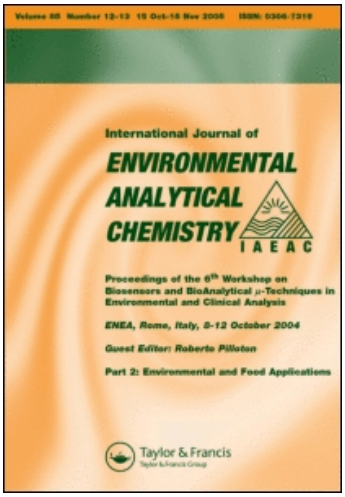

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Torazzo, Annamaria , Zelano, Vincenzo and Ostacoli, Giorgio(1990) 'A Comment on the Use of Colorimetric Determinations of Phenolic Compounds in Surface Water', International Journal of Environmental Analytical Chemistry, 38: 4, 599 — 605
To link to this Article: DOI: 10.1080/03067319008026962
URL: http://dx.doi.org/10.1080/03067319008026962

Intern. J. Environ. Anal. Chem., Vol. 38, pp. 599–605
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A COMMENT ON THE USE OF COLORIMETRIC DETERMINATIONS OF PHENOLIC COMPOUNDS IN SURFACE WATER

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(*Received 1 October 1988; in final form 1 July 1989*)

Total phenolic compound values were determined in samples of river water by means of the Folin–Ciocalteu colorimetric method, while HPLC with UV detection was used to identify individual phenols. Preconcentration was needed in all cases to meet the required detection limits. Given the absorption coefficients of the components, the error incurred when a curve calibrated against phenol only is used to evaluate a mixture was calculated.

KEY WORDS: Phenolic compounds, Folin–Ciocalteu, colorimetry, surface water

INTRODUCTION

Several methods exist for the determination of phenols and substituted phenols in natural water.[1,2] Chromatography undoubtedly provides a good general solution. However, when all that is needed is an overall evaluation of the compounds present, chemical methods based on a selective reaction with the active group often constitute a useful alternative.

The tried and tested aminoantipyrine colorimetric method is perhaps the most widely employed technique: non-*p*-substituted phenols undergo oxidative condensation with 4-aminoantipyrine (4AAP), giving rise to a coloured product.[3] The Folin–Ciocalteu (FC) method[4] can also be used for the determination of total phenols. This method is based on the development of colour between the FC reagent—a mixture of phosphomolybdic and phosphotungstic heteropolyacids —and phenols, with subsequent oxidation of the phenates and formation of a blue molybdenum and tungsten complex in proportion to the phenol concentration.

These colorimetric methods, however, are subject to a very important limitation. The nature of phenolic compounds in a given real sample is unpredictable. It is thus impossible to prepare a standard phenol mixture applicable to all samples. For this reason, phenol itself is chosen as the standard in these methods and the

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colour produced by the reaction of other compounds is reported as phenol. Since substitution of phenol generally reduces the response, the value observed represents the minimum concentration of the phenolic compounds present. In less frequent cases, however, the opposite may be the case.

Chromatography, on the other hand, allows individual compounds to be discriminated. HPLC is one of the most versatile techniques, and is fast, reproducible and sensitive.[5] This paper compares FC and HPLC findings in water samples taken from the Turin stretch of the river Po, so as to provide an indication of the discrepancy due to the limitation just described. Some determinations were also performed with the 4AAP method for comparison purposes.

EXPERIMENTAL

Materials and methods

Eleven phenols were investigated: phenol, *o*-, *m*- and *p*-cresol, 2-chlorophenol, 4-chlorophenol, 2-, 3- and 4-nitrophenol, 4,6-dinitrorthocresol, and catechol. Some are those whose determination in water analysis is recommended by the Environmental Protection Agency (EPA). The phenols were obtained from different manufacturers and were of 98% purity or better.

4AAP method Aqueous solution (0.2 ml, 2% w/v) of 4-aminoantipyrine and 0.2 ml of an 8% w/v aqueous solution of potassium hexacyanoferrate were added to 10 ml of sample at pH 10.0 ± 0.2 ($NH_3 + NH_4Cl$). The mixture was stirred after each addition. After 15 min, the absorbance of the red solution was measured at 510 nm. The calibration curve ran from 10 to 50 μg phenol.

FC method The reaction was conducted in a medium rendered basic by the addition of 1 ml sodium carbonate (20% w/v) together with 0.2 ml Folin–Ciocalteu reagent to 8 ml of sample; this mixture was shaken and brought to 10 ml with water. The absorbance at 675 nm was measured after 40 min. The calibration curve ran from 5 to 50 μg phenol.

HPLC The separation was performed on a LiChrosorb RP-18 (Merck) column with a water–acetonitrile (72:28) as eluent at a flow rate of 1.8 ml/min. An Altex model 110A pump and a variable-wavelength Philips UV detector were used. The phenols were monitored at 268 nm.

Clean-up and Preconcentration

Condensation and reduction reactions also occur with amines and reducers. A separation step is therefore required. Steam distillation of volatile phenols from a solution acidified with 0.5 ml 85% phosphoric acid per 500 ml sample and containing 5 ml of a 10% $CuSO_4$ solution per 500 ml to hold back sulphide results

in satisfactory separation and recovery, if most of the original water is distilled over. Blank samples spiked with from less than 1 μg/ml to 10 μg/ml of phenol in drinking water were run to provide conditions with the closest possible resemblance to the real sample; the recovery for this range is between 98 and 102%.

Since the methods adopted are not sensitive enough to detect phenols at their probable concentrations in river water, samples must first be preconcentrated. Liquid–liquid extraction was chosen for this purpose because the river Po contains several mg/l of particulate matter consisting of at least 50% organic matter. This means that part of the organic pollutants may be associated with the particulate, so that recovery of phenols through the use of liquid–solid sorption systems may not lead to correct results. The method proposed by Afghan[6] was therefore adopted to concentrate the samples by means of two extractions. Five hundred millilitres of water are extracted at pH 2.0 ± 0.2 with three 60 ml *n*-butylacetate portions; the organic phase is then extracted with 8 ml 6% w/v NaOH. The extract is neutralized and then analysed by means of the FC method (10 ml final volume). This results in 50-fold concentration. The yield from this procedure was checked by means of tests on mixtures consisting of from 0.01 to 3 mg/l of phenol, *p*-cresol, 4-chlorophenol or 2,4-dichlorophenol in drinking water that has an organic matter content (total organic carbon (TOC)) of about 1–2 mg/l and so allows the preparation of standard solutions where the matrix is roughly comparable with those of the samples. Yields of 93–98% of the theoretical value were obtained.

Since phenol concentrations in the river Po are of the order of a few μg/l, they are below the detection threshold for HPLC with UV detection, even after 50-fold concentration. Further preconcentration was therefore necessary next to the concentration by extraction. This was done by distillation under vacuum at not more than 30 °C. In this way, two litres of sample were concentrated to the 500 ml sample used for the extraction procedure. *n*-Butylacetate, which has a higher boiling point than water, was added to the balloon flask used to collect the distillate to recover any phenol compounds that might have escaped from the boiler. Tests with phenol mixtures and with 0.5–10 μg/l phenol-only solutions gave deviations from the theoretical value of 4%.

RESULTS AND DISCUSSION

The colorimetric response obtained with the FC reagent varied considerably from one phenol to another as regards the time taken for the colour to develop and the value of the extinction coefficient. For this reason colorimetric determinations using a calibration curve constructed with phenol only result in discrepancies with regard to the real value as a function of the type of compound present in the sample. The preliminary experimental results also indicate that both nitrophenols and pentachlorophenol develop a very pale colour with the FC reagent and are therefore not suitable for determination with this method.

As an example, the total true and observed concentrations of a phenol mixture analysed by means of the FC method are compared in Table 1. It can be seen that, using a calibration curve constructed with phenol only, the quantity

Table 1 FC determinations of four phenol mixtures calibrated versus phenol only (in mg/l)

Phenolic compounds	*Mixt. 1*	*Mixt. 2*	*Mixt. 3*	*Mixt. 4*
p-Cresol	1.28	1.60	0.64	0.96
4-Chlorophenol	2.33	2.91	1.16	1.75
2,4-Dichlorophenol	1.04	1.29	0.52	0.78
Total true concentration	4.65	5.80	2.32	3.49
Total observed concentration[a]	2.39	2.89	1.15	1.79
Error (%)	−49	−50	−51	−49

[a]Values obtained using a calibration curve constructed with phenol only.

Table 2 FC determination of four phenol mixtures with concentration correction (in mg/l)[a]

Phenolic compounds	*f*	*Mixt. 1*	*Mixt. 2*	*Mixt. 3*	*Mixt. 4*
p-Cresol	0.702	0.899	1.123	0.450	0.675
4-Chlorophenol	0.546	1.272	1.588	0.633	0.956
2,4-Dichlorophenol	0.310	0.322	0.400	0.161	0.241
Total true concentration	—	2.493	3.111	1.224	1.872
Total observed concentration	—	2.39	2.89	1.147	1.786
Error (%)	—	−4	−7	−6	−4

[a]Same solutions as in Table 1. For details, see text.

experimentally determined is about half the true value. This large difference stems from the fact that the phenols chosen for the mixture have FC extinction values that are significantly lower than the phenol used as reference.

The errors observed with other mixtures varied according to their composition. The error is, of course, completely eliminated when the composition of a mixture is known and the concentrations of its components are expressed as phenol equivalent, in relation to their absorptiometric response relative to phenol. In order to do this the concentrations in Table 1 are multiplied by a factor, f, that takes this response into account and is obtained from the ratio between the extinction coefficient, k, of the compound and that of phenol itself; the results are very close to the true values (see Table 2).

The chromatographic separation of eight phenols is presented as an example in Figure 1; only *m*- and *p*-cresol are poorly separated. Our experiments showed that the UV response is linear to 5 mg/l with a detection limit ranging from 0.1 to 1 mg/l, depending on the type of compound.

Real Samples

River water samples were analysed as described. To evaluate the quantity of compounds interfering with the colorimetric methods of analysis, FC and 4AAP determinations were performed on both the distillate and the non-distillate. A

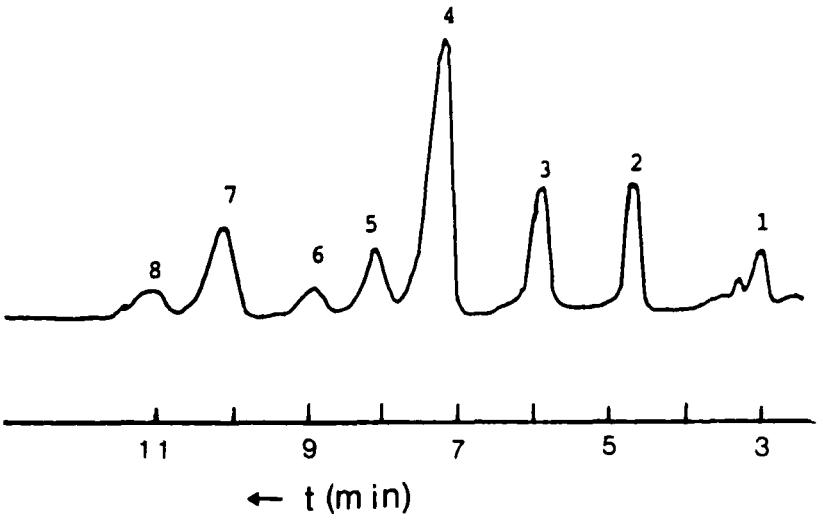

Figure 1 HPLC separation of phenols. Column: LiChrosorb RP-18 (Merck). Eluent: water–acetonitrile (72:28). Flow rate: 1.8 ml/min. Detector: UV at 268 nm. Sample of 20 μl of mixture of: 4.5 μg/ml of catechol (1); 2.0 μg/ml of phenol (2); 5.5 μg/ml of 4-nitrophenol (3); 4 + 4 μg/ml of *p*- and *m*-cresol (4); 3 μg/ml of 2-chlorophenol (5); 0.7 μg/ml of *o*-cresol (6); 6.3 μg/ml of 4- chlorophenol (7); 1.0 μg/ml of 3-nitrophenol (8).

typical finding shows that the FC reagent has a greater detection capacity than 4AAP both before distillation (26 and 15 μg/l, respectively) and after distillation (16 and 8 μg/l, respectively). In addition, the interfering compounds represent about 50% of the absorbance when 4AAP is used. HPLC was used to identify and determine the major components, while with FC the total phenol content was determined.

In order to compare the results of FC and HPLC determinations, a total absorbance has been calculated for each sample of river water: the contribution of each phenolic compound identified by HPLC has been calculated through FC extinction coefficients and the resultant value has been compared with the corresponding value obtained with the FC reagent. Table 3 lists the results obtained for three of the river water samples examined. As can be seen the differences between measured and calculated absorbance range from −1 to 32%. To check the meaning of these figures, tests on phenolic mixtures of known composition in drinking water were carried out using the complete procedure employed for the river water samples. Typically, the difference between absorbances calculated from the HPLC data and determined by means of the FC method vary from 5 to 12 %. In Table 3 a similar value is found for sample 3. As for sample 2, a striking agreement has been found between the measured and calculated absorbances; however, this can well be due to a rather simple composition of the mixture. On the other hand, a difference of 32% is shown for sample 1, which possibly indicates that HPLC was not able to identify all the

Table 3 Data on phenolic constituents for three river water samples[a]

(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
Sample 1				
Phenol	0.6	8.60×10^{-2}	0.052	0.003
Catechol	1.4	9.14×10^{-2}	0.128	0.007
4-Chlorophenol	1.0	4.70×10^{-2}	0.047	0.005
Total absorbance. Measured (A_{Meas}): 0.334; calculated (A_{Calc}): 0.227				
Difference ($A_{Meas} - A_{Calc}$) = 32%				
Sample 2				
Phenol	0.2	8.60×10^{-2}	0.017	0.001
Catechol	1.3	9.14×10^{-2}	0.119	0.006
Total absorbance. Measured (A_{Meas}): 0.134; calculated (A_{Calc}): 0.136				
Difference ($A_{Meas} - A_{Calc}$) = −1%				
Sample 3				
Phenol	0.5	8.60×10^{-2}	0.043	0.002
Catechol	1.0	9.14×10^{-2}	0.091	0.005
4-Chlorophenol	0.6	4.70×10^{-2}	0.028	0.003
Total absorbance. Measured (A_{Meas}): 0.175; calculated (A_{Calc}):[b] 0.162				
Difference ($A_{Meas} - A_{Calc}$) = 7%				

[a](a) Compounds identified by HPLC; (b) concentration in the extract in mg/l; (c) absorptivity values in $1\,cm^{-1}\,mg^{-1}$; (d) absorbance values calculated; (e) concentration in mg/l in the sample itself.

[b]See text for details.

phenolic compounds present, or that some interferents remained in the mixture despite the clean-up procedure.

It may be pointed out that phenol and catechol were found in all examined real samples. 4-Chlorophenol was present in some samples and traces of 4-nitrophenol in one sample not listed in Table 3. The presence of catechol explains that the total concentration determined by FC colorimetry was larger and not smaller than that observed in many of the blank tests. This result can be linked to the fact that catechol is present in a rather high concentration and that its extinction coefficient in FC is higher than that of phenol (9.1×10^{-2} vs. $8.6 \times 10^{-2}\,l\,cm^{-1}\,mg^{-1}$). Its presence in water of the river Po was unexpected and may be the result of photodegradation of phenolic compounds,[7] a result that is in itself of a certain interest because it points to the existence of conditions favouring photo-oxidation, and hence the ability of the river to purify itself.

CONCLUSION

The data presented in this work are useful to demonstrate that the FC

colorimetric method employed for the routine determination of phenolic compounds may lead to considerable errors when the analysis is not supported by a precise knowledge of the phenolic constituents of the sample.

Acknowledgement

We thank Ministero della Pubblica Istruzione and Regione Piemonte for financial support.

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