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Gas chromatography fractionation platform featuring parallel flame-ionization detection and continuous high-resolution analyte collection in 384-well plates

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ABSTRACT

Gas chromatography (GC) is a superior separation technique for many compounds. However, fractionation of a GC eluate for analyte isolation and/or post-column off-line analysis is not straightforward, and existing platforms are limited in the number of fractions that can be collected. Moreover, aerosol formation may cause serious analyte losses. Previously, our group has developed a platform that resolved these limitations of GC fractionation by post-column infusion of a trap solvent prior to continuous small-volume fraction collection in a 96-wells plate (Pieke et al., 2013 [17]). Still, this GC fractionation set-up lacked a chemical detector for the on-line recording of chromatograms, and the introduction of trap solvent resulted in extensive peak broadening for late-eluting compounds. This paper reports advancements to the fractionation platform allowing flame ionization detection (FID) parallel to high-resolution collection of a full GC chromatograms in up to 384 nanofractions of 7 s each. To this end, a post-column split was incorporated which directs part of the eluate towards FID. Furthermore, a solvent heating device was developed for stable delivery of preheated/vaporized trap solvent, which significantly reduced band broadening by post-column infusion. In order to achieve optimal analyte trapping, several solvents were tested at different flow rates. The repeatability of the optimized GC fraction collection process was assessed demonstrating the possibility of up-concentration of isolated analytes by repetitive analyses of the same sample. The feasibility of the improved GC fractionation platform for bioactivity screening of toxic compounds was studied by the analysis of a mixture of test pesticides, which after fractionation were subjected to a post-column acetylcholinesterase (AChE) assay. Fractions showing AChE inhibition could be unambiguously correlated with peaks from the parallel-recorded FID chromatogram.

1. Introduction

Chromatographic isolation of target compounds from mixtures is predominantly carried out by (preparative) liquid chromatography (LC) fractionation. Collection of liquid fractions emerging from an LC column is rather straightforward and liquid-fraction collection systems are widely available. Still, for many compound classes, gas chromatography (GC) yields superior, highly efficient separations. GC fractionation exploits this separation power for isolation of compounds from complex mixtures in order to allow their further characterization by subsequent off-line analysis. GC fractionation has proven useful for a number of applications, such as the analysis of flavors/odors [1–5] and synthetic products [6,7], the structure elucidation of drug impurities [8], isotope analysis [9–11], natural product screening [3,12–14], and for the detection and identification of environmental toxicants via effect-directed analysis (EDA) [15,16]. In the latter approach, fractions from the GC column eluate are collected and subjected to bioassay testing, and compounds in 'active' fractions may be further assessed to reveal their molecular structure. Despite its usefulness for separation of numerous (potential) toxic compounds, so far, GC fractionation has been used only scarcely for bioactivity screening [15–17]. Most probably, this is due to the technical difficulty to fractionate full GC separations in an efficient and easy way.

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Several GC fractionation approaches have been developed over the years. For example, fractions have been collected with sorbent or solvent traps [1,8,15], by rapid cooling [15], by open tubular traps [9,18], or a combination of these. Common limitations of these methods, however, are the restricted number of fractions that can be collected and the technical complexity of the used GC-fractionation platforms. An additional drawback may be the risk of aerosol formation when cold traps are used, resulting in the loss of analytes. In order to resolve these issues, our group recently has introduced a new GC-fractionation concept that enables continuous collection of GC separations in 96-well plates [17]. This platform utilizes post-column infusion of a trap solvent via a y-piece positioned in the GC oven. The infusion solvent provides on-line trapping of eluting analytes in a liquid stream which is directed outside the GC oven via a capillary. Subsequently, the liquid is continuously deposited by moving the capillary outlet step-wise over a 96-well plate collecting discrete GC fractions of a preset size. This new fractionation approach encompasses efficient trapping of eluting analytes and collection of a high number of fractions. However, as the full GC eluate was fractionated, on-line detection by e.g. flame-ionization detection (FID) or electron-capture detection (ECD) was not possible and, consequently, fractions collected could not be correlated to detected compounds (i.e. peaks) in the chromatogram. Moreover, the post-column infusion of liquid caused significant peak broadening for late-eluting peaks spreading them over multiple wells during fractionation.

In this manuscript we describe the development and evaluation of an improved GC fractionation platform featuring FID parallel to collecting fractions in 384-well plates and maintenance of GC resolution during fractionation, also for late-eluting peaks. To this end, a split was implemented at the column outlet in order to direct a small part of the GC eluate towards FID while the larger part was led towards an inverted y-piece where it was mixed with trap solvent for fraction collection. A trap-solvent heater was introduced with the objective to enhance mixing of the analytes in carrier gas with the trap solvent and thereby reduce band spreading. The fraction collection capillary was mounted on an aluminum unit and a PAL robotic system was programmed to pick-up and move this unit over a 384-well plate for fraction collection. Platform optimization was done with an *n*-alkane mixture after which fractions were analyzed for characterization of the platform performance.Several types of trap solvents at different flow rates were investigated to establish their trapping efficiency and effect on peak broadening. The capability of the system to maintain GC resolution during fractionation in a repeatable fashion was assessed, and the possibility to carry out bioassay testing on GC fractions was investigated. For the latter, a mixture of test pesticides was fractionated and subsequently analyzed with an acetyl cholinesterase assay commonly applied in drug discovery and environmental toxicant screening

2. Materials and methods

2.1. Chemicals

Method optimization and platform evaluation was done by analysis of a *n*-alkane mixture (analytical standard, Sigma Alldrich, Zwijndrecht). Pentane, hexane, heptane, octane (all >99% from Sigma-Aldrich, Zwijndrecht) and acetonitrile (MS grade, Biosolve, Valkenswaard) were used as trap solvents. Bioaffinity experiments for acetylcholine esterase (AChE) inhibition were performed with the test compounds carbaryl and aldicarb (>99%, Sigma-Aldrich, Zwijndrecht) and bioassay reagents AChE, acetylthiocholine iodide, 5,5′-dithiobis-(2-nitrobenzoicacid), K_2HPO_4, and KH_2PO_4 (Sigma Alldrich, Zwijndrecht).

2.2. Instrumentation

Fig. 1 presents a schematic overview of the GC fractionation platform which is operated as follows. The sample is injected on the GC column using the PAL robotic system (position 1) and the temperature program is started. The GC eluate is split (A) towards the FID detector and the inverted Y-piece (B) where preheated/vaporized trap solvent is infused. This trap solvent is delivered to the inverted Y-piece by a LC pump (C). Between the LC pump and inverted Y-piece a solvent heater (D) vaporizes the solvent to enhance mixing with the GC eluate. After injection, the PAL robotic system grabs a smart grip unit (F) to which the transport capillary (dotted purple line) is connected and holds the waiting position for a preset time (position 2). In order to start fraction collection, the robotic system moves the unit towards the well plate (position 3) where it subsequently moves the transport capillary outlet (E, now depicted as a continuous purple line) in serpentine fashion over a 384-well plate for fraction collection. Because the transport capillary is situated outside the GC oven at ambient temperature, the vaporized trap solvent and analytes condense and elute in the liquid state.

The GC oven, split/splitless injector and flame ionization detector were from Agilent Technologies (Palo Alto, CA, USA). The injector was equipped with a Sky 4.0 mm I.D. single taper/gooseneck inlet liner (4.0 mm × 6.5 mm × 78.5 mm). A PAL robotic system (CTC analytics, Zwingen, Switzerland) was used for injection and fraction collection. The instrument components were controlled by Agilent Chemstation 7.0 software. The PAL robotic system was controlled by a DaVinciEurope Smart PAL Interface (version 0.09). Separation was performed on an Agilent FactorFour VF-5 ms column (5% phenyl/95% dimethylpolysiloxane; 30 m × 0.25 mm × 0.25 μm). The end of the column was connected to a Y-union (1/32′, 0.15 mm bore) that split the eluate 1:9 towards, respectively, the FID detector via a 50-μm i.d. deactivated fused-silica capillary and an inverted Y-piece (1/16′, 0.25-mm bore) via a 250-μm deactivated fused-silica capillary. A LC20AD LC pump (Shimadzu's Hertogenbosch) was used to deliver trap solvent to a custom developed trap solvent heating device. This device was developed by mounting a stainless steel tube in the heating block of a FID detector. To both ends of this tube metal capillary tubing was connected. One end functioned as solvent inlet through which solvent was delivered to the tube for preheating. The trap solvent was vaporized at 300 °C and the vapor left from the other end and was directed to the inverted y-piece for mixing with the eluting analytes carried by helium gas. The gaseous mixture was directed outside the GC oven using a 320-μm i.d. deactivated fused silica capillary. All flow splitters and deactivated fused silica capillaries were from Restek (Bellefonte, PA, USA). Outside the GC oven at ambient temperature, the trap solvent condenses, thereby desolving the eluting analytes prior to fractionation. Fractionation was achieved with the PAL robotic system that was programmed to pick-up and move a smart grip unit (DaVinciLaboratorySolutions B.V., Rotterdam) to which the capillary outlet was connected over a 384-well plate in serpentine fashion (7 s/fraction).

Analysis of the fractions for platform optimization and evaluation was done on a standard GC-FID system consisting of an Agilent HP6890 GC Oven, HP 6890 series autoinjector, an split/splitless injection port and flame ionization detector (Palo Alto, CA, U.S.A). An Agilent DB-5 column (30 m × 0.25 mm × 0.25 μm) was used for separation.

2.3. GC fractionation program

Injection was performed at 300 °C in splitless mode. The sample purge was set at 2 min with a flow of 20 ml/min. The temperature program started at 40 °C and was maintained for 2 min. Next, the temperature was increased at 20 °C/min to 300 °C and held constant