Optimization Of Method For Determination Of Caffeine, Ibuprofen, And Amitriptyline In Urban Effluents By SPE/GC-MS Method

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Abstract:
This article details the optimization of an analytical method for the simultaneous detection of pharmaceutical residues, specifically ibuprofen, caffeine, and amitriptyline, in urban effluents. The method combines solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). The research is justified by the growing concern over the presence of contaminants of emerging concern in aquatic environments, resulting from the improper disposal of pharmaceuticals. The study details the development and validation of the method, including the derivatization steps, analysis of calibration curves, and evaluation of precision, accuracy, limit of detection, and quantification parameters. Derivatization promoted greater fragmentation, facilitating compound identification, improving peak symmetry and response factor, resulting in lower detection limits and higher analytical sensitivity. SPE was applied to treated sewage and fortified water samples, using different pH levels (2 and 7) to evaluate matrix effects and analyte recovery. The results demonstrate that the LODs (0.004 to 0.028 µg L^{-1}) and LOQs (0.012 to 0.080 µg L^{-1}) are appropriate for detecting ibuprofen (IBU), caffeine (CAF), and amitriptyline (AMI) in aqueous matrices. Additionally, they are suitable for the preliminary screening of contaminants of emerging concern in extracts from treated domestic sewage samples. Therefore, the optimized method is effective in identifying and quantifying the target compounds in treated domestic sewage samples, providing a useful tool for environmental monitoring of these contaminants.
Key Word: *Pharmaceuticals; Wastewater Treatment Plants (WTP); Contaminants of Emerging Concern; Environmental Monitoring, GC-MS..*

I. Introduction

The lack of basic sanitation is one of the most complex obstacles affecting the quality of natural water resources. Inadequate management of domestic effluents often results in the discharge of untreated sewage into rivers, lakes, and springs, as shown by studies on contaminants of emerging concern (ECs), which negatively impact biodiversity, public health, and well-being. [1-4]

The use of gas chromatography coupled with mass spectrometry (GC-MS) has opened new avenues for elucidating the structure of matter, thereby increasing the analytical power in chemistry and allowing the detection of low concentrations of these compounds in surface and groundwater. [5-7]

A survey conducted by the Federal Council of Pharmacy (CFF) in collaboration with the Datafolha research institute found that 77% of Brazilians reported self-medicating, including with prescription drugs. Even after consulting a healthcare professional for a diagnosis, many do not follow the prescribed medication instructions. Nearly half of this population self-medicates at least once a month, and 25% do so daily or at least once a week. Another study by the Federal Council of Pharmacy observed that analgesics were the second most sold class of drugs between 2021 and 2020, second only to antidepressants. [8,9]

Several studies have reported the presence of amitriptyline, caffeine, and ibuprofen in environmental samples, with their presence correlated with high consumption by the population and the detection of these compounds described in other studies in wastewater samples. [10-15]
These compounds are only partially removed in conventional wastewater treatment plants (WTPs), primarily due to their physicochemical properties, which allow them to penetrate biological barriers easily, exerting

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pharmacological effects even at low concentrations. They are widely dispersed in aquatic ecosystems and can contaminate aquifers when released directly or indirectly into the soil. [6]

The pollution caused by these pharmaceutical products has opened a new field of research on pharmaceuticals in the environment, which may be as harmful or even more so than the widely discussed plastics, waste, and industrial residues. A study published in the journal Environmental Toxicology and Chemistry revealed that 43.5% of the 1,056 rivers analyzed worldwide had high concentrations of pharmaceutical residues. The most frequent drug in the samples was carbamazepine (an anticonvulsant), followed by metformin (used to treat type 2 diabetes) and caffeine (a stimulant and lifestyle chemical), all detected in more than half of the monitored systems. [16]

Considering the above, the aim of this study was to optimize an analytical method for the simultaneous determination of pharmaceutical residues, specifically ibuprofen (IBU), caffeine (CAF) and amitriptyline (AMI), using solid phase extraction (SPE) for preconcentration and clean up, followed by silylation derivatization and GC-MS analysis.

II. Material And Methods

Reagents

For the development of the study, the following standards were used: amitriptyline hydrochloride 100 mg - DR. EHRENSTORFER® Germany (Carvalhaes® https://www.carvalhaes.net) purity ≥ 98.00% GC; caffeine 1GR - Aldrich® 99.98% (Merck® https://www.sigmaaldrich.com) purity ≥ 99.00%; ibuprofen 1GR - ACROS® Belgium (Carvalhaes® https://www.carvalhaes.net) purity ≥ 99.00%. The analytes were selected due to the high consumption of drugs containing these active ingredients by the population and the detection of these compounds described in other studies in wastewater samples. [10-15]

Stock solutions of each standard were prepared using acetonitrile (ACN), with a purity grade of 99.98% (Merk® https://www.sigmaaldrich.com). From these solutions, a Stock Standard Mixture (MP-10 mg L^{-1}) was prepared. Working solutions were prepared by diluting this solution in Dichloromethane (DCM-HPLC PLUS), with a purity grade of 99.5% Êxodo Científica® (https://www.glasslab.com.br). Derivatization was performed using N-Methyl-N-trimethylsilylyltrifluoroacetamide - MSTFA (Merk® https://www.sigmaaldrich.com). Solid-phase extraction (SPE) cartridges OASIS HLB (Hydrophilic-Lipophilic Balanced) 500 mg/6 mL were purchased from Waters Corp® (https://www.waters.com). For conditioning the SPE cartridges, methanol with a purity grade of 99.80% was used, acquired from Synth® (https://www.lojasynth.com). Acetone with a purity grade of 99.80% was purchased from Merk® (https://www.sigmaaldrich.com). Concentrated hydrochloric acid (HCl) and sodium hydroxide (NaOH) were also purchased from Merk® (https://www.sigmaaldrich.com). Anhydrous sodium sulfate (Na_2SO_4), used for drying organic extracts, with a purity grade ≥ 99.00%, was acquired from Honeywell/Fluka®, Germany (Carvalhaes® https://www.carvalhaes.net).

Optimization of Chromatographic Method

To develop the chromatographic method, a standard of 1000 µg L^{-1} in dichloromethane (DCM) containing the three pharmaceuticals: amitriptyline (AMI), caffeine (CAF), and ibuprofen (IBU) was used.

The standard was derivatized with MSTFA, where the initial volume of the MP-10 mg L^{-1} was added to a vial, receiving 60 µL MSTFA and 0.5 mL of DCM, sealed, and heated to 80°C in a water bath for 30 minutes. The volume was then adjusted to 1.0 mL with DCM, and the mixture was separated in SCAN mode to determine the retention times and identify the main ions in the mass spectrum. For qualitative analysis, similarities greater than 80% were considered when compared to the NIST (National Institute of Standards and Technology) library. The GC-MS Solution 2.6 software (SHIMADZU, JAPAN) was used to process the results. [17,18]

The study was carried out using a gas chromatograph coupled with a mass spectrometer (Model QP2010 ULTRA – SHIMADZU). The oven was programmed as follows: initial temperature of 100°C (held for 2.0 min), ramped at 8.0°C min^{-1} to 300°C (held for 13.0 min), based on the studies of COLL et al., (2023)[18] and Ferreira & Sanches Filho (2020)[19], with an Rtx-5MS column, injector temperature at 280°C, injection (1µL) in splitless mode with 2.0mL min^{-1} purge. Ultra-pure helium was used as the carrier gas at 1.0 mL min^{-1}. The interface temperature was set at 280°C, with an ion source temperature of 200°C, an ionization energy of 70 eV, and a scan range from 40 to 550 m/z. [18,19]

Based on the data obtained from the SCAN mode analysis (scan mode), the method for analysis in SIM mode (single ion monitored) was developed. Under these conditions, calibration curves were constructed in two ranges: from 5 to 100 µg L^{-1} and from 100 to 2000 µg L^{-1}.

The significance analysis of the angular and linear coefficients was obtained using Statistica® software (STATSOFT, USA), with a significance level of 5% and a p-value below 0.05. A low p-value provides strong evidence that the model obtained is statistically significant. [18-21]

The accuracy of the results is largely dependent on the data's normality. To assess whether the responses were normally distributed, the Shapiro-Wilk normality test was employed. [20]

To assess the model's fit to the experimental data, analysis of variance (ANOVA) was performed. This involved examining the coefficient of determination (R^2), the linear correlation coefficient (r), and conducting Fisher's F tests. Furthermore, the relative error between the model's predicted values and the observed experimental data was calculated, as illustrated in Equation 1.

$$(\%)E = (Ev-p)/Ev \times 100 \qquad (1)$$

Where: (%)E: error in percentage; Ev experimental value; P: predicted value.

For the validation of the chromatographic method, the following parameters were used: linearity, precision, accuracy, selectivity, limit of detection (LOD), and limit of quantification (LOQ). Precision studies were determined by calculating the relative standard deviation (RSD) of the areas, expressed as a percentage, obtained from repeated analyses of the standards at 50 µg L-1 and 1000 µg L-1 (n=7). Accuracy was evaluated by the relative error (RE%), considering the values of 50 µg L-1 and 1000 µg L-1 as the correct values.

The detection limit (LOD) and quantification limit (LOQ) were determined by analyzing the signal corresponding to the average baseline area in the chromatograms of blank samples. This analysis was performed using a minimum of 50 baseline areas for each ion selected for quantification, focusing on the retention time windows specific to each compound. Taking ten times the standard deviation for LOQ and three times the standard deviation for LOD, each divided by the slope of the calibration curves.[23,22]

Selectivity was evaluated through the analysis of treated domestic sewage samples fortified with 50 µg L-1 (FTDS), and the matrix effect was also assessed through recovery studies. The matrix in this study was generated from the LLE extraction of 100 mL of treated domestic sewage obtained from the sewage treatment plant (STP – Fragata 31°45'09,82" S / 52°21'30,64" W), Pelotas-RS (Brazil), using the EPA method (1698). In 100 mL of the sample, 10% NaCl was added, followed by LLE with three portions of 15 mL of DCM. The extracts were pooled, and residual water was retained in anhydrous sodium sulfate columns. The DCM extracts were concentrated under a gentle N_2 flow to less than 1.0 mL, derivatized with MSTFA, and the volume was corrected to 1.0 mL before being analyzed by GC-MS (SCAN/SIM mode). [18,24]

Blanks were prepared using 60 µL MSTFA and DCM, in the same manner as the standards.

Study of the Solid Phase Extraction (SPE) Method

For the development of the SPE method, 100.0 mL of synthetic aqueous samples (SAS) (ultrapure water fortified with 10 µg L-1 of the three analytes), 100 mL of fortified treated domestic sewage (FTDS), and non-fortified treated domestic sewage (NTDS) were used. For the conditioning of the HLB cartridges, 3.0 mL of methanol was followed by 3 mL of ultrapure water at pH 2 (adjusted with NaOH/HCl 0.1 mol L-1). To evaluate pH and matrix effects, 100 mL of the FTDS sample with pH adjusted to 2.0 and 7.0 were percolated continuously under vacuum, followed by washing the cartridge with 20 mL of ultrapure water, drying under vacuum for 5 minutes, and eluting the analytes with 5 mL of DCM, based on previous studies by Negreira et al. (2009) and Bratkovics & Sapazhnikova (2011). The fractions were dried using anhydrous sodium sulfate, and their volume was subsequently reduced to 0.5 mL. Afterward, the extract was derivatized with MSTFA, and the volume was brought up to 1.0 mL using DCM.[24-26]

In parallel, blank analyses were performed using 100.0 mL of ultrapure water as described. Precision and accuracy were obtained through triplicate analysis of the SAS samples. Accuracy was assessed by recovery, considering the standard value of 1000 µg L-1 as 100%, in relation to the value found by the SPE/GC-MS method, was determined by measuring the percentage of relative standard deviation across triplicate samples.

The ions used for quantification were m/z: 165 and 58 (amitriptyline); 194 (caffeine); and 263 and 73 (ibuprofen). Matrix effects (ME) can lead to decreased sensitivity and compromised reproducibility, often due to co-eluting components from the matrix or through competition and saturation within the extraction phase. In this research, MEs were quantified using Equation 2, which compares recoveries achieved in the standard addition solution (SAS) with those obtained from fortified wastewater samples (FTDS) at pH levels 2 and 7 (Equation 2): [27]

$$ME (\%) = ((SAS - FTDS)/SAS \times 100 \qquad (2)$$

The optimized method was extended beyond the effluent wastewater from the WTP-Fragata (NTDS) to include samples gathered from the Santa Bárbara and Pepino stormwater drainage channels, both of which are tributaries feeding into the São Gonçalo canal.

III. Results And Discussion

Optimization of the Chromatographic Method

Figure 1 shows the comparison between the chromatograms obtained from the SCAN mode analysis for the standard solution of the compounds at 1000 µg L-1, both derivatized and non-derivatized.
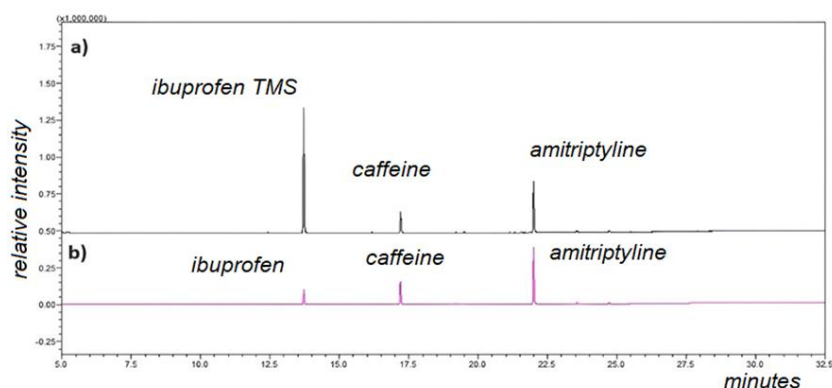

Figure 1: Chromatogram of 1000 µgL-1 standard solution in SCAN mode, derivatized (a) versus non-derivatized (b).

The presence of peaks corresponding to the three analytes in the standard indicated the complete derivatization of IBU. For this analyte, there was practically no change in the retention time (RT 13.705 min) due to derivatization. However, an improvement in peak symmetry and an increase in the response factor were observed, resulting in lower detection limits and more sensitive and precise analyses. Derivatization facilitates greater fragmentation, allowing for clearer identification of the compounds. IBU contains an acidic OH functional group, which is derivatizable and can add a trimethylsilyl (TMS) group to the molecule. This reduces polarity, improves interaction with the stationary phase (polydimethylsiloxane with 5% phenyl groups), and minimizes thermal degradation of the hydroxyl (OH) functional group. The silylation reaction of IBU can be seen in Figure 2. [18,28,29]
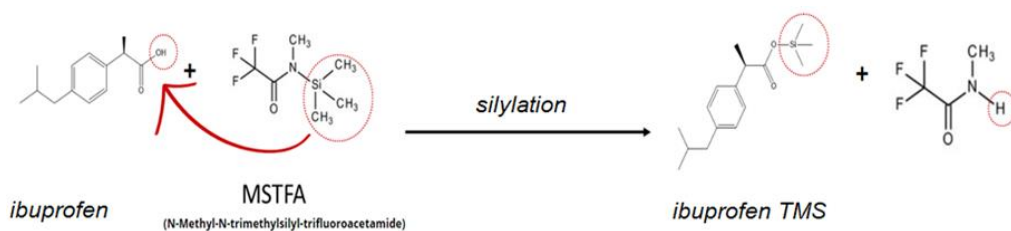

Figure 2: Silylation reaction of ibuprofen with MSTFA. Source: the author, structures Chem3D Pro 12.0.
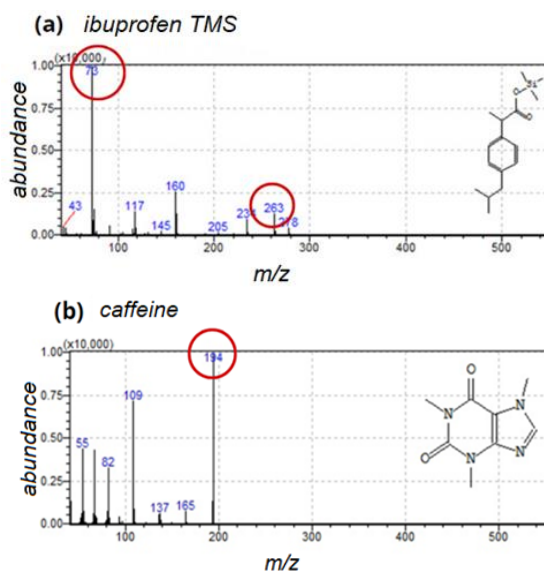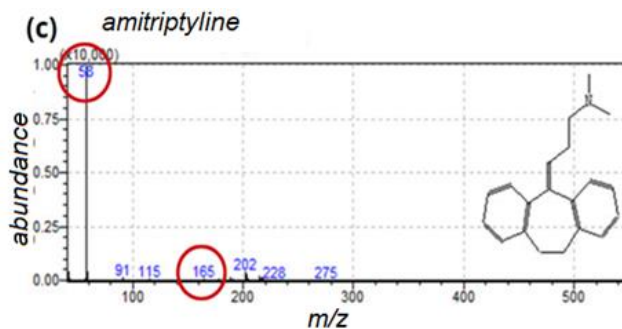
Figure 3: Mass spectra of ibuprofen TSM (derivatized) (a), caffeine (b) and amitriptyline (c) in SCAN mode highlighting their main fragmentation ions.

Figure 3 presents the mass spectra for the analytes. In the mass spectrum of IBU-TMS (Fig. 3-a), we observe the molecular ion 278 [M]+ (with lower intensity), the ion 263 [M−15]+ corresponding to the loss of a methyl group, and the fragment m/z 73 characteristic of the trimethylsilyl derivative $Si(CH_3)_3+$ (as the base ion). Caffeine (b) features the molecular ion itself (m/z 194) as the base ion, characteristic of compounds exhibiting aromaticity30. The strong signal intensity of the molecular ion is a result of its exceptional stability, given that it comprises four nitrogen atoms and two oxygen atoms with lone pair electrons. These non-bonding electrons facilitate the conversion into the detectable molecular ion (M- C=O+), thereby amplifying the signal. (Figure 4).
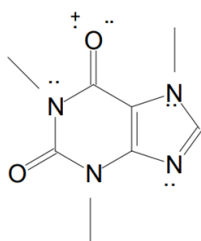
**Figure 4:** Molecular ion (m/z 194). Source: the author, structures Chem3D Pro 12.0.

In amitriptyline (c), the ion m/z 58 appears as the base ion corresponding to the fragment m/z C3H8N+.[31]

To increase sensitivity and avoid interference from overlapping peaks present in the sample matrix, and based on the mass spectra obtained in SCAN mode, time windows and confirmation and quantification ions for SIM mode analysis were defined, as presented in Table I.

Table no 1: Ions defined for the Time Window used for SIM mode.

Analyte	Analyte Window (min)	m/z quantification	m/z confirmation
IBU-TMS	5 – 20	73; 263	160; 263 e 278
CAF	5-20	194	109 e 82
AMI	20-39	58;165	91; 202; 215

Figure 5. Chromatogram obtained under the conditions outlined in Table I.
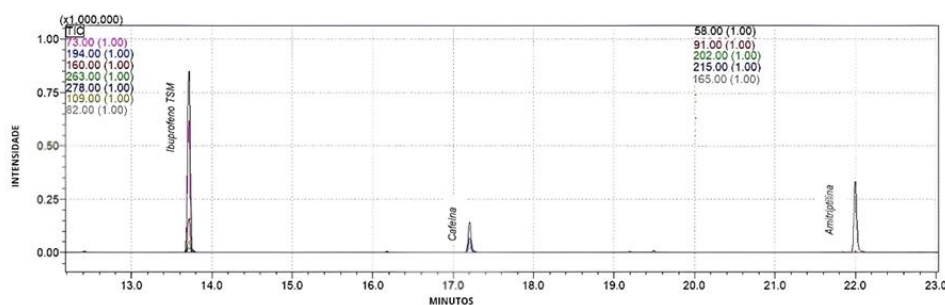
Figure 5: Chromatogram in selected ion monitoring mode for the analytes (mode – SIM).

Method Validation

Table II presents the performance characteristics of the chromatographic method in SIM mode. The results indicated a normal distribution, with Shapiro-Wilk test W values ranging from 0.888 to 0.900 in the low

concentration range and from 0.889 to 0.898 in the high concentration range. These W values exceed the critical threshold of 0.818, which is defined for a significance level of 5% (n=8).

From the analysis of the slope (a), it is observed that the method shows higher sensitivity for IBU at ion 73 compared to ion 263, due to the greater abundance of the fragment, a similar behavior is noted for amitriptyline (58 and 165), observed in both concentration ranges evaluated. Although the slopes for ions 73 (IBU) and 58 (AMI) were higher, ions 165 (AMI) and 263 (IBU) were also evaluated for their greater specificity, eliminating possible interference from co-eluting matrix compounds that might present the same fragments, thus enhancing selectivity.

Table II illustrates that the low p-values validate the significance of the regression coefficients, while the calculated F value for the ratio between the mean squares (MS) of regression and the MS of pure error surpassed the tabulated F value, considering the degrees of freedom (1:2) for all analytes. This result confirms the existence of a linear relationship between the two variables. Additionally, the F value derived from the ratio of the MS of lack of fit to the MS of pure error was lower than the corresponding tabulated F value, with degrees of freedom (4:2), indicating that the model does not exhibit a lack of fit. The coefficients of determination explain more than 99.9% of the overall result. Considering these data, the models can be considered linear, significant, and predictive for all analytes. The equipment demonstrated a linear response with correlation coefficients exceeding 0.9.

The accuracy and precision were acceptable for all analytes, with ER% between -0.4% and -4.9% and RSD% between 0.2% and 3.8% for both concentration ranges. These were evaluated in the µg L^-1 range, which allows for RSD% up to 20% and ER% up to 15%. The precision is comparable to the RSD values described by Melchor-Martínez et al. (2021)[32] for AMI. The cited authors used LC-MS/MS equipment, which is less available in environmental analysis laboratories compared to GC-MS. [22,32]

Both the RSD values calculated for the repeatability of the derivatization process (<7.2%) and the relative errors obtained from analysis at levels of 50 and 1000 µg L-1 (<13.2%) met the analytical quality requirements for the working range (µg L-1), with RSDs < 20% and ER < 15%, according to IMETRO (2016).[22]

The values found are consistent with those described by Sanches Filho et al. (2024)[24], Ferreira and Sanches Filho (2020)[19], and Coll et al. (2023)[18] for pharmaceutical analysis by SPE/GC-MS and sewage samples, and similar to those described by Peña-Alvarez & Castillo-Alanis (2015)[5]. The LD values indicate the need for pre-concentration steps for levels typically found in real samples. The relationship between the fragment abundance used for quantification and the LD values and sensitivity is observed for both IBU and AMI. [18,19,24,33]

Selectivity was verified by analyzing the quality of the mass spectra acquired in SCAN mode, where similarities exceeded 80%. Matrix effect was evaluated through recoveries, which remained within the range of 60 to 115%, with only IBU-TMS - 73.00 presenting a value above this range, indicating a possible matrix effect. [22]

Table no 2: Parameters for validation of analytical methodology.

Low curve	IBU-TMS-263.00 5 a 100 µg L⁻¹		IBU-TMS-73.00 1 a 100 µg L⁻¹		CAF-194 1 a 100 µg L⁻¹		AMI-165.00 20 a 200 µg L⁻¹		AMI-58.00 1 a 100 µg L⁻¹	
Coefficient	Value	p	Value	p	Value	p	Value	p	Value	p
b	10.25819	0.002816	264.3286	0.0001119	95.82034	0.028024	54.2	0.000018	207.2335	0.039834
a	6.35373	0.0000003	69.5855	0.0000001	24.12521	0.000202	3.1	0.00001	38.0520	0.000550
R²	0.99979		0.99997		0.9865		0.992		0.99008	
r	0.999895		0.99985		0.993227		0.996		0.995028	
adj	0.99975		0.99997		0.9842		0.991		0.98843	
$F_{Calculated}$ model	309470.2	0.0000003	1325686	0.0000001	4944.667	0.000202	3061.228	0.000013	1816.370	0.000550
$F_{calculated}$ lack of fit	15.9	0.059955	9.4	0.099032	16.417	0.058240	9.0	0.000304	4.049	0.207768
Freference (95% 1.2)	18.513				Freference (95% 4.2)	19.247				
RSD%*	0.3		0.2		2.5				3.8	
RSD%**	2.4		4.1		4.8				1.9	
Wcalculated	0.888		0.890		0.892		0.927		0.900	
RE%*50 ug L-1	-0.9		-0.4		-7.2				-0.4	
RE%**50 ug L-1	-7.4		4.0		7.8				7.7	
Matrix Effect % Recovery ±RSD %	79.9±4.8		136.4±8.2		114.7±5.8		114.3±9.0		139.0±12.7	

Range 100 to 2000 µg L⁻¹ (High Curve)	IBU-TMS 263.00		IBU-TMS 73.00		CAF 194		AMI 165.00		AMI 58.00	
Coefficient	Value	p	Value	p	Value	p	Value	p	Value	p
b	-392.900	0.008583	-210.940	0.00965	304.3494	0.0395836	-16.6438	0.041926	-427.77	0.0288516
a	27.340	0.0000002	342.088	0.0001522	44.3856	0.000039	0.7880	0.0004200	144.03	0.000413
R^2	0.999		0.993		0.998		0.991		0.992	
r	0.999		0.996		0.999		0.995		0.996	
adj	0.999		0.992		0.997		0.989		0.990	
$F_{Calculated}$ model	580750.1	0.0000002	6569.622	0.0001522	25165.42	0.000040	2379.238	0.0004200	2422.699	0.000413
$F_{calculated}$ lack of fit	12.8	0.073298	13.771	0.0684599	18.84	0.050812	6.559	0.135153	5.795	0.150693
$F_{reference}$ (95% 1.2)	18.513				$F_{reference}$ (95% 4.2)	19.247				
RSD%*	0.2		2.1		1.0		3.3		3.2	
RSD%**	2.9		4.1		2.3		4.6		2.8	
$W_{calculated}$	0.898		0.894		0.889		0.892		0.892	
RE %* 1000 ug L⁻¹	-0.5		-4.9		-2.3		1.1		3.4	
RE%**1000 ug L-1	-12.5		-3.7		7.5		-13.2		-12.2	
Matrix Effect % Recovery ±RSD %	83.4±20.0		109.1±14.2		98,9±6.9		117,9±8,9		123.3±4.7	
LOD/LOQ (µg L⁻¹)	0.3/1.1		0.1/0.2		0.2/0.6		2.0/6.6		0.3/0.9	

a: Slope coefficient; b: Intercept coefficient; RSD%: Relative standard deviation for repeated measurements on the equipment; RSD%: Relative standard deviation for the repetition of the derivatization procedure (n=7); ER%: Relative error based on the observed and predicted values from the statistical model; ER%: Relative error expressed as a percentage for the derivatization and analysis procedure (n=3) at 50 µg L⁻¹ and 1000 µg L⁻¹; LOD: Limit of detection; LOQ: Limit of quantification. F-reference for regression (1,2) = 18.513; F-reference for regression (4,2) = 19.242, according to the F-Fisher table at a 95% confidence level; the critical W value at a 5% significance level (n=8) = 0.818; R^2: Coefficient of determination; r: Correlation coefficient; adj: Adjustment coefficient.

Table no 3: Parameters of merit for the SPE/GC-MS method.

Analytes	RT (min)	m/z quantification	Recovery (%)- distilled water fortified pH 2	RSD %	Recovery (%) Fortified Sewage pH2	RSD %	EM	Recovery (%) Fortified Sewage pH7,0	RSD %	ME (%)	LoD ug/L	LoQ ug/L
IBU	13.707	263.00	73.4	7.4	61.1	6.1	16.8	7.9	11.3	89.2	0.005	0.016
IBU	13.705	73.00	78.8	9.8	41.4	10.9	47.5	5.6	10.2	92.9	0.015	0.048
CAF	17.172	194.00	110.7	3.1	89.0	1.4	19.6	88.0	13.6	20.5	0.014	0.046
AMI	22.003	165.00	25.4	5.0	30.8	18.4	-19.6	ND	ND		0.025*	0.080*
AMI	22.003	58.00	3.0	29.0	15.2	13.1	-406.2	299.8	28.8	9893.5	0.004	0.012

RT: retention time; %RSD: relative standard deviation; ME(%) matrix effect; LoD: Limit of detection of the SPE/GC-MS method; LoQ: Limit of quantification of the SPE/GC-MS method; * considering an average recovery of 25%.

Study of the Solid Phase Extraction (SPE) Method

Figure 6 presents the SIM mode chromatogram of the compounds eluted from the ASA using DCM.
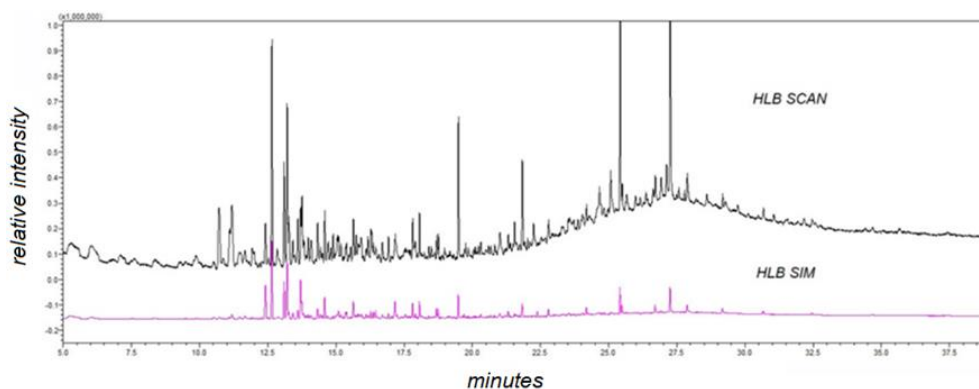
Figure 6: Chromatograms in SCAN vs. SIM mode of FTDS eluted with DCM

From the spectra obtained after analyzing the elution of FTDS from HLB cartridges, it can be observed through the similarity (>80%) of the mass spectra that both ibuprofen and caffeine were not affected by the compounds eluted from HLB and the matrix by DCM, demonstrating the method's selectivity for these compounds. However, amitriptyline did not meet validation requirements, and the compound could not be identified in the HLB eluate using SCAN mode analysis, as confirmed by the low recovery levels presented in Table III.

This situation can be explained by the high $LogK_ow$ (octanol-water partition coefficient) value of this compound (4.5) compared to ibuprofen (3.7) and caffeine (0.07), which restricts the compound's interaction with the HLB phase at this pH. $LogK_ow$ is an important physicochemical parameter inversely related to the chemical's solubility in water. High $LogK_ow$ values (above 4) indicate high hydrophobic properties with a greater tendency to associate with solid organic matter, while $LogK_ow$ < 2.5 indicates lower hydrophobicity with lower adsorption potential. [34]

The analysis underscores the significance of addressing the matrix effect on peak intensity, as matrix-induced suppression or enhancement can lead to either a decrease or an increase in chromatographic peak intensities. Specifically for amitriptyline, the method failed to achieve acceptable precision, highlighting the need for a more thorough evaluation of matrix effects during method validation, as well as careful consideration of the pH used in the pre-concentration process. [35]

For IBU-TMS (263, 73) and CAF, recoveries in ASA at pH 2 align with method validation requirements, consistent with results described by Gros et al. (2006), where recovery ranged from 99% in surface waters to 90% in raw sewage and 63% in treated sewage using HLB. Benotti et al. (2007) reported 64% recovery for caffeine, lower than the 88% for FTDS samples in this study. It is observed that pH 2.0 in FTDS favored recoveries for both IBU-TMS and AMI, while CAF remained with similar recoveries of 90%. However, it was possible to verify through matrix effects (EM) that ions m/z 73 and m/z 58 (for IBU and AMI) are subject to interference, confirming the use of ions 263 and 165 as more selective. RSDs below 20% for the SPE/GC-MS method at pH 2, except for ion 58 for amitriptyline, characterize the method as precise. At pH 7.0, a strong matrix effect and reduced recoveries for both IBU and AMI were observed, likely due to competition from matrix compounds for the HLB sorptive groups. [22,36,37]

As reported by Wode et al. (2012), EM% values under 20% are typically regarded as insignificant and do not impact the overall analytical performance. [38]

Both IBU and CAF exhibited acceptable recoveries in the range of 60-115%, with precision below 21% for the 10 ppb (µg/kg) concentration range, chosen based on studies by Boogaerts et al., (2019) and a review by Morin-Crini et al., (2022), which provides a comprehensive review of emerging contaminant concentrations. These findings align with Sodré & Sampaio (2020), which reports recoveries of 79%, 91%, and 26% for IBU, CAF, and AMI, respectively, using pH 2.9. [28,35,39]

Chromatographic Characterization of Treated Sewage Sample by SPE

The implementation of the optimized method under optimal conditions enabled the quantification of compounds in samples from the Pelotas region, RS, Brazil, using SIM mode, as detailed in Table IV. It also enabled screening of the main classes of compounds present in treated domestic sewage samples from the WTP effluent.

Table no 4: Levels of analytes (µg L^{-1} ± RSD%) found in treated domestic sewage on different sampling dates.

Sampling dates	20/05/2023	20/05/2023	17/11/2023	17/06/2024*
Sampling site Analytes/ Ion	Santa Barbara canal	Pepino canal	Treated Sewage WTP-Fragata	Treated Sewage WTP-Fragata
IBU 263	0.21±16.0	0.46±18.6	1.75±15.4	1.23±14.3
CAF 194	1.96±13.6	1.06±20.2	0.83±9.2	1.4±19.0
AMI 165	ND	ND	ND	1.16±19.0

WTP:Wastewather treatment plant: IBU: Ibuprofen; CAF: Caffeine; AMI: Amitriptyline. *Sampling carried out after the flood of May/2024. Santa Barbar Canal - Output: 31°45'09.82" S / 52°21'30.64" W; ** Canal Pepino Canal: Output: 31°47'23"S / 52°20'56"W e ** WTP-Fragata - Saída: 31°46'54"S / 52°19'30"W.

It was possible to quantify IBU and CAF in all samples, while AMI was only quantified in the treated sewage collected on 16/06/2024, likely due to low recovery in the SPE procedure.

The sampling points at Santa Bárbara and Pepino, which are stormwater drainage channels passing through the urban areas of Pelotas, are situated near the entrance of the São Gonçalo channel. These channels not only receive urban runoff but also untreated domestic sewage. The levels detected in both the drainage channels and sewage effluents during the collections align with the findings reported by Peña-Guzmán et al. (2019)[14] in wastewater (CAF 0.91 µg L^{-1} and IBU 2.90 µg L^{-1}) and in sanitary sewage (CAF 0.01 µg L^{-1} and IBU 0.90 µg L^{-1}), as well as Campanha & Souza (2015)[40] in surface waters (CAF 3.54 µg L^{-1}) and sanitary sewage (CAF 2.79 µg L^{-1} and IBU 1.38 µg L^{-1}). It is also observed that the AMI levels were corrected, considering a recovery of 30%, and were higher than those reported by Lajeunesse et al. (2012)[41] (AMI 0.046 – 0.283 µg L^-1) in wastewater in Canada and (AMI 0.015 – 0.759 µg L^{-1}) in wastewater in Belgium. [13,40,41]

Table V presents the composition of the treated sewage collected in November 2023 from WTP - Fragata and analyzed by SPE-HLB/GC-MS.

Table no 5: Main compounds determined in TDS: sample analyzed by SPE/GC-MS in SCAN mode (areas > 0.4%).

Compound	Relative Area	Function
Nonanoic acid	0.5	Acid
4-Phenylbutan-2-ol	0.6	Alcohol
NI	2.0	NI
2,6-di-tert-Butylphenol	2.5	Phenol
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	0.5	Ester
Dodecanoic acid	0.5	Acid
Tetradecanoic acid	1.8	Acid
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	5.0	Phthalate
n-Pentadecanoic acid	0.5	Acid
Hexadecanol	0.5	Alcohol
Hexadecanoic acid	26.0	Acid
Dodecanoic acid	0.9	Ester
Hexadecanoic acid, 2-methylpropyl ester	1.6	Ester
Octadecanol	1.9	Alcohol
cis-9-Octadecenoic acid	3.3	Acid
trans-9-Octadecenoic acid	0.7	Acid
Octadecanoic acid	13.8	Acid
Decyl oleate	1.0	Ester
Oleic acid, butyl ester	0.4	Ester
Octadecanoic acid, 2-methylpropyl ester	0.8	Ester
Eicosanol	0.7	Alcohol
Diisooctyl adipate	0.7	Ester
Dehydroabietic acid	0.6	Acid
Pentacosane	1.0	HC
Myristyl myristate	0.7	Ester
Hexacosane	0.6	HC
Bis(2-ethylhexyl) phthalate	2.7	Phthalate
Hexadecanoic acid, 2,3-dihydroxypropyl ester	1.8	Ester
Docosanoic acid	1.7	Acid
Heptacosane	1.5	HC
1,3-Benzenedicarboxylic acid, ester	0.9	Phthalate
Octadecanoic acid, 2,3-Dihydroxypropyl ester	2.3	Ester
Octacosane	1.5	HC
Decanedioic acid, bis(2-ethylhexyl) ester	1.0	Ester
Triacontane	1.4	HC
Heneitriacontane	1.2	H
Coprostan-3-ol	1.9	CPPF

Cholesterol	2.5	CPPF
(3β,5α)-Cholestan-3-ol	0.6	CPPF
Cholestan-3-one, 1,2-ethanediol acetal cyclic, (5α)-	1.1	CPPF
Beta-Sitosterol	0.4	CPPF

CPPP: cyclopentaneperhydrophenanthrene; HC: hydrocarbons; NI: not identified
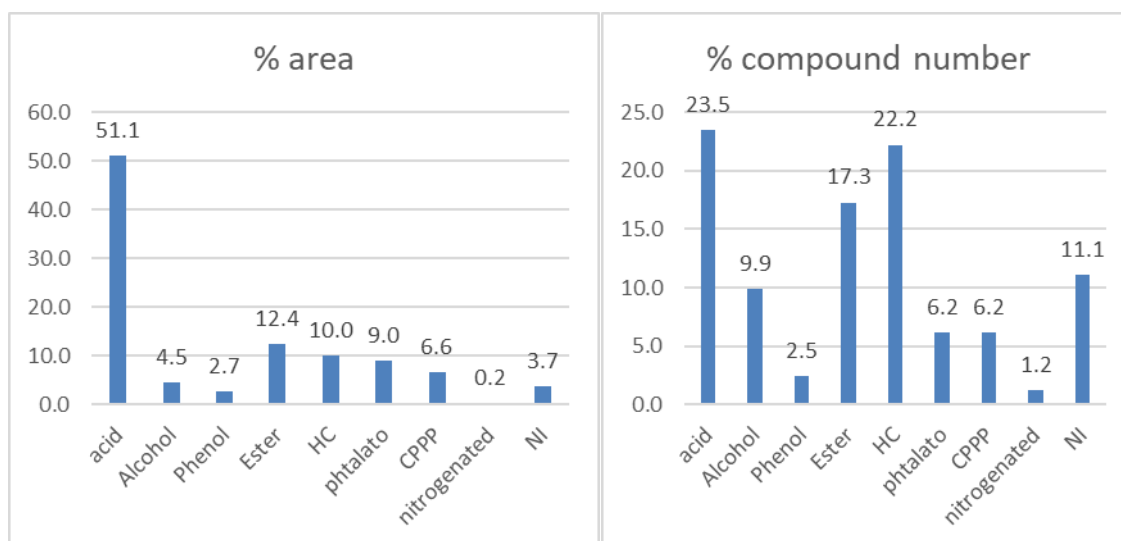

Figure 8: Percentage composition of area and number of compounds, according to chemical classes.

A major presence of organic acids (51.1%) was observed, followed by esters (12.4%) and hydrocarbons (10.0%). Although they represent more than 50% of the area, fatty acids account for 23.5% of the compounds, hydrocarbons 22.2%, and esters 17.3%. Alcohols, although representing only 4.5% of the area, make up 9.9% of the compounds. Only 11% of the compounds were unidentified by the method.

With emphasis on the acid group, hexadecanoic acid (26%), also known as palmitic acid, was highlighted as one of the main saturated fatty acids, present in both animal-derived (butter, cheese, beef, etc.) and plant-derived (palm oil) sources, followed by octadecanoic acid (13.8%), another abundant fatty acid in nature after palmitic acid. These results are consistent with previous studies at the same site by Coll et al., (2023)[18]. Compounds such as caffeine, ibuprofen, carbamazepine, and octocrylene were also identified in SCAN mode with relative areas below 0.4% after the wastewater treatment process. [18]

This can be attributed to the fact that WTP – Zona Fragata uses a stabilization lagoon system, which consists of biological treatment systems where organic matter stabilization is carried out by bacterial oxidation (aerobic oxidation or anaerobic fermentation) and/or photosynthetic reduction by algae. This operational mechanism can lead to the reduction of long-chain groups to their precursors. It is important to consider that the microorganisms present may have difficulties degrading some organic compounds compared to others. [42]

Acids may be more easily degraded by the microorganisms present, resulting in a decrease in their concentration throughout the treatment. On the other hand, compounds like hormones are more resistant to biological degradation and may persist in the treated effluent, leading to an increase in their concentration. These compounds come from various sources (cleaning products, cosmetics, pharmaceuticals, and industrial activities) and are present in significant quantities in the system. [43]

Seasonality of the samples should also be considered, as cooler temperatures tend to reduce microbial activity, as well as the potential undersizing of the system, where the inflow might exceed the station's capacity and other parameters might be below optimal levels.

IV. Conclusion

The refined analytical method, which integrates SPE using HLB cartridges with MSTFA derivatization followed by GC-MS analysis in SIM mode, demonstrated adequate selectivity, precision, and accuracy. The method's instrumental detection and quantification limits align with the standards set by regulatory bodies such as INMETRO (2016). With detection limits ranging from 0.004 to 0.028 µg/L and quantification limits from 0.012 to 0.080 µg/L, the method is well-suited for the determination of ibuprofen (IBU), caffeine (CAF), and amitriptyline (AMI) in aqueous matrices. It is also effective for preliminary screening of contaminants of emerging concern (CEs) in extracts from treated domestic wastewater samples, making it a valuable approach for monitoring these pollutants in wastewater treatment plants (WTPs).

MSTFA derivatization improved the response factor for ibuprofen in the GC-MS analysis, enhancing peak symmetry and increasing the response factor, resulting in lower detection limits and more sensitive and precise analysis.

The detection of these compound classes in treated wastewater samples indicates a clear necessity for enhanced treatment processes to effectively remove these persistent substances in WTPs. Their presence in treated effluent highlights the shortcomings of current treatment methods and underscores the risk of these contaminants entering the environment, potentially disrupting the ecological balance..

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