W) and after cooling to ~50 °C the mixtures were diluted with 0.5 ml MeCN through the septa using a needle. Subsequently, the HPLC/GC vials were directly placed into the autosampler of the HPLC-UV instrument for analysis. To test the stability of indomethacin in various organic solvents, 20 HPLC/GC vials were filled with 0.1 ml of the indomethacin stock solution and the MeCN was evaporated. Thereafter several HPLC/GC vials were equipped with stir bars and filled with 1 ml of water, methanol, ethanol, isopropanol, butanol, hexane, cyclohexane, decane, toluene, tetrahydrofuran, acetonitrile, ethyl acetate, acetone, chloroform, ethylene glycol, NMP, DMA, DMF, DMSO, and chlorobenzene, respectively. Subsequently, the vials were sealed and the mixtures were heated to 150 °C and 160 °C (preset IR temperatures of 144 °C and 155 °C), the temperature was kept constant for 30 min, applying active stirring at 600 rpm. After cooling, the mixtures were diluted with 0.5 ml MeCN through the septa and directly analyzed by HPLC-UV (230 nm). An additional set of multimode microwave-assisted experiments was performed exposing solid indomethacin to different gases (argon, nitrogen, air, oxygen and ammonia). Therefore, 20 HPLC vials were filled with 0.1 ml of the indomethacin stock solution and the solvent was evaporated. After that, the vials were sealed, flushed with the five gases mentioned above for ~30 s and subsequently heated for 30 min at 150 °C and 180 °C in the multimode microwave instrument ($n = 4$). After cooling the SiC platform, 0.5 ml MeCN was filled through the septa into the HPLC/GC vials and samples were directly analyzed by HPLC-UV.

3. Results and discussion

3.1. Preliminary optimization of the forced degradation protocol

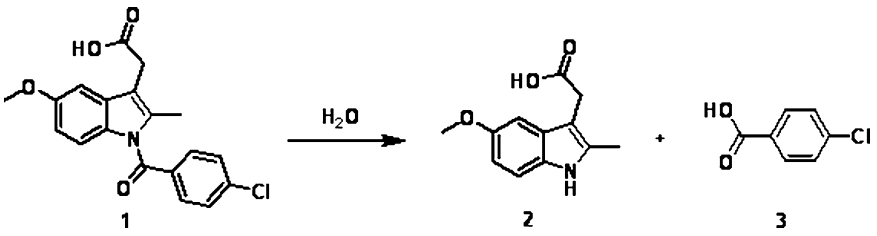
Indomethacin (**1**) [29,30], a non-steroidal anti-inflammatory drug, was selected as model compound to evaluate the concept of performing high-throughput microwave-assisted forced degradations in SiC platforms. As the degradation pathways of indomethacin (**1**) under a variety of conditions are very well established [31–39], analytical thoroughness and a strict chromatographic method validation was not the primary concern in this study. Therefore, a precise quantification of degradation products using internal standards was not performed, rather a comparison of peak areas at a certain wavelength (230 nm) utilizing HPLC-UV analysis was deemed sufficient for the purpose of validating the microwave-assisted forced degradation concept. However, all microwave-assisted degradation experiments were repeated at least 3 times in order to ensure the statistical relevance of the method. Indeed, the decomposition products of indomethacin (**1**) are well known and extensive studies on the degradation can be found in the literature [31–39]. The main products resulting from hydrolysis are 5-methoxy-2-methyl-3-indoleacetic acid (**2**) and 4-chlorobenzoic acid (**3**) (Scheme 1) [31–37], with additional degradation products being formed when oxidative stress conditions are applied, emerging from an epoxide intermediate that is subsequently rearranging to the decarboxylation (4-chloro-N-[4-methoxy-2-(1-methylene-2-oxopropyl)phenyl]-benzamide) and the cyclization 8-(4-chlorobenzoyl)-3a-hydroxy-5-methoxy-8a-methyl-3,3a,8,8a-tetrahydro-2H-furo[2,3-b]indol-2-one products **5** and **6**, respectively (see Scheme S2) [38].

Table 1
Acceleration of indomethacin (**1**) degradation using elevated temperature microwave conditions.

T (°C)	Time (min)	Degradation (%)
100	60	47
110	30	47
120	15	43
130	8	40
140	4	36
150	2	31
160	1	27

Single-mode microwave reactor (Biotage Initiator). Experiments were performed in 0.1 M HCl solutions. % degradation refers to decomposition of indomethacin to the hydrolysis products **2** and **3** (Scheme 1).

Traditionally, forced degradation studies are performed in solution, using concentrations of the target compounds ranging between 1 and 10 mg/ml, aiming for a degradation level of approximately 5–20% [1–7]. In most instances, the analytical methods of choice are LC–MS as well as HPLC-UV analysis [1–7]. The main drawback of these classical forced degradation protocols is the time factor, because the targeted decomposition of many drug molecules or active pharmaceutical ingredients (APIs) requires many hours or even days applying room temperature or reflux conditions (~100 °C) [7]. One of the main aims of this study was to speed up the forced degradation protocol of the model API indomethacin (**1**) using sealed vessel microwave technology. For this purpose, initial control experiments were performed at room temperature (0.1 M HCl was used as reagent), revealing that the desired level of indomethacin degradation was obtained after 24 h (11%) and 72 h (24%), respectively (HPLC-UV at 230 nm). Apart from the experiment using 0.1 M HCl, various other reagents (acids, bases, and peroxide) were tested for their ability to decompose indomethacin (**1**) in a subsequent screening performed under condition of reflux heating (100 °C) for 10–120 min. Similar degradation rates were obtained for 0.1 M HCl and H_2SO_4 after 1 h compared to the 72 h room temperature experiment. Complete degradation of indomethacin (**1**) was obtained after 30 min applying basic stress conditions (0.1 M NaOH and $NaHCO_3$) and 7% degradation was observed after 120 min using 0.02% hydrogen peroxide (results of the complete screening are given in Table S1). After reducing the decomposition time from days to hours by simply increasing the temperature from room temperature to reflux conditions, microwave-assisted heating under sealed vessel conditions was utilized to increase the temperature above the boiling points of the used reagents [21], aiming for a further decrease of heating time required for an adequate decomposition rates of indomethacin (**1**). Sealed vessel single-mode microwave heating experiments at 100–160 °C in 10 °C increments (Table 1) demonstrate that microwave heating for 1 min at 160 °C delivered the same decomposition level compared to the initially performed experiments at room temperature lasting 72 h (27% compared to



Scheme 1. Main decomposition pathway of indomethacin (**1**). Hydrolysis of the amide group leads to the formation of indole **2** and benzoic acid **3**.

24%). These results are in good agreement with the Arrhenius relationship indicating that a temperature increase of 10 °C leads to a reduction of the reaction time by a factor of 2 [21]. Naturally, this rule of thumb involving a doubling of the rate of degradation is only an approximation, and the rate of increase of a reaction as a function of temperature is clearly dependent on the energy of activation (E_a). For the above rule to hold true in these temperatures ranges, the E_a would need to be in the 12–15 kcal/mol range, whereas for solution degradations, the average appears to be around 23–24 kcal/mol for many pharmaceuticals [3]. A temperature/time fine tuning for other HCl concentrations and H_2O_2 is shown in Table S2.

As far as the hydrogen peroxide stress testing is concerned it should be noted, however, that the high temperatures (up to 160 °C) used in these degradation studies are likely to induce the formation of hydroxyl radicals which are very strong oxidants. Therefore, such high temperature stress conditions using hydrogen peroxide may in some cases lead to artifactual and non-predictive degradation pathways [3,6]. In the case of indomethacin, the peroxide-induced degradation at higher temperatures does lead to the formation of the non-hydrolytic, additional decomposition products **5** and **6** derived from the epoxidation of the indole 2,3-double bond consistent with the results obtained previously [38], as confirmed by careful HPLC-UV monitoring (Scheme 2).

3.2. Investigation of microwave effects

A reliable reaction temperature control in microwave-assisted experiments is absolutely essential in order to obtain meaningful results [18,19]. In general, temperature monitoring in microwave-heated reactions is a not trivial affair, in particular in pressurized reaction vessels at elevated temperatures [18,19]. In order to confirm that the observed rate accelerations are the consequence of purely thermal/kinetic effects on the basis of the Arrhenius equation (and do not involve so-called nonthermal microwave effects as often claimed) [18–20] a set of control experiments was performed using accurate internal fiber-optic temperature monitoring in combination with single-mode microwave heating (see Fig. S1 in the Supporting Information). The degradation of indomethacin (**1**) was thus performed at 150 °C with 0.1 M HCl in standard Pyrex vessels heating the reaction mixtures contained inside the vessels directly by microwave irradiation and in specially designed vessels made out of strongly microwave absorbing silicon carbide (SiC) where the vessel itself is absorbing most of the microwave energy which leads to a conventional heat transfer from the vessel wall to the reaction mixture inside [20]. An overlay of the temperature profiles using both vessel types assured identical inside temperature conditions during the forced degradation runs (Fig. 1).

Comparison experiments were performed at 150 °C, heating the indomethacin reaction mixtures for 5 min in 0.1 M HCl solution. Since the degradations rates obtained in both vessel types applying different heating mechanisms were almost identical (~50%), the contribution of a nonthermal microwave effect to the decomposition of indomethacin can be excluded.

3.3. Parallel processing in SiC heating platforms

After demonstrating the ability to significantly reduce the time required for the decomposition of indomethacin (**1**) using closed vessel microwave conditions at elevated temperatures, our next goal was the parallelization and miniaturization of the forced degradation protocol. Therefore, the degradation protocols were transferred to the microtiter platform described above using sealed HPLC/GC vials as reaction vessels (Fig. 2).

In initial control experiments, the temperature inside the HPLC/GC vials was determined using internal fiber-optic probes as shown in Figs. S3 and S4 in the Supporting Information. Subsequently, the 20 position SiC microtiter platform was employed for a parallel degradation screening of indomethacin (**1**) evaluating five different reagents at four different concentrations applying 150 °C

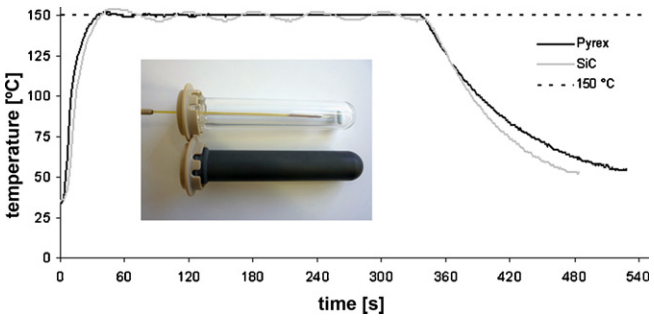


Fig. 1. Comparison of internal temperature profiles for the microwave-assisted degradation of indomethacin with 0.1 M HCl at 150 °C in Pyrex and SiC vessels (Monowave 300 reactor). An image of the vessels is shown in the inset.

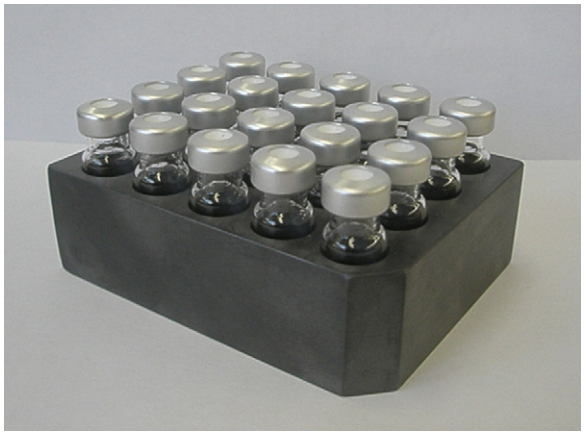
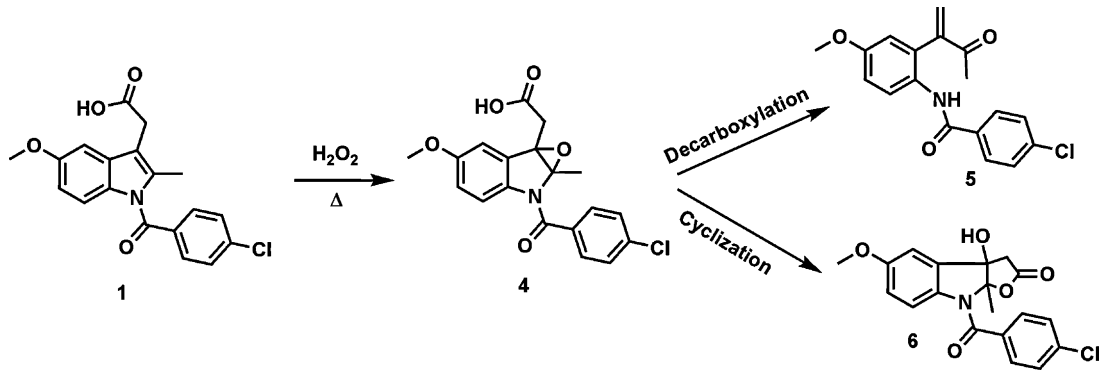


Fig. 2. Heating platform made out of sintered silicon carbide (SiC, grey reaction block), holding 20 standard HPLC/GC vials [22,23]. The vials are sealed with standard aluminum crimp tops in combination with PTFE coated silicone septa for high-temperature and pressure operation.



Scheme 2. Decomposition products of indomethacin (1) applying oxidative stress conditions. Apart from the hydrolysis products (5-methoxy-2-methyl-3-indoleacetic acid (**2**) and 4-chlorobenzoic acid (**3**), shown in Scheme 1) additional peaks with *m*/*z* values of 330 and 374 were detected utilizing LC–MS analysis. Under oxidative and thermal stress conditions an unstable indole-2,3-epoxide intermediate (**4**) is formed, that subsequently rearranges to the decarboxylation (**5**, *m*/*z* 330) and cyclization (**6**, *m*/*z* 374) products [38].

for 5 min. Apart from the time saving aspect due to the elevated temperatures and the significant increase in sample throughput, an additional advantage of the miniaturized setup is the direct utilization of the HPLC/GC analysis vials as reaction vessels. Thus, after cooling the setup to ambient temperature, the vials can be directly transferred to the corresponding autosampler of the analytical instruments (HPLC-UV, GC–MS, LC–MS) reducing handling effort and avoiding sample loss caused by an extra transfer step. The results of the parallel degradation screening provide a rapid overview over several stress parameters in less than 20 min, compared to relatively long reactions times (30 min–20 h) which are observed when lower temperatures (25–100 °C) are applied, utilizing basic stress conditions (pH 8–11) in combination with conventional heating [31–33]. Degradation levels for the various applied stress conditions are summarized in Fig. 3.

Apparently, indomethacin follows the same degradation pathways in a high-temperature regime as compared to the more traditional protocols operating at significantly lower temperatures [31–39]. Careful monitoring by HPLC-UV did not reveal any additional degradation being formed for any of the investigated stress conditions. Clearly, this will not be the case for other, more complex or labile drug molecules [3,40]. In these instances, the high-temperatures typically applied in sealed-vessel microwave processing may induce alternative degradation pathways not predictive of degradation pathways at lower temperatures or room temperature.

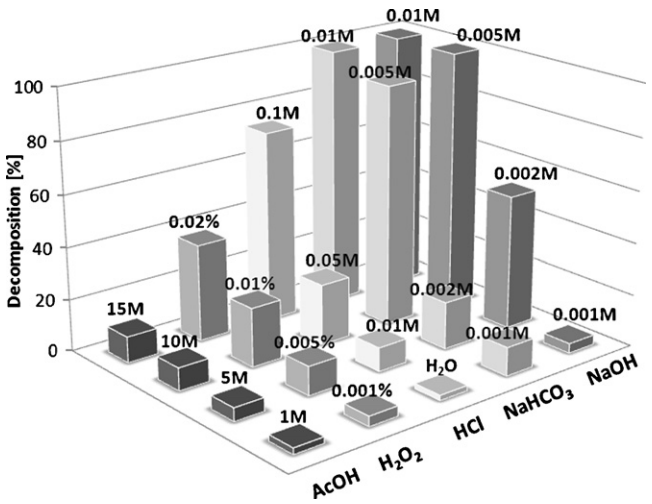


Fig. 3. Parallel indomethacin degradation evaluating testing different stress conditions (HPLC-UV at 230 nm). The SiC platform (Fig. 2) was heated at 150 °C for 5 min (~19 min total processing time). Mean values of three experiments are shown.

In addition to the classical forced degradation screening, solvent stability tests were performed, exposing indomethacin (**1**) to 20 different, mostly organic, solvents at 150 °C and 160 °C for 30 min. The highest decomposition rates were observed in water (20%) and ethylene glycol (15%) at 160 °C (30 min), whereas for all the other solvents decomposition was comparatively low (only hydrolysis products were detected, for the results of the complete screening see Table S3 in the Supporting Information). It should be stressed that, because of the strongly microwave-absorbing nature of the SiC ceramic, solvents with vastly different microwave absorption characteristics can be used in the individual vials of the microtiter plate reaching the same temperature [23]. Also, because of the 20 bar pressure limit, these experiments tolerate low boiling solvents such as acetone, tetrahydrofuran (THF), or methanol.

As a final set of experiments 20 HPLC/GC vials containing 0.5 mg of indomethacin as a solid were sealed with aluminum crimp caps and flushed with five different gases (nitrogen, argon, air, oxygen, and ammonia). To make use of the 5 × 4 matrix provided by the SiC platform, experiments were performed in four replicates (5 gases, $n = 4$) heating the SiC setup for 30 min at 150 °C and 180 °C, respectively. After cooling to ~50 °C, to each vial 0.5 ml MeCN was added and the vials were directly transferred to the HPLC-UV autosampler for analysis (230 nm). No degradation was observed for the vessel treated with dry nitrogen and argon for both temperature regimes, whereas hydrolysis of the indomethacin amide bond was observed when the solid drug was exposed to air and oxygen. At the moment no satisfactory explanation can be given for this phenomenon, but it appears likely that the relative humidity/water activity of the used gases (air and oxygen) is playing a major role. Apart from the known hydrolysis products (Scheme 1), a different compound was detected when the API was exposed to ammonia which was identified as an anion of the ammonium salt [39] (*m*/*z* value of 373 utilizing LC–MS analysis, Scheme S1) appearing

Table 2
Exposure of indomethacin to different gases applying elevated temperatures (150 °C and 180 °C for 30 min).

Gases	Degradation (%)	
	150 °C	180 °C
Nitrogen	0	1 ± 1
Argon	0	1 ± 0
Oxygen	5 ± 1	85 ± 1
Air	2 ± 0	26 ± 1
Ammonia	46 ± 5	97 ± 0

at both temperature regimes. Generally, the degradation rate was high when indomethacin was exposed to ammonia, similar to the results obtained for the basic reagents in solution presented in Fig. 3 (a summary of data for the gas exposure screening is presented in Table 2).

4. Conclusions

In summary, we have demonstrated that sealed vessel microwave-assisted forced degradation can be carried out efficiently in a silicon carbide-based microtiter plate fitted with standard disposable HPLC/GC autosampler vials utilizing reaction volumes from 0.5 to 1.5 ml. Using an appropriate sealing mechanism inside a dedicated multimode microwave instrument with accurate online temperature monitoring, processing can be performed at temperatures and pressures up to 200 °C and 20 bar, respectively. Since the SiC plate material itself is strongly microwave absorbing, the reagent mixtures contained inside the HPLC/GC vials are essentially heated by conduction phenomena from the SiC plate. Thus, the microwave absorbance characteristics of individual reagents/solvents become irrelevant and due to the 20 bar pressure limit also low boiling solvents can be used providing the same internal reaction temperatures. Since this platform employs standard HPLC/GC autosampler vials as reaction vessels, a subsequent analysis by either HPLC-UV or LC–MS (or even GC–MS) can in general be performed directly from the HPLC/GC reaction vial. The platform was evaluated for the parallel forced degradation of indomethacin assessing different reagents at various concentration levels, in addition to organic solvents and reactive gases. These techniques allow the execution of parallel forced degradation studies within minutes in a highly parallelized and miniaturized format. Using only 0.5–1.5 ml of reaction volume, a rapid evaluation of different stress conditions at a significantly expanded temperature and pressure regime is possible. Once suitable forced degradation parameters were discovered, 80 different samples can be treated simultaneously under the exact same temperature/time conditions.

The use of high-temperatures in predictive degradation studies assumes that the drug molecule will follow the same pathway of decomposition at all temperatures. This assumption may not hold true for all drug molecules, and therefore great care must be taken in using the extreme temperatures easily accessible in a sealed-vessel microwave experiment for predictive degradation studies. Examples have been reported in the literature where stressing at temperatures above 80 °C has led to different decomposition pathways for some compounds [3,40]. The risk of such changes is clearly higher with sealed-vessel microwave-assisted degradation experiments at temperatures in excess of the reflux temperature.

On the other hand, the forcing conditions resulting from the microwave-assisted high-temperature technique may also be useful for isolating preparative amounts of pharmaceutical impurities formed by degradation of APIs that otherwise would be difficult to obtain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.07.042.

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