



ORIGINAL ARTICLE

Trace analysis of mefenamic acid in human serum and pharmaceutical wastewater samples after pre-concentration with Ni–Al layered double hydroxide nano-particles



Hossein Abdolmohammad-Zadeh^{a,*}, Fatemeh Morshedzadeh^b,
Elaheh Rahimpour^a

^aDepartment of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University,
35 Km Tabriz-Maragheh Road, P.O. Box 53714-161, Tabriz, Iran

^bDepartment of Chemistry, Payame Nour University of Tabriz, P.O. Box 19395-3697, Tabriz, Iran

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Ultraviolet spectroscopy

Abstract In this work, the nickel–aluminum layered double hydroxide (Ni–Al LDH) with nitrate interlayer anion was synthesized and used as a solid phase extraction sorbent for the selective separation and pre-concentration of mefenamic acid prior to quantification by UV detection at $\lambda_{\max} = 286$ nm. Extraction procedure is based on the adsorption of mefenamate anions on the Ni–Al(NO₃⁻) LDH and/or their exchange with LDH interlayer NO₃⁻ anions. The effects of several parameters such as cations and interlayer anions type in LDH structure, pH, sample flow rate, elution conditions, amount of nano-sorbent and co-existing ions on the extraction were investigated and optimized. Under the optimum conditions, the calibration graph was linear within the range of 2–1000 µg/L with a correlation coefficient of 0.9995. The limit of detection and relative standard deviation were 0.6 µg/L and 0.84% (30 µg/L, $n=6$), respectively. The presented method was successfully applied to determine of mefenamic acid in human serum and pharmaceutical wastewater samples.

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1. Introduction

Mefenamic acid (MFA, 2-(2,3-dimethyl phenyl) aminobenzoic acid) is a prevailing non-steroidal anti-inflammatory drug which is used as a potent analgesic and anti-inflammatory agent in the treatment of several pathologies such as osteoarthritis, nonarticular rheumatism, sport injuries, and other painful musculoskeletal illnesses

*Corresponding author. Tel.: +98 4124327500; fax: +98 4124327541.

E-mail addresses: h.abdol@azaruniv.edu,

h_abdol@yahoo.com (H. Abdolmohammad-Zadeh).

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[1,2]. Overdose of mefenamic acid produces toxic metabolite accumulation that causes nausea, vomiting and occasionally bloody diarrhea [1]. On the other hand, MFA is a diphenylamine derivative pollutant and the third compound on the European Union list of priority pollutants [3]. Many studies have revealed that MFA cannot be effectively removed by conventional sewage treatment plants and that it has been detected at trace level in the effluent of wastewater treatment plants [4–7]. Due to the vital importance and widespread use of MFA, the need for the development of simple and sensitive analytical methods for trace analysis of drug is increasing.

So far, various analytical methods regarding MFA determination in pharmaceutical formulations and biological fluids have been published in literature. Some reported methods are spectrophotometry [8–11], fluorimetry [12–14], potentiometry [15–18], chromatography [19–23], chemiluminescence [24,25] and capillary electromigration [26,27]. However, to determine the trace levels of drug, spectrophotometry may be used, especially in combination with extraction for separation of special purpose component from the main admixture. Solid-phase extraction (SPE) techniques have recently been among the most popular separation methods for the enrichment of analytes prior to their determination. The basic principle of SPE is the pre-concentration and purification of analytes from solution by sorption on a solid sorbent [28]. SPE has several advantages over other techniques, such as low cost, low consumption of organic solvents, high enrichment factor, high recovery, safety with respect to hazardous samples and the ability of combination with different detection techniques in the form of on-line or off-line mode [29]. Recently, nano-meter sized materials have been used as sorbents in SPE procedures.

Layered double hydroxides (LDHs) are a class of synthetic ionic inorganic compounds with a similar structure to clays and with the general formula of $[M_1^{2+}_x M_2^{3+}_y (\text{OH})_2]^{x+y} [A^{n-}]_z \cdot m\text{H}_2\text{O}]^{x-y-z}$, where M^{2+} is a divalent metal ion like Zn, Mg, Cu, Co or Ni, M^{3+} is a trivalent metal ion like Al, Fe or Cr, x is the ratio of $M^{3+}/(M^{2+}+M^{3+})$ and A^{n-} is a n -valent anion [30]. The layer structure of LDHs is based on that of brucite $[\text{Mg}(\text{OH})_2]$, which is typically associated with small polarizing cations and polarizable anions. Their structure is based on a series of layers, where a divalent metal cation is located in the center of octahedron, and two-dimensional infinite layers are formed by edge-sharing of octahedral. The partial substitution of divalent cations by trivalent ones generates a positive charge on the layers that is balanced by

anions or molecules of solvent. Interlayer anions can be exchanged with various kinds of inorganic or organic anions by ion exchange reaction or surface adsorption [31,32]. Scheme 1 shows ion exchange mechanism of mefenamate anions, as organic anions, with the interlayer anions in a LDH structure.

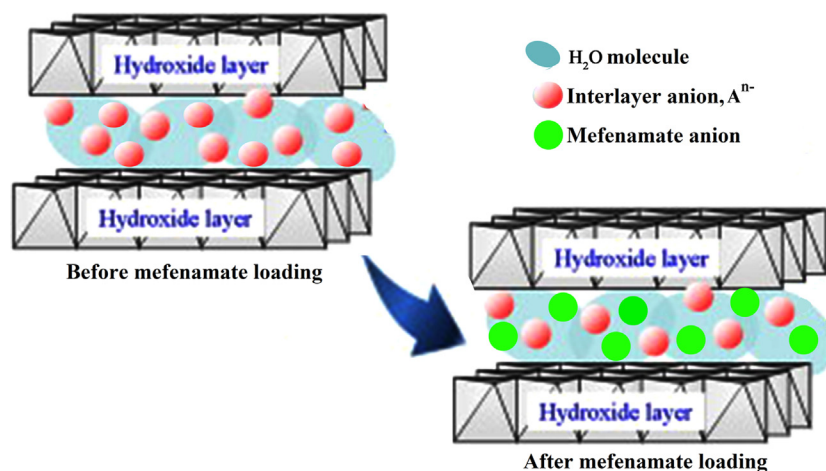
In the present work, a simple SPE system based on Ni–Al(NO_3) LDH was developed for the separation and pre-concentration of MFA prior to determination by spectrophotometry. To the best of knowledge, there is no report concerning the application of the LDHs in SPE of MFA. The effect of various experimental parameters on the extraction efficiency of MFA was investigated and the presented method was successfully used for trace analysis of MFA in various real samples.

2. Experimental

2.1. Apparatus and instruments

The UV–vis absorption spectra and intensity measurements are recorded on a 1601 PC UV–vis spectrophotometer (Shimadzu, Japan). A 2 mL polypropylene cartridge (30 mm \times 7 mm i.d.) (Shafa Co., Iran) containing 250 mg of Ni–Al(NO_3) LDH with cotton-fitted ends is used for the extraction of the analyte. A vacuum pump model DV-85N-250 (Platinum Co., USA) is used for controlling the flow rate of solution throughout the column.

In order to obtain better insight into the structural properties of LDH, XRD data were collected on a Bruker-D8 advance X-ray powder diffractometer using $\text{Cu}_{K\alpha}$ radiation source ($\lambda = 0.154$ nm) operating at 40 kV and 30 mA. The patterns were recorded at 2θ from 2° to 70° at room temperature. Also, Fourier transform infrared (FT-IR) spectra (4000 – 400 cm^{-1}) were recorded using a Shimadzu FT-IR Spectrometer, model 8400 (Japan). The samples were mixed with KBr with a sample/KBr weight ratio of 1/100 and pressed into a disk. Morphological characterization of the synthesized Ni–Al(NO_3) LDH was performed using a scanning electron microscope (SEM) model Hitachi S 4160 and a transmission electron microscope (TEM) model PHILIPS SM10. A centrifuge (Shimifann CE. 86) with a relative centrifugal force of 2810g (4000 rpm) was used to accelerate the phase separation. The pH was adjusted using a Motrohm pH-meter (model 827, Switzerland) with a precision of ± 0.01 . An electrical furnace (Exciton Co.,



Scheme 1 Ion exchange mechanism of mefenamate anions with the interlayer anions in a LDH structure.

Iran) with an accuracy of ± 1 °C was applied to control the temperature in LDH synthesis process.

2.2. Standard solutions and reagents

All the chemicals were of analytical grade and all solutions were prepared with high-purity deionized water (Shahid Ghazi Co., Tabriz, Iran). A 1000 mg/L stock solution of mefenamic acid was prepared by dissolving appropriate amount of reagent in deionized water. Working standard solutions were prepared daily by suitable stepwise dilution of the stock solutions with deionized water and shaking them just prior to use. All salts used for the interference study, NaOH, NaCl, and LDH precursors, i.e., purified nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 99%) and aluminum nitrate nonahydrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, 99%) were purchased from Merck (Darmstadt, Germany). All the plastic and glassware was properly aged in 15% (v/v) nitric acid at least overnight, and subsequently washed three times with deionized water prior to use.

2.3. Preparation of nickel–aluminum layered double hydroxide

The Ni–Al(NO_3^-) LDH was prepared by the co-precipitation method with controlled pH, and followed by hydrothermal treatment according to our previous work [33] with some modifications. 0.581 g $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.375 g $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ were dissolved in 30 mL deionized water under vigorous stirring at room temperature. The pH of the reaction mixture was adjusted to 9.6 by addition of 1 M NaOH solution. The reaction continued for another 30 min under nitrogen protection. Then, the obtained slurry was subjected to hydrothermal treatment at a constant temperature of 90 °C for about 24 h. The obtained product was separated by centrifugation at 4000 rpm for 10 min, washed several times with deionized water and dried at 60 °C.

2.4. Column preparation

The column was prepared by introducing 250 mg of synthesized nano-sorbent into a 2 mL polypropylene cartridge. The ends of the column were plugged with a small portion of cotton wool to retain the nano-sorbent in the column. Before loading the sample, 2.5 mL of 1 M NaOH solution was passed through the column to clean it. Then, the column was conditioned by passing 5 mL of deionized water prior to each use.

2.5. Sample preparation

2.5.1. Wastewater

The wastewater samples were collected in pre-washed (with detergent, deionized water, dilute HNO_3 and deionized water, respectively) polyethylene bottles from different effluents of Zahravi Pharmaceutical Manufactory (Tabriz, Iran). These samples were filtered through black band filter paper and centrifuged to remove any suspended particulate. Then, aliquots of 200 mL from samples were analyzed within 24 h of collection without previous treatment.

2.5.2. Human serum

Human blood samples were obtained from healthy volunteers and patients that consumed the mefenamic acid at Ali-Nasab hospital (Tabriz, Iran). To prepare serum samples, they were drawn into the

test tube, centrifuged at 3000 rpm for 10 min and then allowed to stand at 4 °C until the phase separation was done. The serum samples were kept in a freezer (-80 °C) until analysis. A 500 μL of each serum sample was transferred into a 25 mL volumetric flask and diluted to the mark with deionized water. Finally, the concentration of mefenamic acid in the obtained sample solution was determined as described in Section 2.6.

2.6. General procedure

An aliquot of 200 mL from aqueous standard or sample solution containing MFA (pH 7) in the range of 2–1000 $\mu\text{g/L}$ was passed through the Ni–Al(NO_3^-) LDH nano-sorbent in a column at a flow rate of 2 mL/min. After sample loading, 2.5 mL of 1 M NaOH solution was used for the elution of the retained analyte from the column. The concentration of mefenamic acid was subsequently determined spectrophotometrically by measuring the absorbance of the solution at $\lambda = 286$ nm.

3. Results and discussion

3.1. Selection of layered double hydroxide

The charge density and anion exchange capacity of the LDHs were controlled by varying the type of di- and trivalent cations and their ratios in the LDH structure. The nature of the layer cations can be changed among wide possible selection (almost restricted by size and charge), and because of weak LDHs interlayer bonding, the nature of interlayer anions can also be freely selected [34]. Therefore, seven LDHs with different cations i.e., Zn^{2+} , Ni^{2+} , Mg^{2+} , Al^{3+} and Fe^{3+} and same interlayer anion (nitrate) were synthesized and used as nano-sorbents in SPE of MFA. The results are displayed in Fig. 1A. As can be seen, the best recovery was achieved in the case of Ni–Al LDH with 1:1 $\text{Ni}^{2+}:\text{Al}^{3+}$ molar ratio. The type of interlayer anion is important and can affect the retention efficiency of analytes. Therefore, three Ni–Al LDHs with different interlayer anions such as SO_4^{2-} , NO_3^- and CO_3^{2-} were synthesized and tested for SPE of MFA. As shown in Fig. 1B, the highest recovery was obtained in the case of NO_3^- interlayer anion. So, Ni–Al(NO_3^-) LDH was used as a nano-sorbent in further SPE experiments.

3.2. Characterization of nano-sorbent

The powder X-ray diffraction (XRD) is a very powerful technique for characterizing the structure of materials. Fig. 2A shows XRD pattern of the Ni–Al(NO_3^-) LDH nano-sorbent. It can be seen that the synthesized LDH has the characteristic structure of hydroxalite-like compounds. This fact was verified by the existing characteristic reflection peaks of (003), (006), (009) planes and the typical doublet of (110)–(113) planes in XRD pattern of LDH. The Fourier transform infrared (FT-IR) spectrum which is used for identifying the nature and symmetry of interlayer anions and the presence of impurity phases was included in Fig. 2B. The broad band around 3517 cm^{-1} can be assigned to the stretching vibration of the hydroxyl groups of LDH layers and interlayer water molecules. Also, the weak band at 1766 cm^{-1} is due to the bending mode of interlayer water molecules and the band with maximum peak at 1379 cm^{-1} belongs to the stretching vibration of NO_3^- ions intercalated in the interlayer gallery. Finally, bands at lower wavenumbers (400–800) are due to vibrational modes of

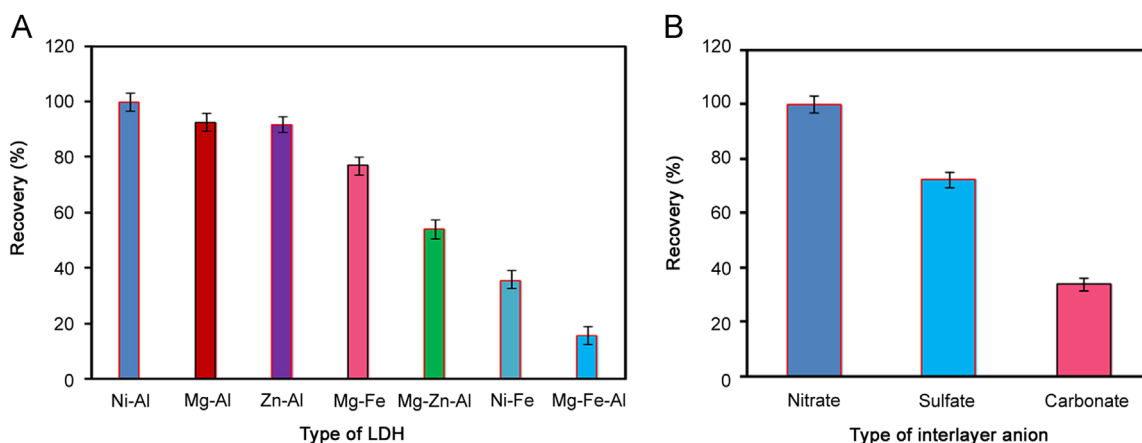


Fig. 1 Effect of (A) LDH type with the same interlayer anion, and (B) interlayer anion type between the hydroxide layers in Ni-Al LDH nano-sorbent, on the retention of MFA.

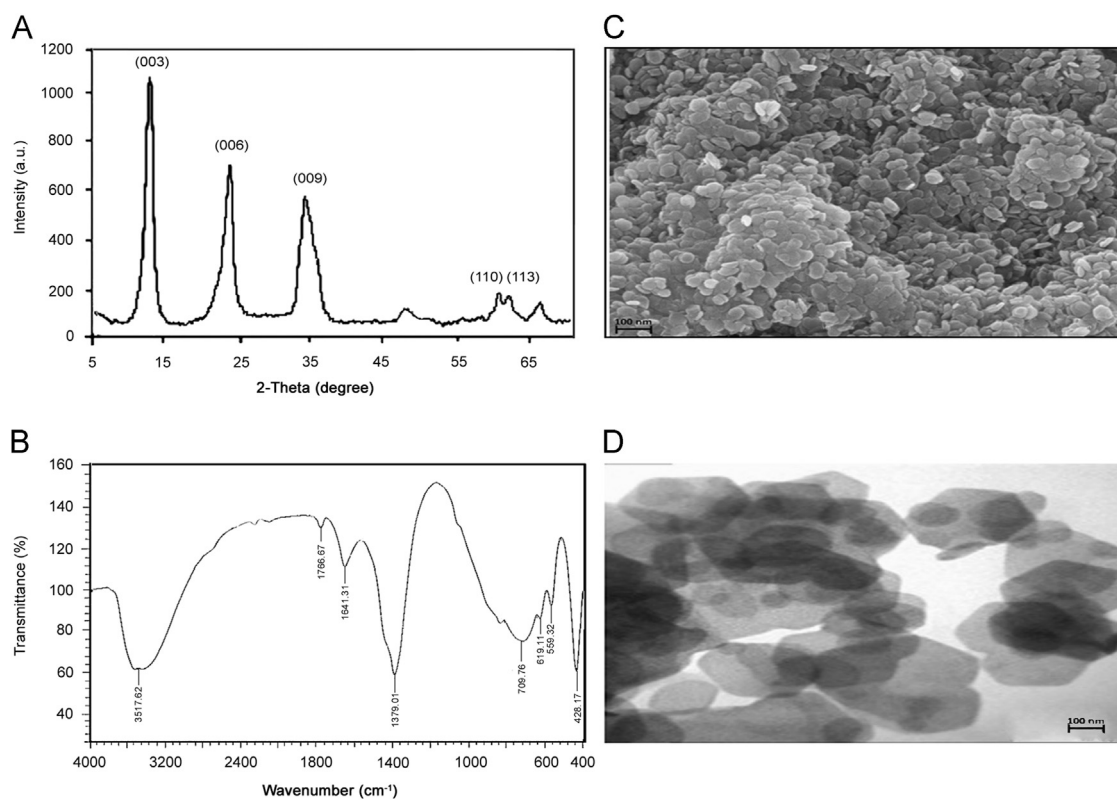


Fig. 2 (A) XRD pattern, (B) FT-IR spectrum, (C) SEM image and (D) TEM image of Ni-Al(NO₃⁻) LDH.

M-O, M-O-M, and O-M-O species. The scanning electron microscopy (SEM) was employed to explore the morphology of the nano-sorbent. SEM image of Ni-Al(NO₃⁻) LDH (Fig. 2C) shows an aggregate due to the collection of crystallites as small pseudo-hexagonal platelets after thermally treated at 90 °C for about 24 h. The approximate sizes of the particles fall in the 10–60 nm range. Transmission electron microscopy was also employed to explore the morphology and distribution pattern of the nano-structured Ni-Al(NO₃⁻) LDH in a colloidal suspension. As can be seen from Fig. 2D, the particles are all generally hexagonal plate-like in shape forming a roughly mono-dispersed suspension.

3.3. Optimization of solid phase extraction conditions

3.3.1. Effect of pH

The effect of pH was studied as the first important factor for the quantitative measurement of analyte. The retention of analyte by nano-sorbent depends on the pH at which electrostatic interaction between LDH layers and analyte was facilitated. The influence of pH on the analyte recovery was tested over the pH range of 4.0–12.0. The resulting solutions' pH(s) were adjusted with minimal volume of diluted HNO₃ and/or NaOH solutions. The results illustrated in Fig. 3 show that the highest recovery could be

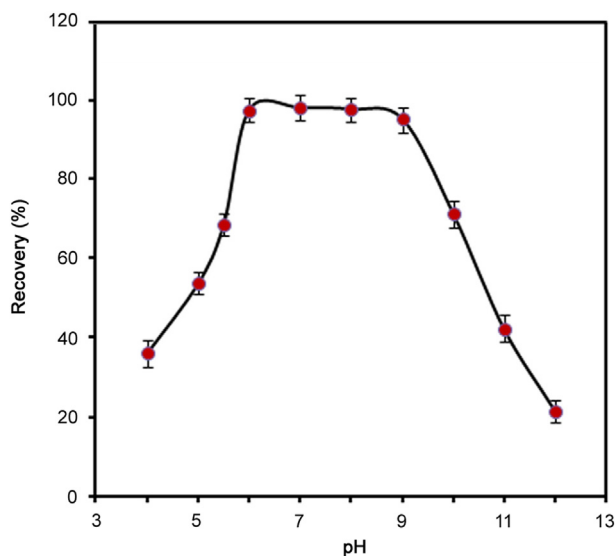


Fig. 3 Effect of sample pH on the retention of MFA on the Ni–Al(NO₃⁻) LDH nano-sorbent.

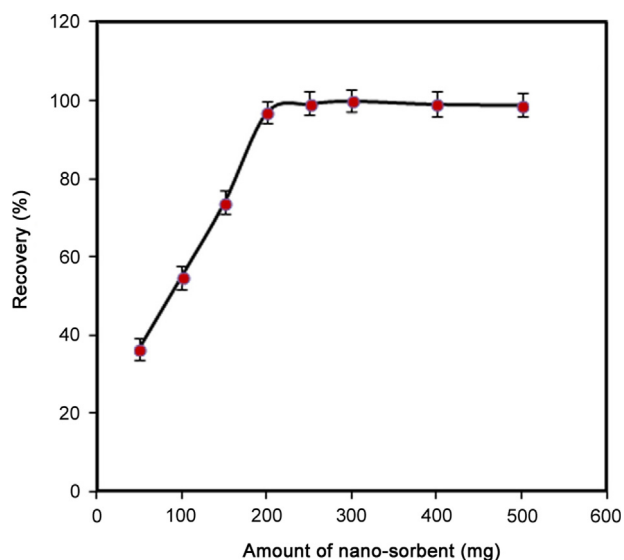


Fig. 4 Effect of the Ni–Al(NO₃⁻) LDH amount on the retention of MFA.

achieved in the pH range of 6–9. For pH values below 6, significant fractions of mefenamate ion ($pK_a=4.2$) changed to the corresponding protonated form which is not intercalated into the LDH gallery by ion exchanging. Also, at pH above 9.0, an increase in the concentration of the competing OH⁻ anions is responsible for the observed decrease in the recovery. Therefore, pH 7 was chosen as optimum value.

3.3.2. Effect of the nano-sorbent amount

To test the effect of the nano-sorbent amount on the quantitative extraction of MFA, the extraction was conducted by varying the amounts of Ni–Al(NO₃⁻) LDH from 50 to 500 mg. As shown in Fig. 4, the recovery of MFA was not affected by the nano-sorbent amount in the range between 200 and 500 mg. Consequently, 250 mg of the nano-sorbent was used in all further experiments.

3.3.3. Optimization of elution conditions

In order to choose the best reagent for stripping of the retained mefenamate ions on Ni–Al(NO₃⁻) LDH, various eluents such as NaOH, NaCl, NaF, NaBr and Na₂CO₃ were tested. Among these reagents, NaOH solution provided the highest recovery (Fig. 5A). The concentration of the NaOH solution was also investigated in the range of 0.5–3.0 M. Based on the obtained results, 1 M NaOH was sufficient for complete elution of the retained analyte on the nano-sorbent. The volume of eluent is important for obtaining the high enrichment factor. So, the effect of elution volume on the analyte recovery was studied in the range between 0.5 and 3.5 mL. As shown in Fig. 5B, the minimum volume of NaOH required for the quantitative elution was 2.5 mL. As a result, 2.5 mL of 1 M NaOH was employed as an eluent in further experiments.

3.3.4. Effect of sample loading flow rate

The sample flow rate through the packed column is a very important parameter, since the retention of analyte on the sorbent depends on the flow rate of sample solution. In fact, the sample flow rate not only affects the retention of analyte but also is one of the variables that control the analysis time. The influence of the sample loading rate on the recovery was investigated between 0.5 and 4.5 mL/min. Fig. 6 shows that the sample solution flow rate in the interval 0.5–2.5 mL/min had no significant influence on the recovery of MFA. However, at flow rates higher than 2.5 mL/min the recovery of the analyte reduced. Thus, all subsequent experiments were performed at a sample flow rate of 2.0 mL/min.

3.3.5. Sorption capacity

To determine the sorption capacity, 250 mg of the nano-sorbent was added to 10.0 mL of solution containing 70 mg/L of MFA. The mixture was then magnetically stirred for 60 min and the supernatant was separated by a filter paper. Loaded MFA in the LDH nano-particles was stripped with 2.5 mL of 1.0 M NaOH and concentration of the analyte was then determined spectrophotometrically after appropriate dilution. As a result, capacity of Ni–Al(NO₃⁻) LDH for MFA was found to be 30.0 mg/g.

3.3.6. Reusability of the nano-sorbent

The potential regeneration and stability of the nano-sorbent were investigated. The column packed with 250 mg sorbent was rinsed with 2.5 mL of 1.0 M NaOH and 5.0 mL deionized water, before application in the next one. After at least 250 times of recycling, there was no obvious decrease in the recovery of the analyte. In fact, this is one of the advantages of the LDHs as solid phase extraction sorbents.

3.4. Study of interferences

The influence of some potentially interfering ions on the determination of 60 µg/L MFA using the developed method was investigated. The tolerable limit was defined as the highest amount of interfering ion that produced an error not exceeding ±5%. The obtained results are given in Table 1. It can be seen that most of the examined ions did not interfere with the extraction and determination of mefenamic acid. Additionally, the influence of frequently encountered excipients and additives on the determination of 60 µg/L MFA was studied by adding different amounts of possible interferents to sample. No interference was observed from the presence of lactose, glucose, citrate, saccharose, starch, talk and stearate in the ratios commonly used in pharmaceutical preparations.

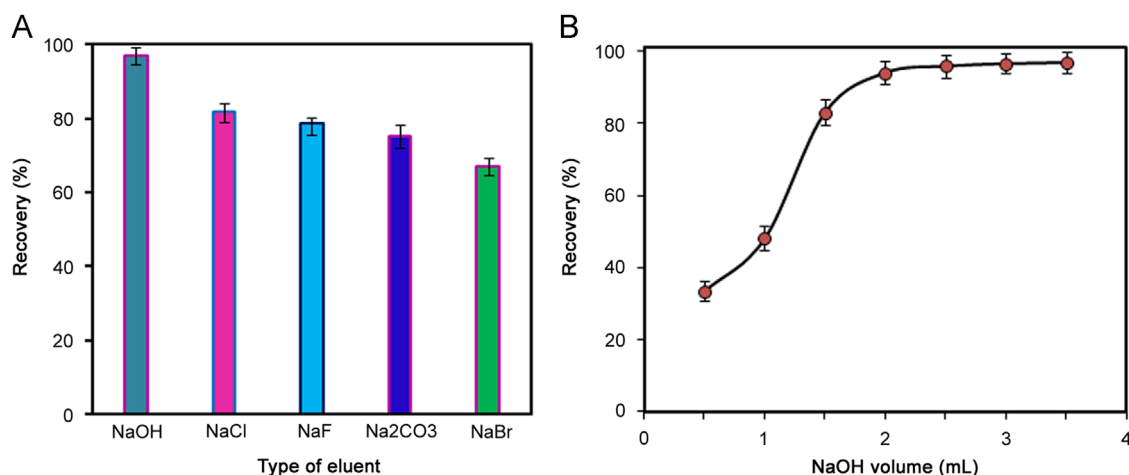


Fig. 5 Effect of (A) type of eluent and (B) eluent volume on the elution of mefenamate ions from the Ni-Al(NO₃)₃ LDH nano-sorbent.

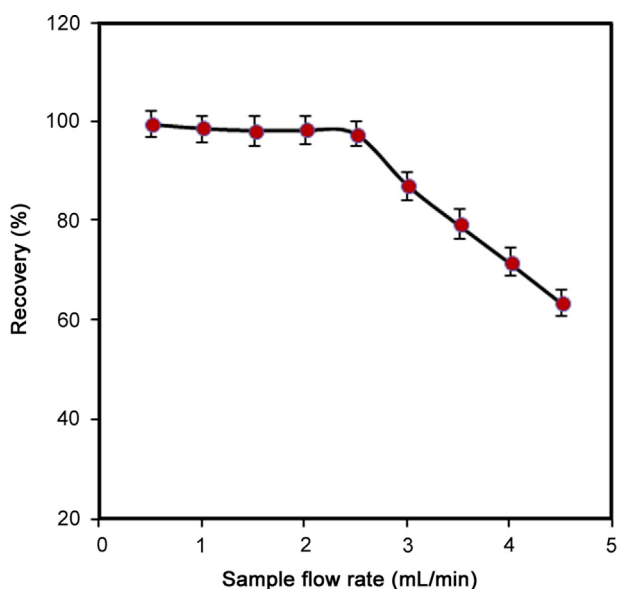


Fig. 6 Effect of sample loading flow rate on the retention of MFA on the Ni-Al(NO₃)₃ LDH nano-sorbent.

3.5. Method validation and analysis of real samples

In the optimum conditions, a calibration curve was plotted for MFA by using a series of standard solutions. The linear concentration range was between 2.0 and 1000.0 µg/L, with a correlation coefficient of 0.9995. The regression equation was $\Delta A = 0.031C + 0.011$, where ΔA is the blank-corrected absorbance intensity and C is the concentration of MFA in µg/L, respectively. The limit of detection (LOD) and limit of quantification (LOQ), defined as $3S_b/m$, and $10S_b/m$ (where S_b is the standard deviation of the blank and m is the slope of the calibration curve) were 0.6 µg/L and 2.0 µg/L, respectively. The relative standard deviation (RSD) resulting from the analysis of 6 replicates of 200 mL solution containing 30 µg/L of MFA was 0.84%. The enrichment factor, defined as the ratio between the volume of the initial aqueous solution and the final elution volume, was 80. The optimum experimental conditions and analytical characteristics of the presented method are summarized in Table 2.

To explore the reliability of the method, the presented method has been successfully applied to determine MFA in pharmaceutical

Table 1 Tolerance limits of interfering ions in the determination of 60 µg/L of mefenamic acid.

Coexisting ion	Foreign ion to analyte ratio
Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , Fe ²⁺ , Ni ²⁺ , Cu ²⁺ , Zn ²⁺ , Co ²⁺ , Al ³⁺ , Cr ³⁺ , Fe ³⁺	1000:1
SO ₄ ²⁻ , CH ₃ COO ⁻ , NO ₃ ⁻	500:1
H ₂ PO ₄ ⁻ , HPO ₄ ²⁻ , Br ⁻	200:1
F ⁻ , CO ₃ ²⁻ , Cl ⁻	50:1

Table 2 Optimum SPE conditions and analytical characteristics of the presented method for MFA separation and determination.

Condition and parameter	Value
<i>SPE condition</i>	
pH	7.0
Amount of sorbent (mg)	250
Eluent volume (mL)	2.5
Eluent concentration (M)	1
Sample flow rate (mL/min)	2
Maximum sample volume (mL)	200
<i>Analytical parameter</i>	
Linear range (µg/L)	2 – 1000
Intercept	0.011
Slope	0.031
Correlation coefficient	0.9995
Limit of detection (µg/L) ^a	0.6
RSD (%) ($n=6$) ^b	0.84 (30)
Enrichment factor ^c	80

^aCalculated as three times the standard deviation of the blank signal divided by the calibration curve slope.

^bValue in parentheses is the MFA concentration (µg/L) for which the RSD was obtained.

^cEnrichment factor calculated as the ratio between the volume of the initial aqueous solution and the final elution volume.

wastewater and human serum samples. The results are given in Table 3. The accuracy of the established procedure was verified by the analysis of the samples spiked with different levels of the

Table 3 Determination of MFA in real samples (results of recoveries of spiked samples analysis).

Samples	Added MFA ($\mu\text{g/L}$)	Found MFA ($\mu\text{g/L}$)	Recovery (%)
<i>Wastewater samples^a</i>			
Sample 1	–	135.0 ± 3.1	–
	125	262.0 ± 2.0	101.6
Sample 2	–	135.0 ± 3.1	–
	125	265.0 ± 3.2	104.0
Sample 3	–	121.0 ± 2.6	–
	125	247.0 ± 4.5	100.8
Sample 4	–	160.0 ± 2.6	–
	125	289.0 ± 2.4	103.2
<i>Serum samples^b</i>			
Sample 1	–	51.3 ± 0.3	–
	50	103.0 ± 0.3	103.4
	100	154.0 ± 1.0	102.7
Sample 2	–	45.3 ± 0.6	–
	50	93.7 ± 1.7	96.8
	100	140.0 ± 1.9	94.7
<i>Control sample^c</i>			
	–	–	–
	50	48.6 ± 1.1	97.2
	100	99.0 ± 2.1	99.0

^aCollocated from different effluents of Zahravi Pharmaceutical Manufactory, Tabriz, Iran.

^bObtained from Ali-Nasab hospital, Tabriz, Iran.

^cHealthy volunteer.

Table 4 Comparison of analytical characteristics of the presented method with other techniques for determination of mefenamic acid.

Method	Linear range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	RSD (%)	Reference
Spectrophotometry	2–10	1.10	2.07	[8]
Spectrofluorometry	0.39–3.90	–	1.11	[12]
DPV	0.048–36.190	0.019	4.60	[18]
HPLC/UV	0.025–2.000	0.015	11.7	[19]
GC	1–25	–	–	[23]
CL	0.05–6.00	0.05	1.1	[25]
CZE	0.4–40.0	0.0003	<0.6	[27]
SPE–LC–MS	0.005–0.500	0.0016	1.3	[37]
SPE–Spectrophotometry	0.002–1.000	0.0006	0.84	This work

DPV: Differential pulse voltammetry, HPLC: high-performance liquid chromatography, GC: gas chromatography, CL: chemiluminescence detection, CZE: capillary zone electrophoresis, SPE: solid phase extraction, LC–MS: liquid chromatography–mass spectrometry.

known amount of MFA prior to preparation and analysis according to the general procedure. The obtained relative recoveries between 94.7% and 104.0% confirmed the accuracy of the presented method. In addition, the presented method was successfully applied to the analysis of MFA in its pharmaceutical dosage form (250 mg per capsule). It was found that the MFA content measured by the presented method (252.3 ± 3.4) was in good agreement with those obtained by the standard method [35] (250.8 ± 2.6), which involves the direct titration of MFA with NaOH in an ethanolic medium. Statistical analysis [36] of the results using the Student *t*-test showed no significant difference at 95% confidence level between the performance of the two methods as regard to accuracy and precision.

3.6. Comparison with other methods

A comparison of analytical features of the presented method with those of some previously reported methods for MFA determination

is shown in Table 4. The results indicate that LOD and RSD of the developed method are better than or comparable with most of the other methods. The presented method shows good accuracy and repeatability for determination of MFA in pharmaceutical formulation, pharmaceutical wastewater and serum samples.

4. Conclusions

Layered double hydroxides can be regarded as a class of materials that are simple to synthesize in the laboratory. In this study, Ni–Al(NO₃) LDH was employed as a nano-sorbent for the separation and pre-concentration of MFA prior to determination by spectrophotometry. It was found that Ni–Al(NO₃) LDH showed a good adsorption capacity for MFA, and the retained analyte can be easily stripped with NaOH solution. This method is simple, accurate with good recovery and low detection limit, repeatable and matrix-independent at the low levels. The presented method, which greatly improves the enrichment factor, can be applied for

the determination of MFA in various samples with complicated matrices involving pharmaceutical wastewater and biological samples. Moreover, the method represents a low cost, sensitive and environment-friendly technique in the area of pharmaceutical monitoring that can be recommended for the routine analysis of MFA in quality control laboratories.

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