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Note

High-performance liquid chromatographic determination of phenols as 4-aminoantipyrine derivatives: silver chloride as oxidizing agent in the derivatization reaction

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The determination of phenols as 4-aminoantipyrine derivatives by high-performance liquid chromatography (HPLC) has recently been described[1]. The method consists of the well known Emerson reaction[2] between phenols and 4-aminoantipyrine (4-AAP) with potassium hexacyanoferrate(III) as the oxidizing agent and of the HPLC separation of the dyes with detection in the visible range (460–500 nm). Even if the dye derivatives showed sufficient absorbance to be detected at 254 nm (see Table I), detection in the visible region became necessary because of the large number of reaction by-products that absorb that in the UV region. In order to overcome this restriction and to extend routine applications further, the replacement of the potassium hexacyanoferrate(III) as the oxidizing agent was considered.

Of the possible oxidizing agents, silver chloride was chosen as it is known to produce good dye yields (90–98%) in oxidative coupling reactions of phenols with *p*-aminodimethylaniline without attacking the phenols[3,4].

Preliminary results obtained using silver chloride, formed *in situ* by addition of potassium chloride to an aqueous reaction mixture containing silver nitrate, in the presence of a dispersion stabilizer (dextrin), were promising. In fact, as one can see in Fig. 1b, the chromatographic separation, recorded in the UV region (254 nm), does not reveal many number of reaction by-products and those which occur do not interfere because of their very short elution times.

In this work, optimum conditions for the oxidative coupling reaction between 4-AAP and the phenol, in the presence of silver chloride, were studied. Comparison with the potassium hexacyanoferrate(III) method is discussed and the response to the 4-AAP test for other phenols, under the same conditions, is also reported.

EXPERIMENTAL

The liquid chromatograph was a Spectra-Physics Model 3500B, equipped with a Model 770 spectrophotometric detector and a Varian Aerograph UV detector, connected in series and operating at 480 and 254 nm, respectively.

The reagents and the procedure for synthesizing the phenol derivatives with potassium hexacyanoferrate(III) as oxidizing agent, and also the chromatographic conditions, have been reported previously[1]. The reagents used for phenol derivati-
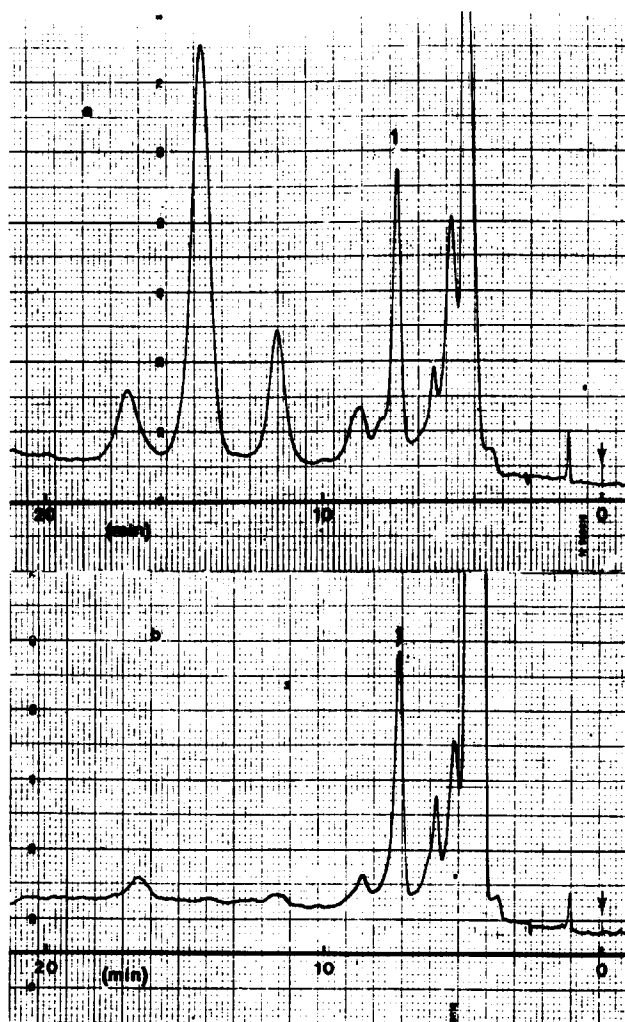
Fig. 1. Interference of 4-AAP reaction by-products in the HPLC determination of phenol using detection at 254 nm UV: (a) $K_3Fe(CN)_6$; (b) AgCl. Column, μBondapak-phenyl; mobile phase, methanol–water (60:40); flow-rate, 1 ml/min; detector, UV (254 nm, 0.16 a.u.f.s.); Sample: peak 1, phenol (2 ppm).

zation with silver chloride as the oxidant were purchased from Carlo Erba (Milan, Italy) and used in aqueous solution: 4-AAP (2%), potassium chloride (0.05 M), silver nitrate (0.08 M), borax buffer[5] (pH range 8–10), carbonate–hydrogen carbonate buffer[5] (pH range 9–10), dextrin (BDH, Poole, U.K.) as a 0.6% solution.

The optimum reaction conditions, established for phenol, were as follows: to the water sample (100 ml) containing phenols in the concentration range 0.5–10 ppm, 2 ml of KCl solution, 1 ml of dextrin solution, 1 ml of AgNO₃ solution, 2 ml of borax buffer (pH 9), 0.5 ml of 4-AAP solution and 10 ml of chloroform were added. The reaction time was fixed at 40 min and the temperature at 40°C. The chloroform extract was then treated and analysed as described previously[1].

RESULTS AND DISCUSSION

As the reaction is a two-phase one, the coupling of phenols with 4-AAP in the presence of AgCl is not instantaneous but requires 30–60 min for completion. This finding, with the use of silver ion as the oxidizing agent, is not uncommon (*cf.*, aldehyde determinations[6]).

In order to obtain linearity, reproducibility and the highest reaction yields in a shorter time, many variables were considered. First, the reaction temperature must be controlled because both the induction time (*ca.* 5 min.) and rate are affected. The reaction temperature, established at 40°C, provides the best yield in a 40-min reaction time. The pH of the medium, the type and amount of buffer and the amounts of 4-AAP and AgCl were then considered. Optimum conditions for these variables are reported under Experimental. A 4-AAP concentration higher than that suggested does not improve the reaction yields. At a phenol concentration of 1 ppm, the optimum molar ratio between the phenol, KCl and $AgNO_3$ was found to be 1:100:80. Also, the presence of dextrin colloid protector and the introduction of chloroform extractant at the beginning of the reaction improve the efficiency of the AgCl oxidant.

Under the suggested experimental conditions a linear calibration graph with
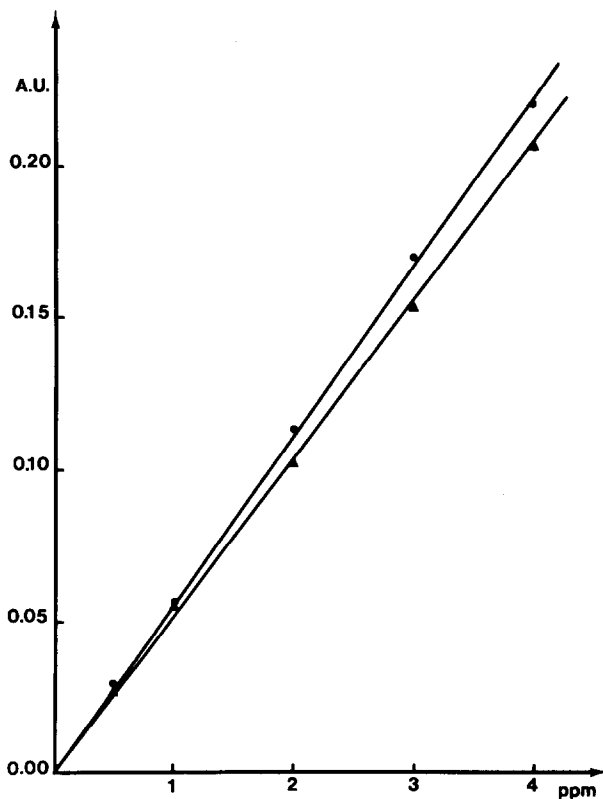

Fig. 2. Calibration graphs for the 4-AAP phenol derivative with $K_3Fe(CN)_6$ (●) or AgCl (▲) as oxidizing agent.

TABLE I

COMPARISON BETWEEN THE AgCl AND K₃Fe(CN)₆ METHODS

A, Relative reaction yields (%); B, relative detection sensitivities; C, overall relative sensitivities (%) using AgCl and UV detection together.

Compound	A^*	B^{**}	C^{***}
Phenol	95	9.1	10
2-Methylphenol	55	6.1	9
3-Methylphenol	65	7.3	9
2-Chlorophenol	100	12.6	8
3-Chlorophenol	92	11.5	8
4-Chlorophenol	61	9.4	7
2,4-Dichlorophenol	44	11.5	4
2-Nitrophenol	11	1.5	7
3-Nitrophenol	15	31.8	0.5

$$* \text{ Relative reaction yield} = \frac{\text{yield with AgCl (480 nm)}}{\text{yield with K}_3\text{Fe(CN)}_6 \text{ (480 nm)}} \cdot 100.$$

$**$ $\varepsilon_{480\ nm}/\varepsilon_{254\ nm}.$
$***$ Overall relative sensitivity $= A/B.$

a slope nearly equal to that observed using potassium hexacyanoferrate(III) was obtained for the phenol in the concentration range 0.5–5 ppm (Fig. 2). The response of other monohydric phenols to the 4-AAP test was studied and the reaction yields in comparison the potassium (hexacyanoferrate(III) method are reported in Table I.

The use of detection at 254 nm together with the silver chloride oxidant reduces the overall sensitivity of the 4-AAP methods[1] to about 10–20% for all phenolic compounds (excepted 3-nitrophenol). However this lower sensitivity is not disadvantageous in most applications, provided that the phenol concentration is in the parts per million range[7].

It should be noted that specificity of the method towards phenolic compounds is preserved using silver chloride; the response of aniline to the 4-AAP test is 0.12, taking the response of the phenol as 100.

Using either silver chloride or potassium hexacyanoferrate(III), the reaction yields given by *para*-substituted phenols are lower than those given by the corresponding non-*para*-substituted phenols. As the decrease in yield is enhanced with silver chloride, this may be useful for identification purposes, bearing in mind that these compounds react with 4-AAP by elimination of the *para*-substituent and give the same dye products as the corresponding non-*para*-substituted phenols[1]. The use of both oxidizing agents and a comparison of chromatographic responses at 480 nm permit the identification of *para*-substituted phenols in the presence of corresponding non-*para*-substituted phenols.

We conclude that using silver chloride, UV detection of the derivative is possible and the selectivity of the method in the determination of *para*-substituted phenols is improved.

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