

Article

# Comparison between Exhaustive and Equilibrium Extraction Using Different SPE Sorbents and Sol-Gel Carbowax 20M Coated FPSE Media

Angela Tartaglia <sup>1</sup>, Marcello Locatelli <sup>1,\*</sup> , Abuzar Kabir <sup>2,\*</sup> , Kenneth G. Furton <sup>2</sup>, Daniela Macerola <sup>1</sup>, Elena Sperandio <sup>1</sup>, Silvia Piccolantonio <sup>1</sup>, Halil I. Ulusoy <sup>3</sup> , Fabio Maroni <sup>1</sup> , Pantaleone Bruni <sup>1</sup> , Fausto Croce <sup>1</sup>  and Victoria F. Samanidou <sup>4</sup> 

<sup>1</sup> Department of Pharmacy, University of Chieti–Pescara “G. d’Annunzio”, Via dei Vestini 31, 66100 Chieti, Italy; angela.tartaglia@unich.it (A.T.); daniela.macer@gmail.com (D.M.); sperandioelena94@gmail.com (E.S.); silvia.piccolantonio@studenti.unich.it (S.P.); fabio.maroni@unich.it (F.M.); pantaleonebruni@libero.it (P.B.); fausto.croce@unich.it (F.C.)

<sup>2</sup> Department of Chemistry and Biochemistry, International Forensic Research Institute, Florida International University, 11200 SW 8th St, Miami, FL 33199, USA; furtonk@fiu.edu

<sup>3</sup> Department of Analytical Chemistry, Faculty of Pharmacy, Cumhuriyet University, Sivas 58140, Turkey; hiulusoy@yahoo.com

<sup>4</sup> Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; samanidu@chem.auth.gr

\* Correspondence: m.locatelli@unich.it (M.L.); akabir@fiu.edu (A.K.);

Tel.: +39-0871-3554590 (M.L.); +1-305-348-2396 (A.K.); Fax: +39-0871-3554911 (M.L.); +1-305-348-4172 (A.K.)

Academic Editor: Victoria Samanidou

Received: 13 December 2018; Accepted: 20 January 2019; Published: 22 January 2019



**Abstract:** This paper reports the performance comparison between the exhaustive and equilibrium extraction using classical Avantor C18 solid phase extraction (SPE) sorbent, hydrophilic-lipophilic balance (HLB) SPE sorbent, Sep-Pak C18 SPE sorbent, novel sol-gel Carbowax 20M (sol-gel CW 20M) SPE sorbent, and sol-gel CW 20M coated fabric phase sorptive extraction (FPSE) media for the simultaneous extraction and analysis of three inflammatory bowel disease (IBD) drugs that possess logP values (polarity) ranging from 1.66 for cortisone, 2.30 for ciprofloxacin, and 2.92 for sulfasalazine. Both the commercial SPE phases and in-house synthesized sol-gel CW 20M SPE phases were loaded in SPE cartridges and the extractions were carried out under an exhaustive extraction mode. FPSE was carried out under an equilibrium extraction mode. The drug compounds were resolved using a Luna C18 column (250 mm × 4.6 mm; 5 μm particle size) in gradient elution mode within 20 min and the method was validated in compliance with international guidelines for the bioanalytical method validation. Novel in-house synthesized and loaded sol-gel CW 20M SPE sorbent cartridges were characterized in terms of their extraction capability, breakthrough volume, retention volume, hold-up volume, number of the theoretical plate, and the retention factor.

**Keywords:** FPSE; in-house loaded SPE; HPLC-PDA; method validation; IBD; extraction

## 1. Introduction

Sample preparation and ‘clean-up’ have the aim of improving the analytical parameters to enhance detectability and to protect the performance of analytical equipment. However, these steps are time consuming, utilize large volumes of toxic and hazardous organic solvents, and are prone to error. For these reasons, novel extraction technologies are continuously being developed to overcome these drawbacks [1] in order to improve the extraction efficiency, reduce the sample handling, and enhance the reproducibility.

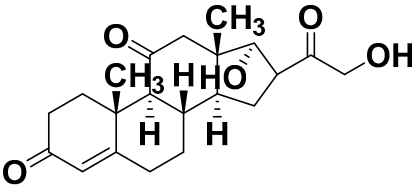
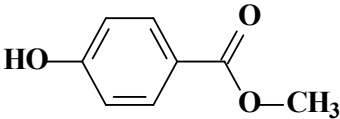
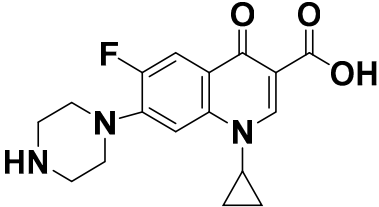
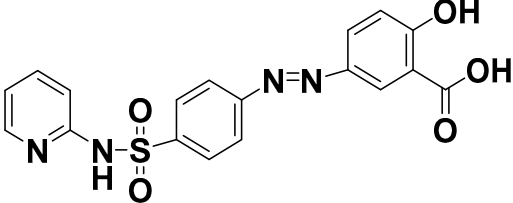
Novel extraction strategies focus on improving the quality of extraction, decreasing the use of solvents to become environmentally friendly, reducing the time for extraction, and the associated costs. Solid-phase extraction (SPE) is the most popular sample preparation technique used for extraction, clean up, and pre-concentration of both environmental and biological samples. In addition, SPE is fast, easy, and green due to its reduced consumption of solvent compared to liquid–liquid extraction, its relative inexpensiveness and ability for easy automation as well as shorter analysis time. The primary advantage is that SPE is a non-equilibrium, exhaustive extraction procedure; the problem with using an equilibrium process is that the analyst may never know when equilibrium has been reached, and the equilibrium distribution may necessitate multiple extractions [2].

Fabric phase sorptive extraction (FPSE) is a new generation solvent-free/solvent-minimized microextraction technique developed by Kabir and Furton [3]. FPSE is a simple, rapid, and green sample preparation technique, which not only simplifies the sample preparation by eliminating many unnecessary steps such as filtration, protein precipitation, solvent evaporation, and sample reconstitution, it also substantially reduces the organic solvent consumption [4]. The permeable fabric substrate used to chemically bind sol-gel derived high performance sorbents in FPSE mimics the flow-through extraction mechanism used in SPE even when the extraction is carried out under equilibrium extraction mode, resulting in a faster mass transfer and extraordinarily high analyte recovery. The flexibility of the FPSE media allows its direct insertion into the sample container, reducing possible analyte loss [1] and potential cross contamination stems from the containers. The main advantages of FPSE are its high contact surface area for rapid sorbent-analyte interactions and the possibility of enhancing the diffusion of analytes through the FPSE media with magnetic stirring or sonication to obtain a fast extraction equilibrium [5].

Among different treatment regimens used in inflammatory bowel disease (IBD), cortisone, ciprofloxacin, and/or sulfasalazine are orally administrated among other drugs; these drugs are generally present at trace level concentrations in physiological samples including plasma, urine, and saliva. As such, a preconcentration step is necessary to obtain advantageous signal-to-noise ratios in chromatographic separation and detection [6,7]. The logP values of the target analytes ranging from 1.66 for cortisone, to 2.30 for ciprofloxacin, and 2.92 for sulfasalazine (Table 1), coupled to their widespread range of pK<sub>a</sub> values pose a great challenge for their simultaneous extraction and analysis. Particularly these differences could influence the extraction efficiency of the analytes. These differences necessarily guide the selection of possible sample preparation and clean-up procedures that can be applied, and failure to select an appropriate sample preparation technique may lead to low analytical performances during traces analysis.

Both the exhaustive extraction technique (such as solid phase extraction) and equilibrium extraction technique (such as solid phase microextraction, fabric phase sorptive extraction, stir bar sorptive extraction) not only utilize different extraction mechanism, but also use mutually exclusive extraction sorbents. Among SPE sorbents, hydrophilic-lipophilic balance (HLB) and C18 are the most popular, whereas for equilibrium extraction, poly(dimethyl siloxane), poly(ethylene glycol), and polyacrylate are noteworthy. As such, it is important to compare the extraction performances between the two major extraction modes as well as between different sorbent chemistries. Only a few sorbent chemistries are available in both the exhaustive and equilibrium extraction formats. However, our research group has recently introduced a large number of traditional microextraction sorbents including poly(dimethyl siloxane) and poly(ethylene glycol) in solid phase extraction format. In the current study, sol-gel CW 20M SPE sorbent and sol-gel CW 20M coated FPSE media are used for extraction performance evaluation and comparison among other sorbents. These phases were newly synthesized in our laboratory and herein tested in order to compare them in terms of the main analytical parameters (for SPE breakthrough volume ( $V_B$ ), retention volume ( $V_R$ ), hold-up volume ( $V_M$ ), retention factor ( $k$ ), and theoretical plates number ( $N$ ), and for both SPE and FPSE the enrichment factors).

**Table 1.** Molecular structures and other pertinent physicochemical properties of selected inflammatory bowel disease (IBD) drugs and the internal standard.

Drug Name	CAS No.	Molecular Weight (g/mole)	Molecular Structure	LogP	pK <sub>a</sub>
Cortisone	53-06-5	360.44		1.66	12.6
Methyl- <i>p</i> -hydroxy benzoate	99-76-3	152.15		1.96	8.4
Ciprofloxacin	85721-33-1	331.34		2.30	5.76
Sulfasalazine	599-79-1	398.39		2.92	3.3

To the best of our knowledge, this is the first comprehensive comparison between exhaustive and equilibrium extraction modes utilizing multiple C18 SPE sorbents, HLB sorbent, novel sol-gel CW 20M SPE sorbent, and sol-gel CW 20M coated FPSE membrane for the determination of IBD drugs, possessing logP values (polarity) of 1.66 for cortisone, 2.30 for ciprofloxacin, and 2.92 for sulfasalazine. Due to the difference in their physicochemical properties, especially their polarity, selected IBD drugs pose a challenge for their simultaneous extraction and preconcentration at their trace and ultra-trace level concentrations.

## 2. Results and Discussion

### 2.1. Determination of SPE Parameters: Breakthrough Volume, Retention Volume, Hold-Up Volume, Retention Factor, and Theoretical Plate Number for SPE Devices

To understand the extraction mechanism and to utilize their full potential as the extraction media, it is important to characterize the sorbents available for the extraction and clean-up process. When the solid phase extraction (SPE) procedure is adopted, the main parameters that need to be considered are the breakthrough volume ( $V_B$ ), retention volume ( $V_R$ ), hold-up volume ( $V_M$ ), retention factor ( $k$ ), and the theoretical plates number ( $N$ ).

As reported in the literature [8,9], for the determination of the above parameters, *off-line* SPE can be followed by applying the sigmoid shape breakthrough curve. Particularly, from this model obtained by fitting of the experimental points by Boltzmann's function:

$$Y = A_2 + \frac{A_1 - A_2}{1 + e^{\frac{x-x_0}{dx}}} \quad (1)$$

and be calculated by the breakthrough volume

$$V_B = x_0 + dx \cdot \ln \left[ \frac{100}{99} \left( 1 - \frac{A_1}{A_2} \right) - 1 \right] \quad (2)$$

hold-up volume:

$$V_M = x_0 + dx \cdot \ln \left[ 99 - 100 \cdot \frac{A_1}{A_2} \right] \quad (3)$$

The retention volume, defined as the inflection point of the curve, corresponds to the  $x_0$  value. The retention factor ( $k$ ) is defined using the following equation:

$$V_M = V_R(1 + k) \quad (4)$$

and the theoretical plates number:

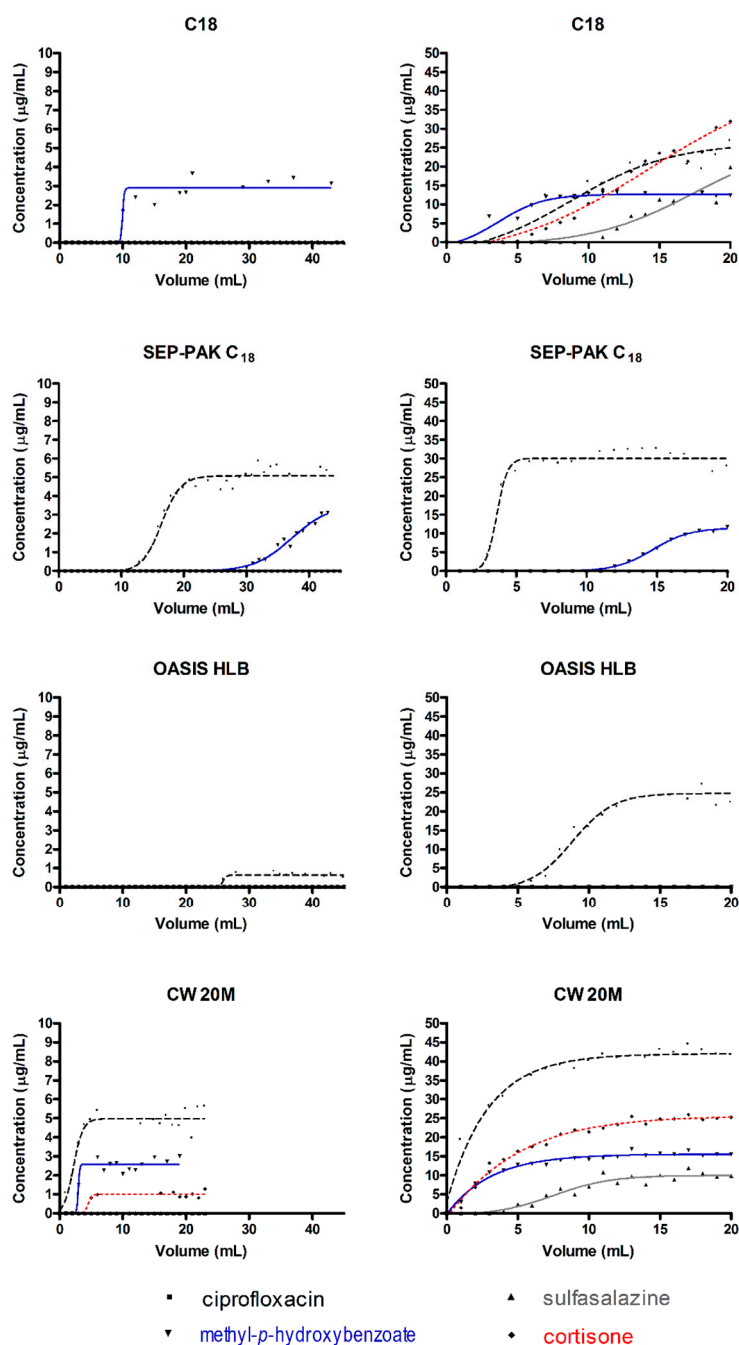
$$N = \frac{V_R(V_R - \sigma_V)}{\sigma_V^2} \quad (5)$$

were  $\sigma_V$  was obtained by:

$$V_B = V_R - 2\sigma_V \quad (6)$$

In the current study, following our research on innovative extraction procedures [4,10–13] and devices, we compare four different SPE sorbents (both commercially available and in-house produced) and the innovative fabric phase sorptive extraction (FPSE) media.

Using these equations, it was possible to evaluate the breakthrough volume, retention volume, hold-up volume, retention factor, and theoretical plate number for the herein considered stationary phases loaded on the SPE cartridges. In Figure 1 we reported the experimental curves and the fittings used for the parameters calculation.



**Figure 1.** Comparison of breakthrough curves determined for the analytes on the different solid phase extraction (SPE) sorbents and at 10 µg/mL (left) and 50 µg/mL (right).

The Boltzmann's functions shown in Figure 1 allows us to evaluate the breakthrough volume ( $V_B$ ), retention volume ( $V_R$ ), hold-up volume ( $V_M$ ), retention factor ( $k$ ), and theoretical plate number ( $N$ ) as reported in Tables 2 and 3 (by using the above reported equations). In these Tables, it was highlighted that for the tested compounds, some of these materials are not available in a "universal" phase. In fact, some analytes were not reported (both in Figure 1 and Tables 2 and 3) because no quantitative data were obtained after the SPE steps and, consequently, it was not possible to perform the analysis by Boltzmann's function (like all the other compounds). Similarly, in other published papers [8,9,14], this model could not reach a complete analysis (due to a "floating point error" applying Boltzmann's function) and evaluation of the above-mentioned parameters.

**Table 2.** Parameters determined for the analytes on different sorbents in frontal analysis at the concentration of 10 µg/mL.

Sorbent	Analyte	Breakthrough Volume (mL)	Retention Volume (mL)	Hold-Up Volume (mL)	Retention Factor ( <i>k</i> )	Number of Theoretical Plates ( <i>N</i> )
Avantor C <sub>18</sub>	Ciprofloxacin	>50	-	-	-	-
	Sulfasalazine	>50	-	-	-	-
	Methyl- <i>p</i> -hydroxybenzoate	11.3	9.9	11.9	0.20	2000
	Cortisone	>50	-	-	-	-
Sep-Pac Vac C <sub>18</sub>	Ciprofloxacin	23.6	16.4	30.6	0.87	16
	Sulfasalazine	>50	-	-	-	-
	Methyl- <i>p</i> -hydroxybenzoate	52.8	37.3	66.8	0.79	18
	Cortisone	>50	-	-	-	-
Oasis HLB	Ciprofloxacin	27.6	25.9	28.8	0.11	845
	Sulfasalazine	>50	-	-	-	-
	Methyl- <i>p</i> -hydroxybenzoate	>50	-	-	-	-
	Cortisone	>50	-	-	-	-
CW 20M	Ciprofloxacin	5.26	2.2	8.7	2.95	1
	Sulfasalazine	>50	-	-	-	-
	Methyl- <i>p</i> -hydroxybenzoate	4.4	2.9	4.9	0.66	13
	Cortisone	6.5	4.5	7.9	0.75	17

**Table 3.** Parameters determined for the analytes on different sorbents in frontal analysis at the concentration of 50 µg/mL.

Sorbent	Analyte	Breakthrough Volume (mL)	Retention Volume (mL)	Hold-Up Volume (mL)	Retention Factor ( <i>k</i> )	Number of Theoretical Plates ( <i>N</i> )
Avantor C18	Ciprofloxacin	15.1	8.8	32.2	2.68	5
	Sulfasalazine	29.7	17.4	46.5	1.67	5
	Methyl- <i>p</i> -hydroxybenzoate	6.7	3.7	14.3	2.91	3
	Cortisone	25.7	14.2	49.6	2.48	4
Sep-Pac Vac C18	Ciprofloxacin	5.4	3.6	7.2	0.99	12
	Sulfasalazine	>20	-	-	-	-
	Methyl- <i>p</i> -hydroxybenzoate	21.4	14.7	27.4	0.87	15
	Cortisone	>20	-	-	-	-
Oasis HLB	Ciprofloxacin	13.9	8.8	20.7	1.34	8
	Sulfasalazine	>20	-	-	-	-
	Methyl- <i>p</i> -hydroxybenzoate	>20	-	-	-	-
	Cortisone	>20	-	-	-	-
CW 20M	Ciprofloxacin	>20	-	-	-	-
	Sulfasalazine	13.2	7.5	22.7	2.02	4
	Methyl- <i>p</i> -hydroxybenzoate	>20	-	-	-	-
	Cortisone	>20	-	-	-	-

All tested SPEs show high retention values for sulfasalazine that do not allow us to evaluate the principal parameters, also due to the fact that with the obtained quantitative data no Boltzmann's function can be performed. Within the four tested phases, the sol-gel CW 20M shows the ability to retain all compounds with a good number of theoretical plates, and in the meantime allow the use of a small elution volume. These findings follow the general principles of the Green Analytical Chemistry. In this case, unfortunately, the calculated breakthrough volume, retention volume, hold-up volume, retention factor, and theoretical plate number were lower than the values obtained for the other tested phases.

Interestingly, commercially available phases show superior results at higher concentration levels for the tested compounds, while the new sol-gel CW 20M are at low concentrations. This finding could be observed from the comparison of Tables 2 and 3 where the evaluated parameters can be calculated for high and low concentrations, respectively.

## 2.2. Mechanism of Extraction in FPSE

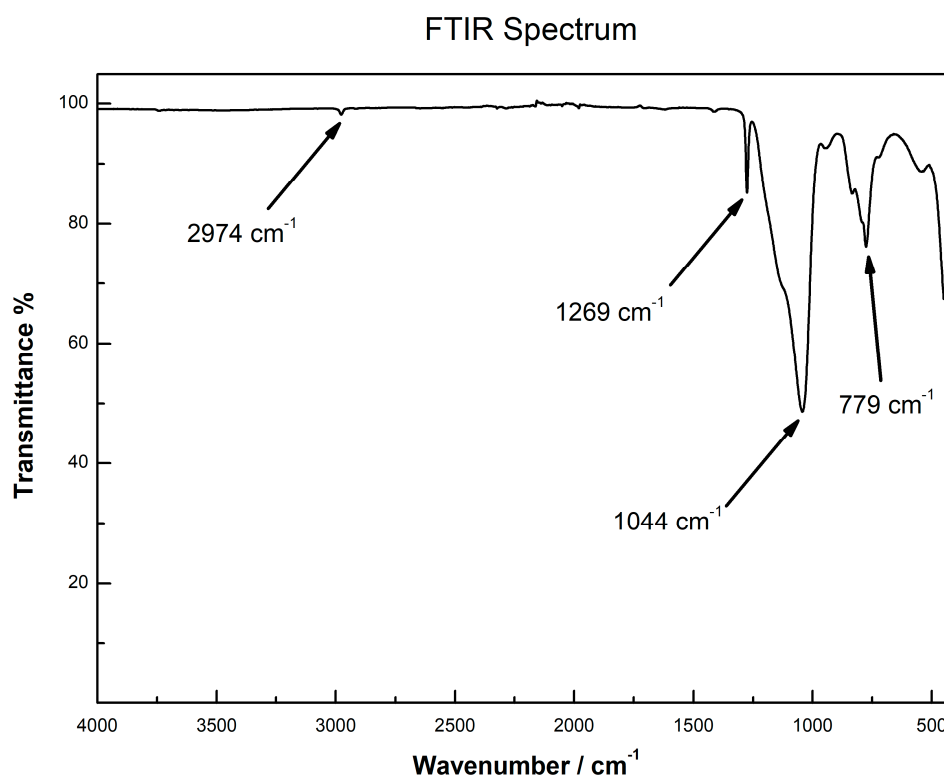
FPSE has combined two major sample preparation techniques: SPE (governed by exhaustive extraction principle) and SPME (governed by equilibrium driven extraction principle) into a single sample preparation technique. Due to the geometrical advantage and the combination of both the equilibrium and exhaustive extraction mechanism [15], FPSE can perform exhaustive extraction under equilibrium extraction conditions. The fabric substrates are inherently permeable. The permeability of the fabric substrate remains intact even after the sol-gel sorbent coating. When the FPSE media is immersed into the liquid sample matrix, the aqueous solution rapidly permeates through the porous bed of sol-gel sorbent-coated FPSE media and rapidly interacts with the sorbent via different intermolecular interaction mechanisms. As such, the FPSE membrane behaves like a SPE disk. At the same time, when the FPSE media is introduced into the aqueous solution, it behaves similarly to the SPME thin film format, analytes continue to accumulate onto the FPSE media based on the partition coefficient of the analyte(s) between the sol-gel sorbent and the sample matrix until the extraction equilibrium is reached.

## 2.3. Characterization of Sol-Gel CW 20M SPE Sorbent Using FTIR and TGA Analysis

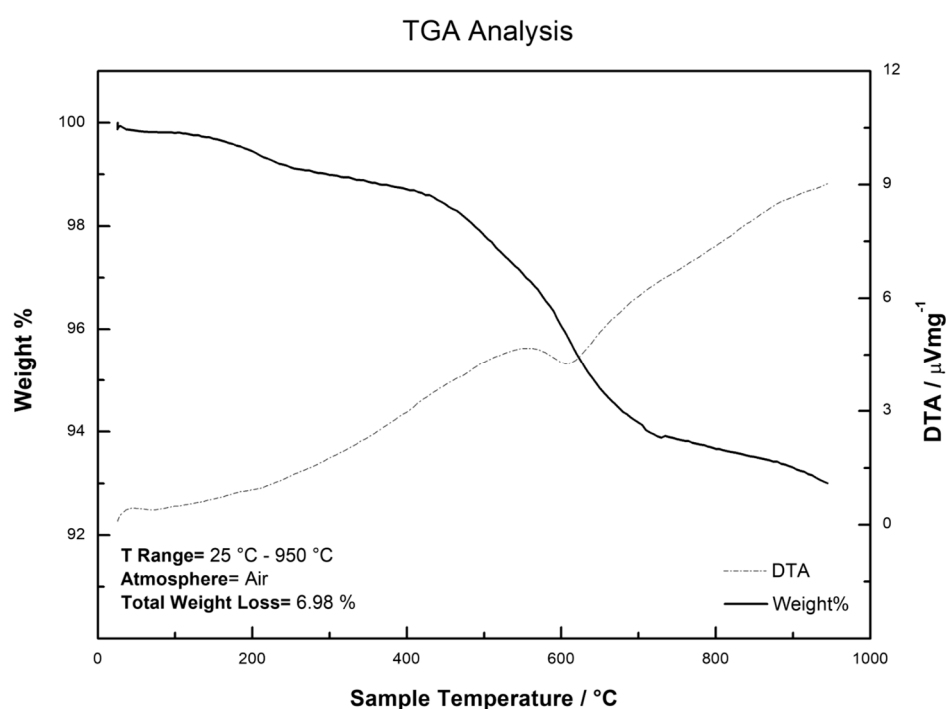
The experimental FTIR spectrum shows a very weak band at  $2974\text{ cm}^{-1}$ , indicating the symmetric stretching of a C-H bond. Then a medium sharp band at  $1269\text{ cm}^{-1}$ , which can be assigned to a Si-CH<sub>3</sub> bond and is indicative of the successful integration of methyl trimethoxysilane (MTMS) into the sol-gel network. Then a broad band with a visible shoulder, centered at  $1044\text{ cm}^{-1}$ , indicative of vibrations related to Si-O-Si bonds. These features are likely connected to the siloxane nature of the sample [16]. All these findings are reported in Figure 2. One very important point that can be noted here, is the absence of any band around  $3690\text{ cm}^{-1}$  that corresponds to free silanol (Si-OH). This indicates that even though the sol-gel CW 20M SPE sorbent was not treated with any endcapping (post synthesis derivatization to minimize unreacted surface silanol groups of the silica substrate), the novel SPE sorbent does not possess any residual surface silanol groups.

Thermogravimetric analysis shows a 2% weight loss up to  $250\text{ }^{\circ}\text{C}$ , probably due to loss of moisture and volatile species entrapped inside the sol-gel CW20M particles. Then, as indicated by differential thermal analysis (DTA) data, between  $400\text{ }^{\circ}\text{C}$  and  $600\text{ }^{\circ}\text{C}$  a slightly exothermic process is visible, probably due to the loss of unreacted reaction products trapped inside the sol-gel particles. The sol-gel CW 20M sorbent was synthesized using two catalysts, an acid catalyst during hydrolysis and a base catalyst during polycondensation. Due to the use of two catalysts in the sol-gel synthesis, the CW 20M polymer becomes chemically integrated into the sol-gel network and was able to endure the high temperature without converting into CO<sub>2</sub> during the thermogravimetric analysis. It should also be noted that the stoichiometric carbon loading of sol-gel CW 20M was approximately 35% compared to 9–10% carbon loading in commercial C18 sorbents. The total weight loss at the end of the experiment was 6.99%. All these findings are reported in Figure 3.





**Figure 2.** FTIR spectrum for the CW 20M stationary phase.



**Figure 3.** TGA analysis for the CW 20M stationary phase.

#### 2.4. Enrichment Factors Determination

In Table 4 we reported the enrichment factors observed for the tested compounds. As highlighted within the SPE format, Oasis HLB shows the highest values (up to 607-folds), even if sol-gel CW 20M shows more reproducible values for all compounds, especially for cortisone. Contrary to the speculation made in Section 3.6, sol-gel CW 20M did not provide superior extraction performance

compared to its commercial counterparts including C18 and HLB sorbents. The unexpected low performance can be attributed to the insufficient cleaning of the sol-gel CW 20M sorbent after the synthesis and conditioning (the TGA profile of sol-gel CW 20M corroborates this hypothesis). As such, the matrix still contains trapped solvent and unreacted reaction byproducts. Therefore, interaction between the target analytes and the sorbent occurred preferentially on the outer surface. Further experimentation is needed to evaluate actual extraction performance of the new sorbent after proper cleaning to ensure that all the trapped solvent and the unreacted reaction byproducts are exhaustively removed.

**Table 4.** Enrichment factors (%) observed at concentrations of 0.8, 2.5, and 8 µg/mL of water standard solutions. The enrichment factors were calculated as the percentage of peak area enhancement (after extraction) with respect to the area of reference standard solutions.

Analyte	QC Concentration (µg/mL)	FPSE CW 20M	SPE Format			
			Avantor C18	Sep-Pac Vac C18	Oasis HLB	CW 20M
Ciprofloxacin	0.8	28.4	<b>740</b>	304	67	321
	2.5	37.0	406	199	<b>607</b>	186
	8	13.6	200	188	<b>367</b>	113
Sulfasalazine	0.8	248	<b>640</b>	100	380	202
	2.5	164	325	206	<b>408</b>	217
	8	278	344	299	<b>493</b>	225
Methyl- <i>p</i> -hydroxybenzoate	0.8	190	204	78	<b>314</b>	59
	2.5	155	310	133	<b>344</b>	113
	8	121	205	216	<b>252</b>	76
Cortisone	0.8	136	80	123	n.a.	<b>171</b>
	2.5	88	160	195	167	<b>199</b>
	8	128	203	240	<b>245</b>	161

n.a. not available, the analyte is not detected during the analysis.

In Table 4 we also reported the enrichment factors observed for sol-gel CW 20M in the FPSE format. These values, in accordance with previously observed ones [6], were lower than the sol-gel CW 20M in SPE format, even if always comparable (or higher) in respect to other SPE commercially available devices.

It should be noted that, even if the enrichment factors calculated for both the SPE and the FPSE (Table 4) can vary a lot, and for the latter they were always lower than the SPE, the greatest advantage of the FPSE technique lies in the fact that it allows extraction of the analytes directly from the matrix of interest without having to resort to preliminary procedures of protein precipitation and/or cleaning up [4,6,7] to avoid the clogging phenomena.

### 3. Material and Methods

#### 3.1. Chemicals, Solvents and Devices

Ciprofloxacin, methyl-*p*-hydroxybenzoate (methyl paraben, IS), sulfasalazine, and cortisone (>98% purity grade), sodium phosphate monobasic and sodium phosphate dibasic (>99% purity grade) and phosphoric acid were purchased from Sigma-Aldrich (Milan, Italy). Acetonitrile (ACN) and methanol (HPLC-grade), DMSO and HCl 37% (RPE-ISO for analysis) were purchased from Carlo Erba (Milan, Italy) and were used without further purification. The water (18.2 MΩ·cm at 25 °C) for HPLC analysis was generated by a Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp, Bedford, MA, USA).

Solid phase extraction devices evaluated in this work were Oasis HLB (Waters, Milford, MA, USA), Sep-Pak Vac C18 (Waters, Milford, MA, USA), Avantor C18 loaded SPE (J.T. Baker, Avantor Performance Materials, LLC., Center Valley, PA, USA), and novel Carbowax 20M (sol-gel CW 20M, highly polar sorbent possessing poly(ethylene glycol), H[OCH(CH<sub>3</sub>)CH<sub>2</sub>]<sub>n</sub>OH as the building block).

All devices were in the format of 1 mL, 30 mg. GraphPad Prism v.4 (GraphPad Software Inc, San Diego, CA, USA) was used for the statistical analysis of experimental data.

### 3.2. Stock Solution and Quality Control Samples

The stock solutions of chemical standards were made at the concentration of 1 mg/mL in the appropriate solvent, referring to solubility tests: ciprofloxacin was solubilized in a mixture MeOH:HCl (0.1 M) 1:1 (*v/v*), sulfasalazine in DMSO, methyl-*p*-hydroxybenzoate and cortisone in MeOH. The combined working solutions (at the concentration of 10 and 50 µg/mL) were prepared by dilution of a mixed solution in MilliQ water. The resulting samples were used to evaluate the enrichment factors, breakthrough volume, retention volume, hold-up volume, retention factor, and theoretical plate number.

### 3.3. Apparatus and Chromatographic Conditions

Analyses were performed using an HPLC Thermo Fisher Scientific liquid chromatography system (Model: Spectra System P2000) coupled to a photodiode array detector (PDA) Model: Spectra System UV6000LP. Mobile phase was directly on-line degassed by using a Spectra System SCM1000 (Thermo Fisher Scientific, Waltham, MA, USA). Excalibur v.2.0 Software (Thermo Fisher Scientific, Waltham, MA, USA) was used to collect and analyze the data. The Luna C18 (250 × 4.6 mm, 5 µm particle size; Phenomenex, Torrance, CA, USA) packing column connected to a security guard column (4.0 × 3.0 mm, 5 µm particle size; Phenomenex, Torrance, CA, USA) were used to separate drugs and internal standard (IS). The column and security guard column were thermostated at 25 °C (± 1 °C) using a Jetstream2 Plus column oven during the analysis. Drugs and IS were detected at the maximum wavelengths of 283 nm (ciprofloxacin), 369 nm (sulfasalazine), 260 nm (methyl-*p*-hydroxybenzoate), 247 nm (cortisone), respectively, following the validated method reported in reference [6].

### 3.4. Preparation of Fabric Phase Sorptive Extraction (FPSE) Media

The preparation of the cellulose fabric substrate for sol-gel coating, the preparation of the sol solution for sol-gel coating, and the sol-gel immersion coating process have been described in detail elsewhere [15].

### 3.5. Synthesis of Sol-Gel CW 20M SPE Sorbent

The sol solution for sol-gel CW 20M SPE sorbent was prepared by mixing Carbowax 20M polymer, tetramethoxysilane (TMOS), methyl trimethoxysilane (MTMS), and isopropanol in a molar ratio 0.015:1:1:20, respectively, as reported by Kabir and Furton [17]. The mixture was first vortexed for 5 min and subsequently sonicated for 30 min to ensure that the resulting solution became homogeneous and free of any trapped bubbles. Afterwards, 0.1 M HCl was added to the mixture in a molar ratio of TMOS: 0.1 M HCl, 1:4. The mixture was mixed again for 3 min and the sol solution was left overnight thus that the hydrolysis of the sol-gel precursors, TMOS and MTMS, continued towards completion. Subsequently, 1 M NH<sub>4</sub>OH was added in droplets to the sol solution under vigorous stirring until the pH of the solution became basic and the sol solution started turning into a gel spontaneously. Once the gelation completes, the sol-gel matrix was allowed to age for 48 h. The sol-gel matrix was then crushed and dried in a vacuum oven at 70 °C for 48 h. The dried sol-gel CW 20M SPE sorbent was then rinsed with 50:50 (*v/v*) methanol: Methylene chloride under sonication to remove unreacted sol solution ingredients and reaction byproducts. The sol-gel CW 20M sorbent was dried again at 70 °C for 24 h. Finally, the dried sol-gel CW 20M sorbent was pulverized in a ball mill into fine particles and particles between 40–52 µm diameter were collected in a mesh screen for using as the SPE sorbent.

### 3.6. Preparation of the SPE Media

The SPE cartridges used in this work were both commercial (Sep-Pak Vac C18, Oasis HLB, and Avantor C18) and in-house loaded with the sol-gel CW 20M (this latter stationary phase was synthesized as previously reported in paragraph 3.5). All devices were obtained in the format of 1 mL, 30 mg in order to compare the analytical performances directly. The principal SPE phase characteristics are reported in Table 5.

**Table 5.** Physical characteristics of SPE sorbent phases.

Sorbent	Sorbent Mass (mg)	Surface Area (m <sup>2</sup> /g)	Pore Diameter (Å)	Pore Volume (cm <sup>3</sup> /g)	Particle Size (µm)
Oasis HLB	30	800	80	1–3	30
Sep-Pac Vac C <sub>18</sub>	30	325	125	-	55–105
Avantor C <sub>18</sub>	30 *	320–350	60	-	40
Carbowax 20M	30	990	71	1.8	40

\* Commercially available in the size of 1 mL/100 mg sorbent phase; to compare cartridges with the same amount of sorbent phase, we have emptied and reconstituted them with 30 mg of their sorbent phase.

Compared to Brunauer–Emmett–Teller (BET) adsorption isotherm values (surface area, pore diameter, and pore volume) obtained for sol-gel CW 20M SPE sorbent with commercially available HLB and C18 sorbents, sol-gel CW 20M SPE sorbent demonstrated higher surface area and pore volume and is expected to provide superior extraction efficiency compared to their commercial counterparts.

### 3.7. SPE Sorbents Characterization Using FTIR and TGA

FTIR spectroscopy was conducted on a Perkin-Elmer spectrum TWO instrument, operating in Attenuated Total Reflectance (ATR) mode in the 4000 cm<sup>-1</sup>–450 cm<sup>-1</sup> wavenumber range. Thermogravimetric analysis was carried out using a Netzsch Regulus 2500 thermobalance, in air atmosphere, in the 25–950 °C temperature range.

## 4. Conclusions

In these researches, it was highlighted that for the tested compounds nowadays there is not available a “universal” phase. In fact, for some analytes it was not possible to evaluate the breakthrough volume ( $V_B$ ), retention volume ( $V_R$ ), hold-up volume ( $V_M$ ), retention factor ( $k$ ), and theoretical plates number ( $N$ ), because no quantitative data were obtained after the SPE steps. Consequently, it was not possible to perform the analysis by Boltzmann’s function due to a “floating point error”.

Comparing the breakthrough volume and enrichment factors data allowed us to evaluate the performance of both the FPSE and SPE techniques. The SPE technique showed the highest enrichment factors; consequently, this method is more suitable for samples with low analytes concentration. Indeed, breakthrough volumes and the number of theoretical plates data from the SPE method are higher for analytes concentration at 10 µg/mL, than the analytes concentration at 50 µg/mL. Generally, the deployment of silica sorbents (e.g., Avantor C18 and Sep-Pac-Vac C18) is not that advantageous relative to polymeric sorbents (e.g., Oasis HLB and sol-gel CW 20M) due to their instability in broader pH ranges. Additionally, silanol groups in silica sorbents bind irreversibly with target components, thus requiring more eluting solvents and a large consumption of time [18,19].

Among the different sorbent phases tested, Oasis HLB cartridges have the best performances, furthermore, against expectations, we found that even C18 cartridges showed better results than sol-gel CW 20M cartridge for that kind of analytes.

**Author Contributions:** Data curation, A.T., D.M., E.S., S.P., F.M. and P.B.; investigation, A.T., D.M., E.S., S.P., F.M. and P.B.; methodology, M.L., A.K., K.G.F., H.I.U., F.C. and V.F.S.; project administration, M.L., A.K., K.G.F., H.I.U., F.C. and V.F.S.; supervision, M.L., A.K., K.G.F., H.I.U., F.C. and V.F.S.

**Funding:** This research was funded by grant MIUR ex 60%, University of Chieti–Pescara “G. d’Annunzio”, Chieti, Italy.

**Acknowledgments:** The authors want to thank Enrico Marcantoni and Serena Gabrielli at the Chemical Science Department—School of Science and Technology at the university of Camerino (Italy) for making available the FTIR and TGA instrumentations.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Samanidou, V.; Kaltzi, I.; Kabir, A.; Furton, K.G. Simplifying sample preparation using fabric phase sorptive extraction technique for the determination of benzodiazepines in blood serum by high-performance liquid chromatography. *Biomed. Chromatogr.* **2016**, *30*, 829–836. [[CrossRef](#)] [[PubMed](#)]
2. Wells, M.J.M. Handling large volume samples: applications of SPE to environmental matrices. In *Solid-Phase Extraction: Principles, Techniques, and Application*, 1st ed.; Simpson, N.K.J., Ed.; CRC Press: Boca Raton, FL, USA, 2000.
3. Kabir, A.; Furton, K.G. Fabric Phase Sorptive Extractors. United States Patents 9557252, 31 January 2017.
4. Locatelli, M.; Kabir, A.; Innosa, D.; Lopatriello, T.; Furton, K.G. A Fabric Phase Sorptive Extraction-High Performance Liquid Chromatography-Photo Diode Array Detection Method for the Determination of Twelve Azole Antimicrobial Drug Residues in Human Plasma and Urine. *J. Chromatogr. B* **2017**, *1040*, 192–198. [[CrossRef](#)] [[PubMed](#)]
5. Aznar, M.; Alfaro, P.; Nerin, C.; Kabir, A.; Furton, K.G. Fabric phase sorptive extraction: An innovative sample preparation approach applied to the analysis of specific migration from food packaging. *Anal. Chim. Acta* **2016**, *936*, 97–107. [[CrossRef](#)] [[PubMed](#)]
6. Kabir, A.; Furton, K.G.; Tinari, N.; Grossi, L.; Innosa, D.; Macerola, D.; Tartaglia, A.; Di Donato, V.; D’Ovidio, C.; Locatelli, M. Fabric Phase Sorptive Extraction-High Performance Liquid Chromatography-Photo Diode Array Detection Method for Simultaneous Monitoring of Three Inflammatory Bowel Disease Treatment Drugs in Whole Blood, Plasma and Urine. *J. Chromatogr. B* **2018**, *1084*, 53–63. [[CrossRef](#)] [[PubMed](#)]
7. Locatelli, M.; Tinari, N.; Grassadonia, A.; Tartaglia, A.; Macerola, D.; Piccolantonio, S.; Sperandio, E.; D’Ovidio, C.; Carradori, S.; Ulusoy, H.I.; et al. FPSE-HPLC-DAD method for the quantification of anticancer drugs in human whole blood, plasma and urine. *J. Chromatogr. B* **2018**, *1095*, 204–213. [[CrossRef](#)] [[PubMed](#)]
8. Bacalum, E.; Tanase, A.; David, V. Retention mechanisms applied in solid phase extraction for some polar compounds. *Analele Universitatii Bucuresti–Chimie* **2010**, *19*, 61–68.
9. Bielica-Daszkiwicz, K.; Voelkel, A. Theoretical and experimental methods of determination of the breakthrough volume of SPE sorbents. *Talanta* **2009**, *80*, 614–621. [[CrossRef](#)] [[PubMed](#)]
10. D’Angelo, V.; Tessari, F.; Bellagamba, G.; De Luca, E.; Cifelli, R.; Celia, C.; Primavera, R.; Di Francesco, M.; Paolino, D.; Di Marzio, L.; et al. MicroExtraction by Packed Sorbent and HPLC-PDA quantification of multiple anti-inflammatory drugs and fluoroquinolones in human plasma and urine. *J. Enz. Inhibit. Med. Chem.* **2016**, *31*, 110–116. [[CrossRef](#)] [[PubMed](#)]
11. Locatelli, M.; Ciavarella, M.T.; Paolino, D.; Celia, C.; Fiscarelli, E.; Ricciotti, G.; Pompilio, A.; Di Bonaventura, G.; Grande, R.; Zengin, G.; et al. Determination of Ciprofloxacin and Levofloxacin in Human Sputum Collected from Cystic Fibrosis Patients using Microextraction by Packed Sorbent-High Performance Liquid Chromatography Photo Diode Array Detector. *J. Chromatogr. A* **2015**, *1419*, 58–66. [[CrossRef](#)] [[PubMed](#)]
12. Locatelli, M.; Cifelli, R.; Di Legge, C.; Barbacane, R.C.; Costa, N.; Fresta, M.; Celia, C.; Capolupo, C.; Di Marzio, L. Simultaneous determination of Eperisone Hydrochloride and Paracetamol in mouse plasma by High Performance Liquid Chromatography-Photo Diode Array Detector. *J. Chromatogr. A* **2015**, *1388*, 79–86. [[CrossRef](#)] [[PubMed](#)]
13. Campestre, C.; Locatelli, M.; Guglielmi, P.; De Luca, E.; Bellagamba, G.; Menta, S.; Zengin, G.; Celia, C.; Di Marzio, L.; Carradori, S. Analysis of imidazoles and triazoles in biological samples after MicroExtraction by Packed Sorbent. *J. Enz. Inhibit. Med. Chem.* **2017**, *32*, 1053–1063. [[CrossRef](#)] [[PubMed](#)]

14. Bielica-Daszkiwicz, K.; Voelkel, A.; Rusińska-Roszak, D.; Zarzycki, P.K. Estimation of the breakthrough volume of selected steroids for C-18 solid-phase extraction sorbent using retention data from micro-thin layer chromatography. *J. Sep. Sci.* **2013**, *36*, 1104–1111. [[CrossRef](#)] [[PubMed](#)]
15. Kabir, A.; Mesa, R.; Jurmain, J.; Furton, K. Fabric Phase Sorptive Extraction Explained. *Separations* **2017**, *4*, 21. [[CrossRef](#)]
16. Smith, A.L. Infrared spectra-structure correlations for organosilicon compounds. *Spectrochim. Acta* **1960**, *16*, 87–105. [[CrossRef](#)]
17. Kabir, A.; Furton, K.G. Sol-Gel Polymeric Stationary Phases for High-Performance Liquid Chromatography and Solid Phase Extraction: Their Method of Making. U.S. Patent 9925518, 27 March 2018.
18. Raza, N.; Kim, K.-H.; Abdullah, M.; Raza, W.R.; Brown, J.C. Recent developments in analytical quantitation approaches for parabens in human-associated samples. *TrAC* **2018**, *98*, 161–173. [[CrossRef](#)]
19. Pavlović, D.M.; Babić, S.; Horvat, A.J.M.; Kaštelan-Macan, M. Sample preparation in analysis of pharmaceuticals. *TrAC* **2007**, *26*, 1062–1075.

**Sample Availability:** Samples of the compounds are available from the authors under request.



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).