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Comparison of the performance of different silica hydride particles for the solid-phase extraction of non-volatile analytes from dark chocolate with analysis by gas chromatography–quadrupole mass spectrometry

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ABSTRACT

The extraction capabilities of a Diamond Hydride™ phase, as well as silica hydride phases modified with bidentate octadecyl (BDC_{18}), phenyl or cholesteryl groups, were evaluated for the analysis of fatty acids, amino acids, sugars and sterols in a dark chocolate extract. These batch adsorption performances were investigated using either methanol or aqueous methanol as the solvent. The compositions of the extracted fractions were assessed by gas chromatography interfaced with quadrupole mass spectrometry (GC–qMS). The batch binding propensities of the various compound classes with silica hydride particles modified with immobilised phenyl groups or larger ligands followed trends predicted from linear solvation energy relationships. Both prediction and experiment revealed that better extraction results could be obtained with the phenyl, BDC_{18} and cholesteryl hydride particles for the major chocolate components. Based on these results, separations in micro-pipette tip format with these three types of stationary phase particles have been undertaken.

1. Introduction

Silica-hydride-based stationary phases are well known in liquid chromatography for the separation of peptides, proteins, nucleotides, metabolites, pharmaceuticals and other compound classes (Buszewski & Noga, 2012; Ge, Liu, Holmes, Ostrander, & Li, 2012; Hellmuth, Koletzko, & Peissner, 2011; Pesek, Matyska, Boysen, Yang, & Hearn, 2013; Soukup & Jandera, 2013; Weisenberg, Butterfield, Fischer, & Rhee, 2009; Zhang, Creek, Barrett, Blackburn, & Watson, 2012). Compared to type-B silica, silica hydride phases, with ~95% of the surface silanols replaced by Si–H groups, as confirmed by ^{1}H HR/MAS NMR measurements (Yeman, Nicholson, Matyska, Pesek, & Albert, 2013), are less polar and adsorb a lower amount of water onto their surfaces (Pesek et al., 2013; Soukup, Janas, & Jandera, 2013; Soukup & Jandera, 2013). However, silica hydride materials become more negatively charged in aqueous organic solvents, as shown from zeta potential measurements, and this affects the separation performance of several types of basic analytes (Kulsing et al., 2014; Yang, Matyska, Boysen, Pesek, & Hearn, 2013). The surface of silica hydride materials can be further chemically modified to result in highly stable stationary phases with utility in a large array of aqueous-organic solvent systems (Pesek & Matyska, 2009). Depending on the water content of the mobile phases, these silica hydride materials can display either a reversed-phase or aqueous normal-phase (ANP) characteristics, thus broadening the selectivity range for the separation of polar analytes in the gradient elution mode (Boysen et al., 2011), as well as extending the capabilities of two-dimensional liquid chromatography (Gilar, Olivova, Daly, & Gebler, 2005).

One objective of analytical green chemistry is rapid high throughput analyses at the small (micro- or nano-) scale, with simultaneous reduction in the number of experiments, time of analysis, energy and reagent consumption. The use of micropipette

Abbreviations: ANP, aqueous normal-phase; BSTFA, *N,O*-bis(trimethylsilyl) trifluoroacetamide; LSER, linear solvation energy relationship; MeOH, methanol; TMCS, trimethylchlorosilane.

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tip format for the extraction and separation of analytes allows only small quantities of solvents to be used and can be readily multiplexed. High resolution analysis by gas chromatography hyphenated with mass spectrometry (GC–MS) increases the number of volatile analytes that can be studied in a single analysis (Nolvachai, Kulsing, Boysen, Hearn, & Marriott, 2014), whilst derivatization techniques allow the analysis of non-volatile compounds (Nolvachai & Marriott, 2013).

The aim of this study was to investigate the performance of silica hydride particles packed into micropipette tips for the separation of analytes present in an extract of dark chocolate, focusing on the sterols and fatty acids, which were chosen because of the high content of these health beneficial compounds (Gao, Williams, Woodman, & Marriott, 2010). To this end, comparative investigations of the batch extraction performance were carried out by material screening methods (with four types of silica hydride stationary phases). The results were compared to the predictions based on the linear solvation energy relationship, which were used to provide either a prediction of the extraction result or an explanation of the probable extraction mechanism. The particles with best performance were selected for the separation-in-tip experiments and subsequent GC–qMS analysis.

2. Material and methods

2.1. Chemicals and materials

Epicatechin, catechin and stigmasterol were obtained from Sigma Aldrich (St. Louis, MO). *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% (v/v) trimethylchlorosilane (TMCS) was purchased from Supelco (Bellefonte, PA). Methanol (HPLC grade) was purchased from Biolab Scientific Pty Ltd. (Sydney, Australia). Water was distilled and deionised with a Milli-Q system (Millipore, Bedford, MA). Four different stationary phases, namely Diamond Hydride™ (DH) and silica hydride-based particles modified with cholesteryl, phenyl and bidentate (with two separate points of attachment) octadecyl (BDC_{18}) groups, were gifted from MicroSolv Technology (Eatontown, NJ). All silica hydride phases had a particle size of 4 μm.

2.2. Extraction of standards and dark chocolate

Dark chocolate (1 g, 85% cocoa, Lindt, Switzerland) was dissolved in 50% or 100% (v/v) methanol/water (45 mL) and sonicated for 1 h. Each solution (200 μL) was loaded in triplicate into Eppendorf tubes each containing a different stationary phase (20 mg of particles). The Eppendorf tubes were vortexed and the samples left to reach equilibrium for 3 h. The Eppendorf tubes were centrifuged and an aliquot (100 μL) of the supernatant from each tube was transferred into vials for derivatization. The extractions were each performed in three replicates. The authentic standard solutions (50 ppm in methanol) of epicatechin, catechin and stigmasterol were prepared for further confirmation of these compounds.

2.3. Dark chocolate sample separation in micropipette tips

Micro-pipette tips (200 μL) (Eppendorf, Germany) with glass wool frits (Agilent Technologies, Mulgrave, Australia) were slurry packed in triplicate with the phenyl, BDC_{18} or cholesteryl-modified stationary phase particles (40 mg each) dispersed in 50% (v/v) methanol/water solution. The dark chocolate extract (100 μL in 50% (v/v) methanol/water solution) was loaded into each tip, and after equilibration for 10 min a 50% (v/v) methanol/water solution (100 μL) was applied as the eluent and pushed through manually using a disposable syringe (Nolvachai et al., 2014). The eluent was then changed to 100% methanol (200 μL) in order to elute more strongly retained analytes. The collected fractions (50 μL) from each of these tip experiments could be captured within 5 min. All fractions were transferred into vials for derivatization.

2.4. Sample derivatization

Vials containing either sample or standard aliquots from the extraction or the separation-in-tip studies were dried under N_2. Pyridine (50 μL) and BSTFA containing 1% (v/v) TMCS (50 μL) were added. The derivatization was performed at 100 °C for 30 min. The derivatized solutions were transferred into micro-insert vials (100 μL) prior to GC–qMS analysis.

2.5. GC–qMS analysis and instrumentation

For compound identity confirmation, the standard solutions and samples were analysed by gas chromatography hyphenated with quadrupole mass spectrometry using an Agilent Technologies 7890A GC–5975C qMS system. The GC was installed with an Agilent Technologies HP-5ms column (30 m × 0.25 mm × 0.25 μm film thickness). The oven program commenced at 100 °C, heated to 280 °C at 10 °C/min (held for 10 min), before increasing to 320 °C at 10 °C/min (held for 5 min). Samples (1 μL) were injected at 280 °C with a split ratio of 50:1. The flow rate of carrier gas (He) was 1.5 mL/min. The transfer line and ion source temperatures were maintained at 300 and 230 °C, respectively. Electron ionisation (EI) voltage was −70 eV. Mass spectra were scanned over the mass range of 70–700 Da with scan rate of 1.6 Hz. Data acquisition and analyses were conducted using Agilent Technologies Mass Hunter Qualitative Analysis B.05.00 software. Compounds in dark chocolate not available as standards were tentatively identified by comparison of their mass spectra and retention data with those contained in the NIST11 library.

3. Theoretical considerations

Linear solvation energy relationship (LSER) approaches have been reported to be useful methods to characterise newly developed stationary phases (Abraham, Ibrahim, & Zissimos, 2004; Vynuchalova & Jandera, 2011). Previously, these approaches were applied to explain and predict liquid chromatographic separation mechanisms of flavonoids and related analytes found in beverages (Jandera, Hajek, Skerikova, & Soukup, 2010; Soukup & Jandera, 2012). With a careful choice of descriptors and selection of suitable standard analytes, the impact of different interaction processes between a particular stationary phase and the analytes, such as hydrogen bonding, hydrophobic or steric interaction can be identified, thus allowing a comparison of different types of stationary phases (Wilson et al., 2002). In this investigation, LSER theory was applied in order to permit the prediction of the partition coefficients (K_{cal}) of analytes between each stationary phase (silica hydride particles) and mobile phase. For extraction phenomena, K_{cal} for each mobile phase condition can be predicted from:

$$\log\ K_{cal} = Ee + Ss + Ab + Ba + Vv + (\log\ k)_0 \quad (1)$$

where $(\log k)_0$ is a correlation factor, e, s, a, b and v are stationary phase descriptors, which represent the contributions of the stationary phase to the interactions and are related to dispersity, dipolarity, hydrogen bond acidity, hydrogen bond basicity and dispersion/cavity formation effects, respectively. The corresponding analyte descriptors (representing the contributions of the analyte to the interactions) are E, S, A, B and V. More positive values of $\log K_{cal}$ indicate stronger analyte–particle interactions (higher affinities), thus correlating with better extraction performance. For many analyte