# Analysis of Active Compounds Using the New, Highly Inert HP-5MSi Column

Application

**Environmental Analysis**


## Authors

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## Abstract

**Quantitating low levels of very active compounds can be facilitated with the use of a highly inert capillary column. The use of the highly inert HP-5MSi column in conjunction with the Agilent 6890/5973 inert gas chromatograph/ mass spectrometer (GC/MS) system allows the measurement of pesticides and environmental contaminants at low levels while maintaining response linearity over a wide concentration range.**


## Introduction

The analysis of active compounds by gas chromatography (GC) continues to be challenging in such areas as pesticide [1], environmental [2], and drug analysis [3]. Adding to this problem is the need to reduce detection limits (DLs) to the low nanogram level. To meet these demanding requirements, almost every GC column manufacturer has developed a low bleed column. Low column bleed reduces detector noise and increases signal-to-noise, which improves DLs. However, lowering column bleed alone does not address the problem of peak tailing that results from interactions of polar compounds with active sites within the sample flow path of the GC.

Active compounds will adsorb on active sites throughout the sample flow path including the injection port, liner, and seal in a split/splitless inlet, along with the capillary column and any metal detector parts. Various deactivations were used to deactivate glass liners and capillary columns, and different metal passivation techniques have had similar effects on seals and mass spectrometer sources [4]. Unfortunately, treating these parts in such a manner does not completely remove all the unwanted active sites.

One of the major sources of active sites can be the capillary column. Keeping in mind the large surface area of a standard column and long residence time of an analyte in the column, it is not surprising that peak tailing and response-loss can occur even with the most highly deactivated columns. Further gains in detection sensitivity could be realized if the number of column active sites was reduced by more complete deactivation procedures.

We report here the use of a new HP-5MSi (MS Inert) column that has low column bleed and is specially deactivated for the analysis of polar compounds that show noticeable adsorption, especially at low concentrations. The column performance was evaluated using a specialized testing protocol and then subsequently tested with different active pesticides and environmental compounds.

### Sample Preparation

As a qualitative test for column inertness, an application-specific test sample (Test Mix #1) was prepared. Individual components were obtained from Sigma-Aldrich (St. Louis, MO) and diluted in dichlorobenzene.

Pesticide samples were obtained from AccuStandard (New Haven, CT) at 1000 µg/mL in different solvents and diluted as needed. Linearity studies were performed using a sample of terbofos, malathion, endrin aldehyde, and DDT over the range 0.8–100 ng/µL in methanol (0.4–50-ng injected).

A number of substituted phenols and semivolatile amine samples were obtained from AccuStandard (New Haven, CT) at 2000 µg/mL in different solvents and diluted as needed. Linearity studies were performed using a sample of 2,4-dinitrophenol, pentachlorophenol, and n-nitrosodipropylamine over the range 5–200 ng/µL in methanol.

### Chromatographic Conditions

All the HP-5MSi columns used in these tests have met the requirements for low column bleed and selectivity of the standard HP-5MS columns. Chromatographic conditions are detailed in Table 1. Additional equipment used is summarized in Appendix A.

Test Mix #1 was then developed as a stringent but simple test for column activity. The chromatographic conditions were chosen such that the active compounds had sufficient retention and resolution. Peak tailing of the polar compound would be an indication of column activity.

Initial testing of the HP-5MSi column with pesticides and environmental compounds was performed using a GC configured with an on-column injector and an inert source MSD. These conditions indicated how well the column performed when identifying these compounds at low levels.

Detailed linearity studies of selected pesticides and environmental compounds used a GC configured with an on-column injector and an FID. This instrument configuration reduced the potential for compound interactions with metal surfaces and allowed us to more effectively evaluate the performance of the column alone.

Lastly, all pesticide samples were run using the standard chromatographic conditions used with the Retention Time Locking (RTL) pesticide data base [1]. Pressure was adjusted to lock chlorpyrifos at 16.596 minutes.

## Results

### Column Evaluation

The column performance for active compounds was evaluated first using Test Mix #1. The presence of active sites was indicated by a loss in peak height/area or evidence of peak tailing of the active pesticide, acids, or bases relative to an inactive compound like octane.

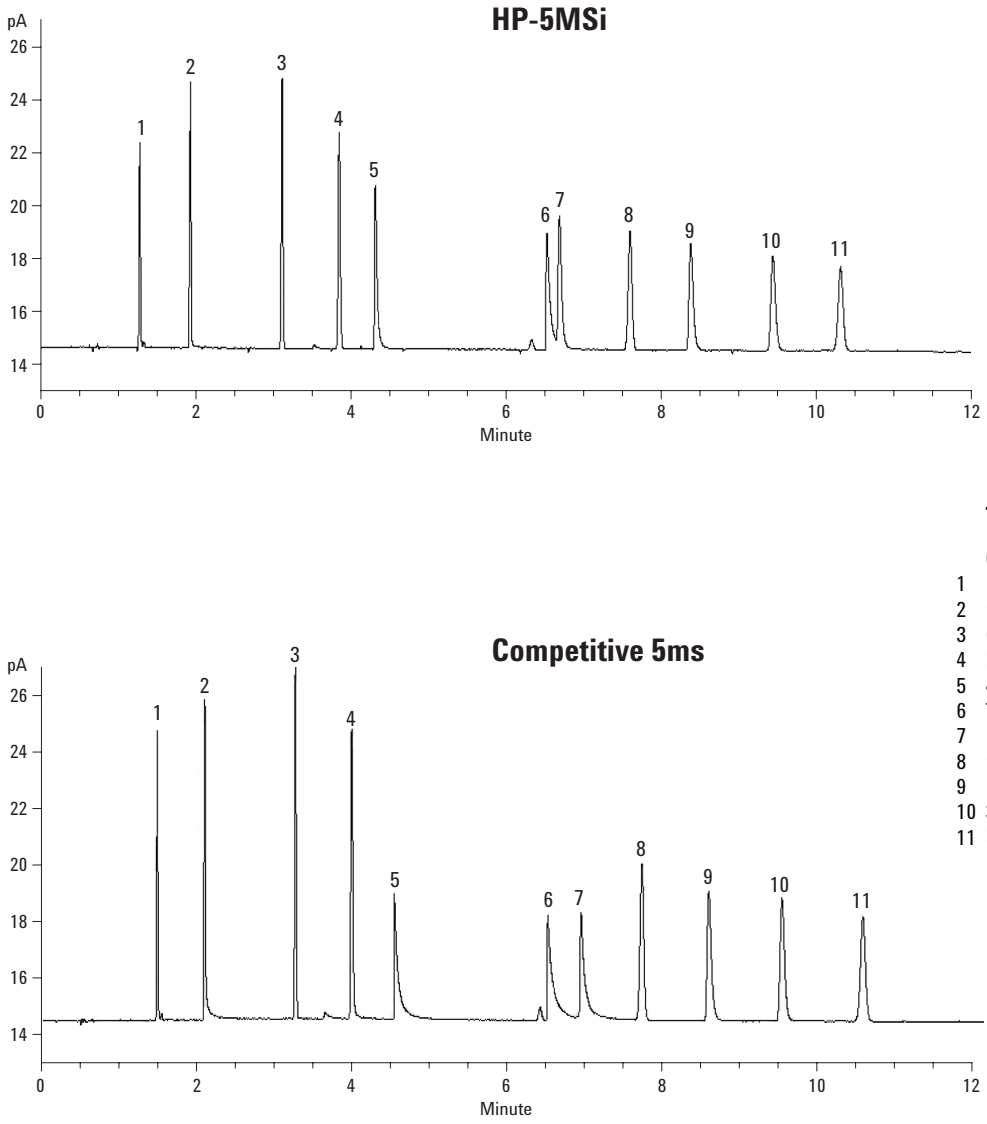
**Table 1. Chromatographic Conditions**

| Application | Test Mix #1 | Pesticides | Semivolatile phenols and amines |
|---|---|---|---|
| Column: | HP-5MSi, 30 m x 0.25 mm x 0.25 µm | | |
| Carrier gas: | $H_2$, 1 mL/min | He, 0.7 mL/min, RTL on chlorpyrifos (16.596 min) | He, 1.3 mL/min |
| Injector | 250 °C, 50:1 split, 0.5 µL | 0.5 µL, cool-on-column, oven track | 0.5 µL, cool-on-column, oven track |
| Oven | 135 °C (15 min), 15 °C/min to 270 °C | 70 °C (2 min), 25 °C/min to 150 °C, 3 °C/min to 200 °C, 8 °C/min to 280 °C (10 min) | 50 °C (1 min), 12 °C/min to 250 °C (1 min), 25 °C/min to 300 °C (10 min) |
| Detector | FID, 320 °C | MSD, 280 °C, scan 50–500 amu or FID, 320 °C | MSD, 280 °C, scan 50–500 amu or FID, 320 °C |

A column performance comparison of the new HP-5MSi column and a standard 5ms column is shown in Figure 1. The 5ms column from another manufacturer shows severe peak tailing for the more active compounds. The new HP-5MSi column exhibits much improved peak shapes for this challenging analysis and demonstrates the benefits a more complete deactivation can have for the analysis of a wide range of active compounds.

**Pesticides**

A series of pesticide samples were prepared to further evaluate the performance of the HP-5MSi column. Figure 2 shows a total ion chromatogram (TIC) of a number of different pesticides at a level of 0.1-ng on-column.




**Test Mix #1**

| | Compound | ng injected |
|---|---|---|
| 1 | Methane | |
| 2 | Propionic acid | 7.0 |
| 3 | Octane | 2.4 |
| 4 | Nitrobutane | 4.9 |
| 5 | 4-Picoline | 2.7 |
| 6 | Trimethyl phosphate | 12.7 |
| 7 | 1,2-Pentanediol | 5.4 |
| 8 | Propylbenzene | 2.2 |
| 9 | 1-Heptanol | 3.3 |
| 10 | 3-Octanone | 3.0 |
| 11 | Decane | 2.2 |

**Figure 1. A performance comparison of the new HP-5MSi column and a competitive 5ms column with Test Mix #1**

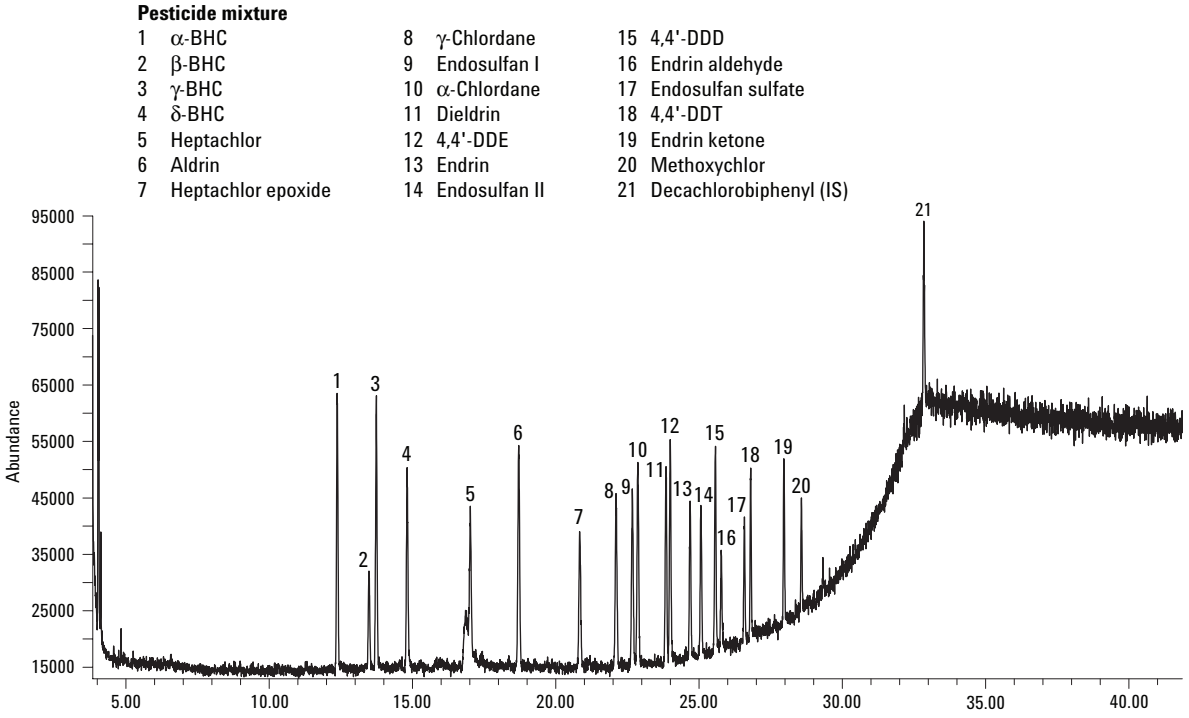



**Figure 2. Pesticide sample containing 0.1 ng of each component on an HP-5MSi column.**

Even at this concentration level in scan mode, it is possible to determine compound identity with greater than 85% confidence after baseline subtraction by spectral matching alone. Retention time locking (RTL) and matching retention times (RTs) to the pesticide database confirmed compound identification.

To further demonstrate the performance of the HP-5MSi, it is important to have linear calibration curves over a broad range of concentrations. A series of tests were performed on the HP-5MSi column using selected pesticides that are known to adsorb strongly to active sites within the column. Standard samples were prepared containing terbofos, malathion, endrin aldehyde, and DDT at different concentrations, and used to prepare a calibration curve for each compound.

Table 2 summarizes the deviation in Response Factors (RFs) as percent relative standard deviation (%RSD) and correlation coefficient ($r^2$) for these compounds over the concentration range tested. The HP-5MSi column shows superior performance when compared with the competitive 5ms column for linearity over the concentration range tested and deviation in response factors.

**Table 2. Calibration Results of Pesticides on HP-5MSi and Another 5ms Column**

| | HP-5MSi | | Competitive 5ms | |
|---|---|---|---|---|
| | %RSD | $r^2$ | %RSD | $r^2$ |
| Terbofos | 5.2 | 0.9999 | 8.9 | 0.9994 |
| Malathion | 5.2 | 0.9998 | 23.8 | 0.9990 |
| Endrin aldehyde | 3.7 | 0.9997 | 29.1 | 0.9994 |
| DDT | 7.1 | 0.9998 | 3.7 | 0.9995 |

### Semivolatiles

It is very important that the capillary column be highly inert for the analysis of semi-volatile phenols and amines especially at low levels. Many of these semivolatile compounds typically show severe loss of response at trace levels. Figures 3 and 4 show both acidic and basic compounds on the new HP-5MSi column. Even at 1-ng, on-column peak shape is still very good, and the strong response allows for accurate quantitation. Calibration curves were linear from 100 to 2.5 ng, with 10%–15% relative standard deviation (RSD) for difficult active compounds like 2,4-dintrophenol and pentachlorophenol.

Acid Mixture

| | | | | | |
|---|---|---|---|---|---|
| 1 | Methyl methanesulfonate | 8 | 2,4-Dimethylphenol | 15 | 2,4,5-Trichlorophenol |
| 2 | Ethyl methanesulfonate | 9 | Benzoic acid | 16 | 2,4-Dinitrophenol |
| 3 | Phenol | 10 | 2,4-Dichlorophenol | 17 | 4-Nitrophenol |
| 4 | 2-Chlorophenol | 11 | Naphthalene (IS) | 18 | 2,3,4,6-Tetrachlorophenol |
| 5 | o-Cresol | 12 | 2,4-Dichlorophenol | 19 | 4,6-Dinitro-2-methylphenol |
| 6 | p-Cresol | 13 | 4-Chlor-3-methylphenol | 20 | Pentachlorophenol |
| 7 | 2-Nitrophenol | 14 | 2,4,6-Trichlorophenol | | |

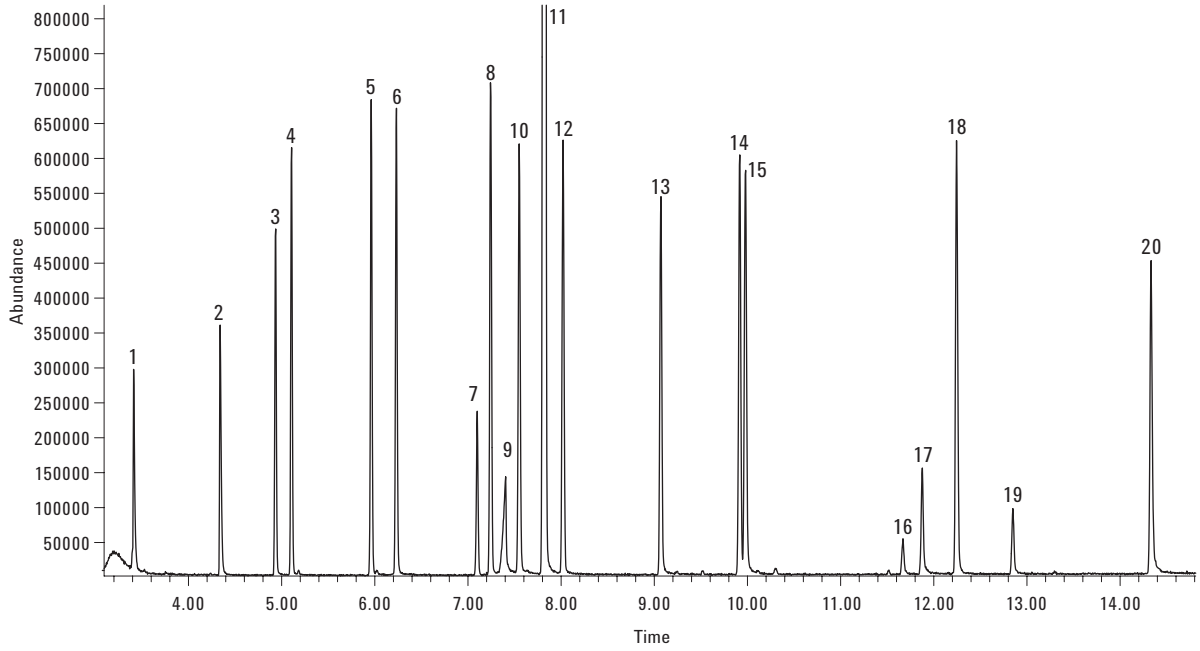



**Figure 3. Acidic semivolatiles sample containing 1 ng of each component on an HP-5MSi column.**

Amines Mixture

1 2-Picoline
2 Acetophenone
3 N-Nitrodopiperidine
4 α,α-Dimethylphenethylamine
5 Naphthalene (IS)
6 N-Nitrosodi-n-butylamine
7 1,2,4,5-Tetrachlorobenzene
8 1-Chloronaphthalene
9 Pentachlorobenzene
10 1-Naphthylamine
11 2-Naphthylamine
12 4-Aminobiphenyl
13 Phenacetin
14 Diphenylamine
15 Pentachloronitrobenzene
16 Pronamide
17 p-Dimethylaminoazobenzene
18 7,12-Dimethylbenz[a]anthracene
19 3-Methylcholanthrene

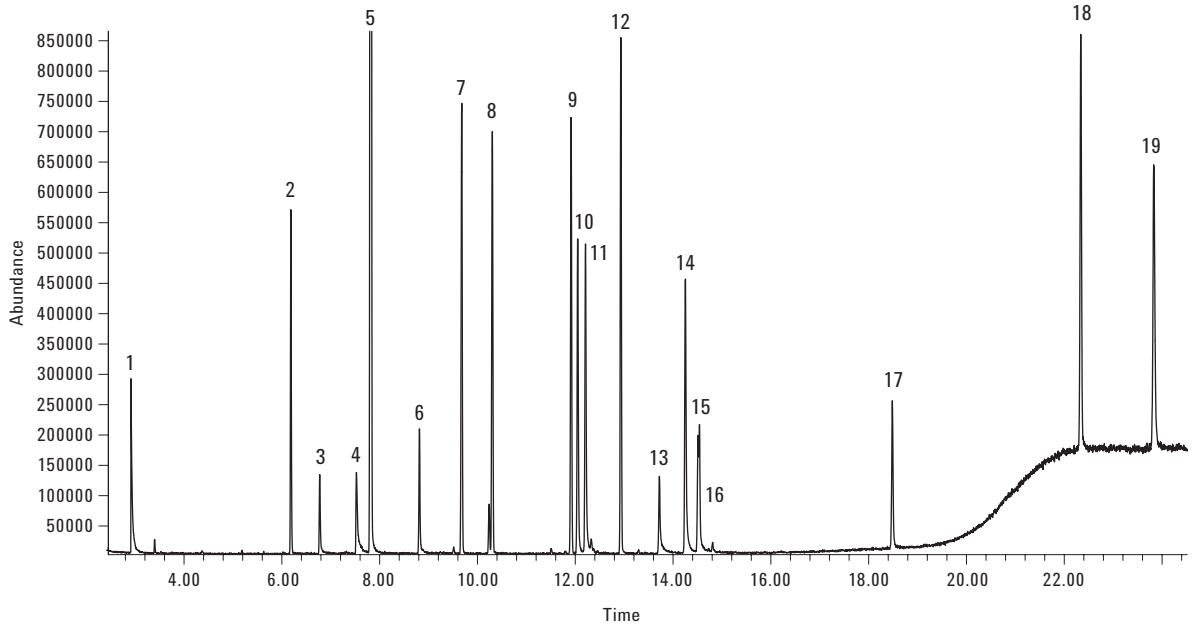



**Figure 4. Basic semivolatiles sample containing 1 ng of each component on an HP-5MSi column.**

## Conclusions

The new HP-5MSi column permits the analysis of low levels of pesticides and other active compounds, such as semivolatile phenols and amines because of its highly inert surface. Peak tailing is minimized thereby allowing low levels of these polar compounds to be determined. In addition, the resulting calibration curves are linear with low variation in response factors.

## References


1. P. Wylie, M. Szelewski, C-K. Meng, and C. Sandy, "Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software," Agilent Technologies, publication 5989-1157EN www.agilent.com/chem.
2. M. Szelewski, W. Wilson, and P. Perkins, "Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270," Agilent Technologies, publication 5968-3072EN www.agilent.com/chem.
3. F. Feyerherm and H. Prest, "Fast GC/MS Method for Routine Drug Analysis," Agilent Technologies, publication 5868-4780E www.agilent.com/chem.
4. M. Szelewski, "Fast USEPA 8270 Semivolatiles Analysis Using the 6890/5973 inert GC/MSD with Performance Electronics," Agilent Technologies, publication 5989-1510EN www.agilent.com/chem.


## For More Information

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# A
# Critical Supplies Needed

| Description | Part number |
|---|---|
| HP-5MSi column | 19091S-433i |
| Column nut | 5181-8830 |
| ALS needle support | G1513-61295 |
| Syringe, 5 µL | 5182-0836 |
| Syringe needle | 5182-0833 |
| Septum | 5183-4758 |
| Septum nut | 109245-80521 |
| Inlet insert (6 rings) | 19245-20515 |
| Graphite ferrule | 5080-8853 |
| Preconditioned ferrules (for 0.25-mm column) | 5062-3508 |
| MS interface nut | 05988-20066 |

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