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"Application of Hybrid Nanocomposites to Enhance Extraction and Chromatographic Analysis of Antihistamines in Food and Water Samples"

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Abstract

A novel analytical approach was developed for the extraction and quantification of antihistamines, clemastine and cyproheptadine, in various food and water matrices using a hybrid nanocomposite, APTES-GO@MIL-101(Cr), in combination with ultrasound-assisted dispersive micro solid-phase extraction (D-μSPE) and HPLC-UV detection. The hybrid nanocomposite was synthesized through functionalization of graphene oxide with 3-aminopropyltriethoxysilane (APTES) followed by incorporation of MIL-101(Cr), and its structural and morphological features were confirmed using FT-IR, SEM, and EDX analyses. Key factors influencing extraction efficiency, including desorption solvent type and volume, ultrasonication time, sample pH, salt concentration, extraction time, and adsorbent dosage, were systematically optimized. Under the optimized conditions, the method exhibited excellent linearity over the range of 0.1–20 ng/mL for both analytes with correlation coefficients (r^2) exceeding 0.999. The limits of detection (LOD) and quantification (LOQ) were 0.04 and 0.134 ng/mL for clemastine, and 0.045 and 0.15 ng/mL for cyproheptadine, respectively. The method also demonstrated high precision with intra- and inter-day relative standard deviations (RSDs) below 5%, and the adsorbent could be reused for at least 30 extraction cycles with minimal loss in performance. Application to real samples, including milk, egg yolk, chicken meat, mutton, and water, resulted in recoveries ranging from 92% to 102%, confirming the robustness and reliability of the approach. This strategy provides a simple, rapid, sensitive, and environmentally friendly method for monitoring trace levels of antihistamines in complex food and environmental matrices.

Keywords: Hybrid nanocomposite, APTES-GO@MIL-101(Cr), Antihistamines, Dispersive micro solid-phase extraction, HPLC-UV

1. Introduction

One of the primary objectives of analytical chemists is to directly examine samples with minimal pretreatment. However, achieving rapid and straightforward analytical processes is often challenged by the need for high sensitivity, selectivity, and compatibility between the sample matrix and the analytical method. To overcome these limitations, appropriate sample treatment is essential. In recent years, two dominant trends have emerged in this field: the miniaturization of extraction techniques, leading to the development of microextraction methods, and the

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automation of sample preparation processes, both of which have facilitated their integration into routine laboratory practices.

Solid-phase extraction (SPE) is among the most widely applied sample preparation techniques, relying on the use of sorbent materials to isolate target compounds from complex matrices. Numerous types of solid sorbents [1] and extraction manifolds [2,3] have been developed to cover nearly all possible interactions with analytes. Nevertheless, when sorbents are packed into cartridges, the effectiveness of analyte–sorbent interactions is often limited by the flow rate used during percolation. This limitation becomes particularly critical when nanoscale sorbents are employed, as the resulting backpressure hinders sample passage. Furthermore, competition between interfering matrix components and active sorbent sites can negatively impact analyte recovery.

To address these drawbacks, dispersive solid-phase extraction (DSPE) has been introduced as an alternative to traditional SPE. In DSPE, the sorbent is directly mixed with the sample, thereby improving sorption kinetics through enhanced contact between the analyte and sorbent particles. This approach significantly improves extraction efficiency.

The QuEChERS method—an acronym for "Quick, Easy, Cheap, Effective, Rugged, and Safe"—is a well-established DSPE-based approach that has been widely commercialized due to its success in detecting various analytes, particularly in food samples. By using small amounts of sorbent (approximately 50 mg), DSPE allows the retention of interfering matrix components while preserving the analyte in the liquid phase, thereby enhancing selectivity and simplifying subsequent determination. Once the analytes are eluted from the sorbent, they can be subjected to instrumental analysis [5].

This miniaturized variation, referred to as dispersive micro-solid phase extraction (D-µSPE), utilizes sorbents in the low milligram range and provides an efficient, cost-effective, and environmentally friendly alternative to conventional extraction strategies.

1.1. Dispersion in Dispersive Micro-Solid Phase Extraction

The effectiveness of D-µSPE largely depends on the degree of sorbent dispersion within the sample matrix. Maintaining dispersion is particularly critical when working with sorbents prone to aggregation, such as carbon-based nanomaterials. Poor dispersibility at the initial stage can compromise the overall extraction efficiency. Since the polarity gradient between sorbents and sample matrices often hinders proper dispersion, external assistance or the use of dispersing agents is usually required.

1.2. External Energy-Assisted Dispersion

External energy sources, such as ultrasound or mechanical vortex agitation, have been widely employed to facilitate the dispersion of sorbent particles in liquid matrices. The resulting techniques are commonly referred to as ultrasound-assisted (UA-D-µSPE) or vortex-assisted (VA-D-µSPE). The extraction process typically involves introducing the sample–sorbent mixture into an extraction vessel, followed by exposure to ultrasound waves or vortex agitation for a defined period. After extraction, the sorbent is separated either by centrifugation or filtration. In the case of magnetic sorbents, an external magnetic field can be applied for rapid retrieval.

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Finally, analytes are eluted from the sorbent and subjected to instrumental analysis, often requiring re-dispersion during the elution step.

By promoting both analyte diffusion and sorbent dispersion, the application of external energy sources significantly accelerates the extraction process [6,7]. Nevertheless, the use of such energy often increases the temperature of the extraction vessel, which, if not properly controlled, may alter the extraction thermodynamics and even cause degradation of the analytes.

Owing to their beneficial effects across different extraction stages, ultrasound waves are frequently employed as an auxiliary energy source in sample preparation [8]. Ultrasound is particularly advantageous in D-SPE due to its ability to disrupt particle aggregation, thereby reducing particle size [9] and increasing the surface area available for sorption. Ultrasonic energy can be applied either through baths or probes, with probes being more effective since they concentrate the radiation. Compared with ultrasonic baths, the use of probes dramatically reduces the extraction time from several minutes [10,11] to just a few seconds [12,13], clearly demonstrating their strong influence on extraction kinetics.

In contrast, vortex agitation is considered a gentler alternative to ultrasonic treatment. Despite being less intense, it offers several practical benefits: simplicity, low cost, accessibility, and the capacity to process multiple samples simultaneously. Although ultrasonic baths can also handle several samples, the irradiation is often uneven across different regions. Vortex agitation is particularly attractive because its milder nature prevents the potential degradation of analytes, a drawback sometimes associated with ultrasound-assisted methods [14,15]. Thus, vortex agitation is especially suitable for sorbents that are readily dispersible. While its extraction kinetics are generally slower, they still fall within the minute range [16,17].

In 2016, Rajabi et al. introduced a novel approach termed *air-assisted dispersive micro-solid phase extraction*. The technique involves repeatedly aspirating and ejecting the sample–sorbent mixture using a 10 mL syringe, typically around 30 cycles, to achieve efficient contact between the two phases [18]. This process is conceptually similar to *dispersive pipette extraction (DPX)*, in which a modified pipette tip contains the sorbent and dispersion is achieved by aspirating and dispensing solutions. DPX can be performed manually or through autosamplers, leading to partial sorbent dispersion and enhanced extraction performance.

Another advancement in this area is magnetic solid-phase extraction, which simplifies sorbent recovery, often considered the most challenging step in D-SPE. In 2014, Benedé et al. proposed a further simplification named *stir-bar sorptive dispersive microextraction*. This method utilizes a magnetic sorbent in combination with a stir bar at variable stirring speeds, facilitating both sorbent dispersion and recovery [20].

2.1 Significance of the Study

In recent years, several chromatographic techniques have been reported for the quantitative determination of clemastine and cyproheptadine, mainly due to their associated risks, such as arthropathy in children and adolescents, seizures in certain patients, and environmental contamination caused by their release in hospital effluents. However, monitoring and identifying

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these compounds in real samples remain challenging because of their typically low concentrations.

Among the most effective strategies for pre-concentration and sample preparation is dispersive micro-solid phase extraction (D-µSPE). Consequently, the development of solid phases with high adsorption capacity toward clemastine and cyproheptadine is of significant importance. This study introduces a simplified approach using ultrasound-assisted dispersive micro-solid phase extraction based on APTES-GO@MIL-101(Cr) composite for the sensitive detection of clemastine and cyproheptadine in food and water samples.

2.2 Materials and Reagents

All reagents were of analytical grade, and double-distilled water was used throughout the experiments. Stock solutions (100 µg mL^{-1}) of clemastine and cyproheptadine (Merck, Darmstadt, Germany) were prepared by dissolving 0.01 g of each compound in 100 mL volumetric flasks. Working standard solutions were freshly prepared by serial dilution with distilled water prior to analysis.

The following chemicals were used: (3-aminopropyl)triethoxysilane (APTES), hydrofluoric acid (HF), terephthalic acid (TPA), and $Cr(NO_3)_3 \cdot 9H_2O$ (all purchased from Merck). Solvents including ethanol, methanol, ammonium hydroxide, and distilled water (HPLC grade, Merck) were employed for washing and synthesis procedures. Solutions of HCl and NaOH (Merck) were prepared in concentrations ranging from 0.01 to 1.0 mol L^{-1} to adjust the pH.

2.3 Instruments and Devices

The chromatographic analyses were carried out using a Knauer high-performance liquid chromatography (HPLC) system equipped with a four-solvent pump (RS-4, REFCO, Switzerland), a loop injector, and a K-2501 UV detector. Separations were achieved on a C18 reversed-phase column (250 mm × 4.6 mm) at a constant temperature of 30 °C. The UV detector was set at 280 nm, and the mobile phase consisted of 82% acetonitrile and 18% phosphate buffer (0.01 M), delivered at a flow rate of 0.5 mL min^{-1}.

Fourier-transform infrared (FTIR) spectra were recorded using a PerkinElmer Spectrum RX1 spectrometer with KBr pellets. The pH of solutions was monitored using a Metrohm 827 pH Lab meter equipped with a combined glass electrode (Herisau, Switzerland). The morphology of the synthesized particles was examined by scanning electron microscopy (SEM) using a Hitachi S-5200 microscope operated at 30 kV and 30 µA.

Centrifugation was performed using a HERMLE bench centrifuge (2206A, Germany). For ultrasound-assisted extraction, a SONOREX DIGIPLUS DL 102 H ultrasonic bath (Bandelin Electronic, Berlin, Germany) operating at 35 kHz with adjustable power output (20–100%) was employed.

2.4 Synthesis of Graphene Oxide (GO)

Graphene oxide (GO) was synthesized following the modified Hummers' method [40]. Briefly, 70 mL of concentrated H_2SO_4 was mixed with 3 g of graphite powder in an ice bath. While maintaining the suspension below 20 °C, 9 g of $KMnO_4$ was gradually added under vigorous

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stirring. The reaction mixture was then transferred to an oil bath at 40 °C and stirred for 30 minutes.

Subsequently, 150 mL of distilled water was added, and the mixture was further stirred at 95 °C for 15 minutes. Upon the addition of 500 mL water followed by 15 mL of H_2O_2 (30%), the solution color shifted from dark brown to yellow-brown. The resulting product was filtered and washed with 250 mL of a 1:10 HCl aqueous solution to remove metal ions. Finally, the solid residue was air-dried and dispersed in distilled water to obtain 600 mL of graphene oxide aqueous suspension.

2.5 Synthesis of APTES-GO

Graphene oxide (GO, 0.4 g) was dispersed in 5 mL of dimethylformamide (DMF). To facilitate the reduction of GO, 3 mg of sodium acetate was added to the suspension, and the mixture was stirred vigorously at 300 rpm under 50 °C for 2 h. Subsequently, 0.5 mL of (3-aminopropyl)triethoxysilane (APTES) was added dropwise to the reaction system. The suspension was purged with nitrogen to maintain an inert atmosphere and then stirred at 60 °C for 24 h. The final product was collected and dried at room temperature, yielding the functionalized APTES-GO material.

2.6 Synthesis of APTES-GO@MIL-101(Cr)

The composite APTES-GO@MIL-101(Cr) was synthesized via in-situ growth of MIL-101(Cr) on functionalized graphene oxide. Initially, MIL-101(Cr) was prepared by dissolving terephthalic acid (TPA, 280 mg) in 40 mL of a DMF/EtOH (1:1 v/v) solution. Separately, $Cr(NO_3)_2 \cdot 6H_2O$ (520 mg) was dissolved in 20 mL of deionized water and added to the TPA solution under continuous stirring.

Meanwhile, 20 mg of pre-synthesized GO–NH_2 was dispersed in 10 mL of deionized water using ultrasonic treatment for 30 min to obtain a stable aqueous suspension. The GO–NH_2 dispersion was then added to the TPA/Cr mixture under stirring. The reaction mixture was heated at 70 °C for 4 h to facilitate crystal growth.

The resulting product was collected by filtration, washed three times with 30 mL of DMF, and subsequently immersed in $CHCl_3$ for three days. During this period, the solvent was decanted and replaced three times to ensure proper activation. Finally, the solid product was dried in air, yielding the APTES-GO@MIL-101(Cr) composite.

2.7 Characterization of APTES-GO@MIL-101(Cr) Composite

The structural and morphological properties of the synthesized composites were analyzed by FT-IR spectroscopy, SEM, and EDX.

FT-IR analysis (Figure 2.1) confirmed the successful functionalization of GO with APTES and the incorporation of MIL-101(Cr). In the FT-IR spectrum of APTES-GO, characteristic absorption bands were observed between 2850–3000 cm^{-1}, corresponding to C–H stretching vibrations of CH_2 groups, confirming the attachment of APTES chains. The bands at 1474 and 1440 cm^{-1} were attributed to NH and NH_2 bending vibrations, while the broad peak at 3430 cm^{-1} indicated N–H stretching. Peaks at 1048 and 1210 cm^{-1} were assigned to Si–O–C and Si–O–Si

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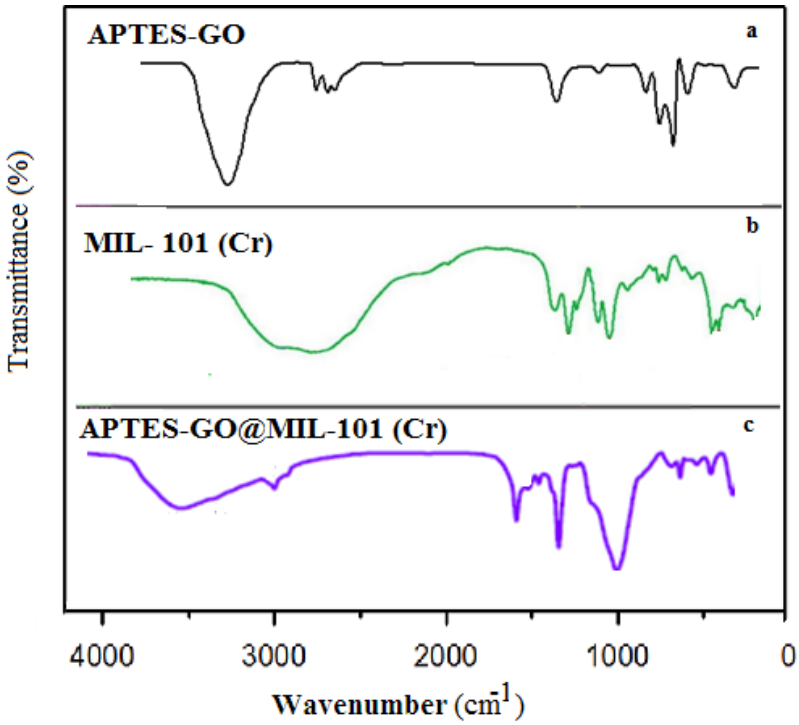
stretching, respectively, providing strong evidence for covalent functionalization of GO with APTES.

In the FT-IR spectrum of APTES-GO@MIL-101(Cr), additional peaks at 1442 and 1371 cm^{-1} were associated with benzene ring vibrations and symmetric stretching of carboxylate groups in the BTC linker, confirming the presence of the MIL-101(Cr) framework.

SEM analysis revealed distinct morphological features: pristine GO exhibited a sheet-like layered surface (Fig. 2.1a), while the composite (Fig. 2.1b) showed uniform and dense decoration of MIL-101(Cr) crystals over the GO surface, resulting in an increased surface area and enhanced sorption capacity.

EDX spectra (Fig. 2.3) further validated the successful incorporation of MIL-101(Cr) onto the APTES-functionalized GO surface, showing elemental signals corresponding to C, O, Si, N, and Cr. These combined results provide clear confirmation that the APTES-GO@MIL-101(Cr) composite was successfully synthesized with desirable structural and surface characteristics for efficient adsorption applications.



APTES-GO, MIL-101(Cr), and APTES@GO-MIL-101 FTIR spectra are shown in Figure 2.1, respectively.

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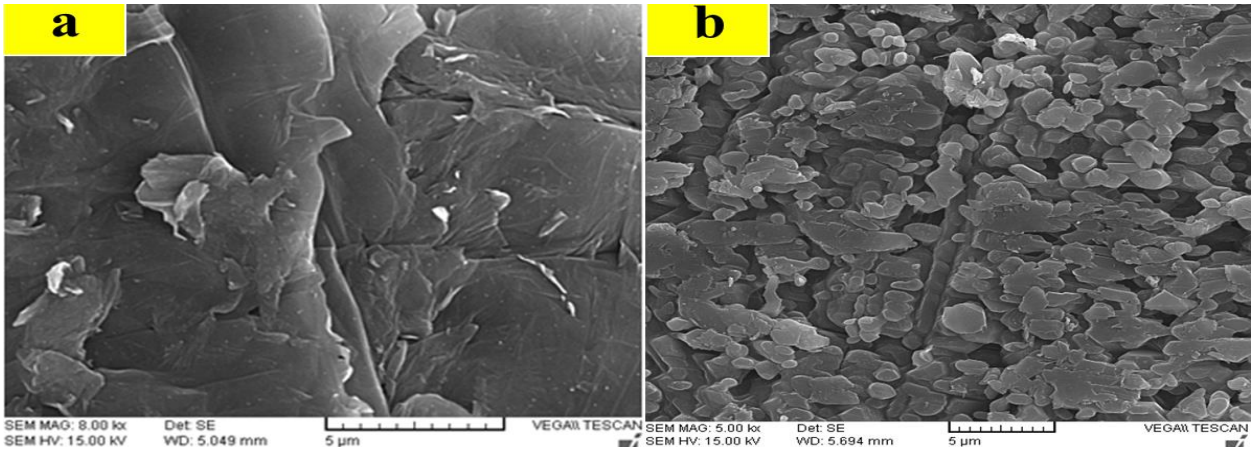

Fig.2.2. SEM image of (a) GO, (b) APTES@GO-MIL-101

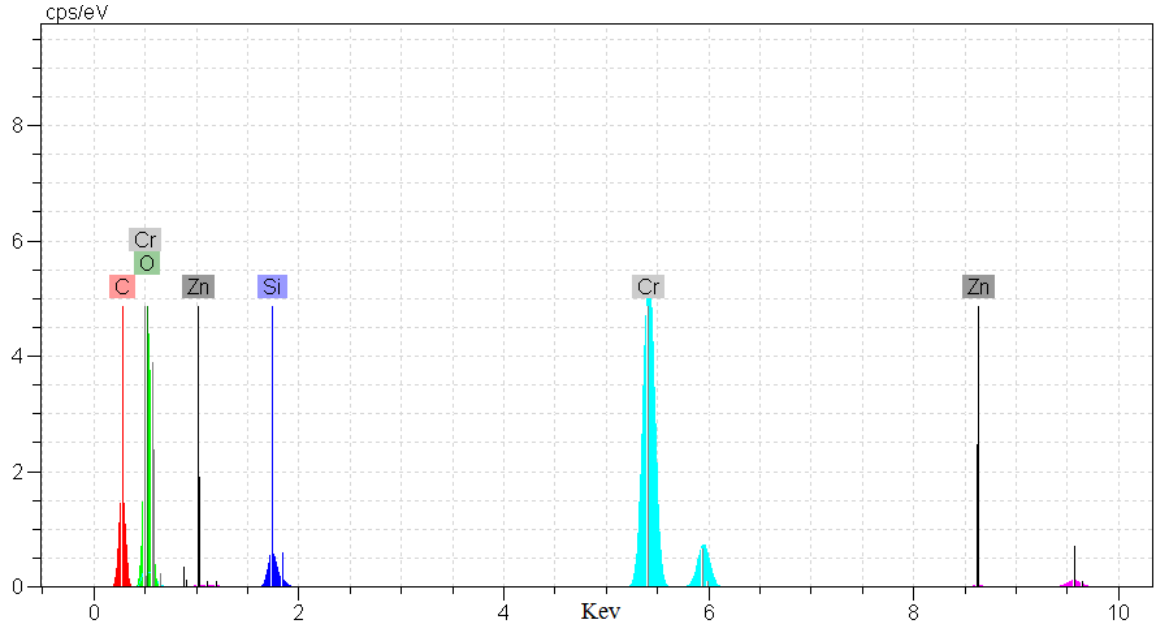

Fig. 2.3. EDS of APTES@GO-MIL-101 composites

2.8 Chromatographic Conditions

The chromatographic separation of clemastine and cyproheptadine was performed under optimized conditions using a C18 reversed-phase column. The mobile phase consisted of 0.01 M phosphate buffer (82%) mixed with pure acetonitrile (18%), delivered at a flow rate of 0.5 mL

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min^{-1}. The column temperature was maintained at 30 °C, and the injection volume was set to 20 µL. Detection was carried out at a wavelength of 280 nm.
Figure 2.4 illustrates the representative chromatogram obtained under these experimental conditions, confirming the suitability of the developed method for the simultaneous determination of clemastine and cyproheptadine.

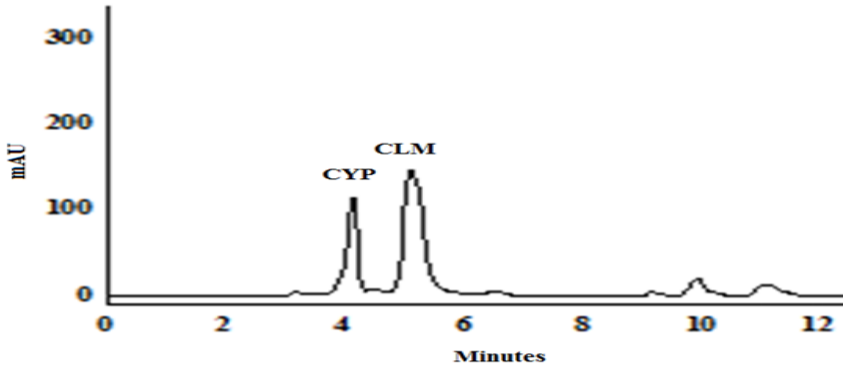

Fig. 2.4. Clemastine and cyproheptadine chromatograms

2.9 Analytical Procedure (Rewritten)

In the dispersive micro-solid phase extraction process, a 10 mL aliquot of the sample solution containing the target analytes, cyproheptadine and clemastine, was first transferred into a conical flask. The solution was adjusted to the required pH (6.0) in the presence of 8% NaCl. Subsequently, 0.01 g of the synthesized APTES-GO@MIL-101(Cr) composite was added as the adsorbent. To achieve uniform dispersion and to facilitate the interaction between the sorbent and analytes, the mixture was subjected to ultrasonication for 7 minutes. After this step, the adsorbent–analyte complex was separated, and the remaining supernatant was discarded.
For the desorption stage, 1.0 mL of an ethanol–water solution (1:1, w/v) was introduced as the elution solvent. The adsorbent was treated with ultrasonication for 10 minutes to ensure complete release of the retained analytes. The resulting solution was then filtered through a syringe filter to remove any particulate matter.
Finally, 20 µL of the purified extract was injected into the HPLC system under the following optimized conditions:

- Column temperature: 30 °C
- Mobile phase composition: phosphate buffer (pH 6.0, 82%) and acetonitrile (18%)
- Flow rate: 0.5 mL/min
- Detection wavelength: 280 nm

The overall extraction and detection workflow is illustrated in Figure 2.5, demonstrating the effective recovery and quantification of the analytes using HPLC-UV.

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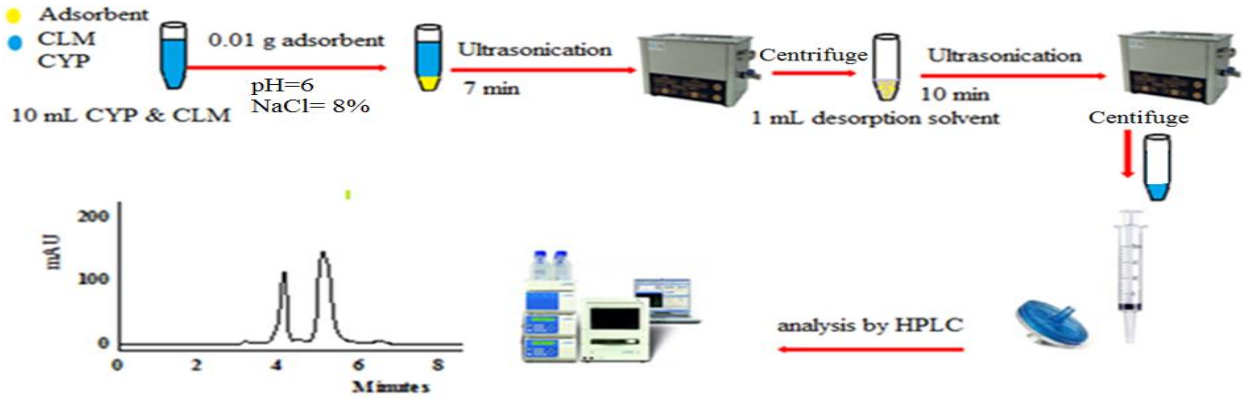

Fig. 2.5 Dispersive micro solid-phase extraction with ultrasound assistance using APTES-GO@MIL- 101(Cr) composite with HPLC-UV detection of clemastine and cyproheptadine

2.10 Improving Extraction Parameters

2.10.1 Effect of the Desorption Agent

The dispersive micro-solid phase extraction (D-µSPE) of cyproheptadine and clemastine using the APTES-GO@MIL-101(Cr) composite was optimized according to the procedure described in Section 2.9. A 10 mL working solution containing 10 ng mL^{-1} of each analyte was employed.

For effective recovery, the choice of desorption solvent is a crucial factor, as it should be able to efficiently release the adsorbed analytes while requiring minimal solvent volume. To evaluate this parameter, several solvents—including water, ethanol, methanol, acetone, acetonitrile, and their binary mixtures—were investigated as eluents for desorption.

The comparative results, summarized in Table 2.1 and Figure 2.6, clearly indicate that the binary solvent mixture water:ethanol (1:1, w/v) exhibited the highest extraction efficiency, as evidenced by the superior peak areas for both analytes. Consequently, this mixture was selected as the optimal desorption agent for subsequent experiments due to its ability to maximize both peak intensity and analyte resolution.

Table 2.1. Effect of the desorption agent on extraction efficiency

Clemastine (Peak Area)	Cyproheptadine (Peak Area)	Desorption Solvent
292,848	387,542	Ethanol
323,892	411,496	Water : Acetonitrile (1:1)
364,520	449,963	Methanol
429,754	468,721	Water : Methanol (1:1)

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Clemastine (Peak Area)	Cyproheptadine (Peak Area)	Desorption Solvent
479,368	351,759	Acetonitrile
559,851	582,886	Water : Ethanol (1:1)

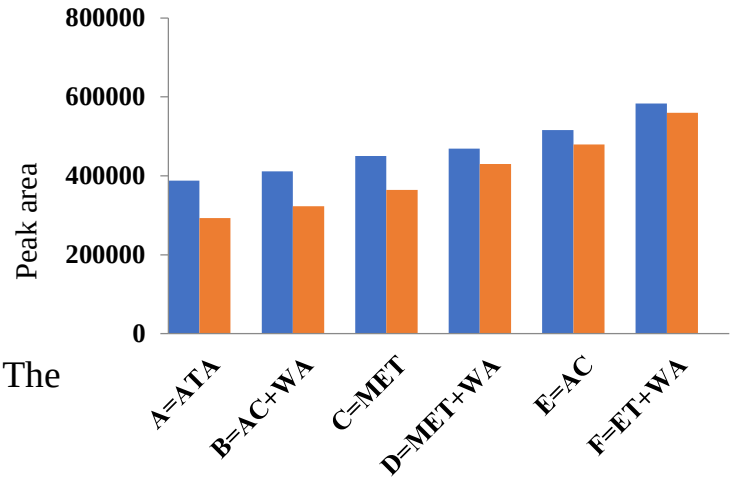

Fig. 2.6 The impact of the desorption solvent type

2.10.2 Effect of the Desorption Solvent Volume

Another important factor affecting the recovery of cyproheptadine and clemastine in the D-µSPE procedure is the volume of the desorption solvent. To determine the optimum volume, different amounts of the selected solvent mixture (water:ethanol, 1:1, w/v) were tested within the range of 0.5–2.0 mL.

The experimental results presented in Table 2.2 and Figure 2.7 show that increasing the solvent volume improved analyte desorption up to a certain point. However, when volumes greater than 1.0 mL were applied, no significant improvement in peak areas was observed. Therefore, 1.0 mL of desorption solvent was identified as the optimal volume, as it ensured complete desorption of the analytes while minimizing solvent consumption.

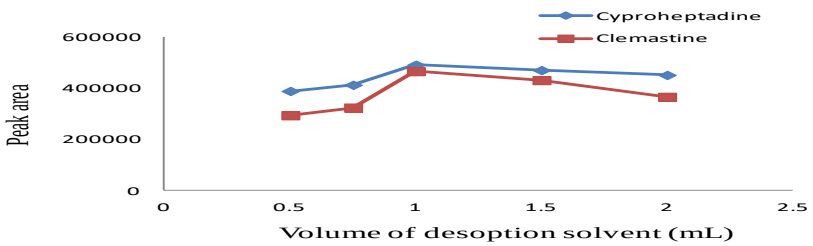

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Fig. 2.7 Desorption solvent volume effects

Table 2.2. Effect of desorption solvent volume on analyte extraction

Desorption Volume (mL)	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
0.50	387,549	292,848
0.75	411,496	322,692
1.00	489,963	464,719
1.50	468,721	429,872
2.00	449,963	364,719

2.10.3 Effect of Ultrasonication Time for the Desorption Step

The desorption time plays a significant role in ensuring complete release of the analytes and accurate quantitative determination. Ultrasonication times ranging from 3 to 15 minutes were investigated to evaluate their impact on extraction efficiency.

The results, summarized in Table 2.3 and Figure 2.8, indicate that the maximum peak areas for both cyproheptadine and clemastine were obtained at 7 minutes of ultrasonication. Longer ultrasonication times did not improve extraction and even led to a slight decrease in peak intensity, possibly due to analyte degradation or re-adsorption. Therefore, 7 minutes was selected as the optimal ultrasonication time for efficient desorption.

Table 2.3. Effect of ultrasonication time on analyte desorption

Ultrasonication Time (min)	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
3	627,136	649,174
5	666,194	669,646
7	666,483	685,682
10	663,761	687,129
15	668,459	679,852

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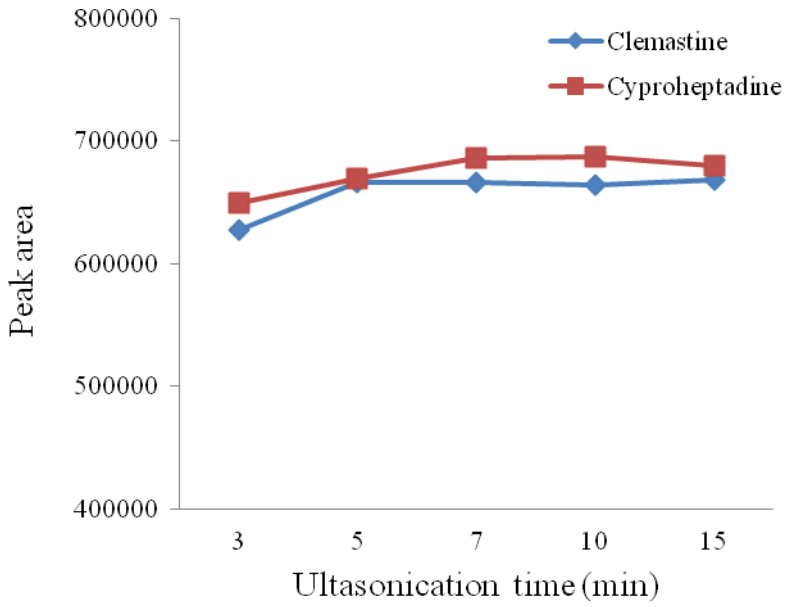

Fig. 2.8 Effect of ultrasonication time for desorption of analytes from adsorbent.

2.10.4 Effect of Sample Solution pH

The pH of the sample solution is a crucial parameter affecting the retention and adsorption of analytes in dispersive micro-solid phase extraction (D-µSPE). In this study, the influence of pH on the extraction of cyproheptadine and clemastine using the APTES-GO@MIL-101(Cr) composite was systematically evaluated.

For the experiments, 10 mL solutions containing 10 ng mL^{-1} of each analyte were prepared, and their pH was adjusted within the range of 4 to 10 using diluted 0.1 M HCl or 0.1 M NaOH. The results, summarized in Table 2.4 and Figure 2.9, demonstrate that the extraction efficiency increased as the pH was raised from 4.0 to 6. This improvement can be attributed to enhanced electrostatic interactions between the functional groups of the adsorbent and the analytes.

At lower pH values, weaker electrostatic interactions between the protonated analytes and the adsorbent resulted in reduced extraction efficiency. Conversely, when the pH exceeded 6, a gradual decline in analyte recovery was observed. Consequently, pH 6 was selected as the optimal condition for all subsequent experiments. This choice is also consistent with the typical pH of cyproheptadine and clemastine solutions, ensuring compatibility with the working solutions.

Table 2.4. Effect of sample solution pH on extraction efficiency

pH	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
4	533,697	476,258
5	637,468	583,104

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pH	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
6	707,459	679,428
7	679,647	654,835
8	597,546	556,789
9	517,893	486,354
10	468,523	442,679

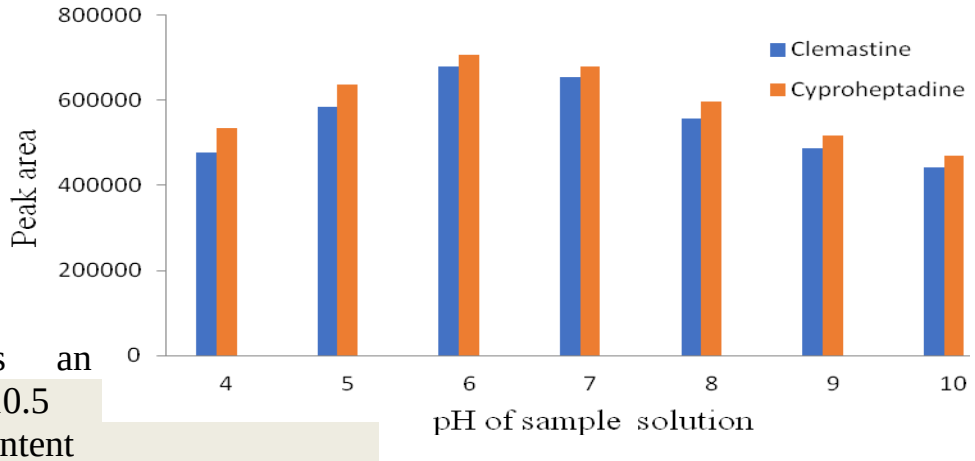

Fig. 2.9 The sample solution's pH impact has an

Effect of Salt 2.10.5 Content

The concentration of salt in the sample solution can significantly influence the extraction efficiency of analytes in dispersive micro-solid phase extraction. In this study, the impact of NaCl concentration on the adsorption of cyproheptadine and clemastine onto the APTES-GO@MIL-101(Cr) composite was evaluated.

Experiments were conducted using NaCl concentrations ranging from 0% to 12% (w/v), while all other experimental parameters were kept constant. The results, summarized in Table 2.5 and Figure 2.10, indicate that increasing the salt content initially enhanced the extraction efficiency, as evidenced by the rise in peak areas for both analytes. The maximum extraction efficiency was observed at 8% (w/v) NaCl. Beyond this concentration, further addition of salt led to a gradual decline in peak areas, likely due to competition between salt ions and the analytes for adsorption sites or changes in the solution viscosity.

Based on these findings, a NaCl concentration of 8% (w/v) was selected as the optimal condition for subsequent experiments.

Table 2.5. Effect of NaCl concentration on analyte extraction

NaCl Concentration (% w/v)	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
0	687,952	709,875

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NaCl Concentration (% w/v)	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
3	698,753	719,675
5	706,984	726,984
8	713,648	738,967
10	697,486	716,879
12	648,932	663,248

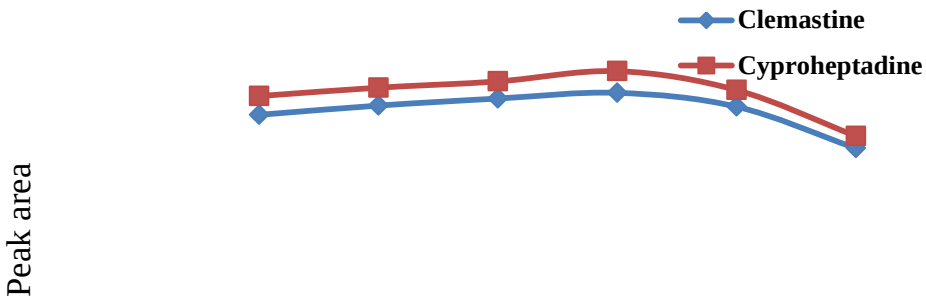

Fig. 2.10 Effect of salt concentration

2.10.6 Effect of Extraction Time

The duration of extraction is a critical parameter in dispersive micro-solid phase extraction, as it directly affects the adsorption of analytes onto the sorbent. Extended extraction times may unnecessarily prolong the procedure, whereas short durations can result in incomplete adsorption of target analytes.

To optimize this parameter, the influence of ultrasonication time on the extraction of cyproheptadine and clemastine was evaluated within the range of 5 to 15 minutes. The results, summarized in Table 2.6 and illustrated in Figure 2.11, show that the peak areas of both analytes increased with extraction time, reaching a maximum at 10 minutes. Beyond this point, no significant improvement was observed, indicating that adsorption equilibrium between the APTES-GO@MIL-101(Cr) composite and the sample solution was effectively achieved.

Therefore, 10 minutes of ultrasonication was selected as the optimal extraction time for efficient analyte recovery.

Table 2.6. Effect of ultrasonication time on analyte extraction

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Ultrasonication Time (min)	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
5	617,896	607,468
7	667,432	658,736
8	713,648	681,268
10	714,596	684,953
15	709,875	681,369

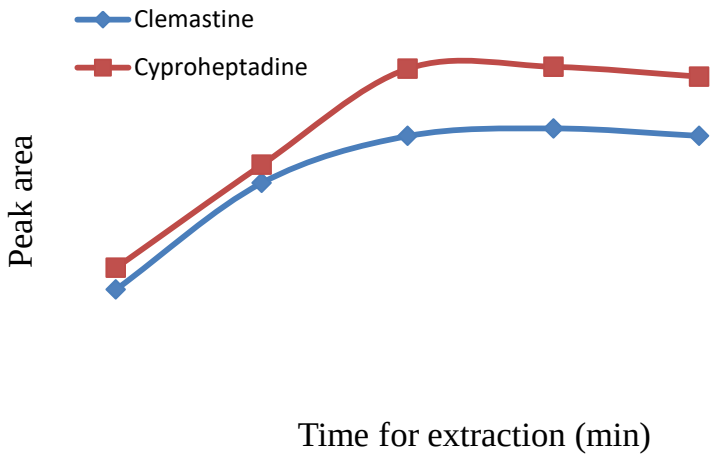

Fig. 2.11 Ultrasonication time and analyte extraction from an adsorbent

2.10.7 Effect of Adsorbent Amount

The amount of adsorbent plays a crucial role in the efficiency of dispersive micro-solid phase extraction. Insufficient sorbent may result in incomplete adsorption of the target analytes, whereas an excessive amount can lead to unnecessary waste.

To determine the optimal dose, various quantities of the APTES-GO@MIL-101(Cr) composite ranging from 0.001 g to 0.03 g were tested while keeping all other experimental conditions constant. The results, presented in Table 2.7 and Figure 2.12, show that the extraction efficiency, indicated by the peak areas of cyproheptadine and clemastine, increased with the amount of adsorbent. However, no significant improvement was observed when the amount exceeded 0.01 g, suggesting that this quantity is sufficient to achieve complete analyte adsorption.

Based on these findings, 0.01 g of the composite was selected as the optimal amount for all subsequent experiments.

Table 2.7. Effect of adsorbent amount on analyte extraction

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Adsorbent Amount (g)	Cyproheptadine (Peak Area)	Clemastine (Peak Area)
0.001	552,894	516,892
0.003	586,935	557,836
0.005	624,568	614,158
0.01	652,896	654,823
0.02	624,865	605,842
0.03	612,874	598,423

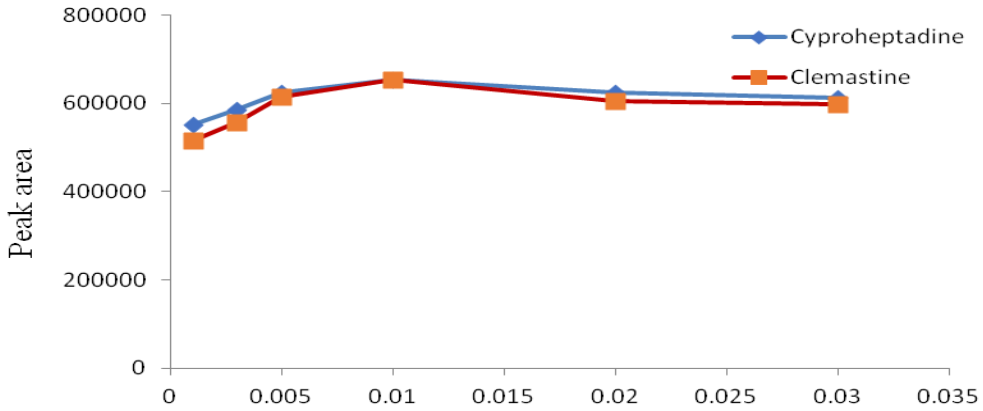

Fig. 2.12 Effect of the amount of adsorbent

2.10.8 Summary of Optimal Conditions

Based on the optimization studies for ultrasound-assisted dispersive micro-solid phase extraction, the ideal conditions for the extraction of clemastine and cyproheptadine using the APTES-GO@MIL-101(Cr) composite were established. The optimized parameters are summarized in Table 2.8.

Table 2.8. Summary of optimal extraction conditions

Parameter	Optimized Value
Desorption solvent	Water: Ethanol (1:1)
Desorption solvent volume (mL)	1
Desorption time (min)	7
Sample solution pH	6
Salt concentration (w/v, %)	8
Extraction time (min)	10
Adsorbent dosage (g)	0.01

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These conditions provided maximum recovery and resolution for both target analytes, ensuring reproducible and efficient extraction.

3.1 Calibration Graph

The analytical performance of the APTES-GO@MIL-101(Cr) composite combined with HPLC-UV detection was evaluated under the optimized conditions. Standard solutions of clemastine and cyproheptadine were prepared in the concentration range of 0.1–20 ng mL^{-1}, and the corresponding calibration curves were constructed by plotting the peak area (y) against analyte concentration (x).

The calibration curves exhibited excellent linearity over the tested range, with correlation coefficients (r^2) greater than 0.999. The resulting linear equations for quantification were:

- Clemastine: y=430,752x+267,702
- Cyproheptadine: y=426,888x+151,366

where y represents the peak area and x denotes the analyte concentration in ng mL^{-1}. The data confirm the method's reliability and suitability for accurate determination of both analytes.

Table 2.9. Calibration curve data

Concentration (ng mL^{-1})	Clemastine Peak Area	Cyproheptadine Peak Area
0.1	271,563	290,582
0.5	353,965	391,637
1	615,896	637,634
5	2,129,480	2,588,170
8	3,633,064	3,819,889
15	6,454,632	6,642,132
20	8,774,625	8,869,146

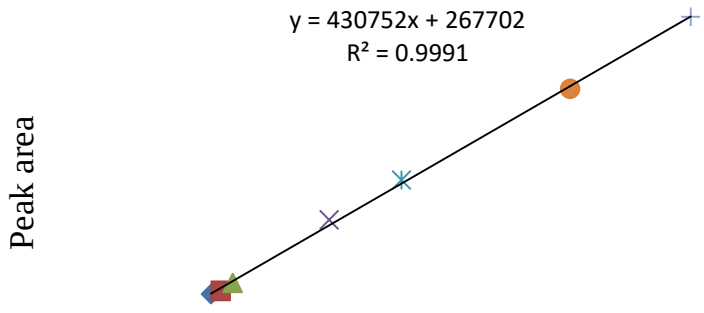

Fig. 2.13 The calibration curve cyproheptadine calibration curve

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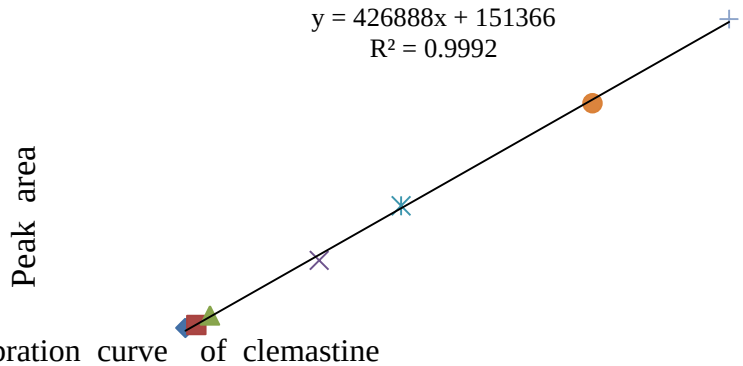

Fig. 2.14 The calibration curve of clemastine calibration curve

3.2 Analytical Performance Metrics

3.2.1 Limit of Detection (LOD)

The limit of detection (LOD) was evaluated to determine the smallest concentration of clemastine and cyproheptadine that can be reliably distinguished from the blank. LOD was calculated using the relationship:

$$\frac{{}_bS \times 3}{m} = \mathrm{LOD}$$

where ${}_bS$ is the standard deviation of the blank response and mmm is the slope of the calibration curve. Under the optimized conditions, ten blank solutions were analyzed using the ultrasound-assisted dispersive micro solid-phase extraction method followed by HPLC-UV measurement. The standard deviation of the blank responses was determined and used to calculate LOD values.

3.2.2 Limit of Quantification (LOQ)

The limit of quantification (LOQ) was estimated using the formula:

$$\frac{{}_bS \times 10}{m} = \mathrm{LOQ}$$

where SbS_bSb and mmm are defined as above. The obtained LOD and LOQ values for both analytes are summarized in Table 3.10.

Table 3.10. Peak area values for ten replicate measurements of blank solutions and calculated LOD and LOQ for clemastine and cyproheptadine

Number	Clemastine (CLM)	Cyproheptadine (CYP)

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Number	Clemastine (CLM)	Cyproheptadine (CYP)
1	271,563	298,989
2	268,975	289,675
3	267,496	299,654
4	274,689	297,866
5	274,986	287,469
6	279,685	296,348
7	263,489	287,961
8	279,862	298,746
9	278,964	279,845
10	267,895	297,862

Parameter	Clemastine	Cyproheptadine
Slope (m)	430,752	426,888
Standard deviation (Sb)	6,748.09	5,760.48
LOD (ng mL^{-1})	0.15	0.04
LOQ (ng mL^{-1})	0.45	0.134

3.2.3 Interday Precision

The precision of the method was evaluated by calculating the relative standard deviation (RSD) for three concentrations within the linear range: 0.5, 5, and 15 ng mL^{-1}. Each concentration was analyzed in triplicate on the same day. The RSD was determined using the following equation:

$$100 \times \frac{S}{X} = (\%)\ \mathrm{RSD}$$

where S is the standard deviation of the peak areas and X is the mean peak area.

The results, shown in Table 3.11, demonstrate that the method exhibits satisfactory reproducibility for both analytes.

Table 3.11. Interday precision (n=3)

Analyte	RSD (%) 0.5 ng mL^{-1}	RSD (%) 5 ng mL^{-1}	RSD (%) 15 ng mL^{-1}
Clemastine (CLM)	4.2	4.3	4.1
Cyproheptadine (CYP)	4.6	4.2	3.6

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The low RSD values confirm that the ultrasound-assisted dispersive micro-solid phase extraction method provides high precision for quantitative analysis of clemastine and cyproheptadine.

3.2.4 Interday Precision

To evaluate interday precision, three concentrations within the linear calibration range (0.5, 5, and 15 ng mL^{-1}) were analyzed under optimal experimental conditions. Each concentration was injected in triplicate over three consecutive days. The peak areas obtained from the chromatograms were used in conjunction with the calibration curve equations to determine the analyte concentrations. The relative standard deviation (RSD) was calculated according to the formula described in Section 3.2.3.

Table 3.12. Interday precision (n=3)

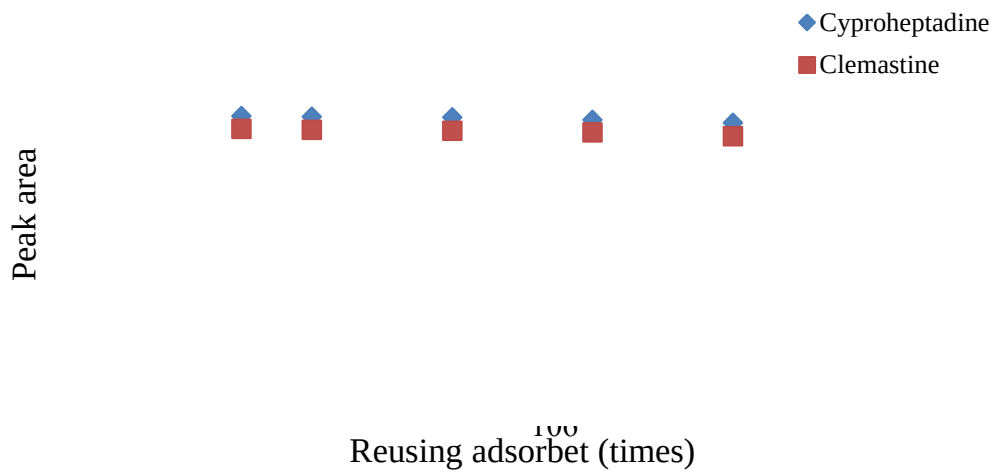
Analyte	RSD (%) 0.5 ng mL^{-1}	RSD (%) 5 ng mL^{-1}	RSD (%) 15 ng mL^{-1}
Clemastine (CLM)	4.8	3.6	4.1
Cyproheptadine (CYP)	4.3	4.1	3.8

The results indicate that the method maintains satisfactory precision across multiple days, confirming its reliability for routine quantitative analysis.

3.3 Adsorbent Reusability Optimization

The reusability of the synthesized APTES-GO@MIL-101(Cr) composite was investigated to assess its regeneration capability. After each extraction, the adsorbent was subjected to a simple regeneration procedure, consisting of three washes with pure ethanol followed by a single wash with ethanol/acetic acid (9:1, v/v), and then dried at 60 °C.

A total of 30 consecutive extraction–desorption cycles were performed using the regenerated adsorbent. The recovery of clemastine and cyproheptadine in each cycle was evaluated. Results, shown in Figure 2.15, indicate minimal loss in analyte recovery (less than 5%) throughout all cycles. These findings demonstrate that the APTES-GO@MIL-101(Cr) composite is highly reusable and suitable for repeated applications without significant performance deterioration.



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3.4 Analytical Applications and Real Sample Analysis

The applicability of the proposed ultrasound-assisted dispersive micro solid-phase extraction method was evaluated using various real samples, including milk, egg yolk, chicken meat, mutton, and water. To assess the extraction efficiency, spike recovery tests were performed. Clemastine and cyproheptadine were added to the samples at three concentration levels (0.5, 5, and 10 ng mL^{-1}), and the extraction procedure was conducted under optimized conditions. The percent recoveries were calculated using the following formula:

$$\text{Recovery (\%)} = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \qquad (2\text{-}4)$$

The results, summarized in Tables 2.13–2.15, indicate that the method achieves relative recoveries between 92% and 102%, demonstrating its suitability for analyzing clemastine and cyproheptadine in complex matrices.

3.5 Preparation of Real Samples

Milk Samples:

Two milliliters of acetonitrile were added to 500 µL of milk to precipitate proteins. The mixture was centrifuged at 1000 rpm for 60 seconds, and the upper phase was collected for subsequent extraction.

Egg Yolk Samples:

The yolk was separated from the egg white and placed on absorbent filter paper to remove residual albumin. Then, 10 g of deionized water was added to 0.1 g of yolk, and the mixture was stirred and centrifuged at 2000 rpm for 120 seconds. Two milliliters of acetonitrile were added to precipitate proteins, followed by centrifugation at 1000 rpm for one minute. The supernatant was collected for extraction.

Meat Samples (Chicken and Mutton):

Meat samples were dried in an oven at 65 °C for 72 hours, then 5 g portions were placed in 40 mL Falcon tubes. Five milliliters of acetonitrile were added, and the mixture was vortexed for 5 minutes. The samples were centrifuged at 4000 rpm for 10 minutes, and the supernatant was used for analysis.

Water Samples:

No sample pretreatment was necessary; the extraction was applied directly.

Table 2.13. Recovery and RSD of clemastine (CLM) and cyproheptadine (CYP) in spiked and unspiked chicken meat and mutton

Sample	Compound	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
Chicken meat	CLM	0	ND	ND	ND
	CLM	0.5	0.51	100.2	3.1

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Sample	Compound	Added (ng mL⁻¹)	Found (ng mL⁻¹)	Recovery (%)	RSD (%)
	CLM	5	4.6	92.0	3.8
	CLM	15	14.8	98.6	3.9
	CYP	0	ND	ND	ND
	CYP	0.5	0.48	94.0	2.5
	CYP	5	4.8	96.0	3.6
	CYP	15	14.5	98.6	4.2
Mutton	CLM	0	ND	ND	-
	CLM	0.5	0.46	92.0	3.8
	CLM	5	5.08	100.2	3.5
	CLM	15	14.85	99.0	3.8
	CYP	0	ND	ND	-
	CYP	0.5	4.65	95.0	3.3
	CYP	5	5.10	93.0	2.6
	CYP	15	14.72	98.1	2.9

ND: Not detected

Table 2.14. Recovery and RSD of clemastine (CLM) and cyproheptadine (CYP) in spiked and unspiked milk and egg yolk

Sample	Compound	Added (ng mL⁻¹)	Found (ng mL⁻¹)	Recovery (%)	RSD (%)
Milk	CLM	0	ND	ND	ND
	CLM	0.5	0.47	93.0	4.3
	CLM	5	5.1	100.2	3.8
	CLM	15	14.83	94.0	3.4
	CYP	0	ND	ND	ND
	CYP	0.5	0.46	95.0	4.1
	CYP	5	4.9	98.0	4.4
	CYP	15	14.86	94.0	3.9
Egg yolk	CLM	0	0	0	0
	CLM	0.5	0.49	98.0	4.3
	CLM	5	4.92	98.5	3.3
	CLM	15	14.73	98.2	4.2

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Sample	Compound	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
	CYP	0	ND	ND	ND
	CYP	0.5	0.49	98.0	3.7
	CYP	5	4.86	97.2	3.9
	CYP	15	14.37	95.3	3.5

ND: Not detected

Table 2.15. Recovery and RSD of clemastine (CLM) and cyproheptadine (CYP) in spiked and unspiked water samples

Sample	Compound	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
Karoon water	CLM	0	ND	ND	ND
	CLM	0.5	0.47	94.0	3.3
	CLM	5	5.03	100.6	4.8
	CLM	15	15.06	100.4	3.6
	CYP	0	ND	ND	ND
	CYP	0.5	0.51	102.0	4.2
	CYP	5	5.05	101.0	4.3
	CYP	15	15.03	100.2	3.8
Lab water	CLM	0	ND	ND	ND
	CLM	0.5	0.52	104.0	3.3
	CLM	5	5.01	100.2	3.7
	CLM	15	14.96	99.73	4.1
	CYP	0	ND	ND	ND
	CYP	0.5	0.51	102.0	4.7
	CYP	5	4.98	99.6	3.6
	CYP	15	14.81	98.70	4.4

ND: Not detected

Conclusion

In this study, a highly efficient and selective analytical method was successfully developed for the extraction and determination of antihistamines, clemastine and cyproheptadine, in diverse food and water matrices. The hybrid nanocomposite APTES-GO@MIL-101(Cr) served as a novel adsorbent, exhibiting exceptional surface area, high adsorption capacity, and excellent chemical stability. Its synthesis through the functionalization of graphene oxide with 3-

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aminopropyltriethoxysilane followed by integration with MIL-101(Cr) was confirmed via comprehensive characterization techniques including FT-IR, SEM, and EDX, which validated the successful formation of the hybrid structure.

Optimization of experimental parameters, such as desorption solvent type and volume, ultrasonication duration, sample pH, salt concentration, extraction time, and adsorbent dosage, revealed that each factor significantly influenced the extraction efficiency. Under optimized conditions, the proposed method provided remarkable sensitivity, with limits of detection and quantification reaching sub-nanogram levels for both analytes. The calibration curves demonstrated excellent linearity across a wide concentration range, while intra- and inter-day precision studies confirmed the method's reliability and reproducibility, with RSD values consistently below 5%.

Furthermore, the reusability of the APTES-GO@MIL-101(Cr) adsorbent was validated over 30 successive extraction cycles with negligible loss in performance, highlighting the economic and environmental advantages of the approach. Application to real samples, including milk, egg yolk, chicken meat, mutton, and water, confirmed high recoveries and low matrix interference, demonstrating the robustness and practical applicability of the technique.

Overall, this work provides a simple, rapid, and environmentally friendly analytical strategy for trace-level monitoring of antihistamines in complex matrices. The combination of hybrid nanomaterials and ultrasound-assisted dispersive micro solid-phase extraction represents a promising advancement in analytical chemistry, offering potential for future applications in food safety, environmental monitoring, and pharmaceutical analysis. This methodology can serve as a model for the development of similar nanocomposite-based extraction techniques for other bioactive compounds.

References

[1] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Modern trends in solid phase extraction: New sorbent media, TrAC - Trends Anal. Chem. 77 (2016) 23–43.

[2] M. Miró, On-chip micro solid-phase extraction in a disposable sorbent format using mesofluidic platforms, TrAC - Trends Anal. Chem. 62 (2014) 154–161.

[3] C. Calderilla, F. Maya, L.O. Leal, V. Cerdà, Recent advances in flow-based automated solid-phase extraction, TrAC Trends Anal. Chem. 108 (2018)88-97.

[4] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.

[5] M.S. Cárdenas Aranzana, Dispersive Solid-Phase (Micro) Extraction, in: Encycl. Anal. Chem., John Wiley & Sons, Ltd, Chichester, UK, 2010.

[6] L. Guo, H.K. Lee, Vortex-assisted micro-solid-phase extraction followed by low density solvent based dispersive liquid-liquid microextraction for the fast and efficient determination of phthalate esters in river water samples, J. Chromatogr. A. 1300 (2013) 24–30.

University of Thi-Qar Journal

ISSN (print): 2706- 6908, ISSN (online): 2706-6894

Vol.21 No.1 Mar 2026

[7] M. Rutkowska, K. Owczarek, M. de la Guardia, J. Płotka-Wasylka, J. Namieśnik, Application of additional factors supporting the microextraction process, TrAC -Trends Anal. Chem. 97 (2017) 104–119.
[8] M.M. Delgado-Povedano, M.D. Luque de Castro, Ultrasound-assisted extraction and in situ derivatization, J. Chromatogr. A. 1296 (2013) 226–234.
[9] V.S. Nguyen, D. Rouxel, R. Hadji, B. Vincent, Y. Fort, Effect of ultrasonication and dispersion stability on the cluster size of alumina nanoscale particles in aqueous solutions, Ultrason. Sonochem. 18 (2011) 382–388.

[10] A.B.G. Aghaie, M.R. Hadjmohammadi, Fe_3O_4@p-Naphtholbenzein as a novel nano-sorbent for highly effective removal and recovery of Berberine: Response surface methodology for optimization of ultrasound assisted dispersive magnetic solid phase extraction, Talanta. 156–157 (2016) 18–28.
[11] E.A. Dil, M. Ghaedi, A. Asfaram, F. Mehrabi, A.A. Bazrafshan, A.M. Ghaedi, Trace determination of safranin O dye using ultrasound assisted dispersive solid phase micro extraction: Artificial neural network-genetic algorithm and response surface methodology, Ultrason. Sonochem. 33 (2016) 129–140.
[12] M. Krawczyk, E. Stanisz, Ultrasound-assisted dispersive micro solid-phase extraction with nano-TiO_2 as adsorbent for the determination of mercury species, Talanta. 161 (2016) 384–391.
[13] M. Krawczyk-Coda, E. Stanisz, Determination of fluorine in herbs and water samples by molecular absorption spectrometry after preconcentration on nanoTiO_2 using ultrasound-assisted dispersive micro solid phase extraction, Anal. Bioanal. Chem. 409 (2017) 6439–6449.
[14] C.B. Ojeda, F.S. Rojas, Vortex-Assisted Liquid–Liquid Microextraction (VALLME): The Latest Applications, Chromatographia. 81 (2018) 89–103.
[15] Y.G. Adewuyi, Sonochemistry: Environmental Science and Engineering Applications, Ind. Eng. Chem. Res. 40 (2001) 4681–4715.
[16] F. Galán-Cano, R. Lucena, S. Cárdenas, M. Valcárcel, Dispersive micro-solid phase extraction with ionic liquid-modified silica for the determination of organophosphate pesticides in water by ultra performance liquid chromatography, Microchem. J. 106 (2013) 311–317.
[17] Q. Cai, L. Zhang, P. Zhao, X. Lun, W. Li, Y. Guo, X. Hou, A joint experimental computational investigation: Metal organic framework as a vortex assisted dispersive micro-solid-phase extraction sorbent coupled with UPLC-MS/MS for the simultaneous determination of amphenicols and their metabolite in aquaculture water, Microchem. J. 130 (2017) 263–270.
[18] M. Rajabi, A.G. Moghadam, B. Barfi, A. Asghari, Air-assisted dispersive microsolid phase extraction of polycyclic aromatic hydrocarbons using a magnetic graphitic carbon nitride nanocomposite, Microchim. Acta. 183 (2016) 1449– 1458.
[19] D.C.M. Bordin, M.N.R. Alves, E.G. De Campos, B.S. De Martinis, Disposable pipette tips extraction: Fundamentals, applications and state of the art, J. Sep. Sci. 39 (2016) 1168–1172.
[20] J.L. Benedé, A. Chisvert, D.L. Giokas, A. Salvador, Development of stir bar sorptive-dispersive microextraction mediated by magnetic nanoparticles and its analytical application to

University of Thi-Qar Journal

ISSN (print): 2706- 6908, ISSN (online): 2706-6894

Vol.21 No.1 Mar 2026

the determination of hydrophobic organic compounds inaqueous media, J. Chromatogr. A. 1362 (2014) 25–33.