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QUANTITATION OF 1-NITROPYRENE IN DIESEL EXHAUST PARTICULATES BY CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY AND CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Obtaining accurate quantitation of 1-nitropyrene in a complex mixture is difficult because of variable decomposition occurring in the analytical system at the low 10–100 ng quantities involved. The procedure described here involves pre-separation of the extract by high-performance liquid chromatography and analysis by both capillary gas chromatography and capillary gas chromatography–mass spectrometry. A modified cool on-column injection technique and bonded phase fused silica capillary columns were used to minimize the decomposition and improve the precision for the quantitative analysis. The method is demonstrated using four different diesel particulate extracts containing 1-nitropyrene in the 10–100 ng/μl range.

INTRODUCTION

Nitroaromatics have been found in some environmental samples such as airborne and diesel exhaust particulate matter[1–4]. Those identified nitroaromatics include nitronaphthalene, nitroanthracene or nitrophenanthracene, nitromethylanthracene or phenanthrene, 6-nitrobenzo[*a*]pyrene, 9-nitroanthrancene, 3-nitrofluoranthene and 1-nitropyrene. The identification and quantitation of those compounds have recently received increasing attention because some of them are believed to be direct-acting mutagens (Ames *Salmonella typhimurium* assay without activation, TA98-S). Several investigators have indicated that a small amount of 1-nitropyrene (1-NP) accounts for about 30% of direct-acting mutagenicity of total extract of the diesel particulate matter[1] and accounts for about 20% of the direct-acting mutagenicity exhibited by the total dichloromethane extract of diesel exhaust particulates[5]. Chemical quantitation of 1-NP and other nitroarmatics has been required in order to assess their behaviour as mutagens. The method of choice for quantitation of such compounds is to fractionate the sample using techniques such as high-performance liquid chromatography (HPLC) or column chromatography[1,2], followed by gas chromatographic (GC)–mass spectrometric (MS) analysis. MS–MS techniques have also been used for quantitation of 1-NP[6]. However, the decomposition of 1-NP has been shown to occur during GC–MS analysis[1,6,7]. This decomposition greatly limits

the accuracy, precision and sensitivity obtainable in quantitation of 1-NP at low 10–100-ng quantities.

In this study, the 1-NP content in four different samples of diesel exhaust particulate extract has been determined by capillary GC and capillary GC–MS analyses following HPLC fractionation. It has been found that the decomposition of 1-NP during analysis appears to be a catalyzed thermal decomposition and that the quantitation result of 1-NP critically depends on the cleanliness of the analytical system used. A modified cool on-column injection technique and bonded phase fused silica capillary column were used to minimize the decomposition of sample and improve the precision for the quantitative analysis. The four diesel exhaust particulate extracts show a 1-nitropyrene content in the 10–100 ng/μl range. The quantitative results obtained from GC and GC–MS are consistent.

EXPERIMENTAL

Sample collection and extraction

Four dichloromethane extracts of the diesel particulate matter collected from the in-use diesel automobiles were received from the New York State Department of Environmental Conservation. The vehicle testing, sample collection and filter extraction procedure for those in-use diesel automobiles have been reported previously[8,9]. These samples came from different testing vehicles, driving cycles, fuel and lubricant combinations. Table I lists the sample name, vehicle type and their values of the soluble organic fraction (SOF) of particulate matter.

TABLE I

DIESEL PARTICULATE EXTRACT

Sample	*Vehicle type*	*SOF (%)*
VW-1	Volkswagen	11.1
VW-2	Volkswagen	14.1
VW-3	Volkswagen	12.1
VW-4	Volkswagen	21.2

Solvent and standard

All solvents were "distilled in glass", UV grade from Caledon Labs. (Georgetown, Canada). A 1-nitropyrene and a [$^2H_9$]1-nitropyrene standard were received from Ford Motor Company (Dearborn, MI, U.S.A.).

HPLC pre-separation

Each dry sample received was dissolved in dichloromethane–acetone (3:1) at a concentration of about 20 mg/ml. A modified HPLC procedure previously described by Schuetzle *et al.*[1] was used to separate these complex diesel particulate extracts into six fractions according to the polarity of the component in the extract. The instrument consisted of a Spectra-Physics SP-8000 liquid chromatograph equipped with a SP-8400 UV–VIS detector and SP-4100 integrator. The monitoring wavelength was 254 nm. A 10-μm, semipreparative Spherisorb silica column (250 × 9.4 mm; Terochem, Toronto, Canada) was employed with a 140-μl sample loop. Each sample was frac-

tionated four times and the corresponding fractions were composited. The sample VW-3 was subjected to the HPLC fractionation procedure in duplicate in order to test the reproducibility of HPLC pre-separation. The solvent of all fractions was reduced to dryness. Based on previous works, fraction 3 contains all the 1-NP and its residual was finally dissolved in 100 μl of a mixture of dichloromethane and benzene (70%) for GC and GC–MS analyses.

Capillary GC analysis

GC analysis was done on a Hewlett-Packard HP-5880 A gas chromatograph equipped with a flame ionization detector and cool on-column injection. A 30 m × 0.32 mm I.D. Durabond DB-5 fused silica capillary column (J & W Scientific, Rancho Cardova, CA, U.S.A.) was mainly used. The GC conditions were: injection port < 50°C; column temperature programmed from 80°C for 1 min to 300°C at a rate of 3°C/min; detector temperature 320°C; and helium carrier gas flow-rate measured at 200°C, 1.5 ml/min.

Capillary GC–MS analysis

GC–MS analyses were performed on a Hewlett-Packard HP-5992 quadrupole gas chromatograph–mass spectrometer equipped with HP-59916A glass capillary effluent splitter interface and on-column injection. Two bonded-phase fused-silica capillary columns, 50 m × 0.32 mm I.D. SE-54 cross-linked column (Hewlett-Packard, Avondale, PA, U.S.A.) and 30-m DB-5 column described under *Capillary GC analysis* were used. The GC condition was similar to that described therein. The helium carrier gas flow-rate was 3 ml/min at room temperature. The capillary effluent splitter allowed approximately 0.5 ml/min to enter the MS analyzer. Electron impact ionization with 70 eV was used in GC–MS.

On-column injection at low temperature

A modified injection technique was used for on-column injection at room temperature in both GC and GC–MS analyses. For the Hamilton 701 RN 10-μl microsyringe with a fused-silica needle (0.18 mm O.D., *ca.* 0.5-μl needle dead volume; Hewlett-Packard), the syringe filling sequence is: pure solvent (*ca.* 0.8 μl) followed by an air space (*ca.* 0.8 μl), then a desired volume of sample solution (0.5–5 μl) to be taken. The sample solution is pulled into the syringe barrel and an empty needle is left. An accurate reading of sample solution in the syringe barrel is taken. This read amount of sample solution can be exactly delivered onto the column when the syringe needle is inserted into the column, then the syringe bar is pushed all the way in.

RESULTS AND DISCUSSION

The presence of 1-nitropyrene in these four diesel particulate extracts was indicated by carefully comparing the retention time in the gas chromatogram of HPLC fraction 3 for each sample with that of 1-nitropyrene external standard. Fig. 1 shows this comparison and a good agreement in retention time between 1-NP in sample and 1-NP standard is observed. Small deviations in retention time (±0.05 min) were caused by the deviation of injection time from sample to sample. This

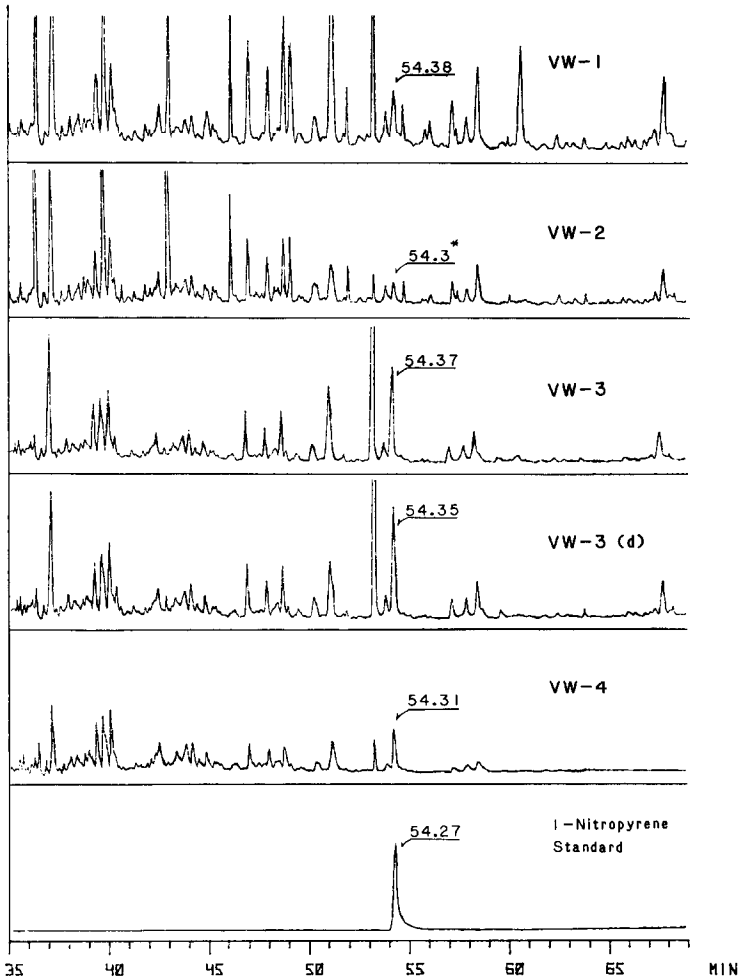

Fig. 1. Gas chromatograms of 1-nitropyrene standard and HPLC fraction 3 of four diesel particulate extracts (sample VW-3 in duplication). GC conditions: 30 m × 0.32 mm I.D. DB-5 fused-silica capillary column; flame ionization detector; cool on-column injection operated at room temperature; temperature 80°C for 1 min, programmed to 300°C at 3°C/min; 66-ng injection for 1-nitropyrene standard and 0.6-μl injection for HPLC fraction 3. *Estimated retention time due to failure in terminal print.

presence of 1-nitropyrene in the sample determined was further confirmed by GC–MS analysis based on the matching in mass spectrum and retention time obtained from the sample to that obtained from the 1-NP standard which was injected separately. Fig. 2 shows the mass spectrum obtained from sample VW-3 compared to the mass spectrum of 1-nitropyrene standard. Slight difference in the minor fragment ion peak may be caused by the impurities. Owing to operation under similar column and temperature programming conditions, the injected samples gave quite similar patterns of gas chromatogram and total ion current trace of GC–MS. It was easy to identify correctly the peak representing 1-NP in the sample GC chromatogram by comparison of two traces.

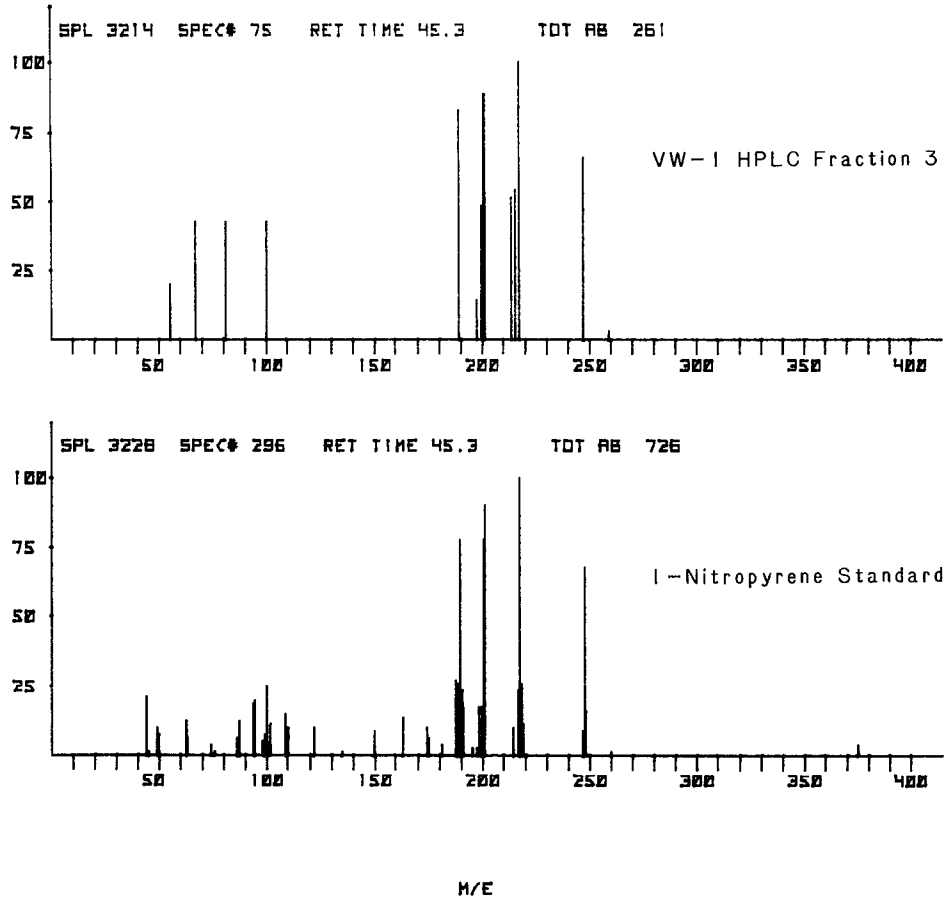

Fig. 2. Mass spectra of 1-nitropyrene obtained from HPLC fraction 3 of diesel particulate extract and from standard.

As shown in Fig. 1, the GC-peak of 1-NP in these complex matrices has been reasonably separated from the adjacent peaks with a high-resolution capillary column. This separation makes a correct integration of GC peak area of 1-NP possible. The baseline of the peak was constructed between the two adjacent valleys and the integrated area of 1-NP peak was automatically printed on the HP-5880A terminal. Quantitation of 1-NP in the sample determined by GC was based on the comparison of integrated peak area of 1-NP obtained from the samples with that obtained from an external standard of 1-NP. The calibration curve of 1-NP standard showed good linearity in the analysis range. The GC result of 1-NP in HPLC fraction 3 is listed in Table II.

In cases where other components interfere with the GC peak of 1-NP and especially when low concentrations are involved, the data obtained solely from GC are not enough for positive quantitation. This has been encountered in the analysis of samples VW-1 and VW-2. From the GC trace of sample VW-1 in Fig. 1, a relatively large error in the integrated peak area of 1-NP was expected because the 1-NP peak

TABLE II

1-NITROPYRENE CONCENYTRATION IN HLPC FRACTION 3 OF DIESEL PARTICULATE EXTRACT DETERMINED BY CAPILLARY GC

Sample	*No. of injections*	*Concentration (μg/μl)*	*Standard error*
VW-1	2	46.9	2.1
VW-2	3	13.3	0.9
VW-3	2	81.6	2.3
VW-3 (duplicate)	2	90.3	1.8
VW-4	5	36.7	1.0

was not well-separated from the neighboring peaks. In the GC analysis of sample VW-2, the value of integrated peak area of 1-NP was very close to the integration threshold set in the instrument. Two of the values of integrated peak area of 1-NP in three GC injections were estimated from the values given by the neighboring peaks because the instrument failed to print out the peak value of 1-NP peak. GC–MS with selected ion monitoring (SIM) was employed to cross-check the result obtained from the GC analysis. [$^2H_9$]1-nitropyrene (1-NP-d_9) standard was used as an internal standard in GC–MS–SIM analysis. Three characteristics ions of 1-NP and 1-NP-d_9, $(M)^+$, $(M - NO)^+$ and $(M - NO_2)^+$, were selectivity monitored. Fig. 3 shows a GC–MS–SIM trace of an injection of 1-NP standard and 1-NP-d_9 internal standard.

The quantitation of 1-NP by GC–MS–SIM was carried out by comparison of the ion current response of $(M)^+$ (*m/z* 247.1 and *m/z* 256.1) of 1-NP in samples and 1-NP-d_9 internal standard with a known concentration. The heights of ion current response were used for the quantitative comparison. By doing so the empirical baseline could be easily constructed for the ion of *m/z* 256.1 which was contaminated by some other component in some samples. A typical GC–MS–SIM trace from the sample VW-1 and 1-NP-d_9 internal standard is shown in Fig. 4. The ions at *m/z* 217.1, 226.1, 201.1 and 210.1 were used to confirm the position of 1-NP in each SIM trace and also served as the reference to construct the baseline for the ion current response of $(M)^+$. These four ions were not used for the purpose of quantitation because they contained interference in some samples and showed less regular behavior in GC–MS. In previous studies, it has been found that the relative abundance among the $(M)^+$, $(M - NO)^+$ and $(M - NO_2)^+$ ions of 1-NP depends on the amount of 1-NP injected at low quantity level[1,7]. This concentration dependence of relative abundance can be observed by comparison of Figs. 3 and 4, and thus creates a difficulty in quantitation work. It is important to match the injected amount between the sample and internal standard for obtaining a reliable result.

The degradation of 1-NP during GC and GC–MS analyses appears to be a catalyzed thermal decomposition. It has been found that the result of 1-NP determination critically depends on the cleanliness of the analytical system. A column contaminated after a certain number of injections of complex samples or a hot glass injection port easily lead to an irregular behavior of 1-NP occurring in GC and GC–MS analyses, such as a tailing peak of 1-NP in GC analysis or an unexpected change in relative abundance of fragment ions of 1-NP in GC–MS analysis. Those phenom-

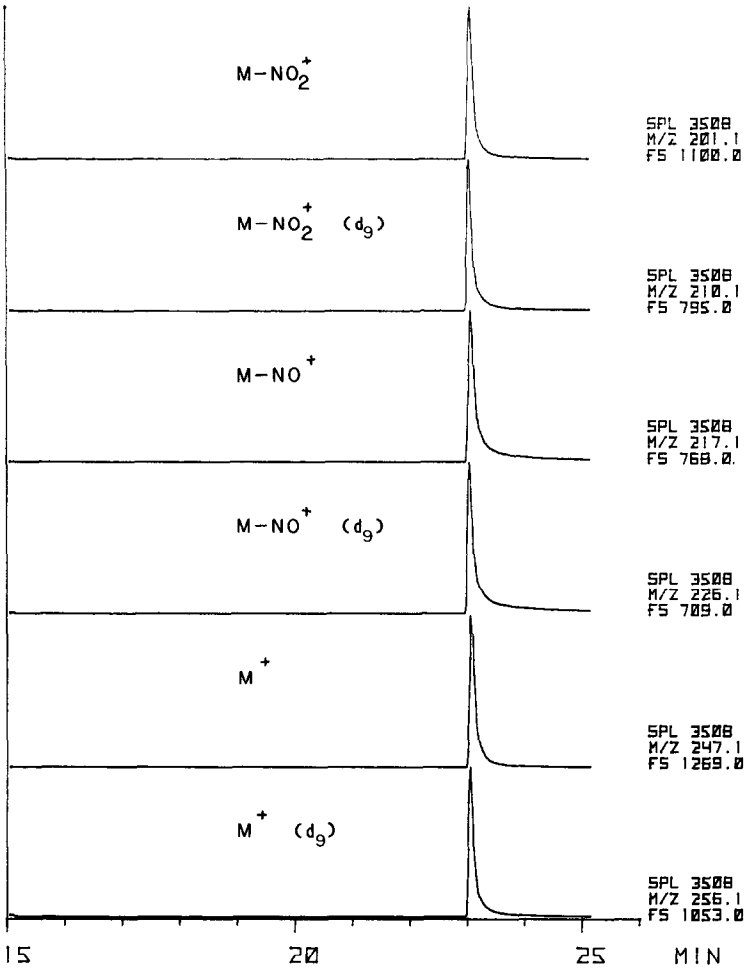

Fig. 3. SIM data for a co-injection of 1-nitropyrene (53 ng) and 1-NP-d_9 (43 ng), monitoring $(M)^+$, $(M - NO)^+$ and $(M - NO_2)^+$. Conditions: 30 m × 0.32 mm I.D. DB-5 fused-silica capillary column; on-column injection at room temperature; temperature 50°C programmed to 300°C at 10°C/min. Subscript (d_9) indicates ions of 1-NP-d_9. FS = Full-scale value.

ena make the quantitation of 1-NP impossible. On-column injection at low temperature and clean system greatly prevent the decomposition of sample by minimizing the contact of 1-NP with the hot surface of metal or glass. A more predictable behavior of 1-NP can be observed in GC and GC–MS analyses. However, the decomposition of 1-NP is more serious in the GC–MS because it can occur at both the glass interface and the ionization chamber of the mass analyzer even when cool on-column injection has been used. Generally, a more reliable result of quantitation of 1-NP is more easily obtained by high resolution GC.

In on-column injection at low temperature, the solvent injected may strip the coated stationary phase and leave an active surface at the front of some conventional capillary columns (non-bonded). This is important to some compounds such as 1-NP which is sensitive to adsorption and decomposition. A peak-broadening of 1-NP has been observed in GC analysis after certain numbers of on-column injection on

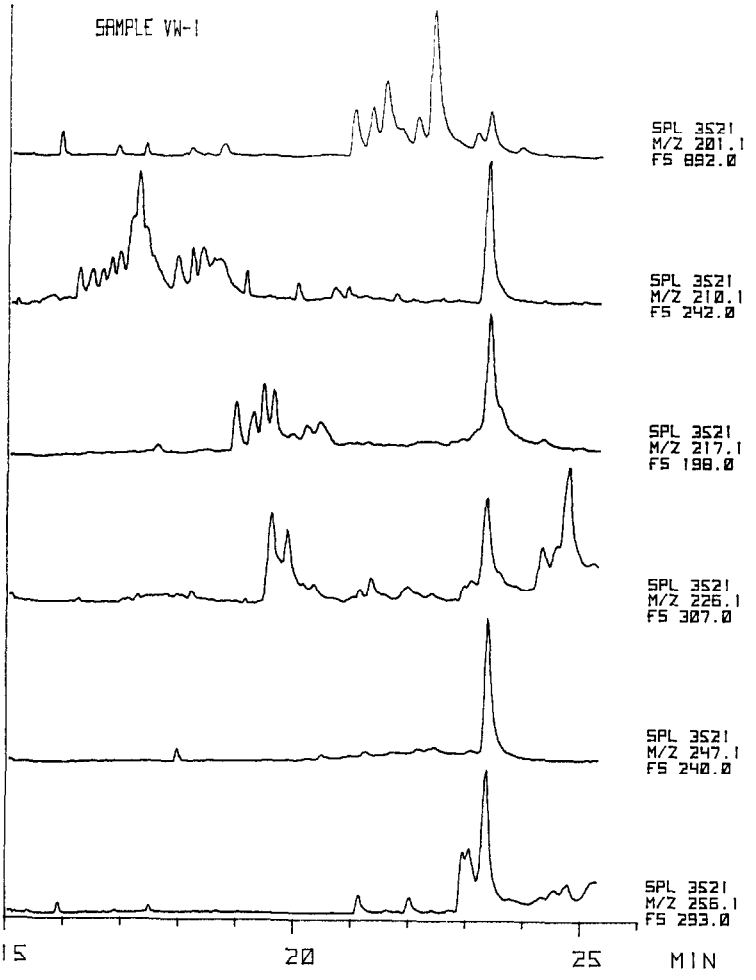

Fig. 4. SIM data for a co-injection of HPLC fraction 3 of diesel particulate extract and 1-NP-d_9 standard. Conditions as in Fig. 2.

a conventional fused-silica capillary column. The use of a bonded-phase fused-silica capillary column overcomes this problem.

For quantitative capillary GC analysis, an accurate delivery of a small volume sample is required. Most conventional injection techniques[10,11] are less satisfactory for the cool on-column injection in terms of controlling the injected amount and reproducibility. In our modified injection technique described in the Experimental section, the empty needle prevents the pre-evaporation of sample solution in the extremely fine needle of the syringe before injection is made. The air space left between the sample solution and the pure solvent plug prevents the sample solution from diffusing into the solvent plug which will exhibit an incomplete evaporation in cool on-column injection. A desired amount of sample solution can be easily taken and accurately read. Using this technique, a controlled small volume of sample so-

TABLE III

1-NITROPYRENE CONTENT IN DIESEL PARTICULATE MATTER

Sample	*No. of injections*	*1-NP/content (ng/mg)*	*Standard error*
By capillary GC			
VW-1	2	49.9	2.3
VW-2	3	17.8	1.1
VW-3	2	92.2	2.6
VW-3 (duplicate)	2	102.0	2.1
VW-4	5	67.2	1.9
By capillary GC–MS			
VW-1	2	42.7	0.5
VW-2	2	22.4	0.3
VW-3 (duplicate)	2	103.2	0.6
VW-4	2	64.9	4.5

lution can be introduced upon the capillary column at low temperature with high reproducibility.

The recovery of 1-NP in the pre-separation procedure was determined in duplicate to be 105%. After taking account the SOF value given in Table I, the content of 1-NP determined in the four diesel exhaust particulate matter is listed in Table III. The results from GC and GC–MS analyses are reasonably consistent.

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