DETERMINATION OF SOME CHEMICAL RESIDUES IN THE EFFLUENTS OF PHARMACEUTICAL INDUSTRY [1]

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ABSTRACT

Widespread occurrence of pharmaceuticals has started to attract attention as aquatic micropollutants that might have been affecting the ecological system in trace amounts. The risks associated with their introduction into wildlife habitats is becoming an important issue for both regulators and the pharmaceutical industry, because of incomplete elimination of pharmaceuticals wastewater and their metabolites. In this work monitoring of some pharmaceutical residues in industrial wastewater such as (caffeine, paracetamol, dexamethasone and naproxen) was carried out. The identification and quantification of chemical and pharmaceutical residues was investigated using GC/MS spectrometer and liquid chromatography-based tandem mass spectrometry LC/MS/MS spectrometer with electrospray ionization (ESI) .The study recorded the presence of different types of pharmaceutical residues with different concentration levels in wastewater .The concentration level of dexamethasone in wastewater were detected at 254 ng/ml, for paracetamol at 7325 ng/ml , for caffeine at 9356 ng/ml and for naproxen at 2 ng/ml . These concentrations representing about 500 fold higher than international safety margin of treated wastewater.

Keywords:- Pharmaceuticals, Micropollutants, Monitoring and wastewater.

INTRODUCTION

Water comprises four-fifths of the human body and is one of the most fundamental factors involved in many vital human interactions and studies have shown that humans can't live without drinking water more than three days (Helmy, 2009).

Drugs and pharmaceuticals have been used for decades in the treatment of diseases, but the surprise was that there are few studies have been made about their environmental and health effect, even if specifications of drinking water does not include any indication of the limits of toxicity and damage to more than 7000 compound drug is described medically (Al-Ananzah,2010).

Pollution with pharmaceutical residues is one form of water pollution and recent researches have revealed that there are pharmaceutical residues in the water which threaten human health as a result of exposure to long-term low doses of pharmaceutical residues, especially since most of these materials are characterized by their resistance to degradation and last for many years to be degraded (Abdel Maged, 2001).

Over the last few decades, the occurrence of micro-pollutants in the aquatic environment has become a worldwide issue of increasing environmental concern. Micropollutants, also termed as emerging contaminants or emerging environmental pollutants, consist of a vast and expanding array of anthropogenic as well as natural substances. These include pharmaceuticals, personal care products, steroid hormones, industrial chemicals, pesticides and many other emerging compounds. They are commonly present in waters at trace concentrations, ranging from a few ng/L to several μg/L. The low concentration and diversity of micropollutants not only complicate the associated detection and analysis procedures but also create challenges for water and wastewater treatment processes (Luo *et al*., 2014).

Among emerging pollutants, a particular attention focuses on pharmaceuticals and hormones because they may exert their activity at the very low ng/L range (Vulliet and Cren-Olivé, 2011). Most of the literature published has been on the treatment of municipal wastewater. However, there is a growing body of research that looks at the presence of active pharmaceutical ingredients in industrial wastewater (Deegan *et al*., 2011).

As pharmaceuticals were consumed in high quantities worldwide, in the range of tons per year per one pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of an improving health care system, discoveries of new drugs and longer life expectations of people (Van der Aa *et al*., 2011). Pharmaceuticals enter the environment via human or animal secretions or disposition of domestic drugs , ends up in wastewater purification plants. Their presence in water can also be attrbiuted to waste of pharmaceutical industry, waste of hospital and therapeutic drugs. They are not only released into the environment after use but some might disposed during manufacture or as unused or expired drugs. Certain pharmaceutical production facilities in pharmaceutical industries were found to be sources of much higher environmental concentrations than those caused by the usage of drugs. (Shalini *et al.*, 2010). It has been estimated that up to half of the pharmaceutical wastewater produced worldwide is released without any treatment (Enick and Moore, 2007), these pollutants are non-biodegradable.

Studies reveled that even small amounts (ng / L) of some pharmaceuticals adverse effect on aquatic communities include endocrine disturbance as the feminization of male fish (Corcoran *et al*., 2010),

development of pathogen resistance or development of antibiotic resistant bacteria (Yu *et al.*, 2009). Kolpin et al., 2002 reported the presence of one or more organic wastewater contaminants (OWCs) in 80% of 139 U.S streams across 30 states during 1999 and 2000 . Barnes *et al.*, 2004 reported the presence of OWCs as lincomycin at conc. 0.05ng/ml in central Oklahoma (well 35).

Thomas and Foster, 2005 and Terns *et al*., 2001 reported the presence of caffeine at conc. 150 ng/ml 42 ng/ml, respectively.

Since little is known about the potential chronic hazards associated with long term ingestion of pharmaceutical compounds through drinking water, so it was necessary to study these residues in industrial wastewater, where the presence of these residues in industrial wastewater may affects all the components of the environment .

The aim of this study was to monitor and determine some chemical and pharmaceutical residues in industrial wastewater using GC-MS and the analytical method was used (EPA625),liquid chromatography-based tandem mass spectrometry LC/MS/MS spectrometer with electrospray ionization (ESI) and the analytical method was used (EPA 1694) .

MATERIALS AND METHODS

Monitoring and Determination of some Chemical and Pharmaceutical residues in the effluent of a Pharmaceutical and Chemical Industries facility

A) Materials

1. Pharmaceutical standards were supplied by The El-Nile Co. for Pharmaceutical and Chemical Industries (El-Nile) Cairo- Egypt . Methanol 99.9% purity , sodium hydroxide, acetonitrile (ACN) and formic acid from Sigma Aldrich. HCl 37% purity from Fisher.Ultra-pure water was used thought the work (MQ) (MilliQ system; Millipore, USA).
2. Water samples were collected from the wastewater of a pharmaceutical and chemical industries facility in pre-rinsed amber glass bottles .

B) Methods of analysis

Samples collected from the effluents of a Pharmaceutical and Chemical Industries facility were determined and monitored for chemical and pharmaceutical residues by (EPA 625) using GC/MS/MS . This method is applicable to the determination of extractable organics in municipal and industrial discharges which collected from field in glass container then the water sample is filtered then a measured volume of sample, approximately 1-L extracted with methylene chloride at a pH 2 using a separatory funnel. The pH is adjusted by sulfuric acid. The organic layer (Methylene chloride) extract dried, concentrated to a volume of 1 ml using Rota vapor instrument. Then analyzed by GC (varian 3800) and mass detector (varian320-MS) in full scan. Qualitative identification of the individuals in the extract is performed using the retention time and the relative abundance of the three characteristics masses (m/z).

Quantitative analysis is performed by using LC/MS/MS.

Samples were collected from the effluents (before treatment) of a pharmaceutical and chemical Industries facility vacuum filtered through a 0.45-µm glass fiber filter then extracted via solid-phase extraction (SPE) (Brosius, 2011) and subsequently analyzed by LC/MS/MS (Agilent's 6410 triple quadruple LC/MS/MS system with ESI). The Agilent MassHunter Workstation software (version: B.01.04) was used for system control and data acquisition. Table (1)

Table (1): Instrument Parameters

<table>
<tr><th colspan="2">HPLC</th><th>MS-MS</th></tr>
<tr><td colspan="2">LC: Agilent 1200 LC system</td><td>MS: G6410A QQQ</td></tr>
<tr><td colspan="2">Column: ZORBAX Eclipse XDB-C18, RRHT, (4.6 × 50 mm, 1.8 µm)</td><td>ionization mode: ESI</td></tr>
<tr><td colspan="2">Column temperature: 40 °C</td><td>Mass range: 125 to 800 amu</td></tr>
<tr><td colspan="2">Mobile phase A: 0.1% Formic acid/H2O</td><td>Scan time: 300 ms</td></tr>
<tr><td colspan="2">Mobile phase B: Acetonitrile (ACN)</td><td>Capillary: 3500 V</td></tr>
<tr><td colspan="2">Flow rate: 0.3 mL/min</td><td>Nebulizer P: 40 psi</td></tr>
<tr><td colspan="2" rowspan="2">Gradient: T=0, A=100%, B=0%
T=15, A=0%, B=100%
T=20, A=0%, B=100%
T=21.5, A=100%, B=0%</td><td>Drying gas: 9 L/min</td></tr>
<tr><td>Gas temperature: 350 °C</td></tr>
<tr><td colspan="2">Injection volume: 1.0 µL</td><td>Skimmer: 35 V</td></tr>
</table>

Source: (Meng, 2008)

RESULTS AND DISCUSSIONS

Qualitative analysis of wastewater showed that there are many organic wastewater contaminants (OWCs) as shown in Figures (1,2,3 and 4) and Table 2 .

Quantitative analysis of selected four compounds of organic wastewater contaminants (OWCs) shown in Figures (5,6 and 7) and Table 3.

Thomas and foster (2005) and Terns *et al*. (2001) reported the presence of caffeine at concentration 150 ng/ml , 42 ng/ml ,respectively. Jones, *et al.* (2005) found paracetamol and naproxen at nanograms per liter and per ml levels, respectively in a large english sewage treatment plant and in Louisiana. Influent concentrations are likely to be quite variable because they are dependent upon various factors such location, socioeconomic status, pharmaceutical cost, and other demographic data .

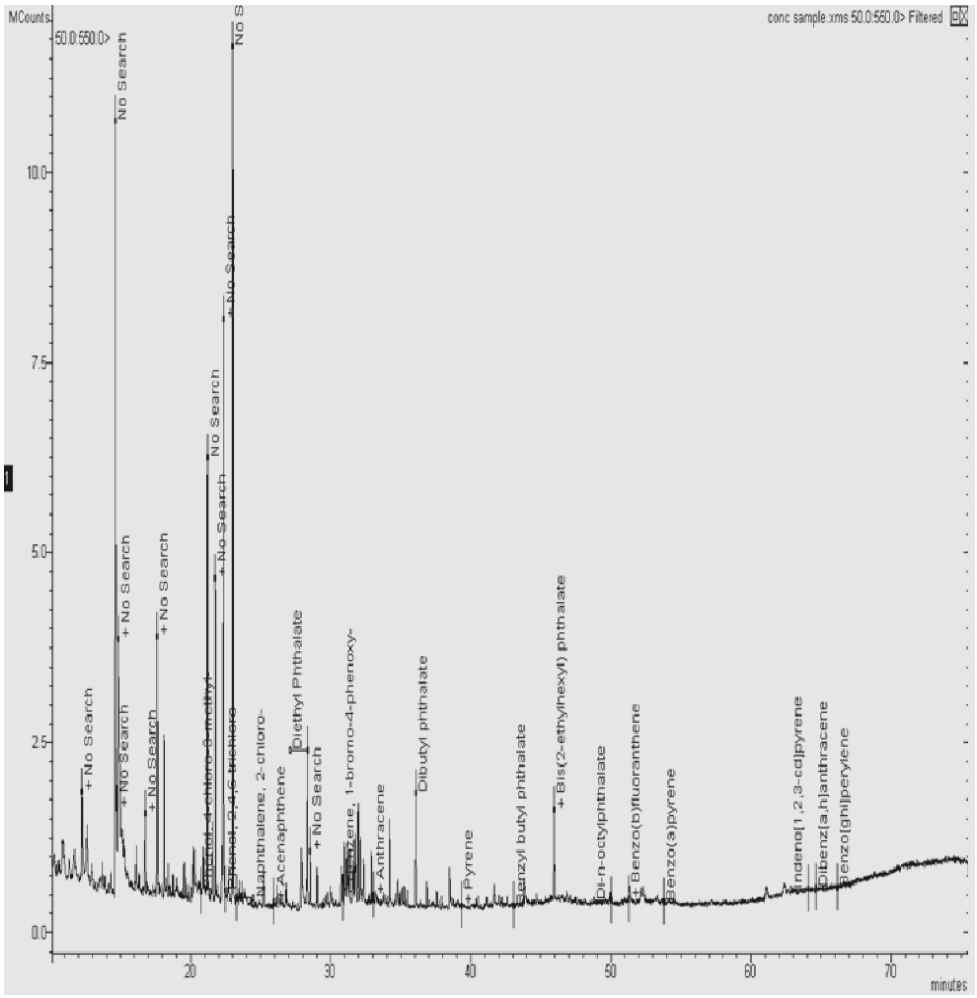

Figure (1):.GC/MS scan for sample (1)

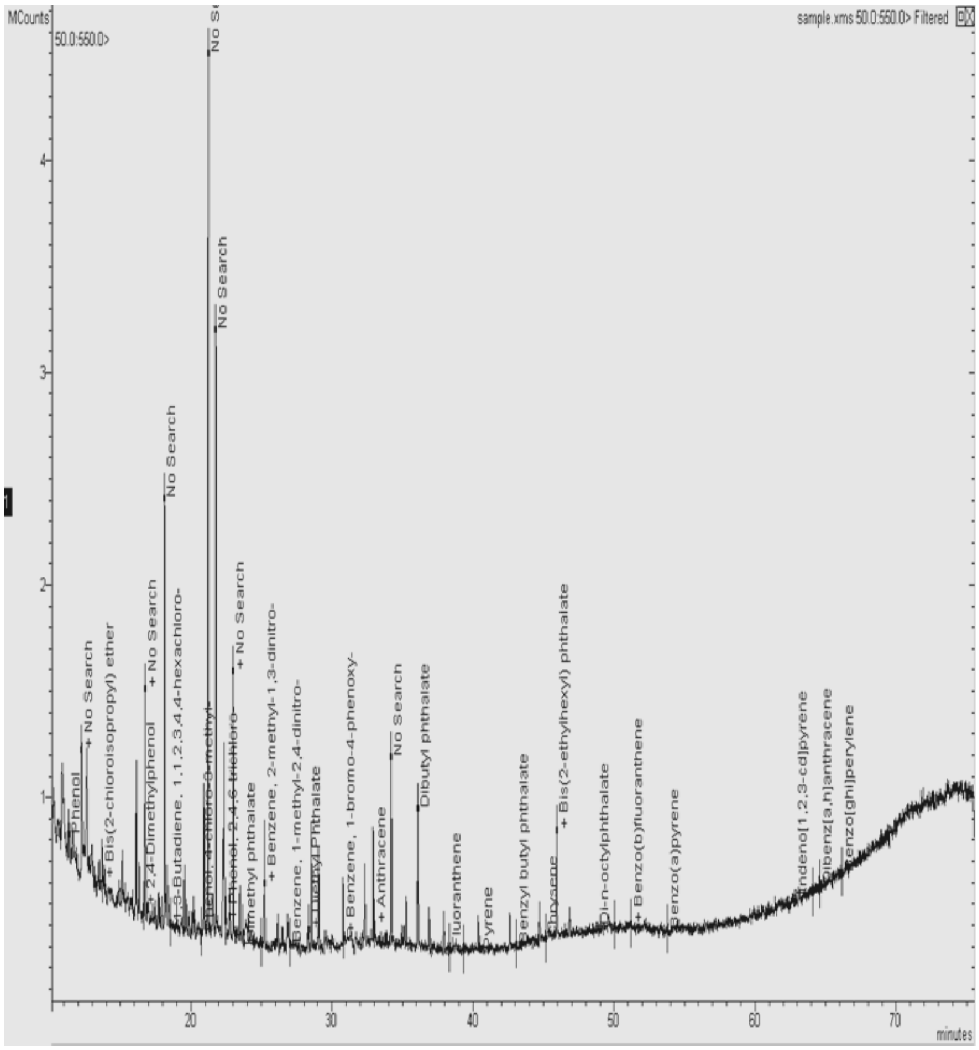

Figure (2):.GC/MS scan for sample (2)

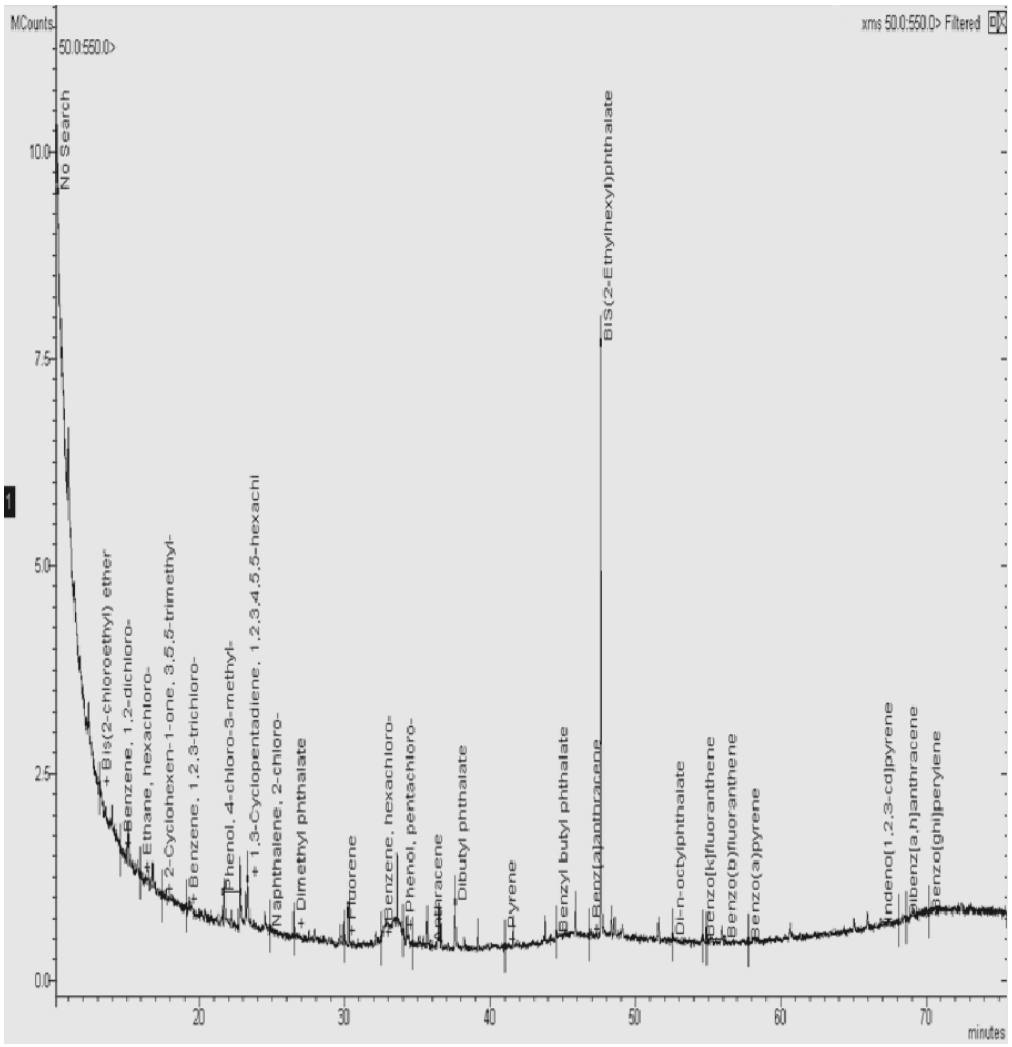

Figure (3):.GC/MS scan for sample (3)

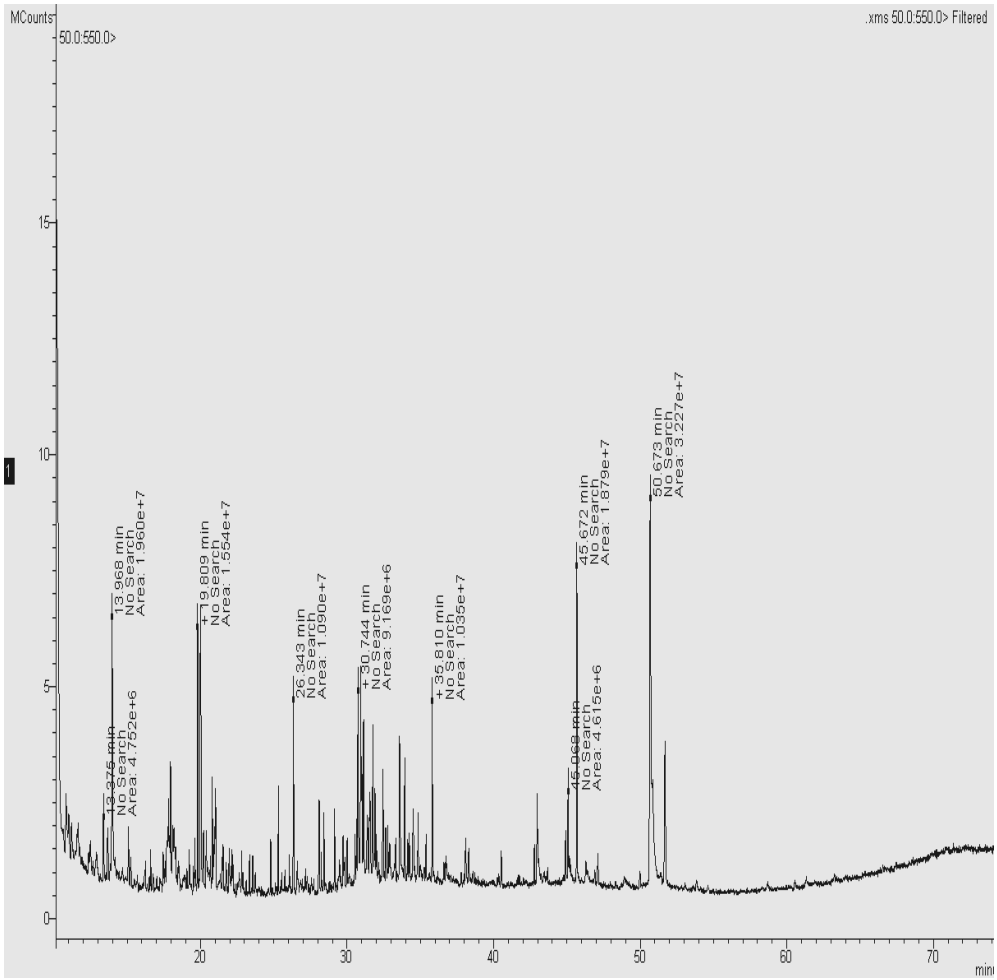

Figure (4):.GC/MS scan for sample (4)

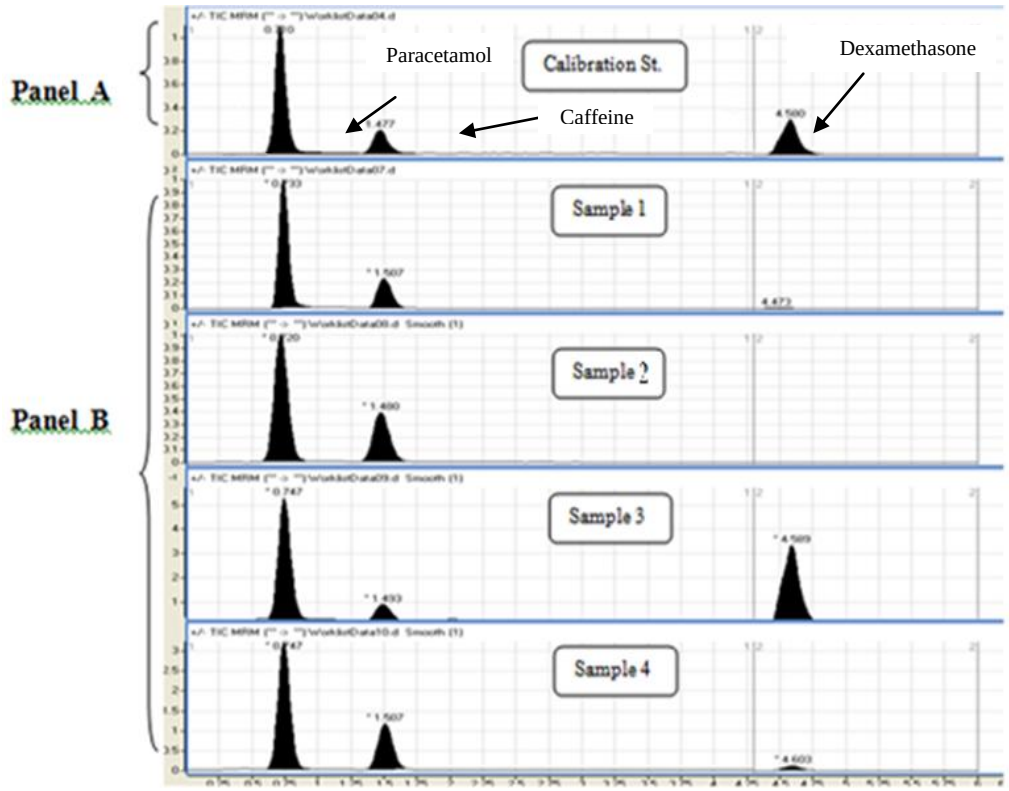

Figure (5): Panel A shows calibration standard of 3 pharmaceutical compounds peaks for (PCM, caffeine and dexamethasone) respectively. Panel B shows 3 industrial wastewater samples compared to panel A in retention time

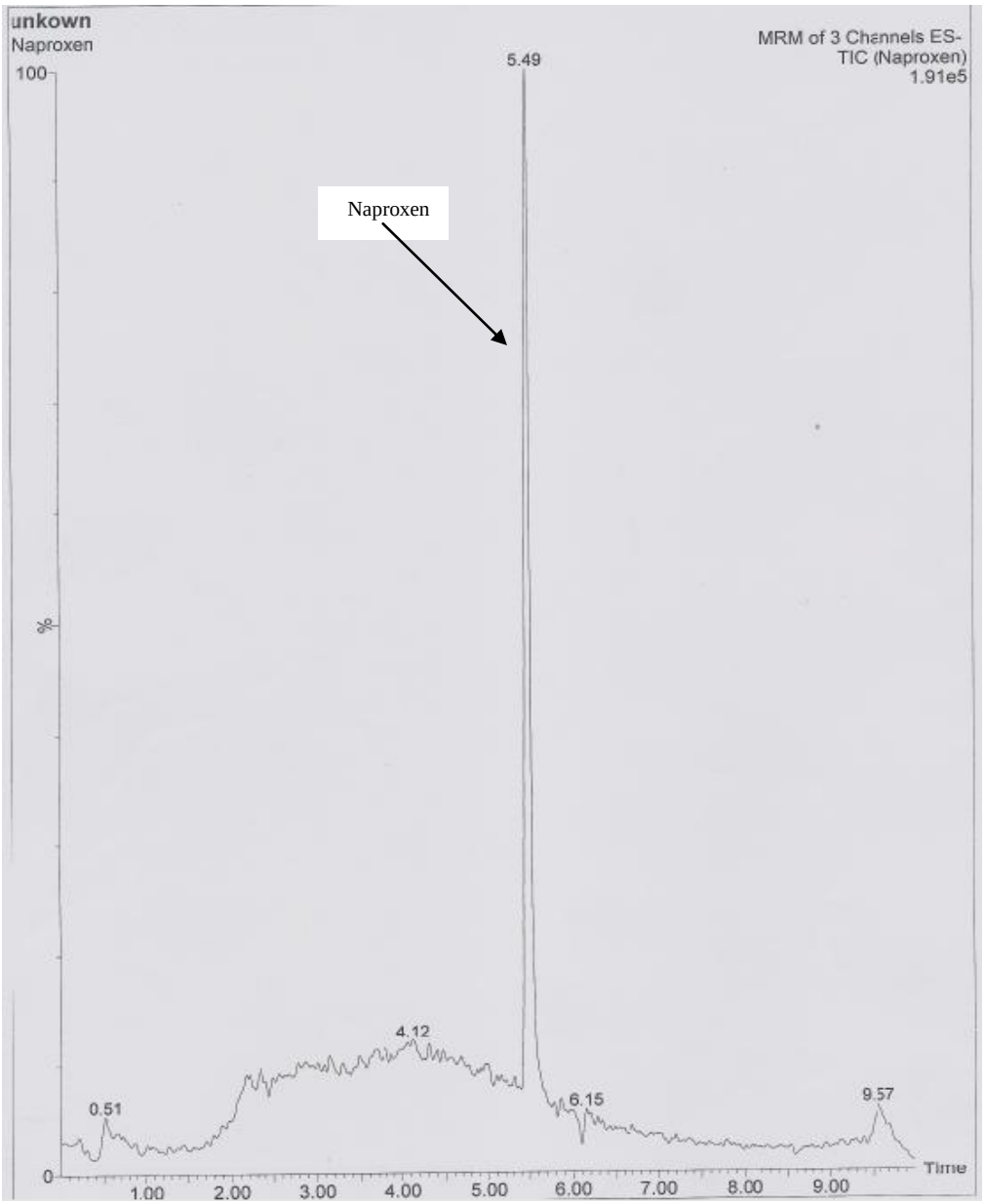

Figure (6): Naproxen chromatogram 1700 ppm

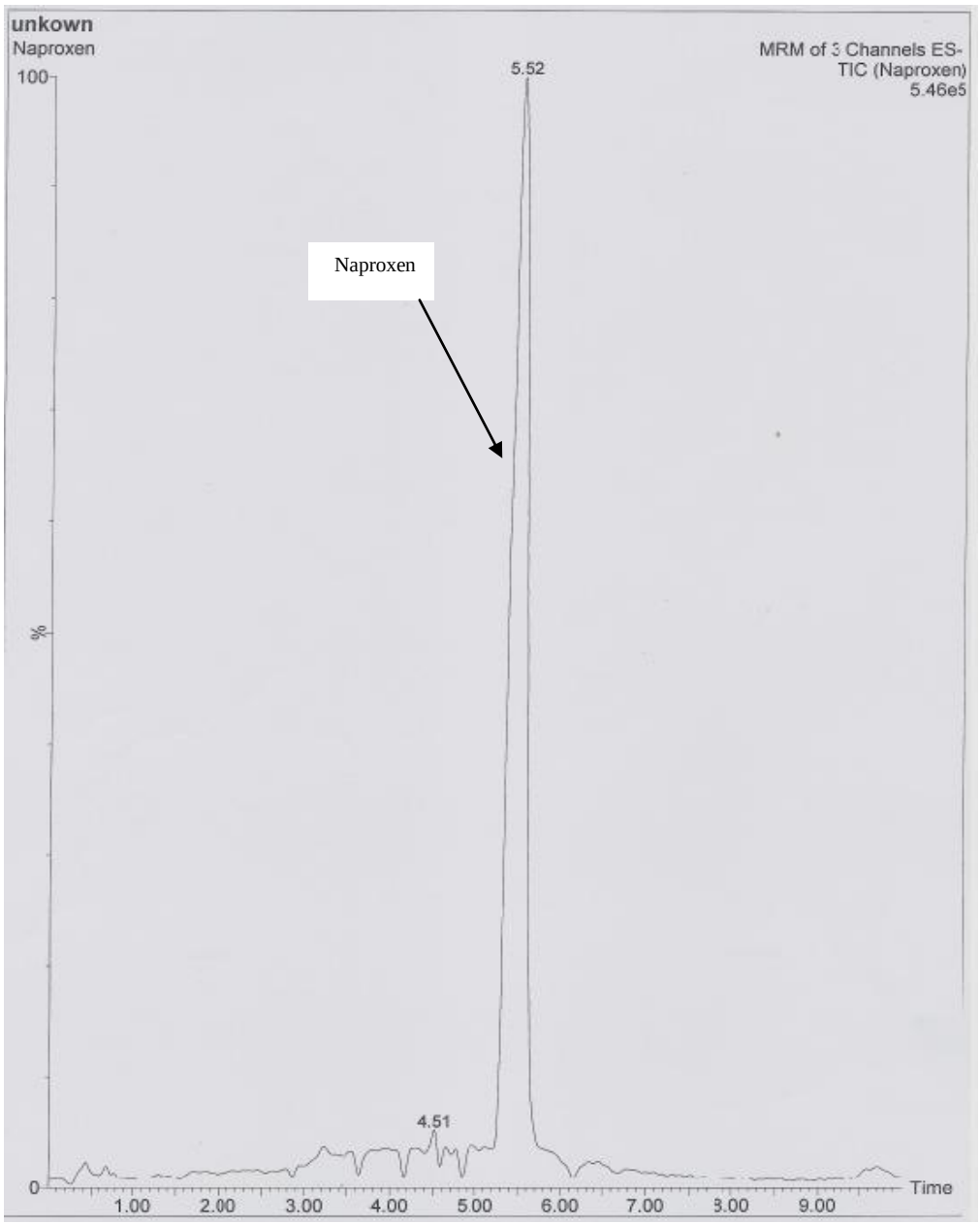

Figure (7): Naproxen chromatogram 2000 pp

Table (2): Compounds found in qualitative analysis of four samples (cont.)

Compounds found in sample 1	Compounds found in sample 2	Compounds found in sample 3	Compounds found in sample 4
1-(2,6,6-Trimethylcyclohex-1-enyl)methanesulfonyl)benzene 2-3-Methylpyridazine 3-4-Chloro-3-methylbut-2-en-1-ol 4-Propane, 2-(2-isopropylidene-3-methylcyclopropyl)-, trans- 5-Phenol, 4-methyl- 6-Phenol, 3-methyl- 7-7-Octen-2-ol, 2,6-dimethyl- 8-L-(-)-Menthol 9-à-Terpieol 10-1H-Imidazole, 2,4,5-trimethyl- 11-3,5-Heptadienal,2-ethylidene-6-methyl- 12-2-(2-Chloro-2,3,3-trifluoro-cyclobutyl)-cyclopropanecarboxylic acid 13-Phenol, 4-propyl- 14-Bicyclo[2.2.1]heptane-1-carboxylic acid, 7,7-dimethyl- 15Tetracyclo[5.2.1.1(2,6).0(3,5)]undecan-10-one, 4,4-dichloro-	1-(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene 2-3-Hexanone, 2,5-dimethyl-4-nitro- 3-Cyclohexanemethanol,à,à,4-trimethyl- 4-3-Cyclohexene-1-methanol, à,à,4-trimethyl- 5-p-Mentha-1,4(8)-diene 6-3,5-Heptadienal, 2-ethylidene-6-methyl- 7-Exo-ketobomeol 8-Benzaldehyde,4-(1-methylethyl) 9-Bicyclo[2.2.1]heptane-1-carboxylic acid, 7,7-dimethyl- 10-7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)- 11-Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester 12-Ascaridole epoxide	1-Pyridin-4-amine, 2,3-dichloro- 2-Benzene, 1-(dichloromethyl)-4-methyl- 3-9-Thiabicyclo[3.3.1]nonane,2,6-dichloro 4-Exo-ketobomeol 5-3-Ethyl-1-heptyne-3-ol 6-Bicyclo[2.2.1]heptane-1-carboxylic acid, 7,7-dimethyl- 7-Phenol, 3,5-bis(1,1-dimethylethyl)- 8-Propanoic acid,2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester 9-8-Hydroxy-2,2,8-trimethyldeca-5,9-dien-3-one 10-9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-diol 11-1,2-Benzenedicarboxylic acid, butyl octyl ester 12- Phthalic acid, butyl isohexyl ester 13-1,2-Benzenedicarboxylic acid, diisooctyl ester 14-lambda.-Cyhalothrin	1-L-(-)-Menthol 2-à-Terpieol 3-Bicyclo[2.2.1]heptane-1-carboxylic acid, 7,7-dimethyl- 4-Propanoic acid,2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester 5- Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester

Table (2): Compounds found in qualitative analysis of four samples (cont.)

Compounds found in sample 1	Compounds found in sample 2	Compounds found in sample 3	Compounds found in sample 4
16-Exo-ketobomeol 17-3'-Hydroxyquinalbarbitone 18-Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester 19-Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester 20-Ethanol, 2-(octadecyloxy)- 21-Benzoic acid, 4-ethoxy-, ethyl ester 22-Formamide, N,N'-[1,3-phenylenebis(methylene)]bis- 23-Diethyl Phthalate 24-Carbamic acid, N-[1,1-bis(trifluoromethyl)ethyl]-, 4-(1,1,3,3-tetramethylbutyl)phenyl ester 25-3-(p-Anisoylhydrazono)-N-(2-ethylphenyl)butyramide 26-Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)- 27-Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo[2.1.0]pentane), 1',4',2,6,6-pen 28- Carbamic acid, N-[1,1-bis(trifluoromethyl)ethyl]-, 4-(1,1,3,3-tetramethylbutyl)phenyl ester 29-Phenol, 3,5-bis(1-methylethyl)- 30-Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl)- 31-Phenol, 4-(1,1,3,3-tetramethylbutyl)- 32-Diisobutyl phthalate	13-Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester 14-4,6-Dimethyl-(1H)pyridone-2, 3-(4-benzyloxyphenylmethyleneamino 15-Glycine, N-(2-hydroxybenzoyl)- 16-Tetradecane 17-Dodecane, 5,8-diethyl- 18-Diisobutyl phthalate 19- Dibutyl phthalate		

Table (3): Concentration in (ng/ml) of 4 analytes in wastewater of a pharmaceutical and chemical industries facility

Sample	Paracetamol	Caffeine	Dexamethasone	Naproxen
	conc. (ng/ml)	conc. (ng/ml)	conc. (ng/ml)	conc. (ng/ml)
Sample1	7325	9437	254	2000
Sample2	890	1750	8	1700
Sample3	38	25	84	0
Sample4	270	450	11	0

As shown in table 3 the Quantitative analysis of water sample in agreement with (Rashed *et al.*, 2015) whom reported the presence of caffeine at conc. 9356 ng/ml , paracetamol at conc. 7216 ng/ml and dexamethasone at conc. 245ng/ml in the wastewater of different pharmaceutical industrial surrounding areas .This results also in agreement with (Salgado *et al.*, 2010; Sim *et al.*, 2011 and Yu *et al.*, 2013)) as they reported the presence of caffeine at conc. 4-19 ng/ml . In our study the effluent concentration of caffeine was 9437ng/ml which reported 500 fold higher than recommended in world wide. In case of paracetamol concentration in wastewater was reported in range from undetected to 97.5 ng/ml (Behera *et al.*, 2011; Sim *et al.*, 2011; Yu *et al.*, 2013) , in our study the effluent concentration of paracetamol was 7325 ng/ml which reported 70 fold higher than recommended in world wide and when naproxen exposed to sunlight it's converted into two related compounds these photodegradants are predicted to be more toxic than naproxen because they have lower polarity .

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تقدير بقايا بعض الكيماويات في المخلفات السائلة للصناعات الدوائية

[١]

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المستخلص

إن الانتشار الواسع المدى للادوية والمركبات الصيدلانية بدأ يجذب الانتباه كملوثات مائية متناهية الصغر يمكن أن تؤثر على النظام البيئى. وأصبحت المخاطر المرتبطة بوجود الأدويه أو المركبات الصيدلانيه فى البيئة قضية أو مسأله هامة للمنظمين البيئيين وصناعة المستحضرات الصيدلانية نتيجة لعدم التخلص الكامل لنفايات الأدوية ونواتج تفاعلاتها الموجودة فى مياه الصرف. وتشمل هذه الدراسة أنواع مختلفة من مجموعات الأدوية.

تهدف الدراسة الي تقدير بعض متبقيات الأدوية والكيماويات في المخلفات السائلة للصناعات الدوائية والتعرف عليها باستخدام جهاز الاستشراب الغازي– مطياف الكتلة والطريقة المستخدمة هي EPA 625 . وكذلك يتم تحديد تركيز هذه المتبقيات فى مياه الصرف الصناعى باستخدام جهاز الفصل الكروماتوجرافى السائلى – مطياف الكتلة والطريقة المستخدمة هي EPA 1694 وسجلت الدراسة تواجد أنواع مختلفة من متبقيات الادوية والكيماويات بتركيزات مختلفة .