Malaysian Journal of Chemistry, 2024, Vol. 26(5), 614-621
(Special Issue: *4th IKMPB Online Symposium 2024 - Development of Chemistry to Accomplish a Sustainable World*)

Dispersive Micro Solid Phase Extraction Using Graphene Oxide for the Pre-concentration of Hydrocortisone in Water Samples by Liquid Chromatography Analysis

Raihana Mohd Yusof[1,2], Wan Norfatin Alya Mazira Wan Kamalludin[1], Nurzaimah Zaini[1], Nor Suhaila Mohamad Hanapi[1,4]*, Ahmad Lutfi Anis[3] and Noor Najmi Bonnia[1]
[1]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Centre of Foundation Studies, Universiti Teknologi MARA, Cawangan Selangor, Kampus Dengkil, 43800 Dengkil, Selangor, Malaysia
[3]Faculty of Applied Sciences, Universiti Teknologi MARA, 94300 Kota Samarahan, Sarawak, Malaysia
[4]Trace Analysis Research Group, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
*Corresponding author (e-mail: norsuhaila979@uitm.edu.my)

Hydrocortisone (HYD) is a synthetic corticosteroid widely used as an anti-inflammatory in human and veterinary medicine. Its presence in environmental water, though in trace concentration, is evident in posing hazardous risks towards aquatic organisms, which leads to numerous health and environmental issues. This contaminant enters environmental water through improper disposal of expired drugs, production residues, and spillage during manufacturing. Dispersive micro-solid phase extraction (D-µSPE) is a solid-phase extraction technique capable of enhancing reproducibility when dealing with low-concentration samples and is excellent in eliminating interferences. In this study, D-µSPE was developed and optimised using graphene oxide (GO) as sorbent coupled with high-performance liquid chromatography (HPLC) to pre-concentrate HYD in water samples. Several parameters, such as sorbent mass, sample pH, extraction time and solvent desorption volume, were investigated using a time (OFAT) approach followed by Liquid Chromatography analysis. Under the optimum conditions, the method provided a good linearity of 0.9882 over a concentration range of 50 and 400 µg L^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) values were 2.77 µg L^{-1} and 8.38 µg L^{-1}, respectively. The relative recovery was calculated within the range of 98 – 120%, and an excellent relative standard deviation (RSD) of $\leq$ 5% (n = 3) was obtained. The D-µ-SPE-GO method was successfully applied to tap water samples. The results showed that the developed method was selective, sensitive, and environmentally friendly to the water samples.

Keywords: Dispersive micro solid phase extraction; graphene oxide; hydrocortisone; water samples; liquid chromatography

Received: January 2024; Accepted: July 2024

Hydrocortisone (HYD) is an anti-inflammatory medication that is widely used in the treatment of allergic conditions, rheumatoid arthritis, asthma, chronic obstructive pulmonary diseases (COPD), and autoimmune disorders [1]The increase of HYD in aquatic environments has been identified worldwide due to uncontrolled prescription and non-prescription usage. This compound, being hydrophilic and polar, is easily transported and distributed through a water system. Removing this contaminant through conventional wastewater treatment is inefficient, causing the effluent of wastewater treatment plants to be the primary source of emerging steroids in environmental water [2].

The occurrence of HYD in aquatic environments, though in trace concentrations (ng L^{-1}), is capable of mimicking or blocking the endogenous hormones [3]. This leads to numerous health risks, such as hormone-dependent cancers, reduced sperm counts, obesity, and fertility deterioration in humans. Whereas for aquatic organisms, the risk posed by this steroid exposure includes a decline in reproduction, sexual malformations, and intersex changes [4].

Given the low concentration of HYD in water samples, sample preparation is considered a crucial step to ensure high accuracy and good reproducibility [5]. Sample preparation's advantages include enriching the target analytes, removing interferences, improving the compatibility of the sample with detection methods, and enhancing analyte detectability [6]. This step profoundly influences the total time required for analysis and the quality of results obtained [6].

To date, solid phase extraction (SPE) has become one of the most popular methods for sample preparation due to its simplicity, shorter processing time, less consumption of organic solvent, and compatibility with various detection methods compared

---

†Paper presented at the 4th IKMPB Online Symposium 2024 – Development of Chemistry to Accomplish a Sustainable World

to liquid-liquid extraction (LLE). However, the SPE method is unable to carry simultaneous extractions and has a tedious sample-loading procedure [7]. With regards to these limitations, dispersive micro solid phase extraction (D-µ-SPE) has become an excellent approach to simplify the procedure and concurrently reduce the extraction time [7]. This method is acknowledged as a green extraction technique (GreETs) as miniaturisation of SPE reduced sample consumption and waste generation drastically [8]. Moreover, D-µ-SPE is proven to be an excellent approach for complex samples with different physical states, offering high sensitivity on trace and ultra-trace level [9].

The triumph of the D-µ-SPE technique depends significantly on the proper sorbent choice. Graphene-based materials are new materials with great diversity in analytical chemistry applications due to their intrinsic characteristics, such as large surface area and functionalisation possibility. They comprised sp^2 hybridised carbons bonded in a hexagonal honeycomb crystal structure by covalent bond (σ) and parallel pi (π) bond [10]. To date, graphene has been acknowledged as the strongest and thinnest nanomaterial, having a sheet thickness of 0.34 nm [11]. However, due to the hydrophobicity and aggregation problem, this nanomaterial is not a proper choice to be implemented in the D-µ-SPE technique for aqueous sample [11].

Graphene oxide (GO) is one of the graphene derivatives produced through the oxidation of graphite using Hummer's method [9]. The presence of abundant oxygen-containing functional groups in GO, namely hydroxyl, carboxyl, and epoxy, associated with the hydrogen bonding and electrostatic interactions between GO and organic compounds [12]. These functional groups also provide more active properties to GO, resulting in high dispersibility in aqueous medium [12]. Owing to its outstanding characteristics, the implementation of GO as sorbent using D-µ-SPE as the pre-concentration technique for trace concentration compounds is of high potential [12].

With the exponential growth of pharmaceutical industries since the last decade, this study is significant to explore the green sample preparation techniques, which is D-µSPE as a preconcentration and separation method using a high potential sorbent to achieve rapid determination of trace amount of HYD in the water sample. Furthermore, methanol was used as a greener solvent substitution to accommodate toxic acetonitrile as the mobile phase to reduce environmental impact.

EXPERIMENTAL

Chemicals and Materials

The HYD standard was purchased from Sigma-Aldrich. HPLC-grade methanol (MeOH) was purchased from Merck (Darmstadt, Germany). Barnstead Nanopure (Thermo Scientific) produced ultrapure water. Graphene Oxide powder was purchased from GO Advance Solution (UPM, Malaysia). Water samples were taken from tap water in the laboratory, UiTM Shah Alam.

Chromatographic Conditions

The instrumental analysis of HYD was performed using the Agilent Technologies 1200 series HPLC system (Agilent Technologies, USA) equipped with solvent degasser, autosampler with 5µL loop, quarternary pump, column thermostat, and diode-array (DAD) detector. The chromatographic separation was carried out on a Zorbax Eclipse C18 column (10 cm x 2.1 mm, 3.5 µm); the mobile phase consisted of (A) methanol and (B) ultrapure water at 50:50 (A: B) composition of isocratic elution. Before the analysis, the chromatographic system was stabilised for 45 to 60 min. The flow rate was set at 0.5 mL min^{-1}, the injection volume was 5 µL, and the column thermostat was set at 38˚C. The detector wavelength was set at 254 nm. The chromatographic data was processed using Agilent Chemstation software.

Preparation of Stock and Standard Solutions

Adding MeOH prepared a 1000 mg L^{-1} of HYD stock solution. Next, working solutions were formed in a series of dilutions from stock solutions. A 100 mg L^{-1} stock solution dilution using MeOH yielded 10 mL of standard solution with spiked concentrations of 2, 4, 6, 8, and 10 mg L^{-1}. All standard solutions were stored in amber glass bottles at 4 ˚C. Tap water samples were collected from a laboratory in Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, Malaysia.

Dispersive Micro Solid Phase Extraction (D-µ-SPE) Procedure

The extraction method of D-µSPE was adopted by Zaini et al. (2022), illustrated in Figure 1. About 20 mg of GO was dispersed in 10 mL of spiked water sample, followed by agitation using a vortex mixer for 10 seconds. This step enhanced the sorption of target analytes onto the sorbent. Next, the partitioning of sorbent and supernatant was performed using a centrifuge at the speed of 8000 rpm for 10 minutes. The supernatant was later discarded. Then, the extracted analytes were eluted with 0.6 mL of MeOH, followed by agitation using a vortex mixer for 2 minutes. The desorption solvent and sorbent were partitioned using a centrifuge, adopting the same previous setting. Later, the eluent was collected by running it through a 0.45 µm disposable nylon filter (13 mm i.d.) into a vial, followed by liquid chromatography analysis.

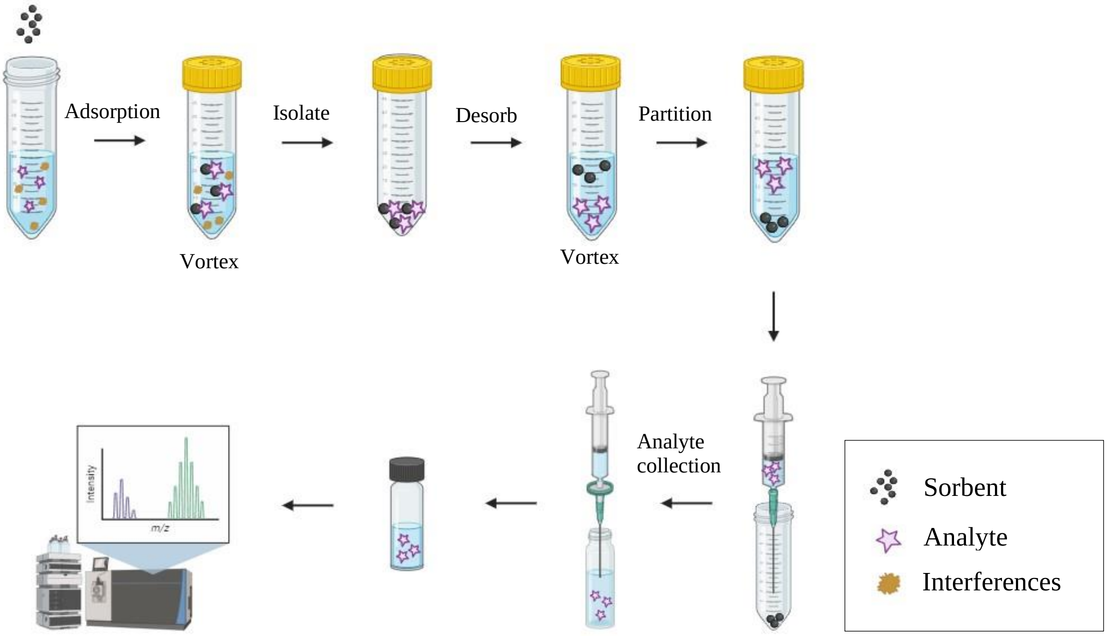

Figure 1. D-µ-SPE procedure of HYD extraction from water sample using GO nanosorbent.

Optimisation Parameters of D- µ-SPE Procedure

Four significant parameters were studied, employing one-factor-at-a-time (OFAT): mass of sorbent, volume of desorption solvent, extraction time, and sample pH. The reproducibility of D-µ-SPE was increased before optimising extraction conditions.

Method Validation and Data Analysis

The D-µ-SPE technique's validation includes determining the limit of detection (LOD), the limit of quantification (LOQ), precision, linearity, and the percentage of analyte recovery (% recovery) following the extraction stages. Analytical parameter validation was necessary to guarantee that the processes and instruments employed were excellent for analyte determination.

Calibration Curve

Calibration standard curves were established through linear regression, represented by $y = ax + b$. In this equation, y denotes the peak area, a represents the slope, x represents the respective concentration, and b represents the intercept. The linearity responses of HYD were assessed using a set of standards encompassing five concentrations, ranging from 50 to 400 µg L^{-1}. The requirement for satisfactory linearity was set with a minimum coefficient of determination (R^2) value of 0.95.

Limit of Detection and Quantification

The values for the limit of detection (LOD) and the limit of quantification (LOQ) were established employing linear regression equations: $LOD = 3.3\ \sigma/S$, and LOQ, 3 times higher than LOD, is calculated as $LOQ = 10\ \sigma/S$. Here, σ denotes the standard deviation of the lowest concentration, and S represents the slope of the calibration graph.

Repeatability

The proposed method's precision was characterized by repeatability (intra-day precision) and reproducibility (inter-day precision), expressed as relative standard deviation (RSD). Intra-day precision was assessed by triplicate measurements of the lowest concentration level prepared on the same day. Conversely, inter-day precision was evaluated by repeating the procedure for three consecutive days.

Recovery

The accuracy of the method, indicated by % recovery (R%), was assessed by spiking real samples with HYD, reaching a final concentration of 100 µg L^{-1}. While there is no official guideline stipulating the recovery of spiked pharmaceuticals in water, the acceptable range is typically between 70%−120%, accompanied by a relative standard deviation (RSD) of $\leqslant$ 20% [17].

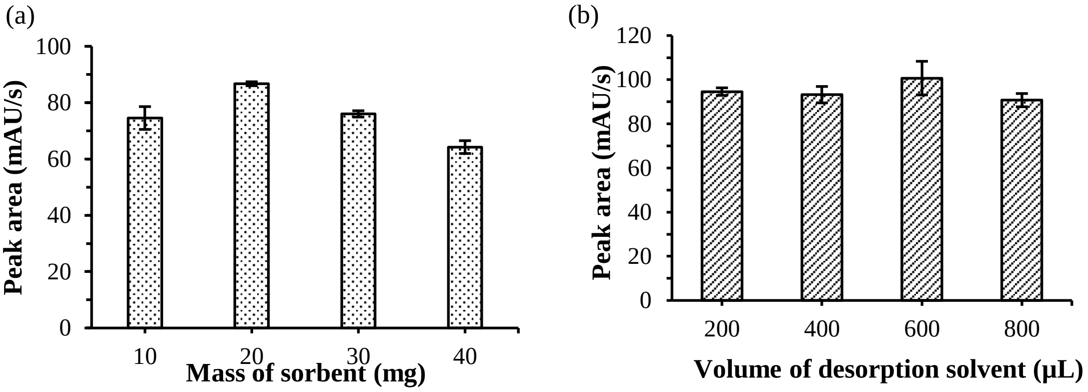

Figure 2. The effect of (a) mass of sorbent, and (b) volume of desorption solvent on the extraction of HYD using GO nanosorbent.

RESULTS AND DISCUSSION

System Performance

The calibration curve, depicting peak area (y-axis) against the concentration of HYD in parts per million (ppm) (x-axis), was constructed using five concentrations of standard HYD injected into the HPLC. The calibration curve demonstrated linearity within the 2 - 10 mg L^{-1} range. The linear regression equation of the calibration curve was determined, and a good level of linearity was confirmed with a correlation coefficient (R^2) of 0.9624.

Optimisation of D- µ-SPE Procedure

Hydrocortisone (HYD) was utilised in the optimisation process to optimise the sorbent mass, desorption solvent volume, extraction time, and sample pH. These four parameters were varied, and the peak area responses were observed to identify the optimum conditions for extracting HYD from water. The conditions with the highest peak area were selected for each parameter. All parameters underwent testing in distilled water, spiked beforehand with target analytes, each at a concentration of 4 mg L^{-1}.

Effect of Sorbent Mass and Desorption Solvent Volume

The quantity of sorbent employed plays a crucial role in determining the effectiveness of D-µSPE extraction. Initially, the optimisation of the sorbent mass involved varying the mass of graphene oxide (GO) used for analyte adsorption. This investigation aimed to identify the minimal sorbent required to achieve the maximum extraction efficiency for the target analyte. The results were then graphically represented to determine the optimal GO mass. Figure 2 (a) illustrates that the mass of 20 mg of GO exhibits the highest peak area. The peak area increased to 20 mg but began declining to 30 mg. This outcome suggests that 20 mg of GO provides sufficient active sites for the adsorption of HYD. Moreover, the chromatogram signals decreased for higher quantities of adsorbent, likely attributed to poorer sorbent dispersion, as noted in the study by Reyes-Gallardo, 2013 [13].

The effect of desorption solvent volume was studied to identify the solvent needed for the complete elution of the trapped analyte. MeOH was selected as the disperser solvent due to its lower toxicity. The optimised volume of desorption solvent was determined to be 0.6 mL, yielding a maximum peak area of 100.7047 mAU/s and significantly enhancing HYD extraction recovery compared to other volumes. Figure 2 (b) depicts the substantial increase in total chromatographic areas as the volume of desorption solvent is increased to 0.6 mL. However, beyond this optimised volume, the chromatographic peak experiences a decline. This phenomenon can be attributed to the increased affinity of HYD to the aqueous solution due to the aqueous solution's polarity reduction. Consequently, this diminishes HYD extraction recovery. As a result, 0.6 mL of MeOH was utilised for subsequent tests.

Effect of Extraction Time and Sample pH

The investigation into the effect of extraction time aimed to determine the duration needed for the complete adsorption of HYD onto the surface of the GO sorbent. Achieving adsorption equilibrium is crucial in the D-µ-SPE system, where adsorption and desorption rates stabilize. In this study, vortex agitation was employed to facilitate the adsorption of HYD. The relationship between extraction time and the interaction of the analyte with the adsorbent was explored by varying the agitation time from 5 to 25 seconds. The findings suggested that the duration of vortexing did not significantly impact the recovery. This lack of influence could be attributed to the extensive contact surface area between the GO nanosorbent and the sample solution. Consequently, the swift mass transfer of the analyte from the aqueous phase to the sorbent surface occurs at such a rapid pace that equilibrium is promptly reached [14]. The rapid adsorption of HYD was associated with the unique morphology of GO, which features both sides of its planar sheets accessible to analyte adsorption [15]. In all subsequent stages of this study, a 10-second extraction time was adopted to obtain a well-dispersed mixture of GO and sample.

The pH levels of the water samples were assessed within the range of 5 to 11. The findings revealed that altering the water sample to pH 9 resulted in the strongest signal, indicated by the response area of the chromatographic peak depicted in Figure 3 (d). This figure highlights the optimised pH for extracting HYD from water samples. Notably, the form of the analytes can vary based on the pH of the aqueous phase. The adsorption onto the sorbent and solubility in the aqueous phase may be influenced by protonation or deprotonation. In this study, the optimised pH was determined to be pH 9. This choice is attributed to the protonation of carboxyl groups in GO at low pH, leading to reduced hydrophilicity and aggregates in GO sheets. Conversely, at high pH, the deprotonated carboxyl groups exhibit high hydrophilicity, causing individual GO sheets to dissolve in bulk water, resembling a regular salt [16].

Method Validation

Several criteria, including linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, and percentage recovery, were tested to determine the application of the proposed technique for assessing hydrocortisone in water samples. The linearity test was performed with five concentrations: 50, 100, 200, 300, and 400 µg L^{-1}. The calibration graph was created by plotting peak area (mAU/s) as a function of concentration under optimum conditions. The calculated coefficient of determination (R^2) is 0.9882. LOD and LOQ are fundamental parts of method validation that establish an analytical technique's limits. LOD specifies the most minor concentration that HPLC-DAD can determine. This study validated the strategy using the signal-to-noise (S/N) ratio methodology. The LOD in chromatography is the amount injected that results in a peak with a height at least three times greater than the baseline noise level (S/N: 3/1). In this investigation, the LOD for hydrocortisone was 2.77 µg L^{-1}. The lowest concentration of an analyte in a sample that can be detected with acceptable precision and accuracy under specified test circumstances is referred to as the limit of quantification (LOQ). The LOQ was calculated using the standard hydrocortisone concentration with a peak height ten times greater than the baseline noise level (S/N: 10/1). The LOQ for hydrocortisone in a water sample was 8.38 µg L^{-1}. The obtained RSD for hydrocortisone were 3.96 for intra-day and 4.24 for inter-day ($n = 3$) at the lowest injection concentration. Table 1 shows the analytical parameters for the developed method of HYD.

Application of D-µSPE on Real Sample

The developed GO-D-µSPE technique was successfully applied to tap water samples. The tap water samples were obtained from the chemistry laboratory at UiTM Shah Alam. The water samples were spiked to achieve final 100 and 400 µg L^{-1} concentrations for relative recovery tests. Table 1 displays the quantitative outcome. The relative recovery of HYD from a spiked sample of 100 µg L^{-1} was 118.49%, with an RSD value of 6.22%. Meanwhile, the relative recovery of the same analyte at 400 µg L^{-1} was 98.41% with an RSD of 2.53%. It demonstrates that good relative recoveries of 70% to 120% were attained [17]. Table 2 summarizes the relative recovery study. The findings indicate that dispersive micro-solid-phase extraction is an effective method for extracting steroids from aqueous matrices.

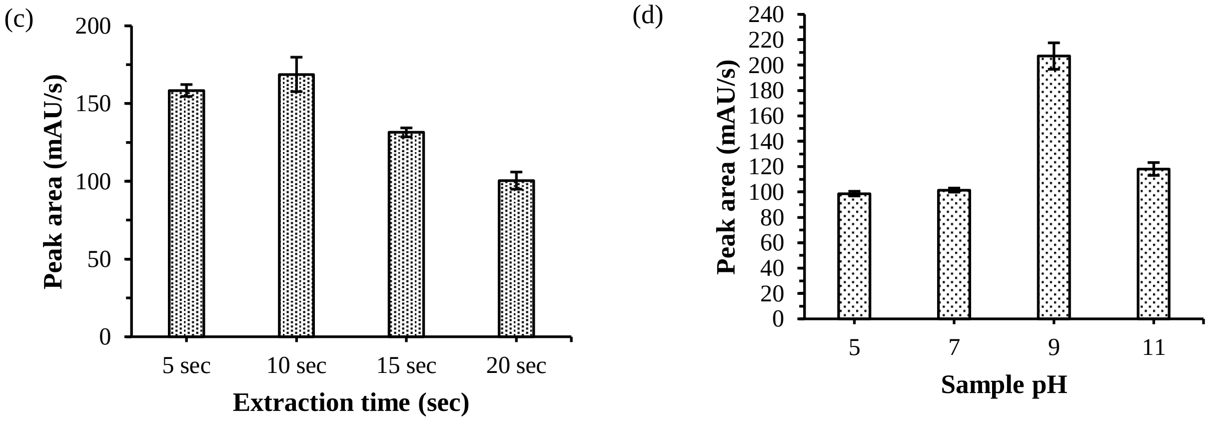

Figure 3. The Effect of (c) Extraction Time, and (d) Sample pH on the Extraction of HYD Using GO Nanosorbent.

Table 1. Analytical data of the D-µ-SPE-GO method for the determination of HYD in water.

Linearity (µg L^{-1})	**R^2**	**LOD (µg L^{-1})**	**LOQ (µg L^{-1})**	**RSD (*n* = 3)**	
				Intra-day	**Inter-day**
5 - 400	0.9882	2.77	8.38	3.96	4.24

Table 2. Analysis of HYD in tap water samples.

Analyte	Spiked concentration (µg L^{-1})	Relative recovery (%)	RSD (%)
Hydrocortisone	100	118.49	6.22
	400	98.41	2.53

Table 3. Comparison of the analytical performance of the developed method with other methods for the determination of HYD steroid drugs using HPLC.

Analyte	Sorbent	Retention time (min)	Matrix	LOD (µg mL^{-1})	Recovery (%)	Analytical technique	Reference
Glucocorticoids	GO	9 - 14	River water	7.5 x 10^{-9} – 1.6 x 10^{-7}	79.6 - 119	HPLC-MS/MS	[18]
Hydrocortisone, cortisone, dexamethasone, 6α-methylprednisolone, prednisone, prednisolone.	GO	7 - 10	WWTP sludge	–	73 - 99	UHPLC-ESI-MS/MS	[19]
Hydrocortisone, dexamethasone, fluocinolone acetonide	C$_{18}$	4 - 8	Topical cream	0.08 – 0.25	100.2 – 102.6	HPLC-DAD	[20]
Hydrocortisone	C$_{18}$	3.5 – 12.5	Topical cream	9 - 12	–	HPLC-UV	[21]
Hydrocortisone and clotrimazole	–	2.5 – 7.5	Topical cream	11.23 – 14.39	–	HPLC-UV	[1]
Hydrocortisone	GO	3	Tap water	2.77	98 - 119	HPLC-DAD	This work

The D-µ-SPE performance using GO nanosorbent in extraction from water was evaluated and compared with other methods, considering factors such as LOD, recovery percentage, and retention time (refer to Table 3). The developed method demonstrated a recovery percentage comparable to conventional SPE using a C$_{18}$ cartridge and µ-SPE based on GO nanosorbent. The LOD for the developed method gives comparable value to other HPLC-UV and HPLC-DAD methods. However, it is essential to note that lower LOD values might be attributed to the sensitivity of the quantification instruments rather than the adsorbent's extraction capability. Nevertheless, the developed adsorbent presents notable advantages over previously reported methods, including rapid extraction time (assisted by 10 seconds of vortex) and having the lowest retention time, 3 minutes.

CONCLUSION

The sorbent used, GO, was employed in the D-µ-SPE technique before determining HYD in tap water. The appropriate quantity of GO for extraction was 20 mg, which was then utilised to optimise the volume of desorption solvent, extraction time, and sample pH to get the best peak area of the HYD signal. In this study, the best conditions for extraction were 20 mg of GO, extracted for 10 seconds using vortex. The pH of the sample was 9, and the desorption solvent used was 0.6 mL of MeOH. These ideal conditions were then used to validate linearity, LOD, LOQ, precision, and accuracy methods using HPLC-DAD. Linearity was obtained between 50 and 400 µg L^{-1}. A good coefficient of determination of 0.9882 was obtained. The LOD and LOQ values were 2.77 µg L^{-1} and 8.38 µg L^{-1}, respectively. The optimised and validation step was used to test the amount of HYD in a tap water sample. The sample was spiked with HYD standard solution at 100 and 400 µg L^{-1}. The relative recovery was calculated within the range of 98 - 120%. The GO sorbent demonstrated high selectivity for HYD. Implementing the GO-D-µ-SPE method in actual samples exhibits a simple, sensitive, selective, and environmentally friendly extraction approach with a high possibility of being employed for regular water sample analysis.

ACKNOWLEDGEMENT

The authors thank Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia, for the facilities and financial support under the research grant 600-RMC/KEPU 5/3 (009/2023).

REFERENCES

1. Iqbal, D. N., Ashraf, A., Iqbal, M. and Nazir, A., (2020) Analytical method development and validation of hydrocortisone and clotrimazole in topical dosage form using RP-HPLC. *Future J. Pharm. Sci.*, **6(1)**.

2. Kamilya, T., et al. (2023) Emerging impacts of steroids and antibiotics on the environment and their remediation using constructed wetlands: A critical review. *Chemical Engineering Journal*, **451**, 138759.

3. Cui, X., et al. (2021) Development of a highly sensitive imprinted electrochemical sensor for detecting hydrocortisone in wastewater. *J. Electrochem. Soc.*, **168(5)**, 057508.

4. Shen, X., Chang, H., Sun, Y. and Wan, Y. (2020) Determination and occurrence of natural and synthetic glucocorticoids in surface waters. *Environ. Int.*, **134**, 105278.

5. Câmara, J. S., et al. (2022) Green extraction techniques as advanced sample preparation approaches in biological, food, and environmental matrices: A Review. *Molecules*, **27**, 2953–2982.

6. Ramos, L. (2012) Critical overview of selected contemporary sample preparation techniques. *Journal of Chromatography A.*, **1221**, 83.

7. Socas-Rodríguez, B., Herrera-Herrera, A. V, Asensio-Ramos, M. and Hernández-Borges, J. (2015) Dispersive Solid-Phase Extraction. *Analytical Separation Science, New Jersey*.

8. Armenta, S., Garrigues, S., Esteve-Turrillas, F. A. and de la Guardia, M. (2019) Green extraction techniques in green analytical chemistry. *TrAC - Trends in Analytical Chemistry*, **116**, 248–253.

9. Treviño, M. J. S., Zarazúa, S. and Płotka-Wasylka, J. (2022) Nanosorbents as Materials for Extraction Processes of Environmental Contaminants and Others. *Molecules*, **27(3)**, 1067.

10. Borsatto, J. V. B. and Lanças, F. M., (2023) Recent Trends in Graphene-Based Sorbents for LC Analysis of Food and Environmental Water Samples. *Molecules*, **28(13)**, 5134.

11. Yu, W., Sisi, L., Haiyan, Y. and Jie, L. (2020) Progress in the functional modification of graphene/graphene oxide: A review. *RSC Advances*, **10(26)**, 15328–15345.

12. Ran, C., Chen, D., Ma, H. and Jiang, Y. (2017) Graphene oxide adsorbent based dispersive solid phase extraction coupled with multi-pretreatment clean-up for analysis of trace aflatoxins in traditional proprietary Chinese medicines. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **1044**, 120–126.

13. Reyes-Gallardo, E. M., Lasarte-Aragonés, G., Lucena, R., Cárdenas, S. and Valcárcel, M. (2013) Hybridization of commercial polymeric microparticles and magnetic nanoparticles for the dispersive micro-solid phase extraction of nitroaromatic hydrocarbons from water. *J. Chromatogr. A.*, **1271(1)**, 50–55.

14. Mahpishanian, S. and Sereshti, H. (2014) Graphene oxide-based dispersive micro-solid phase extraction for separation and preconcentration of nicotine from biological and environmental water samples followed by gas chromatography-flame ionization detection. *Talanta*, **130**, 71–77.

15. Khezeli, T. and Daneshfar, A., (2017) Development of dispersive micro-solid phase extraction based on micro and nano sorbents. *TrAC - Trends in Analytical Chemistry*, **89**, 99–118.

16. Chih-Jen, S., Shangchao, L., Richa, S., Strano, M. S. and Blankschtein, D. (2012) Understanding the pH-Dependent Behavior of GO Aqueous Solutions: A Comparative Experimental and Molecular Dynamics Simulation Study. *Langmuir*, **28**, 235–241.

17. Ungku, A. A. U. A., et al. (2021) Pre-Concentration and Determination of Tetracyclines Antibiotics Residues in Water Samples Using RGO/Fe_3O_4

Nanocomposite as Extraction Sorbent. *Indones. J. Chem*., **21(5)**, 1196-1121.

18. Huang, Y., Li, Y., Luo, Q. and Huang, X. (2021) One-step preparation of functional groups-rich graphene oxide and carbon nanotubes nanocomposite for efficient magnetic solid phase extraction of glucocorticoids in environmental waters. *Chemical Engineering Journal*, **406**, 126785.

19. Chang, H., Hu, J. and Shao, B. (2007) Occurrence of natural and synthetic glucocorticoids in sewage treatment plants and receiving river waters. *Environ. Sci. Technol.*, **41(10)**, 3462–3468.

20. Arif, S. and Ata, S. (2020) Stability-indicating HPLC-DAD assay for simultaneous quantification of hydrocortisone 21 acetate, dexamethasone, and fluocinolone acetonide in cosmetics. *Open Chem.*, **18(1)**, 962–973.

21. Hájková, R., Solich, P., Dvořák, J. and Šícha, J. (2003) Simultaneous determination of methylparaben, propylparaben, hydrocortisone acetate and its degradation products in a topical cream by RP-HPLC. *Journal of Pharmaceutical and Biomedical Analysis*, **(32)**, 921–927.