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An evaluation of microwave-assisted derivatization procedures using hyphenated mass spectrometric techniques

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ABSTRACT

The potential of microwave-assisted derivatization techniques in systematic toxicological analysis using gas chromatography coupled with mass spectrometry (GC–MS) was evaluated. Special emphasis was placed on the use of dedicated microwave reactors incorporating online temperature and pressure control. The use of such equipment 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 and LC–MS/MS) techniques. These studies revealed that for standard derivatization protocols such as acetylation (exemplified for codeine and morphine), pentafluoropropionylation (for 6-monoacetylmorphine) and trimethylsilylation (for Δ^9-tetrahydrocannabinol) 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) indicated that the 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. The results suggest that microwave derivatization procedures can significantly reduce the overall analysis time and increase sample throughput for GC–MS-based analytical methods.

1. Introduction

Bioanalytical applications for the identification and quantification of drugs and their metabolites in various biological matrices generally rely on hyphenated techniques that provide both high levels of specificity and sensitivity. Among the broad range of analytical techniques available, gas chromatography coupled with mass detection (GC–MS), especially in the full scan electron impact (EI) mode, still is the method of choice for most screening procedures in forensic and clinical toxicology, and in doping control [1]. Despite the many advantages of the GC–MS method, time consuming derivatization steps are often required in order to obtain desirable chromatographic characteristics or to improve the stability and detectability of the target analytes [2,3]. These derivatization processes typically require reaction times from 30 min up to several hours. As a consequence, sample derivatization often is the rate limiting step in the overall GC–MS-based analysis procedure.

In recent years, heating by microwave irradiation has become a popular tool in the scientific community for enhancing chemical processes [4–13]. Taking advantage of efficient microwave dielectric heating mechanisms [4], polar reaction mixtures can be rapidly heated using microwave energy. In combination with sealed vessel technology, the temperature of the reaction mixture can be raised far above the boiling point compared to conventional atmospheric pressure conditions [5,6]. As a consequence, for many synthetic transformations reaction times can be reduced from several hours to a few minutes using sealed vessel microwave heating [5,6]. Importantly, microwave irradiation produces efficient internal heating (in core volumetric heating) by direct coupling of microwave energy with the molecules that are present in the reaction mixture. 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. Although many of the early pioneering experiments in microwave chemistry have been carried out in domestic microwave ovens without temperature or pressure control, the current trend undoubtedly is to use dedicated instruments [5,6]. Today's commercially available microwave reactors for chemical synthesis feature built-in magnetic stirrers, direct tem-

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perature control of the reaction mixture with the aid of infrared sensors, and software that enables on-line temperature/pressure control by regulation of microwave power output [5,6].

The advantages of this high speed technology have not only been exploited for organic synthesis [5,6] and in the context of medicinal chemistry/drug discovery [7,8], but have also penetrated fields such as polymer synthesis [9], material sciences [10], nanotechnology [11] and biochemical processes [12]. In the field of analytical chemistry, microwave-assisted digestion and extraction methods can today be considered standard operating procedures in many laboratories [13]. Interestingly, the use of microwave heating to enhance the efficiency of GC derivatization protocols has so far found limited use and acceptance in the scientific community. Pioneering contributions by Dasgupta and co-workers in the mid 1990s have already highlighted the potential usefulness of microwave irradiation for several standard GC–MS derivatization reactions such as trimethylsilylation, acetylation, and pentafluorobenzoylation [14–20]. In most of the published examples the time required for complete derivatization could be reduced to less than 5 min [14–20]. Subsequent work by Maurer and co-workers has validated the use of microwave-assisted derivatization techniques for several case studies in the area of clinical and forensic toxicology [2,21]. Despite these promising data, only a comparatively small number of reports describing rapid microwave-assisted GC derivatization protocols have been published in recent years [14–32].

It has to be noted that in the large majority of the published examples [14–31], domestic microwave ovens without temperature or pressure control have been employed for these derivatization experiments. Without access to reaction temperature data a reliable optimization of reaction conditions and a meaningful comparison between microwave and conventionally heated chemistry, however, is not possible [5,6]. Arguably, the lack of suitable instrumentation and of a clear scientific rationale has so far limited the use and acceptance of microwave-assisted derivatization techniques in the scientific community.

Herein we present a detailed investigation and optimization of three of the most important GC derivatization protocols (acetylation, pentafluoropropionylation, and trimethylsilylation), comparing the efficiency of microwave-assisted and conventional methods. For the detection of both the analytes and their derivatized analogs a combination of GC–MS and liquid chromatography coupled with mass detection (LC–MS and LC–MS/MS) techniques was utilized. Controlled microwave heating was performed in an automated single mode (also termed monomode) microwave reactor capable of irradiating small sample volumes (200–500 μL) under sealed vessel conditions with online temperature and pressure monitoring.

2. Experimental

2.1. Chemicals

Standards (1 mg/mL) of morphine, codeine, 6-monoacetylmorphine (6-MAM) in methanol, heroin in acetonitrile and Δ^9-tetrahydrocannabinole (Δ^9-THC) in ethanol and stable isotope labelled standards of morphine-d_3 and 6-MAM-d_3 in methanol, heroin-d_3 in acetonitrile and Δ^9-THC-d_3 in ethanol were obtained from Serobac Handelsgesellschaft (Vienna, Austria). Acetic anhydride (>99%) and pyridine (99.5% extra dry over molecular sieve) were purchased from Acros Organics (Geel, Belgium). Pentafluoropropionic anhydride (PFPA, 99% derivatization grade), pentafluoropropanol (PFP, >97%), *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), methanol and toluene were supplied by Sigma Aldrich (Darmstadt, Germany). Acetonitrile, formic acid and dichlorodimethylsilane were purchased from Merck (Darmstadt, Germany).

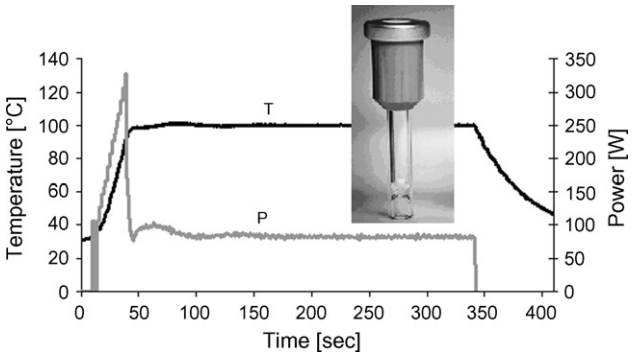

Fig. 1. Temperature (*T*) and power (*P*) profiles for a 200 μL sample of acetic anhydride/pyridine (3:2 v:v) heated under sealed vessel controlled microwave irradiation conditions (set temperature 100 °C). Single mode microwave heating (0–325 W, 10–40 s), temperature control using the feedback from IR thermography (~80 W, 40–340 s), and active gas-jet cooling (340–410 s). The magnetron output power is dynamically adjusted based on feedback from an integrated IR temperature sensor. The inset shows the ultralow volume Pyrex microwave vial (filling volume 200–500 μL) with aluminium crimp and Teflon seal for use in a dedicated single mode microwave reactor.

2.2. Microwave equipment

Microwave-assisted derivatization reactions were performed in an Emrys Initiator 8 EXP 2.0 single mode microwave cavity (Biotage, Uppsala, Sweden), producing continuous (unpulsed) microwave irradiation (0–400 W) at 2.45 GHz. Dedicated Pyrex vessels (200–500 μL filling volume), containing an appropriate ca 4 mm stir bar and sealed with Teflon-coated silicon seals in aluminum crimp tops were used in all derivatization experiments (see Fig. 1). Vials are intended for single-use by the manufacturer. Pressure control up to 20 bars is achieved by means of a non-invasive sensor integrated into the closing lid of the cavity, which senses the deformation of the seal due to internal pressure. Temperature measurement (40–250 °C) is accomplished by means of an infrared sensor perpendicular to the vessel position that reads the surface temperature of the reaction vessel. Efficient cooling of the reaction mixture after microwave heating is achieved by means of a pressurized air supply. All experiments were performed in the temperature control mode (absorption level: "normal") where the magnetron output power is dynamically adjusted between 0 and 400 W to keep the temperature at the preset level using the feedback from the infrared sensor. Reaction times refer to hold times at the temperatures indicated, not to total irradiation times.

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). The GC conditions were as follows: Splitless injection, injection temperature 250 °C, HP-5 MS column (30 m × 0.25 mm ID, 0.25 μm film, Agilent, Waldbronn, Germany); carrier gas helium 5.0, flow 1 mL/min, temperature gradient programmed from 60 to 300 °C at 20 °C/min after an initial time of 6 min. The MS conditions were as follows: positive EI ionization, ionization energy 70 eV, ionization source temperature 280 °C, emission current 100 μA. After verifying the identity of the analytes in full-scan-mode the following SIM parameters were used for quantitative analysis:

2.4. LC–MS instrumentation

A Thermo Quest LCQ DUO ion trap mass (ThermoElectron, Waltham, MA, USA) in combination with a Rheos 2000 HPLC-