Dispersive solid-phase extraction of polyphenols from juice and smoothie samples using hybrid mesostructured silica followed by ultra-high-performance liquid chromatography-ion-trap tandem mass spectrometry

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ABSTRACT

A wormhole-like mesostructured silica was synthesized and modified with octadecylsilane (C18) groups. The resulting hybrid material (HMS-C18) was characterized and evaluated as sorbent for simultaneous extraction of 20 polyphenols from mixed fruit-vegetables juices and smoothies by dispersive solid-phase extraction (dSPE). The samples were first subjected to solvent extraction followed by dSPE procedure. The extraction step was optimized and combined with a reversed-phase ultra-high-performance liquid chromatography method coupled to ion-trap tandem mass spectrometry (UHPLC-IT-MS/MS), which was also optimized. HMS-C18 showed high potential to extract and purify the target analytes, being more effective than commercial C18 amorphous silica. The proposed method was validated for both samples, obtaining average recoveries from 57-99% with relative standard deviations lower than 9%. Its applicability in the analysis of commercial mixed fruit-vegetables juices and smoothies revealed mainly contents of rutin, 4-hydroxybenzoic acid, chlorogenic acid, epicatechin, caffeic acid and naringin in the samples analyzed.

Keywords: Dietary polyphenols, Juices, Smoothies, Dispersive solid-phase extraction, Hybrid mesostructured silica, Ultra-high-performance liquid chromatography tandem mass spectrometry.

Introduction

Nowadays, consumers are becoming more aware and concern about what they eat; consequently, they are increasingly demanding the food industry for healthier food with good nutritional quality. Thereby, in recent years, new types of beverages made of fruits and vegetables, such as smoothies and high-pressure processing (HPP) juices, have appeared in the market as a rich source of antioxidant compounds, so they have become a popular way to consume fruits and contribute to the daily intake of bioactive compounds. The antioxidant properties of these beverages are mainly due to their polyphenol content. Polyphenols are secondary metabolites of plants, vegetables and fruits that comprise a heterogeneous group of compounds with highly diverse structures, including phenolic acids, tannins, flavonoids, stilbenes, lignans, coumarins and phenylethanol analogs.[1-3] These compounds are considered natural bioactive constituents in food that provide multiple health benefits beyond the basic nutritional value of the product, including anti-inflammatory, anti-carcinogenic, anti-thrombotic, anti-atherogenic, anti-microbial, vasodilatory and analgesic effects, which have been strongly associated with risk reduction of several chronic diseases, such as cancer, cardiovascular diseases, diabetes, neurodegenerative diseases and osteoporosis.[4-8] Evidences about their probable role in the prevention of different diseases, the recognition of their antioxidant capacity, their influence on food quality and their abundance in our diet are the main reasons that highlight the interest of recent years in developing analytical strategies for identification and quantification of these compounds in foodstuffs.[9, 10]

Currently, ultra-high liquid chromatography (UHPLC) coupled to mass spectrometry (MS) is one of the most widely employed techniques for the analysis of polyphenols in food samples, since it enables to reduce time analysis without compromising separation efficiency.[1, 10, 11] Additionally, this technique enables the development of multicomponent methods, which allow in a single run to simultaneously determinate a large number of compounds with different chemical nature belonging to different groups or families. However, the high complexity of food matrices, which contain many

components with different chemical properties, such as, proteins, fats, salts, sugars, acids, bases and food additives, may lead to the introduction of interferences into the chromatographic system, what can cause matrix effects, such as, ion suppression or ion enhancement. Hence, despite technology related to chromatographic analysis and MS detection advances, sample preparation is still a crucial part of the analytical procedure and, therefore, the application of effective sample preparation techniques is essential to minimize the effect of these matrix interferences and achieve good analytical results.

In this sense, in order to enhance extraction efficiency and sensitivity, mesostructured silicas can be applied as new sorbent materials in food sample preparation because of their advantageous textural properties, such as large pore volume, well-defined pore size distribution, high surface area and modifiable surface capability,[12] which make them suitable to be used as excellent sorbents for isolation and pre-concentration of analytes and a good alternative to traditional sorbents, such as polymeric materials or amorphous silica, as it has been demonstrated in previous works.[13-15] In this context, up to date, the only mesostructured silica which has been evaluated and employed as sorbent for extraction of polyphenols from food matrices has been a non-functionalized mesoporous silica with hexagonal pore arrangement (SBA-15 type).[16, 17] Nonetheless, wormhole-like mesostructured silicas (for example HMS type) can be also synthesized, presenting some advantages over SBA-15, such as mesostructures with thicker pore walls (which improve stability), spherical morphology and small particle size. These properties allow to afford better access to the wormhole-like framework confined mesopores in adsorption processes and, consequently, promote better retention of analytes.[18] Nevertheless, to best of our knowledge, these silicas have never been evaluated for extraction of polyphenols from food samples.

Regarding sample preparation, solid-phase extraction (SPE) has been one of the most common techniques used for extraction and pre-concentration of polyphenols from food and beverage samples using new sorbent materials, because of its simplicity and low solvent requirement.[16, 19, 20] However,

one drawback of SPE is that cartridges must be uniformly packed to avoid poor extraction efficiency. Additionally, the particle size of the sorbent may negatively influence mass diffusion and cause drop pressure of the extraction device. One way to overcome these problems, is to perform dispersive solid-phase extraction (dSPE). In this extraction technique the sorbent material is directly added into the sample solution, thus improving interaction area between the sorbent and analytes, which allows to use less amount of sorbent and solvents, and reduce time and labor, providing this way cheaper and "greener" analytical approaches. For instance, Soltani et al. successfully performed dSPE of two phenolic acids (p-hydroxybenzoic acid and p-coumaric acid) from fruit juices using as sorbent a porous Ni/Co-NO_3-based layered double hydroxide nanosheet. The procedure required short extraction time and the results obtained revealed good analytical performance, highlighting the advantages of dSPE.[21]

Hence, the purpose of this work was to synthesize a mesostructured silica with wormlike pores modified with octadecylsilane (C18) groups and evaluate its efficiency for the first time as dSPE sorbent for the simultaneous extraction of twenty dietary polyphenols from mixed fruit-vegetables juice and smoothie samples. The samples were first subjected to a solvent extraction procedure, and then the sample extracts were purified by dSPE. For analysis purposes, the extraction was combined with the development of a novel high-throughput, quick and sensitive multicomponent reversed-phase method using UHPLC coupled to tandem ion-trap mass spectrometry detector (UHPLC-IT-MS/MS), which achieves in less than 9 min separation of the twenty target polyphenols. As far as we know, this is the first time that hybrid mesostructured silica with wormlike pores (HMS type) has been applied as sorbent in sample preparation for the simultaneous extraction of different types of polyphenols from food samples. Moreover, the application of the developed method enables to broad knowledge of the nutritional quality of the samples analysed, providing information of the main polyphenols found in this type of products.

Materials and Methods

Reagents and standard solutions.

Dodecylamine 98% (DDA, M = 185.36 g mol^{-1}), tetraethylorthosilicate 98% (TEOS, M = 208.33 g mol^{-1}, d = 0.934 g mL^{-1}), chloro(dimethyl)octadecylsilane (M = 347,09 g mol^{-1}), toluene, diethyl ether and Folin-Ciocalteu reagent were from Sigma - Aldrich (St. Louis, MO, USA). Methanol (MeOH), Acetonitrile (ACN) and ethyl acetate (EtAc) LC-MS grade were obtained from Scharlab (Barcelona, Spain). Ammonium acetate and formic acid LC-MS grade were purchased from Fluka (Busch, Switzerland). Anhydrous sodium carbonate and ethanol were from Panreac Química (Castellar del Vallès, Bacerlona, Spain). A Millipore Milli-Q-System (Billerica, MA, USA) was used to obtain water (resistivity 18.2 MΩ cm). Commercial C18 amorphous silica sorbent (MFE-Pak C18, surface area 300 m^2 g^{-1}, pore size 80Å, particle size 40-60 µm) was from Análisis Vínicos (Tomelloso, Spain).

All analytical standards were of high purity grade (≥90%). Gallic acid monohydrate, epicatechin, ferulic acid, catechin, epigallocatechin, epigallocatechin gallate, *p*-coumaric acid, chlorogenic acid, rutin, protocatechuic acid, naringin, naringenin, *trans*-resveratrol, piceid, caftaric acid, quercetin, quercetin 3-β-D-glucoside and caffeic acid were from Sigma-Aldrich (St. Louis, MO, USA), whereas 4-hydroxybenzoic acid and myricetin were purchased from Acros Organics (Geel, Belgium).

Individual stock standard solutions (1000 mg L^{-1}) in MeOH were prepared and stored at -20 ºC in darkness. For the analytical method development and its validation, working standard solutions with the analytes were prepared daily at different concentration levels (10 mg L^{-1} – 100 mg L^{-1}) by dilution of the individual stock solutions with MeOH.

Synthesis and characterization of HMS-C18 mesostructured silica.

Mesostructured silica with wormhole framework structure (HMS type) was prepared according to Pérez-Quintanilla et al.[22] with slight modifications. DDA (30 g) was dissolved in 388.8 mL of Milli-Q water and 227.4 mL of ethanol. The solution was stirred until its homogenization, and

subsequently 124.8 g of TEOS were added drop by drop. The solution was stirred for 18 h, yielding a thick white suspension that was filtered and dried at 80 ºC for 1 h. Then, the resultant solid was heated at reflux in ethanol with a Soxhlet for 8 h in order to remove the remaining amine. Finally, the residual surfactant was removed by calcination at 550 ºC for 18 h. Subsequently, the resulting material was modified with C18 groups. For this purpose, 8 g of HMS mesostructured silica were heated at 150 ºC for 20 h under vacuum in order to activate the material. Then, 3.5 mmol (1.21 g) of chloro(dimethyl)octadecylsilane were added and the mixture was heated at 80 ºC for 24 h at 500 rpm. The resultant material, denoted as HMS-C18, was recovered by filtration and washed with two fractions of 50 mL of toluene, ethanol, and diethyl ether. The synthesized HMS-C18 mesostructured silica was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), thermogravimetric analysis, elemental analysis and nitrogen adsorption-desorption isotherms (for equipment details see SM1 of Supporting Information).

Juice and smoothie samples

Different commercial mixed fruit-vegetables juices and smoothies were obtained from a local supermarket in Madrid, Spain. According to their producers, the juice samples were treated by high pressure processing (HPP) technique, while smoothies had undergone a mild pasteurization process. Table 1 shows the ingredient details and sample classification. All samples were stored at 4 ºC until analysis, and all of them were extracted and analyzed in triplicate. Juice 1 and smoothie 1 were selected as samples to perform optimization and validation of the analytical method by recovery assays, since there are not standard methods or certified reference materials available for the analysis of polyphenols in this type of samples. For this purpose, in each set of experiments five samples were subjected simultaneously to the extraction procedure in the following way: three samples were spiked with the working standard solutions containing the target analytes, allowing a 30-min period for equilibration prior to the sample extraction procedure, other one (denoted simulated sample) was extracted in the same way than the others but it was spiked with the analytes at the end of the sample treatment procedure, and the last one was extracted without spiking, so it was considered a blank

sample. In this sense, to calculate recovery values, the areas of the spiked samples were compared with the areas obtained for the simulated sample, while the blank samples were used to quantify the target analytes in the samples analyzed.

Solvent extraction procedure

Different solvents, ratios, pH and extraction cycles were tested to optimize the solvent extraction procedure by measuring the total phenolic content (TPC) of the sample extracts by the Folin-Ciocalteu colorimetric method described by Singleton et al.[23] with slight modifications. Briefly, 75 µL of sample extract, 645 µL of Milli-Q water and 30 µL of Folin Ciocalteu reagent were added in an amber Eppendorf tube. Subsequently, 75 µL of 20% (w/v) of sodium carbonate and 675 µL of Milli-Q water were added and mixed thoroughly with a vortex. The reaction mixture was kept 1 h in darkness at room temperature, and then the absorbance was measured at 725 nm. Phenolic compounds in the extracts were estimated with a calibration curve (10 - 500 mg L^{-1}) of gallic acid. Total phenolics were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample. All measurements were performed in triplicate.

For juice samples the optimized extraction procedure was as follows: 10 mL of juice were centrifuged at 6000 rpm for 10 min to separate the aqueous supernatant, which was transferred to a polypropylene tube. Then, the solid residue (0.5 g) was extracted with 5 mL of MeOH (pH 2), vortexed for 1 min and centrifuged at 6000 rpm for 10 min. The resultant supernatant was collected in the same tube containing the aqueous supernatant, and the solid residue was extracted again with 5 mL of MeOH (pH 2), vortexed for 1 min and centrifuged at 6000 rpm for 10 min. The supernatant was collected with the previous ones, obtaining a final sample extract of 20 mL MeOH:aqueous phase (50:50, v/v) which was then purified by dSPE. In the case of the smoothie samples, 0.5 g were directly weighted into a polypropylene tube and 5 mL of MeOH (pH 2) were added, subsequently the extraction procedure was performed in the same way than the juice samples obtaining a sample extract of 10 mL 100% MeOH. Afterwards, 10 mL of acidified water (pH 4.1) were added to the smoothie

extract to achieve a final sample extract of 20 mL MeOH:aqueous phase (50:50, v/v), similar to the one obtained in the juice samples, which was then purified by dSPE.

dSPE procedure

The optimized dSPE procedure was as follows: first 50 mg of HMS-C18 were conditioned with 1 mL MeOH:water (50:50, v/v) and stirred for 10 min at 300 rpm to ease dispersion of the material. Subsequently, 5 mL of sample extract were added, and the suspension was stirred at 300 rpm for 20 min. Then, HMS-C18 concentrated with the analytes was recovered by filtration using a 0.45 μm nylon filter. Finally, the analytes were eluted passing 2x3 mL of MeOH:water (95:5, v/v pH 2) through the nylon filter. The eluate was evaporated to dryness and re-dissolved with 500 μL of MeOH for subsequent chromatographic analysis. For comparison purposes, the same dSPE procedure conditions were performed using different sorbent materials, such as non-modified HMS silica and commercial C18 amorphous silica.

Chromatographic analysis

An UHPLC system (Dionex UltiMate 3000, Thermo Scientific, MA, USA) coupled to an ion-trap mass spectrometer detector (Bruker) was used for chromatographic separation. An ACE Excel 2 C18-PFP column (100 mm x 2.1 mm, 2 μm particle size, ACE, UK) was used as stationary phase and it was set at 30 ºC. the injection volume was 10 μL and the flow rate 0.25 mL min^{-1}. A gradient mode was performed for the mobile phase, which consisted of MeOH (phase A) and Milli-Q water (phase B), both containing 2 mM ammonium acetate and 0.1% formic acid. The initial composition was 20% A and 80% B. Then, phase A increased linearly up to 100% in first 9 min, then returned in 2 min to initial conditions, and was equilibrated for 1 min prior to next injection, yielding a total analysis time of 12 min. MS acquisition was carried out using electrospray ionization interface (ESI) operating in negative ion mode. The end plate offset was -500V and the capillary voltage -4500V. The nebulizer was set at 20 psi, the dry temperature at 200 ºC and the dry gas at 10 L min^{-1}. Multiple reaction monitoring (MRM) mode was employed for all analytes, and direct infusion of standard solutions of

each analyte (5 μg mL^{-1}) at 4 μL min^{-1} was performed to optimize the ESI source parameters. Table 2 shows mass spectrum parameters, product ions and retention time of the target analytes under the chromatographic conditions used.

Analytical method validation

The developed and optimized dSPE method was properly validated in terms of linearity, detection and quantification limits, intra-day and inter-day precision, accuracy and matrix effects (ME). Matrix-matched calibration curves were obtained for each compound to evaluate the linearity of the proposed method. For this purpose, according to sensitivity of the UHPLC-IT-MS/MS system, the samples were spiked with the target analytes at six concentration levels and were extracted by the developed method. Calibration curves were constructed by plotting the peak area of each analyte against the analyte concentration and were fitted by linear regression analysis. Solvent-based standard calibration curves were also constructed by using working standard solutions subjected to the analytical proposed method in order to evaluate ME. The ME was evaluated by comparing the slopes of the matrix-matched and solvent-based standard calibration curves. The method detection limit (MDL) and the method quantification limit (MQL) of each analyte were calculated considering the concentration that produces a signal-to-noise ratio (S/N) of 3 and 10, respectively, for the chromatographic response obtained for the lowest concentration level of the solvent-based calibration curve performed under the optimized conditions of the developed method and applying the ion-suppression caused in the detection by the ME. The accuracy (expressed as recovery percentage) was obtained by spiking the samples in triplicate at three concentration levels (low, medium and high) and subjecting them to the proposed method. Recovery values were calculated by comparing the areas of the spiked samples with the areas of simulated samples (samples spiked at the same concentration levels but at the end of the extraction process prior to UHPLC-IT-MS/MS analysis). Method precision (expressed as relative standard deviation percentage, RSD %) was evaluated in terms of intra-day (repeatability) and inter-day (reproducibility) precision for each fortification level by the analysis of six replicates

(n = 6) of each sample on the same day, to obtain intra-day precision, and six replicates of each sample analyzed daily through three different days (n = 18) to obtain inter-day precision.

Statistical analysis

All juice and smoothie samples were analyzed in triplicate. The data were subjected to one-way analysis of variance (ANOVA) and Duncan multiple range test, considering significant differences at $p \leq 0.05$. The statistical analysis was performed with the SPSS 19.0 software.

Results and discussion

Characterization of HMS-C18 mesostructured silica

The synthesized mesostructured silica was characterized before and after surface modification. TEM micrographs of HMS showed irregularly aligned mesopores throughout the material with relatively uniform pore sizes, what it is known as wormhole-like pore arrangement. SEM micrographs confirmed the spherical morphology of the material, with mean diameters between 0.5-1.0 μm (Fig. 1). After functionalization, it was confirmed that HMS-C18 kept the same morphology, particle size and structure as the non-modified HMS, therefore the modification process did not affect the structural properties of the material. According to the I.U.P.A.C. classification, nitrogen adsorption-desorption isotherms for both materials were of type IV, with a narrow hysteresis loop representative of mesoporous materials. The capillary condensation/evaporation step was not very pronounced, and therefore the pore size was not too uniform, with more than one maximum in the pore-size distribution (Fig. 1). Regarding the textural properties, HMS and HMS-C18 showed a very high Brunauer, Emmett and Teller specific surface area (S_{BET}) of 962 and 889 $m^2 g^{-1}$, respectively, with typical pore volumes (0.88 and 0.71 $cm^3 g^{-1}$) and average Barret, Joyner and Halenda (BJH) pore diameters (25.8 and 23.4 **Å)** values for surfactant-assembled mesoestructures [22] (Fig. 1). These results revealed that surface modification with C18 organic moieties decreased the pore volume and S_{BET}, while it produced low reduction in the pore diameter of the material. This could be attributed to the presence

of C18 groups which, due to their big size, were mainly grafted on the external surface of the mesostructured silica.[24] The presence of C18 groups attached to the HMS-C18 was confirmed by elemental analysis, and the functionalization degree (L_0, mmol/g) was 0.23 mmol g^{-1}. The TGA curves of HMS-C18 revealed exothermic degradation between 200-550 ºC with a weight loss of 5.5% (Fig. 1), what proves the high thermal stability of the material.

Optimization of MS detection and chromatographic conditions

Fragmentation patterns of analytes were first studied by direct infusion of pure individual standard solutions in the ESI source. Better signal intensities of the analytes were achieved in negative mode than in positive mode; therefore, all compounds were ionized in negative mode (Table 2). The most abundant ion was selected as precursor ion to obtain the characteristic product ion spectra (MS^2) of each analyte (Fig. S1). The two most intense product ions were monitored for each compound. MS^3 detection was also performed in those analytes (gallic acid, protocatechuic acid, *p*-coumaric acid, caffeic acid, quercetin 3-β-D-glucoside and rutin) for which it was not possible to determine two sensitive product ions in MS^2 detection (Table 2).

To achieve chromatographic separation of the target polyphenols, different elution gradients and organic solvents (ACN and MeOH) were evaluated in the mobile phase. For this purpose, the elution gradient started with a high aqueous content (95% of water) and gradually the organic solvent increased up to 100%. When using ACN as organic solvent, most of the analytes eluted between 1.5-1.8 min, however this produced selectivity problems due to peak overlap in those analytes with similar precursor ions (such as gallic acid and *p*-coumaric, catechin and epicatechin, or epigallocatechin gallate and quercetin 3-β-D-glucoside). On the other hand, when using MeOH as organic solvent, good resolution was achieved in all peaks, but first analytes eluted at 4 min and the total run-time of the method was 15 min. Therefore, the gradient elution was optimized to bring forward the elution of the most polar compounds, so the run-time analysis could be reduced. In this context, the initial content of MeOH in the mobile phase was increased up to 20%, and then it linearly increased up to

100% in the first 9 min, to finally return in 2 min to initial conditions. This way, the total run-time was 12 min and first compounds eluted at 2.3 min (Table 2 and Fig. S1).

Optimization of the solvent extraction procedure

The first step of the sample treatment involved a solvent extraction procedure. MeOH, ACN and EtAc were tested as extraction solvents at different sample/solvent ratios (1/2, 1/5 and 1/10) using one extraction cycle without adjusting pH. Best results were achieved with MeOH using a 1/10 sample/solvent ratio (TPC 225.37 and 84.70 mg GAE per 100 g of juice and smoothie sample, respectively), while EtAc showed to be the solvent with the less extraction capability (TPC < 10.00 mg GAE per 100 g in both types of samples). Afterwards, MeOH was tested at different pH values (2, 4 and 8). Extraction of analytes was enhanced with acidic pH in both types of samples, what it is in agreement with the literature.[16] Therefore, pH was adjusted to 2 through all the experimental analysis. Finally, the number of successive extraction cycles was evaluated (1, 2 and 3). The TPC increased until the second extraction cycle and then remained constant, the same trend was observed in both samples. Overall, based on these data, the best extraction conditions were: two extraction cycles using MeOH (pH 2) as extraction solvent and a 1/10 sample/solvent ratio.

Optimization of the dSPE protocol

Preliminary studies were performed using dSPE and SPE in order to compare both techniques (see SM2). In these studies we confirmed that the SPE procedure was longer and less effective than the one performed with dSPE. SPE procedure needed approximately 40 min while dSPE was performed in 20 min. Moreover, dSPE technique is more cost-effective, since it uses fewer amounts of sample, solvents and sorbents. Therefore, we decided to use dSPE procedure since it has greater potential to extract the target analytes.

The dSPE procedure was optimized by recovery assays. Firstly, the juice and smoothie samples were extracted two successively times with MeOH (pH 2), according to the solvent extraction

procedure previously optimized, obtaining a total sample extract of 20 mL (MeOH:aqueous phase 50:50, v/v) in the case of the juice sample and 10 mL (100% methanolic extract) in the case of the smoothie sample. Subsequently, these sample extracts were subjected to dSPE. Nevertheless, in the case of the smoothie samples, the recovery values achieved after dSPE were very low (2-30%), probably due to a co-solvent effect of MeOH towards the analytes during the filtration step. In this sense, poplyphenols could be directly eluted without being retained in the filter as a result of the high content of MeOH in the sample extract (approximately 100% MeOH), which is almost the same solvent than the one used for elution (MeOH:water 95:5, v/v). This co-solvent effect has been reported in previous works in the case of wine samples,[27, 28] where it was necessary to dealcoholize first in order to obtain aqueous extracts to overcome the influence of ethanol on the extraction efficiency. Taking into account this effect, and since recovery values obtained in the juice samples were better than in the smoothie sample, 10 mL of acidified water (pH 4.1) were added to the smoothie extract, obtaining a final sample extract of 20 mL MeOH:aqueous phase (50:50, v/v), similar to the one obtained in the juice samples, which was then purified by dSPE, as it has been described in the materials and methods section, confirming that the addition of water to the sample extract improved recovery values of the target analytes.

For the dSPE procedure different extraction times were studied in the range 10-30 min using different HMS-C18 amounts (50 and 100 mg). With 50 mg of sorbent the extraction of analytes increased almost twice from 10 to 20 min, but then extraction efficiency sharply decreased in a 65% from 20 to 30 min. On the other hand, with 100 mg of sorbent the total peak areas of analytes increased in the first 10 min, but then a significant reduction of almost a 50% in the extraction capability was observed upon increasing time extraction, reaching an extraction plateau at 20 min (Fig. S2). The reduction in the extraction performance when increasing the extraction time could be due to the back-extraction effect that arose as a result of the prolonged period of extraction, as it has been reported elsewhere.[25] Regarding the amount of sorbent, the peak areas of the extracted analytes were higher using 50 than 100 mg of HMS-C18 at 20 min, probably due to a possible aggregation of the

material.[25, 26] According to the above results, 50 mg was chosen as the optimal HMS-C18 amount and 20 min as the extraction time for the dSPE procedure.

For elution, according to the literature,[27-29] the combination MeOH:water (95:5, v/v, pH 2) was selected as elution solvent at different volumes: 4 mL (2x2 mL) and 6 mL (2x3 mL). The combination of EtAc with MeOH:water (95:5, v/v, pH 2) was also tested in two different ways: passing first 2 mL of EtAc followed by 2x3 mL of MeOH:water (95:5, v/v, pH 2), and the other one by passing 2 mL of EtAc after 2x3 mL of MeOH:water (95:5, v/v, pH 2). Using first a less polar solvent negatively affected the recovery of the target analytes, which were in the range 12-66%. On the other hand, 4 mL of MeOH:water (95:5, v/v, pH 2) were not enough to obtain high extraction efficiency in all analytes, since recovery values were low for gallic acid, caftaric acid, myricetin, piceid, protocatechuic acid, 4-hydroxybenzoic acid, resveratrol and epigallocatechin, ranging between 29-60%. Conversely, increasing the elution volume to 6 mL improved the recovery values, ranging all of them between 62-103% in both samples, except for gallic acid (48% and 61%) and 4-hydroxybenzoic acid (49% and 63%) in the juice and smoothie sample, respectively, and resveratrol (56%) in the smoothie sample. Nevertheless, in general, no significant improvements were observed by the addition of 2 mL of EtAc after passing 6 mL of MeOH:water (95:5, v/v, pH 2), achieving recovery values between 49-105%. Therefore, based on these observations, 6 mL of MeOH:water (95:5, v/v pH 2) were selected as the optimal elution conditions for the dSPE procedure.

Comparison of HMS-C18 with other sorbent materials

The good performance achieved using HMS-C18 as dSPE sorbent was compared with non-functionalized HMS silica and a commercial C18 amorphous silica with similar L_0 (0.17 mmol g^{-1}), which were also tested as sorbents under the same optimized extraction conditions. Results for both types of samples are shown in Fig. 2

. Regarding mesostructured silicas, functionalization with C18 groups improved the retention of analytes in both types of samples. However, this effect was more pronounced in the smoothie sample

(recovery values 6-79% with HMS non-modified) than in the juice sample (25–83% with HMS non-modified), probably because of the differences that exist among both sample extracts. The smoothie sample has a more complex matrix than the juice sample, since it has a higher content of fiber, which may interfere in the extraction step. Additionally, the juice sample has been processed by HPP technique, which causes the rupture of plant cell structures, releasing compounds and making them more accessible and available for extraction.[30, 31] Therefore, extraction of polyphenols from HPP-juice is easier than in the case of the smoothie sample, where the polyphenols extracted are subjected to more interferences. Although functionalization of the material with hydrophobic groups improved the retention of analytes, it is noteworthy to highlight that these results also demonstrated the good adsorption capability of non-functionalized HMS towards some of these analytes thanks to its specific textural characteristics. Nevertheless, in the case of the smoothie sample, the matrix interferences hinder the efficiency of the material and, consequently, its functionalization was required to achieve satisfactory recovery values. Cao et al.[16, 17] also proved the good adsorption capability of hexagonal mesostructured silicas towards polyphenols using non-modified SBA-15 as sorbent. However, they only used it for the extraction of 6 flavanones[16] and 3 flavonoids,[17] not for a multicomponent analysis, like the one proposed in this work, where 20 polyphenols belonging to different types of families are extracted simultaneously. In this sense, to perform a multicomponent analysis, it has been demonstrated that functionalization of the sorbent material is necessary, so its extraction efficiency could cover and be effective in a wide range of compounds with different physicochemical properties. Results also revealed that HMS-C18 mesostructured silica was clearly more effective than commercial C18 amorphous silica in the extraction of the target analytes (Fig. 2). This improvement in the extraction efficiency of HMS-C18 over the commercial C18 silica was also mainly observed in the smoothie sample than in the juice sample, due to the existing differences among both types of sample extracts. Thus, HMS-C18 was more effective to extract analytes from complex sample extracts than commercial C18 amorphous silica, overcoming problems caused by matrix interferences. Additionally, the better extraction performance of HMS-C18 over commercial C18

amorphous silica could be ascribed to the fact that HMS-C18 mesostructured silica presents higher surface area (889 m^2 g^{-1}) than the commercial C18 material (300 m^2 g^{-1}). Thus, in the HMS-C18 silica, the C18 functional groups are probably more accessible, since they can be more homogenously distributed without pore blocking effect on the silica surface, as it has been checked in the material characterization, compared to commercial C18 amorphous silica, as it has been described previously. [13, 15] Moreover, according to previous studies,[13, 15, 16] HMS-C18 can interact with analytes in two different ways as a hydrophilic-lipophilic balance: by reversed-phase sorption with the C18 group moieties (hydrophobic interactions) and by polar secondary interactions with the residual non-modified silanol groups on the silica surface (hydrogen bonding interactions) (Fig. 3), what enhances retention of the most polar analytes in comparison with commercial C18 amorphous silica, since the commercial material does not presents residual non-modified silanol groups on its surface. Hence, based on these results, HMS-C18 mesostructured silica could be an alternative sorbent to commercial C18 amorphous silica since it has proved to be more effective in the extraction of the target polyphenols.

Method validation

Validation parameters of the dSPE method are shown in Table 3 (juice samples) and Table 4 (smoothie samples). Matrix-matched calibration curves provided good linear regression for all compounds in both samples, with coefficient of determination (R^2) values > 0.999 (Tables 3 and 4). The slope values of the matrix-matched calibration curves were lower than the slopes of the solvent-based calibration curves (Table S1), what indicates ion suppression in the detection of the analytes because of the adverse influence of the matrix. Therefore, to quantify the target analytes in the samples, matrix-matched calibration curves should be used to compensate the errors associated with matrix suppression. MDLs ranged from 0.01-0.5 μg mL^{-1} (except 4-hydroxybenzoic acid, which MDL was 1.7 μg mL^{-1} for both samples), while MQLs ranged between 0.02-1.7 μg mL^{-1} (except 4-hydroxybenzoic acid, which MQL was 5.6 and 5.5 μg mL^{-1} for the juice and the smoothie sample,

respectively). The accuracy (expressed as recovery %) was almost in the same range at the three different validation levels (high, medium and low) achieving good mean recovery values (> 72%) for all analytes in both samples, except for 4-hydroxybenzoic acid and gallic acid, which showed mean recovery values between 57-67%, and also piceid (67%) and *trans*-resveratrol (63%) in the smoothie sample (Tables 3 and 4). Method precision was assessed in terms of intra-day repeatability and inter-day reproducibility at each fortification level for both matrices, achieving satisfactory results with RSD values lower than 7% for intra-day precision and lower than 9% for inter-day precision in both samples. Therefore, the proposed method can be successfully used for extraction and quantification of polyphenols from juice and smoothie samples.

Method application to real samples

The dSPE method coupled to UHPLC-IT-MS/MS was applied for determination of the target polyphenols in 3 commercial juices and 3 commercial smoothies. Each sample was analyzed in triplicate. Analytes were identified by their retention time and mass spectrum, and for quantification purposes, their peak areas were subjected to correction with the recovery values established for them and then interpolated into their corresponding matrix-matched calibration curve. Table 5 lists the results obtained for all the samples analyzed considering the pre-concentration factor of the sample-treatment (mg per 100 mL of juice and mg per 100 g of smoothie).

All the samples analyzed had apple as core ingredient of their composition (Table 1), which is something usual in this type of beverages according to Keenan et al.[32] Esters of hydroxycinnamic acids, such as chlorogenic acid, and monomers of flavan-3-ols, such as epicatechin, are the most predominant polyphenols found in apples, while the monomer catechin is usually found at lower concentration.[33] Probably this is the reason why both juice and smoothie samples analyzed showed in all cases significant contents of chlorogenic acid and epicatechin (Table 5). However, in the smoothie 2, the content of chlorogenic acid was a little bit lower than in the other samples, what according to Keenan et al.[32] could be due to the presence of banana in its composition, since this fruit

contains significant quantities of the enzyme polyphenol oxidase, which may degrade some hydroxycinnamic acids such as chlorogenic acid.[32, 34] As well, in all samples, the content of catechin was lower than the content of epicatechin, what it is in accordance with other authors.[33, 35] Nevertheless, in the smoothie samples, rutin showed a higher content than chlorogenic acid and epicatechin (Table 5). Rutin is a polyphenol mainly found in citrus fruits, such as lemon and orange.[1,2,36] Hence, its abundance in the smoothies could be due to the presence of citric fiber as ingredient in the formulation of these samples (Table 1). Regarding the juice samples, only the juice 2 showed a higher content of rutin, probably as a result of the significant presence of orange juice (15%) in its composition, compared to the lower presence of lemon juice (0.5 and 1%) in juice 1 and 3, respectively (Table 1).

Regarding in general the juice samples analysed, chlorogenic acid, epicatechin, rutin, caffeic acid, naringin, catechin and 4-hydroxybenzoic acid were the main polyphenols found, ranging between 0.08 – 5.8 mg per 100 mL of juice (Table 5), since these compounds are extensively predominant in fruits and vegetables, especially in apples,[1, 2] which is the common ingredient of all the samples analyzed (Table 1), as it has been indicated before. Nevertheless, in juice 2, apart from rutin, it is worth highlighting the higher content of other compounds compared to the other juices analyzed, such as gallic acid, quercetin 3-β-glucoside, quercetin and myricetin (Table 5).

In the smoothie samples, apart from rutin, the main polyphenols found were chlorogenic acid, naringin, epicatechin, ferulic acid, *p*-coumaric acid, quercetin 3-β-glucoside, quercetin, caffeic acid and gallic acid, ranging between 0.12-2.3 mg per 100 g of smoothie (Table 5). In the smoothie 1, rutin was the most predominant polyphenol, probably because of the citric fiber and the high presence of orange juice (39.5%) as ingredient of this sample (Table 1). Rutin was also the main polyphenol quantified in the smoothie 2, followed by epicatechin, catechin, *p*-coumaric and quercetin, probably due to its content in banana, apple and berries (Table 1), according to previous reports.[1, 2, 37, 38] On the other hand, epigallocatechin gallate, 4-hydroxybenzoic acid, rutin, epigallocatechin, chlorogenic acid

and epicatechin were the main polyphenols found in the smoothie 3 (Table 5), probably as a result of its content in apple, grape juice and green tea matcha (Table 1), since these compounds are predominant in this type of products.[1, 2, 37]

Regarding the literature, in general, the works which analyzed this type of samples do not perform an individual analysis of polyphenols in the product, instead they provide a total content of polyphenols through spectrophotometric analysis.[39, 40] There are only a few works which perform individual characterization of polyphenols in juice or smoothie samples treated by HPP technique.[32, 35] However, in these studies they do not analyze a high number of polyphenols such as in the present work, and they use diode array detection (DAD) instead of mass spectrometry, which is less sensitive and selective to perform a correct and precise identification and quantification of analytes. Also, in many cases the studies focus on the influence of the processing treatment (HPP o heat treatment) on the sensory attributes of the products, and not in the individual polyphenol fractions.[39, 40] It is true that the processing treatment, as well as the extraction procedure, may vary the initial composition of the product according to the raw materials employed, therefore it is difficult to establish a comparison among the data obtained with other works. Thus, it is not possible to extrapolate from works on individual fruits and vegetables to mixtures, as well as it is not viable to compare the results with the ones obtained for similar products but with different formulation or processing treatment.

Nevertheless, from the results obtained in this work it can be concluded that the consumption of these foodstuffs contributes to the intake of polyphenols in the diet, highlighting in particular the contribution of rutin, 4-hydroxybenzoic acid, epicatechin, chlorogenic acid, caffeic acid and naringin, what helps to promote antioxidant and beneficial healthy effects in our body Therefore, the proposed method can be used for extraction of polyphenols in food samples.

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Table 1. Declared ingredients for the commercial juice and smoothie samples analyzed according to their labels.

Type of processing treatment	Sample classification	Ingredients
High pressure processing (HPP) technique	Juice 1	Apple juice (59%), carrot juice (33.47%), passion fruit juice (5%), ginger juice (2%), lemon juice (0.5%), ginseng extract (0.03%) and ascorbic acid.
	Juice 2	Apple juice (57%), orange juice (15%), aloe vera puree (7%), blueberries puree (6%), raspberry puree (4%), pomegranate juice (5%), strawberry puree (4%) and ascorbic acid.
	Juice 3	Apple juice (77.5%), spinach juice (10%), celery juice (10%), ginger juice (1.5%), lemon juice (1%) and ascorbic acid.
Mild pasteurization process	Smoothie 1	Orange juice (39.5%), apple juice, carrot (15%), pineapple (11%), pumpkin (7%), agave syrup, citric fiber and guarana (0.2%).
	Smoothie 2	Strawberry (41%), banana, concentrated apple juice, beet juice (10%), raspberry (6%), currant (4%), agave syrup (2%), pea protein, citric fiber, carrot concentrate and hibiscus.
	Smoothie 3	Apple, apple juice, concentrated grape juice, concentrated melon juice (8%), pear (6.5%), iceberg lettuce, celery (3%), spinach (2.5%), kale (2.5%), citric fiber and green tea Matcha powder (0.5%).

Table 2. Mass spectrum parameters and retention time for the target polyphenols using the developed UHPLC-IT-MS/MS method.

Analyte	Ionization mode	Retention time (min)	Precursor ion (*m/z*)	Fragmentation amplitude	MS^2. Daughter ions [a] (*m/z*)	Fragmentation amplitude	MS^3. Granddaughter ions (*m/z*)
Gallic acid	ESI (-)	2.4	169	0.70	124*	0.6	106, 96
Caftaric acid	ESI (-)	3.5	311	0.60	178, 148*		
Protocatechuic acid	ESI (-)	3.7	153	0.50	108*	0.4	90, 80
Catechin	ESI (-)	4.4	289	0.60	244*, 204		
Epigallocatechin	ESI (-)	4.4	305	0.55	220, 178*		
Chlorogenic acid	ESI (-)	4.6	353	0.70	190*, 178		
4-Hydroxybenzoic acid	ESI (-)	4.7	137	0.50	106, 93*		
Epigallocatechin gallate	ESI (-)	5.2	547	0.40	330, 168*		
Caffeic acid	ESI (-)	5.3	179	0.50	134*	0.7	116, 106
Epicatechin	ESI (-)	5.4	289	0.50	244*, 204		
Piceid	ESI (-)	6.1	389	0.30	226*, 158		
p-Coumaric acid	ESI (-)	6.2	163	0.70	118*	0.6	100, 92
Ferulic acid	ESI (-)	6.4	193	0.60	177, 133*		
Naringin	ESI (-)	6.8	579	0.50	459*, 270		
Quercetin 3- β -D-glucoside	ESI (-)	7.0	463	0.40	300*	0.4	178, 150
Rutin	ESI (-)	7.0	609	0.50	300*	0.5	178, 150
Trans-resveratrol	ESI (-)	7.4	227	0.50	184*,142		
Myricetin	ESI (-)	7.8	317	0.50	178*, 150		
Quercetin	ESI (-)	8.6	301	0.30	178*, 150		
Naringenin	ESI (-)	8.7	271	0.40	176, 150*		

[a] Predominant product ions.
* Ions used for quantitation.
Isolation width (*m/z)* is 4.
Chromatographic conditions with the optimized gradient elution: initial composition 20% phase A – 80% phase B, from 0 to 9 min phase A increased linearly up to 100%, from 9 to 11 min the mobile phase returns to initial conditions 20% phase A – 80% phase B, using MeOH as mobile phase A and water as mobile phase B, both containing 0.1% formic acid and 2 mM ammonium acetate. The flow rate was 0.25 mL min^{-1}.

Table 3. Validation parameters of the dSPE method for the determination of the target polyphenols in juice samples.

Analytes	Linear range (μg mL^{-1})	Linearity, R^2 Matrix-matched calibration	Spiked levels (μg mL^{-1})	Accuracy		Precision		MDL (μg mL^{-1})	MQL (μg mL^{-1})
				Recovery (%)	Average Recovery (%)	Intra-day precision (RSD %)	Inter-day precision (RSD %)		
Gallic acid	0.1 – 50	y = 268444 x – 27044	0.1 [a]	57	57	2.5	5.0	0.04	0.1
		0.999	25 [b]	48		2.5	5.5		
			50 [c]	67		4.5	8.6		
Caftaric acid	1.5 – 50	y = 59669 x - 19702	1.5 [a]	76	72	6.3	7.6	0.5	1.5
		0.999	25 [b]	63		4.8	8.8		
			50 [c]	78		2.7	4.6		
Protocatechuic acid	1.7 – 50	y = 87085 x - 44991	1.7 [a]	70	79	1.8	6.4	0.5	1.7
		0.999	25 [b]	87		1.9	2.3		
			50 [c]	81		3.2	5.6		
Catechin	1.0 – 50	y = 211796 x - 319927	1.0 [a]	78	87	3.7	4.4	0.3	1.0
		0.999	25 [b]	87		5.0	6.4		
			50 [c]	97		2.0	4.1		
Epigallocatechin	0.6 – 50	y = 57129 x - 26380	0.6 [a]	82	86	3.2	6.9	0.2	0.6
		0.999	25 [b]	81		3.2	4.3		
			50 [c]	94		6.2	7.8		
Chlorogenic acid	0.8 – 100	y = 256982 x - 1942043	0.8 [a]	79	93	5.4	8.5	0.3	0.8
		0.999	50 [b]	103		1.4	7.8		
			100 [c]	98		4.6	6.1		
4-Hydroxybenzoic acid	5.6 – 50	y = 15213 x + 201	5.6 [a]	58	58	4.9	7.3	1.7	5.6
		0.999	25 [b]	49		4.2	4.6		
			50 [c]	68		3.6	7.0		
Epigallocatechin gallate	0.5 – 50	y = 153569 x - 175034	0.5 [a]	74	76	1.9	3.4	0.2	0.5
		0.999	25 [b]	73		5.5	7.9		
			50 [c]	81		2.4	6.5		
Caffeic acid	0.3 – 50	y = 417054 x - 463975	0.3 [a]	90	92	3.5	6.8	0.09	0.3
		0.999	25 [b]	102		2.1	4.7		
			50 [c]	84		2.9	4.3		
Epicatechin	0.8 – 50	y = 156908 x – 402641	0.8 [a]	96	98	2.4	3.8	0.3	0.8
		0.999	25 [b]	98		1.5	8.3		
			50 [c]	99		2.0	3.2		
Piceid	0.07– 50	y = 108827 x - 17701	0.07 [a]	83	77	1.4	6.4	0.02	0.07
		0.999	25 [b]	74		1.9	4.5		
			50 [c]	74		1.7	4.8		
p-Coumaric acid	0.3 – 50	y = 61308 x - 15947	0.3 [a]	77	75	3.3	6.1	0.08	0.3
		0.999	25 [b]	74		2.7	3.1		
			50 [c]	74		2.4	3.3		
Ferulic acid	0.7 – 50	y = 27479 x - 35902	0.7 [a]	79	81	2.5	6.0	0.2	0.7
		0.999	25 [b]	77		4.1	6.6		
			50 [c]	86		1.3	6.1		
Naringin	0.08 – 50	y = 144574 x + 17454	0.08 [a]	71	72	3.4	6.3	0.02	0.08
		0.999	25 [b]	76		1.5	3.7		
			50 [c]	68		1.8	6.1		

Quercetin 3- β -D-glucoside	0.05 – 50	y = 241393 x - 119807 0.999	0.05 [a] 25 [b] 50 [c]	85 78 88	84	1.2 1.1 6.7	7.4 1.3 8.0	0.02	0.05
Rutin	0.08 – 100	y = 226705 x - 316786 0.999	0.08 [a] 50 [b] 100 [c]	83 98 102	94	2.3 1.4 1.8	6.8 7.6 5.3	0.02	0.08
Trans-resveratrol	0.09 – 50	y = 181806 x - 169864 0.999	0.09 [a] 25 [b] 50 [c]	80 72 81	78	1.3 4.8 3.6	8.5 5.1 6.2	0.03	0.09
Myricetin	0.07 – 50	y = 403427 x – 177300 0.999	0.07 [a] 25 [b] 50 [c]	72 78 67	72	2.9 4.8 2.6	3.6 6.6 4.5	0.02	0.07
Quercetin	0.1 – 50	y = 461721 x + 11529 0.999	0.1 [a] 25 [b] 50 [c]	85 103 90	93	2.5 1.0 1.1	7.3 3.9 1.5	0.04	0.1
Naringenin	0.02 – 50	y = 750558 x - 384005 0.999	0.02 [a] 25 [b] 50 [c]	75 65 76	72	2.7 2.6 1.5	5.0 3.2 1.8	0.01	0.02

[a] Low fortification level.
[b] Medium fortification level (spiked level used for method optimization)
[c] High fortification level.

Table 4. Validation parameters of the dSPE method for the determination of the target polyphenols in smoothie samples.

Analytes	Linear range (µg mL^{-1})	Linearity, R^2 Matrix-matched calibration	Spiked levels (µg mL^{-1})	Accuracy		Precision		MDL (µg mL^{-1})	MQL (µg mL^{-1})
				Recovery (%)	Average Recovery (%)	Intra-day precision (RSD %)	Inter-day precision (RSD %)		
Gallic acid	0.1 – 10	y = 1099846 x - 177414 0.999	0.1 [a] 5 [b] 10 [c]	68 61 71	67	1.1 1.7 1.0	7.5 6.5 6.6	0.03	0.1
Caftaric acid	1.4 - 10	y = 179467 x - 54593 0.999	1.4 [a] 5 [b] 10 [c]	78 73 70	74	1.1 1.2 1.8	3.5 7.2 6.2	0.4	1.4
Protocatechuic acid	1.6 – 10	y = 383223 x - 159610 0.999	1.4 [a] 5 [b] 10 [c]	80 69 76	75	2.9 1.4 3.0	3.9 6.9 6.1	0.5	1.6
Catechin	1.0 – 10	y = 470821 x - 134007 0.999	1.0 [a] 5 [b] 10 [c]	89 68 81	79	1.6 3.1 3.9	3.3 5.2 5.7	0.3	1.0
Epigallocatechin	0.6 – 10	y = 131792 x - 33633 0.999	0.6 [a] 5 [b] 10 [c]	79 62 84	75	2.0 3.8 6.9	2.3 4.5 8.6	0.2	0.6
Chlorogenic acid	0.8 – 10	y = 1172201 x - 518642 0.999	0.8 [a] 5 [b] 10 [c]	98 96 99	98	4.6 2.9 1.2	8.6 6.4 2.8	0.2	0.8
4-Hydroxybenzoic acid	5.5 – 50	y = 211397 x - 336938 0.999	5.5 [a] 25 [b] 50 [c]	69 63 63	65	1.1 3.5 3.7	4.0 6.4 6.8	1.7	5.5
Epigallocatechin gallate	0.5 – 10	y = 294837 x - 25857 0.999	0.5 [a] 5 [b] 10 [c]	73 75 75	74	3.6 1.9 4.7	5.8 7.6 7.6	0.1	0.5
Caffeic acid	0.3 – 10	y = 1542085 x - 388124 0.999	4.0 [a] 5 [b] 10 [c]	85 77 92	85	1.4 2.2 1.1	7.0 4.1 5.1	0.1	0.3
Epicatechin	0.8 – 10	y = 326890 x - 127041 0.999	0.8 [a] 5 [b] 10 [c]	93 90 90	91	3.8 3.2 3.9	6.1 4.1 5.5	0.2	0.8
Piceid	0.1 – 10	y = 465636 x - 28106 0.999	0.1 [a] 5 [b] 10 [c]	74 65 63	67	1.5 1.5 2.2	6.5 2.2 7.9	0.02	0.1
p-Coumaric acid	0.3 – 10	y = 254087 x - 75688 0.999	0.3 [a] 5 [b] 10 [c]	77 73 78	76	4.5 1.3 1.5	5.9 6.4 2.7	0.1	0.3
Ferulic acid	0.6 – 10	y = 103969 x - 44892 0.999	0.6 [a] 5 [b] 10 [c]	83 85 82	83	5.1 3.6 1.5	6.1 4.5 5.4	0.2	0.6
Naringin	0.1 – 10	y = 686227 x - 41158 0.999	0.1 [a] 5 [b] 10 [c]	88 79 75	81	2.9 3.6 2.3	8.2 8.5 7.0	0.02	0.1

Quercetin 3- β -D-glucoside	0.1 – 10	y = 1022660 x - 175795 0.999	0.1 [a] 5 [b] 10 [c]	98 101 99	99	3.7 2.0 1.5	2.9 2.5 7.0	0.02	0.1
Rutin	0.1 – 10	y = 1412948 x – 6577494 0.999	0.1 [a] 5 [b] 10 [c]	99 97 94	97	2.3 4.6 1.6	4.7 6.4 6.2	0.02	0.1
Trans-resveratrol	0.1 – 10	y = 517275 x - 23251 0.999	0.1 [a] 5 [b] 10 [c]	64 56 69	63	5.0 4.8 1.7	8.4 6.1 6.8	0.02	0.1
Myricetin	0.1 – 10	y = 1821482 x - 174892 0.999	0.1 [a] 5 [b] 10 [c]	70 93 81	81	3.5 3.7 4.4	7.8 6.6 6.2	0.02	0.1
Quercetin	0.1 – 10	y = 1733196 x - 153685 0.999	0.1 [a] 5 [b] 10 [c]	80 102 94	92	1.4 1.2 2.6	4.3 2.7 5.4	0.04	0.1
Naringenin	0.02 – 10	y = 3510155 x - 351141 0.999	0.02 [a] 5 [b] 10 [c]	77 72 76	75	5.7 1.2 1.0	6.7 2.5 5.0	0.01	0.02

[a] Low fortification level.
[b] Medium fortification level (spiked level used for method optimization)
[c] High fortification level.

Table 5. Content of the target polyphenols in the commercial juice and smoothie samples analysed by the dSPE proposed method. Values expressed as mean ± sd (n=3).

Polyphenols	**Juice samples (mg per 100 mL of juice)**			**Smoothie samples (mg per 100 g of smoothie)**		
	Juice 1	**Juice 2**	**Juice 3**	**Smoothie 1**	**Smoothie 2**	**Smoothie 3**
Gallic acid	$0.055 \pm 0.008^{a,b}_{a}$	$0.6 \pm 0.1^{c,d}_{d}$	$0.036 \pm 0.006^{a,b}_{a}$	$0.12 \pm 0.01^{a}_{a}$	$0.32 \pm 0.05^{a,b}_{c}$	$0.21 \pm 0.01^{a}_{b}$
Caftaric acid	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Protocatechuic acid	$0.05 \pm 0.01^{a,b}_{a,b}$	$0.07 \pm 0.02^{a,b}_{b}$	$0.033 \pm 0.006^{a,b}_{a}$	<MQL	<MQL	<MQL
Catechin	$0.11 \pm 0.01^{a,b}_{a}$	$0.18 \pm 0.05^{a,b}_{a}$	$0.08 \pm 0.01^{a,b,c}_{a}$	<MQL	$1.6 \pm 0.3^{d}_{b}$	<MQL
Epigallocatechin	$0.027 \pm 0.006^{a,b}_{a}$	$0.028 \pm 0.007^{a,b}_{a}$	$0.020 \pm 0.005^{a}_{a}$	n.d.	<MQL	$2.1 \pm 0.3^{c}_{b}$
Chlorogenic acid	$1.5 \pm 0.3^{d}_{c}$	$2.1 \pm 0.5^{e}_{d}$	$0.8 \pm 0.2^{e}_{a,b}$	$0.6 \pm 0.1^{b}_{a}$	$0.40 \pm 0.03^{a,b}_{a}$	$1.2 \pm 0.1^{b}_{b,c}$
4-Hydroxybenzoic acid	$0.17 \pm 0.07^{b}_{a}$	$0.12 \pm 0.03^{a,b}_{a}$	$0.13 \pm 0.02^{b,c}_{a}$	<MQL	<MQL	$2.3 \pm 0.1^{c}_{b}$
Epigallocatechin gallate	$0.045 \pm 0.007^{a,b}_{a}$	$0.036 \pm 0.002^{a,b}_{a}$	$0.038 \pm 0.003^{a,b}_{a}$	<MQL	<MQL	$9 \pm 1^{d}_{b}$
Caffeic acid	$0.16 \pm 0.03^{a,b}_{b}$	$0.12 \pm 0.04^{a,b}_{a,b}$	$0.09 \pm 0.02^{a,b,c}_{a}$	$0.15 \pm 0.02^{a}_{b}$	$0.24 \pm 0.03^{a,b}_{c}$	$0.17 \pm 0.01^{a}_{b}$
Epicatechin	$0.33 \pm 0.07^{c}_{a}$	$0.4 \pm 0.1^{b,c}_{a}$	$0.27 \pm 0.09^{d}_{a}$	$0.33 \pm 0.07^{a,b}_{a}$	$2.3 \pm 0.7^{e}_{c}$	$1.0 \pm 0.1^{b}_{b}$
Piceid	$0.015 \pm 0.007^{a}_{a}$	$0.008 \pm 0.003^{a}_{a}$	$0.013 \pm 0.002^{a}_{a}$	$0.043 \pm 0.004^{a}_{b}$	$0.048 \pm 0.006^{a}_{b}$	$0.05 \pm 0.01^{a}_{b}$
p-Coumaric acid	$0.054 \pm 0.009^{a,b}_{a}$	$0.06 \pm 0.01^{a,b}_{a}$	$0.07 \pm 0.02^{a,b}_{a}$	$0.23 \pm 0.03^{a,b}_{b}$	$0.9 \pm 0.2^{c}_{c}$	$0.30 \pm 0.03^{a}_{b}$
Ferulic acid	$0.06 \pm 0.01^{a,b}_{a}$	$0.09 \pm 0.03^{a,b}_{a}$	$0.056 \pm 0.007^{a,b}_{a}$	$0.27 \pm 0.04^{a,b}_{b}$	$0.28 \pm 0.05^{a,b}_{b}$	$0.26 \pm 0.02^{a}_{b}$
Naringin	$0.17 \pm 0.04^{b}_{a}$	$0.120 \pm 0.004^{a,b}_{a}$	$0.17 \pm 0.02^{c}_{a}$	$0.13 \pm 0.04^{a}_{a}$	$0.35 \pm 0.08^{a,b}_{b}$	$0.20 \pm 0.04^{a}_{a}$
Quercetin 3-β-D-glucoside	$0.06 \pm 0.01^{a,b}_{a}$	$0.8 \pm 0.2^{d}_{c}$	$0.06 \pm 0.01^{a,b}_{a}$	$0.14 \pm 0.04^{a}_{a,b}$	$0.6 \pm 0.2^{b,c}_{c}$	$0.35 \pm 0.05^{a}_{b}$
Rutin	$0.12 \pm 0.02^{a,b}_{a}$	$5.8 \pm 0.6^{f}_{d}$	$0.10 \pm 0.02^{a,b,c}_{a}$	$5.8 \pm 0.8^{c}_{d}$	$3.4 \pm 0.5^{f}_{c}$	$2.2 \pm 0.1^{c}_{b}$
Trans-resveratrol	$0.037 \pm 0.004^{a,b}_{a}$	$0.031 \pm 0.005^{a,b}_{a}$	$0.031 \pm 0.002^{a,b}_{a}$	$0.035 \pm 0.005^{a}_{a}$	$0.05 \pm 0.01^{a}_{b}$	$0.05 \pm 0.01^{a}_{b}$
Myricetin	$0.029 \pm 0.002^{a,b}_{a}$	$0.06 \pm 0.01^{a,b}_{b}$	$0.020 \pm 0.001^{a}_{a}$	$0.057 \pm 0.004^{a}_{b}$	$0.052 \pm 0.006^{a}_{b}$	$0.056 \pm 0.007^{a}_{b}$
Quercetin	$0.05 \pm 0.02^{a,b}_{a}$	$0.29 \pm 0.06^{a,b,c}_{b}$	$0.05 \pm 0.02^{a,b}_{a}$	$0.129 \pm 0.009^{a}_{a,b}$	$0.7 \pm 0.2^{b,c}_{c}$	$0.14 \pm 0.03^{a}_{a,b}$
Naringenin	$0.030 \pm 0.003^{a,b}_{a}$	$0.025 \pm 0.003^{a}_{a}$	$0.023 \pm 0.001^{a}_{a}$	$0.063 \pm 0.002^{a}_{b}$	$0.078 \pm 0.007^{a}_{c}$	$0.082 \pm 0.008^{a}_{c}$

n.d. = analytes non-detected in the samples

<MQL = analytes detected at a concentration level under their MQL.

Different superscript letters in the same column indicate significant differences ($p < 0.05$) among polyphenols in each sample. Different subscript letters in the same row indicate significant differences ($p < 0.05$) among samples.

Figure captions

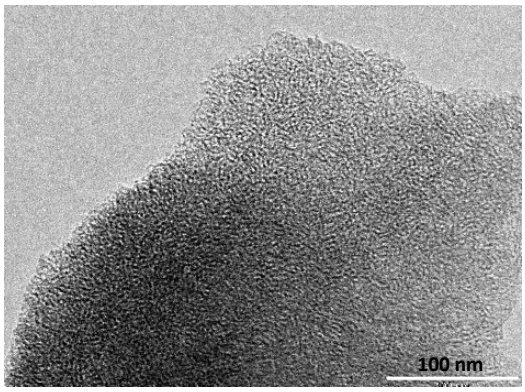
Fig. 1 (a) SEM and (b) TEM images of HMS, (c) N_2 adsorption-desorption isotherms and (d) pore size distribution of HMS and HMS-C18, (e) TGA curves of HMS-C18.

Fig. 2 Comparison of the recovery percentages obtained from the analysis of spiked juice (A) and smoothie (B) samples extracted by the optimized dSPE method using different types of sorbents. Error bars represent the standard deviation of sample replicates (n = 3).

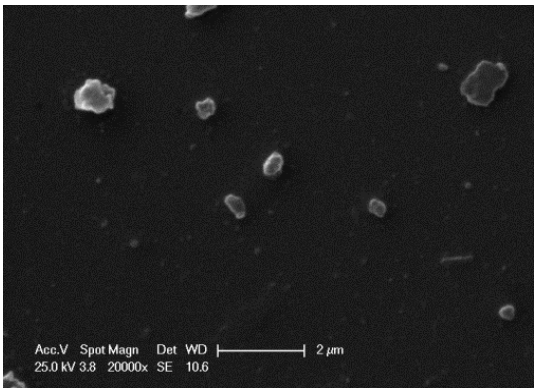
Fig. 3 Possible interaction mechanism by HMS-C18.

Fig. 1

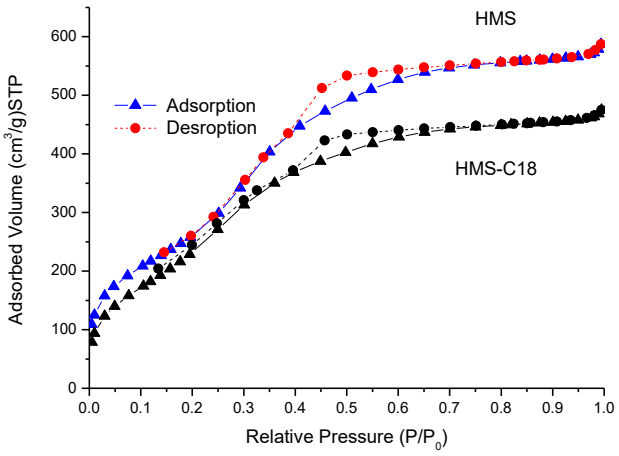
a)


100 nm

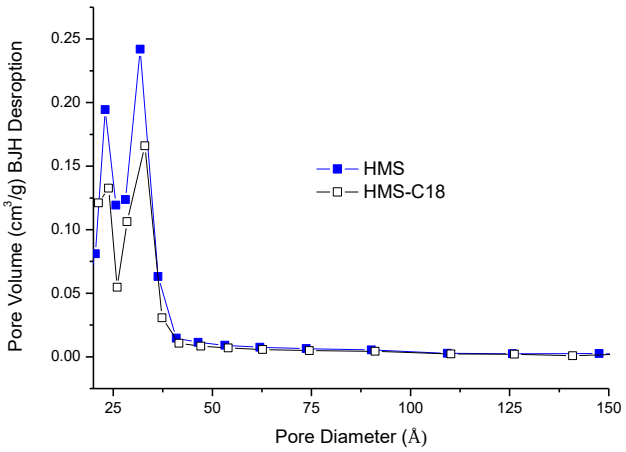
b)


Acc.V Spot Magn Det WD 2 μm
25.0 kV 3.8 20000x SE 10.6

c)


HMS
Adsorption
Desroption
HMS-C18
Adsorbed Volume (cm³/g)STP
Relative Pressure (P/P0)

d)


HMS
HMS-C18
Pore Volume (cm³/g) BJH Desroption
Pore Diameter (Å)

e)

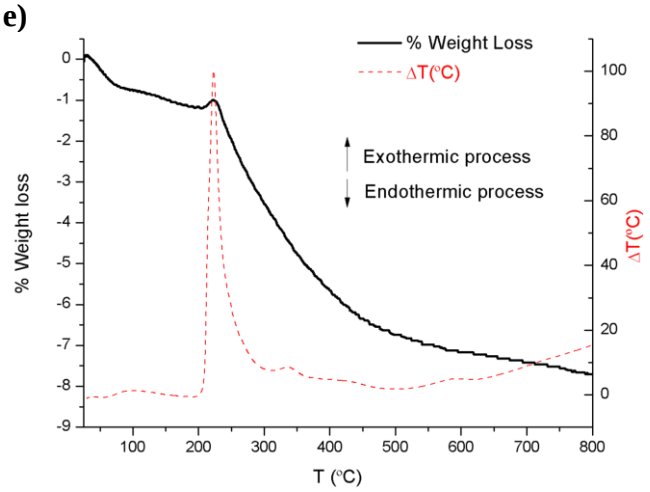

% Weight Loss
ΔT(°C)
Exothermic process
Endothermic process
% Weight loss
ΔT(°C)
T (°C)

Fig. 2

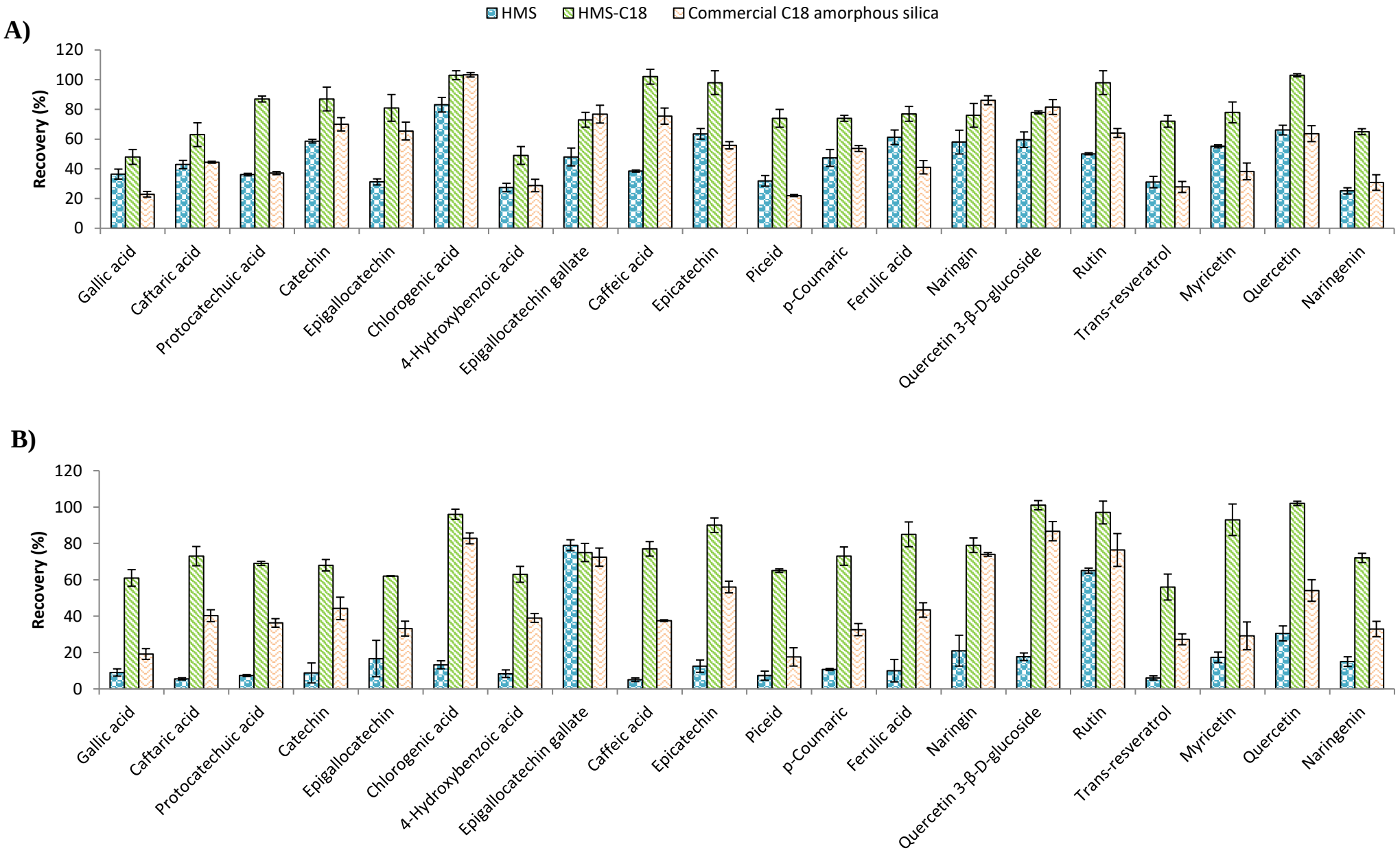
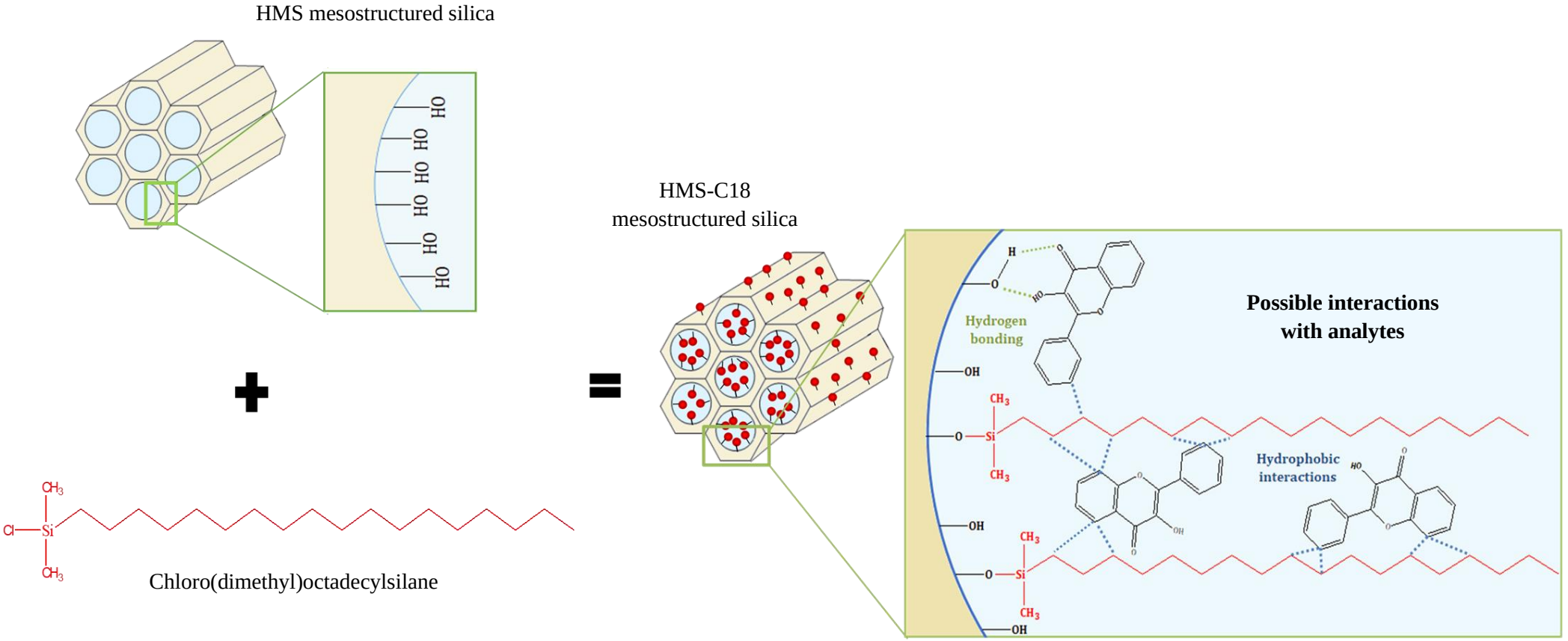
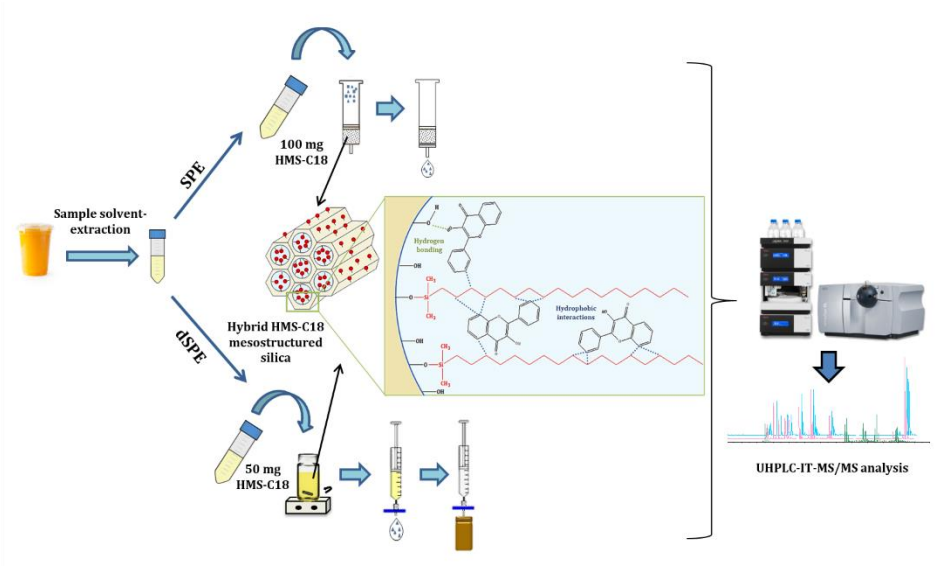

A)
HMS
HMS-C18
Commercial C18 amorphous silica
Recovery (%)
120
100
80
60
40
20
0
Gallic acid
Caftaric acid
Protocatechuic acid
Catechin
Epigallocatechin
Chlorogenic acid
4-Hydroxybenzoic acid
Epigallocatechin gallate
Caffeic acid
Epicatechin
Piceid
p-Coumaric
Ferulic acid
Naringin
Quercetin 3-β-D-glucoside
Rutin
Trans-resveratrol
Myricetin
Quercetin
Naringenin
B)
Recovery (%)
120
100
80
60
40
20
0
Gallic acid
Caftaric acid
Protocatechuic acid
Catechin
Epigallocatechin
Chlorogenic acid
4-Hydroxybenzoic acid
Epigallocatechin gallate
Caffeic acid
Epicatechin
Piceid
p-Coumaric
Ferulic acid
Naringin
Quercetin 3-β-D-glucoside
Rutin
Trans-resveratrol
Myricetin
Quercetin
Naringenin

Fig. 3


HMS mesostructured silica
HO
HO
HO
HO
HO
HO
HMS-C18
mesostructured silica
Possible interactions
with analytes
Hydrogen
bonding
Hydrophobic
interactions
OH
CH3
Si
O
Cl
Chloro(dimethyl)octadecylsilane

TOC


Sample solvent-extraction
SPE
100 mg HMS-C18
dSPE
Hybrid HMS-C18 mesostructured silica
50 mg HMS-C18
Hydrogen bonding
Hydrophobic interactions
UHPLC-IT-MS/MS analysis