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Gas–liquid chromatographic determination of nitroglycerine in pharmaceutical preparations

A gas–liquid chromatographic (GLC) method recently developed for the determination of nitroglycerine (glyceryl trinitrate) and other nitrate esters in nitrocellulose-base propellants[1] has been successfully applied, with modifications, to the assay of nitroglycerine (NG) in commercially produced 10 % (nominal) NG–β-lactose powder and in sublingual NG tablets prepared from the same mixture. This technique appears to offer the simplest, most rapid and most accurate means devised to date for the routine quality control of such pharmaceutical preparations.

The standard assay method specified in the *U.S. Pharmacopeia*[2], *i.e.*, column chromatographic separation of the NG and subsequent quantitative determination by absorption spectrometry, is involved and time-consuming. Thin-layer chromatography, which has been used extensively for similar analyses[3], offers a simple and rapid procedure for separating the NG, but precise and accurate quantification requires an additional step by another method. Other available methods, described by CAMERA and co-workers[4,5], are unsuitable or undesirable for this application. One GLC method reported by CAMERA and co-workers was applied only to the determination of NG and other nitrate esters in admixtures, and featured flash vaporization of an acetone solution of NG, separation on short columns at low temperatures and thermal conductivity detection. A method similar to this but using an ethanol extract and lightly-loaded 7 ½-ft. columns was reported by FOSSEL[6] for the assay of NG in pharmaceutical preparations.

The GLC procedure described here is considered to be an improvement over these methods in several important respects. The use of a flame ionization detector increases the NG detection sensitivity, thus permitting smaller samples to be precisely assayed; the total time for sample preparation and assay is substantially reduced by using a semi-micro extraction procedure followed by aliquot analyses on a short, lightly-loaded column; and the precision and accuracy of the assay are improved by using (1) high quality columns; (2) on-column injection of aliquots with column temperature programming; (3) an electronic digital integrator for measuring peak areas; and (4) an internal standard technique.

Experimental

Apparatus and conditions. The chromatograph used was a Hewlett-Packard Model 7624A equipped with a 3370A electronic digital integrator. The eluted components were detected with a dual hydrogen flame ionization detector operated at 135°. The dual columns were 1/8 in. × 2 ft. stainless-steel tubing (Hewlett-Packard) packed with 3 % (w/w) UCW-98 on 80–100 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.). The flow-rate of the helium carrier gas was 40 ml/min.

The packing was prepared by a filtration–fluidization procedure[7] so as to obtain a uniform, high quality coating of the stationary phase on the solid support. The packed columns were conditioned overnight at 250° and a constant helium flow of 10–15 ml/min was maintained during conditioning. For each newly prepared column, different sized aliquots of the calibration mixture were analyzed to ensure

that detector response as a function of the weight of NG was linear with zero intercept. All aliquot samples were injected onto the column at a column oven temperature of 70°, and this temperature was maintained for 1 min after injection. The oven temperature was then linearly programmed to 130° at the rate of 15 °/min, and maintained at this temperature until the internal standard eluted.

Chemicals. A stock solution of the internal standard, diethyl phthalate (DEP), was prepared by dissolving 25 mg of reagent grade DEP (Eastman Organic Chemicals) in reagent grade 1,2-dichloroethane (Eastman) and diluting this solution to 50 ml in a volumetric flask. The same stock solution was used in preparing both the calibration mixture and samples for each series of analyses. Neat NG for calibration was obtained from a 78% (w/w) solution of NG in acetone (Hercules, Inc.) by evaporating the acetone from a 0.5-ml volume at room temperature. The 10% NG–β-lactose powder and sublingual NG tablets were obtained from commercial sources.

Procedure. A single calibration mixture was prepared as follows. A 9–10 mg sample of the neat NG was weighed, with a precision of ±0.001 mg, on a Cahn Gram Electrobalance, into a differential scanning calorimeter (DSC) aluminum pan (Perkin-Elmer Corporation). The pan was placed in a 1 dram, polyethylene-stoppered, glass vial, 2 ml of the internal standard solution was added by pipet and the sealed vial was vigorously shaken on a No. 5000 mixer/mill (SPEX Industries, Inc.) for 1 min. Four 2-μl aliquots of the calibration mixture were analyzed to establish the NG calibration factor, F, which is the inverse of the relative response factor. A new calibration factor was established each day before beginning a series of analyses.

The NG–lactose powder samples were taken from 3 g of a lot that, according to the manufacturer, contained a nominal 10% (w/w) of NG. Each sample consisted of approximately 0.1 ± 0.001 g of the powder weighed into a 1 dram glass vial. A 2-ml volume of the internal standard solution was added by pipet, the sealed vial was shaken on the mixer/mill for 2 min and the slurry was centrifuged before the extract was analyzed.

The tablet samples were prepared from three nominal concentrations (0.3, 0.4 and 0.6 mg) of NG tablets, each from a different bottle of 50 tablets. The approximate weight of each tablet, which was not used in the calculations, was 350 mg. The extract was prepared by placing 20 NG tablets and an empty DSC aluminum pan in a 1 dram glass vial, adding 2 ml of the internal standard solution by pipet, shaking the sealed vial on the mixer/mill for 10 min to pulverize the tablets and extract the NG, and then centrifuging the slurry.

The analysis procedure was the same for the powder and tablet extracts. Each series of measurements analyzed replicate 2-μl aliquots of the extract, and the total weight of NG in milligrams was calculated by the expression

$$\text{NG (mg)} = \frac{(\text{NG peak area})\,(F)}{(\text{DEP peak area})}.$$

Results

The high quality of NG separation that is achieved by this method is indicated by the sharpness and symmetry of the NG peak in Fig. 1, which is typical of the gas chromatograms obtained in the study. The adjusted retention times for NG

and DEP were 3.25 and 5.15 min, respectively. The chromatograms were recorded using an electrometer range setting of 1000 and recorder full-scale settings of 1 mV for NG and 10 mV for DEP.

NG–lactose powder. For the determination of NG in the 10% NG–lactose powder, three extract aliquots from each of four samples of the powder were assayed. The results of the analysis of variance[8] are given in Table I. The mean square (variance) among samples was more than twice as large as the mean square for replicate aliquots within samples; however, there was no significant difference between these two mean squares at the 5% ($\alpha = 0.05$) significance level, which indicates that the variation among sample averages was not significantly greater than the variability of the GLC technique itself. Consequently, the results show that the powder is a homogeneous mixture.

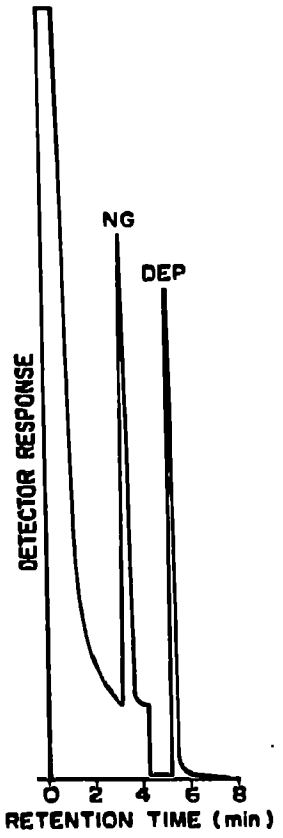

Fig. 1. Gas chromatogram for the separation of nitroglycerine (NG) and diethyl phthalate (DEP).

TABLE I

ANALYSIS OF VARIANCE FOR 10% NG–LACTOSE POWDER

Source of variation	*Degrees of freedom*	*Sum of squares* ($\times 10^2$)	*Mean square* ($\times 10^2$)
Among samples	3	5.267	1.756
Within samples	8	6.572	0.822
Total	11	11.839	

The average analysis results and estimated standard deviations for individual aliquot analyses are given in Table II. The two mean squares in Table I were pooled to estimate the random error variance used in calculating the confidence limits. The confidence interval on the average assay would be expected to include, with 95% confidence, the true average NG percentage of the lot (population). As the resulting nominal percentage falls within this interval, the 10% concentration given by the manufacturer is considered to be correct within the experimental error of the assay.

TABLE II

GLC DETERMINATION OF NG IN 10% NG–LACTOSE POWDER

Sample No.	*NG (% w/w)*	*Standard deviation*[a]
1	10.01	0.098
2	9.88	0.108
3	10.06	0.084
4	9.94	0.068
Average[b]	9.97 ± 0.07	

[a] Two degrees of freedom.
[b] 95% confidence limits with eleven degrees of freedom.

TABLE III

ANALYSIS OF VARIANCE FOR NG TABLETS

Source of variation	*Degrees of freedom*	*Sum of squares* (× 10^4)	*Mean square* (× 10^4)
Among concentrations	2	2991.697	1495.849[a]
Between samples within concentrations	3	20.922	6.974[a]
Within samples	18	6.263	0.3479
Total	23	3018.882	

[a] Significant at 1% ($\alpha = 0.01$) significance level.

NG tablets. In the assay of the tablets for NG, analyses were made of four extract aliquots from each of two samples at each of the three concentrations. The analysis of variance results are given in Table III. Both the mean square among concentrations and the mean square between samples within concentrations were significantly larger than the mean square within samples. The large value for the mean square among concentrations was expected because three different nominal concentrations were combined in the calculation. As the mean square between samples within concentrations is an estimate of the variability between average sample assays at each concentration, the significant variance in this instance was due to the manufacturing process.

The confidence intervals for the sample analysis results, given in Table IV, were calculated from the mean square within samples (Table III). As indicated by the 95% confidence intervals, all the NG percentages except for one of the 0.3-mg

TABLE IV

GLC DETERMINATION OF NG IN NG TABLETS

Nominal (mg/tablet)	*GLC (mg/tablet)*	*95% confidence interval*	*GLC average (mg/tablet)*
0.3	0.282	0.276—0.288	0.288
	0.295	0.289—0.301	
0.4	0.390	0.384—0.396	0.378
	0.367	0.361—0.373	
0.6	0.548	0.542—0.554	0.557
	0.566	0.560—0.572	

tablet samples were significantly smaller than nominal; however, all the percentages were well within the 80–120% of nominal limits specified in the *U.S. Pharmacopeia*[2].

The GLC method described here is not only simple and rapid, but offers a high degree of precision and accuracy. The average analysis time per sample was 15 min. The estimated relative standard deviations of individual aliquot determinations for the calibration mixture, the 10% NG–lactose powder and the NG tablets were 0.513%, 0.909% and 1.45%, respectively. The fact that there was no significant difference between NG determination and nominal values for the basic 10% NG–lactose powder indicates that this assay technique provides accurate results.

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