

Method development for the analysis of Phenols in the ambient air using automatic thermal desorption-gas chromatography-mass spectrometry (TD-GC/MS)

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Abstract. The study presents the development and validation of a method for analyzing phenolic compounds in ambient air using TD-GC/MS. Phenols in the air are known for their toxic and carcinogenic properties, making accurate analysis crucial for assessing their distribution and potential impacts on human health and the environment. The TD-GC/MS technique offers significant advantages over traditional methods by automating sample preparation and improving reproducibility and accuracy. This approach enables sensitive and precise analysis of 9 phenols compounds in ambient air. The optimized method demonstrated high sensitivity, allowing for the detection and quantification of phenols at trace levels in the low parts-per-trillion range. It also exhibited excellent selectivity, distinguishing target phenols from potential interferences in air samples. The validation parameters, such as LOD, LOQ, linearity, and recovery, confirmed the method's reliability and performance. Overall, the developed method, in conjunction with appropriate materials and instrumentation, provides a reliable and efficient approach for analyzing phenolic compounds in ambient air. It contributes to the advancement of air quality monitoring and risk assessment strategies by enabling the identification of pollution sources, evaluation of exposure levels, and formulation of effective mitigation measures to protect human health and the environment from the harmful effects of phenols.

Introduction

Phenolic compounds in ambient air pose a significant risk to human health and the environment due to their toxic and carcinogenic properties [1, 2]. Therefore, the accurate and reliable analysis of phenols in air samples is crucial for understanding their distribution, sources, and potential impacts. In recent years, automatic thermal desorption-gas chromatography-mass spectrometry (TD-GC/MS) has emerged as a powerful technique for the sensitive and precise analysis of volatile and semi-volatile organic compounds, including phenols.

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The traditional methods for the analysis of phenols in air samples often involve multiple steps, including sampling, sample preparation, and chromatographic analysis, which can be time-consuming, labour-intensive, and prone to errors [3, 4, 5]. The introduction of automatic thermal desorption (ATD) systems in conjunction with GC/MS has revolutionized the analysis of volatile and semi-volatile compounds in complex matrices, offering significant advantages over conventional methods [6].

ATD systems enable the automated extraction and preconcentration of analytes from air samples, eliminating the need for manual sample preparation. This automation not only improves sample throughput but also enhances the reproducibility and accuracy of the analysis. The ATD system uses a sorbent tube or cartridge to capture the target analytes, which are subsequently thermally desorbed and transferred to the GC column for separation and detection by MS.

Gas chromatography provides excellent separation capabilities, enabling the resolution of individual phenolic compounds from complex air matrices [3, 7]. The choice of a suitable GC column and optimized chromatographic conditions plays a crucial role in achieving efficient separation and peak resolution. The subsequent detection by mass spectrometry provides reliable identification and quantification of the phenolic compounds based on their characteristic mass spectra. The application of TD-GC/MS for the analysis of phenols in ambient air offers several key advantages. Firstly, it provides high sensitivity, allowing for the detection and quantification of phenols at trace levels, often in the low parts-per-billion (ppb) or even parts-per-trillion (ppt) range [8, 9, 10]. This sensitivity is crucial for accurate environmental monitoring and risk assessment of phenolic compounds. Secondly, the TD-GC/MS method offers excellent selectivity, enabling the differentiation between target phenols and potential interferences present in air samples [11].

In this scientific article, we present the development and validation of a method for the analysis of phenols in ambient air using automatic thermal desorption-gas chromatography-mass spectrometry (TD-GC/MS). We focus on the optimization of key parameters such as sorbent selection, desorption temperature, desorption time, GC column selection, temperature programming, and mass spectrometric settings. The developed method aims to achieve enhanced sensitivity, selectivity, and accuracy for the analysis of phenolic compounds in ambient air samples. By establishing a robust and reliable TD-GC/MS method for phenol analysis, this study aims to contribute to the advancement of air quality monitoring and risk assessment strategies. The developed method will facilitate the identification of pollution sources, evaluation of exposure levels, and formulation of effective mitigation measures to protect human health and the environment from the detrimental effects of phenols in ambient air.

Materials and Methods

TD-GC/MS is an analytical which involves three main steps: thermal desorption, gas chromatography, and mass spectrometry (Figure 1). In the thermal desorption step, the phenols are trapped in an adsorbent material and released by heating and transferred into a cryogenic trap. The trap is connected to a gas chromatograph (GC) system; thus, the desorbed compounds are transferred onto a GC column, where they are separated based on their physical and chemical properties. The GC column contains a stationary phase that interacts differently with each compound, allowing for their separation. The compounds are eluted from the column and carried by a carrier gas through the system. The eluted compounds are directed into a mass spectrometer (MS) for detection and identification. The mass spectrometer ionizes the compounds, generating charged fragments. These charged fragments are separated based on their mass-to-charge ratios (m/z) in the mass analyser. The

detector measures the abundance of the ions, producing a mass spectrum that represents the molecular composition of the compounds. By comparing the mass spectra with reference databases, the compounds can be identified.

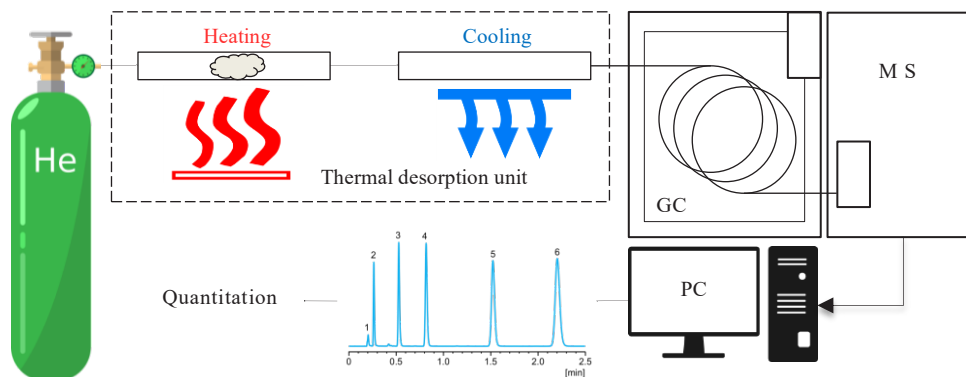


Figure 1. Description of thermal desorption process in GC/MS data analysis

The system configuration for the GC/MS analysis employed a Shimadzu GCMS-TQ8050 NX system, with Helium 6.0 serving as the carrier gas. The TD-30R pretreatment unit, controlled by pressure, was utilized in the analysis. The GC was equipped with an SH-I-5MS column (30 m x 0.25 mm I.D., $df=0.25\ \mu\text{m}$) to facilitate separation of the phenolic compounds. Sample injections were performed in split mode with a ratio of 1:20 and a column flow rate of 1.10 mL/min. The column oven temperature was programmed from 40°C to 280°C at a ramp rate of 10°C/min, allowing for efficient elution of analytes.

In the MS component of the system, the TD-30R was employed for tube desorption, with a desorption temperature of 280°C maintained for 10 minutes, and a desorption flow rate of 60 mL/min to ensure efficient release of the trapped compounds. The trap cooling temperature was set to -20°C to maintain the integrity of the analytes during the desorption process. The subsequent trap desorption was performed at 250°C for 5 minutes to transfer the analytes into the GC/MS system. The joint temperature, valve temperature, transfer line temperature, and interface temperature were set to 250°C, 225°C, 225°C, and 250°C, respectively, to facilitate smooth flow and transfer of the analytes. The ion source temperature was maintained at 200°C to ensure efficient ionization of the compounds. The detector voltage was set to 0.2 kV (relative) to achieve optimal signal intensity.

The measurement mode was configured as Scan/SIM, allowing for both scanning and selective ion monitoring. A scan mass range of 35-450 m/z was selected to cover the desired mass range for phenolic compounds. The scan event time was set to 0.2 seconds, providing sufficient time for data acquisition at each data point. The scan speed was set to 1428 u/sec to ensure rapid data acquisition while maintaining sufficient resolution.

The system was controlled and operated using the GCMSsolution™ software version 4.54 for the GC, which provided comprehensive control and data analysis capabilities. The TD unit was controlled using the dedicated TD-30 Control Software, enabling efficient operation and control of the thermal desorption process.

Overall, this system configuration, comprising the GCMS-TQ8050 NX system, TD-30R pretreatment unit, SH-I-5MS column, and appropriate temperature settings, along with the utilization of GCMSsolution™ software and TD-30 Control Software, provided an optimized setup for the analysis of phenols in ambient air samples using the TD-GC/MS method.

Furthermore, the materials used in the research, such as the Restek TD Glass tubes, Restek Deactivated Wool, Aluminum caps, Shimadzu microsyringe, Supelco Methanol,

single channel Micropipettes, and Restek Phenols Calibration Mix, played crucial roles in developing and validating the TD-GC/MS method for analysing phenols in ambient air. The Restek TD Glass tubes and Deactivated Wool facilitated sample collection and trapping of phenolic compounds, while the aluminium caps ensured sample integrity during the storage in TD30 carousel. The Shimadzu syringe enabled precise and consistent sample injections into the glass tubes filled with glass wool. The methanol of gas chromatography MS purity was used for the dilution of standard solution in order to make multiple reference materials for calibration and quantification in multiple points. Single Channel MicroPipettes ensured accurate dispensing of samples and standards, while the Restek Phenols Calibration Mix provided a mixture of 11 phenolic compounds for calibration purposes. Collectively, these materials enabled the accurate and reliable analysis of phenols in ambient air, contributing valuable insights into the presence and concentrations of these compounds in the environment.

Results and Discussions

The Restek 604 phenols Standard, which had an initial concentration of 2000 mg/L, was diluted using methanol by the specified dilution factors: 1:100, 1:200, 1:400, 1:1000, and 1:2000. Each dilution involved mixing the standard with methanol in the appropriate ratios. This process allowed for the devise of different concentration levels of the phenol's standard for analytical or calibration purposes. These diluted solutions span a range of concentrations was labeled PhA, PhB, PhC, PhD and PhE. Each of these solutions represents a specific concentration level within the range of dilutions.

To analyse the prepared solutions, a specific procedure was followed:

- a glass tube was prepared and filled with glass wool, occupying a length of 3-5 cm in the middle of the tube. This glass wool acted as a packing material to ensure proper vaporization and distribution of the sample;
- next, a volume of 2 μ L of the PhC solution was injected into the glass tube. The injection was carried out using the Shimadzu microsyringe equipped with a needle, for delivering the precise volume of the sample into the tube.
- after the injection, the glass tube containing the sample was desorbed and transferred into the capillary column of the Gas Chromatography-Mass Spectrometry instrument for analysis.

During the GC-MS analysis in scan mode, the instrument scans a specific mass range to detect and identify the compounds present in the sample. In this particular case, the GC-MS instrument was set to scan the mass range of 35-450 atomic mass units (amu). This mass range is carefully selected to cover a broad spectrum of molecular fragments that can be generated during the ionization process in the mass spectrometer. As the analysis progresses, the injected sample undergoes vaporization, and the phenolic compounds present in the PhC solution are separated and carried through the GC column by the mobile phase gas. The GC column plays a crucial role in the separation process by selectively interacting with different compounds based on their physical and chemical properties. This separation allows for the individual identification and quantification of the phenolic compounds present in the sample.

Figure 2 provides a visual representation of the chromatographic separation process during the analysis. As the sample components move through the GC column, they experience different levels of interaction with the stationary phase of the column. This interaction leads to variations in the retention time of each compound, which corresponds to the time it takes for a compound to travel through the column and reach the detector.

By the time the separated compounds reach the mass spectrometer, they undergo ionization, typically through electron ionization (EI), resulting in the generation of charged fragments. These charged fragments are then separated based on their mass-to-charge ratios

(m/z) within the mass analyzer of the instrument. The mass spectrometer detects and measures the abundance of these ions, producing a mass spectrum that represents the molecular composition of the compounds present in the sample.

The scan mode allows the mass spectrometer to sequentially scan through the specified mass range, capturing the mass spectra of the detected ions. This comprehensive scanning process enables the identification and characterization of a wide range of compounds present in the sample. By comparing the observed mass spectra with reference libraries or databases, each phenolic compound present in the PhC solution can be identified based on their characteristic mass spectra patterns.

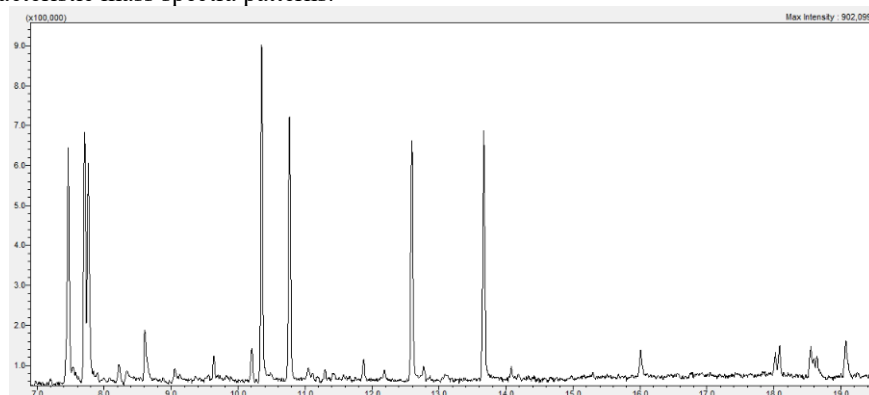


Figure 2. Chromatographic separation of phenols in SCAN mode

The compounds are then introduced into the mass spectrometer and undergo electron ionization (EI) generating charged fragments. These charged fragments were separated based on their mass-to-charge ratios (m/z) in the mass analyser. During the scan mode, the mass spectrometer scans the entire specified mass range, from 35 amu to 450 amu. It records the abundance of ions at different m/z values, creating a mass spectrum for the analysed sample.

By comparing the observed mass spectra with reference libraries or databases, each phenolic compound present in the PhC solution was identified based on their characteristic mass spectra patterns. Thus, were successfully separated at a specific time (retention time) nine phenolic compounds (Table 1) from the standard solution. Transitioning from scan mode to selected ion monitoring (SIM) mode in GC-MS analysis involves specifying specific ions for quantification and confirmation of the presence of each compound. The transition allows for improved signal-to-noise ratio and enhanced sensitivity for the targeted compounds. For each phenolic compound listed in Table 1, specific ions have been designated for quantification and confirmation purposes.

Table 1. Separation of phenols from the PhC solution.

ID	Compound	Retention time (min)	Quantification ion (m/z)	Confirmation	
				Ion 1 (m/z)	Ion 2 (m/z)
1.	Phenol	7.467	94.00	66.00	65.00
2.	2-Chlorophenol	7.712	128.00	64.00	130.00
3.	2-Nitrophenol	10.206	139.00	65.00	109.00
4.	2,4-Dimethylphenol	10.352	107.00	122.00	121.00
5.	2,4-Dichlorophenol	10.766	162.00	164.00	63.00
6.	4-Chloro-3-methylphenol	12.593	107.00	140.00	77.00
7.	2,4,6-Trichlorophenol	13.672	196.00	198.00	97.00
8.	4-Nitrophenol	16.016	139.00	109.00	65.00
9.	Pentachlorophenol	19.071	266.00	268.00	269.00

The quantification ion (m/z) is used to measure the concentration of the compound, while the confirmation ions (m/z) are used to verify the presence of the compound. During the analysis in SIM mode, the mass spectrometer will selectively monitor the specified quantification ion and confirmation ions for each compound. This focused monitoring enables increased sensitivity and reduced noise, as only the targeted ions are analysed. By comparing the observed intensity of the quantification ion with calibration standards, the concentration of each compound can be determined accurately. Additionally, the confirmation ions provide further evidence of the presence of the compound, as they should exhibit similar retention times and relative intensities as the quantification ion. By transitioning from scan mode to SIM mode and utilizing the specific ions for quantification and confirmation, the analysis becomes more selective, sensitive, and efficient for the detection and quantification of the nine phenolic compounds listed.

A calibration curve was constructed to analyse the phenolic compounds listed in Table 1. This involved injecting 2 μL of each PhA, PhB, PhC, PhD, and PhE solution into ten glass tubes, with two tubes assigned to each concentration level. The tubes were filled with glass wool to facilitate the analysis. Using the developed SIM method, each tube was individually analysed. By plotting the data obtained from these analyses, a calibration curve was generated. This curve will be used as a quantitative reference for determining the concentrations of the phenolic compounds in future sample analyses. The concentrations will be determined based on the respective peak areas of the compounds obtained from the analysis.

The data points obtained from the calibration curve were subjected to a linear regression analysis. This statistical technique helped establish a mathematical relationship between the peak area and the concentration of each phenolic compound. By fitting the data points with a linear regression function, a straight line was determined that best represents the relationship between the two variables.

Figure 3 demonstrates the linearity of the Phenol and 2,4-Dimethylphenol compounds. The data points obtained for these compounds were plotted, and a straight line was fitted to the data using linear regression analysis. This linearity indicates a proportional relationship between the concentration of the compounds and their respective peak areas. The linear relationship allows for accurate quantification of the concentrations of phenol and 2,4-Dimethylphenol based on the peak areas obtained from the analysis.

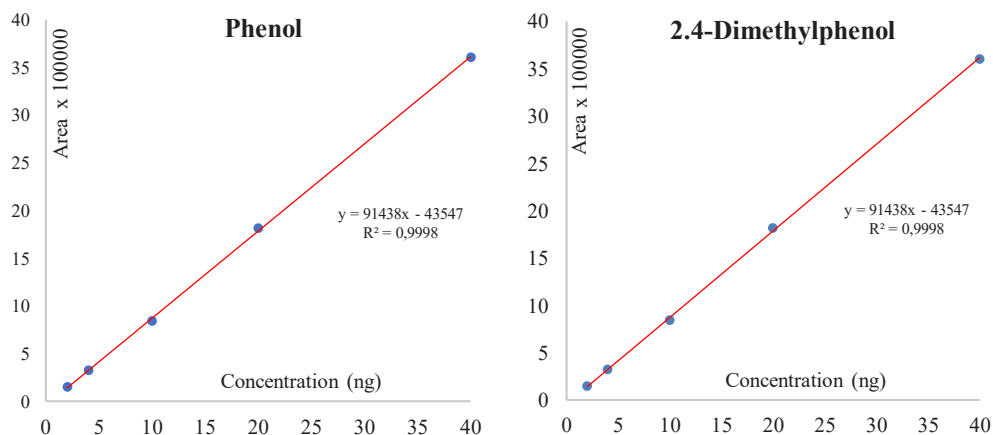


Figure 3 Linearity of the Phenol and 2,4-Dimethylphenol

The Limit of detection (LOD) was calculated as a signal-to-noise ratio of 3.3:1, by injecting progressively lower concentrations of the compound until the signal (peak area) was

three times greater than the average baseline noise level. The lowest concentration that meets this LOD criteria was calculated by equation 1.

$$LOD = (3.3 \times SD \text{ of Blank Signal}) / \text{Slope of Calibration Curve} \quad (1)$$

Likewise Limit of quantification (LOQ) was calculated as a signal-to-noise ratio of 10:1. Similar to the LOD determination, by injecting progressively lower concentrations of the compound until the signal (peak area) is ten times greater than the average baseline noise level. The lowest concentration that meets this LOQ criteria was calculated by equation 2.

$$LOQ = (10 \times SD \text{ of Blank Signal}) / \text{Slope of Calibration Curve} \quad (2)$$

The standard deviation of the blank signal is based on the average baseline noise level measured from the blank samples, and the slope of the calibration curve is determined from the linear regression analysis. These values provide important information about the sensitivity and limit of detection of the method for each compound.

The sample recovery of phenolic compounds was performed by preparing a set of known concentrations of the phenolic compounds to be added as spikes to the sample matrix. These spike solutions covered a range of concentrations to assess the recovery at different levels. The spike solutions to a set of representative samples from the same matrix type as the samples being analyzed. The spike solutions were added in a known volume and thoroughly mixed with the sample matrix.

The calculation of recovery of the phenolic compounds by comparing the measured concentrations in the spiked samples with the known concentrations added as spikes. The recovery is typically expressed as a percentage and can be calculated using the equation 3.

$$\text{Recovery (\%)} = (\text{Concentration in spiked sample} / \text{Known spike concentration}) \times 100 \quad (3)$$

Table 2 provides a summary of the validation parameters for the analysis of phenolic compounds in ambient air. These parameters are crucial for ensuring the accuracy, precision, and reliability of the analytical method employed. The table presents a comprehensive overview of the validation parameters, which include LOD, LOQ, linearity and recovery. These parameters are essential for assessing the method's sensitivity, dynamic range, reproducibility, and suitability for quantifying phenolic compounds in ambient air samples.

Table 2. Validation parameters for analysis of phenols in ambient air.

ID	Compound	Linearity R ²	LOD (ng)	LOQ (ng)	Recovery (%)
1.	Phenol	1	0.473	1.435	100.59
2.	2-Chlorophenol	0.9996	1.741	5.276	83.72
3.	2-Nitrophenol	0.9995	2.018	6.184	80.84
4.	2,4-Dimethylphenol	0.9998	1.336	4.049	82.76
5.	2,4-Dichlorophenol	0.9994	2.112	6.398	71.87
6.	4-Chloro-3-methylphenol	0.9997	1.486	4.506	82.24
7.	2,4,6-Trichlorophenol	0.9997	1.484	4.498	82.16
8.	4-Nitrophenol	0.9627	8.304	17.261	128.69
9.	Pentachlorophenol	0.9981	3.984	12.075	78.44

The validation parameters listed in Table 2 are determined through rigorous testing and validation procedures, following established guidelines and protocols. These parameters are evaluated using appropriate reference standards and quality control samples to ensure the

method's performance meets the required criteria. By reviewing the values and criteria specified in Table 2, researchers and analysts can assess the validity and reliability of the analytical method for the analysis of phenolic compounds in ambient air. The validation parameters provide critical information necessary for assessing the quality of data generated from the analysis and for making informed decisions regarding the presence and concentration of phenolic compounds in the air samples.

Statistical Analysis: Perform statistical analysis on the recovery data, such as calculating the mean recovery and evaluating the precision of the recovery values using standard deviation or relative standard deviation (RSD). The sample recovery assessment provides insights into the accuracy and efficiency of the analytical method for extracting and quantifying the phenolic compounds from the sample matrix. It helps evaluate the method's capability to recover and measure these compounds accurately in real-world samples.

Conclusions

The analysis of phenolic compounds in ambient air is of utmost importance due to their detrimental effects on human health and the environment. The development and validation of a robust analytical method for the accurate and reliable analysis of phenols in air samples are essential. In this study, we utilized the automatic thermal desorption-gas chromatography-mass spectrometry (TD-GC/MS) technique, which offers numerous advantages over traditional methods.

The TD-GC/MS method allows for sensitive and precise analysis of volatile and semi-volatile organic compounds, including phenols, in complex matrices. It eliminates the need for manual sample preparation and improves the reproducibility and accuracy of the analysis. By automating the extraction and preconcentration of analytes from air samples, the TD-GC/MS method significantly enhances the efficiency and reliability of phenol analysis.

Gas chromatography provides excellent separation capabilities, enabling the resolution of individual phenolic compounds from complex air matrices. The choice of a suitable GC column and optimized chromatographic conditions play a crucial role in achieving efficient separation and peak resolution. The subsequent detection by mass spectrometry allows for reliable identification and quantification of the phenolic compounds based on their characteristic mass spectra.

The developed TD-GC/MS method demonstrated high sensitivity, allowing for the detection and quantification of phenols at trace levels. The method offers excellent selectivity, enabling the differentiation between target phenols and potential interferences in air samples. The method's linearity was confirmed through the construction of a calibration curve, establishing a mathematical relationship between the peak area and the concentration of each phenolic compound.

The calculation of the Limit of Detection (LOD) and Limit of Quantification (LOQ) further characterized the method's sensitivity and limit of detection for each compound. These values are crucial for assessing the method's capability to detect and quantify phenolic compounds accurately at low concentrations.

Additionally, the assessment of sample recovery provided insights into the method's accuracy and efficiency in extracting and quantifying phenolic compounds from the sample matrix. Statistical analysis of the recovery data, such as calculating the mean recovery and evaluating precision using standard deviation or relative standard deviation (RSD), allows for a comprehensive evaluation of the method's performance.

Overall, the validation parameters summarized in Table 2 provide crucial information about the sensitivity, linearity, and accuracy of the TD-GC/MS method for the analysis of phenolic compounds in ambient air. This method offers significant advancements in the field of air quality monitoring and risk assessment, enabling the identification of pollution sources,

evaluation of exposure levels, and formulation of effective mitigation measures. The developed TD-GC/MS method, coupled with the appropriate materials and instrumentation, provides a reliable and efficient approach to the analysis of phenols in ambient air, contributing to the understanding and management of their potential impacts on human health and the environment.

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