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THE USE OF BENTONE 34*-COATED SUPPORTS IN COLUMN CHROMATOGRAPHY AND THEIR POTENTIAL APPLICATION IN THE FIELD OF ORGANIC POLLUTION ANALYSIS

D. W. GRANT, R. B. MEIRIS and M. G. HOLLIS

The British Carbonization Research Association, Wingerworth, Chesterfield (Great Britain)

SUMMARY

The use of columns containing Bentone 34 as a stationary phase in gas–solid chromatography (GSC) and high-performance liquid chromatography (HPLC) is discussed. Particular reference is made to potential applications in the separation of polynuclear hydrocarbons (PNHs) and of monohydric and dihydric phenols. A rapid separation of monohydric phenols with a high degree of *meta–para* selectivity is achieved in GSC columns packed with glass beads coated with a liquid-modified Bentone 34. Similarly, highly selective HPLC columns are obtained by using a Zipax support coated with Bentone 34 only. The latter give symmetrical peaks for PNHs and phenols and measurement at the ppm level is possible using a UV monitor.

INTRODUCTION

There are various analytical problems associated with the monitoring of gaseous or aqueous wastes from coal carbonization and tar refining operations, particularly where these wastes enter the atmosphere or are discharged into rivers. Perhaps the most important of these problems are the individual determinations of trace amounts of polynuclear hydrocarbons (PNHs) in atmospheric samples and of phenolic compounds in aqueous effluent samples. The extent of investigations into PNH analysis is well known as numerous publications have appeared in the literature in recent years. The determination of phenols in aqueous effluents has also received attention and a valuable routine method based on gas chromatography (GC) of the trimethylsilyl ether derivatives after solvent extraction has been evolved by Cooper and Wheatstone[1]. Nevertheless, the continuing improvement of methods in relation to speed, sensitivity and accuracy is an important feature of the research effort in the carbonization industry, particularly as legislation on minimum consent levels becomes more demanding.

It is within this general context that both GC and liquid chromatographic (LC) techniques are being studied and the possible use of high-selectivity stationary

* Dimethyldioctadecylammonium montmorillonite. Bentone 34 is a trademark of F. W. Berk and Company Limited, London, Great Britain.

phases is being assessed. Organic derivatives of the clay minerals have been used extensively in GC since White and Cowan[2] first demonstrated linearity of adsorption for alkanes on Bentone 34. The usefulness of these organo-clay derivatives evolved originally from the high degree of selectivity they exhibited towards *meta-* and *para-*substituted aromatic compounds. This selectivity was first noticed by Hughes *et al.*[3], although tailing peaks were obtained when Bentone 34 alone was used. Mortimer and Gent[4], however, showed that the addition of a liquid modifier improved the peak symmetry for low-polarity compounds, but polar compounds still gave a degree of tailing. Taramasso and Timidei[5] have studied organic derivatives of other clay materials, particularly those of beidellite and vermiculite, and found that symmetrical peaks could be achieved on these materials without the addition of a liquid modifier. One of the present authors later showed that Bentone 34 coated onto glass beads, but without a liquid modifier, also produced symmetrical peaks for a wide range of compounds, including several of high polarity[6]. In a later publication[7] it was shown that glass beads coated with modified or unmodified Bentone 34 gave efficient columns with high effective plate speeds and plate heights of 0.3–0.4 mm. These columns exhibited selective properties to various types of isomeric compounds including anthracene and phenanthrene, *cis-* and *trans-*methylcyclohexanols, quinoline and isoquinoline and *meta-* and *para-*aromatic isomers. These results suggested that Bentone 34 was potentially a far more versatile stationary phase than had previously been realized. The selectivity between anthracene and phenanthrene is particularly significant as it indicates that useful differences would also be obtained between higher-molecular-weight PNHs if the technique could be extended to cover this region. Unfortunately, Bentone 34 starts decomposing at about 180° and consequently high-molecular-weight materials cannot be reasonably eluted. This problem was overcome by employing the material as a stationary phase in LC columns, but coated onto Zipax support.

Thus, it is felt that Bentone 34 can find useful applications as a stationary phase in both GC and LC. GC applications would include highly selective separations of monohydric phenols without the need for derivatization, while LC applications would include selective separations of PNHs, monohydric and dihydric phenols.

GC ANALYSIS OF MONOHYDRIC PHENOLS

Discussion

The use of Bentone 34-coated glass beads (BCGB) as a GC packing for the analysis of phenols offers several advantages in principle over the more conventional method based on derivatization. The main advantage is that aqueous solutions can be injected directly onto the column, hence avoiding the need for solvent extraction and derivatization with their attendant errors.

Another clear advantage is the improved plate speed of BCGB columns[7] in comparison with conventional columns. This follows from the expression:

$$\text{Effective Plate Speed } (EPS) = \frac{\bar{u}}{h}\left[\frac{k'^2}{(1+k')^3}\right]$$

where $\bar{u}$ is the mean linear carrier gas velocity, h is the true plate height at $\bar{u}$, and k' is the capacity ratio. The k' values on the BCGB columns are between one and two

orders of magnitude smaller than on conventional columns because of the low phase loading. Also, the ratio $\bar{u}/h$ is higher in value because of the rapid rate of mass transfer of the solute in the thin stationary phase layer.

Another advantage arising from the low phase loading is that relatively low column temperatures can be employed, which ensures stability of column operation and allows higher separation factors to be achieved.

Experimental

A Pye 104 FID gas chromatograph (Pye Unicam, Cambridge, Great Britain) was employed with a Smith's Servoscribe Type RE511.20 potentiometric recorder (Smith Industries, Wembley, Middlesex, Great Britain). Standard glass columns were used, 1.5 m × 6.4 mm I.D., and were packed with 100 BS mesh glass beads (BDH, Poole, Great Britain) coated with 0.12% (w/w) of the liquid-modified Bentone 34.

The packing was prepared and the columns packed by the procedures described in an earlier publication[7]. Before use, however, the commercial Bentone 34 (F. W. Berk and Co., London, Great Britain) was washed exhaustively with water until the washings were free from halide. The material was then filtered and dried. The most effective stationary phase for the separation of phenols was found to be a three component blend of Bentone 34, trimer acid and either silicone OV-17 or squalane as a non-polar additive. The presence of trimer acid was necessary to prevent serious tailing with phenols and the non-polar additive improved the plate characteristics of the column. The latter effect is attributed to improved gelation of the Bentone 34, which should favour a more even dispersion of the stationary phase over the glass bead surfaces. The effect of the non-polar additive is shown in Fig. 1, which is a plot of plate height *versus* the amount of OV-17 added to a constant ratio of Bentone 34 to trimer acid. There is clearly an optimum value for the amount of OV-17 added at about 0.02% (w/w). Above this level the plate height increases owing to the diminishing overall concentration of Bentone 34.

The extreme selectivity of the BCGB columns towards phenolic isomers is demonstrated by the results plotted in Fig. 2. This shows relative retention volumes at a column temperature of 120° plotted against the ratio of trimer acid to Bentone 34 in a stationary phase in which the non-polar additive was omitted. The presence of

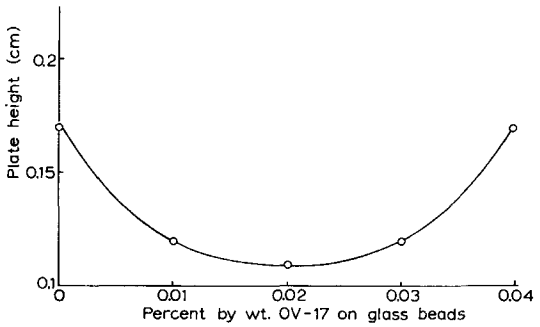

Fig. 1. Effect on plate heights of non-polar additive to BCGB columns. Stationary phase loading: 0.08% of trimer acid–Bentone 34 + additive (2:1).

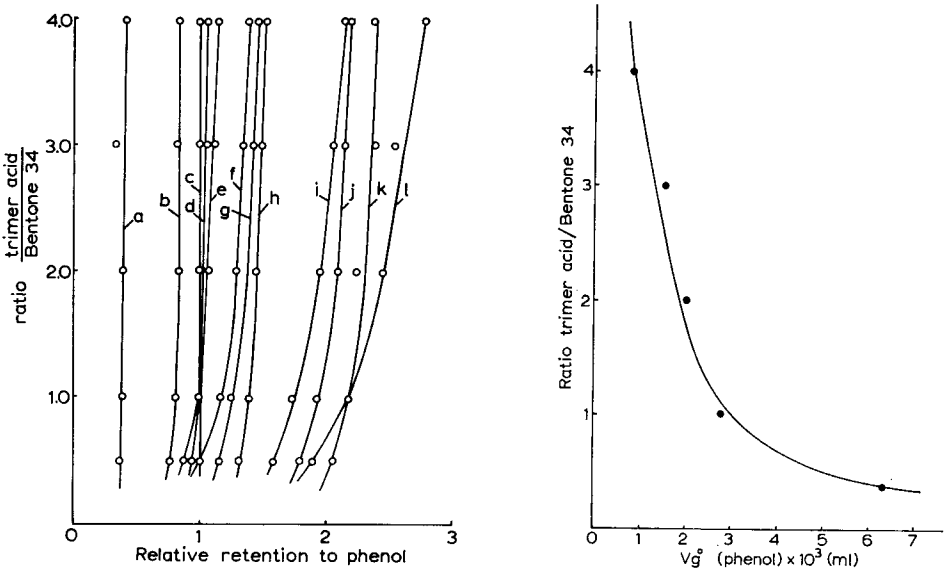

Fig. 2. Relative retention characteristics of monohydric phenols on Bentone 34–trimer acid column. Column temperature, 120°. a = 2,6-Xylenol; b = *o*-cresol; c = phenol; d = 2-ethylphenol; e = 2,4- + 2,5-xylenol; f = *p*-cresol; g = 2,3-xylenol; h = *m*-cresol; i = 4-ethylphenol; j = 3-ethylphenol; k = 3,5-xylenol; l = 3,4-xylenol.

Fig. 3. Effect of Bentone 34 concentration on V_g^0 values for phenol. Stationary phase loading: 0.08% (w/w) on glass beads. Column temperature, 122°.

this additive in subsequent columns modified the effects slightly, but had little effect on the *meta–para* selectivity.

The selectivity is seen to increase rapidly with increasing Bentone 34 content but, perhaps surprisingly, the lines tend to converge with increasing selectivity. The specific retention volumes, however, also increase rapidly with increasing Bentone 34 content, as is shown by Fig. 3 for phenol.

It is clear that at high Bentone 34 concentrations the effects of differing vapour pressure become almost insignificant and the main factors influencing the separation are of steric origin, which give highly specific interactions with the adsorbent. Conversely, at high trimer acid concentrations both hydrogen bonding and vapour-pressure effects play a significant part in the separation. This balance of interactive forces explains the reversals in the elution order shown by the curves as the composition is changed.

The degree to which the use of Bentone 34 imparts selectivity to the separation is illustrated by the results in Table I, which gives boiling points and retention characteristics for *meta-* and *para*-substituted isomeric pairs. The ratio of the activity coefficients was calculated from:

$$\frac{\gamma_{meta}}{\gamma_{para}} = \frac{t_{meta} \cdot p^s{}_{meta}}{t_{para} \cdot p^s{}_{para}}$$

where t_{meta}, t_{para} are the adjusted retention times and $p^s{}_{meta}$, $p^s{}_{para}$ are the saturation

TABLE I

SELECTIVITY DATA OF BCGB COLUMNS TOWARDS *meta*- AND *para*-SUBSTITUTED ISOMERS OF MONOHYDRIC PHENOLS

Ratio Bentone 34 to trimer acid = 3:1; stationary phase loading = 0.08%.

Compound	*Boiling point* (°C)	*Separation factor* t_{meta}/t_{para}	$\gamma_{meta}/\gamma_{para}$
meta-Cresol	202.23	1.33	1.45
para-Cresol	201.94		
2,5-Xylenol	211.13	1.06	1.06
2,4-Xylenol	210.93		
2,3-Xylenol	216.87	1.48	1.26
2,4-Xylenol	210.93		
3,5-Xylenol	221.69	1.07	1.62
3,4-Xylenol	226.95		
3-Ethylphenol	218.2	1.14	1.14
4-Ethylphenol	219		

vapour pressures for the *meta*- and *para*-components, estimated by interpolation of available data.

The isomeric pairs 2,3- and 2,4-xylenol and 3,5- and 3,4-xylenol have appreciable boiling-point differences and consequently are easy to separate by conventional GC on a low-selectivity column. For the first pair the stationary-phase selectivity has the effect of increasing the separation already caused by the vapour-pressure difference, but with the second pair the selectivity acts against the effect of vapour pressures to the extent that the elution order is reversed. The strong degree of *meta–para* selectivity experienced with Bentone 34 has not been satisfactorily explained mechanistically, but there is undoubtedly a stronger affinity between the organo-clay surface and *meta*-substituted phenols than with other phenols. The results in Table I show that this affinity increases substantially when there are two *meta*-substituted groups with respect to the hydroxyl group, but decreases when there is an *ortho*-substituted group present.

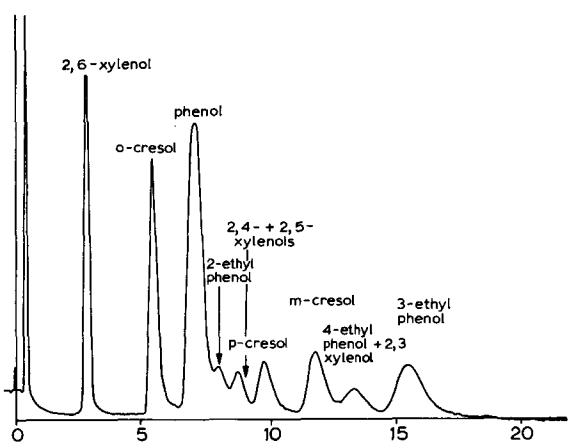

Fig. 4. Chromatogram of monohydric phenols in aqueous solution on BCGB column. Column: 0.12% Bentone 34–trimer acid–OV-17 on glass beads (1:1:1); temperature, 122°.

If we refer again to the curves shown in Fig. 2 it is clear that an optimum separation of the C_6 to C_8 monohydric phenols should occur at a ratio of about 2:1, trimer acid to Bentone 34. Squalane or OV-17 is added to this, as explained earlier, to improve the plate characteristics, hence the final composition chosen for the GC of phenols was 0.04% Bentone 34, 0.02% trimer acid and 0.02% squalane on the glass beads. Fig. 4 is a chromatogram of an aqueous injection of monohydric phenols.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) APPLICATIONS OF BENTONE 34

Discussion

The use of Bentone 34 as a stationary phase in HPLC suggested itself when attempts to apply BCGB columns to high-molecular-weight PNHs failed because of the thermal instability of the organo-clay above 180°. However, by coating a recognized HPLC pellicular support with Bentone 34 from a benzene dispersion a column packing was obtained that gave stable and selective characteristics.

As the Bentone 34 is essentially a solid adsorbent, the columns appeared to be useful for separating both polar and non-polar materials by an appropriate choice of mobile phase. Such columns have the additional advantage over alumina or silica gel adsorption columns that aqueous or partly aqueous mobile phases can be employed.

Experimental

A Chromatronix 3100 liquid chromatograph was employed (Spectro-Physics, Harpenden, Hertfordshire, Great Britain) equipped with 20-μl sample valve and UV monitor allowing measurement at 254 nm and 280 nm. Stainless-steel columns were used, 500 mm × 2.1 mm bore, and these were polished internally before being packed.

The packing was prepared by dispersing 50 mg of Bentone 34 in 2–3 ml of benzene and adding the resulting mixture to 10 g of Zipax (DuPont, Hitchin, Great Britain), and thoroughly stirred on a water bath until the benzene had been removed. This left a dry, homogeneous, and free-flowing packing identical in appearance to the original Zipax.

Columns were packed after inserting a porous Teflon* plug into one end by a "dry-pack" technique. A steel rod of slightly smaller diameter than the column I.D. was inserted down the bore after each small addition of the prepared packing and the column was vibrated electrically to give a gentle tamping effect under the weight of the rod. The column was also tapped vertically onto a solid surface until no further contraction of the packing volume occurred, as observed by marking the rod. After completion of the packing procedure a further Teflon plug was placed in the upper part of the column.

The columns were run with methanol–water mixtures as the mobile phase. For PNHs the composition was methanol–water (2:1) and for phenols, the composition was methanol–water (1:4). All solvents were degassed under vacuum before use.

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* Trademark of E. I. Du Pont de Nemours, Wilmington, Del., U.S.A.

Separation of PNHs

The UV detector was set to 254 nm and the performance of the column was assessed with regard to the separation of PNHs. Samples of these compounds at approximate concentrations of 1 mg per 100 ml for each component were injected via the sample valve with the monitor sensitivity set to 0.16 O.D. unit for f.s.d. The resulting peaks were symmetrical, indicating that adsorption on the Bentone 34 was linear for these compounds under the conditions used.

Fig. 5 shows plots of plate height (h) *versus* mobile phase flow-rate (F_m), for the three compounds naphthalene, fluoranthene and chrysene.

The anomalous results for naphthalene are probably due to the effect of the sample valve on rapidly eluted peaks. Thus, it seems possible that much lower plate heights would be achieved for rapidly eluted components if a more efficient injection technique were to be used. A more detailed study of these columns is being carried out with regard to their plate performance and effective speed and these results will be published in a later paper.

Table II lists retention characteristics for a number of PNHs that can occur in atmospheric samples, determined on both a Bentone 34 HPLC column and a conventional column packed with Corasil C18 (Waters Ass., Stockport, Great Britain).

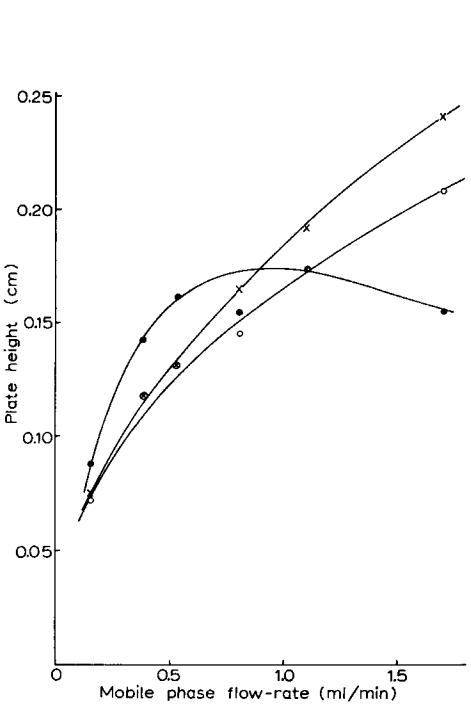
There are considerable differences in the elution characteristics of the two columns. The Corasil C18 column gives a separation based mainly on molecular weight, but the Bentone 34 column shows several wide divergencies, notably for phenanthrene, fluoranthene, benzo(k)fluoranthene and 1,2,3,4-dibenzanthracene, thus, there is apparently a strong steric effect, which should be of considerable advantage in this type of separation. For instance, benzo(k)fluoranthene is not separated on the Corasil column from benzo(a)pyrene and the UV detector cannot be set to detect either of these compounds specifically in the presence of the other. This problem does not exist with the Bentone 34 column, which gives a separation factor of greater than 2 to 1 for these compounds.

Fig. 6 shows a separation of 50 ng each, of several PNHs. This does not show

TABLE II

COMPARISON OF RETENTION CHARACTERISTICS OF PNHs ON BENTONE 34 AND CORASIL C18 COLUMNS

Compound	*Mol. wt.*	*k' Values*	
		Bentone 34	*Corasil C18*
Fluorene	$C_{13}H_{10} = 166$	0.16	0.44
Anthracene	$C_{14}H_{10} = 178$	1.00	0.56
Phenanthrene	$C_{14}H_{10} = 178$	0.88	0.54
Pyrene	$C_{16}H_{10} = 202$	1.70	0.87
Fluoranthene	$C_{16}H_{10} = 202$	2.1	0.77
Chrysene	$C_{18}H_{12} = 228$	4.7	1.52
1,2-Benzanthracene	$C_{18}H_{12} = 228$	4.6	1.55
Benzo(a)pyrene	$C_{20}H_{12} = 252$	10.1	3.21
Benzo(e)pyrene	$C_{20}H_{12} = 252$	9.7	2.47
Benzo(k)fluoranthene	$C_{20}H_{12} = 252$	24.4	2.84
1,2,5,6-Dibenzanthracene	$C_{22}H_{14} = 278$	13.5	5.12
1,2,3,4-Dibenzanthracene	$C_{22}H_{14} = 278$	41.3	4.17



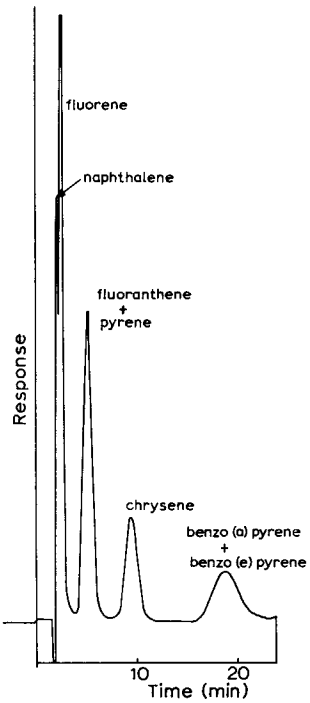


Fig. 5. Plate characteristics of Bentone 34-coated HPLC column towards PNHs. Mobile phase: methanol–water (2:1); UV monitor: 254 nm. ●—●, Naphthalene ($k' = 0.17$); ○—○, fluoranthene ($k' = 0.83$); ×—×, chrysene ($k' = 1.62$).

Fig. 6. Separation of PNHs on Bentone 34–Zipax column. O.D. = 0.16 f.s.d.

a separation of benzo(*a*)pyrene from benzo(*e*)pyrene, which in this instance indicates that the selectivity acts disadvantageously. These two isomers are normally easy to separate by HPLC. However, this is not necessarily serious as the carcinogenic benzo(*a*)pyrene can be measured selectively in the presence of benzo(*e*)pyrene at *ca.* 403 nm. Alternatively, of course, separate columns could be employed, connected in parallel for convenience.

Separation of monohydric and dihydric phenols

The Bentone 34 column was tested for potential application to the analysis of monohydric and dihydric phenols in dilute aqueous solution. The mobile phase was changed to the water–methanol (4:1) composition and the pressure adjusted to give a flow-rate of 0.7 ml/min. The eluate was monitored at 280 nm. Dilute solutions containing about 10 ppm of phenols in water were chromatographed using the 20-μl sample valve for injection.

The column did not exhibit the same degree of *meta–para* selectivity that is normally achieved in GC and, in fact, no separation was obtained between the *meta*- and *para*-cresols. Nevertheless, the peaks showed only a low degree of tailing, which suggested that adsorption isotherms, even for the polar compounds, were almost linear. This apparent loss of *meta–para* selectivity is probably a result of the increase

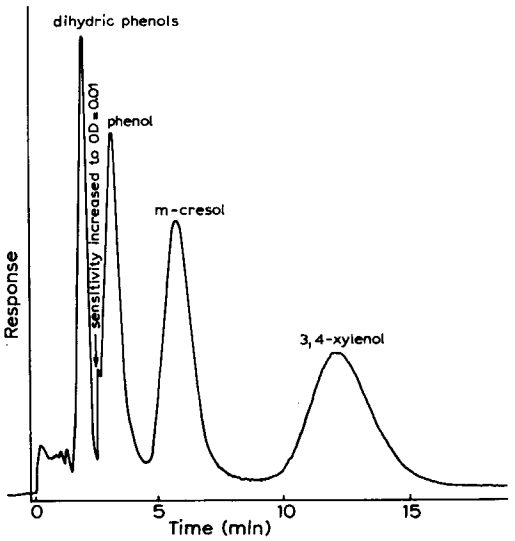


Fig. 7. Separation of monohydric and dihydric phenols on Bentone 34–Zipax column.

in the organo-clay basal spacing at low temperatures first noted by Taramasso and Veniale[8]. Perhaps of greater importance was the effect on dihydric phenols, which were eluted very quickly and before any of the relevant monohydric phenols. This is illustrated by Fig. 7 for a simple mixture containing 10 ppm of each component. Thus, it appears that the total dihydric phenols can be separated from other phenols present and measured together.

This result also indicates that a fast analysis would be possible giving phenol, total cresols and total xylenols and this would be sufficient for many purposes. A more specific analysis can, of course, be carried out using the GC technique. There was no difficulty in detecting the 10-ppm level of individual phenols and direct detection down to less than 1 ppm was possible by using higher sensitivities.

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