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Microwave-assisted on-spot derivatization for gas chromatography–mass spectrometry based determination of polar low molecular weight compounds in dried blood spots☆

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ABSTRACT

Dried blood spot (DBS) sampling and analysis is increasingly being applied in bioanalysis. Although the use of DBS has many advantages, it is also associated with some challenges. E.g. given the limited amount of available material, highly sensitive detection techniques are often required to attain sufficient sensitivity. In gas chromatography coupled to mass spectrometry (GC–MS), derivatization can be helpful to achieve adequate sensitivity. Because this additional sample preparation step is considered as time-consuming, we introduce a new derivatization procedure, i.e. "microwave-assisted on-spot derivatization", to minimize sample preparation of DBS. In this approach the derivatization reagents are directly applied onto the DBS and derivatization takes place in a microwave instead of via conventional heating. In this manuscript we evaluated the applicability of this new concept of derivatization for the determination of two polar low molecular weight molecules, gamma-hydroxybutyric acid (GHB) and gabapentin, in DBS using a standard GC–MS configuration. The method was successfully validated for both compounds, with imprecision and bias values within acceptance criteria (<20% at LLOQ, <15% at 3 other QC levels). Calibration lines were linear over the 10–100 μg/mL and 1–30 μg/mL range for GHB and gabapentin, respectively. Stability studies revealed no significant decrease of gabapentin and GHB in DBS upon storage at room temperature for at least 84 days. Furthermore, DBS-specific parameters, including hematocrit and volume spotted, were evaluated. As demonstrated by the analysis of GHB and gabapentin positive samples, "microwave-assisted on-spot derivatization" proved to be reliable, fast and applicable in routine toxicology. Moreover, other polar low molecular weight compounds of interest in clinical and/or forensic toxicology, including vigabatrin, beta-hydroxybutyric acid, propylene glycol, diethylene glycol, 1,4-butanediol and 1,2-butanediol, can also be detected using this method.

1. Introduction

Dried blood spot (DBS) sampling has been associated with many advantages. It is a minimally invasive sampling technique enabling rapid (home-)sampling and convenient transport and storage of samples [1,2]. Moreover, it offers a reduced risk of infection and in many instances leads to improved compound stability. Furthermore, DBS are a convenient sample preparation strategy: they may simplify sample preparation procedures and they are suitable for automation of sample processing and analysis [3,4]. Consequently, DBS analysis is an increasing field of research, which can be deduced from the rapidly increasing number of published studies on DBS in the last decade [2,3].

Abbreviations: 1,2-BD, 1,2-butanediol; 1,4-BD, 1,4-butanediol; % RE, % residual error; BHB, beta-hydroxybutyric acid; DBS, dried blood spot(s); DEG, diethylene glycol; GBL, gamma-butyrolactone; GC–MS, gas chromatography coupled to mass spectrometry; GHB, gamma-hydroxybutyric acid; Hct, hematocrit; HFB, heptafluorobutanol; IS, internal standard(s); LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; LLOQ, lower limit of quantification; LOD, limit of detection; PG, propylene glycol; QC, quality control; TFAA, trifluoroacetic anhydride; ULOQ, upper limit of quantification.

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DBS have been applied in many disciplines such as preclinical and clinical studies, epidemiological research, phenotyping, therapeutic drug monitoring and toxicology [3,5–8]. In these applications, mostly liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has been used [9–11]. Adequate sensitivity has also been achieved with other analytical methods, such as direct MS/MS, LC coupled to fluorescence detection, LC with ultraviolet detection or gas chromatography coupled to (tandem) mass spectrometry (GC–MS(/MS)) [10].

GC–MS based analysis of DBS has been described for a wide range of analytes, amongst which pesticides, drugs of abuse, antiepileptic and antidepressant drugs [11–16]. Also for metabolic profiling of DBS, GC–MS has been used [14]. GC–MS also still has its place in many forensic toxicology laboratories, for a variety of reasons. First, GC–MS remains an important confirmation method in systematic toxicological analysis, complementing LC–MS/MS results [17–19]. Second, one single configuration can be used for a variety of compounds with widely varying polarity, not requiring a dedicated configuration for determination of a given compound. E.g., while LC–MS/MS procedures for the determination of gamma-hydroxybutyric acid (GHB) have been described, these typically require the use of hydrophilic interaction liquid chromatography, although reversed phase C_{18} columns with acidified mobile phases have been used as well. However, the latter poses limitations with respect to method sensitivity and selectivity [20]. Third, the use of a standard GC–MS configuration also offers the advantage that it is a robust and highly selective technique which is widely available at reasonable prices, requiring less specialized handling than LC–MS. Additionally, in emerging countries, some laboratories cannot afford buying or maintaining expensive LC–MS equipment but do often have a GC–MS system at their disposal.

In many GC–MS based procedures (including those starting from DBS), a derivatization reaction is needed to improve the chromatographic properties of the analytes of interest and to achieve adequate method sensitivity [10,21]. Although the integration of derivatization techniques may offer several advantages –higher molecular weight compounds can more easily be discerned from interfering signals and the chromatographic and/or mass spectrometric properties of the target analyte may be improved- this additional sample preparation step is often experienced as laborious and tedious. In order to overcome this rate limiting step, we further simplified the concept of “on-spot derivatization” that we introduced previously [22]. In this concept, we add the derivatization reagents directly to a DBS, without the use of a separate extraction step. Here, we extend this concept towards “microwave-assisted on-spot derivatization”. Microwave derivatization is increasingly being applied in a toxicological context [23–30]. To the best of our knowledge, we are the first to combine microwave derivatization with “on-spot derivatization”. By doing so, the derivatization step should no longer be experienced as a rate-limiting step of the sample workup protocol. An approach bearing some resemblance to the “microwave-assisted on-spot derivatization” used here is actually already being applied in proteomics, where DBS are subjected to a direct enzymatic digestion in a microwave, allowing quantification of therapeutic proteins [31].

We evaluated the validity of “microwave-assisted on-spot derivatization” in a real setting for the GC–MS based determination of two distinct polar low molecular weight compounds, GHB and gabapentin, in DBS. GHB and its precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) are well-known illicit club and date-rape drugs which are often abused in combination with other drugs of abuse [32–35]. Consequently, quantification of GHB is important in forensic and clinical toxicology. While determination of the anti-epileptic drug gabapentin may be done in the context of therapeutic drug monitoring, this drug is also increasingly encountered in the forensic lab, given the increased illegal use of gabapentin [36]. Additionally, to demonstrate that our methodology is not limited to these two compounds, we also assessed the applicability of our method for the determination of some other polar low molecular weight molecules with relevance in forensic and clinical toxicology, including the ketone body beta-hydroxybutyric acid (BHB), the GHB precursor 1,4-BD and its isomer 1,2-butanediol (1,2-BD), as well as the glycols propylene glycol (PG) and diethylene glycol (DEG) and the anti-epileptic vigabatrin, which is often prescribed together with gabapentin.

2. Materials and methods

2.1. Chemicals and reagents

1,4-BD, 1,2-BD, DEG, PG, vigababatrin, gabapentin, the sodium salt of BHB and GHB, as well as the derivatization reagents (trifluoroacetic anhydride (TFAA), acetic anhydride, pyridine and heptafluorobutanol (HFB)) were purchased from Sigma-Aldrich (Diegem, Belgium). Suprasolve methanol, ethyl acetate, toluene and hexane were provided by Merck (Darmstadt, Germany). The internal standards (IS) GHB-d6 and gabapentin-d10 were obtained from Lipomed (Arlesheim, Switzerland) and Sigma-Aldrich (Diegem, Belgium), respectively.

2.2. Preparation of calibrators and quality control samples

Stock solutions were prepared by dissolving respectively 50 mg of BHB, 10 mg of GHB and 10 mg of gabapentin in one mL of methanol. For DEG, PG, 1,2-BD and 1,4-BD, we prepared 100 mg/mL stock solutions in methanol. Vigabatrin was available as a 1 mg/mL solution. Quality control samples (QCs) were prepared from independent stock solutions. All these stock solutions were stored at −20 °C. At the day of analysis, working solutions were prepared by dilution of the stock solutions with methanol. These working solutions were used to prepare a multi-analyte mix, i.e. a mix containing all of the above-mentioned analytes, being PG, DEG, 1,2-BD, 1,4-BD, GHB, BHB, vigabatrin and gabapentin. Using this mix, 6 calibration standards (5, 20, 50, 100, 150 and 200 μg/mL for PG, 1,2-BD and 1,4-BD; 2.5, 10, 20, 40, 75 and 100 μg/mL for DEG; 5, 15, 20, 50, 100 and 300 μg/mL for BHB; 10, 15, 25, 50, 75 and 100 μg/mL for GHB; 1, 5, 10, 17.5, 22.5 and 30 μg/mL for gabapentin; 5, 10, 12.5, 17.5, 20 and 30 μg/mL for vigabatrin) and 4 QCs (5, 15, 85 and 175 μg/mL for PG, 1,2-BD and 1,4-BD; 2.5, 7.5, 50 and 90 μg/mL for DEG; 5, 10, 125 and 250 μg/mL for BHB; 10, 12.5, 30 and 85 μg/mL for GHB; 1, 2.5, 15 and 25 μg/mL for gabapentin; 5, 7.5, 15 and 25 μg/mL for vigabatrin) were prepared in blood. The percentage organic solvent used to prepare calibrators and QCs did not exceed 5%. Finally, DBS were prepared by spotting 25 μL of venous whole blood, which was spiked with the above-mentioned polar low molecular weight molecules, onto filter paper. For quantification of GHB and gabapentin, we used the IS GHB-d6 and gabapentin-d10, which were mixed to obtain final concentrations of 60 and 12 μg/mL, respectively. For the quantification of BHB, GHB-d6 was used as IS, whereas for PG, DEG, 1,2- and 1,4-BD and vigabatrin we used gabapentin-d10.

2.3. Instrumentation

Analytical standards and QCs were prepared using an AT261 DeltaRange balance of Mettler Toledo (Zaventem, Belgium). Three different filter papers, being Whatman 903, Munktell 2460 and Ahlstrom 237, were evaluated. Microwave-assisted derivatization was performed in a Samsung ME711 K household microwave. Samples were centrifuged at room temperature and at 4 °C in respectively a MSE Mistral 2000 (Anderlecht, Belgium) and a 5804R Eppendorf centrifuge (Hamburg, Germany). A Branson 1510