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GAS CHROMATOGRAPHIC DETERMINATION OF 1,2-PROPANEDIOL DINITRATE IN BLOOD

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SUMMARY

A method is described for determination of 1,2-propanediol dinitrate in blood at concentrations ranging from 10 ng/ml up to 25,000 ng/ml. It uses double ether extraction with manual shaking in order to complete sample preparation within 5 min. Samples are analyzed via gas chromatography–electron-capture detection using a column of 3% base deactivated SP-2250 on Supelcoport. This column provides excellent separation and little 1,2-propanediol dinitrate tailing.

INTRODUCTION

1,2-Propanediol dinitrate (PGDN) was originally considered as a replacement for 1,2-ethanediol dinitrate in the manufacture of antifreeze dynamite[1] due to the latter's toxicity. It was later found that the effects of vasodilation, hypotension and methemoglobinemia were also present during PGDN intoxication[2,3]. Since PGDN is now used as a principal torpedo fuel (Otto Fuel II) component, further toxicologic investigations were undertaken. These studies required a method of analysis for PGDN in blood that was both rapid and extremely sensitive.

Various analytical techniques have been developed for measuring the organic dinitrate ester compounds. A well established method is alkaline hydrolysis followed by colorimetric determination[4]. This procedure has several drawbacks in that it is time consuming, insensitive with a demonstrated lower detection limit of only 1 μg/ml and relatively non-specific. Polarography is another method which has been applied to PGDN determinations[5]. This equipment is generally not available in most toxi-

cology laboratories. Gas chromatography is by far the most versatile and useful method for PGDN analysis and there are several published methods. Most[6,7] are insensitive or too time consuming and presented technical problems such as peak tailing[8]. In view of our needs and the available methodology, a more sensitive and rapid gas chromatographic technique was developed.

EXPERIMENTAL

Materials

PGDN was obtained as "spirits" in methanol. The neat PGDN was recovered by slowly passing helium gas over the liquid to evaporate the methanol. High-performance liquid chromatography and density measurements were made and compared to published values[9] to ensure adequate PGDN purity for making standard solutions.

The ethenyloxyethene (diethyl ether) used to prepare standards and extract blood samples was purchased as absolute, A.C.S. reagent grade.

Equipment

The gas chromatograph was a Hewlett-Packard Model 5880A, equipped with a modulated-flow thermal conductivity detector (TCD), a ^{63}Ni electron-capture detector (ECD) and a Level Four data integrator. The carrier gas was 30 ml/min helium for the TCD and 30 ml/min methane–argon (5:95) for the ECD.

The column was nickel, 1 m × 2 mm I.D., containing 3% SP-2250 DB on 100–120 mesh Supelcoport (Supelco). This material is a methyl phenyl silicone, similar to OV-17, which has been deactivated for basic compounds. The column was temperature programmed from 70°C to 120°C at 10°C/min. Following each analysis, the temperature was increased to 200°C for 1 min to elute less volatile residues off the column. The injection port was maintained at 135°C and the detector at 165°C. Although Otto Fuel II decomposition begins above 120°C and becomes rapid above 145°C[10], lowering the injector and detector temperatures to 110°C did not improve the chromatograms.

Sample preparation

A 1-ml sample of freshly drawn whole blood was added to 1 ml distilled water and 5 ml diethyl ether in a capped (PTFE lined) 20 ml glass test tube. The mixture was vigorously shaken manually for 15 sec before centrifuging at 3000 RCF (gravities) for 30 sec to aid separation of aqueous and organic layers. The upper layer was withdrawn and transferred to a capped (PTFE lined) 10-ml graduated centrifuge tube. An additional 5 ml of diethyl ether was used to extract the sample a second time. The extracts were combined and the total volume noted. A 2-μl aliquot of the combined extracts was then injected into the gas chromatograph.

Calculations

The Hewlett-Packard data integrator determined both retention time and area of the generated peaks. Based upon a daily run of nine external standards, the total extraction volume and the PGDN peak area of the sample, a concentration expressed as ng/ml was automatically generated by the integrator.

RESULTS AND DISCUSSION

The use of SP-2250 DB column material permitted excellent separation of PGDN from solvents (Fig. 1). Less volatile constituents which might elute during a following run were removed by using a 1-min post-run column bake-out at 200°C. An additional 2 min were required to reequilibrate the column at 70°C thereby allowing one sample to be analyzed every 10 min. Another advantage of this column was that tailing associated with nitrate esters was greatly reduced. It was also found that recommended daily preconditioning of the column with glycerol trinitrate[4] was not only unnecessary, but undesirable, in that traces of nitrates continued to elute even after many injections.

Since the range of concentration of PGDN in blood was expected to be from 10 ng/ml to 25,000 ng/ml, a nine-point calibration curve was constructed each day. Comparison of the response factors showed a non-linear portion of the calibration curve at the lower concentrations. Use of 1,2,3-propanetriol trinitrate as an internal standard did not prove successful because of significant metabolism in whole blood.

Precision

Several samples were repetitively analyzed in order to demonstrate that the instrument would reliably integrate and report the PGDN values. Since these samples

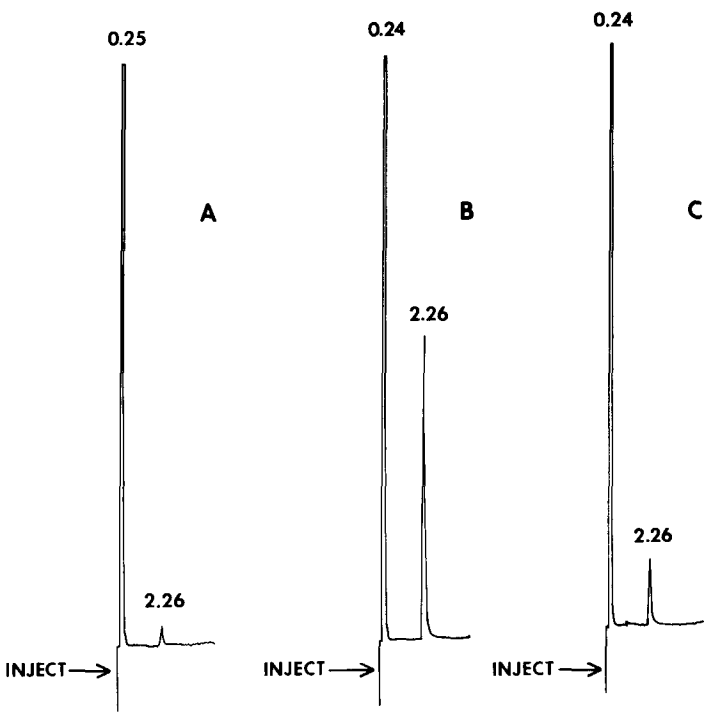

Fig. 1. Chromatograms of diethyl ether standards and sample extract. A, Standard of 10 ng PGDN/ml ether (PGDN retention time of 2.26 min); B, Standard of 100 ng PGDN/ml ether; C, Extract of blood which contained 27 ng PGDN per ml.

TABLE I

PRECISION OF ANALYSIS IN PGDN DETERMINATION

Repetitive determinations of different sample media containing several PGDN concentrations.

Sample type	*PGDN concentration (ng/ml)*	*Number of analyses*	*Mean area counts (± S.D.)*
Blood extract	104	10	8941 (±7%)
Blood extract	7580	10	715753 (±2%)
Ether standard	10	10	1808 (±2%)

were injected manually rather than by mechanical means, the results also reflect operator variability. Table I presents the data for both samples and a standard. Approximately 95% of the samples to be collected during toxicokinetic studies are expected to be within the range of concentrations covered by the two blood extract samples.

Sensitivity

Minimum amounts of PGDN that could be determined were routinely observed for both thermal conductivity and electron capture detectors. The limit of detectability for ECD was 1 pg injected, whereas 75,000 pg was the value on the TCD. These results correspond to concentrations of 5 ng PGDN/ml blood and 150,000 ng PGDN/ml blood, respectively. No interfering peaks were present in extracts of PGDN free blood samples. Concentration of the volume of combined sample extracts to increase sensitivity further was impractical due to variation in recovery.

Reliability

The procedure was tested for two types of reliability. The first was an observation of the number of samples a column could process before PGDN tailing made quantitation difficult. This point was taken to be when the data integrator showed an aborted area count for a 2500 ng/ml standard. Approximately 400 analyses could be

TABLE II

LONG-TERM STABILITY OF A PREPARED EXTRACT

An extract of blood containing PGDN was stored in a capped (PTFE-lined) glass test tube for several days. The GC was calibrated daily before analyses were made.

Time of analysis after sample preparation (h)	*Analyzed concentration (ng PGDN/ml blood)*
0.0	2887
1.0	2964
17.5	2714
20.5	2932
22.5	2972
24.5	3053
42.5	2872
	$\bar{x}$ = 2913 ± 4% (S.D.)

TABLE III

COMPARISON OF TWO SAMPLE PREPARATION METHODS

Blood samples were spiked to contain 4420 ng PGDN/ml and prepared by either rotary or manual shaking extraction. Each succeeding 5-ml extract was analyzed separately, rather than combining extracts as is normally done.

5-ml extraction	*PGDN found (ng/ml)*	
	Rotary shaking for 5 min	*Manual shaking for 15 sec*
1st	2487	3456
2nd	69	134
3rd	7	7
4th	None	None
Composite	2573 (58% recovery)	3597 (81% recovery)

run on this type column before replacement was necessary.

Due to the number of samples taken for analysis in this laboratory, extracts frequently must be stored and run several days following preparation. To test stability of the PGDN in the extract, a sample was prepared and successive analyses made over 42.5 h. These data are presented in Table II and show that no decrease in concentration occurred within the expected limits of variability over that time frame.

Extraction efficiency

Several methods[4,11] for extracting dinitrate ester compounds from biological media incorporate multiple extractions on rotary shakers for periods up to 5 min thereby extending sample preparation to 15 min or more. Since PGDN is rapidly metabolized by blood *in vitro*[2,12] it was desirable to reduce preparation time as much as possible without sacrificing efficiency. Samples of blood containing PGDN were prepared either by shaking the mixture on a rotary shaker for 5 min for each extract or manual shaking for 15 sec. Each extract was analyzed for PGDN. The results, listed in Table III, indicate that all recoverable PGDN has been removed by the third

TABLE IV

ACCURACY OF PGDN DETERMINATION IN SPIKED BLOOD SAMPLES

1-ml aliquots of blood were spiked with known quantities of PGDN before sample preparation and analysis.

PGDN added (ng/ml)	*Number of samples*	*Mean recovery (% ± S.D.)*
10,000	3	83 ± 7
5000	10	89 ± 3
1000	3	72 ± 4
500	3	65 ± 1
100	10	64 ± 5
50	3	47 ± 8
10	3	19 ± 2

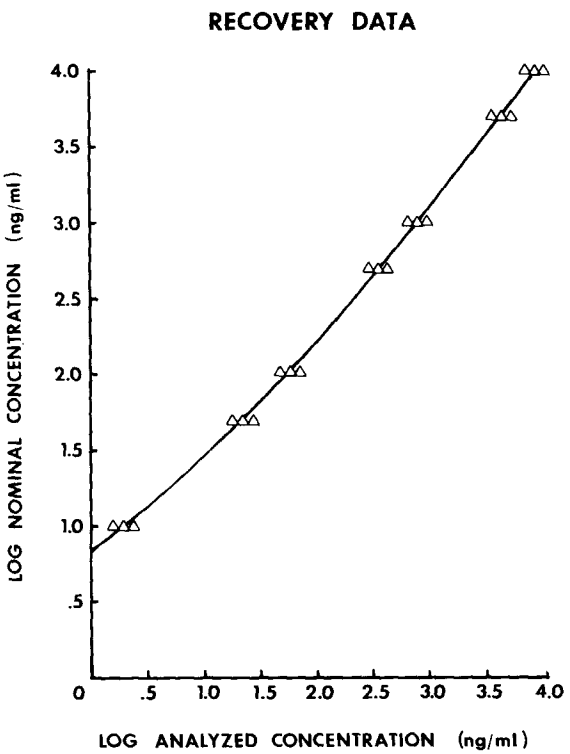

Fig. 2. Plot of PGDN recovery for 35 blood samples spiked to contain various concentrations.

extraction and that the manual technique is more efficient with a 23 % higher recovery of PGDN. Sample preparation time, including centrifugation, was reduced to within 5 min.

Accuracy

The accuracy of the PGDN determination was assessed by analyzing 35 blood samples spiked to contain various PGDN concentrations. The analyses, listed in Table IV, indicate recoveries approach 90 % at higher concentrations but drop below 50 % at levels below 50 ng/ml. Inasmuch as the determined values will be converted to the logarithm of the concentration for toxicokinetic uses, a curve was drawn representing recovery (Fig. 2). A second degree polynomial regression model was fitted to these data. The equation is:

$$y = 0.0664x^2 + 0.54795x + 0.82919$$

This relation has been applied to all raw data before further use.

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