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Note

Determination of 2,4,7-trinitro-9-fluorenone in workplace environmental samples using high-performance liquid chromatography

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Recent interest in 2,4,7-trinitro-9-fluorenone began when it was reported that this compound, which is used as part of a charge-transfer complex in several models of business machines, was not only a suspect carcinogen, but was also reported to give positive results in mutagenicity assays¹. Possible exposure to trinitrofluorenone may occur during maintenance and repair of the business machine, during the manufacture of the photo conductor drum, and during manufacture of trinitrofluorenone. No sampling and analytical method was available to determine the amount of worker exposure to airborne trinitrofluorenone. The equilibrium vapor concentration of this solid at room temperature, based on available vapor pressure data², would be only $1.9 \cdot 10^{-3} \mu\text{g}/\text{m}^3$. Therefore a method for the determination of particulate trinitrofluorenone air concentration was needed. The method presented here uses normal-phase high-performance liquid chromatography (HPLC) with UV detection for the quantitative analysis of trinitrofluorenone in particulate air samples and in bulk spent toner*.

EXPERIMENTAL

Equipment

The liquid chromatograph used consisted of a Waters 6000A reciprocating pump, a Waters Intelligent Sample Processor 710B autosampler, a Waters 440 UV detector equipped with a 280-nm filter, and a Waters Model RCM 100 radial compression module with a Radial-Pak B adsorption cartridge. The detector signal was recorded on a Soltec Model B281 dual-channel strip-chart recorder and integrated by a Hewlett-Packard Model 3354B laboratory automation system. Filters investigated for recovery of trinitrofluorenone were 37 mm polytetrafluoroethylene filters (Millipore, Fluoropore, FH) and glass fiber filters (Gelman A/E).

Reagents

Dilute standards of 2,4,7-trinitro-9-fluorenone (reagent grade, Matheson, Coleman & Bell) were prepared from a concentrated $1\text{-}\mu\text{g}/\mu\text{l}$ stock solution of the compound in toluene (Burdick & Jackson). The mobile phase solvents were methylene chloride and isoctane (Burdick & Jackson).

* Business machine toner is a finely powdered pigment and plastic resin. The toner is contaminated with trinitrofluorenone during contact with the photo-conductor drum. The excess toner not used in producing the printed page is discarded as spent toner.

Procedure

All filter samples were placed in 8-ml glass screw-cap test tubes and extracted with 2 ml of toluene. Bulk quantities of spent toner (0.3–1.0 g) were extracted with 10 ml of toluene in 20-ml glass screw-cap vials. The trinitrofluorenone was extracted from the spent toner in bulk or on a filter with the aid of ultrasonic agitation for 5–10 min. Toner particulate was then separated by centrifuging at approximately 1300 *g* for 20–30 min. A portion of the clear extract was transferred to an autosampler vial and a 100- μ l aliquot of the clear extract was then injected into the HPLC system with no further preparation and analyzed under isocratic conditions of isooctane–methylene chloride (20:80) at 2 ml/min and ambient temperature. The chromatograph was calibrated with standard solutions of trinitrofluorenone in toluene.

RESULTS AND DISCUSSION

Although normal-phase chromatography was determined to be the best analytical technique, others were investigated first. Gas chromatography with a nitrogen-selective detector was investigated because of the possibility of enhanced sensitivity. However, the thermal instability of the trinitrofluorenone made this approach unsatisfactory, even when using an all-glass gas chromatographic system. Reversed-phase liquid chromatography and electrochemical detection with various types of C_{18} columns were also tried. However, the trinitrofluorenone eluted too slowly when using the high percentages of water in the mobile phase necessary for operation of the available electrochemical detector. In addition, when an aliquot of the toner extract was injected into the LC system, the system became plugged. This was traced to the precipitation of some of the extracted toner compounds from the sample solvent, tetrahydrofuran, upon dilution of the aliquot with the aqueous mobile phase.

Resolution of trinitrofluorenone from toner components was adequate with normal-phase liquid chromatography when an isooctane–methylene chloride mobile phase was used. The all-organic mobile phase also eliminated the problem of precipitation of the toner components. The trinitrofluorenone, however, exhibited some interesting characteristics with this technique. When the strength of the mobile phase solvents was changed, the elution order of trinitrofluorenone with respect to the toner components changed, as shown in Fig. 1. This permitted more maneuvering of the separation, and good separation of trinitrofluorenone from the other toner components could be obtained. In addition, when working over a wide range of concentrations, the retention time of trinitrofluorenone at a constant mobile phase strength changed with the amount injected. Representative data are shown in Table I. The greater the quantity of trinitrofluorenone injected in the same injection volume, the shorter the retention time tended to be. However, over the recommended range of the method, 12 to 80 ng was injected, and the variation of the retention time during a day's analyses was usually around 1% relative standard deviation.

Peak area was used for quantitation to minimize effects due to any variation in retention time of the peak of interest. The average slope of nine calibration curves with a range of 120–800 ng/ml was 1035 area counts-ml/ng with a standard deviation of ± 90 . The average *y*-intercept and its standard deviation for these calibration curves was 1489 ± 3283 area counts, which is not significantly different from zero. The limit of detection was 20 ng/ml and the limit of quantitation, where the standard deviation was no worse than ± 12 , was 120 ng/ml.

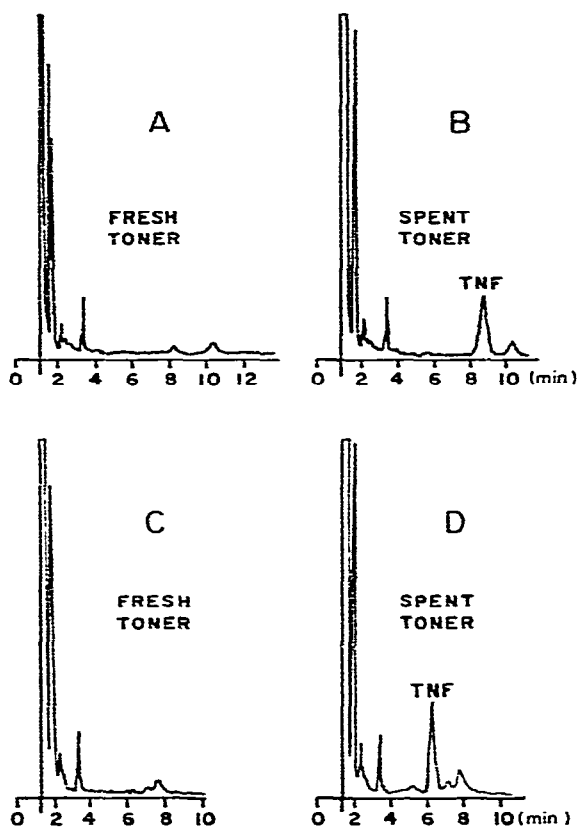


Fig. 1. Chromatograms of the toluene extract of fresh toner (A and C) and spent toner (B and D) showing the change in elution order of trinitrofluorenone (TNF) relative to the other components of the toner with a change in mobile phase strength. For chromatograms A and B the mobile phase was methylene chloride-isooctane (70:30). For chromatograms C and D the mobile phase was methylene chloride-isooctane (80:20).

Since trinitrofluorenone is a solid at room temperature, where it is reported to have a vapor pressure of only $1.1 \cdot 10^{-10}$ Torr², a filter sampling medium for particulate is appropriate for the determination of breathing zone and general air concentrations. Glass fiber and polytetrafluoroethylene filters were investigated for recovery of trinitrofluorenone. Six samples for each filter type at two levels, 0.4 and 1.6

TABLE I

CHANGE OF RETENTION TIME WITH AMOUNT OF TRINITROFLUORENONE INJECTED

Amount injected (ng)	Retention time (min)
2	7.16
6	7.02
10	6.82
20	6.75
200	6.55

TABLE II
RECOVERY OF TRINITROFLUORENONE FROM FILTER MEDIA

<i>Type</i>	<i>Loading (μg)</i>	<i>Recovery (average % \pm S.D.)</i>
Glass fiber	1.6	82.5 \pm 2.9
Glass fiber	0.4	79.7 \pm 5.4
Polytetrafluoroethylene	1.6	101.3 \pm 1.1
Polytetrafluoroethylene	0.4	93.7 \pm 2.4

μg , were prepared. After overnight storage, the samples were analyzed giving results listed in Table II. From these results polytetrafluoroethylene filters were selected as more suitable.

Two 2-week stability studies with samples prepared by spiking blank filters with either pure trinitrofluorenone or spent toner were used to further evaluate method recovery (see Table III). From the data collected when trinitrofluorenone only was present stability on polytetrafluoroethylene filters seemed to be adequate for at least 2 weeks at room temperature. However, when trinitrofluorenone was in the presence of toner, it may not have been as stable. Because of the imprecision of the spent toner data and the limited number of data, it was difficult to assess the stability of trinitrofluorenone in this type of sample.

TABLE III
STABILITY OF TRINITROFLUORENONE ON POLYTETRAFLUOROETHYLENE FILTERS

<i>Loading (ng)</i>	<i>Number of samples</i>	<i>Time stored (days)</i>	<i>Average recovery (% \pm standard deviation)</i>
400	6	1	86.7 \pm 7.6
400	6	7	93.0 \pm 3.6
400	6	14	87.7 \pm 6.3
417*	6	1	108.2 \pm 7.7
417*	6	7	84.2 \pm 8.9
417*	6	14	87.8 \pm 13.7

* The amount of trinitrofluorenone in spent toner loaded on filters was determined by analyzing five aliquots of the spiking suspension, spent toner in toluene, and had a relative standard deviation of 10.6%.

CONCLUSION

From the work presented, normal-phase liquid chromatography can provide an adequate analysis of trinitrofluorenone in workplace environmental samples. The method is quantitative over the range of 0.48–1.6 $\mu\text{g}/\text{m}^3$ with an air sample volume of 500 l and exhibits a pooled relative standard deviation of 5.6% over this range. Stability of trinitrofluorenone in samples with toner present may not be as good as samples with only pure trinitrofluorenone, but use of control samples prepared with spent toner would aid in determining the validity of samples stored for any period of time.

REFERENCES

- 1 *Fed. Reg.*, Vol. 45, No. 157, August 12, 1980; Notices; Department of Labor, Part II.
- 2 *Interoffice communication*, IBM, San Jose Research Laboratory, San Jose, CA, December 16, 1969 (submitted in response to EPA follow up request A DC No. 8EHQ-0680-0339).