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Short communication

Microwave-assisted high-throughput derivatization techniques utilizing silicon carbide microtiter platforms

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ABSTRACT

Parallel microwave-assisted gas chromatography (GC) derivatization protocols utilizing a silicon carbide (SiC)-based microtiter plate platform fitted with screw-capped GC vials were developed. For three selected standard derivatization protocols such as acetylation (exemplified for morphine), pentafluoropropionylation (for 6-monoacetylmorphine) and trimethylsilylation (for Δ^9-tetrahydrocannabinol) complete derivatization was achieved within 5 min at 100 °C in a dedicated multimode microwave instrument using online temperature monitoring. Microwave irradiation leads to rapid and homogeneous heating of the strongly microwave-absorbing SiC plate, with minimal deviations in the temperature recorded at different positions of the plate. The current platform allows the simultaneous derivatization of 80 reaction mixtures under strictly controlled temperature conditions. Similar results can also be obtained using a standard hotplate as heating source, although heating to the target temperature of 100 °C is slightly slower. The results demonstrate that parallel microwave derivatization procedures can significantly reduce the overall analysis time and increase sample throughput for GC–MS-based analytical methods.

1. Introduction

The use of microwave heating for performing gas chromatography (GC) derivatization protocols in forensic/clinical toxicology and doping control is steadily increasing [1–12]. In general, microwave irradiation produces efficient and rapid internal heating (in core volumetric heating) by direct coupling of microwave energy with the molecules that are present in the reaction mixture [13]. In contrast to conventional heating by conduction phenomena using a heating block or drying oven, this process does not require the slow and energy inefficient heating of the reaction vessel itself and subsequent heat transfer to the reaction mixture by convection currents. Therefore, in most of the published examples on microwave-assisted derivatization protocols the time required for complete derivatization could be significantly reduced, typically to less than 5 min [1–12]. However, it has to be noted that in the overwhelming majority of publications in this field, domestic microwave ovens without temperature control have been employed for these derivatization experiments. Clearly, without access to accurate reaction temperature data, reliable and reproducible derivatization protocols are difficult to establish.

In a recent publication we have evaluated the potential of microwave-assisted derivatization techniques applying a dedicated single-mode microwave reactor with online temperature and pressure control [14]. The use of this equipment has allowed a detailed analysis of several microwave-assisted derivatization protocols comparing the efficiency of microwave and conventional heating methods utilizing a combination of GC–MS and liquid chromatography coupled with mass detection (LC–MS) techniques. These studies revealed that for standard derivatization protocols such as acetylation, pentafluoropropionylation and trimethylsilylation a reaction time of 5 min at 100 °C in a microwave reactor was sufficient to allow for an effective derivatization. Control experiments using standard operating procedures (30 min at 60 °C conventional heating) have indicated that faster derivatization under microwave irradiation is a consequence of the higher reaction temperatures that can rapidly be attained in a sealed vessel and the more efficient heat transfer to the reaction mixture applying direct in core microwave dielectric heating [13].

While these studies have provided both a clear scientific rationale and optimized conditions for the use of microwave technology in GC derivatization chemistry, the applied single-mode microwave technique required the use of specialized microwave process vials and did not allow an easy adoption of the derivatization protocols to

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a high-throughput format, since each reaction vessel needed to be irradiated separately. We herein describe the efficient parallelization and miniaturization of these derivatization methods applying a dedicated high-throughput reactor platform that uses standard GC vials as reaction vessels in a microtiter plate made out of silicon carbide (SiC).

2. Experimental

2.1. Chemicals

The derivatization reagents and morphine, 6-monoacetylmorphine (6-MAM) and Δ^9-tetrahydrocannabinol (Δ^9-THC) standards used in this work are identical to those used in our previous publication [14].

2.2. Heating equipment and temperature monitoring

Microwave-assisted derivatization reactions using a high-throughput SiC experimentation platform were performed in a Synthos 3000 multimode microwave reactor (Anton Paar GmbH) producing microwave irradiation (0–1400 W) at 2.45 GHz [15,16]. Details regarding the use of the 5×4 deep-well silicon carbide plates in combination with standard GC vials (deactivated amber screw neck fitted with a 200 μL inlet, QsertVial, Waters, MA, USA) were previously reported [16]. Microwave irradiation experiments in the Synthos 3000 reactor using infrared and/or internal fiber-optic probe temperature monitoring technology and conventional heating experiments using a hotplate were performed as recently described [16].

2.3. GC–MS instrumentation

GC–MS analysis for the detection of derivatized analytes was performed on a Trace-GC Ultra–DSQ II-MS system (ThermoElectron, Waltham, MA, USA) as previously reported [14].

2.4. Calibration standards and quality control samples

Calibration curves of morphine, 6-MAM and Δ^9-THC standards were constructed in the same manner as described in our recent publication [14].

2.5. Derivatization reactions

For derivatization the prepared solutions described in Section 2.4 were transferred into deactivated GC-autosampler vials (QsertVial, Waters, MA, USA) and brought to dryness under a gentle stream of nitrogen (5 min). 200 and 50 μL derivatization reagent were added, respectively, for the microwave irradiation and hotplate experiments: acetic anhydride/pyridine (3:2, v:v) for morphine, PFPA/PFP (7:5, v:v) for 6-MAM, and MSTFA for Δ^9-THC derivatization.

After derivatization the samples were brought to dryness under a gentle stream of nitrogen (ca. 30–45 min) and were dissolved in 50 μL of methanol (MeOH) for GC–MS analysis.

3. Results and discussion

3.1. Parallel microwave derivatizations in SiC platforms

A recent concept in microwave-assisted chemistry is the utilization of SiC-based microtiter plates/rotor systems for high-throughput/parallel synthesis [15,16]. These recently commercialized platforms allow sealed-vessel parallel microwave processing – depending on the specific plate footprint – of up to ~200 reactions on a <3.0 mL scale using multimode microwave reactors under carefully controlled reaction conditions. Microwave irradiation of the strongly microwave-absorbing SiC plates leads to a rapid and homogeneous heating of the entire plate, with minimal deviations in the temperature recorded at different positions of the plate or inside the wells [15,16]. Because of the large heat capacity and high thermal conductivity of SiC, the plates are able to moderate any field inhomogeneities inside a microwave cavity and therefore avoid the problems of "hot and cold spots" frequently experienced in multimode microwave systems [16].

For microwave-assisted derivatization reactions the use of a 5×4 deep-well SiC platform that utilizes standard low volume (maximum filling volume 200 μL, conical design) screw-cap GC vials [16] is particularly attractive, since both the derivatization and the analysis can be performed in the same vessel without the need of sample transfer [15,16]. Regardless of the position in the plate and largely independent on the filling volume the same temperature profiles are obtained [16]. This is in stark contrast to the use of standard (microwave transparent) polypropylene racks fitted with GC vials in combination with a domestic microwave oven. In this case, the heating behavior of the solutions contained in the individual GC vials is rather unpredictable and very dependent on the vial position in the rack [16].

3.2. Validation study

The purpose of the present investigation was to transform our previously validated and optimized standard derivatization protocols (5 min at 100 °C) including acetylation (exemplified for morphine), pentafluoropropionylation (for 6-monoacetylmorphine) and trimethylsilylation (for Δ^9-tetrahydrocannabinol) from single-mode conditions using 200 μL volume [14] to the GC vial/SiC plate format applying 50 μL volume [15]. A detailed comparison between microwave-assisted and conventionally heated transformations was already described in our previous publication and substrate/product stabilities were also addressed by irradiating the reaction mixtures for up to one hour at 100 °C and analyzing morphine against two different calibration curves [14]. One calibration curve was obtained without derivatization by diluting the pure 3,6-diacetylmorphine standard and a second calibration curve was derived by acetylating the pure morphine solutions using the microwave-assisted derivatization protocol. No significant difference using the two different methods was observed [14].

In the current study calibration curves were obtained by derivatizing pure solutions (200 μL of the corresponding derivatization cocktail, see Section 2.5) of the corresponding analytes to give final concentrations of 10, 20, 40, 80 and 160 μg/mL. For validation of the SiC platform the single-mode conditions using specially designed microwave vessels (200–500 μL filling volume) were translated to multimode conditions, where standard GC vials were used as reaction vessels. This implies that the transfer of the derivatized analytes from the microwave reaction vial to the analysis vial can be omitted, which reduces operator errors, potential loss of material, costs and time effort. Employing a single-mode microwave reactor derivatizations need to be performed sequentially, whereas in a larger multimode cavity up to 80 reactions (4 plates) can be performed in parallel utilizing the current SiC platform set-up [15,16]. The temperature in both the single- and the multimode system is controlled with IR temperature measurement. In addition to the IR surface temperature measurement of the SiC block in the multimode cavity, the reaction temperature inside the GC vials (~100 °C) was measured by fiber-optic probes which were inserted into the cavity [16]. After obtaining similar results for both cavity types a downscaling from 200 to 50 μL derivatization volume was performed which proved to be sufficient for derivatization (Table 1).

Table 1
Accuracy and precision data for the acetylation of morphine, the pentafluoropropionylation of 6-MAM and the silylation of Δ^9-THC. Derivatization reactions were performed in a single-mode (SM, Biotage, Initiator) and a multimode (MM, Synthos 3000, Anton Paar) microwave instrument, respectively. All derivatization reactions were irradiated for 5 min (hold time) at 100 °C.

	Derivatized morphine calibration curve		Derivatized 6-MAM calibration curve		Derivatized Δ^9-THC calibration curve	
	MM 200 μL (n = 7)	MM 50 μL (n = 7)	MM 200 μL (n = 7)	MM 50 μL (n = 7)	MM 200 μL (n = 7)	MM 50 μL (n = 7)
Mean [μg/mL]	61.3	60.8	63.0	59.6	61.7	58.8
SD	1.6	1.8	1.3	1.1	2.2	1.4
% CV	−1.3	−0.8	2.1	1.8	3.6	2.4
% of target	102	101	105	99	103	98

For each of the three derivatization protocols 19 samples were heated in the multimode cavity. Five reaction mixtures containing different final concentrations (10, 20, 40, 80 and 160 μg/mL) for the corresponding calibration curve, seven vials for the 200 μL filling volume QC experiments and seven for the 50 μL QC experiments. For the validation of each analyte in the multimode instrument one SiC plate was equipped with the vials from position 1 to 19. Position 20 was kept empty. No stir bars were used for the derivatization reactions. From all sets of experiments similar results were obtained. Calibration curves were linear in the range from 10 to 160 μg/mL with coefficients of determination (r^2 values) ≥ 0.9975. As shown in Table 1 the coefficients of variation (CVs) for all quality control samples were ≤3.6%. The accuracies, referred to as % of target in Table 1, were determined by comparing the mean calculated concentrations with the spiked target concentration of the quality control samples. The accuracies for all analytes were found to be within 98% and 105%, respectively, of the target values. These data indicate, that there is no measurable difference between the irradiation experiments performed in the single-mode [14] and the multimode reactor. Additionally, no adverse effect was observed when the reactions were downscaled from 200 to 50 μL regarding the recoveries of 3,6-diacetylmorphine, 6-MAM-PFP and TMS-Δ^9-THC.

As a consequence, this technology now allows the parallel microwave-assisted derivatization of up to 80 analytes simultaneously under carefully controlled reaction conditions. In agreement with our previous data [14], the higher reaction temperatures (100 °C) allowing a reaction time of only 5 min did not negatively impact on substrate/product stabilities or recovery rates using standard derivatization procedures (30 min at 60 °C). As the microwave absorptivity of the individual derivatization mixtures plays only a minor role when using SiC reactor platforms [15–17], different types of derivatizations can be performed in parallel in the same SiC plate. It should be noted that this is not possible employing conventional microwave heating of GC vials in multimode microwave reactors [16].

3.3. Conductive heat transfer in SiC platforms

The data shown herein and discussed in a recent publication [16] demonstrate that due to the strongly microwave-absorbing SiC plate, the microwave absorption characteristics of the individual reaction mixtures contained in the GC vials will be practically irrelevant, since the semiconducting plate itself absorbs microwave energy much stronger than any organic material contained inside the wells. This implies that "microwave heating" of the reaction mixture using this technology essentially occurs by conventional conduction and convection principles, similar to a standard heating block experiment. Since SiC possesses very high thermal conductivity, the SiC plate itself can be heated rapidly on a standard hotplate [16]. Due to the high thermal effusivity of this material (a measure for the ability to exchange thermal energy with its surroundings), heat transfer through the glass wall of the GC vial to the reaction mixture is also reasonably fast [16,17].

In order to compare the efficiency of the microwave-assisted protocol described above with conventional heat transfer in detail, the three derivatization reactions were additionally performed on a pre-heated standard hotplate using the same SiC reactor set-up. By recording the temperature profiles of the reaction mixtures directly with the fiber-optic probes mentioned above, it was possible to establish the same temperature conditions (100 °C for 5 min) as applied in the multimode microwave cavity (Fig. 1). To reach the desired 100 °C internal reaction temperature inside the GC vials, a hotplate temperature of 107 °C was selected as some heat is lost during the conventional heating process [16]. In general, the internal heating profiles obtained for both heating methods were rather similar (Fig. 1). While the multimode microwave experiment allowed ramping from room temperature to 100 °C within ~140 s, the same temperature was achieved within ~220 s on the hotplate. For comparison purposes, the heating profile for genuine microwave dielectric heating of the morphine acetylation process in a single-mode microwave reactor [14] is also shown in Fig. 1. Clearly, direct microwave heating of the reaction mixture is more rapid than using the SiC plate concept, but the use of the single-mode microwave technology ultimately leads to an overall gain in processing time of only 4–5 min and can not be executed in a parallel fashion.

The accuracy and precision data for the conventionally derivatized quality control samples using the hotplate experiments are summarized in Table 2. The coefficients of variation (CVs) for all compounds were ≤3.1%. The accuracies, referred to as % of target in Table 2, were determined by comparing the mean calculated concentrations with the spiked target concentration of the quality control samples. The accuracies for all analytes were found to be within 98% and 100%, respectively, of the target val-

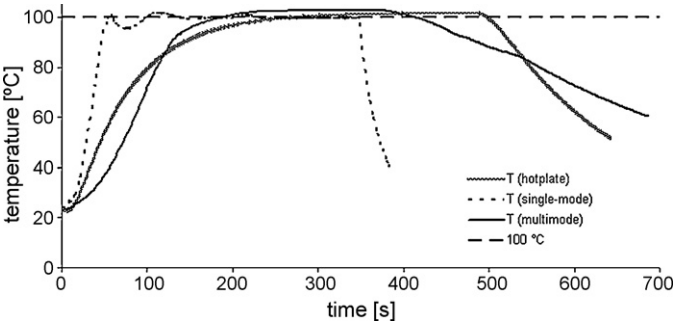

Fig. 1. Comparison of temperature profiles heating an acetic anhydride/pyridine (3:2, v:v) mixture (200 μL) using different heating sources. In all three cases the target temperature was 100 °C and the reaction mixtures were heated for ~5 min at 100 °C (hold time). As expected very rapid heating to the desired end temperature in the single-mode instrument was observed (~40 s) because of the direct in core heating effect. Longer ramp times were obtained when conductive heating, applying the SiC platform, was used. Compressed air is used for cooling the reaction mixture in the single-mode instrument (~30 s cooling time). In the case of the multimode instrument the whole assembly containing vessels and the SiC platform is cooled by a fan and therefore cooling is inefficient (~5 min cooling time). For cooling the vials from the hotplate set-up, they were removed from the SiC platform after the heating period and cooled at ambient conditions (~2.5 min cooling time).

Table 2
Accuracy and precision data for the acetylation of morphine, the pentafluoropropionylation of 6-MAM and the silylation of Δ^9-THC. All derivatization reactions were performed on a pre-heated hotplate (2.5 min to reach 107 °C), heating the reaction mixtures for 5 min (hold time) at 100 °C.

	Derivatized morphine calibration curve 50 μL (n = 7)	Derivatized 6-MAM calibration curve 50 μL (n = 7)	Derivatized Δ^9-THC calibration curve 50 μL (n = 7)
Mean [μg/mL]	58.7	59.6	60.2
SD	1.8	1.2	1.9
% CV	1.3	2.1	3.1
% of target	98	99	100

ues. Combined with our previous results [14] and the information presented above (Table 1), these data indicate that there is no difference between derivatizations performed using single-mode microwave irradiation (genuine microwave dielectric heating) [13], and derivatizations performed in GC vials inserted in SiC plates applying either a multimode microwave reactor format or a standard hotplate. The efficiency of the derivatization process is only a function of the reaction temperature, and not related to any special microwave effect [17].

4. Conclusions

The experimental results presented herein demonstrate that standard GC derivatization processes such as acetylation, pentafluoropropionylation and trimethylsilylation can be conveniently performed within 5 min using microwave-assisted derivatization protocols at 100 °C. For parallel derivatization reactions a microtiter platform based on strongly microwave-absorbing silicon carbide plates fitted with standard disposable GC vials has proven most efficient and cost effective. In this set-up 80 reactions (4 plates fitted with 20 vials each) can be performed simultaneously with excellent temperature control and reaction homogeneity across the plate. Rapid heat transfer from the SiC to the contents inside the GC vials using this platform is ensured by the high thermal conductivity and effusivity of the SiC material. Since the heat transfer occurs mostly by conventional conductive heating, similar results can also be obtained in a standard hotplate experiment at 100 °C using the SiC set-up. We are currently exploring the routine use of the SiC/GC vial platform in the context of a qualitative GC–MS-based systematic toxicological and confirmatory analysis.

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