An-Najah University Journal for Research – A
Natural Sciences

# Eco-Friendly and Efficient Extraction of Parabens from Cream Products Using Magnetic Cellulose Nanoparticles: A Promising Analytical Method

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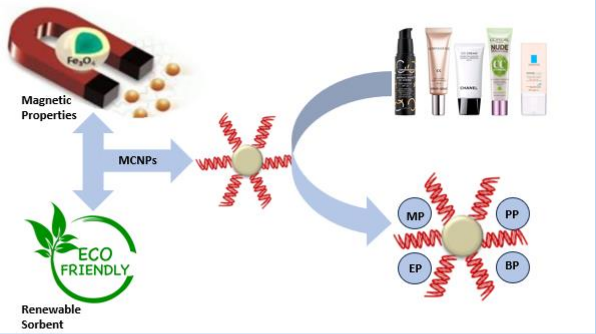
Type: Full Article. Received: 26th Dec. 2024, Accepted: 23rd Mar. 2025, Published: ××××, DOI: https://doi.org/10.xxxx

**Accepted Manuscript, In Press**

**Abstract**: This study presents an eco-friendly and efficient method for the extraction of methylparaben, ethylparaben, propylparaben and butylparaben from cream products using magnetic cellulose nanoparticles as a solid phase extraction sorbent. Parameters that affect adsorption (pH, adsorption time, amount of magnetic cellulose nanoparticles, salt content and sample volume) and desorption (desorption solvent, desorption volume, type of energy and time) were studied. The values that provided the maximum adsorption efficiency and extraction recovery were selected at optimum conditions for adsorption and desorption, respectively. Following the sample preparation stage, high-performance liquid chromatography with a photo-diode array detector was implemented. The intra and inter-day (*n*=5) precision were below 8.7%. Limits of detection and quantification were in the range 0.19–0.31 µg $L^{-1}$ and 0.63–1.1 µg $L^{-1}$, respectively. Analyte extraction in real samples yielded excellent preconcentration factors (141-150) and good recoveries (87.9% to 100.8%). The proposed method is a promising alternative for the analysis of parabens in cream products, as it is simple, rapidly implemented, and environmentally favourable.



**Keywords**: Magnetic Cellulose Nanoparticles, Cream Products, Parabens, Renewable Sorbent, Eco-Friendly Extraction.

## Introduction

Solid phase extraction (SPE) is a widely employed method for preconcentration and clean-up, offering numerous advantages over traditional methods such as liquid-liquid extraction and liquid phase microextraction techniques (1,2). For instance, SPE has the capacity to integrate with a variety of detection techniques and possesses a high enrichment factor. Despite the advantages of SPE, it is susceptible to certain potential drawbacks, including the quality and uniformity of the packaging material, as well as the limited selectivity of certain conventional sorbents and the challenges associated with their separation, particularly in the dispersive mode (3).

The inclusion of nanoparticles effectively addresses these drawbacks by utilizing them as novel sorbents in different working modes. Nanocellulose (NC) has emerged as a significant nanomaterial in recent years. It is considered a green alternative to conventional sorbents because of its distinctive characteristics, including biocompatibility, biodegradability, and chemical stability (4-6). Furthermore, the formation of strong hydrogen bonds with the surface of NC results in a significant increase in extraction efficiency. This effect can be further amplified by combining NC with magnetic nanoparticles (MNPs) (7,8). This coupling introduces a hybrid material, known as magnetic cellulose nanoparticles (MCNPs), which has been used in magnetic solid phase extraction (MSPE), as reported in recent works (9,10).

As a new alternative mode of SPE, MSPE offers significant advantages in sample preparation. It is very efficient and time-saving due to the straightforward isolation process employing a magnet (11). In addition, MCNPs offer significant advantages for preconcentration, clean-up, and extraction processes. These advantages stem from their low surface hydrophilic coatings, exceptional selectivity towards target analytes, and their ability to achieve rapid and effective recovery (12). Furthermore, MCNPs utilize cellulose, the most prevalent renewable polymer, which is non-toxic and has favorable biodegradability (13). Although MCNPs have significant advantages in MSPE, their applications are still very scarce. Thus far, this technique has

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only been used in a limited capacity, mostly for extracting emerging pollutants in some applications (8,13,14). Therefore, it is imperative to conduct additional investigation and expansion of this technique to other domains of application that need the determination of analytes at low concentrations and necessitate preconcentration, such as the detection of parabens in cream samples.

Parabens are a group of chemicals that are commonly used as antimicrobial preservatives in cosmetics, skin care products, foods, and many other pharmaceutical and industrial products (15). They are a family of alkyl esters of para-hydroxybenzoic acid that can take on different forms depending on the various chemical substitutions that can attach on the para position of the benzene ring. Common parabens include methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP). Each chemical substitution provides the corresponding paraben with a different solubility and spectrum of antimicrobial activity (16). The desirability for the use of parabens in industry is related to the unique characteristics of this group of chemicals such as being odourless, tasteless, stable, non-volatile, and efficient under a wide pH range (17). On the hand, recent studies have shown that exposure to parabens may lead to serious diseases, since parabens can be absorbed through the skin and into the human body. For example, parabens may cause irritation to human skin and disrupt the respiratory and reproductive system in humans (18,19). Moreover, parabens were found to cause breast cancer and the development of malignant melanomas due to them acting as endocrine disruptors, shown by the estrogenic activity (20,21).

Hence, there is utmost need to develop a sensitive method for the determination of parabens in cream products. The novelty of this method is that the nanoparticle sorbents, loaded with the analyte, are extracted and pre-concentrated without sample loss prior to sensitive and effective detection of parabens in cream samples with MCNPs-MSPE. This study attempted to develop and apply, for the first time, an efficient procedure of MSPE based on the use of MCNPs, an environmentally friendly sorbent, for the simultaneous extraction and preconcentration of MP, EP, PP, and BP in cream samples for further analysis by high performance liquid chromatography (HPLC) with photo-diode array detector (PDA).

## Materials and Methods

### Standards, solvents and reagents

All reagents used were of analytical grade. MP, EP, PP, and BP were obtained from Sigma-Aldrich (St. Louis, USA). LC-MS grade water, Methanol (MeOH), acetonitrile (MeCN), hydrochloric acid (HCl), phosphoric acid ($H_3PO_4$), and sodium hydroxide (NaOH) were purchased from Scharlab (Barcelona, Spain). Sodium chloride (NaCl), Ferric chloride ($FeCl_3.6H_2O$), ferrous chloride ($FeCl_2.4H_2O$), and cellulose microcrystalline (~50 µm particle size) were purchased from Sigma-Aldrich (St. Louis, USA).

### Instruments

Chromatographic separation was conducted using a Shimadzu Prominence LC-2030C 3D HPLC instrument (Shimadzu Corporation, Japan), which is equipped with a photodiode array detector. The analytical column employed was a hypersil ODS ($C_{18}$) of 250 mm × 4.6 mm I.D. and 5-µm particle size (Thermo Scientific, USA). The mobile phase was filtered using a vacuum filtration system through 0.2 µm polyamide membrane filters (Sartorius Stedim Biotech GmbH, Göttingen, Germany).

For the determination of target analytes, an isocratic program was employed at a fixed flow rate of 1.0 mL min$^{-1}$ of the mobile phase, which was composed of 60:40 (v/v) mixed solution of MeOH and water. The detector was set at 254 nm and the injection volume was 10 µL. An adequate separation is achieved in less than 10 minutes. Data analysis was carried out using Shimadzu lab solutions software (Shimadzu Corporation).

### Synthesis and activation of MCNPs

The synthesis of MCNPs followed previously described procedures [13,20]. Initially, 20 mg of microcrystalline cellulose were immersed in 200 mL of aqueous solutions of $FeSO_4$ and $CoCl_2$ with a molar ratio of [Fe]/[Co] = 2. Next, the mixture was swirled for 15 min to in order to achieve a homogenous distribution. The system was heated at 90 ºC for 3 hours to convert soluble iron/cobalt hydroxides into insoluble iron/cobalt oxyhydroxide complex. After that, 200 mL of a 1.32 mol L$^{-1}$ NaOH solution with $KNO_3$ ($[Fe^{2+}]/[NO^{3-}]$ = 0.44) was added and the mixture was heated again at 90 ºC for 8 hours. Then, the MCNPs were freeze dried in order to produce ferromagnetic nanoparticles. The resulting solid nanomaterial was then transferred to an oven and subjected to drying at 80°C for 8 hours.

The activation process of MCNPs was performed according to previous work (22). At first, 25 mg of MCNPs were vortexed for 1 min with 1 mL of ultrapure water for three times. Then, they were vortexed twice with 1 mL of MeCN.

### Preparation of solutions and real samples

Stock standard solutions of all analytes were prepared in MeOH at a concentration of 1 mg L$^{-1}$ and stored in a dark location at 4°C. Using ultrapure water, the mixed standard solutions were diluted in a precise, step-by-step manner for daily preparation.

Cream samples, including skin cream, sunblock cream, hand cream and foot cream were purchased from a local supermarket (Ramallah, Palestine). A 5 mg sample of each cream was accurately weighed and dissolved in a solution mixture containing 3.0 mL MeOH and 7.0 mL water. After that, 1.0 mL HCl (37 w/w%) was introduced to the solution, which was then sonicated for 10 minutes. The achieved solution was diluted to 150 mL with ultrapure water and its pH was adjusted to 8.0, filtered through a 0.45 µm filter membrane, and stored at 4°C for further determination by MSPE-HPLC/PDA.

### MSPE procedure

In a 15 mL vial, 10 mg of activated MCNPs were initially combined with a 15 mL solution of the analytes (100 µg L$^{-1}$) that had been adjusted to pH 8.0. The vial was then vortexed at 1000 rpm for 10 minutes. An external magnetic field was subsequently employed to separate the MCNPs from the solution. The supernatant was discarded and 100 µL MeCN was added to elute the target analytes, which was sonicated for 10 min. Then, 10 µL of the elute was injected into the HPLC-PDA for analysis.

## Results and Discussion

### Optimization of the MSPE conditions

A univariate strategy was employed to optimize the factors that impact the efficiency of the MSPE technique. The experiments were conducted in triplicates and average data were calculated.

The initial standard conditions were adsorption stage of 50 mg of MCNPs, 10 mL of sample containing 100 µg L$^{-1}$ of analytes and 15 min of vortex agitation with pH adjusted to 7.0. Next, desorption was performed with 500 µL of MeCN for 10 min under sonication. To evaluate the efficiency of the adsorption step, the aqueous supernatant was injected into the HPLC-PDA.

Adsorption efficiency (AE), which was selected for monitoring the adsorption process, was calculated as follows:

$$AE = \left(1 - \frac{C_a}{C_i}\right) \times 100 \qquad \text{Eqn. 1}$$

where $c_a$ is the concentration of the analyte in the aqueous phase after adsorption and $c_i$ is the initial concentration.

Extraction recovery (ER) was calculated as the percentage of total amount of analyse which was desorbed, following this equation 2:

$$ER = \frac{C_f \times V_f}{C_i \times V_i} \times 100 \qquad \text{Eqn. 2}$$

where $C_f$ and $C_i$ are the concentration of the analyte in the extract after desorption and the initial concentration in the sample, respectively. The same applies to the volumes $V_f$ and $V_i$.

## Adsorption

The first parameter to be optimized was the pH level. The pH of an aqueous solution had a notable impact on the extraction of the analytes. This is because the pH affects the form of the analytes, the charge of the species, the surface charge density of the sorbent, and ultimately, AE. An investigation was conducted to examine the impact of pH on the adsorption of analytes within a pH range of 2–10, which was adjusted using 0.5 M HCl and 0.5 M NaOH. The lower end of the pH range yielded unsatisfactory results, with analytes exhibiting AE values of 50-60% at pH range of 2–6, and below 70% at a pH of 7. The target analytes have p$K_a$ values of approximately 9. Therefore, the coupling between analytes and MCNPs is suboptimal at pH values below p$K_a$, as the analytes are uncharged. The AE of analytes were around 85% at pH 8, and then dropped gradually to 70% when pH values increased up to 10 (**Figure 1a**). At pH values higher than p$K_a$, the target analytes are present in their ionized form and have minimal adsorption. Thus, pH 8 was selected for the simultaneous adsorption of the analytes. At the chosen pH, the analytes can interact with MCNPs through many types of interactions, including hydrogen bonding, hydrophobic contacts, electrostatic interactions, and π-π interactions.

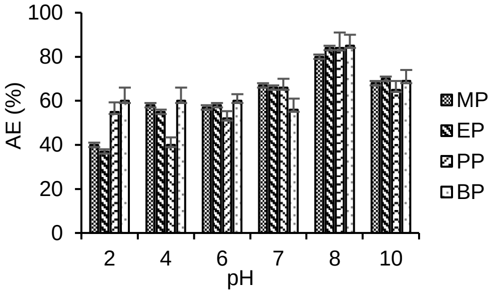

**Figure (1) a.** Influence of pH on adsorption efficiency. Standard deviations are plotted as error bars.

The adsorption time was also examined to seek the minimum time required to obtain quantitative results securely. As a result, and while maintaining other conditions constant, vortex agitation was investigated for periods ranging from 5 to 40 minutes. The AE of analytes manifested an apparent increase as the adsorption time increased, culminating in a maximum value at 10 minutes, with no further significant changes in the studied range (**Figure 1b**). Consequently, the optimal duration was selected to be 10 minutes

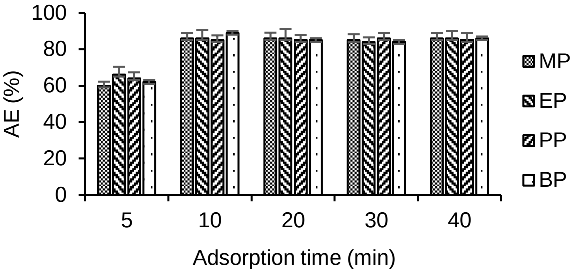

**Figure (1)b.** Influence of adsorption time on adsorption efficiency. Standard deviations are plotted as error bars.

An evaluation was conducted to determine the ideal amount of MCNPs for the quantitative adsorption of all analytes, ranging from 5 to 50 mg. The AE showed a significant increase as the amount of MCNP increased from 5 to 10 mg. Beyond this point, up to 50 mg, the AE remained constant for all analytes. This highlights the potential of MCNPs as a sorbent, even when a small amount is used (**Figure 1c**). Thus, the selection of 10 mg was based on its ability to efficiently adsorb all analytes while minimizing material usage.

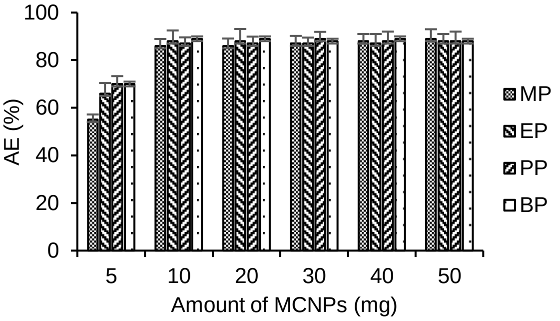

**Figure (1)c.** Influence of amount of MCNPs on adsorption efficiency. Standard deviations are plotted as error bars.

To examine the impact of ionic strength on AE, a series of experiments have explored the influence of varying quantities of NaCl within the range of 0–2.5 g. The findings revealed that higher NaCl concentrations resulted in an AE drop. This is attributed to the action of NaCl on the migration of analytes to the sorbent, which is caused by the salting-out effect. Therefore, it was determined that the addition of NaCl to the sample is not recommended.

To attain high preconcentration factors, a substantial sample volume is necessary. To study the effect of sample volume on the adsorption of the analytes, sample solutions of varying volumes (5, 10, 15, and 20 mL) were prepared and subjected to the procedure. The findings demonstrated that the AE remained fairly constant over the range of 5–15 mL (≥ 90%), but declined to around 80% as the sample volume increased to 20 mL. Therefore, a sample volume of 15 mL was established for subsequent experiments.

## Desorption

The primary characteristic of desorption is to achieve the highest possible ER. A comparison was made between the utilization of sonication and vortex agitation, both for a duration of 10 min. The ERs for MP and BP were comparable regardless of the energy type used. However, the ERs of EP and PP increased when sonication was used. Therefore, the latter option was chosen as the most suitable.

MeOH, MeCN and MeCN:MeOH (1:1, v:v) were tested as desorption solvents. The ERs were from 88 to 90% with MeCN, from 60 to 70% with MeCN:MeOH (1:1, v:v) and negligible with MeOH (**Figure 2**). Consequently, MeCN was selected for further experiments.

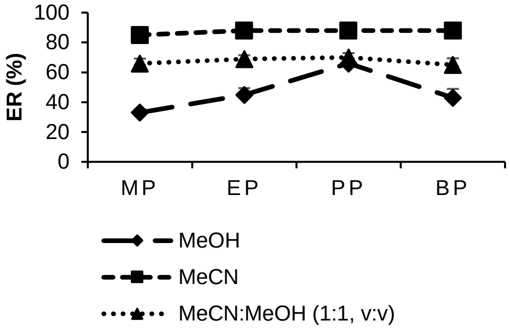

**Figure (2).** Influence of desorption solvent on extraction recovery. Standard deviations are plotted as error bars.

Another crucial aspect is the desorption volume, which should be minimized to maximize the preconcentration factor (PF), defined as the ratio of the initial volume to the final volume. The minimum manageable volume of desorption solvent was 100 µL, hence it was chosen. Given that the initial volume of the sample was 15 mL and the final volume was 100 µL, the analytes could potentially be concentrated by a factor of 150. In order to increase the ER, sonication was evaluated for desorption. The effect of the sonication time from 1 to 30 min on the ER of parabens was investigated (**Figure 3**). The ERs of parabens increased with increasing sonication time up to 10 min. Further increase in sonication time does not result in significant increment in recoveries but leads to a plateau. Therefore, in this study, the desorption time of 10 min was chosen for obtaining the complete extraction.

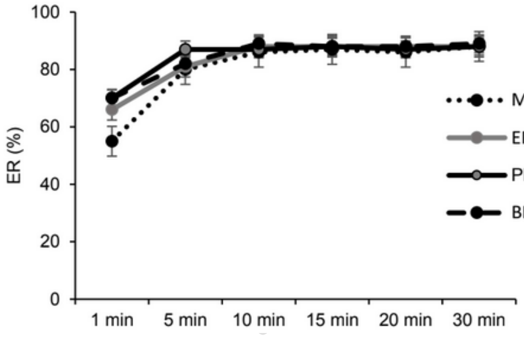

**Figure (3):** Influence of sonication time on extraction recovery. Standard deviations are plotted as error bars.

## Analytical figures of merit

The analytical figures of merit are summarized in **Table 1**. The present method was characterized in terms of sensitivity, linearity and precision. The limits of detection and quantification (LOD and LOQ) were calculated from a chromatogram of a 50 µg L$^{-1}$ standard after the MSPE process, using a S/N ratio of three and ten, respectively. LODs were in the low µg L$^{-1}$ range. The calibration graphs were constructed using aqueous standards between 20 and 1000 µg L$^{-1}$. The determination coefficients (R$^2$) were in the range 0.9949–0.9988 for all analytes. Intra-assay precision was calculated after injections of samples from five independent experiments on the same day, whereas inter-assay precision was assessed across four successive days. The % RSD results showed RSD values lower than 8.7%, demonstrating the adequate repeatability and reproducibility.

## Reusability assessment

The evaluation of the sorbent's effectiveness relies heavily on the possibility of reusing the nanoparticles. The reusability of the MCNPs was investigated by conducting multiple adsorption and desorption cycles consecutively using the optimal experimental conditions. Two sample solutions spiked at 50 and 100 µg L$^{-1}$ each, were analyzed seven times successively with the same sorbent. Following each use, the sorbent was washed with 2 x 1 mL of ultrapure water with vortex agitation for 1 min and dried at 80 ºC [22]. At both levels, the ERs were quantitative up to the fifth cycle, but dropped to 69% in the sixth cycle. Meanwhile, a modest decrease on BP adsorption was already observed after reusing the MCNPs six times and this decreasing trend continued in the seventh cycle.

## Analysis of real samples

To evaluate the applicability of the proposed method in different real samples, the cream samples (skin cream, sunblock cream, hand cream and foot cream) were analyzed by the MCNPs-MSPE/HPLC-PDA technique. To achieve this objective, the samples were prepared according to the instructions provided in the section on real sample preparation. Subsequently, analyses were conducted on both the non-spiked and spiked samples. Since all analytes were below the LODs in all real samples, three independent aliquots of each sample were spiked at 20 µg L$^{-1}$; whereas, three others were spiked at 50 µg L$^{-1}$, and each aliquot was analysed by triplicate. **Table 2** shows that ERs ranged from 87.9 and 100.8%. The results exhibited the great potential of the MCNPs sorbent for the extraction and determination of parabens in real samples. **Figure 4** displays HPLC-PDA chromatograms of non-spiked (a) and spiked (b) skin cream sample after extraction under the optimized conditions.

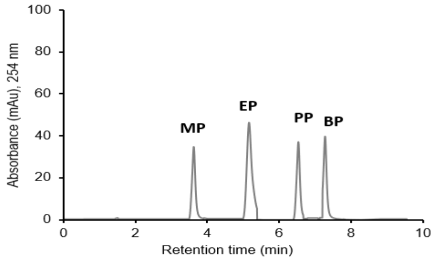

**Figure (4)a.** HPLC-PDA chromatograms of non-spiked skin cream sample after extraction under the optimized conditions.

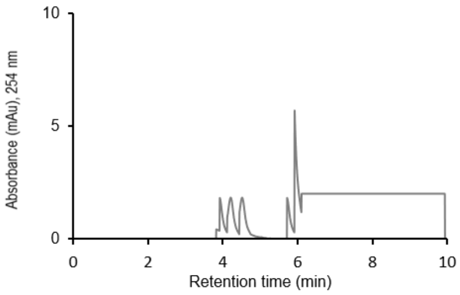

**Figure (4)b.** HPLC-PDA chromatograms of spiked skin cream sample after extraction under the optimized conditions.

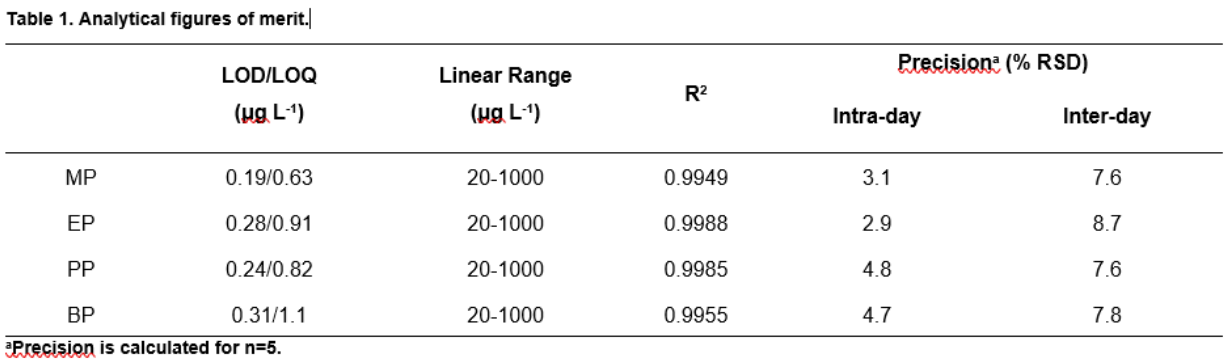

Table 1. Analytical figures of merit.

| | LOD/LOQ (μg L$^{-1}$) | Linear Range (μg L$^{-1}$) | R$^2$ | Precision$^a$ (% RSD) | |
|---|---|---|---|---|---|
| | | | | Intra-day | Inter-day |
| MP | 0.19/0.63 | 20-1000 | 0.9949 | 3.1 | 7.6 |
| EP | 0.28/0.91 | 20-1000 | 0.9988 | 2.9 | 8.7 |
| PP | 0.24/0.82 | 20-1000 | 0.9985 | 4.8 | 7.6 |
| BP | 0.31/1.1 | 20-1000 | 0.9955 | 4.7 | 7.8 |

[a] Precision is calculated for n=5.

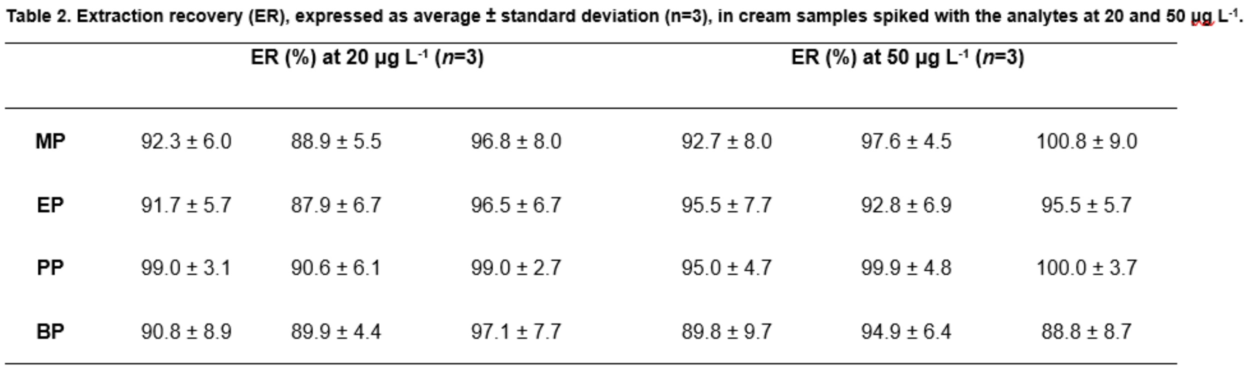

Table 2. Extraction recovery (ER), expressed as average ± standard deviation (n=3), in cream samples spiked with the analytes at 20 and 50 μg L$^{-1}$.

| | ER (%) at 20 μg L$^{-1}$ (*n*=3) | | | ER (%) at 50 μg L$^{-1}$ (*n*=3) | | |
|---|---|---|---|---|---|---|
| **MP** | 92.3 ± 6.0 | 88.9 ± 5.5 | 96.8 ± 8.0 | 92.7 ± 8.0 | 97.6 ± 4.5 | 100.8 ± 9.0 |
| **EP** | 91.7 ± 5.7 | 87.9 ± 6.7 | 96.5 ± 6.7 | 95.5 ± 7.7 | 92.8 ± 6.9 | 95.5 ± 5.7 |
| **PP** | 99.0 ± 3.1 | 90.6 ± 6.1 | 99.0 ± 2.7 | 95.0 ± 4.7 | 99.9 ± 4.8 | 100.0 ± 3.7 |
| **BP** | 90.8 ± 8.9 | 89.9 ± 4.4 | 97.1 ± 7.7 | 89.8 ± 9.7 | 94.9 ± 6.4 | 88.8 ± 8.7 |

### Comparison with literature

**Table 3** provides a summary of the comparison between the MSPE procedures that were previously employed for the extraction of parabens. The current work offers significant advantages in terms of: (i) utilizing a biodegradable and eco-friendly sorbent; (ii) enabling the straightforward synthesis of MCNPs without requiring additional modification or functionalization; (iii) achieving extremely low detection limits; and (iv) utilizing cost-effective and renewable sources. This novel method stands out as the sole approach that utilizes a sorbent composed of magnetic nanoparticles combined with a renewable and eco-friendly nanomaterial such as nanocellulose for MSPE in the extraction of parabens from cream products. The method's overall features have rendered it preferable over previously reported method.

## Conclusion

This study marks the initial application of magnetic cellulose nanoparticles for the extraction of parabens from cream products. This approach offers several advantages compared to the existing magnetic solid phase extraction procedures, namely in terms of utilizing an eco-friendly and renewable sorbent that can be easily synthesized. The MCNPs-MSPE technique is cost-effective because it uses nanocellulose, an abundant and sustainable resource, and eliminates expensive functionalization steps. In contrast to traditional SPE techniques that typically rely on commercially sourced polymeric or silica-based sorbents, MCNPs are produced from inexpensive precursors such as cellulose, iron salts, and cobalt salts. Furthermore, our technique requires less sorbent (10 mg per sample) than typical SPE cartridges, resulting in cheaper sample costs.

The method also combines the benefits of magnetic solid phase extraction, such as the convenient separation of analytes from the sorbent, minimal use of reagents, and low cost, with the analytical requirements of high selectivity for the target analytes, strong anti-interference ability, and extremely low detection limits. In addition, excellent preconcentration factors (141-150) and great recoveries (87.9% to 100.8%) were achieved in real samples. Due to these factors, this procedure presents itself as a novel and advantageous alternative for monitoring the trace amount of preservative chemicals in creams.

## Disclosure Data

- **Ethics approval and consent to participate:** The authors confirm that they respect the publication ethics and that they consent the publication of their work.
- **Consent for publication:** The authors consent the publication of this work. Availability of data and materials Data is available upon the request.
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
- **Author's contribution:** The first version of the document was written by Siouri.
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The data that support the findings of this study are available from the corresponding author, [JF], upon reasonable request.
- **Author's contribution:** Abujaber was in charge of overseeing the organization of research activities and the primary conceptual ideas. Siouri, Jaradat, and Mubarak were the ones who carried out the experiment. Explanation of the data, experimental design, and outcomes by Siouri and Abujaber. Data curation and manuscript editing were done by Siouri. The final draft of the work was reviewed by all authors, who also gave their approval.

- **Funding:** The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Researchers supporting project, Arab American University, Palestine.

- **Conflicts of interest:** The authors declare that there is no conflict of interest regarding the publication of this article.
- **Acknowledgements:** The authors would like to acknowledge the financial support from Arab American University and Birzeit University.

## Open Access

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