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COMPARISON OF GC AND LC FOR DETERMINING SOLANESOL IN ENVIRONMENTAL TOBACCO SMOKE

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ABSTRACT

Solanesol (a high molecular weight isoprenoid alcohol) is the best available tracer of environmental tobacco smoke (ETS) particles in indoor air because of its high specificity for tobacco smoke, extremely low volatility, and relatively large concentration in the particles of ETS. Previously, we have developed and applied GC methods for solanesol determination which require derivatization and on-column injection onto short, thin-film capillary columns. However, there are several limitations of this approach which include tedious sample preparation and lack of automation. Consequently, LC procedures were developed which circumvent these problems. Included are comparisons of the GC and LC analysis methods in terms of recovery, precision, sensitivity, and ease of analysis.

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Environmental tobacco smoke (ETS) is the aged, dilute mixture of sidestream and exhaled mainstream smoke resulting from combustion of tobacco products. An extremely dynamic mixture, ETS is an aerosol consisting of both vapor and particulate phases which has been implicated as one of a myriad of sources impacting air quality in the indoor environment. Numerous chemical tracers have been proposed for estimating this impact. However, most suffer from shortcomings of being either non-specific to tobacco smoke or being present in indoor air at such minuscule concentrations that they cannot be detected in typical real-world settings at realistic smoking rates.

Nicotine and respirable suspended particles (RSP) are two tracers used most often in indoor air quality surveys. Nicotine, a component of the vapor phase of ETS aerosol, is reasonably specific to tobacco, should enter indoor air only from tobacco smoke, and is relatively simple to determine by gas chromatography with thermionic-specific detection.(1)(2)(3)

RSP is also reliably determined (by micro-gravimetry) but is not specific to tobacco smoke. As an ETS indicator, RSP most often results in a gross overestimate, although the magnitude of this bias can be reduced by determining either ultraviolet absorbance (ultraviolet particulate matter, UVPM) or fluorescence (fluorescence particulate matter, FPM) of methanolic extracts of the collected particles.(4)(5)

Solanesol, a high molecular weight trisesquiterpenoid alcohol (Figure 1), is associated solely with the particulate fraction of ETS, is very specific to tobacco smoke, and is the only chemical constituent identified to date which can serve as a reliable tracer of ETS RSP. We were the first to determine solanesol in ETS in 1986 and have since found it to comprise 2 to 3% by weight of RSP from ETS. Solanesol has been used in both laboratory experiments and

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field surveys to apportion indoor RSP into the fraction attributable to ETS.(3)(5)(6)(7)(8)

The methodology developed previously for solanesol determination consists of solanesol derivatization followed by gas chromatography (GC) on short, thin-film capillary columns with on-column injection. This analysis, although reliable, has proven tedious, time-consuming, and incompatible with automated (autosampler) analysis. A liquid chromatography (LC) procedure has been developed and applied to the determination of solanesol in ETS. This procedure has precision and sensitivity comparable to the GC technique while offering considerable advantages in sample throughput due to simplified sample preparation and being conducive to automation.

EXPERIMENTAL

Materials: Solanesol and 1-triacontanol were obtained from Sigma Chemical Co. (St. Louis, MO).

Sample collection: Airborne solanesol was collected by drawing air at 2-4 L/min through Fluoropore (37-mm diameter, 1.0 μm pore size) membrane filters (FALP 03700 from Millipore Corp., Bedford, MA).(5,8)

Gas chromatography: Samples were prepared for GC analysis by extracting filters with pentane, evaporating the extract to dryness, and derivatizing the residue with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine.(6,8) Solanesol determination was performed on a Hewlett-Packard model 5890A GC with flame ionization detection (FID) and on-column injection. The column used was a 15 m x 0.32 mm i.d. fused silica capillary coated with a 0.1 μm film of DB-1 (a 100% methyl polysiloxane) connected to a 2 m x 0.53 mm i.d.

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pre-column coated with a 0.15 μm film of DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed at 7°/min from 100° to 310°C and held 5 min. Carrier gas was helium at 10 psig (ca. 60 cm/sec at 100°). On-column injections of 1 μL were made manually *via* a 10-μL syringe fitted with a fused silica needle. Detector flow rates were helium make-up gas 25 mL/min, hydrogen 30 mL/min, and air 375 mL/min with a detector temperature of 350°C. Quantitation was performed with 1-triacontanol as internal standard. (3,6,8)

Liquid chromatography: Samples were prepared by placing the Fluoropore filter in a 4-mL LC autosampler vial, adding 3 mL methanol, capping and extracting 30 min in an ultrasonic bath. Two sets of LC conditions were evaluated for solanesol determination: (1) C-18 column (5 μm particles, 4.6 mm i.d. x 150 mm, ASTEC Inc., Whippany, NJ) with 100% methanol mobile phase at 1.5 mL/min (retention time, 10.8 min); and (2) C-8 column (5 μm particles, 4.6 mm i.d. x 250 mm, ASTEC) with acetonitrile:methanol (95:5) mobile phase at 1.2 mL/min (retention time, 9.1 min). Injection volumes were 100 μL. Detection was by ultraviolet absorbance at 205 nm with an Hitachi model L-4200 UV-VIS detector (Hitachi Instruments, Danbury, CT). Quantitation was performed by the method of external standards with calibration standards prepared in methanol.

RESULTS AND DISCUSSION

Ever since our early work with ETS solanesol determination, there have been problems associated with the chromatography. We confirmed the earlier work of others showing that solanesol could not be determined by GC without derivatization due to thermal degradation of the underivatized molecule. However, even with derivatization, we noted a gradual increase in

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solanesol decomposition occurring on the GC column with extended use and established procedures for restoring initial performance when necessary.(3,8) In this regard, the DB-1 pre-column is preferred over a deactivated retention gap in that it offers additional (although marginal) resistance to destructive contamination.(8)

We then began experiencing an injection-related degradation of solanesol when using an autosampler for injection even though we were using on-column injection (the most reliable injection mode for labile compounds). This is most likely attributable to either an interaction of the sample with the stainless steel syringe needle or an effect of the injection septum.(3) To date, we have not been completely successful in overcoming this limitation and reliable results are only obtained when performing the on-column injection manually with a syringe equipped with a fused-silica needle.

This inability to automate the analysis presents a serious limitation for any routine determination. The number of samples analyzed is limited to ca. 10/day and sufficient detector calibration cannot be performed on a daily basis. A typical GC chromatogram of a derivatized ETS RSP sample is shown in Figure 2.

In addition to limited sample throughput, another problem is that the sample extraction procedure results in an extract which is incompatible with our UVPM and FPM determinations.(4,5) These determinations are additional RSP apportionment estimators based on total ultraviolet absorbance and fluorescence, respectively, of a methanol extract of filters used to collect RSP. If either UVPM or FPM is to be determined in addition to solanesol (by GC), duplicate samples need to be acquired.

The objectives of the work reported here were to establish an LC method for determining solanesol in ETS which overcomes the automation/decomposition problems encountered in the

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GC method and ensure compatibility with the UVPM and FPM methods enabling any or all three ETS RSP estimators to be determined from a single sample extract.

Lack of a chromophore is the most serious drawback to routine LC determination. With readily available equipment, the only viable option appeared to be UV detection at 205 nm; a wavelength at which nearly all organic compounds absorb. For low wavelength UV detection at trace concentrations, a detector with a deuterium lamp is a necessity. The Hitachi detector (model L-4200; deuterium lamp) was found to be ca. 50 times more sensitive at 205 nm than a Waters (Millipore Corp, Milford, MA) model 490E detector (xenon lamp). As an alternative means of detection, a laser light scattering detector was also evaluated and found to have extremely limited sensitivity.(9)

The wavelength of choice (205 nm) is near the UV cutoff for methanol which results in some detector baseline instability attributable to the mobile phase. Acetonitrile is a better choice for the mobile phase due to its lower UV cutoff, but the limited solubility of solanesol makes it a much weaker solvent in this application although typically, acetonitrile is considered a stronger solvent in reverse-phase LC.

Two sets of LC conditions have been found to be suitable for determining solanesol in methanol extracts of filters used to collect ETS RSP. By using an LC column with a smaller degree of carbon loading (the C-8 column), a mobile phase with a significant percentage of acetonitrile can be used, thus improving baseline stability. However, a small percentage of methanol is necessary to enable solanesol elution within a reasonable time period and to prevent severe peak broadening.

The chromatograms shown in Figure 3 were obtained on the C-18 column with 100% methanol mobile phase. Note the baseline undulation in the standard (top chromatogram).

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The chromatograms shown in Figure 4 are of the same samples shown in Figure 3. Note the more stable baseline and better peak shape with the C-8 column, although overall resolution is not as good as that obtained on the C-18 column. These attributes result in a slightly lower limit of detection (LOD) for the C-8 column, although the practical significance of this difference is minimal.

The graph in Figure 5 illustrates that equivalent results are obtained by these two LC methods for determining solanesol in ETS. The slope is 1 indicating method equivalence and the intercept is not statistically different from 0. The C-8 column with predominantly acetonitrile mobile phase is the preferable method, primarily due to the lower UV cutoff described earlier. Based on this solvent strength argument, the C-8 column with less carbon loading and the stronger mobile phase, should result in less retention, and thus less interference and column contamination, from other organic species present in the sample extract.

Standard addition of solanesol to an ETS sample extract gives results equivalent to quantitation by external standard with an insignificant bias of ca. 2% (Figure 6). Samples were prepared and analyzed (C-8 column) in triplicate at each addition level. This indicates that the peak of interest is pure solanesol; i.e., there are no appreciable co-eluting interferences.

Solanesol recovery from spiked Fluoropore filters is ≥ 90%. Recovery is equivalent for a variety of agitation methods used during extraction (wrist-action shaker, vortex mixer, and ultrasonic bath). Results are also equivalent for 30 and 60 min extraction times and also for 2, 3, and 4 mL methanol extracting volumes. Relative recovery in the GC method is 100% (i.e., relative to the internal standard) although the confidence limits are wider. This is not unexpected due to the greater number of sample handling steps (transfer, evaporation, derivatization, etc.) in the GC procedure.

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The graph in Figure 7 illustrates that equivalent results are obtained by the GC and LC methods (slope and intercept not statistically different from 1 and 0, respectively). For this experiment, duplicate air samples were acquired on eight different occasions in a controlled environment chamber containing ETS. One sample of each matched pair was analyzed by the GC procedure and one by the LC procedure (C-8 column and associated conditions). Concentrations determined were corrected for the apparent recoveries shown in Table I.

Overall method performance for the GC and LC methods is summarized in Table I. LOD for this application appears to be slightly lower for LC when compared to GC. Per given unit of mass, the FID is far more sensitive than UV detection at 205 nm; however, the ability to deliver at least a 100-fold increased mass to the detector (100 *vs* 1 μL injection) results in a sensitivity advantage for LC-UV. It is possible to increase the LC sensitivity with even larger injection volumes, although this is presently deemed not necessary and has not been attempted.

The final comparison between GC and LC is the dramatic difference in separation efficiency. As expected, and evidenced by the chromatograms in Figures 2-4, GC on the open-tubular column provides a tremendous advantage in resolution and separation efficiency. The price for this efficiency is extreme, however, and it appears unnecessary for this application. The LC procedures, which sacrifice high efficiency for selectivity and analysis speed, provide adequate separation of solanesol (illustrated previously with the standard addition experiments, see Figure 6) in less than half the time.

CONCLUSIONS

With roughly equivalent limits of detection and recoveries, the LC procedure is the method of choice due to the significantly higher sample throughput and simplified sample

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preparation. An added benefit of the LC procedure is that the sample extract is compatible with the additional determinations of UVPM and FPM.

ACKNOWLEDGMENTS

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Table I. GC and LC methods performance summary.

	GC	LC
Time/analysis	40 min	15 min
Analyses/day	ca. 10	> 80
LOD; μg/mL	0.2	0.01
LOD; μg/sample	0.04	0.02
LOD; μg/m^3 for 2-hr sampling at 3.25 L/min	0.1	0.05
Recovery from filters (mean ± std. dev.)	104 ± 10 %	90 ± 4 %

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FIGURE CAPTIONS

Figure 1. Solanesol structure (3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaen-1-ol; MW=631).

Figure 2. ETS RSP sample analyzed by GC-FID (derivatized with BSTFA).

Figure 3. Solanesol and ETS RSP samples analyzed by LC-UV (205 nm) on 4.6 x 150 mm C-18 column with 100% methanol mobile phase at 1.5 mL/min.

Figure 4. Solanesol and ETS RSP samples analyzed by LC-UV (205 nm) on 4.6 x 250 mm C-8 column with acetonitrile:methanol (95:5) mobile phase at 1.2 mL/min.

Figure 5. Equivalency of LC methods for ETS solanesol determination (dotted lines indicate 95% confidence interval about the regression line).

Figure 6. Solanesol standard addition to ETS RSP sample extract (μg determined *vs* μg added; dotted lines indicate 95% confidence interval about the regression line).

Figure 7. Comparison of GC and LC methods for ETS solanesol determination (dotted lines indicate 95% confidence interval about the regression line).

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FIGURE 1

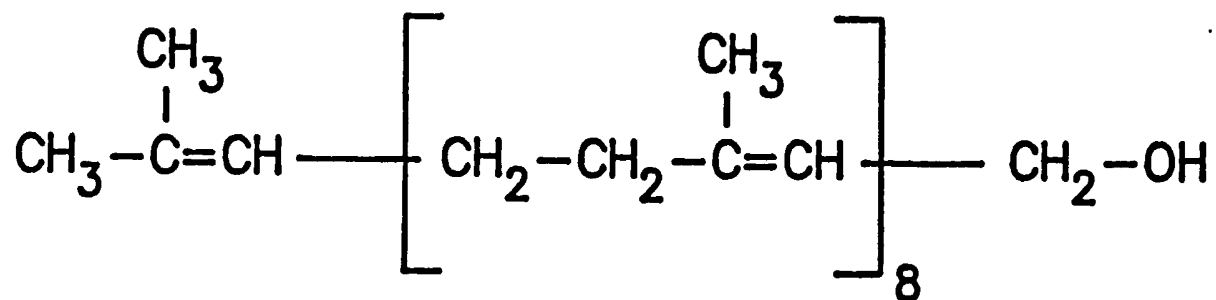

FIGURE 2

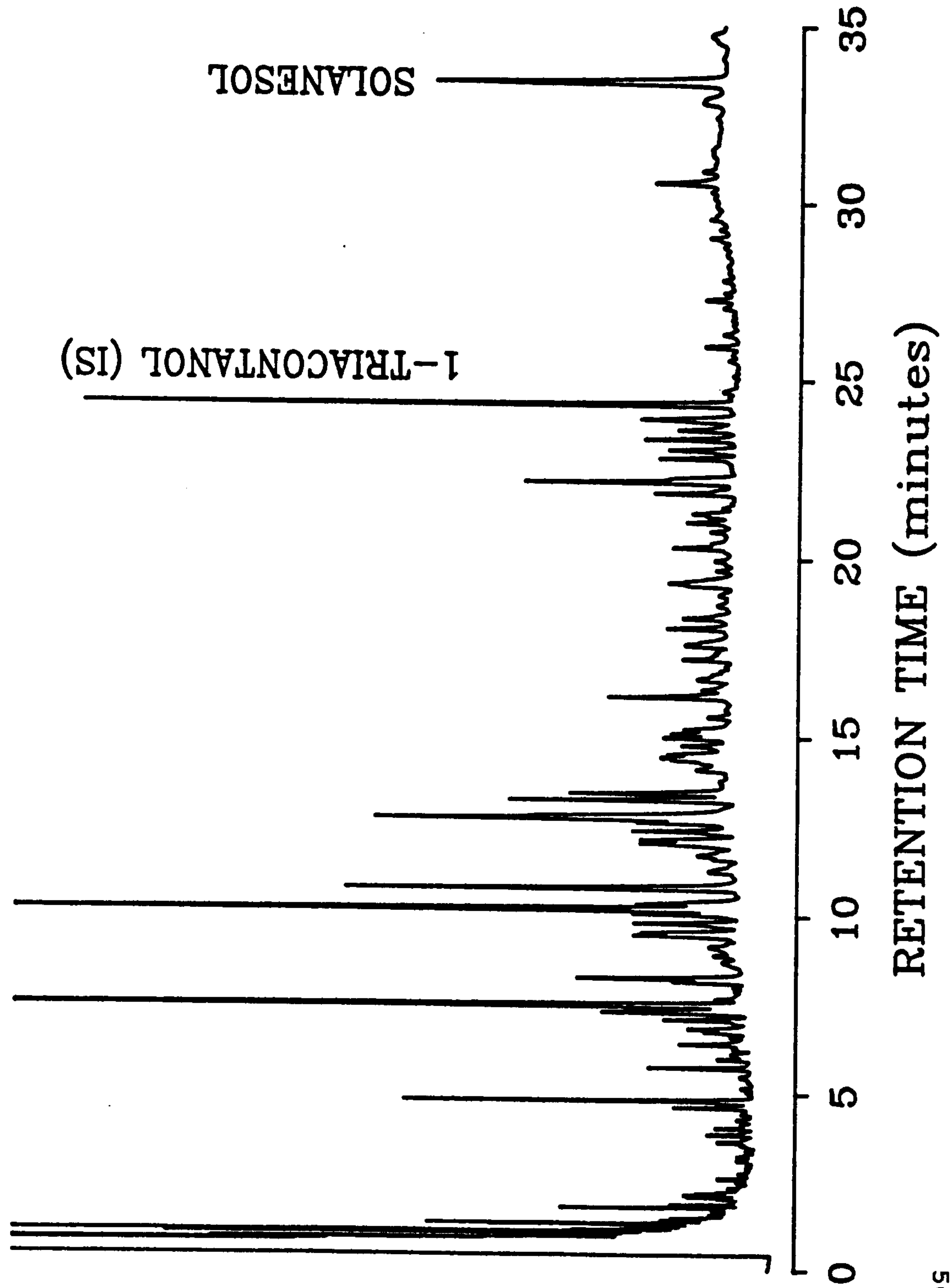

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FIGURE 3

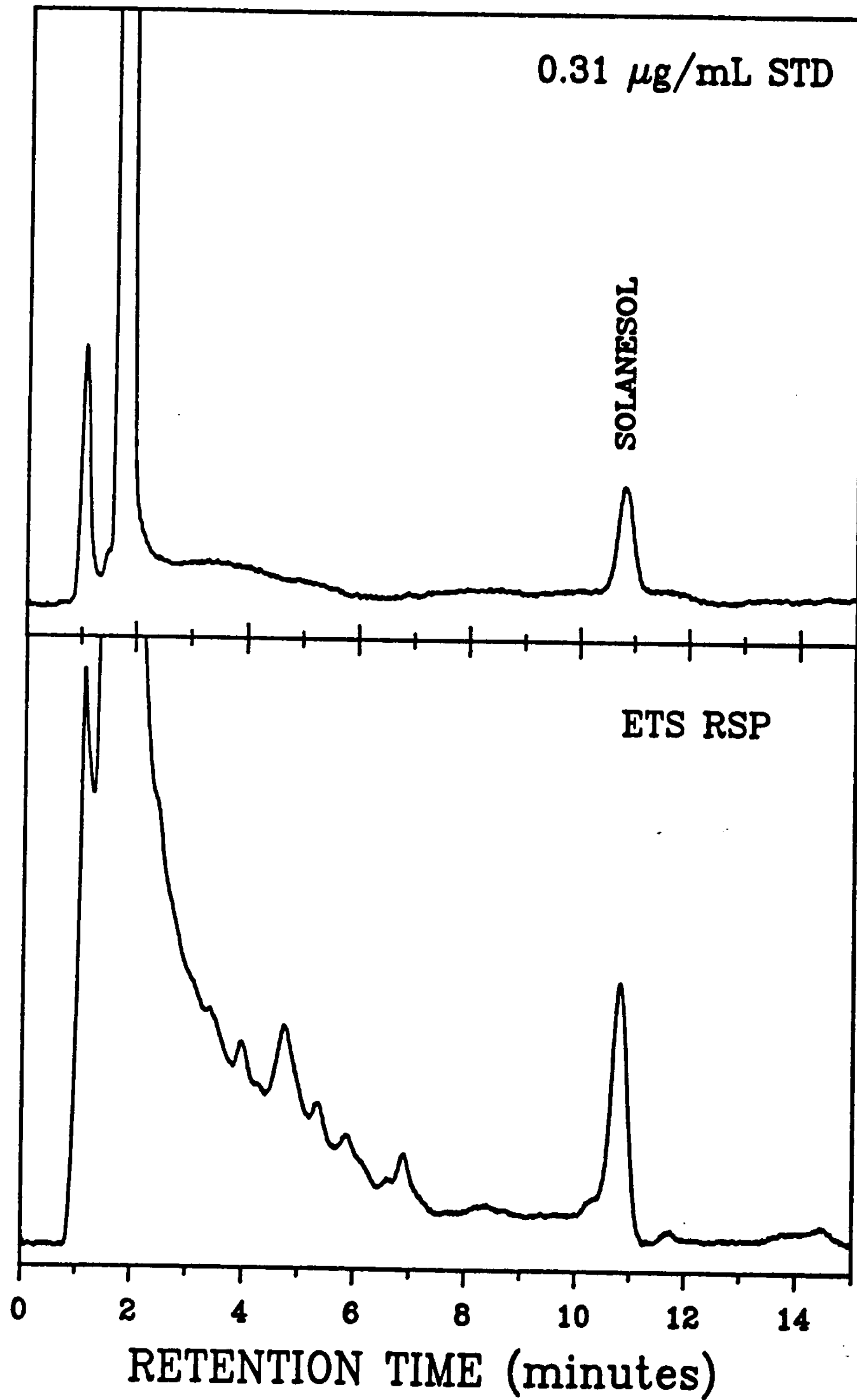

FIGURE 4

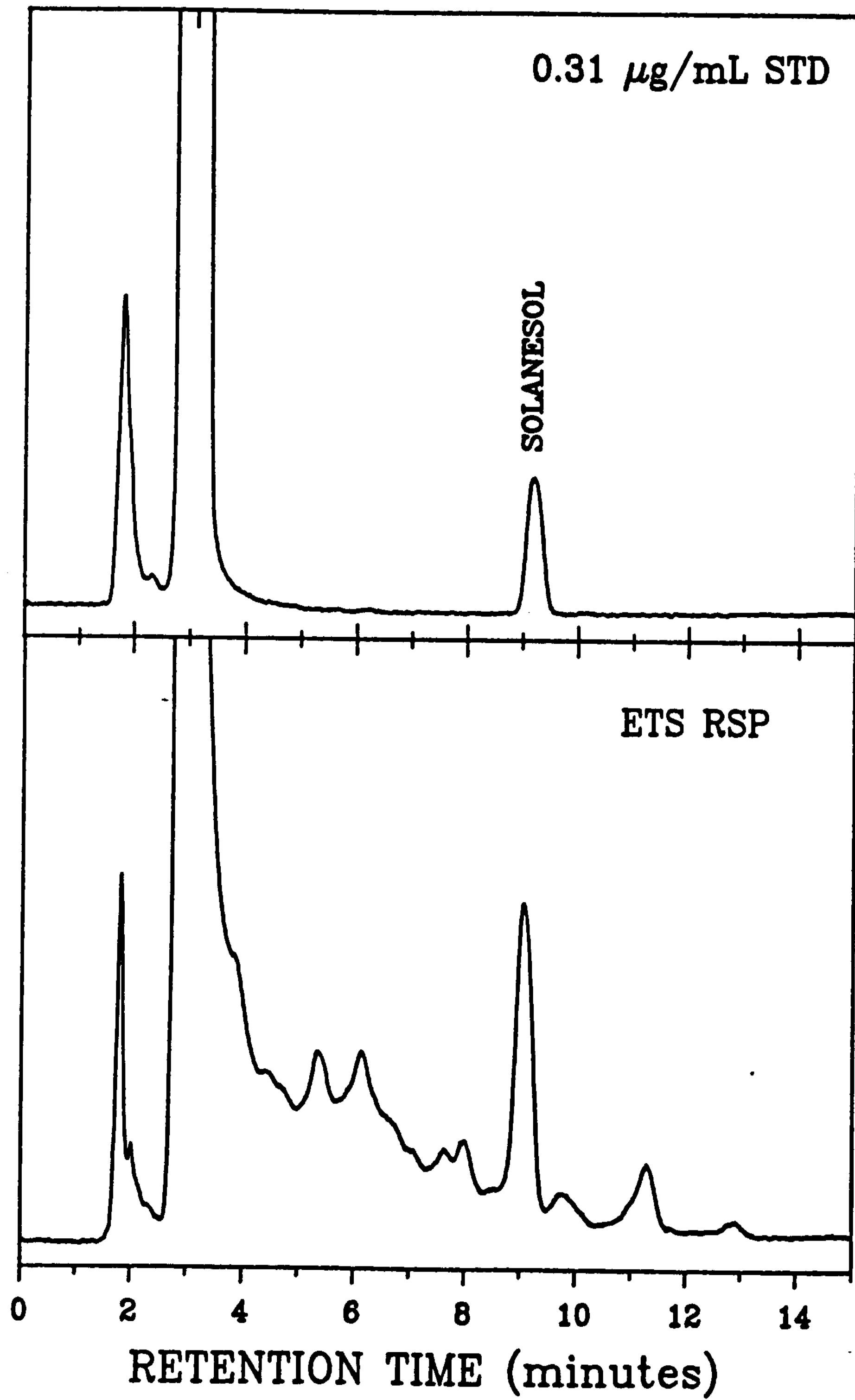

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FIGURE 5

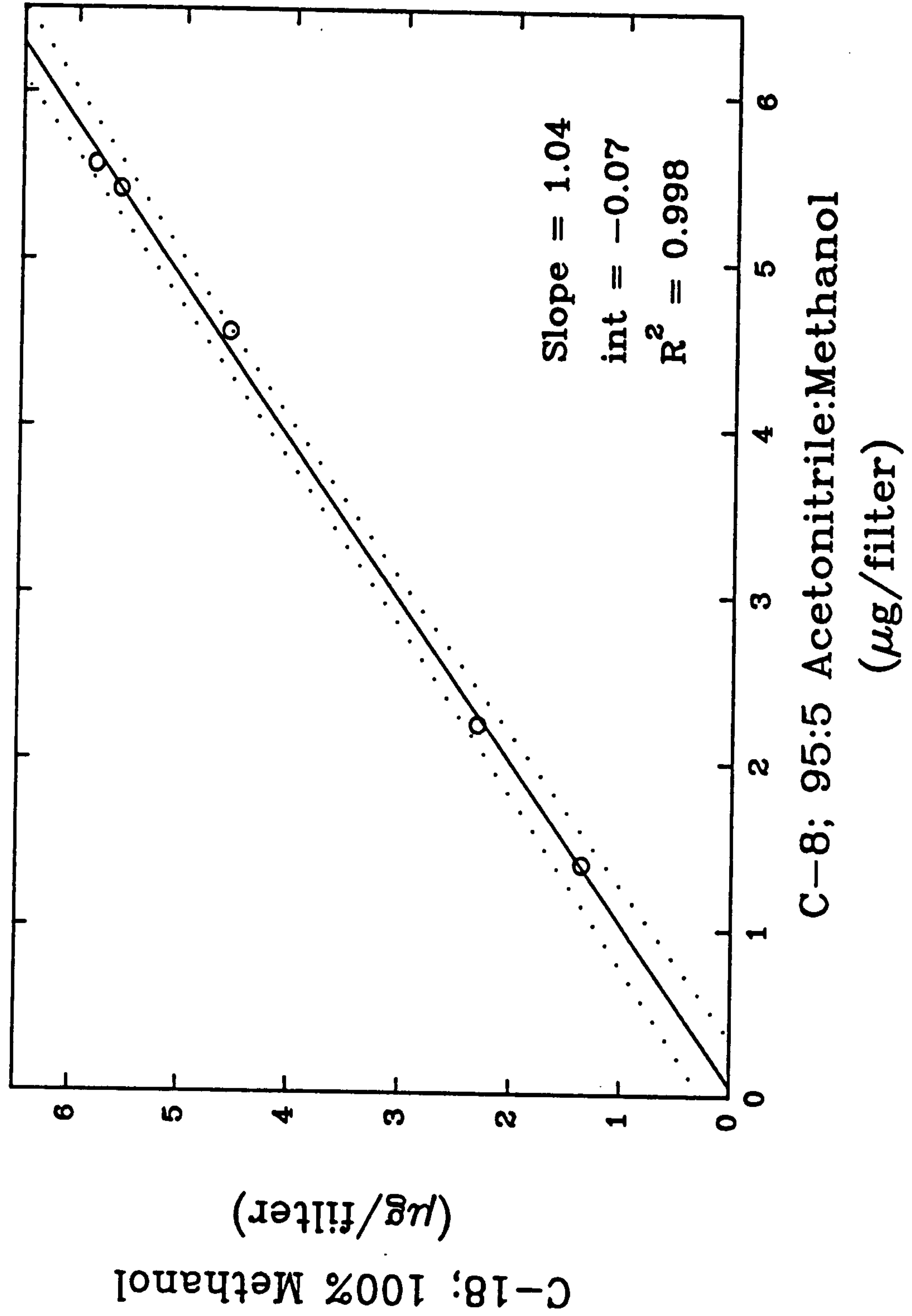

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FIGURE 6

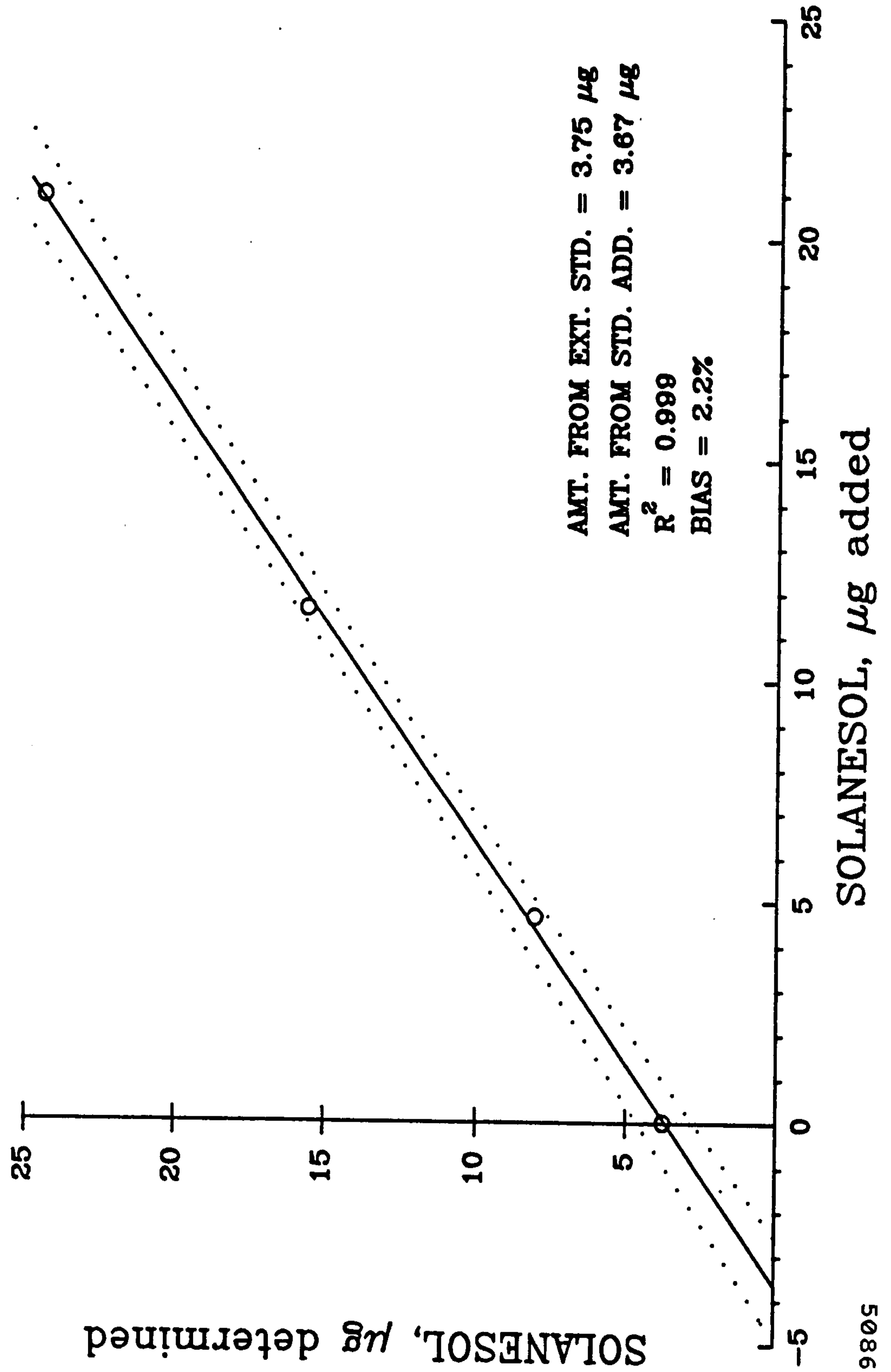

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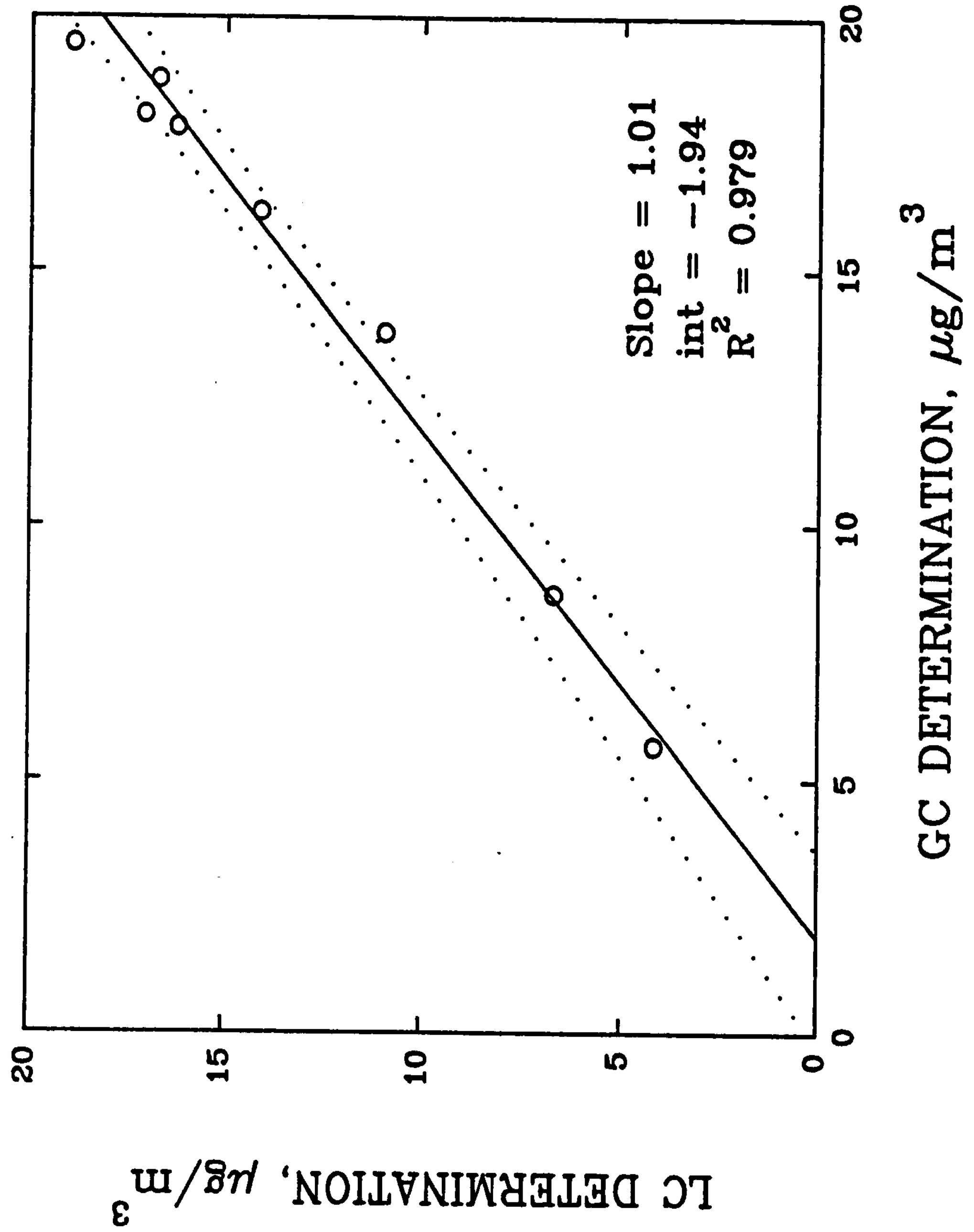
FIGURE 7



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