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Analysis of Nicotine in California Air Samples from XAD-4 Resin

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Nicotine is found abundantly in tobacco, is a major constituent of its smoke, and has been used as a marker for environmental tobacco smoke (ETS), composed of second-hand smoke (Hammond et al. 1987,Caka et al. 1990) and side stream smoke. The Staff of the Air Resources Board of California (ARB), in an effort to improve estimates of Californians' exposures to ETS, conducted an air sampling study with analysis of nicotine as part of on-going ETS studies (Air Resources Board 1996). The Trace Analytical Laboratory (TAL) in the Department of Environmental Toxicology, University of California, Davis was selected to provide air sampler cartridges and analysis of nicotine in air samples.

Nicotine is present at >95% vapor phase in ETS (Oldaker and Conrad 1987). Filter capture, problems with nicotine degradation, extraction, and analysis need to be overcome for efficient estimates of nicotine in air. Several methods of capturing nicotine for analysis from air sampling have been proposed such as filter packs, sorbent beds, annular denuders and passive samplers (Caka et al. 1990). XAD-4 resin has been used successfully for air sampling for nicotine in indoor and outdoor studies (Ogden et al. 1989, Chuang et al. 1990, Pendergrass et al. 2000). XAD-4 has been shown to be more efficient in capturing nicotine than XAD-2 due to its larger surface area (Chuang et al. 1990, Ogden 1989). Efficient extraction of nicotine from the XAD-4 resin with ethyl acetate with 0.01% triethylamine has been accomplished in several studies (Ogden et al. 1989, Chuang et al. 1990, Pendergrass et al. 2000, Ogden 1989). Another study suggested pyridine or triethylamine added to ethyl acetate for full extraction of nicotine from resin (Chuang et al. 1990).

Nicotine has primarily been analyzed by gas chromatography (GC) using nitrogen selective or nitrogen/phosphorus detection (NPD), chemical ionization mass spectrometry (CI/MS), and GC/MS, with or without internal standards (Hammond et al. 1987, Caka et al. 1990, Oldaker and Conrad 1987, Ogden et al. 1989, Chuang et al. 1990, Pendergrass et al. 2000, Ogden 1989, Ogden and Malolo 1992). In the present study, GC/MS is utilized for quantitation.

California ARB staff collected air-samples using XAD-4 resin in five different

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locations in California. Trapping efficiencies, method detection limits, concurrent recoveries and storage stabilities were determined by GC/MS to aid the ARB investigation. The purpose of this study was to develop quantitative nicotine analysis from XAD-4 resin for the ARB samples.

MATERIALS AND METHODS

Nicotine (98.5%, Lot#267-54A) reference standard, was obtained from Chem Service (Cat. #PS-85, 660 Tower Lane, West Chester, PA), and stored frozen at approx. -20°C except when in use. All solvents and reagents were residue grade or better. Stock solution (1mg/mL) was prepared by dissolving 0.0509 g nicotine into 50 mL acetone. Stock solution was stored frozen.

Calibration standards for GC/MS analysis were prepared in the presence of matrix. For each calibration standard solution, solvent was prepared through the method and the resin extract used for final dilution with 40/40/20 acetone/ethyl acetate/methanol. Dilution of 2, 1, 0.5, 0.2, 0.1 and 0.05 mL of stock solution (1 mg/mL) in 10 mL of 40/40/20 acetone/ethyl/acetate resin extract produced 200, 100, 50, 20, 10 and 5 pg/μL calibration standard solutions. Calibration standards were stored in the refrigerator (<5°C) and were prepared fresh every two weeks.

XAD-4 (Rohm and Haas, Amberlite, Philadelphia, PA, 100-120 mesh), a macro reticular resin, was employed as the trapping medium. XAD-4 resin was prepared prior to use according to Seiber et al. 1989, with modifications. Resin was initially rinsed with methanol in an ~ 40 L container. The fines were removed by placing a hose at the bottom of the container, overfilling with deionized water and stirring vigorously. Two liters of 0.25 *N* hydrochloric acid were added and the resin was stirred for 30 min. Again, water was added and fines with excess water was decanted. The water steps above were repeated until the water above the resin was clear and the pH was that of the deionized water. The resin was then transferred with methanol to gallon bottles. The resin was Soxhlet extracted once with fresh methanol and ca. 100mL of pyridine for 24 hours, then extracted again with fresh methanol for 24 hours, then extracted twice with fresh ethyl acetate for 24 hours each. The pyridine step was added specifically for nicotine analysis to enhance extraction of the captured nicotine from the resin. The resin was placed in a vacuum oven (25 in. Hg) until thoroughly dried. Resin batches were numbered and stored at room temperature in clean, dry jars with Teflon®-lined lids. This procedure provided thorough cleanup of the XAD-4 resin.

Preparation of cartridges is described in Hall et al., 1997. The resin cartridge consisted of a resin bed (~30 mL) held in position with a stainless steel mesh screen. The cartridges were connected in tandem with Teflon® tubing. Tygon® tubing was connected to a Staplex high-volume air pump fitted with a manifold that allowed for a flow rate of approximately 15 L per min (Lpm). Nicotine standard solution was applied directly to the resin bed at 50 μg. Air samples were collected for 1 and 8-hr intervals, n=4. Resin was extracted as described below and analyzed as described in the quantitation section.

Eight samples each were fortified with 0.10μg nicotine for a 1-hr and an 8-hr air sampling interval. Samples were extracted and quantified as below in a final volume of 10 mL. The method detection limit (MDL) is considered to be the t-value (2.998 for n=8) times the standard deviation. For quantitation at pg/μL, values below MDL were non-detect (ND), values above or equal to MDL but below or equal to estimated quantitation limit (EQL) were trace (TR), and values above EQL were reported at two significant figures. EQL was determined using 10 mL samples while actual samples were analyzed in 5 mL, so the pg/μL level determined whether non-detect or trace amounts were reported.

For storage stability, glass jars with 30 mL of pyridine washed XAD-4 resin were each fortified with 1 μg of nicotine. Six jars were analyzed through the method below on Day 0 and the remaining jars were stored at –20±6°C. Six jars were removed and analyzed as below on Day 29.

Air sample collection was performed by Air Resources Board personnel using cartridges prepared by the TAL facility, and air samplers similar to those used for trapping efficiency with air flow of approximately 15 L/min. Air samplers were placed at various sites in California and samples were taken for 1-hr and 8-hr periods. Usually 3 air pumps were used at a site, and cartridges changed for the appropriate time interval. Cartridges were stored in freezer boxes after collection and delivered to the TAL facility. Samples were assigned unique numbers and analyzed as below.

To extract the resin, the cap and screen were removed from the resin cartridge and poured into a 4 oz. jar. The remaining resin was transferred by carefully rinsing the cartridge using 75 mL of 0.01% of pyridine in 40/40/20 acetone/ethyl acetate/methanol (extraction solvent) into the jar and capping with a Teflon®-lined lid. Fortifications were prepared at this point by adding appropriate standard to a jar, then adding resin and 75 mL of extraction solvent. Jars are mechanically swirled, on a rotary platform shaker for one hour, at a moderate speed. The extraction solvent was decanted into a 500-mL round bottom flask (RBF) through a funnel with glass wool. The resin was re-extracted with an additional 75 mL of extraction solvent and swirled for 30 min. Pooling the decanted extraction solvent from the jar, resin was re-extracted a final time with 75 mL extraction solvent for 30 min. The pooled extract in the round bottom flask was concentrated to 1-2 mL using a rotary evaporator with an ~35°C water bath. The sample was diluted to an appropriate volume for GC/MS analysis with 40/40/20 acetone/ethyl acetate/methanol.

For nicotine analysis, a Hewlett Packard (HP, Avondale, PA) 6890, equipped with a 30 m x 0.25 mm DB-17ms column (0.25 μm film thickness, J&W Scientific, Folsom, CA) and Mass Selective Detector (MSD) Model 5973, in Selective Ion Monitoring (SIM) Mode was used. The inlet was operating in pulsed splitless mode with the injection pressure pulse 50 psi for 1 min and the injector purge at 50 mL/min for 0.95 min. A HP 6890 autosampler was used to make 3 μl injections. The injector was heated at 250°C, the MSD interface at 280°C, MSD source at 230°C, the MSD quadrapole at 150°C and the column at 80°C for 1 min then 20°C/min to 280°C. The retention time was 6.44 min for nicotine. The quantitation ion (*m/z*) was 84 (Dwell time = 50 ms x 2) and the confirmation ion (*m/z*) was 162. Prior to each analytical set, the analyst performed an autotune and a tune evaluation of the MSD to insure proper function. In addition, calibrations standards were injected with each run to check GC/MS performance.

HP ChemStation® G1701BA version B.01.00 was used for data collection. Peak areas from calibration standards were used to generate a linear standard curve (nicotine response vs. concentration pg/μL). Peak areas from samples were converted to pg/μL by using the linear regression from the standard curve. The sample concentration was multiplied by the final sample volume resulting in μg/sample. Fortified samples yielded a percent recovery by dividing the μg/sample by the fortification amount. If the peak area for nicotine was larger than 100% of the highest standard value, the sample was diluted and reinjected. For sample values above EQL, 10% of the samples were assessed by ion ratio comparison for nicotine. Sample values that were less than or equal to EQL, but greater than or equal to MDL were labeled "trace" at the μg level, and "ND" at the $\mu g/m^3$ level. Sample values less than MDL were labeled "ND" for non-detect at the μg level and the $\mu g/m^3$ level.

RESULTS AND DISCUSSION

Difficulties with trapping efficiencies for nicotine were curtailed by addition of pyridine to the extraction solvent and pre-washing the XAD-4 collection resin with dilute pyridine. Trapping efficiencies were 82 ± 6% for the 1-hour study and 69 ± 3% for the 8-hour study, with 90 ± 1% concurrent recoveries. These trapping efficiencies were sufficient and comparable to other XAD-4 studies (Caka et al. 1990,Ogden et al.1989).

Results for the 1-hour air sampling study showed an average of 9.14 ± 0.35 pg/μL recovered. MDL was calculated as 1.05 pg/μL (0.35 x 2.998). EQL was calculated as 5.25 pg/μL (MDL x 5); thus, non detect (ND) $\leq 1.05 <$ Trace ≤ 5.25 pg/μL. "Trace" value at the pg/μL level would calculate to a "ND" at the $\mu g/m^3$ level. Results for the 8-hour air sampling study showed an average recovery of 10.22 ± 0.18 pg/μL, an MDL of 0.53 pg/μL and an EQL of 2.66 pg/μL. Because the standard deviation for the 8-hour set was significantly less than the 1-hour set, and when calculated was well below our standard curve, the MDL and EQL at pg/μL for the 1-hour set was used throughout the study.

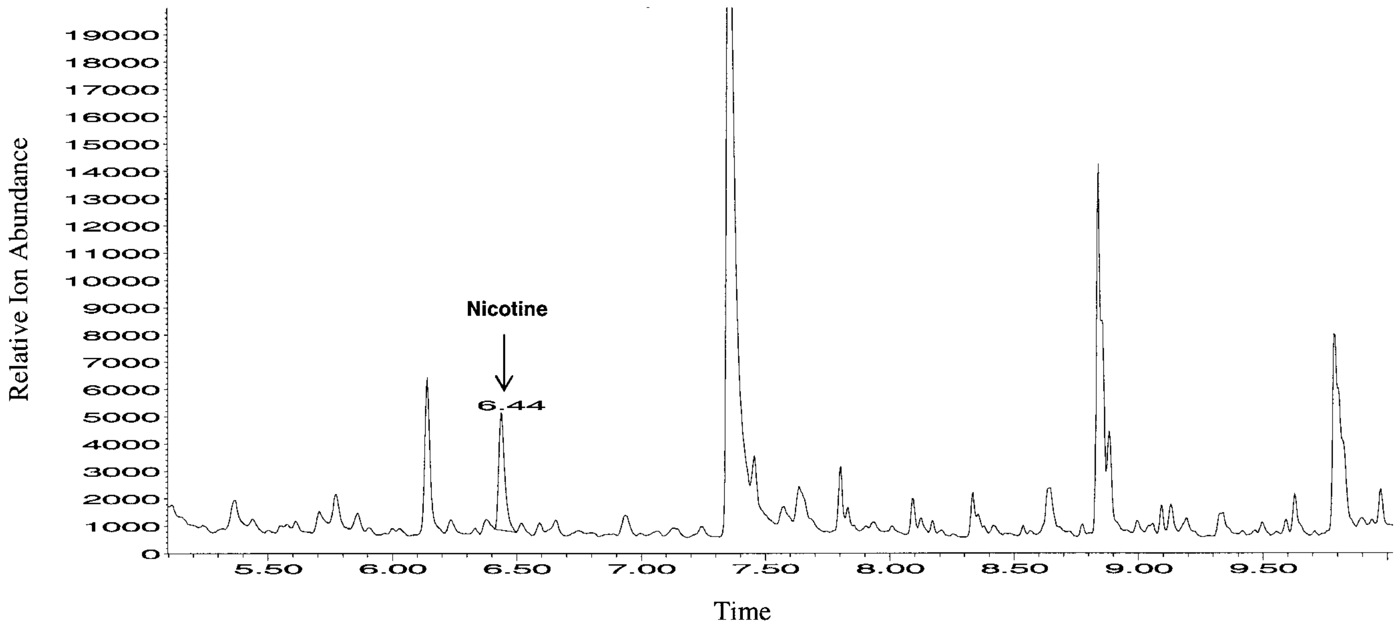

Figure 1. Nicotine Standard, 5 pg/μL, Rt = 6.44 min (SIM *m/z* = 84).

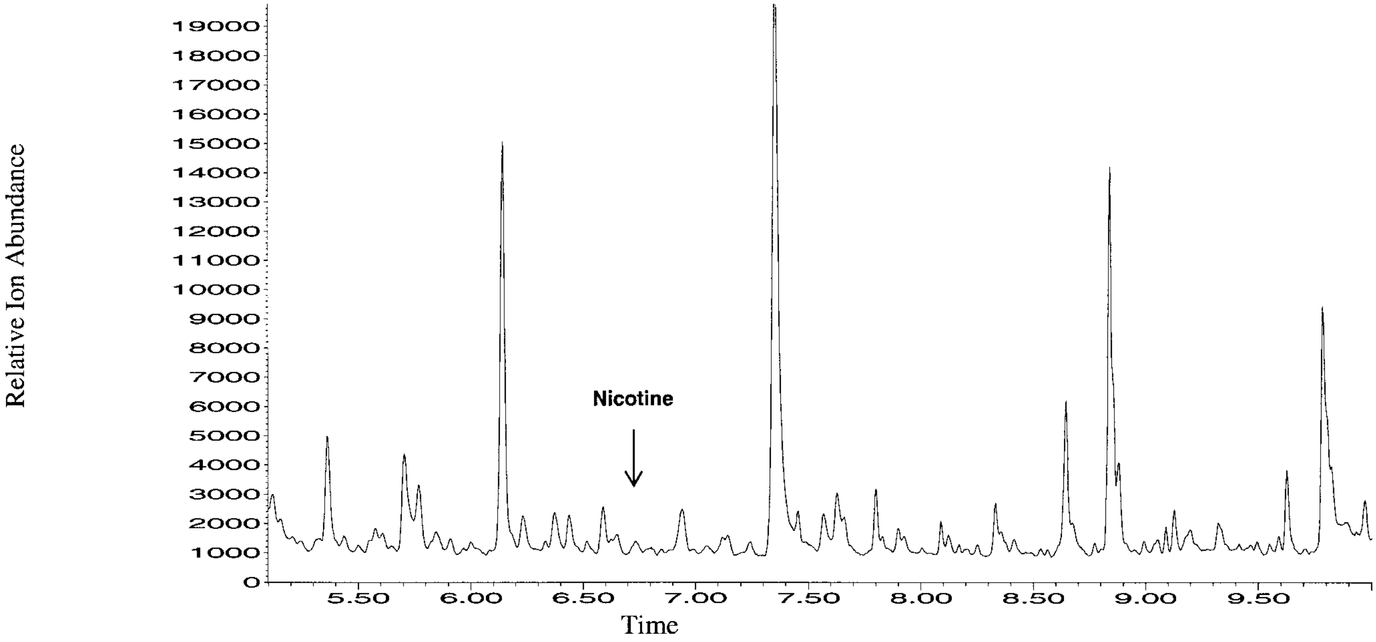

Figure 2. Control Resin blank (SIM $m/z = 84$).

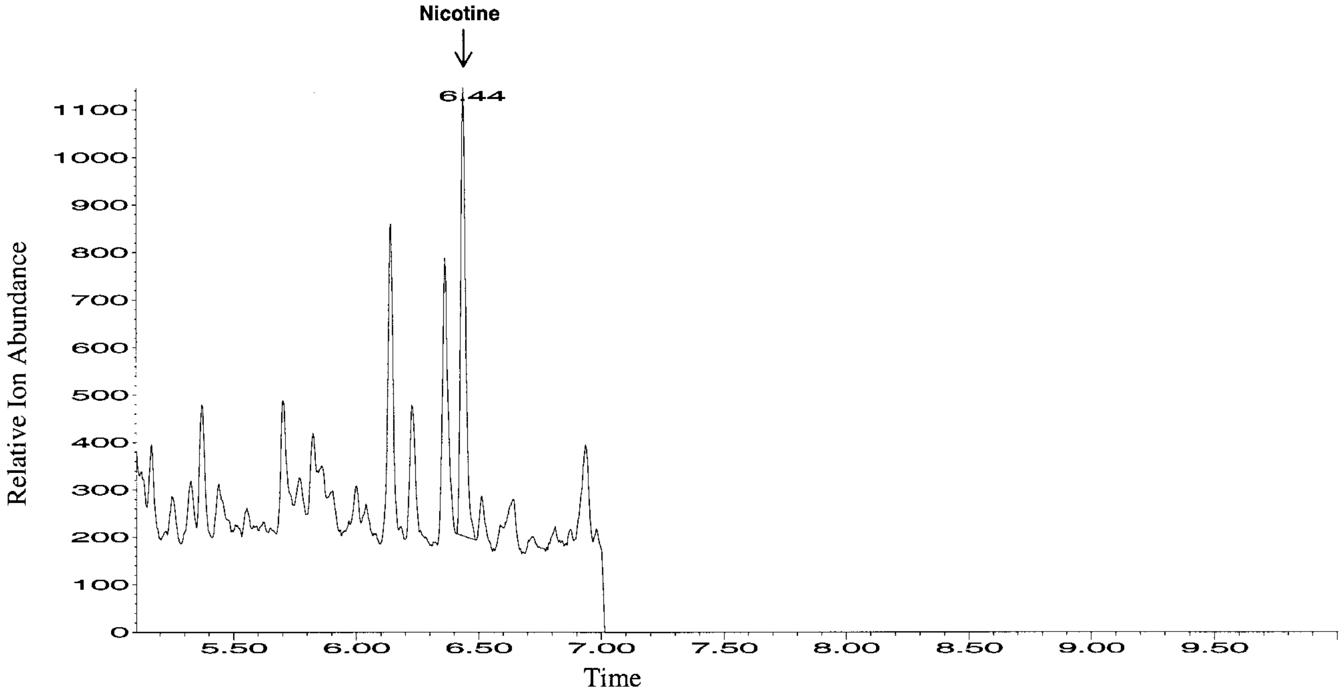

Figure 3. Nicotine recovery, 0.1 μg, 92% recovery. Rt = 6.44 min (SIM $m/z = 84$).

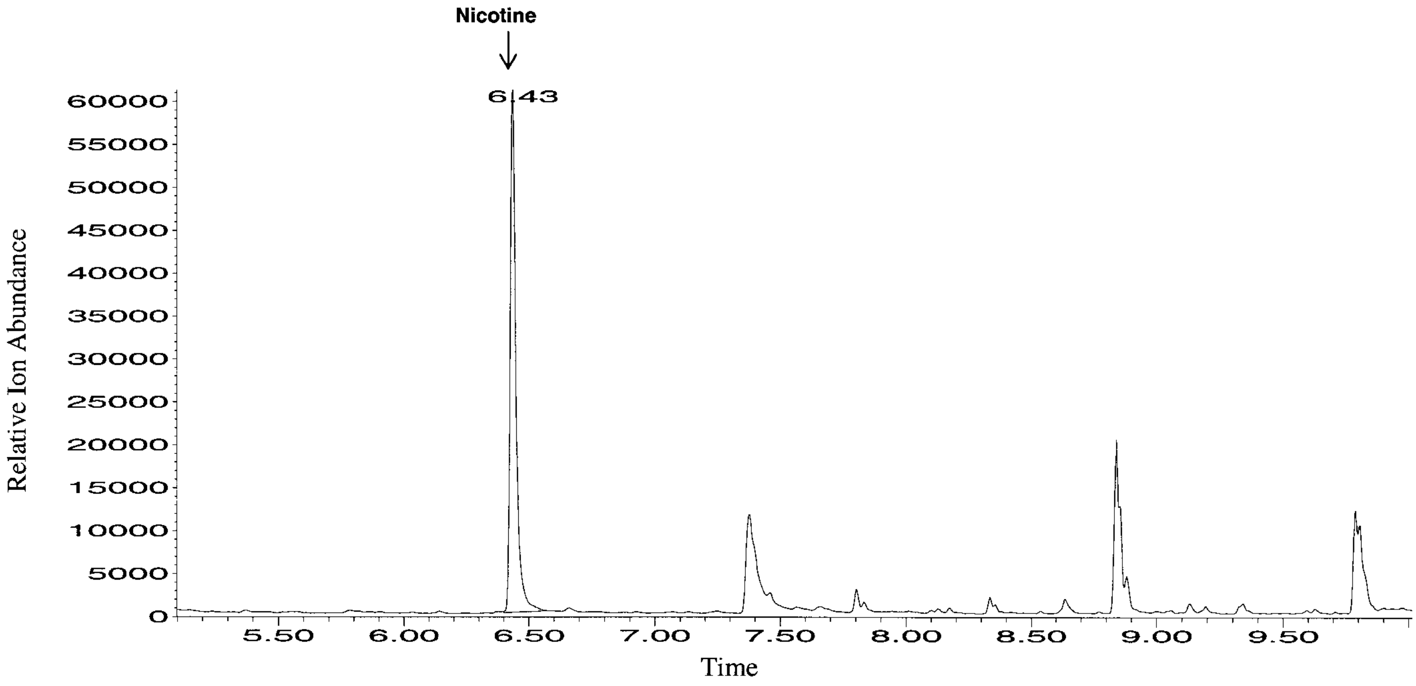

Figure 4. Amusement Park, Pump#2, 1.211 μg/m^3. Rt = 6.43 min (SIM m/z = 84).

Storage stability samples were analyzed 29 days after fortification. Recovery averaged 103 ± 5% for n=6. Concurrent recoveries run with those samples averaged 89 ± 4% for n=6. No apparent degradation of nicotine occurred in spiked frozen cartridges over 29 days.

For each set of samples received from ARB, concurrent recoveries were run. For the entire study concurrent recoveries averaged 85 ± 7%. Trip/field spikes, which were fortified by TAL, averaged 80 ± 9% recovery. Figures 1 to 4 show chromatograms of standard at 5.0pg/µL nicotine, control resin blank, a recovery, 0.1 µg, at 92%, and an air sample containing 1.211 µg/m^3, respectively. Recoveries were comparable to other studies; however, our method is quantitative to approximately 0.005 ug/m^3 MDL (Chuang et al. 1990, Pendergrass et al. 2000, Ogden and Malolo 1992).

Table 1. Nicotine in air samples from 5 different California locations in µg/m^3. *Sites in italics,* **8 hour samples in bold,** all others, 1 hour samples.

Airport			**Junior College**			
1	*2*	*3*	*1*	*2*	*3*	*4*
1.114	0.556	0.0565	TR	TR	0.0359	TR
0.649	0.588	0.0472	TR	TR	0.0377	TR
1.032	0.517	0.0454	0.0434	0.0752	0.0462	TR
0.620	0.553	0.0525	0.0444	0.0484	0.0650	TR
0.663	1.498	0.0417	0.0960	0.1460	0.0525	TR
0.362	0.439	0.0355			0.0556	TR
0.490	**0.990**	**0.0185**	**0.0316**	**0.0437**	**0.0296**	**0.0183**
0.480	**0.731**	**0.0228**	**0.0273**	**0.0151**	**0.0129**	**0.0047**

Local Government Center			**Office Complex**			**Amusement Park**		
1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
0.176	0.145	TR	0.213	0.151	0.0392	0.859	0.660	0.155
0.172	0.0797	TR	0.236	0.282	0.0715	1.980	1.211	0.140
0.0702	0.0601	TR	0.139	0.243	0.0724	3.167	0.881	0.169
NP	0.131	TR	0.121	0.194	0.0728	3.096	2.891	0.134
0.0387	0.0822	ND	0.124	0.187	0.0762	4.607	3.636	0.263
0.0463	0.0702	TR	0.102	0.242	0.0321	3.096	2.134	0.156
0.0592	**0.0731**	**0.0102**	**0.105**	**0.127**	**0.124**	**2.361**	**2.792**	**0.124**
0.0417	**0.0674**	**0.0077**	**0.121**	**0.152**	**0.0495**	**3.087**	**2.547**	**0.122**

ND = non detect , TR = Trace, NP = No Pump (malfunction).

Limits Used for µg/m^3:

1 Hour ND < 0.0058≤ Trace< 0.029 µg/m^3

8 Hour ND < 0.00073≤ Trace< 0.0036 µg/m^3

Results from samples collected by the staff of California ARB are presented in Table 1, for 1-hour and 8-hour sampling intervals. General locations were an airport, a junior college, a government building, an office complex and an amusement park. The second set of samples taken at the junior college has collocated samplers #1 and #2, indicating they were placed for sampling air right next to each other. The collocated samples had comparable levels of nicotine found. Samples taken at the amusement park had the highest levels of nicotine, with the highest collected sample in the study at 4.6 $\mu g/m^3$ nicotine. The lowest values were found in the junior college samples.

California ARB will be utilizing the data provided by TAL in estimating the environmental impact of nicotine levels in ETS. Use of pyridine to wash XAD resin and extract nicotine from the resin insured consistent recovery of nicotine. GC/MS detection was utilized without an internal standard and using matrix standards for accurate identification and quantification of the nicotine captured. This method, developed to quantify nicotine from XAD-4 resin cartridges, is sensitive, efficient, selective and robust.

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