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ANALYSIS OF THE DIAZINON METABOLITES G 27550 AND GS 31144 BY GAS–LIQUID CHROMATOGRAPHY WITH NITROGEN-SPECIFIC DETECTION AFTER DERIVATIZATION

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SUMMARY

The alkylation and silylation of the diazinon metabolites G 27550 (2-isopropyl-4-methyl-6-hydroxypyrimidine) and GS 31144 [2-(1'-hydroxy-1'-methyl)-ethyl-4-methyl-6-hydroxypyrimidine] with methyl iodide/sodium hydride and bis-(trimethylsilyl)-trifluoroacetamide, respectively, were examined for use as confirmation techniques for these metabolites in sample material. The methods proved to be rapid and simple. The derivatives chromatographed well on 4% SE-30/6% QF-1. Electrolytic conductivity detection proved satisfactory for the derivatives with minimum cleanup. The techniques were successfully applied to the analysis of the two metabolites in dog urine in the range of 1 ppm.

INTRODUCTION

A number of authors[1–4] have shown that the major pathway of degradation of diazinon and its oxygen analogue, diazoxon, in several mammalian and plant systems is via the hydrolysis of the pyrimidinyl phosphate bond. Mücke *et al.*[2] have shown in rats that more than 40% of pyrimidine ring-labeled diazinon was excreted in the urine after nine days as a combination of G 27550 (2-isopropyl-4-methyl-6-hydroxypyrimidine) and GS 31144 [2-(1'-hydroxy-1'-methyl)-ethyl-4-methyl-6-hydroxypyrimidine]. No intact diazinon was found in the urine.

Although the pyrimidinyl metabolites G 27550 and GS 31144 are more than tenfold less toxic than the parent diazinon[2], analyses of such compounds in the urine can serve as a good indication of diazinon ingestion in a variety of mammals including humans. Fatal human poisonings by diazinon have been documented[5–8].

Present methods for diazinon analysis include gas–liquid chromatography (GLC) with thermionic detection after methylation of the phosphorus moiety of the molecule[9,10]. However, this method is not specific for diazinon as it is applicable to a large variety of organophosphorus pesticides. Laanio *et al.*[4] used GLC with an electrolytic conductivity detector (ECD) for G 27550 in rice, paddy soil and pea plants as part of a study on the fate of diazinon in these systems.

The sensitivity of the ECD for diazinon has been reported by several workers[11–13]. Palframan *et al.*[14] and Greenhalgh and Cochrane[15] compared the ECD with the alkali flame ionisation detector for the detection of nitrogen-containing compounds. While the responses for nitrogen were of the same order, the electrolytic conductivity system was considered more suitable for routine use since it was more selective and less disturbed by small changes in operating parameters[14,15].

Methyl iodide/sodium hydride alkylation has been used recently for the confirmation of triazine herbicides in foods[16] and is based on the method developed by Greenhalgh and Kovacicova[17] for the herbicides atratone and linuron as well as for some organophosphorus insecticides. An on-column alkylation technique has recently been published for urea herbicide analysis by GLC[18]. These alkylation techniques are well suited for electrolytic conductivity analysis since none of the reagents contain nitrogen and therefore few interferences are encountered upon GLC.

The present work reports on the development of two confirmation techniques for determining G 27550 and GS 31144 in urine by GLC with an ECD.

MATERIALS

Apparatus

An Aerograph HY-FI Model 600C gas chromatograph equipped with a Coulson electrolytic conductivity detector (ECD) (Tracor, Austin, Texas, U.S.A.) and a 6-ft. × 6-mm-O.D. glass column packed with 4% SE-30/6% QF-1 on Chromosorb WHP (80–100 mesh) was used for the analyses. Operating conditions were: transfer unit, 210°; pyrolysis furnace, 780°; helium carrier flow-rate, 60 ml/min; helium sweep, 60 ml/min; hydrogen flow-rate, 50 ml/min; d.c. bridge potential, 30 V. Column temperatures were varied for the different derivatives. A 1-cm plug of strontium hydroxide-coated glass wool was placed 3 cm from the end of the quartz pyrolysis tube and a 2-cm-long flattened coil of nickel wire was placed 5 cm from the end of the tube. The reservoir water level was maintained 1 cm above the pump entrance. A 1.0-mV strip-chart recorder operating at 0.25 in./min was employed.

Mass spectra of the derivatives were obtained on a Hitachi Perkin-Elmer RMS-4.

Reagents

Authentic standards of diazinon [O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidyl)-phosphorothioate], G 27550 and GS 31144 were dissolved in glass-distilled residue-free acetone at a concentration of 1 mg/ml. A dilution series was made from these for calibration purposes. Dimethylsulfoxide (DMSO) (BDH, Poole, Great Britain), methyl iodide (Fisher Scientific, Pittsburgh, Pa., U.S.A.) and the silylating reagents BSTFA [N,O-bis-(trimethylsilyl)-trifluoroacetamide], BSA [N,O-bis-(trimethylsilyl)-acetamide] and MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide) (Pierce, Rockford, Ill., U.S.A.) were used as obtained from the suppliers. Sodium hydride (50% mineral oil dispersion) (J. T. Baker, Phillipsburgh, N.J., U.S.A.) was washed with hexane before use. All organic solvents were glass-distilled residue-free materials. Urine samples were collected from control beagle dogs and pooled for spiking purposes. The method was applied to the analysis of ^{14}C ring-

labeled diazinon and its two major pyrimidyl metabolites in the pooled urine of a beagle dosed orally with 4 mg/kg of diazinon daily for five days.

ANALYTICAL PROCEDURE

Sample extraction

Twenty-five millilitres of urine diluted to 200 ml with distilled water were extracted three times with 75-ml portions of trichloromethane in a 500-ml separatory funnel. Each trichloromethane extract and existing emulsion were centrifuged in a 250-ml centrifuge bottle in a Sorvall Superspeed Centrifuge (SS3) and the aqueous portion returned to the separatory funnel for further extraction. The combined organic extracts were concentrated to about 2 ml by rotary vacuum evaporation at 30°. The concentrate was then transferred to a 5-ml graduated centrifuge tube and brought to 1 ml under a gentle stream of nitrogen for gas chromatography. After injection, the column effluent was vented for at least 1 min before being directed into the pyrolysis furnace.

Alkylation

One-half of the concentrated trichloromethane extract was evaporated to dryness at 35° under a gentle stream of nitrogen. To the residue were added 2 ml of DMSO and about 10 mg of sodium hydride. The tube was gently shaken to disperse the sodium hydride. A 0.1-ml volume of methyl iodide was then added to the mixture. The tube was stoppered, shaken again and permitted to stand at room temperature for 5 min. The tube was opened and 2 ml of benzene were added. The stopper was replaced and the tube shaken. Distilled water (5 ml) was carefully added to the tube to destroy the remaining sodium hydride. The tube was then shaken and the phases permitted to separate. The benzene layer was used for GLC analysis.

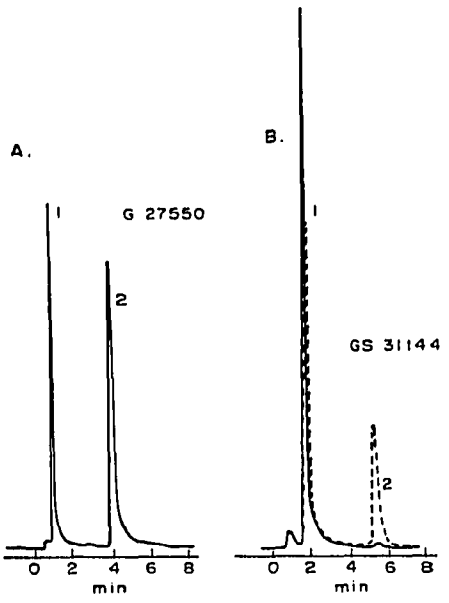
Silylation

The remaining concentrated trichloromethane extract was evaporated to dryness at 35° under a gentle stream of nitrogen. A 25-μl aliquot of BSTFA was added and the tube gently swirled until all the residue was wetted by the reagent. The tube and contents were heated at 40° for 10 min. Ethyl acetate (1 ml) was added and the tube shaken. An aliquot of this solution was used for GLC.

RESULTS AND DISCUSSION

Alkylation

Under the described conditions the alkylation reactions were almost instantaneous. However, two derivatives were consistently formed for each metabolite. Mass spectrometry showed that the two methylated products of GS 31144 had the same molecular ions ($m/e = 182$), which indicated that monoalkylation had occurred for each. The fragmentation patterns for each were very similar and showed that the hydroxyl group on the isopropyl substituent was not methylated. The two peaks were believed to be the pyrimidinyl O-methyl and N-methyl derivatives with the former being the first eluted peak. The same explanation is given for the two products of G 27550. Fig. 1 shows chromatograms of the methylated products of the two



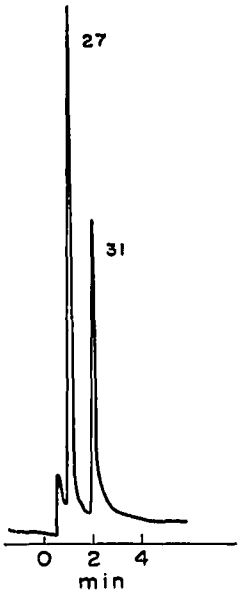


Fig. 1. Chromatogram of: (A) methylated G 27550, (B) methylated GS 31144. Column temperature, 165°. A dashed line indicates methylated products formed when heated at 35° for 90 min. 25 ng of equivalent metabolites were injected. Attenuation, ×2. 1 = O-Methyl derivative; 2 = N-methyl derivative.

Fig. 2. Separation of monosilylated G 27550 (27) and GS 31144 (31) on 4% SE-30/6% QF-1 at 165°. 10 ng of each was injected. Attenuation, ×4.

metabolites. The second peak of methylated G 27550 was much more prominent than that for methylated GS 31144 under the same reaction conditions. Heating the alkylation mixture of GS 31144 for 60 min or more at 35° increased the second peak at the expense of the first. DMSO was found to be the most suitable reaction solvent examined. Tetrahydrofuran was less satisfactory and N,N-dimethylformamide unsuitable since it contains nitrogen and caused a broad solvent peak which obscured the derivative peaks.

Silylation

All three silylating reagents (BSA, BSTFA, MSTFA) gave a single derivative peak with G 27550 under the reaction conditions mentioned in Analytical procedure. With GS 31144, BSTFA gave a single peak (t_R = 2.05 min) under the same reaction conditions while BSA and MSTFA gave two peaks in different proportions. The second peak in each case was the disilyl derivative (t_R = 2.25 min). When the BSA reaction mixture was heated at 80° for 20 min the disilyl derivative was formed exclusively. The BSTFA and MSTFA reactions also proceeded to the disilyl product upon overnight reaction at room temperature. Peak identities were confirmed by combined GLC–mass spectrometry. The silylation occurred at the ring hydroxyl substituents for the mono-derivative. Fig. 2 shows a chromatogram of a separation of the monosilyl derivatives of G 27550 and GS 31144 using BSTFA for silylation.

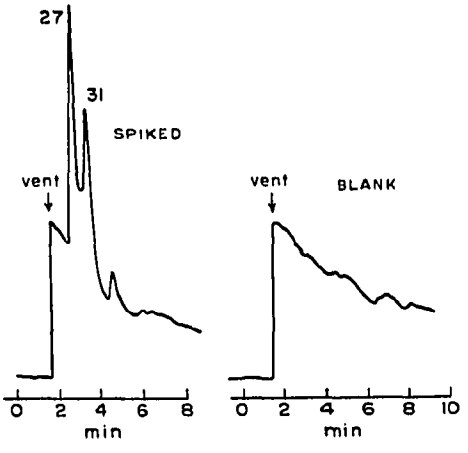
Analysis

The derivatization reactions were found to improve greatly the sensitivity and chromatography of the two metabolites compared to the non-derivatized compounds. Table I compares sensitivities obtained on the Coulson ECD. The direct analysis of G 27550 in dog urine spiked at 1.0 ppm was accomplished under the extraction conditions used. GS 31144 was not detected directly at the spiked level of 1.0 ppm. This was due to the lack of sensitivity for this metabolite as well as to the incomplete extraction from the urine. About 50% of GS 31144 was recoverable using chloroform. Mücke *et al.*[2] used butanol extraction of urine followed by Soxhlet extraction with chloroform to quantitatively recover both metabolites.

The alkylation of the same urine extract indicated the presence of both compounds as their methylated derivatives. Fig. 3 depicts chromatograms of alkylated spiked and blank samples. The peaks shown are the $N-CH_3$ derivatives formed upon alkylation. The $O-CH_3$ derivatives were obscured in the solvent front when only 45-sec venting was attempted.

The silylation of an aliquot of the same spiked extract also proved successful. Fig. 4 illustrates a chromatogram of the two metabolites in a silylated spiked urine extract.

The application of these two derivatization techniques to the analysis of ^{14}C ring-labeled diazinon metabolites in the pooled urine of a dog fed 4 mg/kg of diazinon for five days confirms the presence of both G 27550 and GS 31144 at about 1 ppm, although only G 27550 was found in the extract by direct chromatography without derivatization. No attempts were made to quantitate accurately the metabolites in the



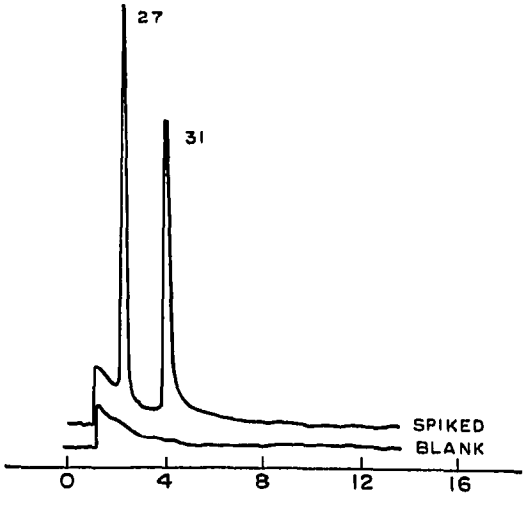


Fig. 3. Alkylated control urine. Spiked with 1 ppm each of G 27550 (27) and GS 31144 (31). 125 mg of equivalent urine sample injected. Attenuation, ×2.

Fig. 4. Silylated control urine. Spiked with 1 ppm each of G 27550 (27) and GS 31144 (31). 50 mg of equivalent urine sample injected. Attenuation, ×4.

TABLE I

COULSON ECD RESPONSE TO DIAZINON METABOLITES AND DERIVATIVES

Column: 4% SE-30/6% QF-1. Carrier flow-rate, helium: 60 ml/min. Temperatures: parents and alkylated products, 185°; silylated products, 135°.

Compound	*Retention time*	*Peak height/ng parent (in.)*
Parent		
Diazinon	5 min	0.7
G 27550	4 min 20 sec	0.04
GS 31144	5 min 15 sec	0.01
Alkylated		
G 27550 peak 1	50 sec	0.44
peak 2	2 min 5 sec	0.38
GS 31144 peak 1	1 min 7 sec	0.41
peak 2	2 min 45 sec	0.16
Silylated (mono)		
G 27550	1 min 10 sec	2.1
GS 31144	2 min 5 sec	1.21

urine via the derivatization reactions. Fig. 5 illustrates chromatograms of both alkylated and silylated extracts. Several other nitrogen-containing peaks which were not present in the control sample (Figs. 3 and 4) were also evident. Scintillation counting of the urine extract before and after alkylation showed that 70% of the radioactivity was extracted into the benzene for GLC analysis.

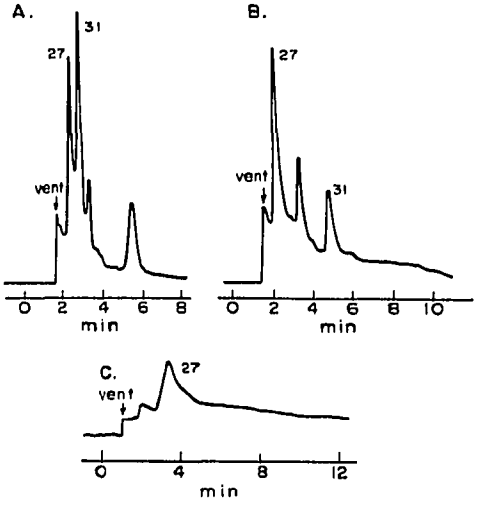


Fig. 5. Analysis of ^{14}C ring-labeled diazinon metabolites in dog urine. (A) Alkylated urine sample; 20 mg of equivalent sample injected; attenuation, ×2; column temperature, 152°. (B) Silylated sample; 20 mg of equivalent sample injected; attenuation, ×4; column temperature, 134°. (C) Untreated urine extract; 125 mg equivalent sample injected; attenuation, ×2; column temperature, 165°.

CONCLUSIONS

Alkylation or silylation of the diazinon metabolites G 27550 and GS 31144 greatly improved sensitivity and peak shape by gas chromatography. The reactions were simple and rapid and made analysis of GS 31144 possible where it was otherwise undetectable.

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