Sustainable Environmental Insight

Research Article
Volume 3(2), 2026, 170−186
https://doi.org/10.53623/sein.v3i2.1062

Optimized Adsorption of Sulfonamide Antibiotics from Milk Using Organically Modified Bentonite Clay

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SUBMITTED: 19 February 2026; REVISED: 15 April 2026; ACCEPTED: 18 April 2026

ABSTRACT: Sulfonamide antibiotic residues in milk are considered highly dangerous to human health and food safety due to their persistence and potential for bioaccumulation. Conventional removal methods were often costly and inefficient, highlighting the need for sustainable and effective alternatives. The study aimed to assess the efficiency of organically modified bentonite clay (OMB-16) in the removal of sulfonamide antibiotics (sulfadimidine sodium, sulfadiazine, and sulfaguanidine) from milk and to optimize the conditions to achieve maximum adsorption capacity. The extraction efficiency of OMB-16 was evaluated in comparison with unmodified bentonite, and key parametersm including solution pH, adsorbent loading, and elution solvent—were optimized. Quantification was performed using high-performance liquid chromatography with an ultraviolet detector (HPLC-UV), which demonstrated that OMB-16 exhibited better extraction performance than untreated bentonite. The recovery percentages using OMB-16 reached 90% for sulfadimidine and sulfadiazine, which were higher than the 80% and 75% recoveries obtained using unmodified bentonite, respectively. The best results were achieved at a mildly acidic pH of 4–5, while methanol was identified as the most efficient desorption solvent. The intra- and inter-day precision of the OMB-16 method was also evaluated, and the results confirmed that the method was reproducible. It was further observed that the HPLC-UV method was sensitive and specific, demonstrating good linearity ($R^2 \geq 0.99$) and low limits of detection. Overall, this study showed that OMB-16 was an effective and reliable adsorbent for removing pharmaceutical residues from complex food matrices such as milk, with potential applications in food safety monitoring and residue analysis.

KEYWORDS: Pharmaceutical residue removal; modified clay adsorption; green adsorbent; food safety

1. Introduction

The increasing proportion of pharmaceutical residues in environmental matrices has been a major concern, particularly regarding the pollution of water bodies and agricultural systems [1]. Among various pharmaceutical substances detected in the environment, sulfonamide

antibiotics such as sulfadiazine, sulfadimidine sodium, and sulfaguanidine have been widely reported. These antibiotics have extensive applications in veterinary medicine for the treatment of bacterial infections in livestock; however, their intensive use has resulted in the unintended release of these compounds into the environment [2]. Sulfonamides in wastewater, surface water, and even milk are potentially hazardous to human health and aquatic ecosystems due to their persistence and bioaccumulative properties. These compounds are not effectively removed by conventional wastewater treatment methods and may persist for extended periods in the environment, posing significant challenges for environmental management strategies aimed at their elimination [3, 4].

Conventional methods for removing pharmaceutical contaminants, such as chemical oxidation and advanced oxidation processes, are often energy-intensive, typically requiring between 0.5 to 5 kWh/m³ for ozonation and 1 to 10 kWh/m³ for UV/H_2O_2 systems, and may also generate toxic by-products. Consequently, researchers have focused on more economical and sustainable treatment approaches, particularly adsorption techniques [5, 6]. Among various adsorbents, organically modified clays have been widely studied and have demonstrated superior adsorption capacity and cost-effectiveness. In particular, organically modified bentonite clay has been identified as a promising material for the removal of organic contaminants from water [7].

Bentonite is a naturally occurring clay mineral characterized by a high surface area and layered structure, which provides a favorable framework for adsorption. However, raw bentonite has limited efficiency in adsorbing many organic contaminants due to its hydrophilic nature [8]. Its surface properties can be modified using surfactants or polymers to enhance the adsorption of hydrophobic molecules such as sulfonamide antibiotics. This modification increases surface area, promotes intercalation of organic molecules, and introduces functional groups that enhance interactions with target pollutants [9, 10].

The adsorption of sulfonamides in milk samples using organically modified bentonite clay represents a promising approach for reducing pharmaceutical contamination in dairy products. Milk, as an important food source, can act as a carrier of pharmaceutical residues; therefore, more efficient removal strategies are required. The adsorption capacity of organically modified bentonite clay for sulfadiazine, sulfadimidine sodium, and sulfaguanidine has the potential to significantly improve the safety and quality of milk. In addition, this approach is consistent with green chemistry principles, as it utilizes natural, biodegradable, non-toxic, and readily available materials [11, 12].

The present study investigated the potential of organically modified bentonite clay for the adsorption of sulfadiazine, sulfadimidine sodium, and sulfaguanidine from milk samples. The study evaluated the efficiency of the adsorbent and examined various parameters, including contact time, pH, temperature, and contaminant concentration. This research provided a practical and timely solution to pharmaceutical contamination in food products, contributing to a cleaner and safer environment. The use of organically modified bentonite clay represented a promising approach for mitigating pharmaceutical contamination in milk and other food matrices.

2. Materials and Methods

2.1.Materials, chemicals and glassware.

Several materials and chemicals were used to conduct the experiment, including sulfadiazine, sulfadimidine sodium, and sulfaguanidine, along with organically modified bentonite clay. Bentonite, an aluminosilicate mineral, served as the base adsorbent [13]. It was modified by incorporating cetyltrimethylammonium bromide (CTAB), a cationic surfactant, to enhance its adsorption capacity for organic pollutants. Sulfadiazine, sulfadimidine sodium, and sulfaguanidine were obtained in pure form and dissolved to prepare stock solutions for use in the study. Methanol was used for the preparation of these stock solutions. The pH of the adsorption experiments was adjusted using hydrochloric acid (HCl) and sodium hydroxide (NaOH), allowing evaluation of the effect of pH on adsorption efficiency. Fresh milk, obtained from a local dairy farm, was used as the sample matrix to assess the removal efficiency of the pharmaceuticals [14].

Various glassware was used, including 250 mL glass bottles for preparing adsorption mixtures, 500 mL beakers for solution preparation, and 250 mL Erlenmeyer flasks for mixing. Precise volumetric measurements were performed using graduated cylinders and pipettes, while Whatman filter paper was used to filter the adsorbent after each experiment. A mechanical shaker was used to mix the adsorbent with pharmaceutical solutions to ensure sufficient contact between phases. pH adjustment was carried out using a digital pH meter, and adsorption analysis was performed using a High-Performance Liquid Chromatography (HPLC) system equipped with a C18 column and a UV detector set at 254 nm. Temperature was controlled using a thermostatic incubator, and sample weights were measured using an analytical balance to ensure accuracy. This methodological framework provided controlled and reproducible conditions for evaluating the efficiency of organically modified bentonite clay in removing pharmaceutical contaminants from milk.

2.2.Experimental procedures.

This section outlines the overall experimental procedures used to evaluate the adsorption efficiency of organically modified bentonite clay for the removal of sulfonamide antibiotics (sulfadiazine, sulfadimidine sodium, and sulfaguanidine) from dairy matrices.

2.2.1. Preparation of drug solutions.

The adsorption experiments began with the preparation of stock solutions of sulfadiazine, sulfadimidine sodium, and sulfaguanidine by dissolving 1 g of each pharmaceutical compound in 1 L of deionized water, resulting in an initial concentration of 1,000 mg/l for each substance. Serial dilutions were then prepared to obtain working concentrations of 10, 25, 50, 100, and 200 mg/L for use in adsorption experiments. This concentration gradient allowed systematic evaluation of removal performance under different contaminant loadings. All solutions were aliquoted into sterile glassware and stored at ambient laboratory temperature [15].

2.2.2. Preparation of organically modified bentonite clay.

Cetyltrimethylammonium bromide (CTAB), a quaternary ammonium surfactant, was used to functionalize bentonite to enhance its affinity for organic pollutants. In a typical modification process, 10 g of pristine bentonite was suspended in 50 mL of 0.1 M CTAB solution and

continuously stirred at room temperature for 2 h to allow intercalation of the surfactant into the Ca-montmorillonite interlayers. After stirring, the composite material was filtered to remove unbound CTAB, washed several times with deionized water, and dried at 60 °C for 24 h. The pH of the solutions was adjusted within the range of 2–10 using 0.1 M HCl and 0.1 M NaOH to systematically evaluate its effect on adsorption performance. The final product, organically modified bentonite, was stored in a sealed container until further use in adsorption experiments [16]. The synthesis mechanism is illustrated in Figure S1 in the supplementary file.

2.3.Pharmaceutical sample preparation and standard solution preparation.

Standard solutions of sulfadiazine, sulfadimidine sodium, and sulfaguanidine were prepared for analytical analysis. Stock solutions (1,000 ppm) of each pharmaceutical were prepared by dissolving 25 mg of each compound in 25 mL of methanol. These stock solutions were stored at 4 °C until further use. A mixed working solution was prepared by combining equal volumes of each stock solution. The stock solution was further diluted to 100 ppm by pipetting 5 mL of stock solution and diluting it to 50 mL with methanol. This solution was subsequently diluted to prepare standard solutions of 1 ppm, 5 ppm, 10 ppm, 20 ppm, and 50 ppm. Dilutions were calculated using the equation:

$C_1V_1 = C_2V_2$

where C_1 and V_1 represent the initial concentration and volume, and C_2 and V_2 represent the final concentration and volume, respectively. These standard solutions were used for HPLC analysis to quantify pharmaceutical concentrations.

2.3.1. Pipette-tip solid phase extraction procedure.

Pipette-tip solid phase extraction was used for the extraction of sulfonamide antibiotics. Ten milligrams of sorbent were packed into a 1000 μL pipette tip, with a filter placed at the end of the tip. The tip was connected to a syringe to control flow rate. The sorbent was activated by passing 100 μL of water followed by 100 μL of methanol. Subsequently, 5 mL of sample solution was passed through the sorbent at a flow rate of 1 mL/min. Interfering substances were removed by washing with 1 mL of water (pH 5.0). The adsorbed pharmaceuticals were then eluted using 1 mL of methanol. The eluent was collected, filtered, and analyzed by HPLC.

2.3.2. Stock solution preparation for HPLC analysis.

For HPLC analysis, stock solutions of each pharmaceutical were prepared by dissolving 10 mg of sulfaguanidine, sulfadimidine sodium, and sulfadiazine separately in 10 mL of water, resulting in 1,000 μg/mL solutions. Equal volumes of each solution were combined to obtain a mixed solution. The stock solution was further diluted to obtain working concentrations suitable for HPLC analysis, minimizing analytical errors in complex matrices.

2.3.3. Spiked milk sample preparation.

Fresh milk obtained locally was stored at 4 °C. Spiked samples were prepared by adding 5 mL of a known concentration of mixed sulfonamide solution to a measured volume of milk. The mixture was stirred for 10 min to ensure homogeneity. Subsequently, 2 mL of acetonitrile was added to 5 mL of homogenized milk. The mixture was transferred into a 15 mL centrifuge tube and centrifuged at 4,500 rpm for 10 min. This step facilitated the separation of pharmaceutical

residues from the milk matrix. The supernatant was then analyzed using HPLC to determine the concentration of target pharmaceuticals.

3. Results and Discussion

3.1.Mechanism of adsorption.

The adsorption of sulfonamide antibiotics (sulfadimidine sodium, sulfadiazine, and sulfaguanidine) onto organically modified bentonite (OMB-16) was a complex process involving both physical and chemical interactions. The mechanism can be summarized as follows. Sulfonamide antibiotics, as organic compounds, were initially adsorbed onto the adsorbent surface through van der Waals forces. Although these interactions are weak, they played an important role in the initial attachment of drug molecules to the adsorbent surface.

The presence of long-chain quaternary ammonium surfactants replacing inorganic exchangeable cations caused organically modified bentonite (OMB-16) to exhibit hydrophobic characteristics. This modification reduced the surface polarity of bentonite by orienting hydrocarbon chains within the interlayers, thereby transforming naturally hydrophilic clay into an organophilic and water-repelling material. The high density of alkyl chains restricted water adsorption and increased affinity toward non-polar compounds, as indicated by higher contact angles and lower water uptake. This hydrophobic transformation was attributed to surfactant intercalation and expansion of basal spacing, which increased the surface coverage of organic functional groups [17].

Since sulfonamide antibiotics exhibit partially hydrophobic characteristics, the hydrophobic surface of OMB-16, enhanced by cetyltrimethylammonium bromide (CTAB), strengthened these interactions. This hydrophobicity facilitated the diffusion of drug molecules into the porous structure of bentonite, particularly within the interlayer spaces.

In addition, the presence of CTAB introduced a positive charge on the surface of OMB-16. This positive surface charge enhanced electrostatic interactions with negatively charged sulfonate groups in sulfonamide molecules, particularly under mildly acidic conditions (pH 4–5), where partial ionization of sulfonamide compounds occurred. Furthermore, hydrogen bonding interactions between functional groups on the adsorbent surface and sulfonamide molecules may have also contributed to adsorption enhancement.

3.2. Characterization of organically modified bentonite.

3.2.1. FTIR analysis.

Figure 1(a) shows the FTIR spectrum of OMB-16, which provides detailed information on its structural and functional properties. The absorption band at 3620 cm^{-1} was attributed to stretching vibrations of hydroxyl groups or bound water molecules within the bentonite lattice, indicating the presence of water or hydroxylated sites typical of clay mineral structures. The band at 1403 cm^{-1} was assigned to C–H bending vibrations of organic moieties, particularly those originating from cetyltrimethylammonium bromide (CTAB), which was used in the modification process. The presence of this band confirmed successful intercalation of CTAB into the bentonite interlayer structure, thereby enhancing its affinity for organic contaminants. The absorption band at 997 cm^{-1} was attributed to Si–O bending vibrations associated with the silicate framework of bentonite, indicating that the fundamental clay structure remained intact after modification. Overall, the FTIR results demonstrated that OMB-16 retained the silicate

structure of bentonite while incorporating organic functional groups that enhanced its adsorption capacity. Therefore, OMB-16 was confirmed to be an effective sorbent for the removal of sulfonamide antibiotics from dairy matrices.

3.2.2 XRD analysis.

Figure 1(b) shows the X-ray diffraction (XRD) patterns of raw bentonite and OMB-16, illustrating structural changes resulting from modification with cetyltrimethylammonium bromide (CTAB). The red curve represents raw bentonite, while the black curve represents OMB-16. The XRD pattern of raw bentonite exhibited sharp diffraction peaks at 2θ values of 8.7°, 26.7°, 45.2°, and 60.7°, with the most intense peak observed at 8.7°. This peak is characteristic of the montmorillonite structure and corresponds to a basal spacing of approximately 10–12 Å, indicating a naturally compact layered structure with minimal intercalation. In contrast, the XRD pattern of OMB-16 showed a shift of the 8.7° peak toward a lower angle, indicating an increase in interlayer spacing due to CTAB intercalation. This shift confirmed that CTAB molecules were successfully inserted between clay layers, increasing basal spacing and enhancing the adsorption capacity for organic contaminants. The modification also increased hydrophobicity, which is essential for adsorption of sulfonamide compounds. In addition to peak shifting, changes in peak intensity and broadening were observed. OMB-16 exhibited reduced crystallinity and more diffuse diffraction peaks compared to raw bentonite, indicating a transition toward a more amorphous structure after modification. Such structural disorder is typical of surfactant-modified clays and contributes to improved accessibility of adsorption sites. Overall, the XRD results confirmed that CTAB modification successfully expanded interlayer spacing, reduced crystallinity, and enhanced the adsorption properties of bentonite, making OMB-16 a more effective adsorbent for sulfadiazine, sulfadimidine sodium, and sulfaguanidine.

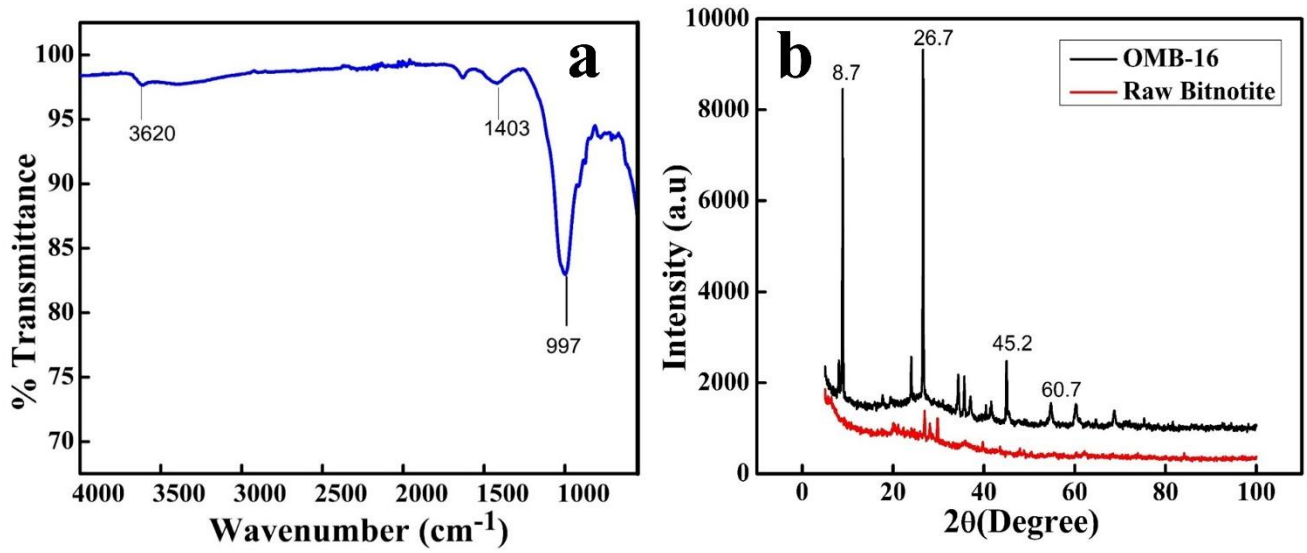

Figure 1. (a) FTIR spectrum of OMB-16 and the known absorption bands. (b) XRD pattern of raw bentonite and OMB-16 showing characteristic peaks.

3.2.3. SEM analysis.

Scanning electron microscopy (SEM) images revealed the surface morphology of raw bentonite (RB) and organically modified bentonite (OMB) at different magnifications, highlighting clear structural differences between the two materials. The surface morphology of

raw bentonite appeared relatively smooth and compact, as observed in micrographs (a3) and (c3). The particles exhibited a well-organized and layered crystalline structure with closely stacked platelets. This morphology is typical of unmodified bentonite, where clay layers are arranged in parallel orientation. Due to its dense microstructure and limited interlayer spacing, raw bentonite exhibited relatively low surface area and reduced capacity for adsorption of organic molecules. In contrast, OMB exhibited a markedly different morphology after modification with CTAB. As shown in micrographs (d) and (f), OMB displayed a rougher, more expanded, and irregular surface structure. This change was attributed to the intercalation of CTAB molecules between clay layers, which increased interlayer spacing and disrupted the original layered arrangement. As a result, OMB exhibited increased porosity and surface roughness, leading to a higher specific surface area and improved adsorption capacity. These structural changes significantly enhanced the material's ability to interact with organic contaminants, particularly hydrophobic sulfonamide antibiotics. The SEM analysis confirmed that surface modification effectively transformed bentonite into a more efficient adsorbent by increasing surface accessibility and adsorption sites.

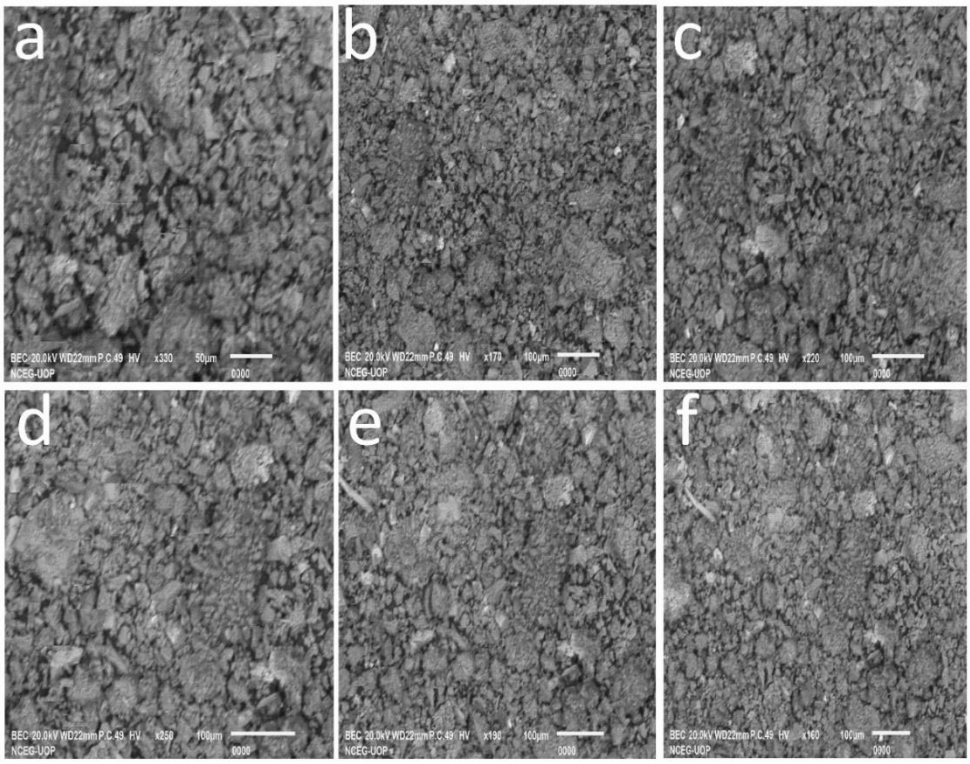

Figure 3. SEM images of raw bentonite (RB) (a-c) and organically modified bentonite (OMB) (d-f) at different magnifications.

3.2.4. SEM EDX analysis.

The scanning electron microscopy (SEM) image (a) and energy-dispersive X-ray (EDX) spectrum (b) provided detailed information on the surface morphology and elemental composition of organically modified bentonite (OMB) (Figure 4). In image (a), the surface topography of OMB at 220× magnification is shown. The surface exhibited an irregular morphology with an expanded and more porous structure. This morphology was consistent with the expected outcome of the modification process, in which cetyltrimethylammonium bromide (CTAB) molecules were intercalated into the clay layers, thereby increasing interlayer spacing. The resulting increase in surface roughness and porosity was critical in enhancing the

adsorption capacity of the material toward organic contaminants, as it provided a greater surface area and improved pore accessibility for contaminant interaction. Spectrum (b) shows the elemental composition of OMB. The analysis revealed the presence of several elements, with oxygen (O) being the most abundant, accounting for 53.77% by mass and 60.49% by atomic percentage, which is typical of silicate-based materials such as bentonite. Silicon (Si) was also present at 18.42% by mass, confirming the silicate framework of bentonite, while aluminum (Al) accounted for 5.89% by mass, which is characteristic of the montmorillonite structure. Sodium (Na), magnesium (Mg), calcium (Ca), potassium (K), and trace amounts of iron (Fe) were also detected, indicating the natural mineral composition of bentonite. The presence of carbon (C) at 12.48% by mass confirmed the successful incorporation of the organic surfactant (CTAB) into the bentonite structure, indicating that the modification process was successfully achieved. Overall, the SEM and EDX results confirmed that OMB underwent significant structural and compositional changes, which enhanced its efficiency as an adsorbent for organic contaminants.

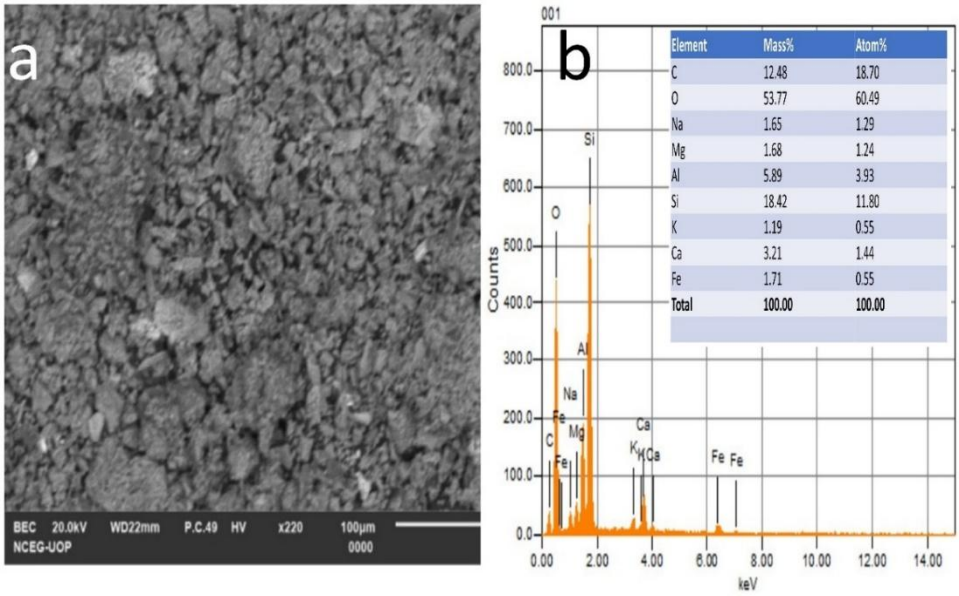

Figure 4. SEM image (a) and EDX spectrum (b) of organically modified bentonite (OMB).

3.3. Optimization parameters.

Several parameters were used for optimization, including concentration, temperature, contact time, pH, adsorbent dosage, and ionic strength. These factors critically influence the efficiency of the adsorption process and play an important role in determining optimal conditions for contaminant removal. Adjusting these parameters significantly affects extraction efficiency and minimizes waste.

3.3.1. Effect of concentration.

The effect of concentration on the extraction efficiency of Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) was evaluated using both raw and organically modified bentonite. Pharmaceutical concentrations were adjusted to 5, 10, 20, and 50 ppm to assess adsorption performance. The results showed that extraction efficiency increased with increasing concentration of pharmaceuticals. This is attributed to the higher number of solute molecules available for interaction with the adsorbent surface. At all tested concentrations,

OMB-16 exhibited higher extraction efficiencies than raw bentonite for all three drugs, indicating that CTAB modification enhanced adsorption performance through increased surface area and improved functional interactions. Raw bentonite showed lower efficiency, particularly at lower concentrations, due to its hydrophilic nature and limited interaction sites. Overall, concentration was found to significantly influence adsorption performance, with higher concentrations favoring improved extraction, especially for OMB-16. Figure 5 shows the comparison of extraction efficiency for SDM, SDZ, and SGD at different concentrations using raw bentonite and OMB-16.

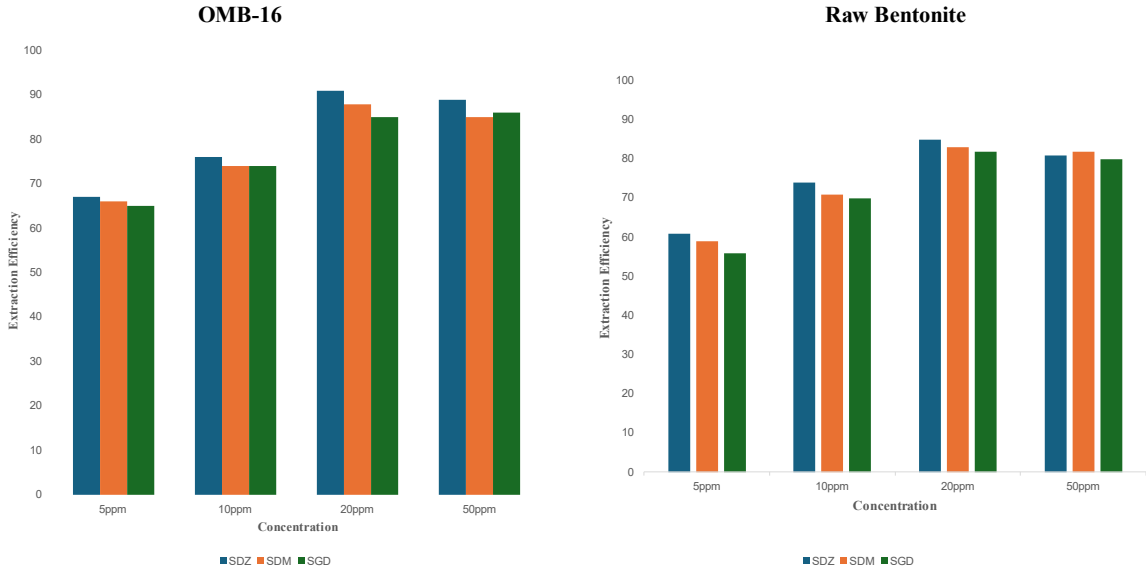

Figure 5. Comparison of extraction efficiency for Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) at varying concentrations using raw bentonite and organically modified bentonite (OMB-16) as adsorbents.

3.3.2. Effect of pH.

The extraction efficiency of OMB-16 that displays a strong deviation with different pHs. The highest extraction efficiencies of all three drugs are shown at both pH 4 and pH 5 after which there is a slight decrease of the extraction efficiencies as the PH increases to greater heights. In particular, Sulfadimidine Sodium (SDM) and Sulfadiazine (SDZ) give the best results at pH 4, whereas Sulfaguanidine (SGD) shows similar results at pH 5. Such observations number that the reaction between the pharmaceuticals and OMB-16 is optimized in a slightly acidic environment, which is probably caused by the positive charge interactions and increased availability of the adsorbent surface. On the other hand, the raw bentonite graph exhibits much lower extraction efficiencies on the whole and the highest extraction efficiencies are also attained at pH 4 and pH 5, which resembles the trend of OMB-16 but with a much weaker response. The higher the pH, the lower is the extraction efficiency especially: Sulfadimidine Sodium (SDM) and Sulfaguanidine (SGD). This tendency indicates that the use of raw bentonite has poorer adsorbent properties to these pharmaceuticals, in particular, at high pH levels. All of these data demonstrates that OMB-16 bentonite outperforms raw bentonite at all the studied PH levels, whereas slightly acidic conditions (pH 4-5) are the most effective in

terms of efficient extraction. Figure 6 illustrates the effect of pH on extraction efficiency of SDM, SDZ, and SGD using raw bentonite and OMB-16.

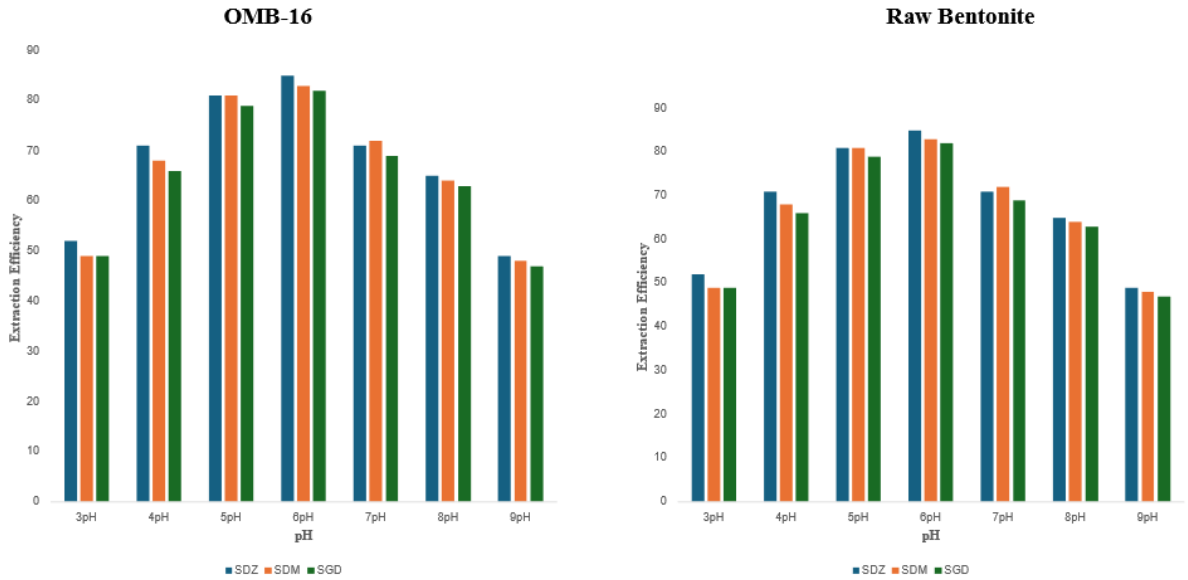

Figure 6. Effect of pH on extraction efficiency of Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) using raw bentonite and OMB-16 bentonite.

3.3.3. Effect of adsorbent dose.

Figure 7 presents the effect of adsorbent dose on extraction efficiency of SDM, SDZ, and SGD using raw bentonite and OMB-16. In the OMB-16 graph, extraction efficiency increases with increasing dose of adsorbent, especially, with Sulfadimidine Sodium (SDM) and Sulfadiazine (SDZ). Optimal results in terms of the extraction efficiencies are achieved at 30 mg and 40 mg of the OMB-16 where all three drugs are near to optimum adsorption. This fact implies that the more an adsorbent dose an adsorbent has, the more the adsorption sites available to it, thus boosting drug removal.

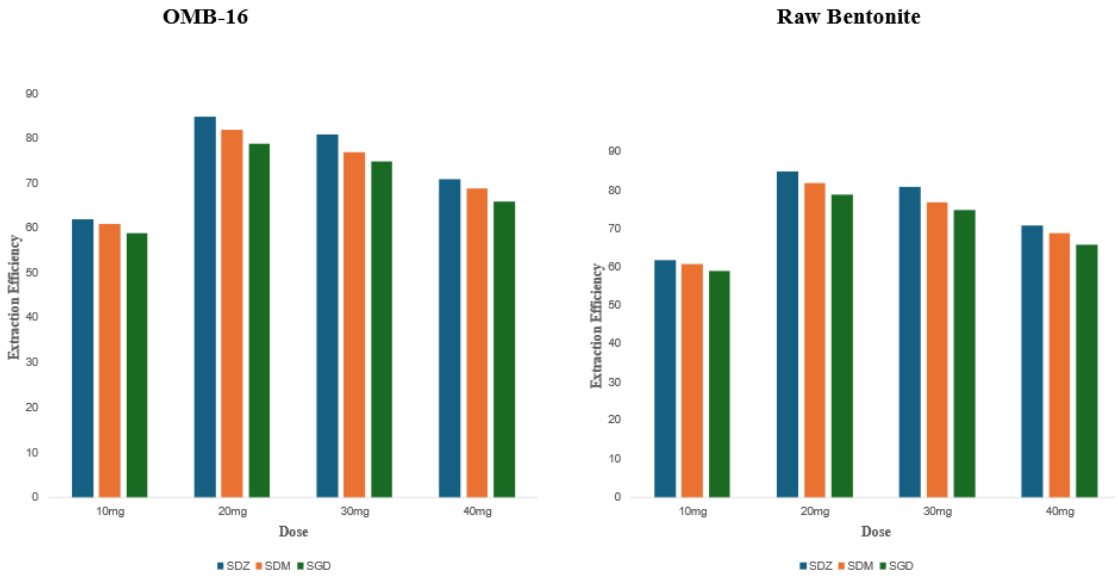

Figure 7. Effect of adsorbent dose on extraction efficiency of Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) using raw bentonite and OMB-16 bentonite.

In the case of Sulfaguanidine (SGD), the efficiency increases with increasing doses, although no longer with as striking an increase as in the other drugs, perhaps due to the special effect on the adsorbent surface. Raw bentonite, conversely, exhibits significantly lower extraction efficiencies at all doses. Although, efficiency changes incrementally with dose, the overall performance is significantly low than OMB-16. This implies that the surface attributes of the crude bentonites are of poorer adsorption capability of the pharmaceutical substances, particularly lower dosages. Therefore, the more the dose of adsorbent used the greater the efficiency of the extraction and OMB 16 performance changed drastically as compared to the performance of raw bentonite in all the doses used.

3.3.4. Effect of solvent.

The OMB -16 graph illustrates that methanol always exhibits the highest extraction efficiency of all the three pharmacologic agents with a specifically high benefit to sulfadimidine sodium (SDM) and sulfadiazine (SDZ). The next most potent solvents, which are much less effective than methanol, are acetonitrile and water, and dimethylformamide (DMF) becomes relatively poor in extraction efficiencies. It is implied in these observations that the high polarity of methanol and the ability to dissolve a wide range of organic components enables the development of good interactions with the adsorbent, thus improving the recovery of the analyte. On the other hand, the graph in the case of the raw bentonite shows constant and decreasing efficiencies of extraction in each of the solvents analyzed. But still, methanol is the most successful one followed by acetonitrile and water. The decrease in efficacy between DMF and OMB -16 follows the curve but with a smaller value difference suggesting that raw bentonite has relatively lower adsorptive properties compared to the organic modified bentonite. In general, methanol is the most effective solvent of OMB-16 and pure bentonite and the organically modified substance works better in all the solvent systems, compared to the raw one. Figure 8 shows the effect of different solvents on extraction efficiency of SDM, SDZ, and SGD using raw bentonite and OMB-16.

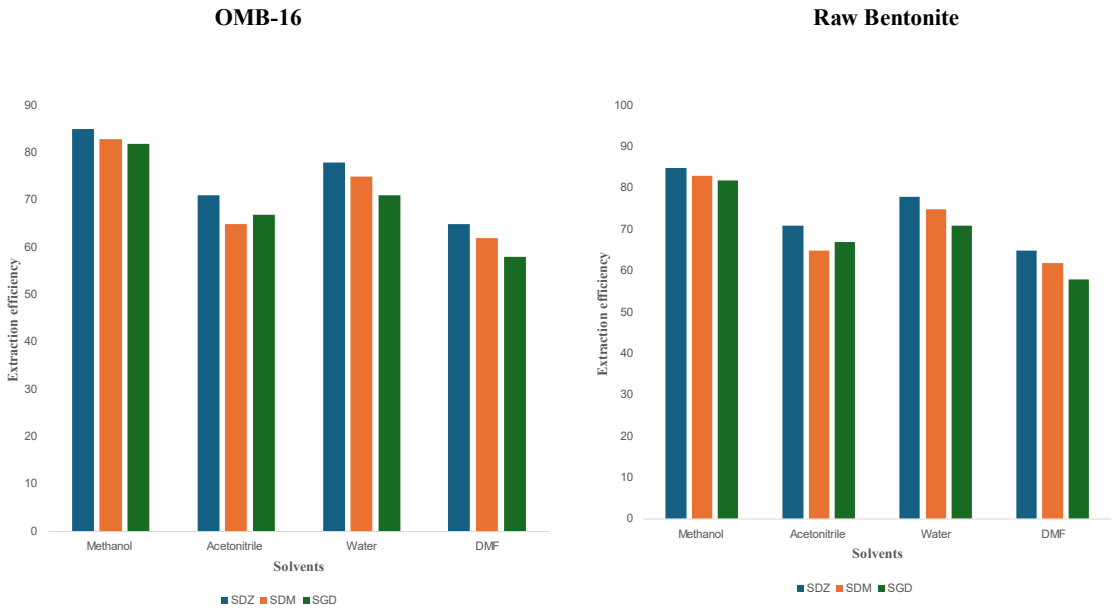

Figure 8. Effect of different solvents on extraction efficiency of Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) using raw bentonite and OMB-16 bentonite

3.3.5. Reproducibility.

The extraction efficiency of OMB 16 is relatively independent of the six replicates but there is a slight decrease in the second and the sixth replicates (R5 and R6). However, the efficiencies of all the three target pharmaceuticals are very high with Sulfadimidine Sodium (SDM) and Sulfadiazine (SDZ) having an efficacy of well above 70%. The consistency in the findings indicates to show that OMB-16 is a reliable performance material as far as reproducibility is concerned making it a mixed adsorbent of pharmaceutical contaminants. The fact that there is no major change in the reproducibility suggests a high degree of repeatability and reliability in adsorption of these compounds under similar experimental conditions. By comparison, the raw bentonite data have lower average extraction efficiencies, particularly of Sulfaguanidine (SGD), and have stronger variation between replicates. Although the overall trend of going downward is the same, the efficiency declines further in later replicates, especially in R6. This trend indicates that unmodified bentonite is less stable and uniform as an adsorbent than OMB-16 thus, emphasizing the benefits that organic modification has. All in all, OMB-16 exhibits better reproducibility and high extraction capability as compared to uncooked bentonite, which makes it a more reliable substance as a remover of pharmaceutical contaminants. Figure 9 illustrates the reproducibility of extraction efficiency for SDM, SDZ, and SGD across multiple replicates using raw bentonite and OMB-16.

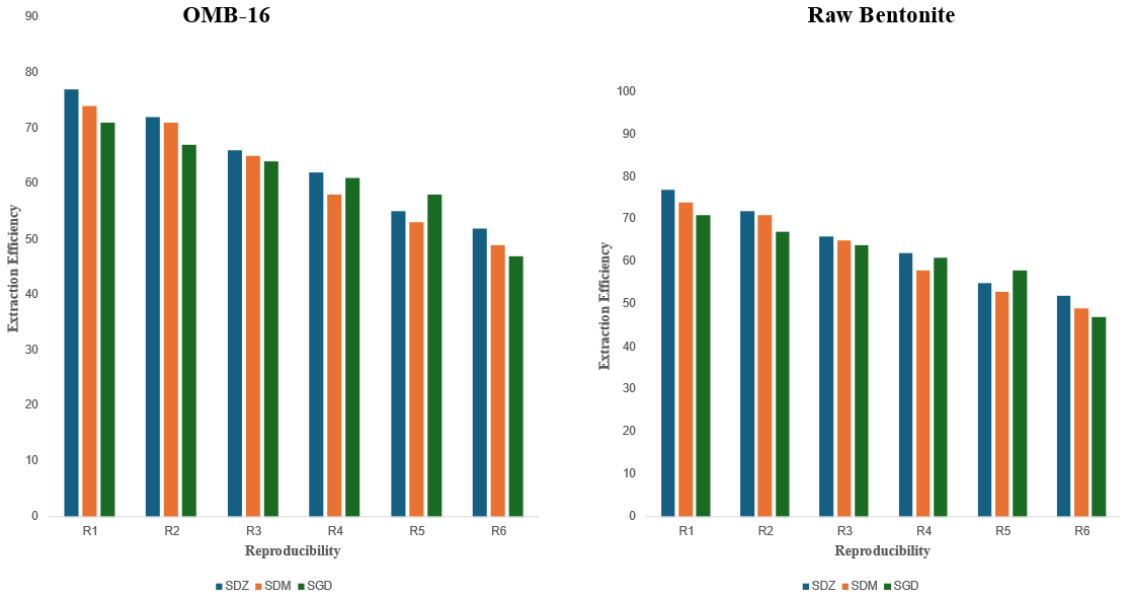

Figure 9. Reproducibility of extraction efficiency for Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) using raw bentonite and OMB-16 bentonite across multiple replicates.

3.4. Method Validation.

The validation of the extraction and quantification of the Sulfonamides, Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) in milk samples was done by evaluating various parameters of fundamental analysis data, such as linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and recovery. The dispatching system was a high-performance liquid chromatography (HPLC) that formed a system with the addition of UV detection, solids The solid-phase extractable material was organically modified bentonite and traditional bentonite. The validation indicated that the three analytes gave a very

good linear calibration curve above correlation coefficient (R 2) of 0.99 over the concentration range investigated, which is a strong response of the analytical process. LOD and LOQ were calculated for each drug, with Sulfadimidine Sodium (SDM) having an LOD of 0.03 µg/mL and a LOQ of 0.10 µg/mL, Sulfadiazine (SDZ) showing LOD of 0.04 µg/mL and LOQ of 0.12 µg/mL, and Sulfaguanidine (SGD) with LOD of 0.05 µg/mL and LOQ of 0.15 µg/mL. The intraday and interday precision for the analysis were calculated using relative standard deviation (RSD), and values were consistently low (less than 5%), confirming high method precision. Recovery tests on spiked milk samples demonstrated the high performance of OMB -16, recoveries of between 87-90%, as opposed to the 75-80% of raw bentonite. These findings validate the precision and accuracy of the method to be used in regular monitoring of sulfonamides in milk.

3.5. Real Sample Analysis

The retrieved chromatogram of the HPLC analysis of the authentic milk sample indicates the existence of sulfonamide drug residues, most likely, Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) drug. There are three different peaks with the retention times of 3.1, 7.1, and 7.4 min respectively, which are related to the elution of SDM, SDZ and SGD. The steepness of these peaks and their focusing implies that the substances were detached successfully off of the milk background, and only a few interferences were present. The maximum values are also associated with the concentration of all the analytes in the milk sample, where increasing values of the maximums correspond to increasing concentrations. There are no sharp peaks in the chromatogram and the baseline is stable, with no strong noise levels, indicating the best chromatography environment and accurate determination of the analytes. The space beneath the two peaks can be used to measure the number of each sulfonamide in the sample and the analysis was consistent and accurate. As a result, the findings suggest that the technique is effective in determining and quantifying the sulfonamides in solid food samples like milk and thus proves the appropriateness of the HPLC-UV technique in the regular monitoring of drug residues in food items. The above chromatographic profile reiterates the effectiveness, and precision of the method in identifying sulfonamide contamination in actual samples, which supports its use in food safety testing. Figure 10 shows the chromatogram of SDM, SDZ, and SGD residues in real milk samples using HPLC-UV.

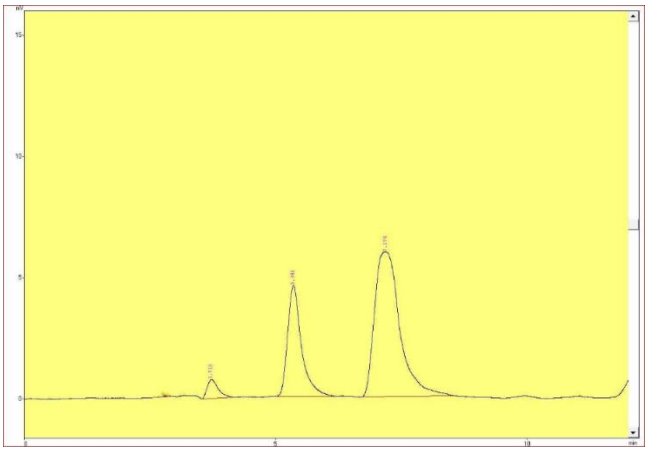

Figure 10. Chromatogram showing the detection of Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD) residues in a real milk sample using HPLC-UV.

3.6. Discussions.

This study demonstrates that organically modified bentonite (OMB-16) exhibits significantly higher adsorption capacity for sulfonamide antibiotics compared to raw bentonite, consistent with previous studies on chemically modified clay adsorbents. The enhanced performance can be attributed to increased surface area, porosity, and the introduction of hydrophobic functional groups through CTAB modification, which promotes stronger interactions with organic contaminants [18]. Similar trends have been reported in earlier studies, where surfactant-modified clays showed improved adsorption of pharmaceutical residues due to enhanced organophilic properties [19]. The observed optimum performance at mildly acidic conditions (pH 4–5) is also consistent with previous findings, where pH plays a key role in controlling the ionization state of both adsorbent surfaces and antibiotic molecules. Under acidic conditions, increased protonation enhances electrostatic attraction and hydrogen bonding, thereby improving adsorption efficiency. In contrast, reduced performance at higher pH values may be due to competition with hydroxide ions and reduced electrostatic affinity between adsorbent and analytes.

The use of methanol as the most effective elution solvent is also well supported by literature, where polar organic solvents are commonly reported to efficiently desorb pharmaceutical compounds from solid-phase materials. The high recovery rates (up to 90%) and strong linearity ($R^2 \geq 0.99$) obtained in this study indicate that the developed method is reliable and comparable to established solid-phase extraction (SPE) techniques using commercial cartridges. However, although OMB-16 shows high adsorption efficiency, its performance may still be lower in some cases compared to advanced adsorbents such as activated carbon and nanomaterials [20]. Nevertheless, OMB-16 remains a promising alternative due to its low cost, environmental friendliness, and simple preparation process. Solvent selection significantly influenced desorption efficiency. Methanol showed the highest elution efficiency, followed by acetonitrile. This is attributed to methanol's higher polarity, which facilitates disruption of adsorbent–analyte interactions and enhances recovery. In contrast, water and dimethylformamide (DMF) showed lower recoveries, likely due to their limited ability to effectively disrupt hydrogen bonding and electrostatic interactions between sulfonamides and the adsorbent surface.

Reproducibility tests confirmed that OMB-16 provides high precision, with low intra- and inter-day variation. Recovery from spiked milk samples consistently showed higher values for OMB-16 compared to raw bentonite, reaching up to 90% for SDM and SDZ, compared to 80% and 75%, respectively, for unmodified bentonite. These findings confirm the superior efficiency and stability of OMB-16 for extracting pharmaceutical residues in dairy matrices.
HPLC-UV analysis further supported the effectiveness of the extraction method, showing well-resolved chromatographic peaks with minimal matrix interference. The method demonstrated strong linearity ($R^2 > 0.99$) and low limits of detection, confirming high sensitivity and specificity for sulfonamide analysis. Overall, this study confirms that OMB-16 is an effective and reliable adsorbent for removing sulfonamide antibiotics from complex food matrices such as milk. The findings highlight its potential application in food safety monitoring as a cost-effective and environmentally friendly alternative to conventional adsorbents. Future studies should explore its applicability to other food matrices and broader classes of pharmaceutical contaminants.

4. Conclusions

The results of this study demonstrate the high efficiency of organically modified bentonite (OMB-16) in removing sulfonamide antibiotic residues from milk compared to raw bentonite. Through systematic optimization of key parameters, including pH, adsorbent dosage, and elution solvent, OMB-16 achieved significantly improved extraction efficiencies and recovery rates for Sulfadimidine Sodium (SDM), Sulfadiazine (SDZ), and Sulfaguanidine (SGD). Optimal performance was observed at slightly acidic conditions (pH 4–5), while methanol was identified as the most effective desorption solvent. OMB-16 also demonstrated excellent reproducibility, with minimal intra- and inter-day variation, confirming its suitability for routine analytical applications. The analytical method using HPLC-UV showed strong performance, with high sensitivity, low limits of detection, and excellent linearity ($R^2 \geq 0.99$). Recovery results further confirmed the superiority of OMB-16, achieving up to 90% recovery for SDM and SDZ, significantly higher than raw bentonite. Overall, OMB-16 presents a promising, cost-effective, and environmentally friendly adsorbent for the removal of pharmaceutical contaminants in food matrices, offering clear advantages over conventional bentonite. The method developed in this study is suitable for food safety monitoring and may be extended to other pharmaceutical classes and food systems. Future research should focus on broader applications and large-scale feasibility.

Acknowledgments

No funding collected for this research work.

Competing Interest

There is no competing interest for this research work.

Author Contribution

All authors contributed equally to this work. They jointly participated in the conceptualization of the study, literature review, experimental design, data analysis, interpretation of results, and manuscript preparation. All authors reviewed and approved the final version of the manuscript.

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