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The sampling and determination of azelaic acid in air

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Air samples of azelaic acid (nonanedioic acid), were collected on polyvinyl chloride (PVC) filters, extracted with ethanol, derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), containing 1% trimethylchlorosilane (TMCS) and analyzed by gas-liquid chromatography. The esterification product is the trimethylsilyl ester, which is different from the methyl ester usually chosen in the esterification processes. A calibration curve was used to determine the azelaic acid content in the filter samples. The detection limit was 1 microgram per sample. Results indicate that the analytical method can be applied to other monocarboxylic and dicarboxylic acids.

The sampling and determination of azelaic acid in air

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introduction

Workers for a major fatty acids producer complained of respiratory and eye irritation, which they believed occurred from the inhalation of a fine white dust present in the bagging operation work area. The NIOSH Hazard Evaluation Services Branch collected air filter samples from the bagging operation area. The white dust consisted of 90% C_9 (azelaic acid), 1% C_8, 2% C_{10} and 6% C_{11} dicarboxylic acids, plus 1% other monocarboxylic and dicarboxylic acids. Air samples were collected on filters using personal sampling pumps. A bulk sample of the product produced on the same day that the samples were collected was also submitted by NIOSH industrial hygienists for analysis.

Azelaic acid and its derivatives are employed widely in the plastics and synthetic fiber industry, in the preparation of hydraulic fluids and lubricants and as plasticizers in rubber.[1] Short term toxicological animal studies have shown that azelaic acid is not toxic but is considered as an irritant.[2] No threshold limit value (TLV) or Federal Standard has been established for this compound.

A titrimetric method, used in early developmental work,[3] was insensitive for the analysis of samples containing less than 10 milligrams azelaic acid. Therefore, gas-liquid chromatography (GLC) was chosen for the present analytical work, because it was considered more sensitive, specific and more rapid than other separation techniques.

experimental

instrumentation

Analyses were performed with Perkin-Elmer (P&E) Model 900 gas chromatograph equipped with a flame ionization detector and linear temperature programmer. A P&E Model PEP-1 GC data system and a strip chart recorder 1.0 mv full scale were used to calculate and plot the chromatographic peaks respectively.

apparatus

The GC column consisted of a 3 meter (10 ft.) silanized glass column with 6.4 mm (1/4 in.) O.D., 2 mm (0.08 in.) I.D. packed with 6% SP2100 on 60/80 mesh Supelcoport. The carrier gas was dried with a molecular sieve column connected in series with the carrier gas line. The filters used in the field and percent recovery studies were 37 mm vinyl Metricel (Gelman VM-1) filters with 5 μm pore size.

reagents

N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), containing 1% trimethylchlorosilane

(TMCS) as a catalyst, obtained from Pierce Chemical Company, Rockford, Illinois 61105.

Pyridine, ACS certified grade, from Fisher Scientific, Fair Lawn, N.J. 07410.

Ethanol, dehydrated U.S.P., from Publicker Industries Company, Linfield, Pa. 19468.

Azelaic acid (Emerox No. 1144), from Emery Industries, Cincinnati, Ohio.

Helium and hydrogen 99.995% minimum purity and *"zero grade" compressed air* from local supplier.

procedure

treatment of standards

Six samples of 1, 2, 4, 6, 8 and 10 milligrams of 90% pure azelaic acid were prepared. Each sample was placed in a glass vial with teflon-lined cap and dissolved with 1 mL pyridine. A 1-mL aliquot of BSTFA/TMCS reagent mixture was added to each vial to derivatize the carboxylic acids. The vials were capped and placed in a constant temperature water bath (70° C) for 20 minutes. The contents of the vials were shaken at five minute intervals. After 20 minutes the vials were removed and allowed to cool to room temperature. At least triplicate injections of 1 microliter aliquots were made into the chromatograph.

The GC conditions were:

Oven (column) temperature 190° − 250° C programmed at 6° / min.
Helium carrier gas flow rate 39 mL/ min. at 60 psi.
Hydrogen flow rate 37 mL/ min at 20 psi.
Air flow rate 500 mL/ min at 30 psi.
Detector manifold temperature 250° C.
Injection port temperature 250° C.

treatment of air filters

The filter samples were carefully removed from the cassettes with tweezers and placed in the glass vials. Five mL ethanol was added to each vial to extract the sample. The capped vials were placed in the 70° C water bath for twenty minutes and were shaken every five minutes. At the end of the period, the filters were lifted with tweezers, so that they were above the ethanol level, and washed ten times with 1 mL portions of ethanol using a 1 mL Eppendorf pipette. The vials were placed in a vacuum oven (15 in. of water at 40° C) until all the ethanol was evaporated. The residue then was treated as described in "treatment of standards" section. Filters for percent recovery studies were treated in the same manner as described above. The chromatographic peak area of each standard was determined and a calibration curve of average peak area *vs* concentration of azelaic acid was constructed. Sample weights were corrected to account for the 90% purity. The best straight line was obtained using the least squares method. The concentration of the unknown air filter samples and percent recovery samples was calculated from the equation of the calibration curve.

results and discussion

Although monocarboxylic acids RCOOH have been analyzed in their free form (underivatized) by gas-liquid chromatography,[4-6] dicarboxylic acids $R(COOH)_2$ have not, because they are highly polar compounds. Such compounds have a significant tendency to react or interact with surfaces of the chromatographic support or even with the interior surface of the column structural material. Furthermore, dicarboxylic acids have a very low volatility which contributes additional difficulty in the gas chromatographic analysis. Attempts to chromatograph 1% azelaic acid in chloroform using a very polar stationary phase, 10% diethylene glycol succinate (DEGS), having a McReynolds x constant value of 496 on Chromosorb W washed with 3% H_3PO_4, were unsuccessful since azelaic acid was retained in the column. Derivatization of the acid, therefore, was necessary to increase its volatility, so that chromatography could be useful in the analysis.

Several derivatization techniques which produce the butyl or methyl ester of the carboxylic acid were investigated. These techniques converted the carboxylic acid into ester via acid catalysis,[7,8] acid chlorination,[8] diazomethane,[9] boron trifluoride/ methanol[10] and tetramethyl ammonium hydroxide/ iodobutane.[11] Many of these procedures were complex and time consuming, some used toxic and explosive reagents, (diazomethane) and most of them required a large quantity of sample. Since the range of interest was less than 10 milligrams, it was inevitable that some sample loss was possible during any analytical step. Crowel *et al.*,[12] indicated that with methylation,

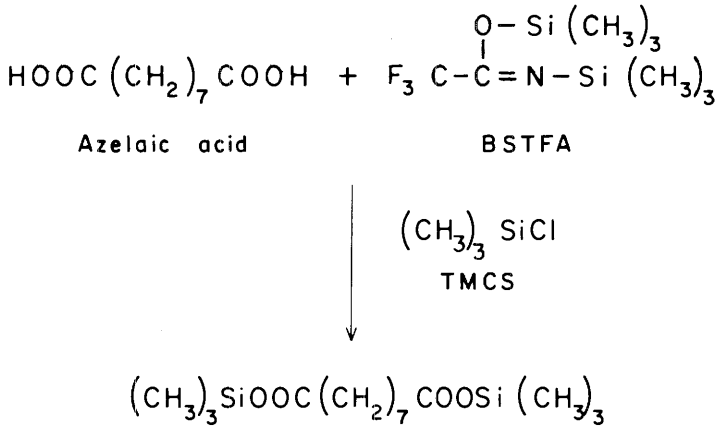

Figure 1 – Esterification reaction of azelaic acid.

low molecular weight acids below C_{12} are partially lost during extraction from the aqueous alcohol layer. Because of these problems, a different esterification method was required, which would be simple, rapid, applicable at trace levels, require minimum sample transfer and have better than 90% recovery efficiency.

Trimethylsilyl esterification met all the above requirements. The carboxylic acids were dissolved in pyridine and then derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA). The BSTFA reagent first synthesized in 1968 by Gehrke and coworkers,[13] proved to be a powerful silylating agent for compounds that contain reactive hydrogen, e.g. amines, amino acids, steroids, carbohydrates, alcohols and carboxylic acids.[14,15] The esterification reaction is shown in Figure 1.

The trimethylsilyl groups $-Si(CH_3)_3$ from both BSTFA and TMCS contribute to the formation of the ester. The reaction shown is a typical esterification of both carboxyl groups by the trimethylsilyl groups. The resulting ester is more volatile than the acid, thus it can easily be chromatographed.

Aliquots taken from the silylation reaction mixture at 1, 5, 10 and 15 minutes were injected into the gas chromatograph. The resulting chromatogram showed that all the acids contained in the sample were converted into their silyl esters within the first five minutes of the reaction.

In general, trimethylsilyl derivatives have excellent thermal stability. It has been reported[15] that no decomposition has been noted with injection and column temperatures up to 300° C. Work performed in our laboratory confirmed this evidence. Since water decomposes both reagents and derivatives, moisture was minimized throughout the experiment. The confirmation of the azelaic ester chromatographic peak was accomplished

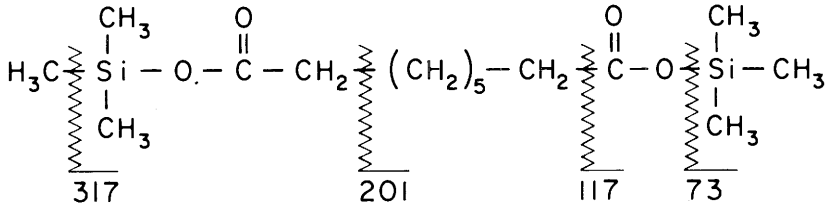

Figure 2 – Fragmentation pattern of azelaic TMS ester.

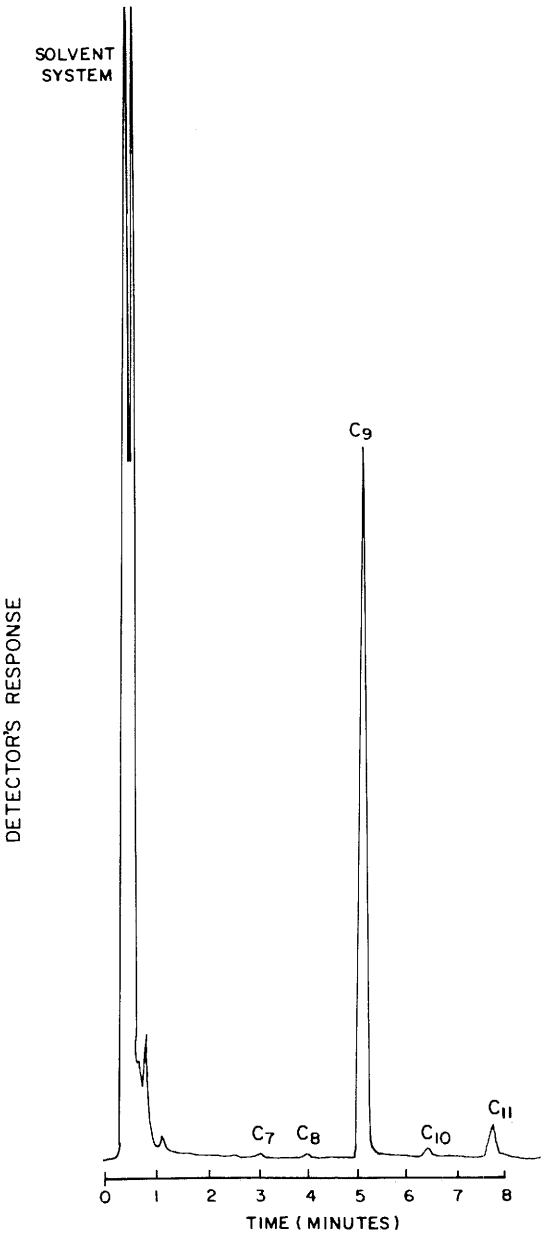

Figure 3 – Gas chromatographic separation of azelaic acid trimethylsilyl ester (C_9) from other aliphatic dicarboxylic acids (matrix).

by GC-mass spectrometry. The ester was identified by its fragmentation pattern. Characteristic of the trimethylsilyl esters is the (M-15) peak which is due to the molecular ion minus a CH_3 group,[16] (see formula below).

In fact, a peak at m/e 317 (332-15) was observed in the spectrum. The largest peaks in the spectrum are due to the trimethylsilyl fragment $-Si(CH_3)_3$, which produces ion peaks at m/e 73 and 75 from the isotopic contribution of Si^{28} and Si^{30}. Other fragments of interest observed are at m/e 117 and at m/e 201 (see Figure 2). The molecular ion peak was too small to be detected. Little attention was given to the mass spectrometric identification of the other carboxylic acids present in the sample.

Gravimetric standard samples of azelaic acid were prepared, treated and derivatized as described in the "treatment of standards" section. A typical chromatogram is shown in Figure 3. The chromatographic peak areas were calculated by a Perkin-Elmer GC data system. The average peak area of the trimethylsilyl ester of azelaic acid varies linearly with concentration; the analytical data and statistical analysis are listed in Table I. Percent recovery data for the analysis are presented in Table II. A calibration curve of the average peak area *vs* concentration of azelaic acid is illustrated in Figure 4.

The range over which the analytical method was tested was 1-10 mg azelaic acid per sample. Preliminary results indicate that the range can be lowered to 0.01 mg per sample and that the analytical method can be applied to monocarboxylic and dicarboxylic acids other than C_9.

The minimum detectable amount of azelaic acid was 0.5 nanogram per one microliter injection at 1X10 attenuation on the Perkin-Elmer Model 900 gas chromatograph. This corresponds to a concentration of 0.001 mg (1 μg) per sample.

summary

The determination of azelaic acid in an air matrix containing aliphatic carboxylic acids has been demonstrated. The analytical method is simple and relatively rapid. It involves extraction of the samples from PVC filters with ethanol, evaporation of the ethanol and derivatization of the residue. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is employed to convert the azelaic acid to its trimethylsilyl ester *in situ* with one derivatizing step. The chromatographic analysis of the trimethylsilyl ester is accomplished in six minutes, utilizing a 6% SP2100 (methylsilicone) on Chromosorb W column. Good chromatographic peak resolution is observed between the azelaic acid and all other aliphatic carboxylic acids which are normally

TABLE I
Results and Statistical Analyses[17,18]

Sample Weight mg	Weight of Azelaic Acid in Sample[a] mg	Average Peak Area	Standard Deviation	Relative[b] Standard Deviation	y-Value[c] from Least Squares Method
1.03	0.93	2.861	0.021	0.73%	2.29
2.01	1.81	5.124	0.111	2.16%	5.23
4.01	3.61	10.846	0.162	1.50%	11.22
6.00	5.40	16.898	0.188	1.11%	17.17
8.01	7.21	22.735	0.533	2.34%	23.19
10.08	9.07	30.016	0.613	2.04%	29.38

The slope and the y-intercept were calculated by the least squares method; $m = 3.33 \pm 0.07$ and y-intercept $= -0.79 \pm 0.37$.

[a]The weight of azelaic acid in the sample was calculated by multiplying the sample weight (from column 1) by 0.90 to correct for sample purity.

[b]The mean analytical Relative Standard Deviation (RSD) is approximately 2%. The total RSD for the method has not been calculated since synthetic atmosphere samples were not generated.

[c]The calculated average peak height (y) was calculated using the formula $y = 3.33x - 0.79$ where x is the concentration of azelaic acid in the sample.

TABLE II
Percent Recovery Data

Sample Weight mg	Weight of Azelaic Acid in Sample mg	Average Peak Area	Weight of Azelaic Acid Recovered mg	Percent Recovery
2.02	1.82	4.71	1.654	90.98%
4.05	3.65	11.53	3.71	101.64%
6.06	5.45	16.01	5.05	92.60%
8.13	7.32	22.67	7.05	96.39%
10.17	9.15	27.44	8.49	92.73%

The average percent recovery was 94.9%.

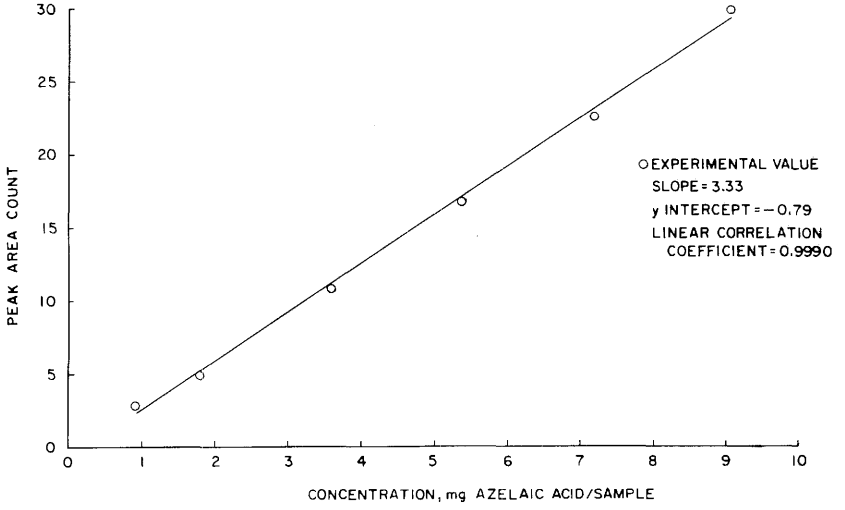

Figure 4 – Azelaic acid chromatographic peak area count versus concentration. Solid line is the least squares fit for all data points.

present in the acid matrix. Results indicate a 2% analytical precision and 95% average recovery. This technique should be applicable for the analysis of other aliphatic monocarboxylic and dicarboxylic acids.

acknowledgment

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references

1. **Snell, F. D. and L. S. Ettre:** *Encyclopedia of Industrial Chemical Analysis 8* p. 400, Interscience, New York (1969).
2. **Health Hazard Evaluation Determination Report #75-154:** U. S. Department of HEW, NIOSH, Cincinnati, Ohio 45226.
3. **Kim, W. S.:** Personal Communication, NIOSH, Cincinnati, 1976.
4. **Ottenstein, D. M. and W. R. Supina:** Improved Columns for the Separation of C_{14}-C_{20} Fatty Acids in the Free Form. *J. Chromatogr. 91*:119 (1974).
5. **Supelco, Inc.:** *Chromatography catalog #10,* p. 9 (1976).
6. **Mlejnek, O. and L. Cveckora:** Evaluation of New Phases for the Gas Chromatography of Dibasic Acids. *J. Chromatogr. 82*:377 (1973).
7. **Gee, M.:** Methyl Esterification of Nonvolatile Plant Acids for Gas Chromatographic Analysis. *Anal. Chem. 37*:926 (1965).
8. **Morrison, R. T. and R. N. Boyd:** *Organic Chemistry,* 2nd Ed. p. 601 Allyn and Bacon, Inc., Boston (1966).
9. **Schlenk, H. and J. L. Gellerman:** Esterification of Fatty Acids with Diazomethane on a Small Scale. *Anal. Chem. 32*:1412 (1960).
10. **Metcalfe, L. D. and A. A. Schmitz:** The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis. *Anal. Chem. 33*:363 (1961).
11. **Greeley, R. H.:** Rapid Esterification for Gas Chromatography: *J. Chromatogr. 88*:229 (1974).
12. **Crowell, E. P., S. M. Aronovic and B. B. Burnett:** Gas Chromatographic Determination of Mono- and Di-Basic Acids. *J. Chrom. Science 9*:296 (1971).
13. **Gehrke, C. W., D. L. Stalling and R. W. Zumwalt:** A New Silylation Reagent for Amino Acids. *Biochem. Biophys. Res. Commun. 31*:616 (1968).
14. **Applied Science Labs., Inc.:** *Technical Bulletin No. 11B.* State College, Penn. 16801.
15. **Pierce Chemical Co.:** *Handbook of Silylation,* Rockford, Ill. 61105.
16. **Shrader, S. R.:** *Introductory Mass Spectrometry,* p. 182, Allyn and Bacon, Inc., Boston (1974).
17. **Crumpler, J. B. and J. H. Yoe:** *Chemical Computations and Errors,* p. 125. p. 202, J. Wiley & Sons Inc., New York (1940).
18. **Gordon, A. J. and R. A. Ford:** *The Chemist Companion,* p. 480, J. Wiley & Sons Inc., New York (1972).

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