

Determination of Some Anti-Inflammatory in the Wastewater of Treatment Pharmaceutical Industries by RP-HPLC

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ABSTRACT

Some pain relievers and non-steroidal Anti-Inflammatory drugs (NSAIDs) were identified simultaneously: Diphenhydramine Hydrochloride (DMH), Sodium Diclofenac (SD), pure state Mefenamic Acid (MA) and wastewater from the pharmaceutical industry using High-Performance Liquid Phase Chromatography inverted RP-HPLC. The proposed method was characterized by good accuracy and health. The proposed method was used to investigate the low concentrations of studied substances produced in the national pharmaceutical industrial facilities and the most productive of such compounds. This study was conducted on different samples of industrial wastewater and sewage at the most important local laboratories and pharmaceutical establishments, in Syria. The results of the analytical study were excellent. It affects both the environment and the human being. Although Very low concentrations of (DH, DS, MA) were identified in the pharmaceutical waste water samples using RP-HPLC technology. The proposed analytical method achieved high accuracy and sensitivity, with Excellent detection limits.

Keywords: Anti-Inflammatory (NSAIDs), Diphenhydramine Hydrochloride (DMH), Sodium Diclofenac (SD), Pure state Mefenamic Acid (MA), RP-HPLC, Wastewater of Treatment Pharmaceutical Industries

INTRODUCTION

The world currently suffers from many different types of diseases and is hardly incurable from them, as the current pharmaceutical preparations no longer have the appropriate resistance to this or that disease, whether at the level of the individual or the spread of the epidemic over the whole society, due to the widespread and multi-source environmental pollutants, especially the surface water resulting from industrial waste Pharmacokinetics, as one without chemical treatment, to reduce the slightest impact, can be transmitted to water, soil, or air, and then to humans at the end of the chain of the food chain. Pain relievers and non-steroidal anti-inflammatory drugs (NSAIDs) are among the most productive and traded pharmaceutical preparations at the local and global levels, due to their high effectiveness in the direction of diseases and pain associated with various infections, but without a controlled “environmental-analytical” process or a clear and controlled action plan for the remnants of the treated pharmaceutical industry. From them and untreated when put outside the industrial establishments to wide land areas and may be agricultural from them and what may result from it from the dangerous impact, which is the presence of a defect in one of the treatment plants and the lack of follow-up in the analysis of its impact, which leads to the transmission of epidemics and

diseases, which It came almost intractable because of the decomposition of modern medicine “chemical-bio” for these diverse chemical compounds and the resultant and then move to man and its impact on society as a whole [1]. Due to the increasing and necessary need to control pollution and pollutants resulting from the pharmaceutical industry’s waste, whether wastewater or wastewater from the pharmaceutical industry, it was necessary to suggest a method for accurate treatment of wastes of the pharmaceutical industry and the need to separate it from wastewater to take into account the environmental conditions necessary to take advantage of this water in irrigation Or recycle it to be suitable for drinking or human consumption without any pollutants or environmental hazards to human and community health. Analytical studies have varied in quantifying the effect of NSAIDs of various

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types. Most of the studies recently went to find an analytical method, simple, accurate, and with a lower cost, especially at the level of manufacturers of the drug locally, and this is what this study meant in finding an analytical chromatographic method that can quantify the effect of these compounds on treatment plants for the pharmaceutical industry at a lower cost. Quantitative study of some pain relievers and NSAIDs, including: Diclofenac sodium (DS) in waste water stations in the Kingdom of Saudi Arabia - Madinah, using high-performance liquid chromatography technology equipped with LC/MS mass spectroscopy, according to the stepping flow technique at length Wave detector 210 nm [2]. Diclofenac sodium (DS), mefenamic acid (MA) and others in the surface waters of an industrial city in East Asia (Vietnam) were identified by the solid phase extraction method (SPE) with container purification columns on gaskets of polystyrene diphenyl using RP-HPLC technology equipped with UV-VIS detector [3]. Chromatographic separation was studied to determine (DS) and mefenamic acid (MA) in treated water plants adopting MBR biofilms using the same RP-HPLC technology with MS detector on C18 separation column (125 × 2.0 mm, 5 µm) [4]. The identification of the same compounds in treated wastewater plants adjacent to some pharmaceutical establishments in (Australia) was studied using purification columns and the method of extracting samples using RP-HPLC technology equipped with UV-VIS detector and dry residue analysis of the sample using gas chromatography technology equipped with GC/MS detector [5]. A high-performance liquid chromatography method with the SPE-UHPLC-MS/MS detector has been developed to determine the DS component and others at seawater desalination plants in Portugal [6]. The (DS) compound was also identified in the treated water stations (Spain) by extractive method using RP-HPLC and X-Ray technology for the rest of the sample [7]. A near-compound (DS) with the same technology RP-HPLC was identified but with a chemical brilliance detector and UV-VIS [8]. Quantification of the compound with others has been studied in surface waters of rivers and treated water plants (Spain) using liquid chromatography technology equipped with HPLC/MS detector [9]. The identification of the compound (DS) in treated water plants in (Jordan) was studied on a separation column [10] C18. (DS, DH, MA) compounds are defined in pharmaceutical preparations according to HPLC technology using SPE purification columns [11,12].

MATERIALS & METHODS

Equipment's and machine of uses

The following laboratory devices and tools were used in this research:

1. A reversible, High Performance Liquid Chromatography device, American-made by Lab Alliance, consisting of Pump Series III pump, Model 5000 visual and UV ray detector connected

to a computer containing a special data-processing program (Clarity). The C₁₈ chromatographic separation column, known as the American Pharmacopoeia, was used in column L1, its dimensions (250 x 4.6 mm, 5 µm, 10A°) produced by the German company Knauer.

2. Sartorius Precision Analytical Scale (German Made) ± 0.1 mg.
3. Sartorius (German-made) pH\mv meter with integrated glass path, and thermal sensor.
4. Ultrasound machine for expelling gases from Ultrasonic solutions.
5. Bio safer TD5-PRP centrifuge.
6. Paper filters whose pores are less than (0.45) µm.
7. SPE (Hand-made) Purification Columns with mg (Octadecylsilane) (activated) 250 g filling and 10 mL capacity.
8. Glass tools (flasks, volumetric liners and standard pipettes).

Materials and solutions for uses

In the implementation of this research, standard reference solutions were used for non-opioid pain relievers and the following non-steroidal anti-inflammatory drugs:

First: The medicinal materials used in the research: Diphenhydramine Hydrochloride (DMH): Parabolic produced 99.98% purity, molecular weight: M.W = 291.80 g/mol. Sodium Diclofenac (SD): Euro hem produced 99.97% purity, molecular weight: M.W = 318.10 g/mol. Mefenamic Acid (MA): Merck's production is 99.99% purity. Molecular weight: M. W = 241.80 g/mol.

All the above-mentioned materials are provided with certificates of the company's production analysis and documented within the purity of each material.

Second: Organic solvents and other solutions used in the research: 99% High-purity stores for HPLC purposes: Methanol, acetonitrile, hexane, and electrolytes produced by Euro lab. Phosphorous acid density: 85%. Merck production. Potassium sulfate and aqueous aluminum for the formation of undissolved deposits: BDH produced 100% purity, a mixture solution of 20 gr/L was prepared. Ascorbic acid to remove suspended sediments: BDH produced 100% purity, a solution of which was prepared at a concentration of 25 mg/L. Under NaOCl to sterilize water: BDH produced 99% purity. A solution of 0.2 mg/L was prepared. Ethylene Diamine Tetrahydric Acid (Na₂EDTA) Salt for Heavy Metal Disposal (Formation of Chelic Complexes): BDH produced 100% purity, a solution of it was prepared at a concentration of 5 g/L. Formic acid: UNI-CHEM produced 99% purity, a solution of 2% (v/v%) was prepared. Ethyl acetate to extract studied materials from the water sample: Chem-Lab produced 99% purity.

Third: Samples Study, the study included both treated water samples: Treated wastewater from the pharmaceutical

industry or mixed with sanitation, which are summarized in **Table 1**.

Table 1. Treated water samples from the pharmaceutical industry.

No	Sample	Source	Temp (°C)
(1)	Treatment of the Wastewater pharmaceutical Industry	Medico Labs	0.5 ±5
(2)	Treatment of the Wastewater and Wastewater pharmaceutical Industry	Medico Labs	0.5 ±7
(3)	Treatment of the Wastewater pharmaceutical Industry	Ibn Hyan Pharma	0.5 ±6
(4)	Treatment of the Wastewater and Wastewater pharmaceutical Industry	Ibn Hyan Pharma	0.5 ±4

Design of Manual Cleaning Columns (SPE)

The fillings of a number of (expired) chromatographic separation columns containing Octadecylsilane were emptied, these fillings were activated with a group of polar phases, filtered and dried at 120°C for a period of 45 min, then weighed 250 mg of the last, and placed in syringes Plastic capacity 10 mL, equipped with glass wool before and after finely weighed filling with a total number (8) purification columns.

Preparation of standard solutions

Both methyl methoxy hydrochloride (DH) was accurately weighed at 100.02 mg, diclofenac sodium (DS) at 100.03 mg, and mefenamic acid (MA) at 100.01 mg. Each calculated weight (with respect to the purity of each separately) was dissolved in standard volumetric flasks of 100 ml each containing 20 mL of a mixture of (high purity distilled water: methanol) at a ratio of (50:50) v/v% (solvent). It was placed on the Ultrasonic device, and the volume was completed with the same solvent up to the standard mark. The prepared solution represents the standard solution with a concentration of 1000 µg/mL, and the solution of each substance was prepared with an appropriate extension solution containing the studied materials with a concentration of 20 µg/mL.

Working method for preparing treated water sample

Water samples were collected from collecting tanks at a volume of 500 mL as shown in **Table 1** and filtered micronically, and kept in the refrigerator at a temperature of (5 ± 0.5°C). $KAl(SO_4)_2 \cdot 12H_2O$, the sample was filtered micronically, then treated with 5 mL of EDTA- Na_2 solution sodium salt of EDTA acid at a concentration of 5 g/L and the sample filtered micronically, then washed with 5 mL of a solution under sodium chloride at a concentration of 0.2 mg/L The sample was filtered micronically and finally it was treated with 5 mL of ascorbic acid solution at a concentration of 25 mg/L, then the sample was micronized and the medium pH was adjusted at 7 with 85% phosphorous acid and passed through soft treatment on purification columns designed manually washed away the rest in columns with 10 mL three batches of mobile phase. Taking 1 mL of the treated sample size and applied by the method of standard additions to get ready for injection into a separation column for your HPLC.

RESULTS & DISCUSSION

The chromatographic separation conditions were set to define each of the standard solutions containing the studied materials: (DH), (DS) and (MA) after making precise chromatographic separation conditions for each one separately and then to separate the mixture until reaching the ideal separation chromatographic conditions suitable for the quantification process. Conditions are listed in **Table 2**.

Table 2. Ideal chromatographic conditions.

Column	C ₁₈ (4.6 × 250 mm, 5 µm, 10A°)
Mobile Phase	(Acetonitrile: Methanol: TEA 1%)
v/v/v %	(45: 20: 35) v/v/v% pH=3 Adjust by H ₃ PO ₄ 85%
Volume Inject (µL)	20
Temperature °C	25
Flow Rate (mL/min)	2
Wavelength (nm)	220

Linear filed of studied compounds

To identify the linear field between the analytical signal (top surface area) and the studied compound concentration. Standard solutions containing the sum of the pain relievers and NSAIDs studied were prepared at the linear field:

(0.0001-20.0000) $\mu\text{g/ml}$. Due to (DH) and (0.0005-25.0000) $\mu\text{g/ml}$ due to (DS) and (0.0002-20.0000) $\mu\text{g/ml}$ is due to the substance (MA), and these solutions were injected successively as shown in **Figures 1-4** and **Table 3**.

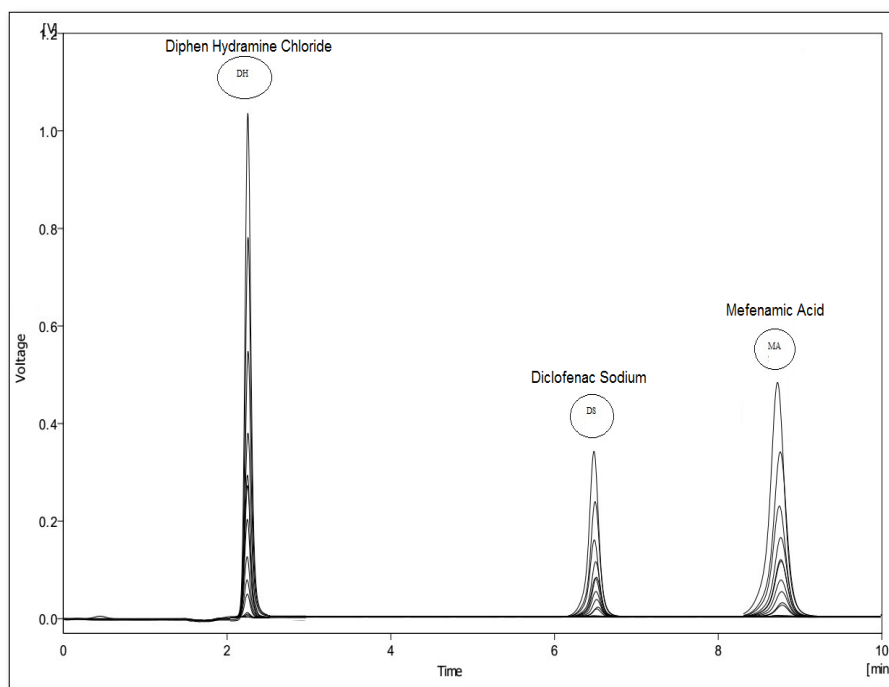


Figure 1. The standard series of total compounds studied at concentrations of standard solutions.

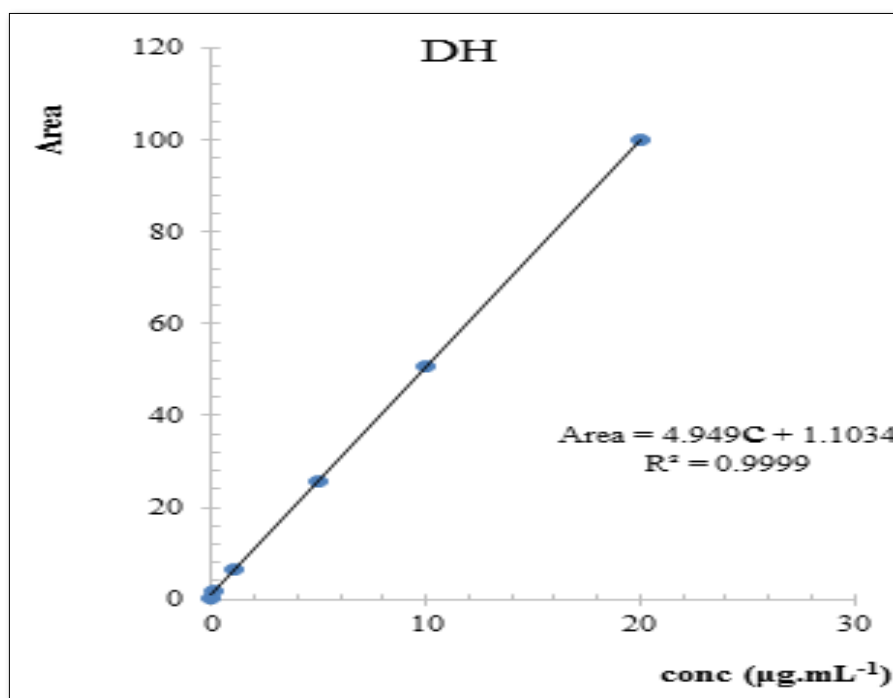


Figure 2. The standard DH line diagram at linear range (0.0001-20.0000) $\mu\text{g/ml}$.

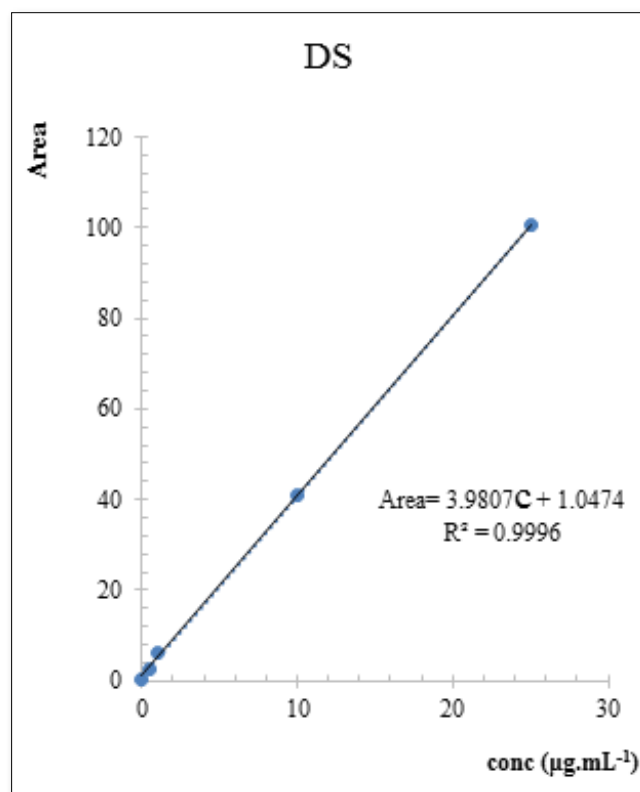


Figure 3. The standard “DS” composite graph at the linear range (0.0005-25.0000) $\mu\text{g/ml}$.

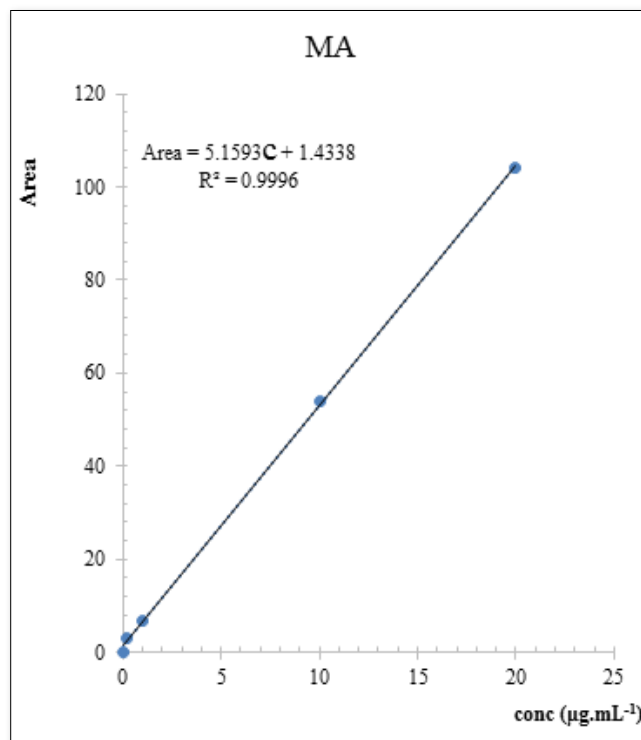


Figure 3. The standard “DS” composite graph at the linear range (0.0005-25.0000) $\mu\text{g/ml}$.

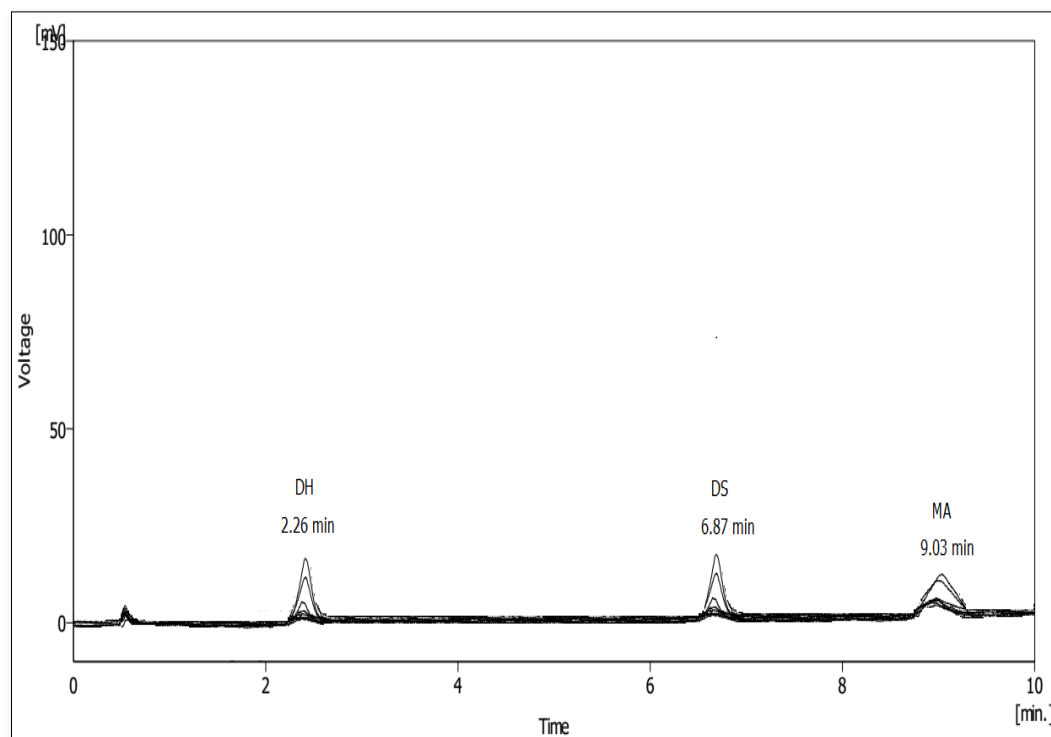
Table 3. Results of chromatographic analysis according to the proposed study.

Parameter	Value Calculate (DH)	Value Calculate (DS)	Value Calculate (MA)
Slope	4.9490	3.9807	5.1593
Intercept	1.1034	1.0474	1.4338
SD from Intercept	0.0010	0.0012	0.0025
Series Rang ($\mu\text{g/ml}$)	0.0001-20.0000	0.0005-25.0000	0.0002-20.0000
R ²	0.9999	0.9996	0.9996
Limit Detection ($\mu\text{g/ml}$)	0.00067	0.00147	0.000484
quantities Limit Detection ($\mu\text{g/ml}$)	0.00203	0.00301	0.00146

Checked the accuracy and validity of the proposed method

To achieve a correct and accurate chromatographic separation method with the ideal approved conditions. Standard laboratory solutions containing different

concentrations of compounds studied in experimental samples located within the studied linear field were prepared. Where three standard solutions were prepared at concentrations (0.01, 0.10, 1.20) $\mu\text{g/mL}$. Based on the parent standard solutions for the studied compounds (**Figure 5**).

**Figure 5.** Chromatogram of experimental samples according to the proposed method (0.01, 0.1, 1.2) $\mu\text{g/ml}$.

The results of injecting these concentrations on the device were approved after five consecutive times, and the values of both Recovery% and RSD% were calculated at each

concentration for four degrees of freedom and a confidence limit of 95%. The tested results were also listed in **Table 4**.

Table 4. Results of the analysis of experimental samples at four degrees of freedom and confidence limit of 95%.

Recovery% $\pm$ SD	RSD%	Real Conc $\mu\text{g/ml}$	Theo Conc $\mu\text{g/ml}$	Compound
96.00 $\pm$ 0.00018	1.87	0.0096	0.0100	DH
95.10 $\pm$ 0.00160	1.68	0.0951	0.1000	
98.33 $\pm$ 0.02300	1.95	1.1800	1.2000	
94.10 $\pm$ 0.00019	2.02	0.00941	0.0100	DS
93.20 $\pm$ 0.00170	1.82	0.0932	0.1000	
99.16 $\pm$ 0.0180	1.51	1.1900	1.2000	
92.30 $\pm$ 0.00020	2.16	0.00923	0.0100	MA
95.70 $\pm$ 0.00170	1.77	0.0957	0.1000	
100.83 $\pm$ 0.02500	2.06	1.2100	1.2000	

It is noted from the results given in the above table that the regression values ranged between 92% for mefenamic acid and 93% for diclofenac sodium and 95% for diphenhydramine hydrochloride and the values for the percentage of standard percentage deviation ranged between 1.95% for the last and 2.02% for sodium diclofenac and 2.16% for mefenamic acid which indicates Good health and accuracy for the proposed method and within the analytically acceptable limits.

Analysis of treated: Water samples in the pharmaceutical industry

The proposed chromatographic conditions were applied to samples of different sources from treated water samples for the pharmaceutical industry after the samples were prepared according to what was mentioned in paragraph (4). **Figure 6** shows the returned chromatogram to determine the studied compounds (DH, DS, MA) in the treated water sample (1).

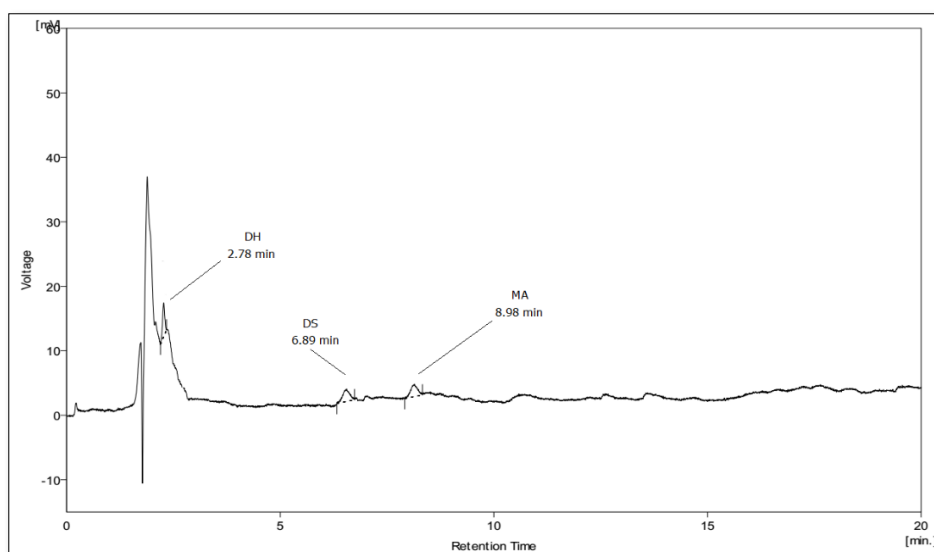


Figure 6. Chromatogram of a realistic sample of a mixture of compounds studied in the sample (1).

Given that the quantity of these materials is relatively low, therefore in order to calculate the actual concentration within the sample, and to enhance the amount of materials studied within the sample and reduce the background noise of the sample on the quantification process, the standard additions method was applied by adding increasing concentrations of

the studied materials, **Figure 7** shows chromatogram The yield for the compounds studied in the treated water sample for the pharmaceutical industry according to the standard additions method.

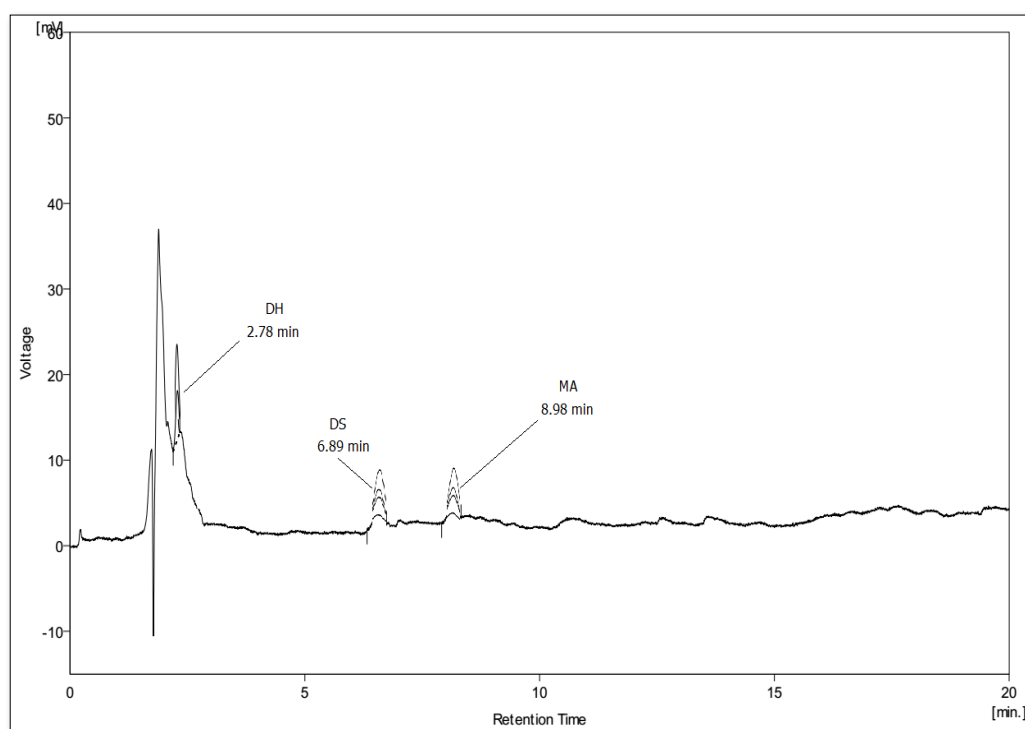


Figure 7. Chromatogram of the realistic sample of the mixture of the compounds studied in the sample (1) with standard additions

The equations of standard line charts for the standard addition's method were obtained by drawing the relationship between the surface area of the chromatographic peak produced for the concentrations after adding specific concentrations of the standard solution containing (DH, DS, MA) as in **Tables 5 and 6**. Determine the content of the treated water sample (1 and 2) from pain relievers and NSAIDs according to the standard additive method.

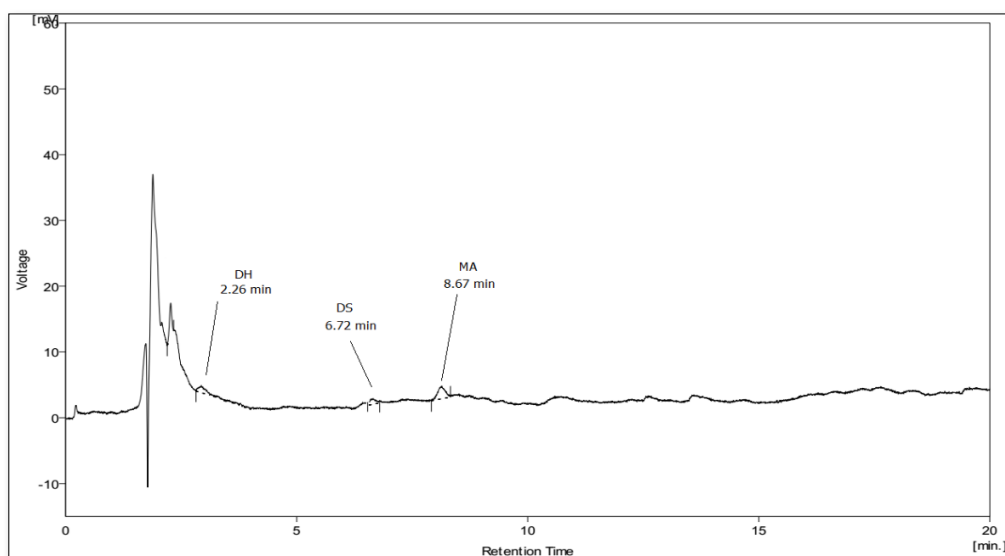
The high content of the studied materials compared to the results in **Table 6**. This is due to the interference of the chromatographic summit due to the studied materials with the background of the sample due to the occurrence of a chemical transformation such as the disintegration of the studied samples during the treatment process in the sample (2), at times Retention of the studied materials As noted from the previous table, the content of the samples from the studied materials was somewhat greater than the values results in **Table 6**. **Figure 8** shows the chromatogram due to determining the studied compounds (DH, DS, MA) in the treated water sample (3 and 4).

Given that the quantity of these substances is relatively low, therefore, in order to calculate the actual concentration within the sample, to enhance the amount of materials studied within the sample and to reduce as much as possible the interference from the sample background, the standard additions method was applied by adding increased concentrations of the studied materials, **Figure 9** shows the returning chromatogram To determine pain relievers and NSAIDs in the treated water sample for the pharmaceutical industry.

Standard line graph equations were obtained for the standard additions method by drawing the relationship between the surface area of the resulting chromatographic peaks of the concentrations after adding specific volumes of the standard solution containing (DH, DS, MA). The treated water sample (3 and 4) from the studied compounds according to the standard addition's method.

Table 5. Equations for standard graphs to determine the compounds studied in sample (1) & sample (2) according to the standard addition's method.

Name Compound		Standard graph line equation in Sample (1)		Correlation coefficient	
DH		Area=0.0392C + 0.0125		0.9703	
DS		Area=0.1593C + 0.0014		0.9982	
MA		Area=0.432C + 0.003		0.9990	
[c] = 0, 0.01, 1.00, 5.00, 15.00 µg/mL					
Name Compound		Standard graph line equation in Sample (2)		Correlation coefficient	
DH		Area=0.8782C + 0.2345		0.9871	
DS		Area=8.6781C + 1.3471		0.9982	
MA		Area=25.985C + 16.321		0.9991	
[c] = 0, 0.01, 1.00, 5.00, 15.00 µg/mL					
Name Compound	Calc conc µg/mL in Sample (1)	Ref Value µg/mL	t-Test	F-Test	
DH	0.000789	0.000214	1.231	1.856	
DS	0.000187		1.754	1.7454	
MA	0.000145		1.345	1.8898	
Name Compound	Calc conc µg/mL in Sample (2)		t-Test	F-Test	
DH	0.00561		2.567	1.9743	
DS	0.00623		3.875	1.9521	
MA	0.00498		2.895	1.9898	

**Figure 8.** Chromatogram of the realistic sample of the mixture of compounds studied in the sample (4).

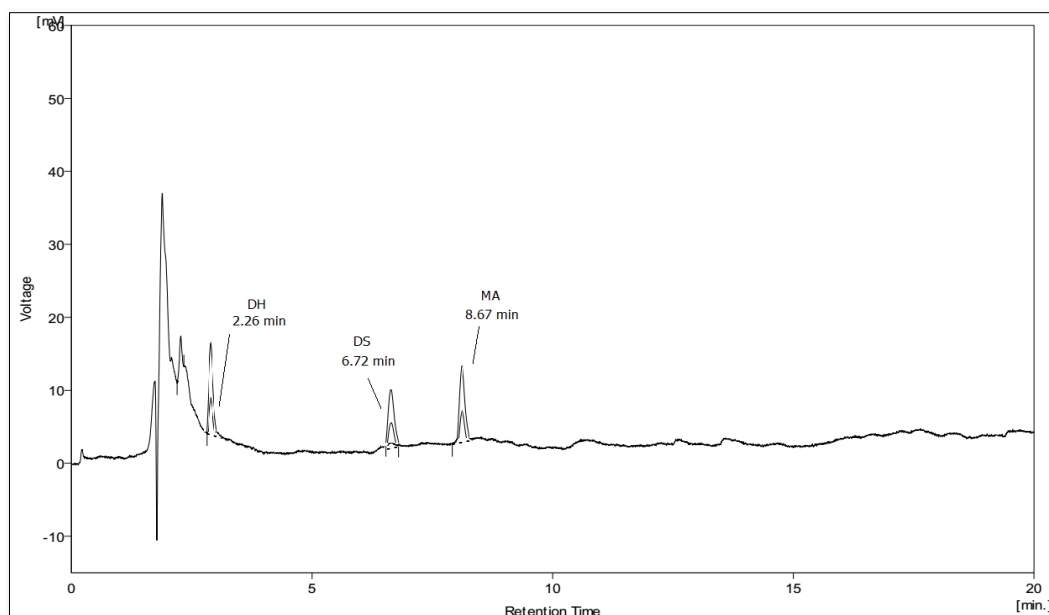


Figure 9. Chromatogram of the realistic sample of the mixture of the compounds studied in the sample (2) with standard additions.

Table 6. Standard graph equations to determine the compounds studied in the sample (3) & Sample (4) according to the standard addition's method.

Name Compound		Standard graph line equation in Sample (3)		Correlation coefficient	
DH		Area=30.439C – 0.4027		0.9386	
DS		Area=5.7749C + 0.069		0.9805	
MA		Area=21.601C + 0.1389		0.9858	
[c] = 0, 0.10, 1.00, 5.00, 20.00 µg/mL					
Name Compound		Standard graph line equation in Sample (4)		Correlation coefficient	
DH		Area=41.231C + 23.566		0.9568	
DS		Area=12.7890C + 5.678		0.9789	
MA		Area=27.9124C + 16.864		0.9987	
[c] = 0, 0.10, 1.00, 5.00, 20.00 µg/mL					
Name Compound	Calc conc µg/mL in Sample (3)	Ref Value µg/mL		t-Test	F-Test
DH	0.000210	0.000214		3.455	3.2211
DS	0.0002354			3.122	6.323
MA	0.000310			2.087	7.2322
Name Compound	Calc conc µg/mL in Sample (4)			t-Test	F-Test
DH	0.00986			4.6743	5.1246
DS	0.00784			4.1235	6.7864
MA	0.01974			3.6743	6.3116

CONCLUSIONS

Very low concentrations of (DH, DS, MA) were identified in the pharmaceutical wastewater samples using RP-HPLC technology. The proposed analytical method achieved high accuracy and sensitivity, with excellent detection limits. The result of the study, and compared with the reference results, shows that the appropriate chemical treatment of the pharmaceutical industry residues containing such compounds is better in relation to the recommended concentrations according to the World Health Organization without being treated by mixing with wastewater that affects little on the background of the sample because of the possibility of a chemical transformation that raises from Quantitative ratios of materials studied in the sample when mixing wastewater with the pharmaceutical industry's waste.

ACKNOWLEDGEMENT

This research aimed to suggest an analytical, chromatographic method: Sensitive, accurate, fast and reliable results, to determine the very low concentrations of months and more pain relievers and NSAIDs used locally and globally in the waters of the pharmaceutical industry wastes, as the latter is dumped in large quantities to agricultural areas And vital to direct contact with the environment, which affects human health and society.

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