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Short Communication

Determination of methacrylonitrile in serum using gas chromatography and flame ionization detection

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ABSTRACT

A gas chromatographic method is described for the quantitation of methacrylonitrile in serum. Methacrylonitrile was extracted from rat serum with diethyl ether and then quantified using a gas chromatograph equipped with a flame ionization detector and a 60-m megabore column coated with polyethylene glycol polymer. The recoveries obtained following a one-step extraction with diethyl ether varied from 60% at 3.2 μg/ml to 70% at 80 μg/ml. The coefficient of variation for the analysis ranged from 2.5% at 400 μg/ml to 15.0% at 3.2 μg/ml.

INTRODUCTION

Methacrylonitrile (MAN) is used as a monomer in the production of plastic homopolymers, copolymers, and as an intermediate in the preparation of acids, amides, amines, esters, and other nitriles [1]. This chemical is known to be toxic in rats, mice, and rabbits [2–5]. MAN has an LD_{50} of 17 mg/kg when given orally to mice and an LD_{50} of more than 200 mg/kg when given by the same route to rats [3]. The metabolism of this compound results in the release of cyanide ions in the blood [6] and depletes glutathione both *in vivo* and *in vitro* [7]. Interaction with erythrocytes has also been reported [6].

Despite its toxicity, few, if any, analytical methods are reported for MAN quantitation in biological samples. Several gas chromatographic (GC) methods are reported for structurally related compounds including tetramethylsuccinonitrile [8] and acrylonitrile [9–11]. These assays are primarily described for the quantitation of the

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aliphatic nitrile in plastics or foodstuffs, but not body tissues or fluids. Although one method is designed for quantification of acrylonitrile in rat plasma, separation of the nitrile from the plasma requires special equipment. The process involves heating the sample, trapping the nitrile on a polymer, and analyzing the sample following desorption of the nitrile from the polymer [9]. The present paper describes the quantitation of methacrylonitrile in serum using simple extraction procedures and without special equipment. The procedure is useful for pharmacokinetic studies in rodents and other small animals because the sample size is small.

EXPERIMENTAL

Apparatus

A Varian Model 6000 gas chromatograph, equipped with a flame ionization detector, was used with a Varian 4700 integrator. The chromatograph injector was fitted to a 60 m × 0.53 mm I.D. megabore column which was coated with a polyethylene glycol polymer stationary phase (1 μm thickness, Stabilwax, Restek, Bellefonte, PA, USA). The column oven temperature was held at 55°C during component separation, then the temperature was increased to 100°C to elute impurities. The capillary injector was run in the split mode (50:1), to reduce solvent interference. Helium was used as a carrier gas, and under optimum conditions, the flow-rate was 23 ml/min.

Chemicals

MAN (99+%) was obtained from Fluka (Ronkonkoma, NY, USA). Isobutyronitrile (IBN, 99+%) was obtained from Aldrich (Milwaukee, WI, USA). Anhydrous diethyl ether used for extractions and water used to dilute standards were obtained from Baker (Phillipsburg, NJ, USA). All these solvents used were the highest quality available.

Methacrylonitrile standard preparation

Two stock solutions were prepared. The first stock solution was prepared by adding 25 μl of MAN to 5.0 ml of water. To make the second stock solution, 15 μl of MAN was added to 5.0 ml of water. Two stock solutions were used so that the quality of the preparation could be evaluated, in addition to the linearity of the standard curve. Standards were prepared by serial dilution of each stock solution. These standards (10 μl) were added to 90 μl of serum to produce samples of the desired concentration. Extraction was accomplished by adding to 100 μl of serum an equal volume of diethyl ether that contained isobutyronitrile (IBN, 152 μg/ml) as an internal standard. Once the diethyl ether was added, the sample vortex-mixed for 10 s, and centrifuged for 5 min at 13 000 *g*, the aqueous layer was frozen by placing the sample in dry ice. This procedure was followed by transferring the organic layer into a separate tube. These extracts were stored at −20°C until injected directly onto the chromatograph.

Methacrylonitrile stability during storage

Serum samples spiked with MAN were stored at −20°C and analyzed over a seven-day period. The stability of MAN was assessed by comparing the calculated concentrations of MAN on each study day to the calculated concentration on day 0. In addition, serum extracts were prepared and stored over this period. Again, the concentrations of MAN were compared to those calculated on the day of preparation.

Linearity

Standards for MAN were added to 90 μl of serum to make concentrations of 400, 240, 133.3, 80, 44.4, 26.7, 14.8, 8.9, 4.9, 3.0, 1.65, and 0.99 μg/ml. Linearity was assessed by regression of the peak-area ratio MAN/IBN *versus* the spiked concentration. DeltaGraph was used to perform these regression analyses [12].

Reproducibility

Two measures of reproducibility were used: the within-day coefficient of variation and the day-to-day coefficient of variation. The within-day coefficient of variation was calculated for 400 and 3.2 μg/ml. Five spiked serum samples were

extracted at each concentration and the extracts were chromatographed. The mean and standard deviation of the peak-area ratios were calculated. The day-to-day variation was the coefficient of variation for the slopes of the standard curves produced on five separate days. These standard curves were in the range of practical applicability for concentrations expected in animals.

Recovery

MAN recovery was evaluated at 80 and 3.2 μg/ml. Serum was spiked with MAN and extracted with an equal volume of diethyl ether containing the internal standard. The peak area of MAN in the extracted sample was compared to the corresponding area obtained from analysis of MAN dissolved in diethyl ether. The peak areas of IBN in both the extracted sample and solvent were compared as well.

RESULTS AND DISCUSSION

Chromatographic separation

Sample chromatograms of MAN separation are shown in Fig. 1. Panels show extracts of (A) blank serum, (B) spiked serum, and (C) serum from a treated animal. In this chromatographic system, MAN consistently eluted at 11.07 min. An unknown peak that occurs in serum eluted at 11.40 min. Although the peaks were approximately 0.34 min apart, close-to-baseline separation can be achieved with the current system configuration. Although this peak appeared in every serum sample from every animal tested, the method used by the 4700 integrator compensated for the interference.

Stability

Storage of the MAN-containing samples in serum or diethyl ether extracts resulted in changes in MAN concentration. The results are summarized in Table I. In this stability study, the apparent concentration of MAN in serum increased 2.6–28.5% over the eight-day period. In contrast, the apparent concentration of MAN decreased 2.7–32.03% in the extracts. Comparison of the two methods over the first two days suggests that storage of MAN as an extract may be slightly better than storage in serum. The best approach to quantification of MAN is to prepare and ana-

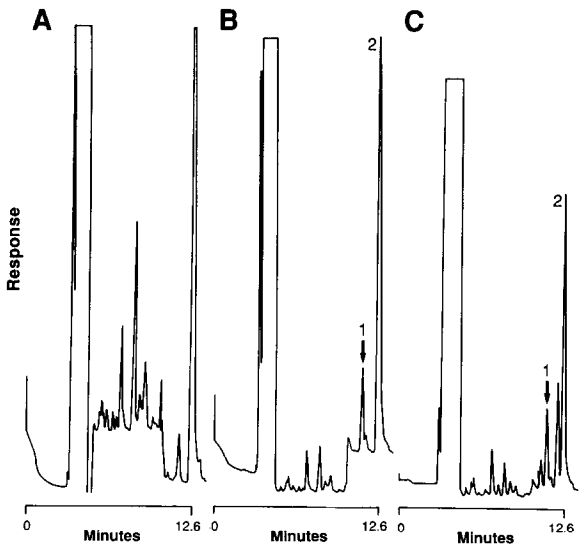

Fig. 1. Typical gas chromatograms of methacrylonitrile extracted from (A) blank serum, (B) spiked serum (32 μg/ml MAN), and (C) serum (40 μg/ml MAN) from a treated animal. Peaks: 1 = methacrylonitrile; 2 = isobutyronitrile (internal standard).

TABLE I

STABILITY OF STORED SAMPLES

This table shows the mean concentration measured in the extracts as well as the percentage change in concentration when compared to values at day 0.

Day	Concentration (μg/ml)	*n*	S.D. (μg/ml)	Change (%)
Extracts				
0	15.89	5	0.996	0
1	13.56	3	1.29	−14.6
2	15.46	3	1.11	−2.71
4	12.21	3	1.29	−23.2
7	10.80	3	1.29	−32.4
Serum				
0	15.89	5	0.719	0
1	18.61	3	0.928	+17.1
2	20.43	3	0.928	+28.5
4	16.32	3	0.928	+2.60
7	18.72	3	0.928	+17.81

lyze samples as soon as possible after collection. In our studies, the samples were analyzed within 24 h of being spiked or taken from the animal.

Linearity and sensitivity

The peak-area of MAN/IBN was linear from 0.99 to 400 μg/ml. The serum sample size was small (100 μl) and only 3 μl of the extract were needed for injection onto the gas chromatograph. The smallest concentration detected represents 0.02 ng of MAN injected on the column at $16 \cdot 10^{-12}$ Af.s. The detection limit of 0.06 μg is defined as twice the baseline noise. This high sensitivity is very important for MAN quantification. Because of this chemical's volatility, simple concentration methods such as extraction followed by solvent removal could not be used to increase sensitivity. A representative standard curve is shown in Fig. 2. Although a linear regression of the data results in a high correlation coefficient, this treatment of the data results in an underestimation of the peak-area ratios at the lowest concentrations. Use of this relationship to predict unknown concentrations would result in an overestimation of the concentrations by 45% at 8.9 μg/ml and by 576% at 0.99 μg/ml. Either two linear standard curves must be used to cover this range or the data can be described by another function. These data were analyzed using a third-degree polynomial. This treatment resulted in all the data being well described by the model.

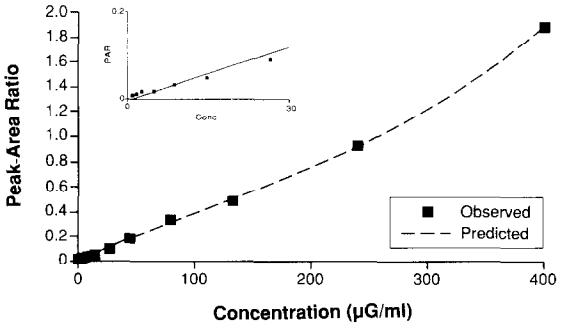

Fig. 2. Typical standard curve for methacrylonitrile. The concentration range measured was 0.99–400 μg/ml. A cubic polynomial was used to describe the data across the entire range: $f(x) = 1.81963 \cdot 10^{-8} x^3 - 6.1530 \cdot 10^{-6} x^2 + 4.260 \cdot 10^{-3} x - 3.791 \cdot 10^{-3}$.

Reproducibility

Table II contains information about the within-days reproducibility. There was a 12.28% variation in the slopes from standard curves obtained from several days of chromatographic operation. The within-day coefficient of variability is listed in Table III. The variability ranges from 2.5% at 400 μg/ml to 15% at 3.2 μg/ml. Both the within- and day-to-day variability are within an acceptable range. Although a lower level of MAN is detectable, we consider 3.2 μg/ml as the minimum quantifiable level because of the high level of variability. In cases where a lower concentration must be measured, multiple extractions and injections may be necessary to increase confidence in the calculated concentration.

Recovery

The data for MAN recovery following extraction is shown in Table III. MAN recovery was 60% at 3.2 μg/ml and 77% at 80 μg/ml. IBN recovery was 127% at 80 μg/ml and 118% at 3.2 μg/ml. The greater than 100% recovery suggests that there may be a phase volume shift during the extraction process and that part of the diethyl ether layer becomes dissolved in the serum layer. This resulted in a higher concentration of MAN in the extracting solvent and improved our ability

TABLE II

BETWEEN-DAY REPRODUCIBILITY

This table shows the squared correlation coefficients and the slopes for the standard curves prepared on five separate study days.

Day	Concentration range (μg/ml)	r^2	Slope
0	3.2–400.0	0.9994	0.00417
1	1.6–400.0	0.9999	0.00542
2	1.6–400.0	1.0000	0.00429
3	1.6–400.0	1.0000	0.00449
4	3.2–400.0	0.9994	0.00417
Mean			0.00451
S.D.			0.00053
Coefficient of variation (%)			11.67

TABLE III

WITHIN-DAY REPRODUCIBILITY AND RECOVERY

This table shows the recovery of MAN at various concentrations. This table also shows the variability inherent in making injections. n = 5 determinations at each concentration.

Concentration (mg/l)	Peak-area ratio (mean ± S.D.)	Coefficient of variation (%)	Recovery (%)	
			IBN	MAN
400.0	0.6837 ± 0.0170	2.49	–	–
80.0	0.3530 ± 0.0071	2.01	130	76
3.2	0.0128 ± 0.0020	15.51	118	60

to quantify MAN at the lower concentrations found in serum.

CONCLUSION

A GC assay has been developed for the quantitation of MAN. The analysis of samples was rapid (< 20 min), only a small sample size was required (100 μl), and a simple, one-step extraction procedure was used to separate MAN from the sample matrix. Although MAN appears to be unstable when stored in serum or serum extracts, consistent results can be achieved if sample extractions are completed and injections are made less than 24 h post collection. This method is very useful for monitoring concentrations of the nitrile in small animals and may be useful in the determination of human exposure to the chemical. It may also be adaptable for the quantitation of other aliphatic nitriles as illustrated by the separation of MAN and IBN.

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