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SELECTIVE GAS CHROMATOGRAPHIC ANALYSIS OF CHLORINATED PHENOLIC COMPOUNDS USING THE NITROGEN–PHOSPHORUS DETECTOR

M. J. BERTRAND*, S. STEFANIDIS, A. DONAIS and B. SARRASIN

Department of Chemistry, University of Montreal, P.O. 6210, Stn.A., Montreal, Quebec H3C 3V1 (Canada)

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SUMMARY

A new gas chromatographic method, using a nitrogen–phosphorus detector to selectively analyse chlorinated phenolic compounds, is described and its usefulness evaluated. In this scheme of analysis the chlorinated phenolic substances are converted into their cyanoethyldimethylsilyl ether derivatives by a one-step reaction with 2-cyanoethyldimethyl(diethyl)aminosilane prior to analysis. Results indicate that the ethers have good gas chromatographic properties and can be detected with high sensitivity by the nitrogen–phosphorus detector. The response is linear over a wide range of concentrations and the limits of detection are $(5 \pm 2) \cdot 10^{-13}$ g N. Furthermore, the relative response factors calculated for chlorophenols and chlorinated hydroxybiphenyls, when using this detector, are close to unity. The mass spectrometric behavior of cyanoethyldimethylsilyl ethers is also discussed with reference to structure specificity and detection at low levels by selected-ion monitoring gas chromatography–mass spectrometry.

INTRODUCTION

The determination of chlorinated phenolic compounds at trace levels is of analytical interest because these substances are found in the environment. Chlorophenols are used mostly as pesticides and fungicides and are considered as toxic contaminants[1–4]. Chlorinated hydroxybiphenyls, which are substituted chlorophenols, are of biological interest because they are involved in the metabolism of polychlorinated biphenyls[5]. In recent years, methods of analysis of these compounds have been developed, many of them using gas chromatography (GC)[6–14] and high-performance liquid chromatography (HPLC)[15–18].

In GC, the selective detection of chlorophenols has been done using flame photometric detection (FPD)[13] and electron-capture detection (ECD)[6,7,11,14]. ECD has the advantage of a high sensitivity and selectivity for the chlorinated phenolic substances but problems can be encountered due to contamination and mostly because this detector has a wide range of response factors for different isomers[6–9,11,12,19,20]. The latter situation is inherent to ECD and can create difficulties

if several isomers of different compounds, present in a wide range of concentrations, are to be analyzed in the same sample.

In order to develop a sensitive method of analysis in which the relative response factors for the chlorinated phenolic substances would be less affected by either the position or the number of chlorine radicals, we have considered the utilisation of another selective detector. The nitrogen–phosphorus detector of the Kolb type[21] offers analytical potential since it has high sensitivity to nitrogen (10^{-13} g N/s), high selectivity (10^4), good stability, a wide dynamic range (10^5) and is fairly resistant to contamination[20]. Chlorinated phenolic compounds are not detected directly by this detector and they must be chemically tagged through derivatization with the proper reagent. Derivatization of the phenolic hydroxy group can be accomplished using a one-step silylating reaction and this approach is depicted in reaction 1.
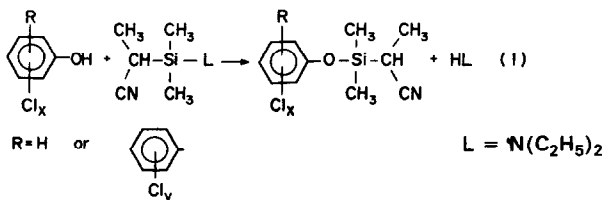
The formation of cyanoethyldimethylsilyl ethers of the compounds of interest should yield derivatives amenable to selective detection by the nitrogen–phosphorus detector with high sensitivity because the cyano radical is believed to be involved in the mechanism of detection of organic compounds by this detector[24]. Moreover, these ethers should have similar relative response factors when analyzed by this detector since they all contain the same "detection site". To evaluate the usefulness of this new approach, chlorinated phenols and hydroxybiphenyls have been studied and the results are presented.

EXPERIMENTAL

Derivatization

All solvents and chemicals used in this study were purified by standard procedures. Chlorophenols were obtained from Aldrich, except for 2,3,4,6-tetrachlorophenol (Pfaltz & Bauer) and they were purified by sublimation prior to use. Chlorinated hydroxybiphenyls were synthesized in this laboratory[22], except for 3-chloro-2-hydroxy- and 3-chloro-4-hydroxybiphenyls, which were obtained from Aldrich. The silylating reagent, 2-cyanoethyldimethyl(diethyl)aminosilane (CEDMSDEA) was prepared in this laboratory according to a procedure published elsewhere[23], and the silylation reactions were done in ethyl acetate under an inert atmosphere of nitrogen. The Reacti-Vials® where pretreated with dimethyldichlorosilane prior to utilization because it was noted that some chlorophenols interact strongly with glass giving irreproductible results. Unless otherwise specified, the reaction time was 15 min and the reagent 80 times more concentrated than the chlorophenols (*ca.* 1 m*M*). In all cases, an aliquot (1 μl) of the reaction mixture was directly injected into the gas chromatograph without prior separation of reactants from the products.

Instrumentation

GC analyses were made on a Perkin-Elmer (Norwalk, CT, U.S.A.) Sigma 2B gas chromatograph equipped with dual detectors; flame ionization and nitrogen–phosphorus. Unless otherwise specified the column was 15 m × 0.23 mm I.D., and the stationary phase was polydimethylsiloxane (DB-1). The temperature was programmed from 120 to 280°C at a rate of 12°C per min. The injector and the detector were maintained at 300°C and the split ratio on the injector was 32:1. In experiments where the nitrogen–phosphorus detector was used, helium was the make-up gas with a flow-rate of 30 ml/min, and the flow-rates of air and hydrogen at the detector were 100 and 2.6 ml/min, respectively. The nitrogen–phosphorus detector was optimized for maximum selectivity[26], and the bead current was kept below maximum sensitivity in order to increase the lifetime of the bead. The carrier gas was helium with an initial flow-rate of 0.7 ml/min.

GC–mass spectrometry (MS) experiments were conducted on a Kratos MS-50TA mass spectrometer. In all cases the electron energy was 70 eV, the source temperature was 220°C and the transfer lines were kept at 260°C. The GC parameters were similar to those described above, scanning speeds varied depending on the separation and helium was the carrier gas.

RESULTS AND DISCUSSION

The kinetic parameters for the silylation reaction of chlorophenols and chlorinated hydroxybiphenyls by CEDMSDEA, were studied at three temperatures in order to optimize the analytical procedure. In these experiments the nineteen chlorophenol isomers and some chlorinated hydroxybiphenyls (see Table I) were treated with CEDMSDEA in ethyl acetate at temperatures of 25, 55 and 75°C. It was found that the compounds reacted readily with an excess of the reagent (80:1) and that varying the temperature had no significant effect on the overall reaction yield. The yield was calculated by measuring the disappearance of the reactant and for all the above mentioned compounds it was found to be >97.5% within experimental error. Fig. 1 shows the variation of the reaction yield with time at different temperatures for 3,4-dichloro- and 2,4,6-trichlorophenols. It can be seen that the reaction is completed within minutes and that the overall yield does not vary when the temperature is increased from 25°C to 75°C. Because of this, a reaction time of 15 min is considered sufficient to derivatize quantitatively these phenolic compounds and was used in other experiments. The results obtained indicate that CEDMSDEA is a good analytical silylating reagent for these phenolic compounds since the reaction is rapid, quantitative and can be performed under very mild conditions.

The GC behavior of the formed ethers of chlorophenols and chlorinated hydroxybiphenyls was examined and found to be similar to that of the corresponding trimethylsilyl ethers. Fig. 2 shows the chromatogram obtained by analyzing a mixture containing the nineteen isomers of chlorophenol with the nitrogen–phosphorus detector. The peaks corresponding to the compounds of interest (numbered 1–19) have a symmetrical shape and no tailing can be observed. Comparison of the retention times obtained for these ethers with those of the corresponding trimethylsilyl homologues analyzed (with the flame ionization detector) on the same stationary phase, indicates that cyanoethyldimethylsilyl derivatives have retention which are 1.6–2.5

TABLE I

MEAN VALUES FOR RELATIVE RETENTION TIMES AND RELATIVE RESPONSE FACTORS
OF THE NITROGEN–PHOSPHORUS DETECTOR FOR CHLORINATED PHENOLIC COM-
POUNDS

| No.	Compound	Relative retention time	Relative response factor
1	2-Chlorophenol	0.585	0.60
2	3-Chlorophenol	0.605	0.77
3	4-Chlorophenol	0.629	0.86
4	2,3-Dichlorophenol	0.788	0.84
5	2,4-Dichlorophenol	0.764	0.82
6	2,5-Dichlorophenol	0.747	0.88
7	2,6-Dichlorophenol	0.754	0.85
8	3,4-Dichlorophenol	0.812	0.81
9	3,5-Dichlorophenol	0.754	0.98
10	2,3,4-Trichlorophenol	0.980	0.96
11	2,3,5-Trichlorophenol	0.916	0.95
12	2,3,6-Trichlorophenol	0.936	0.78
13	2,4,5-Trichlorophenol	0.930	1.03
14	2,4,6-Trichlorophenol	0.905	0.94
15	3,4,5-Trichlorophenol	0.974	1.00
16	2,3,4,5-Tetrachlorophenol	1.122	1.11
17	2,3,4,6-Tetrachlorophenol	1.100	1.07
18	2,3,5,6-Tétrachlorophenol	1.087	1.04
19	2,3,4,5,6-Pentachlorophenol	1.277	1.17
20	Triphenylamine	1.000	—
21	3-Chloro-2-hydroxybiphenyl	1.153	0.78
22	3-Chloro-4-hydroxybiphenyl	1.335	0.65
23	5,4',5',6'-Tetrachloro-4-hydroxybiphenyl	—	0.52
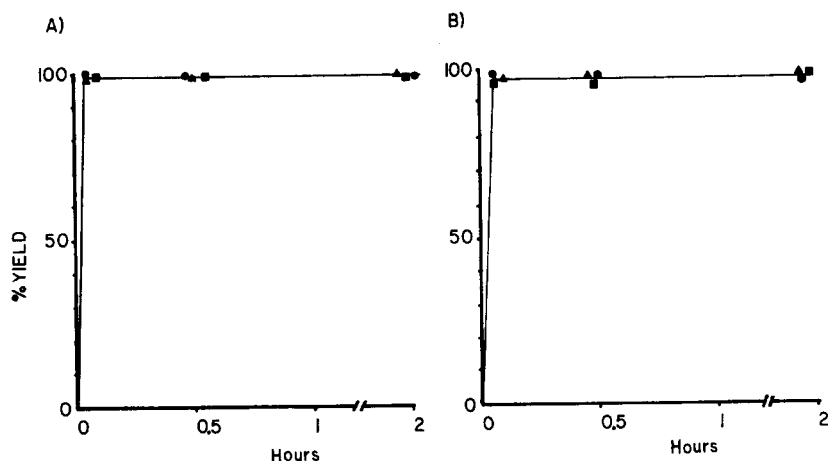
Fig. 1. Kinetic data for the reaction of chlorophenols with 2-cyanoethyldimethyl(diethyl)aminosilane; (A)
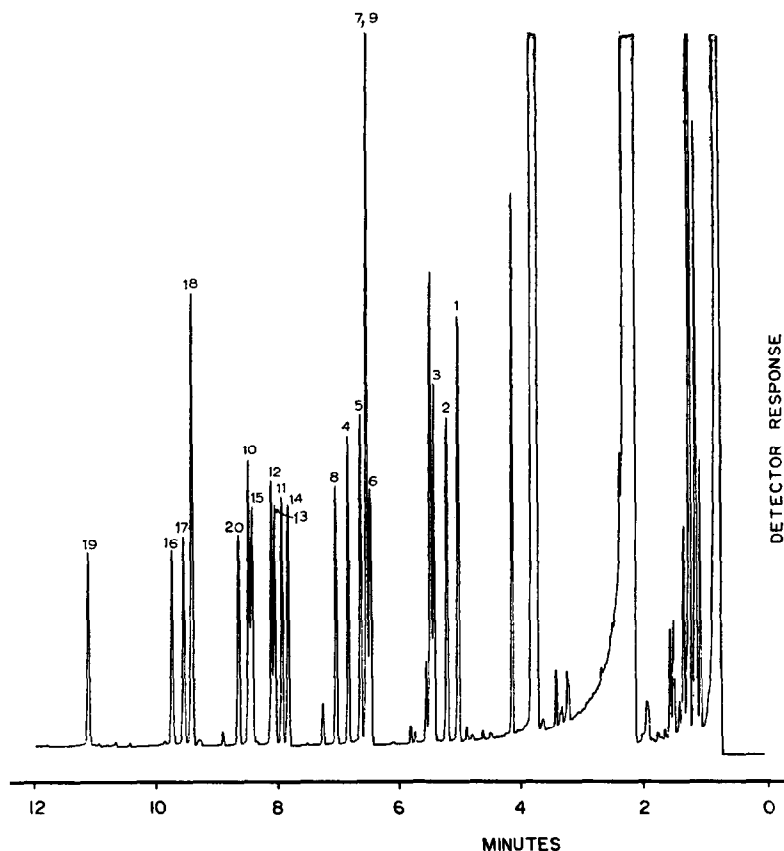3,4-dichlorophenol; (B) 2,4,6-trichlorophenol. Temperatures: ■ = 25°C; ● = 55°C; ▲ = 75°C.

Fig. 2. Chromatogram of a mixture containing the nineteen chlorophenols. Detector; nitrogen–phosphorous column, 15 m DB-1; temperatures, 120°C to 280°C at 12°C min^{-1}. Numbers 1–20 refer to Table I.

times longer. This is to be expected since the cyanoethyl group in the ethers is bulkier than the methyl group in trimethylsilyl and the derivatives are less volatile. One can use this to advantage since it permits the separations of analytes from volatile impurities in the mixture. The chromatographic conditions used to obtain the data shown on Fig. 2 were not optimized for resolution but represent a compromise between resolution and time of analysis. In this example temperature programming at 12°C/min was used and it can be seen that the baseline of the nitrogen–phosphorus detector is not affected thus permitting rapid analysis.

Since one of the objectives of this project was to study the response of the detector to cyanoethyldimethylsilyl derivatives, pertinent analytical parameters involved in the detection of the studied compounds were measured. Parameters that can be used to evaluate the usefulness of the approach are sensitivity, linearity of response over a wide range of concentrations, values of the relative response factors for the isomers and low limits of detection. To calculate relative response factors and verify the linearity of response with concentration, standard calibration curves were plotted for all compounds mentioned in this study. Fig. 3 shows typical response
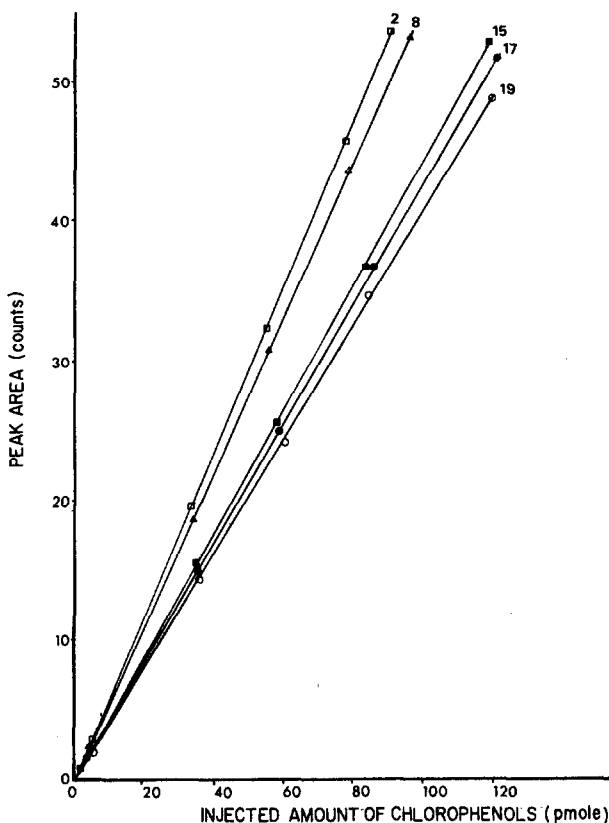
Fig. 3. Concentration response plots for 2-chloro-(2) 3,4-dichloro-(8) 3,4,5-trichloro-(15) 2,3,4,6-tetra-chloro-(17) and pentachloro-(19) phenols.

plots obtained for mono-, di-, tri-, tetra- and pentachlorophenols. The relative response factors calculated from the slope (ratios) are listed in Table I. The response of the nitrogen–phosphorus detector to all phenolic compounds studied was found to be linear from 500 ng (upper amount injected) to the limit of detection which was established at $(5 \pm 2) \cdot 10^{-13}$ g N (signal-to-noise ratio = 5) under our experimental conditions. Lower limits can be obtained by increasing the bead current but the detector becomes unstable and unreliable. It is also possible to tune the detector to achieve a better sensitivity but selectivity, which is an important feature in the experimental signal-to-noise ratio, is lowered.

The relative response factors of the chlorophenols are listed in Table I with those of a few hydroxybiphenyls. It can be seen that the values obtained for the various compounds studied are close to unity. Examination of the relative response factors for chlorophenols and chlorinated hydroxybiphenyls reveals that both the position and the number of chlorine atoms on the molecules have little effect on the response of the detector towards the derivatives. The mean value of the relative response factors is 0.88 with minimum and maximum values of 0.60 and 1.17 for chlorophenols. This is to be expected if the formation of the cyano anion is mostly

responsible for the detection of these substances[24], since the nearby environment of the cyano radical is essentially the same in all compounds and it is isolated from the chlorinated organic moiety. The finding that the detection limits is approximately the same in g N for all substances also supports this rationalization. These results demonstrate that the use of the nitrogen–phosphorus detector permits the quantitative analysis of chlorinated phenols in mixtures without the disadvantages caused by the disparities in response factors observed with the electron-capture detector, which is more sensitive to the number and position of the halogens. A comparison between the detection parameters obtained with the nitrogen–phosphorus and flame ionization detectors was also made and is shown on Fig. 4. The sensitivity of the cyanoethyldimethylsilyl ethers measured with the former is 10–12 times larger than the sensitivity of the cyanoethyldimethylsilyl and the trimethylsilyl ethers to the latter, neglecting the selectivity of the nitrogen–phosphorus detector, which has an important influence on the signal-to-noise ratio and on the limits of detection. Under our experimental conditions the selectivity of the detector was *ca.* 10^4 as measured from benzonitrile and decane, but this value can vary according to the matrix from which the analytes are obtained.
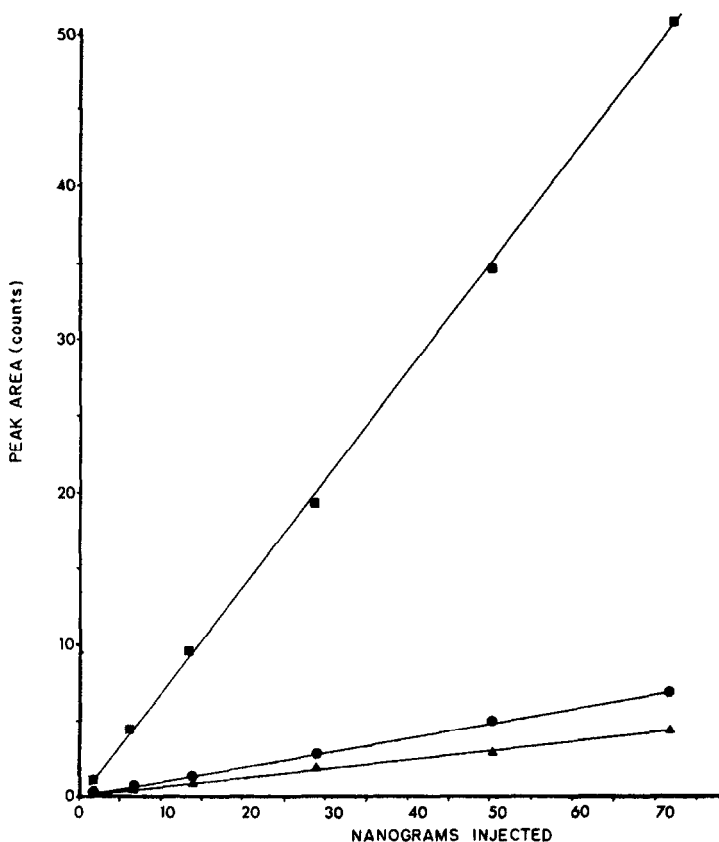

Fig. 4. Concentration response plot for 3,4,5-trichlorophenol derivatives: ■ = cyanoethyldimethylsilyl (NPD); ▲ = cyanoethyldimethylsilyl (FID); ● = trimethylsilyl (FID).

Because MS is often used with GC to confirm the identity or to analyze these compounds at low levels, the behaviour of cyanoethyldimethylsilyl ethers under electron impact ionization was also studied. The mass spectrum of the 3-chloro-4-hydroxybiphenyl derivative is shown in Fig. 5 and typical ions in the mass spectra of the derivatives of phenolic compounds are listed in Table II. The mass spectra of cyanoethyldimethylsilyl ethers are very similar to the trimethylsilyl and *tert.*-butyldimethylsilyl ethers and the differences can be attributed to the presence of the cyanoethyl radical. The molecular ions are always present with variable relative intensities (10–45%) and are seen at odd masses since they contain an odd number of nitrogen. The elimination of the cyanoethyl radical, which is preferred over the methyl, produces important ions observed at (M − 54). These ions, although similar to the (M − 57) encountered in the mass spectra of *tert.*-butyldimethylsilyl ethers, are more specific since a loss of 54 a.m.u. is quite uncommon in the spectra of organic compounds. Whereas the presence of chlorine in the *ortho* position yields an intense rearrangement ion at m/z 93 as can be seen on Table II, the (M − 54) ion is the base peak if there is no chlorine in the *ortho* position. This feature is not specific to cyanoethyldimethylsilyl ethers and has previously been reported for trimethylsilyl ethers[25]. The electron impact fragmentation is structure sensitive and the presence of the molecular ion with the intense ion at (M − 54), combined to the ion at m/z 93 permits the specific detection of these substances using selected-ion monitoring (SIM). The m/z 93 ion can be used as a probe to detect the presence of chlorinated phenolic compounds and the M$^+$ to confirm the identity of the compound.
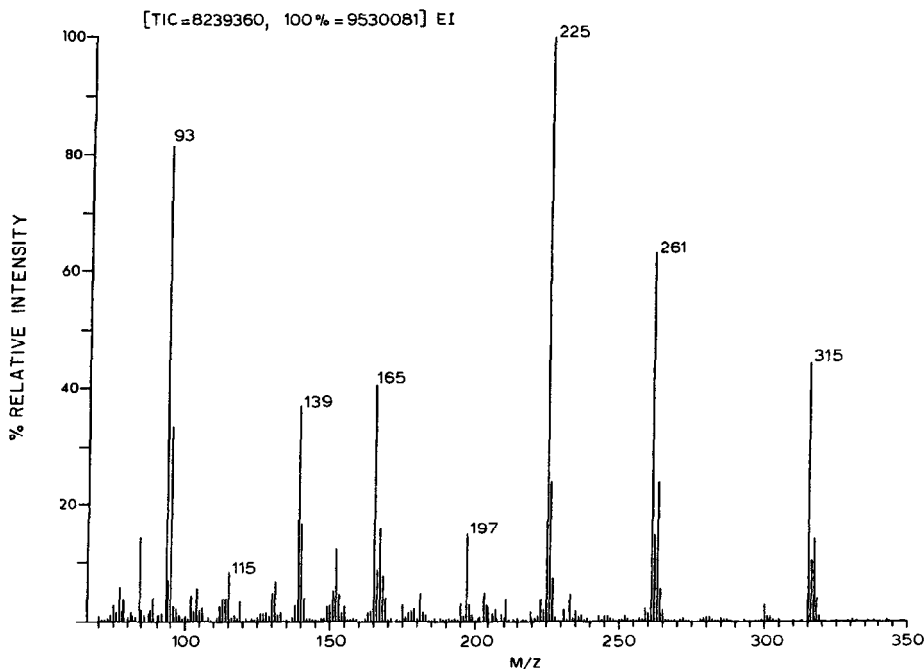

Fig. 5. Mass spectrum of 3-chloro-4-hydroxybiphenyl cyanoethyldimethylsilyl ether.

TABLE II

SPECIFIC IONS IN THE MASS SPECTRA OF CYANOETHYLDIMETHYLSILYL ETHERS OF CHLORINATED PHENOLIC COMPOUNDS

Compound	Mol. wt.	Ion intensity as % of base peak			
		M^+	$(M - 15)^+$	$(M - 54)^+$	93^+
2-Chlorophenol	239	10	3	90	100
3-Chlorophenol	239	25	2	100	22
4-Chlorophenol	239	20	1	100	18
2,3-Dichlorophenol	273	6	3	77	100
2,4-Dichlorophenol	273	13	2	70	100
2,5-Dichlorophenol	273	9	2	86	100
2,6-Dichlorophenol	273	8	4	100	65
3,4-Dichlorophenol	273	33	2	100	27
3,5-Dichlorophenol	273	23	1	100	20
2,3,4-Trichlorophenol	307	6	2	50	100
2,3,5-Trichlorophenol	307	7	2	45	100
2,3,6-Trichlorophenol	307	7	3	85	100
2,4,5-Trichlorophenol	307	6	2	44	100
2,4,6-Trichlorophenol	307	20	4	83	100
3,4,5-Trichlorophenol	307	20	2	100	19
2,3,4,5-Tetrachlorophenol	341	9	2	40	100
2,3,4,6-Tetrachlorophenol	341	15	2	60	100
2,3,5,6-Tetrachlorophenol	341	7	3	56	100
Pentachlorophenol	375	7	4	100*	92
3-Chloro-2-hydroxybiphenyl	315	10	3	100	93
3-Chloro-4-hydroxybiphenyl	315	44	3	64	84

* For this compound the (M + 2) isotope is the base peak.

CONCLUSION

The results presented in this study indicate that the combination of cyano-ethyldimethylsilyl ethers with the nitrogen–phosphorous detector can be useful in the quantitative analysis of chlorinated substances. Use of CEDMSDEA offers a rapid one-step derivatization reaction for these substances under mild conditions and the detector provides a selective and sensitive detection system with a good dynamic range for which the response factors of all phenolic compounds are close to unity. It also offers an alternative to ECD and its operation is not severely affected by contamination from the matrix or from the presence of halogens in the analytes. The cyanoethyldimethyl ethers also provide adequate features for analysis or confirmation by GC–MS since they can be detected by SIM using the M^+, the $(M - 54)^+$ and the m/z 93 ions which are abundant and highly specific to the structure of the chlorinated phenolic compounds.

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