Plackett-Burman Factorial Design for the Optimization of a Spectrophotometric Flow Injection Method for Phenol Determination in Tap and Bottled Water Using 4-Aminoantipyrine

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Abstract. Plackett-Burman and Quarter fraction 2^{5-2} factorial designs were applied to evaluate a spectrophotometric flow injection method in order to determine phenol in water by using 4-aminoantipyrine (4-AAP) as derivatizing reagent. With a minimum number of experiments, the designs enabled the best conditions for phenol analysis: 80 cm and 180 cm reactors; flow-rates being: NH_3 1.0 mL min^{-1}; 4-AAP 0.35 mL min^{-1} and $K_3[Fe(CN)_6]$ 0.35 mL min^{-1}, [NH_3] 0.064 mol L^{-1}, [4-AAP] 9.84×10^{-3} mol L^{-1}, [$K_3[Fe(CN)_6]$] 0.02 mol L^{-1}, and an injection volume of 200 µL. With the optimized method it was possible to increase the lineal range from 0.3 µg mL^{-1} to 30 µg mL^{-1} and also to quantify the maximum allowable phenol concentration in water in comparison with other standard and flow injection methods whose lineal range are from 0.5 µg mL^{-1} to 20 µg mL^{-1} and from 0.5 µg mL^{-1} to 16 µg mL^{-1}, respectively. The detection limit was of 0.13 µg mL^{-1} and the regression coefficient was of 0.9999, making possible a throughput of 36 determinations an hour with a minimum consume of reagent. With the proposed method, a distillation step was not necessary to remove sulfates but, when the sulfate:phenol ratio was higher than 83, the analytical signal for phenol increased 8%, but hypochlorite interfered with the signal when the hypochlorite:phenol ratio was higher than 1.
Key words: Plackett-Burman factorial design, phenol, 4-aminoantipyrine, water, flow injection, spectrophotometry.

Resumen. Se utilizaron diseños factoriales Plackett-Burman y fraccionado 2^{5-2} para evaluar un método espectrofotométrico de inyección en flujo con el objetivo de determinar fenol en agua utilizando 4-aminoantipirina (4-AAP) como reactivo derivatizante. Los diseños establecieron, con la menor cantidad de experimentos, las mejores condiciones para el análisis de fenol fueron: reactores de 80 cm y 180 cm; caudales para NH_3 de 1.0 mL min^{-1}; para 4-AAP de 0.35 mL min^{-1} y para $K_3[Fe(CN)_6]$ de 0.35 mL min^{-1}; las concentraciones óptimas fueron NH_3 0.064 mol L^{-1}, 4-AAP 9.84×10^{-3} mol L^{-1} y $K_3[Fe(CN)_6]$ 0.02 mol L^{-1} con un volumen de inyección de 200 µL. El método una vez optimizado permitió incrementar el intervalo lineal de 0.3 µg mL^{-1} a 30 µg mL^{-1} en comparación con otros métodos estándar y de flujo continuo cuyos intervalos lineales son de 0.5 µg mL^{-1} a 20 µg mL^{-1} y de 0.5 µg mL^{-1} a 16 µg mL^{-1}, respectivamente. El límite de detección fue 0.13 µg mL^{-1} y el coeficiente de regresión 0.9999, con una frecuencia de muestreo de 36 determinaciones por hora y un mínimo consumo de reactivos. Con el método propuesto no fue necesario un paso de destilación para remover sulfatos pero cuando la proporción de sulfato:fenol fue mayor a 83 la señal analítica para fenol incrementó 8%. El hipoclorito interfirió en la cuantificación de fenoles cuando la proporción hipoclorito:fenol fue mayor a 1.
Palabras clave: Diseño factorial Plackett-Burman, fenol, 4-aminoantipirina, agua, inyección en flujo, espectrofotometría.

Introduction

The determination of phenols in the environment is of paramount importance due to their constant presence in industry. Phenolic compounds are used to manufacture insecticides, herbicides, detergents, pharmaceutical products, synthetic resins and disinfectants [1, 2]. Although the main origin of the existence of phenolic compounds in the environment is anthropogenic, they are also produced by seaweed, plants and animal urine [3-5]. Phenolic compounds are sometimes found in surface water from both natural and industrial sources. Their presence in streams and other waterways frequently causes off flavor in fish tissue and other edible sea plants. On the other hand, chlorination of waters containing phenols may produce chlorophenols, which are odoriferous and also cause an objectionable taste.

Phenol is described by the Environmental Protection Agency [6] as a dangerous compound due to its toxicity, its bioaccumulation and persistency in the environment, as well as its carcinogenic properties. In 2011, phenol was given 688.4 points by the Agency for Toxic Substances and Disease Registry (ATSDR), reaching the 179th position on a list of toxic compounds where values are based on three criteria: frequency, toxicity and their potential for human exposure [7]. Phenolic compounds affect taste and odor of natural and tap water. Besides, fish, animals and plants suffer from its toxic effects as phenol is absorbed through skin and cellular membranes [8]. Because of its high toxicity, mainly as chlorophenols, routine controls in water and soil are absolutely necessary. Being phenol concentration very low, its determination requires preconcentration processes using chromatography methods [9] For the determination of phenol in water, several methods have used pervaporation coupled to flow injection analysis with amperometric detection, with lineal range from 1 to 50 µg mL^{-1} [4]. Besides, enzymatic methods for determination in urine have also been used [10]. In Mexico, the concentration of phenol and derivatives in tap water was established by the modification of the Mexican Standard NOM-127-SSA1-1994 [11], when 0.3 µg mL^{-1} was established as the maximum permissible limit.

The Mexican Standard NMX-AA-050-SCFI-2001 [12] and the ASTM method [13] have established the test method for the determination of total phenolic compounds applicable to water containing more than 0.1 µg mL^{-1}, although some laboratories have reported to be able to measure concentration as low as 0.005 µg mL^{-1} by using 10 cm absorption cells. The standard method is based on oxidative coupling of phenols with 4-aminoantipyrine (4-AAP) in alkaline solution to form red antipyrine color, which is measured at 510 nm, so any time that color is produced by the reaction of all phenolic compounds, it is reported as phenol. Phenolic compounds with a substituent in the *para* position may not quantitatively produce color with 4-AAP. However, *para* substituents of phenol such as carboxyl, halogen, hydroxyl, methoxyl or sulfonic acid groups do produce color with 4-AAP [14-15]. Morita and Nakamura [16] have also used an ammonium peroxodisulfate solution as oxidizing reagent for the determination of phenol, instead of potassium hexacyanoferrate (III) on the 4-aminoantipyrine determination. Suitable color development was obtained by the addition of from 1 mL to 5 mL of ammonium peroxodisulfate solution (pH 10, 220 mmol L^{-1}) and 2 mL of 4-aminoantipyrine solution (98 mmol L^{-1}), to a 100 mL sample solution at pH 10 buffered with an ammonium chloride-ammonia solution (9.8 mol L^{-1}).

There are many methods where 4-AAP is used as color reagent, including the official standard methods. By flow injection analysis some methodologies have been able to determine very low phenol concentrations but by using a high injection volume (800 µL) and also high flow-rates, which causes an increase in the volume of the reagents used (0.8 ml min^{-1} for 4-AAP and 1.2 ml min^{-1} for potassium hexacyanoferrate (III) [17-18]. Other authors have evaluated the system used by Frenzel et al. in 1992 by decreasing the injection volume to 400 µL, but the volume of reagents and the sampling throughput was the same [19]. These methods have enabled measuring concentrations of phenol from 0.5 µg mL^{-1}, a concentration higher than the permissible minimum.

The Plackettt-Burman experimental design is used to identify the most important factors early in the experimentation phase, when complete knowledge about the system is usually unavailable. Developed in 1946 by statisticians Robin L. Plackettt and J.P. Burman, it is an efficient screening method for identifying the active factors with as few experimental runs as possible.

In Plackettt-Burman designs, main effects have a complicated confounding relationship with two-factor interactions. Therefore, these designs should be applied to study main effects when it can be assumed that two-way interactions are negligible. In actual use, both the two-level full or fractional factorial designs, as well as the Plackettt-Burman designs are often used to screen the main factors that influence process output measures or product quality. These designs are useful for fitting first-order models (which detect lineal effects) and can provide information on the existence of second-order effects (curvature) when the design includes center points [20].

With the aim of increasing the lineal range for the reaction between phenol, 4-AAP and potassium hexacyanoferrate (III),

a flow injection manifold Plackett-Burman design and Quarter fraction 2^{5-2} factorial ones were used. Main factors such as injection volume, concentration of reagents, flow-rates and length of reactors were studied and optimized. The effect of sulfates and hypochlorite on the reaction of phenol with 4-AAP and potassium hexacyanoferrate (III) was also evaluated. The method was applied to tap and bottled water.

Results and Discussion

Optimization of the Flow Manifold

Plackett-Burman Factorial Design
The flow injection manifold (Fig. 1) was constructed based on the process established by the Mexican standard method, where the sample is first mixed with NH_3 and 4-AAP and then $K_3[Fe(CN)]_6$ is added to this solution. By using the manifold shown in Fig. 1, the first test was carried out under the following conditions: 100 µL as injection volume, flow-rate of every channel at 0.5 mL min^{-1}, $[K_3[Fe(CN)_6]] = 0.25$ mol L^{-1}, [4-AAP] $= 9.84 \times 10^{-3}$ mol L^{-1}, $[NH_3] = 0.5$ mol L^{-1}, and length of the reactors (R_1 and R_2) = 100 cm. For this test the selected reagents concentrations were based on the original concentrations of the standard methods.

The analytical signal obtained was low but the peak was well defined (Gaussian form). Another test was carried out by changing the lengths of the reactors (to 150 cm), which resulted in a small increase of the signal. Therefore, a $2^{7 \times 3/32}$ Plackett-Burman factorial design was constructed in accordance with previous studies, in order to evaluate reagent concentrations, reactors length and flow-rate, as well as injection volume. Table 1 shows the Plackett-Burman experimental design used with three central points, resolution III and three grades of freedom. The ANOVA results are shown in Table 2. The study was carried out at 95% confidence level, so P values are less than 0.05, indicating that they are significantly different from zero. The analysis of residuals showed a random pattern confirming the validity of the model. According to the ANOVA table, at least two effects (injection volume and 4-AAP concentration) are higher than the experimental error for a 95% confidence
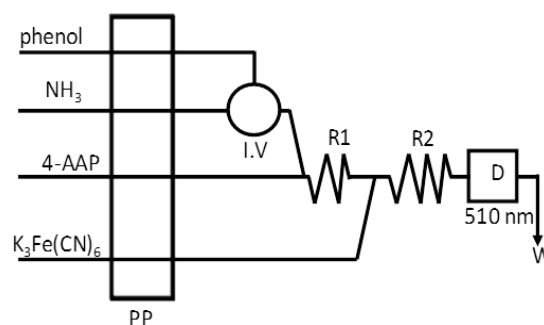
Fig. 1. Flow injection manifold to determine phenol. (4-AAP) 4-aminoantipyryne; R: reactors R_1 = 80 cm, R_2 = 180 cm. V.I. Injection valve; PP: peristaltic pump; W: waste.

Table 1. Plackett-Burman factorial design type III used to evaluate seven variables related with the flow injection manifold.

Test	NH$_3$ (mol L^{-1})	4-AAP (mol L^{-1})	K$_3$Fe(CN)$_6$ (mol L^{-1})	R$_1$ (cm)	R$_2$ (cm)	Flow-rate (mL min^{-1})	V (µL)	Absorbance*
1	+1(0.50)	−1(9.84 × 10^{-4})	+1(0.04)	+1(200)	−1(80)	+1(1.00)	−1(50)	0.7596
2	−1(0.05)	+1(9.84 × 10^{-3})	+1(0.04)	+1(200)	−1(80)	+1(1.00)	+1(200)	1.6617
3	+1(0.50)	+1(9.84 × 10^{-3})	−1(0.02)	+1(200)	+1(200)	−1(0.50)	+1(200)	1.6750
4	−1(0.05)	−1(9.84 × 10^{-4})	−1(0.02)	+1(200)	+1(200)	+1(1.00)	−1(50)	0.8259
5	+1(0.50)	+1(9.84 × 10^{-3})	−1(0.02)	+1(200)	−1(80)	−1(0.50)	−1(50)	0.9130
6	−1(0.05)	−1(9.84 × 10^{-4})	+1(0.04)	+1(200)	+1(200)	−1(0.50)	+1(200)	1.5788
7	0(0.28)	0(5.41 × 10^{-3})	0(0.03)	0(150)	0(150)	0(0.75)	0(125)	1.1078
8	0(0.28)	0(5.41 × 10^{-3})	0(0.03)	0(150)	0(150)	0(0.75)	0(125)	1.0580
9	0(0.28)	0(5.41 × 10^{-3})	0(0.03)	0(150)	0(150)	0(0.75)	0(125)	1.0580
10	+1(0.50)	−1(9.84 × 10^{-4})	+1(0.04)	−1(80)	−1(80)	−1(0.50)	+1(200)	1.6551
11	−1(0.05)	−1(9.84 × 10^{-4})	−1(0.02)	−1(80)	−1(80)	−1(0.50)	−1(50)	0.8756
12	−1(0.05)	+1(9.84 × 10^{-3})	+1(0.04)	−1(80)	+1(200)	−1(0.50)	−1(50)	0.9220
13	+1(0.50)	−1(9.84 × 10^{-4})	−1(0.02)	−1(80)	+1(200)	+1(1.00)	+1(200)	1.5025
14	+1(0.50)	+1(9.84 × 10^{-3})	+1(0.04)	−1(80)	+1(200)	+1(1.00)	−1(50)	0.8955
15	−1(0.05)	+1(9.84 × 10^{-3})	−1(0.02)	−1(80)	−1(80)	+1(1.00)	+1(200)	1.6816

*Mean of three determinations.

interval. The adjusted R-squared, which is more suitable for comparing models with different numbers of independent variables, was 96.68%. The equation of the fitted model is:

$$A = 0.4635 - 0.0545x \text{ [NH}_3] + 10.332x \text{ [4-AAP]} - 0.0275x \text{ [Fe(CN)}_6^{3-}] - 0.00022x \ R_1 - 0.00011x \ R_2 - 0.0983x \text{ Flowrate} + 0.00615x \text{ Volume} \quad (1)$$

The optimum values were: [NH$_3$] 0.064 mol L^{-1} at pH 10.5, [4-AAP] 9.80×10^{-3} mol L^{-1}, [K$_3$[Fe(CN)$_6$]] 0.02 mol L^{-1}, a flow-rate of 0.5 mL min^{-1} and the length of the reactors of 80 cm. As the injection volume was the most important factor, volumes higher than 200 µL were studied. A volume of 400 µL was evaluated under optimum conditions in accordance with the design, but the signal only increased by 3%. 200 µL was the nearest volume to S$_{1/2}$ (189.88 µL), which is the sample volume necessary for the dispersion to be equal to 2. This value was calculated from the following equation (Equation **2**) [20]:

$$-\log\left[1 - \frac{A_{max}}{A_0}\right] = \frac{-0.693}{2.303 S_{1/2}} S_v \quad (2)$$

where:

A_{max} is the absorbance of the dispersed sample, A_o is the absorbance of the sample without dispersion, S_v is volume of sample injected and $S_{1/2}$ is the volume of sample necessary for dispersion to be equal to 2 (dilution 1:1).

Therefore 200 µL was used for the next optimization tests.

Evaluation of Flow-Rate of the Reagents by Using a Quarter Fraction 2$^{5-2}$ Factorial Design

Consequently, a second factorial design (2$^{5-2}$ quarter fraction plus three central points) was carried out in order to evaluate the flow-rate for each reagent. In accordance with the manifold and the reaction, the first reactor (R$_1$) was only used for mixing of NH$_3$ and 4-AAP with the sample. The second reactor R$_2$ proved to be more important as, in that case, a global reaction of all reagents occurred. Therefore, the length of the reactors R$_1$ and R$_2$ as well as the flow-rates of the reagents were included in this design. The used injection volume (200 µL) and the concentration of the reagents were the optimum values found in previous designs. Table **3** shows the matrix used. The ANOVA test and pareto chart showed that there was no clear interaction among the evaluated factors. Fig. 2 shows the pareto chart of the main factors. According to the Fig., at least four effects (length of R$_2$, flow-rate 4-AAP, length of R$_1$ and flow rate K$_3$Fe(CN)$_6$) are higher than the experimental error denoted by the vertical

Table 2. Analysis of variance (ANOVA) at 95% of confidence level from the results of the Plackett-Burman factorial design type III factorial design.

| Source	Sum of Squares	Df	Mean Square	P-Value
A:[NH$_3$]	0.00187194	1	0.00187194	0.5301
B:[4-AAP]	0.0253369	1	0.0253369	**0.0454**
C:[K$_3$Fe(CN)$_6$]	6.75E-08	1	6.75E-08	0.9969
D:R$_1$	0.00253307	1	0.00253307	0.4675
E:R$_2$	0.00100693	1	0.00100693	0.6429
F:Flow-rate	0.00713944	1	0.00713944	0.2381
G:Volume	1.78334	1	1.78334	**0.0000**
Total error	0.0300421	7	0.00429173	
Total (corr.)	1.85125	14		

Table 3. Quarter-fraction 2^{5-2} factorial design used to evaluate five variables related whit the flow injection manifold.

Test	NH_3 (ml min^{-1})	4-AAP (ml min^{-1})	$K_3Fe(CN)_6$ (ml min^{-1})	R_1 (cm)	R_2 (cm)	Absorbance[a]
1	0(0.75)	0(0.60)	0(0.50)	0(140)	0(140)	0.2335
2	−1(0.60)	−1(0.35)	−1(0.35)	+1(200)	+1(180)	0.2848
3	+1(1.00)	+1(0.75)	−1(0.35)	+1(200)	−1(100)	0.2172
4	0(0.75)	0(0.60)	0(0.50)	0(140)	0(140)	0.2342
5	+1(1.00)	−1(0.35)	+1(0.70)	−1(80)	+1(180)	0.2948
6	+1(1.00)	+1(0.75)	+1(0.70)	+1(200)	+1(180)	0.2256
7	−1(0.60)	+1(0.75)	−1(0.35)	−1(80)	+1(180)	0.2733
8	−1(0.60)	+1(0.75)	+1(0.70)	−1(80)	−1(100)	0.2094
9	+1(1.00)	−1(0.35)	−1(0.35)	−1(80)	−1(100)	0.2748
10	−1(0.60)	−1(0.35)	+1(0.70)	+1(200)	−1(100)	0.2139
11	0(0.75)	0(0.60)	0(0.50)	0(140)	0(140)	0.2335

[a]Average of three deteminations

line indicating its 95% confidence interval, where the analytical signal of the product of the reaction shows no dependence on the flow-rate of NH_3, but a strong influence of the length of R_2. With smaller influence, the flow-rate of 4-AAP, the length of R_1 and the flow-rate of $K_3Fe(CN_6)$, gave better signal at lower values. As the length of R_1 had a negative influence on the analytical signal, several experiments were studied at lengths lower than 80 cm. The signal obtained was smaller than 80 cm and the mixing of NH_3 and 4-AAP was not suitable. The flow-rates of 4-AAP and $K_3Fe(CN_6)$ and the length of R_2 were selected in accordance with the results of the codified equation describing the dependency on the variable response (Equation **3**).

$$A = 0.2565 + 0.0155x \text{ flowrate } NH_3 - 0.0714x \text{ flowrate 4-AAP} - 0.0532x \text{ flowrate } K_3Fe(CN)_6 - 0.00023x \, R_1 + 0.00051x \, R_2 \quad (3)$$
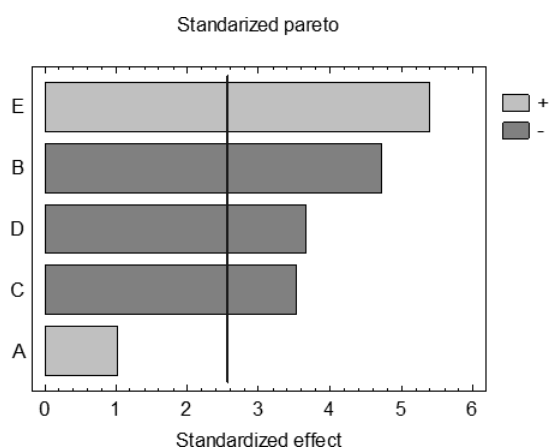
Fig. 2. Pareto chart of standardized effects showing the main effects: A: flow-rate of NH_3, B: flow-rate 4-AAP, C: flow rate $K_3Fe(CN)_6$; D: length of R_1; E: length of R_2 that significantly contribute to absorbance, according the results of the experimental design matrix shown in Table 3.

In the end, the optimum values in accordance with the factorial designs were: R_1: 80 cm; R_2: 180 cm; flow-rates: NH_3 1.0 mL min^{-1}; 4-AAP 0.35 mL min^{-1} and $K_3[Fe(CN)]_6$ 0.35 mL min^{-1}, $[NH_3]$ 0.064 mol L^{-1}, [4-AAP] 9.84×10^{-3} mol L^{-1}, $[K_3[Fe(CN)]_6]$ 0.02 mol L^{-1}, and injection volume 200 μL. Flow-rate values lower than 0.35 mL min^{-1} were not tested because the analysis would had been too time consuming.

Characteristics of the Method

The calibration curve was constructed with an optimized flow injection manifold. The lineal equation was: 0.0336 ($\pm 2.57 \times 10^{-4}$)[phenol] $- 0.0006$ ($\pm 3.16 \times 10^{-3}$). The lineal range is from 0.3 μg mL^{-1} to 30 μg mL^{-1}, with a detection limit of 0.13 μg mL^{-1} and a regression coefficient of 0.9999. The precision of the method regarding repeatability, expressed as relative standard deviation, was 1.37% for 1.0 μg mL^{-1} and 0.82% for 15 μg mL^{-1}. Fig. 3 shows the transient signal of the calibration curve from 0.3 μg mL^{-1} to 30 μg mL^{-1}. Under optimized working conditions, the analysis time was 1.65 min. by each injection, achieving 36 determinations an hour. Some authors have described how a flow system with higher flow-rates shortens the analysis time (50 determinations an hour) with a lineal range from 0.5 μg mL^{-1} to 16 μg mL^{-1} [17-19]. With the proposed method it was possible to increase the lineal range with a similar volume of reagents but with an injection volume of only 200 μL, in comparison with the 400 μL reported by Dolatto et al. [19]. For the official spectrophotometric method, the lineal range was from 0.5 μg mL^{-1} to 20 μg mL^{-1}.

Analysis of Samples
Table 4 shows the results for the water samples analyzed together with their recoveries, which were between 92% and 110% for the two concentrations of phenol studied. The concentration of the samples was lower than the detection limit (0.13 μg mL^{-1}) so the recoveries showed no interference of the matrix.
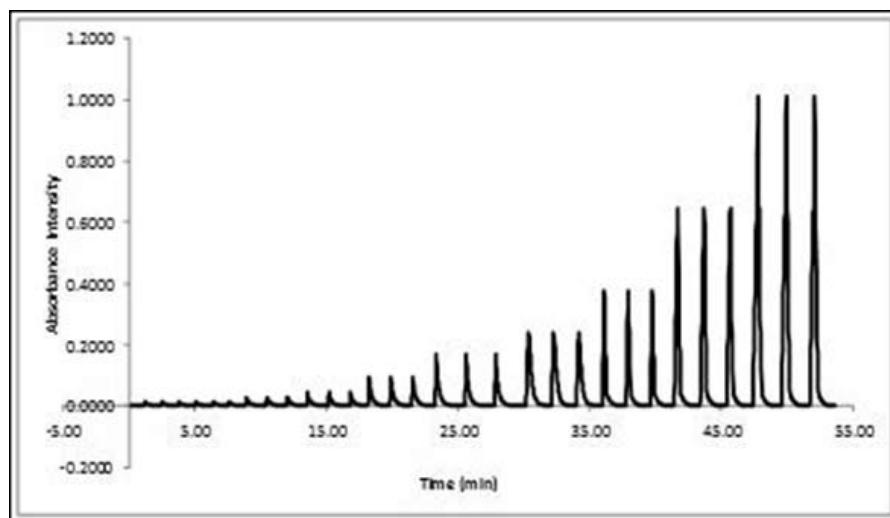
Fig. 3. Transient signals obtained for phenol in a range from 0.3 μg mL^{-1} to 30 μg mL^{-1} (0.3, 0.5, 0.8, 1.0, 4.0, 6.0, 8.0, 10.0, 20.0 and 30 μg mL^{-1}); analysis in triplicate.

Table 4. Analysis of water samples.

	Proposed method			Official method		
Sample	Recoveries (%)			Recoveries (%)		
		Added			Added	
	Found (μg mL^{-1})	1 μg mL^{-1}	15 g mL^{-1}	Found (μg mL^{-1})	1 μg mL^{-1}	15 μg mL^{-1}
Tap water (Coyoacán)	Nd	102.1	104.5 ± 0.1	Nd	96.3	102.3 ± 0.3
Tap water (Iztapalapa)	Nd	93.3	94.0 ± 0.2	Nd	92.1	94.6 ± 0.2
Tap water (Cuahutémoc)	Nd	110.0	99.4 ± 0.1	Nd	103.9	103.2 ± 0.6
Tap water (Miguel Hidalgo)	Nd	96.4	102.3 ± 0.1	Nd	105.2	90.3 ± 0.1
Bottle water (Prial)	Nd	95.8	96.2 ± 0.2	Nd	91.6	93.2 ± 0.4
Bottle water (Aguafiel)	Nd	92.1	101.8 ± 0.3	Nd	90.5	95.2 ± 0.2

Nd: no detected.

Another study was carried out to evaluate the interferences in the flow injection determination, of other possible compounds that could be present in tap water. Table **5** shows the results. The permissible limits of sulfates and hypochlorite are 400 μg ml^{-1} and 1.5 μg mL^{-1}, in natural drinking wastewater and in wastewater treated according with NMX-AA-050-SCFI-2001 [12], respectively. The concentration measured in a phenol solution of 12 μg mL^{-1} was 11.8 ± 0.0025 μg mL^{-1}. The results showed no interference when the concentration of hypochlorite ion was lower than the phenol concentration, (OCl$^-$ ion at 1.5 μg mL^{-1}; phenol: hypochlorite ratio equal to 8), in accordance with the F test for equal variance. However, when the hypochlorite ion concentration was the same or higher than the phenol concentration (from 15 μg ml^{-1} to 240 μg mL^{-1}), the measurement of the phenol concentration decreased by between 15.24% and 54.49% at time cero, and between 14.00% and 79.83% after 60 min. (Table 5).

As for sulfates, the phenol value increased by 8% from 1000 μg mL^{-1} of sulfates with a sulfates phenol ratio equal to 83. For ratios lower than 60, concentrations of sulfates of 240 and 739 μg mL^{-1}, there were no interferences in the measurement of phenol.

Conclusions

1) The optimization of the flow injection determination of phenol with 4-APP by experimental design enabled to reduce the experiments.

2) The proposed method increases the lineal range allowing measuring from 0.3 μg mL^{-1}, which is established as the maximum permissible limit by the Mexican Standard NOM-127-SSA1-1994, to 30 μg mL^{-1}.

3) With the proposed method the possible interferences of sulfates for tap and bottled are avoided, and distillation, as proposed by the standard method, is not necessary.

4) Hypochlorite has to be removed during the sampling. The removal of all hypochlorite ions beforehand is made neces-

Table 5. Study of interferences for the proposed method. The phenol concentration was 12 μg mL^{-1}.

Interference analyzed (μg ml^{-1})	Found phenol concentration (μg mL^{-1})	
	Time (min)*	
	0	60
OCl$^-$		
1.5	11.79 ± 0.003	11.75 ± 0.001
15	10.00 ± 0.002	10.15 ± 0.001
48	7.30 ± 0.003	4.03 ± 0.004
240	5.37 ± 0.005	2.38 ± 0.001
SO$_4^{2-}$		
250	11.75 ± 0.002	11.78 ± 0.001
730	11.73 ± 0.003	11.88 ± 0.002
1000	12.77 ± 0.005	12.66 ± 0.003

*Time passed between the additions of the interferences to the phenol solution and the determination of phenol

sary as, otherwise, it would be an important interference in the analysis of total phenols in tap and bottled water.

Experimental

Instrumentation

In order to determine phenol, an Ismatec 4 channel peristaltic pump, an injection valve (Upchurch Scientific V451) and teflon tubing of 0.8 mm i.d. were used to build the flow injection manifold. A UV-Vis spectrophotometer Cary 3 from Varian (Sydney, Australia) equipped with a flow cell of 118 μL inner volume was used to measure the reaction product at 510 nm. An ultrasonic bath (Branson) was also used. Statgraphics Plus 4.0 software (Statistical Graphics, Rockville, MD) was used for test design and data analysis.

Reagents and Solutions

All reagents were analytical grade. 4-aminoantipyrine (4-AAP) (Fisher Scientific, Suwanee, GA, USA), K$_3$[Fe(CN)$_6$] (99%, Sigma-Aldrich, Toluca, Mexico) and NH$_3$ (Baker-Mallinkroft, Mexico City) were used to carry out phenol quantification. The phenol stock solution was prepared by dissolving 100 mg of phenol (99%, Sigma-Aldrich, Toluca, Mexico) in freshly boiled and cooled water, and diluted to 1000 mL with the same type of water. This solution was used during a 7 day observation period.

Samples
Tap water from different districts of Mexico City and bottled water were directly analyzed in triplicate without any pretreatment.

Procedures
The principle of the reaction is based on the oxidative coupling of phenol with 4-AAP in alkaline solution to form a dye complex, by using a K$_3$[Fe(CN)]$_6$ solution as oxidizing reagent. The reaction product is measured at 510 nm.

Proposed Flow Injection Analysis Method
Fig. 1 illustrates the flow injection analysis manifold designed for method implementation. Phenol standard and sample were injected into a pH 10 NH$_3$ carrier, merged with 4-AAP, and then mixed in reactor R$_1$. Afterwards, the mixture was merged with the K$_3$[Fe(CN)]$_6$ solution to form the red complex along with reactor R$_2$ and it was measured at 510 nm.

Official Spectrophotometric Method
5 mL of a 0.5 N NH$_4$Cl solution were added to each 100 mL of blank, standards and samples. The pH was adjusted between 9.8 and 10.2 with NH$_3$ and then 2.0 ml of 2.5% (w/v) 4-AAP solution was also added and mixed immediately. At this point, 2.0 mL of 8% (w/v) K$_3$[Fe(CN)$_6$] solution were added and also mixed. After 15 min the solutions were transferred to an absorption cell and the absorbance was measured at 510 nm.

Validation of the Proposed Flow Injection Method
First, in order to carry out a screening study of the main factors affecting the reaction, a multifactorial design methodology was built based on a Plackett-Burman factorial design type IV resolution with 4 degrees of freedom, involving 12 randomized runs plus three center points. Seven factors were evaluated, whose top and bottom values were established according to previous studies: NH$_3$ concentration (0.05-0.5 mol L^{-1}), 4-AAP concentration (9.84×10^{-4}-9.84×10^{-3} mol L^{-1}), K$_3$[Fe(CN)]$_6$ concentration (0.02-0.04 mol L^{-1}), R$_1$ (80-200 cm) and R$_2$ (80-200 cm) lengths, flow-rate of the reagents (0.50-1.00 mL min^{-1}) and injection volume (75-200 μL). Each test was done in triplicate.

A second factorial design based on 2^{5-2} quarter fraction type V resolution allowing four freedom degrees and involving 8 randomized runs plus three centre points was also carried out with the aim of finding the optimal values for the flow-rates of each reagent and the lengths of the reactors: NH$_3$ (0.6-1 mL min^{-1}), 4-AAP (0.35-0.75 mL min^{-1}), K$_3$[Fe(CN)]$_6$ (0.35-0.7 mL min^{-1}), R$_1$ (80-200 cm) and R$_2$ (80-200 cm). ANOVA tests were calculated by Statgraphics software.

The precision of the method was evaluated by repeatability injecting 11 standards of 1 μg mL^{-1} and 15 μg mL^{-1}. A study of recoveries by adding 15 μg mL^{-1} and 1 μg mL^{-1} to the water samples was also carried out in triplicate with the aim of demonstrating no interferences in the matrix.

Evaluation of Interferences in the Proposed Flow Injection Method
The most important interferences to measure total phenolic compounds in water are sulfates and hypochlorite. Therefore, different concentration of Na$_2$SO$_4$ and NaOCl were added to 12 μg mL^{-1} of phenol solution and the phenol concentration was measured at 0 min and at 60 min.

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