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Gas Chromatography of Environmentally Active Aromatic Amines in Industrial Dyes Effluents and Human Blood Serum

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Abstract

A GC-FID procedure was developed for the separation and analysis of six isomers of xylidines (di-methylanilines), aniline and 1,4-Phenylenediamine after derivatization via ethyl chloroformate (ECF). GC separation was from column DB-5 (30m x 0.32mm) with the 0.25 µm layer thickness, 90 ˚C column temperature for 3 min, followed via heating rate 10 to 200 ˚C followed by hold of temperature for 7 min. The 1.5 ml /min was nitrogen flow with divided ratio 10:1. Linear calibration range of each of the compound was obtained with 1-20 ng/ml with coefficient of determination (r^2) 0.9969-0.9970. Limits of detections (LOD) calculated as indication to 3:1 noise ratio was within 0.10-0.99 ng/ml. Derivatization, separation and quantitation were replicate in terms of retention time and peak height/peak area with the relative standard deviations within 2.1%. Method was employed for analysis of effluents of dyes manufacturing company and blood samples of workers employed in dyes manufacturing sector. All the six isomers of xylidines and aniline were detected in effluents and human serum samples at the concentration levels within 49-200 µg/ml and 1.7-9.8 ng/ml respectively. Results of analysis were further confirmed by standard addition technique and percent recoveries were calculated within 96-99 and 95-97 along with % RSD within 3.2 and 2.9 from the effluents and the human serum respectively. Central composite design (CCD) was employed to optimise the parameters. The work examines the quantisation of aromatic amines simultaneously in fairly complex matrix of dyes effluents and biological samples (human serum) by simple GC-FID with adequate sensitivity.

Keywords: Aromatic amines; GC; Ethyl chloroformate; Effluents; Serum; Factorial design.

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Introduction

The aniline and its alkyl derivatives (dimethylanilines) as xylidines are industrially important compounds. These have been used in production of many chemicals including pesticides, rubber and azo dyes (1). These are used as intermediates in the preparation of perfumes, drugs, dyes, and synthetic resin (2). These amines are introduced into the ambient environment such as soil, air and water either directly as industrial effluents or indirectly as by product or breakdown of dye stuff components and pharmaceuticals (3). The non-industrial sources include hair dyes, cigarettes smoking, burning of vegetables matter and consumption of cooked meats (4). The commercial aniline and its alkyl derivative xylidines are potent mutagenic and carcinogenic compounds for livings (5-7).

Several methods are employed for measurement of xylidines in ambient environment such as air, water and soil. The methods are applied for the presence of xylidines in the food, industrial effluents, ground water and cigarette smoke. These methods are mostly based on gas chromatography (GC) (8-17), High performance liquid chromatography (HPLC), HPLC-MS, HPLC-MS/MS (18-25) and spectrophotometry (3).

The review of literature indicates that chromatographic approaches are frequently used for separation and simultaneous determination of xylidines and aniline derivatives from environmental and industrial effluents. Among the chromatographic methods GC is easy to operate with less running cost and is free from difficulty of positioning the used solvents. The GC-MS is effective in the determination of aromatic amines, but all the six isomers of xylidines have same molecular mass and some difficulties are encountered in their identifications. The separation of isomers by the GC may be required for their determinations. The GC of the xylidines could be carried out without derivatization (2,14), but a better peak shape is obtained after derivatization (16). A number of derivatizing reagents have been examined to bind amino group of the xylidines including trifloroacetic anhydride (11), pentafluoropropionic anhydride (17), dimethyl chlorothiophosphate (9), n-propyl chloroformate (10) and benzaldehyde (16).

Ethyl chloroformate (ECF) was utilised for GC analysis of primary and secondary amines (26). ECF easily reacts with amines in aqueous-alkaline conditions to form carbamate derivatives with good GC stuffs. ECF also reacts with phenol, thiol, imidazole and carboxylic groups together by amino groups amicable to GC (27-29). Kataoka et al (10) reported a process for analysis of 20 aromatic amines, including the isomers of xylidines by GC as N-propoxycarbonyl derivatives through nitrogen-phosphorus detection. The linear calibration was reported with 20-500 ng of each compound. Present effort observes the GC separation of 8 aromatic amines, including 6 xylidene isomers after pre-column derivatization through ECF. The separation is achieved within 16 min. Method is employed for determination of the analytes simultaneously from industrial effluents and blood serum of the workers within dyes manufacturing industry by GC-FID.

Materials and Methods

1. Chemicals and Reagents

Six isomers of xylidine namely 2,3-xylidene (2,3-DMA) (purity 98%), 2,4-xylidene (2,4-DMA) (purity 95%), 2,5-xylidene (2,5-DMA) (purity 98%), 2,6-xylidene (2,6-DMA) (purity 98%), 3,4-xylidene (3,4-DMA), 3,5-xylidene (3,5-DMA) (purity 97%) including aniline (purity 99.5%) and 1,4-Phenylenediamine (1,4-PDM) (purity 99%) and derivatization reagent ethyl chloroformate (purity 99%) were purchased from Fluka (Switzerland) and Sigma-Aldrich (Switzerland). The chloroformand methyl alcohol were procured from E. Merck (Germany). The buffer solutions of pH 1-11 with 0.1 molar concentration were prepared from KCl: HCl (pH 1-2), CH_3COONa^+: CH_3COOH (pH 3-6), $NH_4CH_3COO^-$: NH_3 (pH 7), $Na_2B_4O_7.10H_2O$: H_3BO_3 (pH 8-9), $NaHCO_3$: Na_2CO_3 for pH 9 and NH_4Cl : NH_3 (pH 10-11).

2. Equipments

GC analysis was performed on Agilent model 6890 (Agilent Technologies Inc. USA) attached with injector (split/splitless) executed in modes of split and detection was carried by flame ionization (FID). The hydrogen generator (Parker Balston, Parker Hanninfin, Havorhill, MA) was used for hydrogen gas generation for FID and Nitrogen gas (pure) was procured from (British oxygen company, Karachi). Chemstation software was used to control equipment. In present studies a column was used DB-5 (J & W Scientific Corporation, USA) (30 m x 0.32 mm id) with layer thickness 0.25 μm. pH meter (Orion research Inc. Boston, USA) 420A model along with glass electrode and internal reference electrode for the pH measurements.

3. GC Analytical Procedure

The (0.1-1.5 ml) solution comprising 1-20 ng/ml of aniline, 1,4-PDM, 2,4-DMA, 2,3-DMA, 2,5-DMA, 3,4-DMA, 2,6-DMA and 3,5-DMA each, 0.5 ml solvent

system made up of water-methanol-acetonitrile-pyridine in ratios 42:42:8:8 (v/v) was added in each, then in sequence carbonate buffer of 9 pH and 0.4 ml ECF were added as derivatizing reagent. The solution was sonicated for 20 min. and added chloroform (0.6 mL). The phases were permitted to distinct and an organic layer (0.5 mL) was moved to sample vial (screw capped) and 1 µL from organic layer was introduced on the column DB-5 (30m x 0.32mm i.d) with the film width 0.25 µm at temperature initial of the column 90 ˚C for 3 min. with the 10 ˚C/min heating rate up to 200 ˚C and hold time was 7 min. Flow rate of nitrogen was 1.5 mL/min. with 10:1 v/v split ratio and detection by FID. Detector temperatures was 280 and the injection port was 270 ˚C. The rate of gas flow (nitrogen) was 45 mL/min., and air flow rates was 450 and hydrogen was 40 mL/min.

4. Analysis of industrial effluents

The number (06) of composite samples (100 mL) each were collected of effluents at different dyes sections using xylidines as intermediate in the formation of dyes from a dye industry, Jamshoro for the analysis of the xylidines. The samples were filtered (2, 29) and appropriate dilutions were made and GC analytical procedure was applied using standard conditions. The linear external calibration curve was used for quantization, based on y = ax+b.

5. Analysis of effluents by standard addition method

The effluent taken from Clarinet industries were treated as above and solutions 0.2 and 0.4 mL were in replica. The mixture of xylidine isomers 5 – 10 ng was added to each solution and GC analytical method was managed for all the solutions. Quantitation was from regression equation of linear calibration curve and by the addition of standards and increases in responses.

6. Analyses of Blood Serum Samples

The samples (5 mL each) of blood were gathered when volunteers, working in dyes section of an industry located at Jamshoro. The blood samples were preserved in clinical tubes with the inoculation of EDTA. The samples were stored at room temperature for 30 min. and centrifuged for 30 min. at 4000 rpm. The supernatant layer was collected after separation was added with the methanol in double of its original volume. Again at 3500 rpm was centrifuged for 40 min. The serum layer (1-3 mL) (upper) was separated and GC analytical procedure was carried out as given above. The quantitative measurements were made from external calibration.

7. Spiked Blood Serum Samples Analyses

Blood samples collected from workers of clarinet, Jamshoro were processed as above and deproteinised serum 0.5 and 1.0 mL were capture in duplicates. Added a mixture of xylidine isomers and aniline 6 – 14 ng each was added to one solution of 0.5 and 1.0 mL, and all solutions were handled as GC process. Quantitation was complete through linear regression calculation of outside calibration curve and growth in response with adding standards.

The blood samples were obtained from volunteers from a dyes industry, Jamshoro who were working in dyes section and had not received medicine for one week. All volunteers were defined objectives of work and they gave verbal permission to collect their blood samples.

8. Factorial Design

8.1. Central composite design (CCD)

The CCD tool was drawn to estimate significant level of parameters used in experiment (31). The three parameters were employed in factorial design like pH (1-11), concentration of analytes (1-20 ng/ml), and

Table 1. Quantitative data of Xylidines isomers, Aniline and 1, 4-Phenylenediamine after derivatization with Ethyl Chloroformate

S.No.	Name of standard	Calibration range ng/ml (n=5)	Limit of detection (LOD) ng/ml	Limit of quantification (LOQ) ng/ml	Liner regression equation	Coefficient of determination R^2
1	Aniline	1-20	0.66	1.98	y = 2.6125x + 2.3333	R^2 = 0.9969
2	1,4-PDM	1-20	0.10	0.30	y = 2.7232x - 1.1048	R^2 = 0.9978
3	2,3-DMA	1-20	0.99	2.97	y = 2.5286x - 0.3238	R^2 = 0.9993
4	2,4-DMA	1-20	0.98	2.94	y = 2.5054x - 0.1524	R^2 = 0.9996
5	2,5-DMA	1-20	0.97	2.91	y = 2.4857x + 5.0286	R^2 = 0.9994
6	2,6-DMA	1-20	0.40	1.20	y = 2.5054x - 0.2857	R^2 = 0.9997
7	3,4-DMA	1-20	0.10	0.30	y = 2.525x - 0.5333	R^2 = 0.9993
8	3,5-DMA	1-20	0.97	2.91	y = 2.625x + 1.8	R^2 = 0.9981

Table 2. Mono Aromatic Amines in Industrial Effluents (µg/ml with %RSD, n=5) after Derivatization with Ethyl Chloroformate

S. No.	2,6-DMA	2,3-DMA	3,4-DMA	3,5-DMA	Aniline	2,4-DMA	2,5-DMA
1	101±1.2	141±0.9	182±1.2	128±0.8	49±1.7	141±2.5	150±1.5
2	182±2.4	122±1.3	103±2.7	170±1.7	174±1.3	101±3.0	110±1.9
3	160±1.9	200±2.0	140±1.8	185±2.1	106±2.0	200±2.8	122±2.3
4	125±2.6	171±2.6	150±0.5	147±0.9	190±2.7	186±1.8	160±2.9
5	62±1.8	58±2.9	102±2.1	108±1.5	68±1.8	141±1.3	180±0.8
6	102±1.0	133±1.8	115±0.4	150±1.5	110±2.1	130±1.9	120±2.5

sonication time (5 to 50) (Table 1). The experimental run (14) was generated by Minitab software and effects of each run was estimated (Table 2).

Results and Discussion

1. Derivatization and separation

The selected six xylidine isomers 2,6-DMA, 3,4-DMA, 2,3-DMA, 2,5-DMA, 3,5-DMA, 2,4-DMA, aniline and 1,4-PDM were inspected for GC elution from column excluding the derivatization and all the compounds eluded from GC column, but all the compounds contained primarily amino groups and responded with ECF and better peak shapes were acquired when derivatized pre-column with ECF. Each of the compound was entertained with ECF separately and formed derivative was deduced via chloroform. The symmetrical peaks were obtained for all the compounds, when eluded from GC column and detached from derivatizing reagent ECF completely. Therefore, conditions were optimized for derivatization and separations of 1,4-PDM, aniline and xylidene isomers. The optimization conditions were examined for effluents pH, derivatizing reagent concentration and sonication time. The solvent media used for derivatization was a combination of distilled water-methanol-pyridine-acetonitrile in ratio of 42:42:8:8 v/v as described for the amino acids (27). The ECF as derivatizing reagent was assorted from 0.1-0.6 ml at an interval 0.1 ml and an addition of derivatizing substance was not dangerous, and comparable feedback was achieved for all concentrations accustomed, but for the maximum derivatization and synergetic extraction in chloroform, 0.4 ml of ECF was selected. The pH of effluents was investigated with buffer solutions within 1-11 at unit interval. The better reaction was marked in alkaline solution of bicarbonate with maximum at pH 9.

The sonication time was altered from 5-50 minutes, an improved response was observed at 20 min. The separation of 1,4-PDM, aniline and xylidine isomers 2,5-DMA, 2,4-DMA, 2,6-DMA, 3,5-DMA, 2,3-DMA, 3,4-DMA, was investigated using different temperatures and flow rates of nitrogen. Complete separation was gained from column DB-5 (30m x 0.32 mM id) when eluted at column temperatures 90 ˚C for 3 min, followed

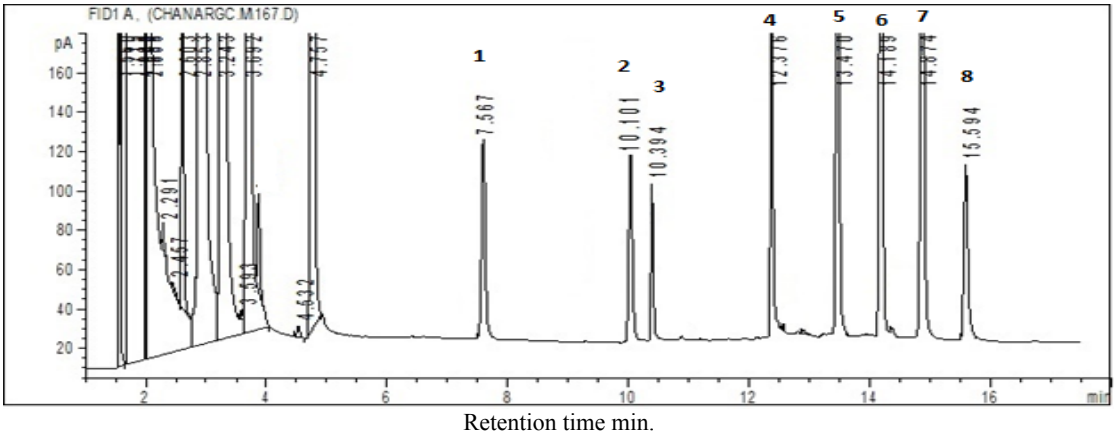


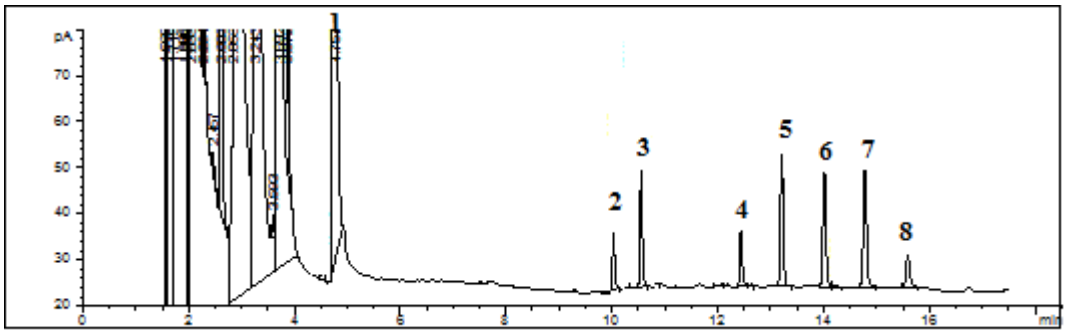
Figure 1. GC elution and separation of xylidines isomers as derivatives of ECF: 1. 1.4-PDM, 2. 2,6-DMA, 3. 2,3-DMA, 4. Aniline , 5. 3,4-DMA, 6. 3,5-DMA, 7. 2,5-DMA, 8. 2,4-DMA

Table 3. Mono Aromatic Amines in Human Serum (ng/ml with ± %RSD, n=5) after derivatization of Ethyl Chloroformate

S. No.	2,6-DMA	2,3-DMA	Aniline	3,4-DMA	3,5-DMA	2,4-DMA	2,5-DMA
1	3.5±1.3	6.8±2.1	3.8±1.9	4.9±0.4	3.1±1.8	1.7±2.1	4.7±1.4
2	3.7±2.1	7.3±0.4	5.9±2.9	5.3±2.5	3.9±2.6	3.1±1.8	1.8±2.9
3	5.0±2.9	6.8±1.3	3.3±0.9	7.0±1.5	5.2±1.3	4.4±0.8	7.0±2.6
4	7.4±0.8	8.3±2.5	5.1±1.8	8.5±2.6	6.5±1.8	6.4±1.9	9.3±2.0
5	7.8±1.7	6.3±2.7	5.3±1.3	7.2±3.0	7.2±1.9	7.1±1.3	9.8±1.3
6	2.9±1.3	3.8±2.4	3.1±1.7	5.2±1.0	1.8±0.9	3.5±1.6	2.9±1.8

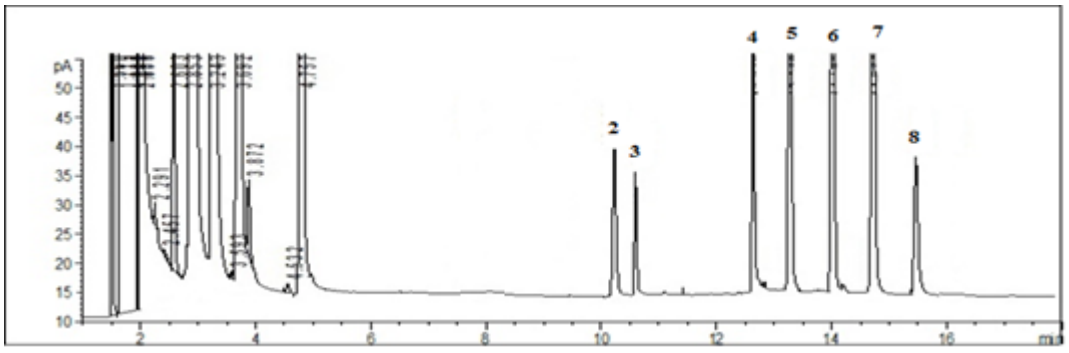
via 10 ºC/min up to 200 ºC heating rate withhold time for 7 min. The flow rate of nitrogen was 1.5 mL/min. with 10:1 split ratio and detection was through FID (Figure 1). The retention times (n=4) RSDs were obtained within 2.2%.

The GC responses were tested for quantitative analyses and linear relations were gained when mean responses (peak area/ peak height) (n=3) were plotted against concentration within 1-20 ng/ml for each compound. The coefficient of determination (r^2) were within 0.9969 to 0.9997 using 8 calibrations. The LOQs and LODs were assessed as signal to noise ratio 10:1 and 3:1 and were acquired within 0.30-2.97 ng/ml and 0.1-0.98 ng/ml individually. Derivatization, quantitation and separation were reproducible in term of retention times and peak heights/ peak areas (n=3) and RSDs were achieved within 1.5-2.2% and 1.7- 2.6% respectively. The test solutions (n=4) were analysed within calibration range and relative error was obtained 2.5%. The variations in GC responses were investigated inter and intra-day (n=5) in terms of peak heights/ peak areas and retention times, and RSDs were obtained within 4.2% (Table 3).



Retention time min.

Figure 2. GC analysis of the xylidines from serum samples of volunteers working in dyes section of a dyes manufacturing industry :1. Reagent 2. 2,6-DMA 3. 2,3-DMA 4. Aniline 5. 3,4-DMA 6. 3,5-DMA 7. 2,5-DMA 8. 2,4-DMA



Retention time min.

Figure 3. GC analysis of the xylidine serum samples of volunteers after spiking with standards: 1. Reagent 2. 2,6-DMA 3. 2,3-DMA 4. Aniline 5. 3,4-DMA 6. 3,5-DMA 7. 2,5-DMA 8. 2,4-DMA

2. Analysis of industrial effluents

The Industrial effluents from a dyes manufacturing industry were collected and analyzed for the determination of xylidines and aniline using GC analytical procedure. Six different samples of effluents were collected at different timings and after appropriate dilutions, were derivatized with ECF and GC elution of the components was carried at optimized parameters. Identification of each component was carried out by relating retention times of standards and by spiking each component in sequence. Results indicated the presence of all six xylidines isomers, including aniline at concentration within 49-200 µg/mL with the RSD within 0.5-3.0% (Table 4). A sample of effluents after appropriate dilution was spiked with 5-10 ng of each standard compound and GC process was followed for the confirmation of xylidines and an increase in the response was calculated by standard addition method and indicated recovery of 96-99% with RSD within 3.2 %.

3. Analysis of Serum and Spiked Samples

Blood samples of six volunteers were composed from workers of dyes section of an industry and analyzed using GC analytical procedure. The blood samples after deproteinizing were derivatized with ECF and quantitation was from linear regression calculation of outside calibration curve and results indicated the presence of all six isomers of xylidines and aniline at the concentration 1.7-9.8 ng/mL with RSD 0.4-3.0% (Figure 2 and Table 5). The blood serum samples after deproteinate were spiked with standard solutions of xylidine isomers and aniline and an increase in the response was calculated by external calibration curve. A similar peak shape was obtained with retention times corresponding to xylidines standards and serum matrix was not interfered with measurement of xylidines (Figure 3). The recovery of xylidines and aniline from human serum was calculated within 95-97% with RSD within 2.7%.

The work reports the GC-FID separation and determination of environmental active compounds xylidines and aniline by precolumn derivatization with ECF. The separation and analysis were replicable with RSD 4.2% (n=5). The industrial effluents of a dyes manufacturing industry indicated the presence of xylidines with highest amount of 2,3-dimethyl aniline 200 µg/ml and lowest of aniline 49 µg/ml. The blood samples of workers in dyes section also indicated the presence of xylidines and aniline at the concentration to

Table 4. Mono Aromatic Amines in Industrial Effluents (µg/ml with %RSD, n=5) after Derivatization with Ethyl Chloroformate

S. No.	2,6-DMA	2,3-DMA	3,4-DMA	3,5-DMA	Aniline	2,4-DMA	2,5-DMA
1	101	141	182	128	49	141	150
	±1.2	±0.9	±1.2	±0.8	±1.7	±2.5	±1.5
2	182	122	103	170	174	101	110
	±2.4	±1.3	±2.7	±1.7	±1.3	±3.0	±1.9
3	160	200	140	185	106	200	122
	±1.9	±2.0	±1.8	±2.1	±2.0	±2.8	±2.3
4	125	171	150	147	190	186	160
	±2.6	±2.6	±0.5	±0.9	±2.7	±1.8	±2.9
5	62	58	102	108	68	141	180
	±1.8	±2.9	±2.1	±1.5	±1.8	±1.3	±0.8
6	102	133	115	150	110	130	120
	±1.0	±1.8	±0.4	±1.5	±2.1	±1.9	±2.5

Table 5. Mono Aromatic Amines in Human Serum (ng/ml with ± %RSD, n=5) after derivatization of Ethyl Chloroformate.

S.No.	2,6-DMA	2,3-DMA	Aniline	3,4-DMA	3,5-DMA	2,4-DMA	2,5-DMA
1	3.5	6.8	3.8	4.9	3.1	1.7	4.7
	±1.3	±2.1	±1.9	±0.4	±1.8	±2.1	±1.4
2	3.7	7.3	5.9	5.3	3.9	3.1	1.8
	±2.1	±0.4	±2.9	±2.5	±2.6	±1.8	±2.9
3	5.0	6.8	3.3	7.0	5.2	4.4	7.0
	±2.9	±1.3	±0.9	±1.5	±1.3	±0.8	±2.6
4	7.4	8.3	5.1	8.5	6.5	6.4	9.3
	±0.8	±2.5	±1.8	±2.6	±1.8	±1.9	±2.0
5	7.8	6.3	5.3	7.2	7.2	7.1	9.8
	±1.7	±2.7	±1.3	±3.0	±1.9	±1.3	±1.3
6	2.9	3.8	3.1	5.2	1.8	3.5	2.9
	±1.3	±2.4	±1.7	±1.0	±0.9	±1.6	±1.8

Table 6. Analysis of Variance.

Source	DF	F-Value	P-Value
Model	9	8.10	0.030
Linear	3	2.37	0.211
pH	1	31.88	0.042
Conc	1	26.87	0.049
Sonication Time	1	4.41	0.01
Square	3	30.93	0.007
pH*pH	1	26.44	0.011
Conc*Conc	1	33.80	0.021
Time*Time	1	28.51	0.043
2-Way Interaction	3	2.20	0.231
pH*Conc	1	25.23	0.044
pH*Time	1	1.67	0.258
Conc*Time	1	0.01	0.929
Error	4		
Lack-of-Fit	1	*	*
Pure Error	3		
Total	13		

trace levels of 1.7-9.8 ng/ml. The recovery of aromatic amines from industrial effluents and human serum was within the range 97-99% by standard addition technique.

4. Factorial design

4.1. Analysis of variance ANOVA

The ANOVA was exploited to weigh the importance of CCD model (Table 6). The p-value <0.05 was significant and >0.05 was treated as non-significant (32).

4.2. Response surface plots

The response plots were programmed to obtain the impacts of variables. The surface plot is powerful toll for estimating the impact of two variables when the recovery increases, proportionality there was increase of surface response (33). The response plot (Figure 4) indicated joint impact of pH and sonication time. Plot indicated response was amplified when increase the pH value up to 9 then response was reduced at higher basic condition. Response plot pH and concentration vs recovery indicated that response was increase at lower concentration (Figure 5).

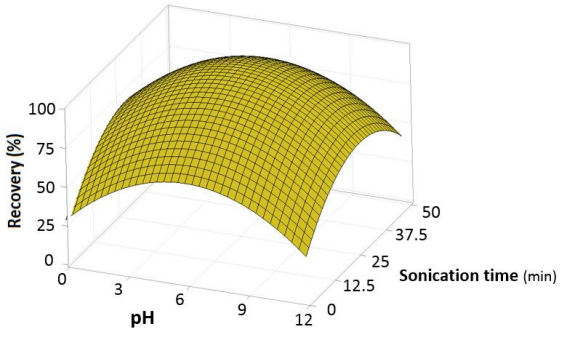


Figure 4. Response surface plot pH and sonication time vs recovery.

4.3. Pareto chart

The Pareto chart was designed to estimate the fitness of model, and fitness of model is dependent on the horizontal bar graph. If horizontal bar graph crosses the vertical line is considered as significant (34). The Figure 6, indicated bar graph with factors of sonication

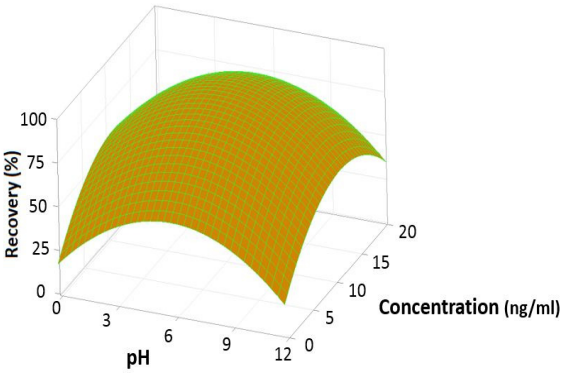


Figure 5. Response surface plot pH and concentration vs recovery.

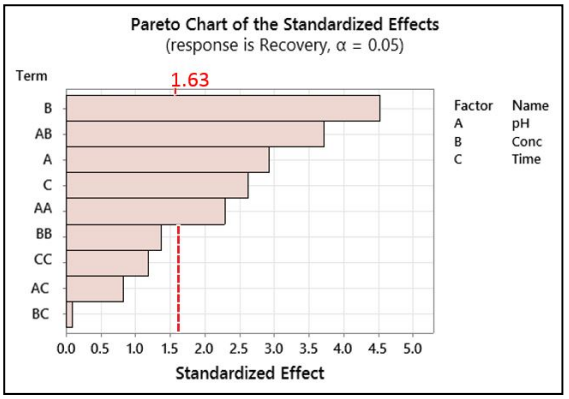


Figure 6. Pareto chart.

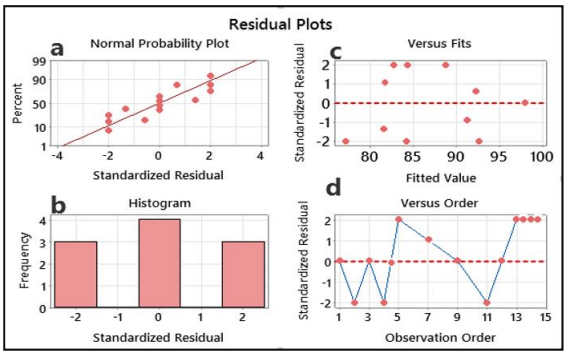


Figure 7. Residuals plots (a-d).

time (B), pH (A), concentration (C), and joint parameters pH with pH (AA) and sonication time with pH (AB), that crossed vertical line and indicated significant impact of factors on model.

4.4. Residuals plots

The residuals plots were extracted to assess the excellence of model and residuals necessity with normally scattered (35). Normal probability plot (Figure 7a) exhibited the half of point falls away from the linear line which indicated significant effects and about half of point with linear which was considered with insignificance. The (Figure 7b) histogram presented that one bar line pointed negative, obviously not significant, one found in positive side which is considered significant, and one bar line found at zero (0) which showed no any effect. The (Figure 7b and 7c) demonstrations majority of points were distributed on both sides and away from line that showed significant effect on model.

5. Comparison study

Finally, the results of the present study were compared with the representative reported procedures (Table 7) based on GC and HPLC methods. The present method indicated similar sensitivity, repeatability and applications to real samples, but was somewhat less sensitive than GC-MS, GC-MS/MS and HPLC-MS/MS procedures. However, the use simple equipment GC-FID available in a number of laboratories throughout the globe is an added advantage of the method.

Conclusion

GC-FID method was developed for investigation of environmentally active xylidenes and aniline from industrial effluents and blood serum of worker in dyes industry, after precolumn derivatization through ethyl chloroformate. Industrial effluents were calculated to contain xylidenes and aniline in the range 49-200 µg/mL and blood serum of worker of dyes industry 1.7-9.8 ng/mL The recovery of xylidenes and aniline was calculated 95-97% within RSD 2.7% from deproteinized serum by standard addition method. The analytical procedure was repeatable with inter and intraday variations with RSD within 4.2%. The effects of variables were optimized by univariate and multivariate (Factorial design by central composite design) techniques.

Table 7 Comparison table of reported procedures with present work.

Instruments	Methods	Analyte	Linear range	LOD	Sample	RSD%	time	References
GC-NPD	GC	Aromatic amines 20 number	200-500 ng	19-139 pg injected	Urine	3.3-14.9	15 min separation time	10
GC-FPD	GC	Aromatic amines	25-2000 ng	30-100 pg injected	Cigarettes smoke	0.9-8.3	20 min separation time	9
GC-FID	GC	Xylenes, phenols and anilines	Quantitation was by peak normalization method		Impurities in m-cresol and o-xylene	3.2-4.4	14 min separation time	12
GC-mass spectrometry	GC	Chloroanilines, methylanilines, methoxyanilines, dimethylanilines.	0.05-50µg/mL	O.006-0.058 µg/mL	Surface. Water samples	0.89-7.69	32 min separation time	16
GC-MS GC-MS/MS HPLC-MS/MS	GC HPLC	Anilines, methylated and chlorinated anilines	0.1-100 µg/L	0.01-0.05 µg/L	Groundwater	2-23 %	GC 16 min HPLC 5 min	25
GC-MS	GC	Xylidenes	0.121-582 µg/mL	0.016-0.044 µg/mL	Workplace air	1.5-8.5%	11 min	13
UHPLC-QTOF-MS	HPLC-MS	Xylazine and 2, 6-xylidene	2-1000 ng/mL	0.1-0.2 ng /mL	Blood and urine	8.6-11.9%	3 min	18
GC-FID	GC after derivatization with ethyl chloroformate	Aniline, dimethylanilines and 1, 4-phenylenediamine	1-20 ng/mL	0.1-0.98 ng/mL	Industrial effluents of dye industry and blood samples	1.5-4.2%	16 min separation time	Present work

NPD=Nitrogen-phosphorus detector, FPD=Flame photometric detector, FID=Flame ionization detector, UHPLC-QTOF-MS=Ultra high-performance liquid chromatography-quadruple time of flight-mass spectrometry.

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