Solid cartridges in Determination of Benzidines in River and Wastewater by HPLC

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Abstract: A solid Phase Extraction (SPE) cartridges followed by HPLC-UV method is described for the simultaneous quantitative determination of benzidine (BZ) and its substituted 3, 3’-dichlorobenzidine (DCB) and 3, 3’-Dimethylbenzidine (DMB). The Benzidines were separated by liquid chromatography using a C-18 column with UV detector at wave length of 280nm. The mode of Flow was isocratic. The mobile phase was consisted of 75:25 methanol: water, column temperature 50C°, and Flow Rate 1.8ml/min**.** Calibration curves were linear ($R^2 = 0.9979$-0.9995). LOD (26.36-33.67) µg/L, LOQ (109.98-186.11) µg/L, the Robustness (2.99-4.35), Ruggedness (2.93-3.65). Conditions of extraction by (SPE) cartridges were optimized, the resin used is Octadecyl silica (ODS), pH (8) and Elution solvent used is methanol. The water samples to be analyzed were taken from six different locations. Three of them inside the station of petroleum refinery waste water treatment unit and the other three were at the Tigris River in Iraq around the station. The results were between (non detected, 246.9) µg/L inside the station and (non detected, 288.7) µg/L at the river.

[Hadi Hassan Jasim, Bahaa Malik Altahir, Muntadhar Salih Sultan. **Solid cartridges in Determination of Benzidines in River and Wastewater by HPLC.** *World Rural Observ* 2018;10(1):52-60]. ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). http://www.sciencepub.net/rural. 7. doi:10.7537/marswro100118.07.

Keywords: petroleum refinery wastewater, Benzidine, 3,3’-dichlorobenzidine, 3,3’-Dimethylbenzidine, Solid phase extraction.

Introduction

Benzidine-based azo dyes are widely used in the dye manufacturing, textile dyeing, color paper printing, and leather industries. In 1980, the National Institute for Occupational Safety and Health (NIOSH) published a survey of the data on the carcinogenicity of BZ-based dyes from tests with experimental animals and epidemiological studies of workers exposed to the dyes (Chung *et al*., 1993; Saeed, 2014; Bandala *et al.,* 2008; Husain et al., 2012). Many of these dyes find their way into the environment via wastewater facilities. They also exhibit a high resistance to microbial degradation in wastewater treatment systems (Zaharia et al., 2009; Bayramoglu et al., 2007). Aromatic amines, such as benzidine, initiate bladder cancer in humans (Lakshmi *et al*, 2003}. Without adequate treatment these dyes can remain in the environment for a long period of time (Hao et al., 2000). Benzidine was listed as a carcinogenic agent for the first time in the First Annual Report on Carcinogens (Tannenbaum, 2000). Benzidine has been found in at least 27 of the 1430 National Priorities List sites identified by the Environmental Protection Agency (EPA) (Muneer *et al*. 2002).

The content of benzidinic compounds in aqueous samples can be determined by various analytical instrumental methods, such as gas chromatography (GC)/mass spectrometric assay (Hing et al.,2004); Jedrzjczak et al.,1993; Sang et al.,2001), rapid colorimetric (Rice et al.1955; Ameera et al.,2011), isotope dilution mass spectrometry- capillary gas chromatography/negative ion chemical ionization (Hsu et al.,1996), Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Nyman et al.,1999), oscillating chemical system (Gao et al.,2004), fluorescence spectrographic method, (Chuan et al.,2009), novel resonance light scattering (RLS) (Chen et al.,2011), Carbon- and platinum-based screen-printed electrodes, in comparison with standard GC and Pt electrodes (Falciola et al., 2012), Direct injection and Ultrahigh-pressure liquid chromatography (UPLC) with fluorescence detection (Chao et al.,2013), high performance liquid chromatographic with various detectors such as UV detector (Ononye et al.,1987; Frank et al.,1999; Shi et al.,1999; Bouzige et al.,1999) mass spectroscopy detector (Aznar et al.,2009; Jei et al.,2014) fluorescence detection (whang et al.,2013) electrochemical detection (HPLC-EC) (whang et al.,2013; Vera-Avila et al.,2001; Riggin et al.,1979; Rice et al., 1978). various pretreatment and extraction technique solvent extraction used to pre-concentration and extraction of benzidines such as, SPE (Hsu et al.,1996; Hing et al., 2004; Jedrzjczak et al.,1993; Aznar et al.,2009) Extraction immunosorbent involving antigen–antibody interactions (Bouzige et al.,1999). HPLC is the preferred technique for benzidines separation, because of its high selectivity.

In addition, SPE is necessary before HPLC to remove interfering components. The proposed method is simple; rapid and being used to identify and simultaneously determine several compounds in short time. Benzidines were determined in various samples such as urine (John et al.,1978; Schulte et al.,1986; Jedrzjczak et al.,1993; Hsu et al.,1996; Shin et al.,2001), soil (Ononye,1987), food (Frank et al.,1999) and environmental samples such as: Lake estuaries, industrial wastewater treatment, sediment samples, basin of lake (Nyman et al.,1999), studies were carried out to evaluate the amount of benzidines in industrial waste water and environmental water samples but no previous study had concerned these compounds in petroleum refinery waste water and river water nearby. Dora oil petroleum refinery station is one of the most important stations in Iraq and has waste water treatment unit. The oil petroleum refinery waste water is the one of the source of aromatic compounds because these compounds are the major components of crude oil (Vazquez et al (2000). The aromatic compounds may have effects on water living organism. Benzidines are hazardous pollutants and aromatic compounds can be found in the petroleum waste water. The end stream of this unit downwards the river after local and simple treatment. The aim of this study is to investigate the water concentrations of benzidines via treatment stages and selected site of rivers around the point of waste water discharge.

Materials and Methods

Materials

Standard solution of [Benzidine, 605 benzidines calibration mix (BZ, DCB), 8270 benzidines mix (BZ, DCB, DMB)] supplied from Restek chromatography product and solutions. All solvents were HPLC grade supplied from (sigma-Aldrich, Hi Media Laboratories (England) & J.T Baker (Netherland). All other chemicals were of analytical grade all having a purity >98.0%) were obtained and purchased either from Sigma Chemical Company Inc., Aldrich Chemical Corporation (Milwaukee, WI) or BDH Inc. SPE cartridges CHROMABOND® {HR-P (polystyrene-Divinylbenzine) (PS-DVS), Octadecyl silica (ODS), Cyclohexyl silica (CHS)} supplied from MACHEREY-NAGEL Company.

Instruments

Shimadzu HPLC (LC-20AD), DGU-20As Degasser, LC 20A four pumps P, N 7725i Sample injector, Shimadzu SPD-20A prominence UV-VIS detector, and Column Oven CTO 20AD. Column type (EC NUCLEdur C18RP) Stainless steel made, Length 250mm, I. D 4.6 mm, Machenery nagel. Column protection system (EC guard column, holder system) id 2 mm Machenery Nagel. A special syringe made for HPLC type (M. SYRINGFE, 122F-LC) was used for injection 20 µl of samples into the mobile phase to the column. Extraction system designed and made manually for extract different samples in the same time.

Mobile phase optimization protocol

All grades HPLC solvent were mixed pre-filtered by 0.45 µm nitro cellulose filter paper. The optimization and selection of mobile phase were done by change the solvent mixture type, Organic solvent percentage, Oven temperature, Flow rate.

Solid phase extraction optimization protocol

Extraction cartridges was conditioned by adding 5 ml methanol, then 5 ml distilled water, 50 ml of samples with 1000 µg/L suctioned under vacuum by tube connected with SPE cartridge fitted with conical flask provided with specific stopper. Extraction conditions were optimized such as: kind of resin, pH, Elution solvent.

Buffer preparation

Different buffers were prepared for experiments to have the desired pH (Gomori 1955). The buffers used in this study were sodium phosphate buffer (pH range 5.6-8.0), Acetate Buffer (pH (3.7-5.6). Carbonate buffer (pH (9.2-10.6). Hydrochloric acid-potassium chloride (pH (1-2)).

Experimental sample collection and preparation

Water samples were collected from six selected sites, three of them inside the treatment station. Specific pumps already operated for sampling (after oil skimmers tanks, after Dissolve air flotation (DAF) UNIT, after biological treatment. Additional three other sites were outside the station in Tigris river: (100m north of point of waste water discharge (control) and 100m, 800m to the south). All samples were collected in closed fitted dark glass bottle of 2.5 L. cleaned by diluted chromic acid, then washed thoroughly with distilled water In site location, containers shake well with river water or waste water couple times then filled with sample. The river Samples were collected by immersing the containers with depth (30cm) and were locked immediately. The samples were examined at the same day of sampling. The samples were pre-filtered by 0.45 µm Millipore filter.1ml per liter of concentrated NaOH was added to adjust the pH >8, 2.5ml of methanol was added to each litter water sample. Extraction of desired benzidines done by passing 1 L of sample water through a cartridge of SPE contains ODS resin (flow rate 25 to 35 ml/min). the extraction column was dried in oven at 50 C° for 10 minute. The desired materials were eluted from the cartridge with a 5ml of methanol and concentrated further by evaporation to less than 1 ml and complete volume to 1 ml. The sample components were separated, identified, and measured by injecting an aliquot of the concentrated methanol solution into a high performance liquid chromatography (HPLC) with UV detector containing a reverse phase HPLC.

Results and Discussions

Chromatographic conditions

The liquid chromatography separation and detection of the three different benzidines mixture was carried out by using (C18) column (stationary phase). The flow was Isocratic. A good separation can be achieved in a short separation time. Retention factors (k) of benzidines was optimized and the percentage of an organic HPLC grade solvent to aqueous percentage was 75:25 v/v% methanol: water was the best for full separation. Flow rate 1.8 ml min^{-1}, temperature of the column oven set at 50ºC. Detection was performed at 280 nm.

HPLC chromatogram

The retention times (tR) and void time (t_M) were used to calculate the Retention factor k of the eluted benzidines (k_1, k_2 and k_3) respectively, by using the equation ($k = (t_R - t_M/t_M)$. tR and tM was obtained from the chromatogram Figure 1. the number of theoretical plates (N) was calculated by using equation ($N=16(t_R/w)^{\wedge 2}$). The selectivity factor (α) was calculated by equation ($\alpha = k_2/k_1$) and the resolution factor by the equation ($RS = 2(t_2-t_1)/(w_1+w_2)$), [skoog, 2004; Anirbandeep, 2014] where (w) are the widths of the two peaks and tR is the retention time of the sample. The results are summarized in Table (1) where the capacity factors (k) was between 1,43-3.79, number of theoretical plates (N) was lied between 1487.1 and 3855.5 respectively, and selectivity factor (α) were 1.368 and 1.832 while the RS 2.258 between BZ and DMB, 6.148 between DMB and DCB. Tailing factor (Tf) was in range of (1.045-1.33).

Table 1. HPLC parameters of separation of Benzidines

	Peak width (min)	tR (min)	tM (min)	k	N	α	Rs	Tf
BZ	0.181	1.745	0.700	1.493	1487.146			1.045
DMB	0.176	2.148	0.700	2.069	2383.215	1.386	2.258	1.042
DCB	0.216	3.353	0.700	3.790	3855.490	1.832	6.148	1.333

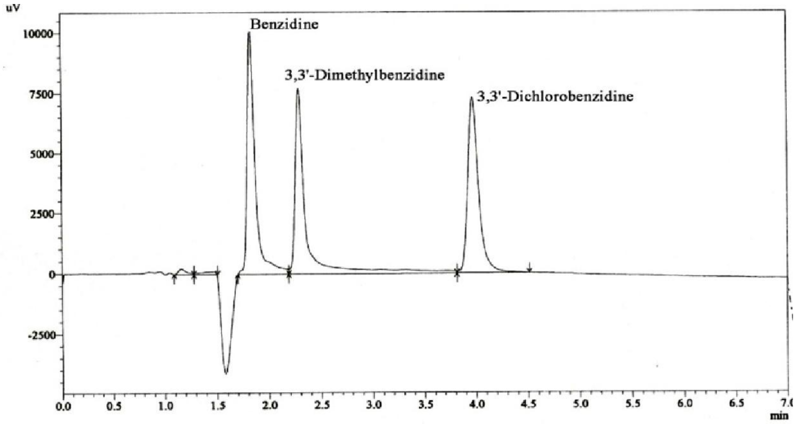


Figure 1. HPLC chromatogram of priority pollutant benzidines (PPB)

Calibration curves and analytical methods validation

Standard solutions were prepared at nine concentrations, typically 800, 600, 400, 200, 100, 50, 25, 12.25, and 6.125. The method of standard preparation and the number of injections will be the same as used in the final procedure. Results were recorded on a data sheet. The mean, standard deviation, and relative standard deviation (RSD) were calculated for each concentration. Concentration (x-axis) versus mean response (y-axis) was plotted for each concentration. The regression equation and coefficient of determination (r2) were calculated. Good linearity was found for all benzidines compounds where the regression coefficients (r^2) were 0.9992, 0.9979, and 0.9994 of BZ, DCB and DMB respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by linear regression method [sangai et al., 2009], the results of LOD were 32.99, 33.67 and 26.39 while values of LOQ were 109.98, 112.26, and 87.89 of BZ, DCB and DMB respectively. Robustness was done by triplicate injections of standard solutions after incubated them in an oven at 50 C° for 30 min. Averages of RSDs were calculated and the results were 3.26, 2.99, and 4.35 of BZ, DCB and DMB respectively. The ruggedness (intraday) was done by triplicate injections of the compounds within 3 days by the average of 3x3 injections. The results of ruggedness were 2.93, 3.56 and 3.36 of BZ, DCB and DMB respectively. Spiked samples were prepared at three concentrations to calculate the error percentages and found to be 0.81,124 and 1.55 of BZ, DCB, DMB respectively. (Table 2).

Table 2. Analytical methods validation (BZ, DCB and DMB)

	r	Slope	Y-intercept	LOD	LOQ	Rubstness	Ruggednes	ER%
BZ	0.9992	81.807	-263	32.99	109.98	3.26	2.93	0.81
DMB	0.9979	66.99	-64.76	33.67	112.26	2.99	3.65	1.24
DCB	0.9994	86.0	-1185.59	26.39	87,89	4.35	3.36	1.55

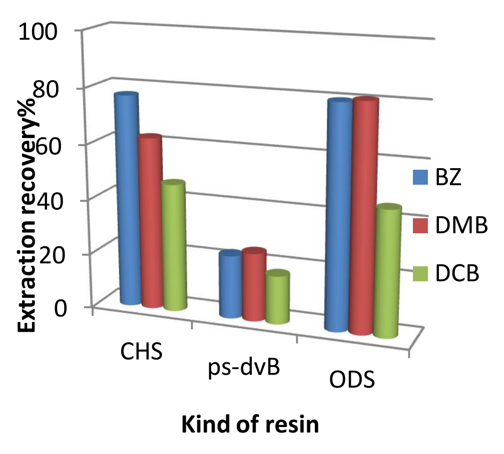


Figure 2. Kind of resin and Extraction Recovery % of (BZ, DMB, and DCB)

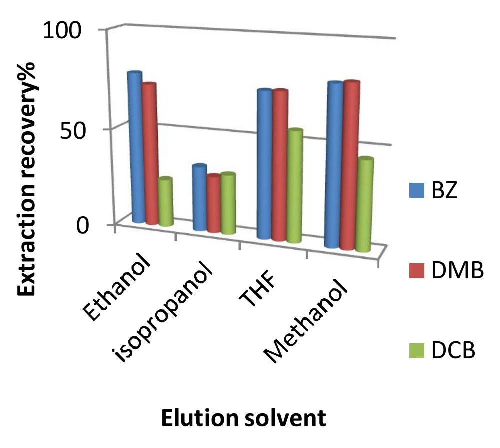


Figure 3. Elution solvents and Extraction Recovery % of (BZ, DMB, and DCB)

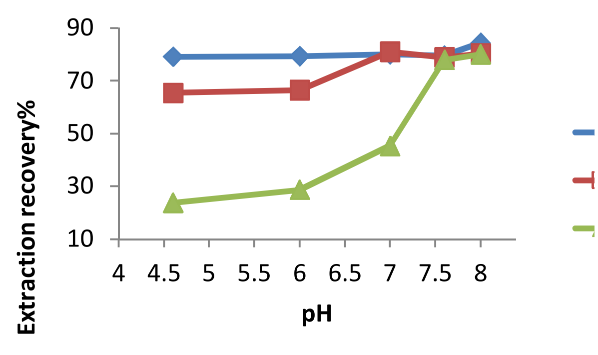


Figure 4. pH and Extraction Recovery % of (BZ, DCB, and DMB)

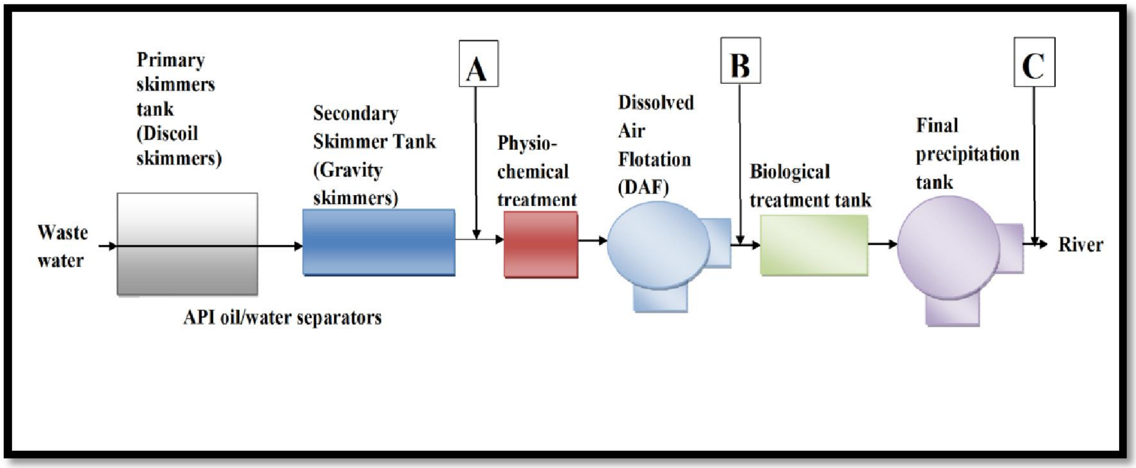
Optimization of extraction

Benzidines were extracted and purified by SPE cartridges which provided by Machinery Nagel Company. The extraction conditions were optimized (resin weight 1 gm, initial benzidines concentrations 1000 µg/L, volume of initial substrate 50 ml, volume of the elution solvent 5ml) and fixed for all optimization experiments. The kind of **cartridge resin** was optimized firstly. Three kinds of solid phase extraction cartridge resins were optimized (polystyrene-divinylbenzine (PS-DVB), octadecyl silica (ODS), cyclohexyl silica (CHS)) at pH 7 and elution solvent methanol. The results are shown in figure2. ODS was the best with extraction recovery percentages (ER %) (80.0, 81.0, and 45.3)% while with PS-DVB (22.94, 24.7, and 17.5) and CHS (77.0, 62.5, and 46.6) of BZ, DCB and DMB respectively. **Elution solvents** were optimized at the previously established kind of resin ODS resin by varying Elution HPLC grade solvent to determine the optimum Elution solvent. The results are shown in Figures 3. Methanol solvent was the best with ER % (80.0, 81.0, and 45.3) % with methanol. (74.1, 74.5, 55.9)% Tetrahydrofuran (78.4, 73.0, 24.7) % Ethanol (33.4, 29.0, 30.7) % Isopropanol. The **pH** was optimized by dilute the pure samples with specific buffer to get the interested pH point. ODS resin and the methanol used as the optimum cartridge resin and elution solvent. Adsorption was active at a wide range of pH (4.6-8.0), the results shown in Figures 4, the optimum pH was 8 the extraction recovery% was in rage of (80.0-84.3). The pKa of the benzidines are {(BZ 4.3 (monoprotonated) 3.3 (diprotonated), DCB pka3.2, and DMB (4.5) (Zierath et al. 1980). At low pH levels the amine groups of benzidines becomes ionic and at high pH would be fully unprotinated and that helps to increase the hydrophobic-hydrophobic interaction with R aliphatic group of c18 aliphatic moiety.

Application to Real Samples

Petroleum refinery industrial waste waters treatment stages

Dora petroleum refinery is located at south of Baghdad in Iraq. This plant contains waste water treatment unit. The waste water streams collected in the main tanks that receive all waste waters from stages of crude oil refinery sites. Also, the sewages include all waters coming from cooling and washing facilities. The waste water treatment unit contains many treatment subunits such as skimmers, physiochemical, DAF, and biological treatment. All of these subunits are open systems and free to air and sun light exposure. The stream goes down to the first treatment subunit (Skimmers and dis-coil skimmers API oil/water separator). In this subunit, the water will be separated from oil by difference in polarity, density, gravity and surface tension (the oil to the surface and water to the bottom). In the second stage, the stream goes down to the physiochemical subunit tank. Some chemicals are added daily to this tank like poly electrolyte and alum that chemicals can help to aggregate the semi soluble materials and to ease separating semi soluble materials for further separating by special skimmers in the next stage called dissolve air floatation DAF tank. In the third stage, the stream goes down to the next subunit called biological treatment tank containing microorganism bacteria for biodegradation of organic and inorganic molecules. Phosphoric acid and urea were added to this tank also some flotation ventilator can help in re-oxygenation of aerobic bacteria. The last tank called final precipitation tank collect the final waste water before streamed goes to the river. In this Study We selected three points in three sites in waste water treatment unit to study the ability of any subunit to remove the aromatic compounds from strain the points were: After skimmers subunit we named it site (A), After dissolve air flotation (DAF) subunit site (B), after biological treatment subunit and final precipitation tank site (C). The end stream of waste water after treatment goes down through the channel to Tigris River. Other three sites were selected to determine and follow up these compounds in the river were: 100m before the point of waste water discharge named site (D) (control),100m after the point of waste water discharge named site (E), and 800m after the point of waste water discharge named site (F) Figure5.



Scheme 1. stages of waste water treatment in the petroleum refinery station with sampling sites A, B and C.

Table 3. Monthly variations in µg/L of (BZ, DCB and DMB) site A after skimmers subunits

	Aug.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.
BZ	9.30	33.44	67.93	ND	155.44	ND	80.83	38.27	32.15
RSD%	2.11	2.13	0.32		1.15		1.76	2.01	3.08
DMB	48.37	178.06	118.91	ND	246.99	186.53	119.69	ND	82.52
RSD%	2.09	3.35	2.01		2.84	3.30	1.11		1.35
DCB	115.46	36.85	ND	ND	131.12	ND	88.49	9.69	1.28
RSD%	0.91	3.61			1.67		2.87	1.75	2.45

Table 4. Monthly variations in µg/L of (BZ, DCB and DMB) site B after physiochemical treatment and DAF unit

	Aug.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.
BZ	3.59	ND	55.92	132.73	42.61	ND	65.63	23.53	4.96
RSD%	1.67		2.56	1.39	2.14		0.31	1.11	2.52
DMB	44.15	163.65	86.12	14.11	29.73	169.06	105.08	ND	12.94
RSD%	1.02	1.70	3.17	1.87	8.69	1.51	2.32		0.43
DCB	50.08	53.37	ND	14.82	27.75	ND	57.03	0.52	5.33
RSD%	1.11	1.43		2.45	2.28		3.88	1.97	3.09

Table 5. monthly variations in µg/L of (BZ, DCB and DMB) at site C after Biological treatment and final precipitation tank.

	Aug.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.
BZ	2.16	1.02	77.14	51.66	104.40	ND	18.58	12.15	2.41
RSD%	1.31	1.47	2.66	4.55	2.11		1.59	1.78	1.11
DMB	10.68	1.11	167.29	68.07	29.54	62.38	17.92	ND	6.14
RSD%	2.45	3.91	1.39	3.13	0.93	3.13	0.92		1.03
DCB	4.37	0.51	ND	14.79	18.82	ND	22.34	1.64	2.05
RSD%	2.13	3.04		1.21	1.24		0.32	0.3	1.31

Benzidines in petroleum refinery industrial waste waters treatment stages.

BZ, DCB and DMB were determined and followed up for one year with seasonally sampling started from the first month of study (August 2013-July2014). **The site A** was at point after skimmers subunit. The sampling was done from special pump that located between the skimmers and physiochemical subunit. The nature of water was turbid. Most of benzidines were found in most samples. The extraction and pre-concentration steps help to detect the trace amount of the compounds. The results are shown in Table 3. Generally, the amounts of all benzidines selected in this study were lower in spring and autumn than other seasons. The higher results were 155.4 µg/L, 246.99 µg/L and 131 in Feb. of BZ, DMB and DCB respectively. The lowest values were ND, ND, ND in March, March, Jan. of BZ, DCB and DMB respectively. The variation of levels of these compounds may be caused by operation conditions, low volatilization in winter being the tanks are opened system and free to air and sun light. **The Site B** was at the point after the DAF subunit. The sampling was from pump located between the DAF unit and biological treatment unit. The nature of water was less turbid than previous skimmers unit and the results are shown in Table 4. The results were lower in summer than the other seasons. The higher results were 132.7 µg/L in Jan. 169.06 µg/L in March and 57.03 µg/L in May. of BZ, DCB and DMB respectively. The lowest amounts were ND in March, June, and March. of BZ, DCB and DMB respectively. The variation of levels of these compounds may be caused by chemicals added before this point, operation conditions, maintenance, molecular inter-conversion changes, and low volatilization in winter. **The Site C** was at the point after biological treatment subunit and final precipitation tank. The sampling was from pump located after the biological treatment and final precipitation tank. The nature of water was clearer than previous DAF unit water. Most of benzidines were found in most samples. The results are shown in table 5. The levels of all benzidines were selected in this study were lower in summer than other seasons. The higher results were 104.4 µg/L in Feb., 167.2 µg/L in Nov., and 22.34 µg/L in May of BZ, DCB and DMB respectively. The lowest amounts were ND in March, June, and Nov. of BZ, DCB and DMB respectively. The variation of levels of these compounds may be caused by treatment effect by previous subunit of physiochemical treatment, and low volatilization in winter. They also exhibit a high resistance to microbial degradation in wastewater treatment systems (Zaharia et al., 2009, Bayramoğlu et.al, 2007).

Benzidines in Tigris River.

BZ, DCB and DMB were determined and followed up for one year with seasonally sampling in Tigris river started from the first month of study (August 2013-July2014) in three sites where the first one named **Site D** and this point was at 100 m before the point of waste water discharge (Control), This point is located at the north against the normal stream of Tigris river because the river stream always comes from north to the south. Most of benzidines were found in most samples. The results are shown in table 6. The higher results were 22.3 µg/L in Feb., 205.0 µg/L in Aug., 12.54 µg/L in Jan. of BZ, DCB and DMB respectively, the lowest amounts were ND in March,, Nov, Oct. of BZ, DCB and DMB respectively. The variation of levels of these compounds may be caused by: waste water sources from other stations such as Al-Dora electricity plant. **The site E** is located at point around 100 m after the point of waste water discharge. This point is at the south with the normal stream of Tigris river. Most of benzidines were found in most samples. The results are shown in table 7. The levels of all benzidines were selected in this study were higher at summer than the other seasons. The higher results were 222.5, 241.6 µg/L in Aug., and 288.6 µg/L in July of BZ, DCB, DMB respectively while the lowest amounts were ND in March, of BZ, DCB and DMB respectively. The variation of levels of these compounds may be due to the effect of petroleum refinery waste water discharge, chemical inter-conversion changes from other compounds coming from waste water, volatilization, UV degradation, dilution. **The site F** is located at point around 800 m after the point of waste water discharge. This point is at the south with the normal stream of Tigris river. Most of benzidines were found in most samples. The results are shown in table 8. The levels of all Benzidines selected in this study were variable and found in most of the months. The higher results were 143.5 µg/L in Jan., 101.8 µg/L, and 88.7 in Aug. for BZ, DCB and DMB respectively. The lowest amounts were ND in March, March, and Nov. of BZ, DCB and DMB respectively. The variation of levels of these compounds may be caused by inter-conversion changes of other compounds coming from waste water, volatilization, UV degradation, dilution.

Figure 5. Tigris River and sampling sites D, E, and F.

Table 6. Monthly variations in µg/L of (BZ, DCB and DMB.) of Tigris River water at 100 m before point of waste water discharge to (-100m) (raw water).

	Aug.	**Oct.**	**Nov.**	**Jan.**	**Feb.**	**Mar.**	**May**	**Jun.**	**Jul.**
BZ	9.02	2.08	17.37	10.59	22.34	ND	5.52	8.81	19.62
RSD%	0.32	2.11	1.76	3.11	1.61		3.44	2.13	1.21
DMB	205.04	8.49	ND	1.91	7.35	ND	4.49	6.61	12.95
RSD%	0.32	1.21		2.12	2.4		1.21	1.22	2.21
DCB	ND	ND	ND	12.54	2.43	ND	9.25	3.56	6.34
RSD%				1.11	0.43		1.11	0.21	0.43

Table 7. Monthly variations in µg/L of (BZ, DCB and DMB) of Tigris River water at site D 100m after point of waste water discharge

	Aug.	**Oct.**	**Nov.**	**Jan.**	**Feb.**	**Mar.**	**May**	**Jun.**	**Jul.**
BZ	222.56	21.84	16.29	6.91	8.37	ND	20.79	8.76	23.24
RSD%	1.21	2.43	0.11	1.56	2.13		1.21	1.22	0.32
DMB	241.64	23.53	7.09	14.59	13.08	ND	17.22	13.61	120.91
RSD%	2.11	0.24	3.95	2.31	2.21		3.11	2.31	1.00
DCB	ND	7.13	6.66	2.08	ND	ND	6.15	ND	288.66
RSD%		2.39	2.40	2.11			1.21		1.00

Table 8. Monthly variations in µg/L of (BZ, DCB and DMB) of Tigris River at site E (800 m after point of waste water discharge).

	Aug.	Oct.	Nov.	Jan.	Feb.	Mar.	May	Jun.	Jul.
BZ	26.92	8.07	ND	143.57	12.06	ND	7.88	15.99	3.39
RSD%	2.89	2.91		2.21	3.81		2.21	1.31	3.12
DMB	101.85	12.16	ND	79.36	10.33	ND	6.34	4.83	9.22
RSD%	1.10	1.25		1.14	1.96		3.95	1.82	1.31
DCB	88.75	1.59	4.74	32.87	1.81	ND	2.57	1.60	7.05
RSD%	1.62	1.23	1.11	2.12	1.21		2.2	3.18	3.47

Acknowledgments:

The Authors thank Dora petroleum refinery waste water treatment laboratory for sampling and Ibn Al-betar Research center/Ministry of industry & minerals for some HPLC measurements.

Conclusions

In the present work, SPE step followed by an HPLC-UV is simple and fast method. Determination was developed for the simultaneous extraction and routine analysis of three benzidines in environmental samples. The proposed method was exhaustively validated in terms of linearity, accuracy, specificity and precision in environmental samples. The SPE step optimized allows not only the elimination of interferences but also an important sample pre-concentration which results in LOD (s) in water samples. It can be a useful tool to determine the amount of these compounds discharged from wastewater treatment plants (WWTPs) to the aquatic environment and to assess the effect of WWTPs in the elimination of benzindinic compounds. Most of benzidines were found in most samples inside the station with significant amounts with range of non detected and 246.99 µg/L. The levels of benzidines in river samples were detected in most samples that range from non detected to 45.81 µg/L. Also, these methods are being developed to enhance the capabilities for measuring emerging chemical contaminants in the environment.

References

1. Chung Kt and Stevens S. Edward Degradation of Azo Dyes by Environmental Microorganisms and Helminths. Environmental Toxicology and Chemistry 1993; 12:2121-2132.
2. Saeed WN,. Removal of Azo Benzidine Reactive Dye from Aqueous Solution by Adsorption onto ZnO Surface. Journal of Kerbala University 2014;1 (12): 11-18.
3. Bandala ER. Pel´aez MA., Lopez JG, Salgado MD, Moeller G. Photocatalytic decolourisation of synthetic and real textilewastewater containing benzidine-based azo dyes. Chemical Engineering and Processing 2008; 47:169–176.
4. Zaharia D, Bilba C, Muresan D, Muresan A, Popescu R, Decolonization wastewaters from the textile industry – physical methods, chemical methods. Industria Textila, (2009);60(5):254-263.
5. Bayramoglu G, Yakup A M. Biosorption of benzidine based textile dyes “Direct Blue 1 and Direct Red 128” using native and heat-treated biomass of Trametes versicolor. Journal of Hazardous Materials 2007;143:135–143.
6. Lakshmi, VM, Hsu FF, Fong-Fu; Zenser TV. Transformation and activation of benzidine by oxidants of the inflammatory response. Chemical Research in Toxicology 2003;16(3): 367-374.
7. Hao OJ, Kim H, Chiang PC Decolorization of Wastewater Critical Reviews in Environmental Science and Technology 2000;30(4):449–505.
8. Muneer M, Singh HK. A, Bahnemann D Semiconductor-mediated photocatalysed degradation of two selected priority organic pollutants, benzidine and 1, 2-diphenylhydrazine, in aqueous suspension. Chemosphere 2002; 49:193–203.
9. Hing-BL, Peart TE, Ken T, Maguire RJ, Determination of 3,3'-Dichlorobenzidine in Industrial Wastewaters Water. Qual. Res. J. Canada 2004;39(1):29–34.
10. Jedrzejczak K, Gaind VS.. Determination of benzidine in urine using capillary gas chromatography and negative-ion chemical ionisation mass spectrometry. Fresenius J Anal Chem 1993;347:136-140.
11. Rice RG, Kohn E J, Rapid Colorimetric Determination of Benzidine. anal. chem 1955;27(10):1630-1631.
12. Hamed AH, Simple Method for Spectrophotometric Determination of Benzidine in Aqueous Solutions by Coupling with B-Naphthol. Journal of Al-Nahrain University 2011; 14 (2):43-50.
13. Hsu FF, Lakshmi V, Rothman N, Bhatnager V. K, Hayes R B, Kashyap R, Parikh D J, Kashyap S K., Turk J, Zenser T, Davis BDetermination of Benzidine, N-Acetylbenzidine, and N, N-Diacetylbenzidine in Human Urine by Capillary Gas Chromatography/Negative Ion Chemical Ionization Mass Spectrometry. Analytical Biochemistry 1996; 234:183–189.
14. Nyman MC, Pérez J, Blatchley ER, Kenttämaa HI, Determination of 3,3'-dichlorobenzidine and its degradation products in environmental samples with a small low-field Fourier transform ion cyclotron resonance mass spectrometer. J Am Soc Mass Spectrom 1999; 10(11):1152-6.
15. Gao J, Sun K, Yang W, Yang F, Zhao G Determination of Benzidine Using the Analyte Pulse Perturbation Technique in BZ Oscillating Chemical System. Pakistan Journal of Biological Sciences 2004, 7(10):1721-1726.
16. Chuannan L, Xueying W, Zhongpeng X, Zhen Lv Determination of Benzidine using Sulfobutyl-ßcyclodextrinand Neutral Red Complexation as a Supermolecule Probe 3rd International Conference on Bioinformatics and Biomedical Engineering, June 2009:1-3.

17. Chen Z, Zhang G, Chen X, Peng Y, Lin Y, Lu S a resonance light scattering amplification system for determination of trace amounts of benzidine in surface water. Anal. Methods 2011;3(8):1845-1850.
18. Falciola L, Pifferi V, Mascheroni E, Platinum-Based and Carbon-Based Screen Printed Electrodes for the Determination of Benzidine by Differential Pulse Voltammetry. Electroanalysis 2012, 24(4): 767 – 775.
19. Chao W, ning ly, jiang TE-, xiarphan J, Renji XU, Determination oTrace Aniline and Benzidine in environmental water by ultra high pressure liquid chromatoghraphy with flourscence Detection. Journal of international analysis. vol 2013;32 (1):32-37.
20. Ononye A I, Graveel JG, Wolt JD, Direct Determination of Benzidine in Unaltered Soil Solution by Liquid Chromatography. Bull. Environ. Contam. Toxicol 1987;39:524-532.
21. Shi X, Wang Q, Wu W, Determination of benzidine in cotton cloth by high performance liquid chromatography (HPLC). Chinese Journal of Chromatography 1999;17(1):75-76.
22. Bouzige M, Legeay P, Pichon V, Hennion MC Selective on-line immunoextraction coupled to liquid chromatography for the trace determination of benzidine, congeners and related azo dyes in surface water and industrial effluents. Journal of Chromatography 1999; 846:317–329.
23. Schulte PT, Zeiske J, Kettrup A, Trace. analysis of selected benzidine and diaminodiphenylmethane derivates in urine by means of liquid chromatography using pre-column sample pre-concentration, UV and electrochemical detection, Chromatographia 1986; 22:138-146.
24. Shin HS, Lee JH, Ahn HS, Hong CP, Choi SN, Simultaneous Determination of Benzidine, Acetylbenzidine and di-Acetylbenzidine in Rat Urine. Bull Korean Chem, Soc 2001;22(7): 685-87.
25. Vazquez, D. and Mansoori, G. A. Identification and measurement of petroleum precipitates. Journal of Petroleum Science and Engineering 2000;26: 49–55.
26. Gomori, G. Preparation of buffers for use in enzyme studies 1955: 138-146.
27. Skoog DA., west D M., F. west JH, Crouch S R. Fundamentals of analytical chemistry. Brooks /Cole cengage learninig, USA, 8th Edition, 2004.
28. Anirbandeep Bose HPLC Calibration Process Parameters in Terms of System Suitability Test Austin Chromatog. 2014; 1 (2):1-4.
29. Sanagi MM, Ling SL, Nasir Z, Hermawan D, Ibrahim WA, Abu Naim A. Comparison of Signal-to-noise, Blank Determination, and Linear Regression Methods for the Estimation of Detection and Quantification Limits for Volatile Organic Compounds by Gas Chromatography. J AOAC Int 2009;92:1833-8.
30. Zierath, D. L., Hassett, J. J., Banwart, W. L., Wood, S. G., and Means, J. C. Sorption of benzidine by sedimentsand soils: Soil Sci (1980);129:277-281.

3/22/2018