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Determination of 1-methoxy-2-propanol and its metabolite 1,2-propanediol in rat and mouse plasma by gas chromatography ☆

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Abstract

A method utilizing capillary GC and flame ionization detection was developed for the simultaneous determination of 1-methoxy-2-propanol (propylene glycol monomethyl ether; PGME) and its metabolite 1,2-propanediol (propylene glycol; PG) in rat and mouse plasma. The calibration graphs for rat and mouse plasma were linear with correlation coefficients at >0.997 over the range 2–700 μg/ml. The limit of quantification was ca. 2 μg/ml (2 ng on-column) for both compounds in plasma of each species. The ranges of the precision and accuracy for PGME were 2.8–8.8% and 3.2–13%, respectively, and for PG were 11–26% and 10–25%, respectively. The recovery of PGME from rat and mouse plasma was ca. 73% and for PG it was ca. 65 and 31% from rat and mouse plasma, respectively. The method was used to study the oral absorption and metabolism of PGME in mice. PGME was readily absorbed and metabolized to PG following oral gavage administration at 90 mg/kg. The maximum concentrations of PGME and PG in plasma were attained at 20 and 30 min following dosing, respectively.

1. Introduction

1-Methoxy-2-propanol (propylene glycol monomethyl ether; PGME), is a moderately volatile industrial solvent that belongs to the glycol ether family. These solvents have been used in paints and lacquers, as intermediates in the production of plasticizers, as ingredients in water-based cleaners and as ingredients in brake fluid formulations [1].

Previous studies [2–4] have demonstrated that PGME is remarkably less toxic than its isomer ethylene glycol monomethyl ether (EGME). The difference in toxicity has been attributed to differences in metabolism between the two compounds. For example, [^{14}C]PGME was metabolized and eliminated primarily as $^{14}CO_2$ (50–60% of dose) and, to a lesser extent, in urine (10–20%), whereas [^{14}C]EGME was metabolized and excreted primarily in urine (50–60% of the same molar dose) and, to a lesser extent, as $^{14}CO_2$ (12%). In addition, EGME was metabolized primarily to the toxic metabolite methoxyacetic acid (80–90% of total urinary radioactivity), while PGME was metabolized to 1,2-propanediol (propylene glycol; PG) and the sulfate

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and glucuronide conjugates of PGME, which were identified in rat urine [5]. Owing to the much lower toxicity of PGME, its production and use are steadily increasing as it replaces the more toxic isomer EGME.

The existing methods for the determination of PGME and PG [6] in plasma involve two separate analyses, one for each compound. These analyses require the use of two separate plasma samples, two different sample preparation procedures and two different GC systems. As a result, the methods are inconvenient in studies where analyses of large numbers of samples are needed, or if the volumes of plasma samples are limited, e.g., in pharmacokinetic studies in mice.

This study was undertaken to develop a method for the simultaneous determination of PG and PGME in plasma of rats and mice. The method was used successfully to investigate the oral absorption of PGME and its metabolism to PG following oral gavage administration of PGME to mice.

2. Experimental

2.1. Materials

PGME (98%), PG (99%) and EGME (99.99%) were purchased from Aldrich (Milwaukee, WI, USA) and absolute ethanol from Pharmco Products (Weston, MO, USA). Water was distilled and deionized using a Milli-Q system (Millipore, Bedford, MA, USA). Frozen rat and mouse plasma used for analytical method development and stability studies were purchased from Taconic Farms (Germantown, NY, USA).

2.2. Analytical method

Gas chromatography

A Hewlett-Packard (Palo Alto, CA, USA) Model 5890A chromatograph equipped with a flame ionization detector and a spilt–splitless injector was used. The column was DB-Wax fused silica, 0.25 μm film thickness (60 m $\times$ 0.32 mm I.D.) (J&W Scientific, Folsom, CA, USA). A deactivated fused-silica guard column (0.5 m $\times$ 0.53 mm I.D.) (J&W Scientific) was connected to the analytical column by a zero-dead-volume glass connector (J&W Scientific) deactivated with Sylon-CT (Supelco, Bellefonte, PA, USA). The glass inlet was also treated with Sylon-CT. Helium was used as both the carrier gas at a flow-rate of 1.6 ml/min and the detector make-up gas at 45 ml/min. Hydrogen and air for the detector were used at flow-rates of 40 and 300 ml/min, respectively. The injector and detector temperatures were both set at 250°C. Sample introduction was via the splitless mode using an injection volume of 1 μl and a helium purge at 60 ml/min, with on and off times of 0.4 and 10 min postinjection, respectively. A continuous helium purge of the inlet septum was maintained at 5 ml/min. The column temperature was programmed as follows: initial temperature 50°C for 0.55 min, followed by a 70°C/min increase to 75°C, which was held for 4 min, then an increase at 5°C/min to 110°C, which was held for 6 min. Sample injection was automated using a Hewlett-Packard Model 7673A autosampler. A Hewlett-Packard Model 3393A integrator was used to determine retention times and peak areas.

Preparation of plasma for analysis

Mouse and rat plasma were spiked with PG and PGME dissolved in ethanol to yield the appropriate concentrations. The volume of ethanol spiking solution added to plasma never exceeded 2% of the plasma volume. Absolute ethanol containing the internal standard EGME at 75 μg/ml was added to spiked plasma samples at a ratio of two volumes of ethanol to each volume of plasma. Samples were mixed and centrifuged at 600 *g* for 10 min at 4°C to precipitate protein. The supernatant was filtered through a Gelman (Ann Arbor, MI, USA) Acrodisc LC-13 PVDC 0.45-μm filter and then analyzed by GC.

Calibration graphs were prepared for PG and PGME in rat plasma and for PGME in mouse plasma over the concentration range 2–700 μg/ml. For PG in mouse plasma the range was 2–250 μg/ml. Each calibration graph was ob-

tained using at least six concentrations with duplicate samples of each. A solvent calibration graph with a concentration range of 0.5–250 μg/ml was also prepared in water–ethanol (1:2, v/v) and used to determine the recovery of PG and PGME from plasma.

2.3. Stability studies

In order to determine the appropriate conditions for storage and analysis of plasma samples, it was necessary to determine the stability of PG and PGME in plasma and in the ethanol supernatant of plasma. Stability determinations in rat and mouse plasma were carried out by spiking duplicate aliquots of plasma at 100 and 500 μg/ml with both PG and PGME. The spiked samples were analyzed immediately and aliquots were also stored either in a refrigerator at 4–8°C for 24 h or kept frozen at −20°C or lower for 7 days, then processed for analysis. On the day of analysis, fresh plasma standards were prepared at the same concentrations and used for comparison with the stored samples. The stability of PG and PGME was also determined in the ethanol supernatant of rat plasma at room temperature. The ethanol supernatant of plasma was spiked with PGME and PG at 45 and 250 μg/ml each. The samples also received EGME at a final concentration of 50 μg/ml. The samples were analyzed immediately (0 h) and at 12, 24 and 54 h after preparation. Fresh samples were also prepared and analyzed with the 12-, 24- and 54-h samples. The stability in the ethanol supernatant of plasma was determined by direct comparison of the concentrations of fresh samples with those following storage.

2.4. Oral absorption study of PGME in mice

Male B6C3F$_1$ mice, weighing 25–29 g at the time of dosing, were purchased from Taconic Farms. The animals were acclimatized to our animal facility for at least 1 week prior to dosing. The temperature, humidity and light cycles were controlled at 18–26°C, 40–70% and 12 h light–12 h dark, respectively. The animals were housed individually in stainless-steel cages and were given pelleted Purina Rodent Chow No. 5009 and municipal tap water ad libitum.

PGME was administered by oral gavage to mice as a 1.8% (w/v) solution in saline at 90 mg (1 mmol)/kg in a dose volume of 5 ml/kg. At each of 2, 5, 7, 10, 20 and 30 min and 1, 1.5, 2, 4 and 5 h after dosing, blood was collected by cardiac puncture through the chest cavity from four mice anesthetized with CO_2. Following blood collection, the animals were killed by CO_2 asphyxiation. Blood was collected in heparinized Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) and centrifuged to separate plasma, which was processed for analysis as described above. The concentrations of PG and PGME in plasma samples were determined using concomitant calibration graphs set up in plasma from undosed animals. In addition, four animals were dosed with saline and killed 10 min after dosing, and plasma samples were analyzed as blanks.

3. Results and discussion

In the method development phase of the study, it was observed that the use of a 2:1 ethanol-to-plasma ratio for protein precipitation was essential in order to eliminate peak splitting. Reducing the ratio of ethanol to plasma to less than 2:1 resulted in extensive splitting of PGME and EGME peaks. This problem was attributed to the presence of a high water content in the supernatant, rather than to incomplete precipitation of protein. This was evidenced by the fact that peak splitting was observed when PGME and EGME were analyzed in ethanol–water with an ethanol-to-water ratio of less than 2:1.

A second persistent problem was encountered when the concentration of PG in plasma was >100 μg/ml. This problem was manifested as a carry-over (ca. 10%) of PG into the subsequent injection. Removal of the guard column and/or switching to on-column injection did not alleviate the problem, suggesting that PG was adhering to the head of the column and/or the injection syringe. Baking the column at 250°C for 5 min at the end of each run did not

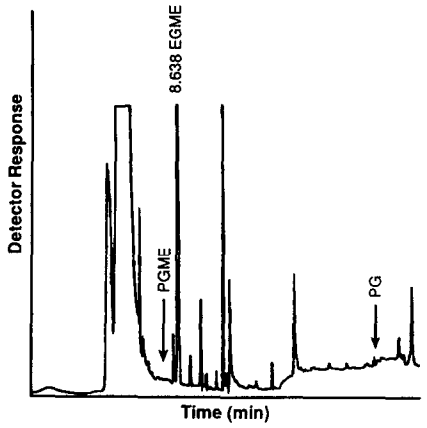

Fig. 1. Typical chromatogram of mouse plasma spiked with the internal standard EGME alone.

completely remove the adhered PG. Only the injection of blank solvent [ethanol–water (2:1)] was successful in alleviating this problem. However, when the concentration of PG was low, the carry-over was not observed. This problem was overcome by either diluting the sample followed by reanalysis or reanalysis with blank solvent between samples.

Figs. 1 and 2 show chromatograms of blank plasma spiked with the internal standard EGME alone and blank plasma spiked with PGME, PG and EGME, respectively. The retention times of PGME, EGME and PG were 7.7, 8.6 and 20.3 min, respectively (Figs. 1 and 2). The chromatogram of blank plasma spiked with EGME (Fig. 1) was very similar to that in Fig. 2 with the absence of the peaks that corresponded to PGME and PG, indicating that the specificity of the method was satisfactory. Owing to the wide concentration range of the calibration graph for both PG and PGME (2–700 μg/ml), it was necessary to divide the data into two ranges in order to obtain the best fit, especially for the lower concentration range. The solvent calibration graph ranges were 0.5–10 and 10–250 μg/ml for both PGME and PG. For rat plasma, they were 2–50 and 50–700 μg/ml for both PG and PGME. The ranges for PGME in mouse plasma were the same as those in rat plasma, whereas for PG the data were inconsistent above 250 μg/ml (precision $\geq$30%). Therefore, the ranges of concentration of PG in mouse plasma were 2–25 and 25–250 μg/ml. Weighted and non-weighted linear regression analyses were performed on the calibration data and the results were overall similar. In all instances, the limit of quantification (LOQ) in plasma was 2 μg/ml, below which the precision was $\geq$30%.

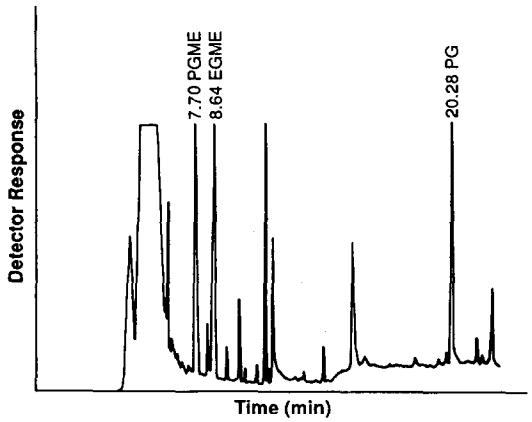

Fig. 2. Typical chromatogram of mouse plasma spiked with PGME, PG and the internal standard EGME. The retention times of the three compounds are shown.

Table 1 gives the parameters of the calibration graphs and the correlation coefficients (r) for the determination of PG and PGME in solvent and in rat and mouse plasma. All calibration graphs had correlation coefficients >0.997.

The recovery of PGME from rat and mouse plasma over the range 2–700 μg/ml was 73.6 $\pm$ 6.2% and 73.4 $\pm$ 3.3%, respectively, the recovery of PG from rat plasma was 65.3 $\pm$ 9.6% over the range 5–700 μg/ml and the recovery of PG from mouse plasma was 30.8 $\pm$ 3% over the range of 25–250 μg.ml.

The precision and accuracy of the method were calculated (in rat plasma only) as the relative standard deviation (coefficient of variation) and the relative error, respectively. Four replicates each of PGME and PG in rat plasma at each of 5, 25, 100 and 500 μg/ml were used in the calculations. The precision for PGME ranged from 2.8 to 8.8% with a mean of 5.5%; for PG the precision range was 11–26% with a mean of 16.2%. The accuracy for PGME ranged from 3.2 to 13%, and for PG from 10.4 to 24.7%. The

Table 1
Calibration graph parameters of PGME and PG

Species	Concentration range (μg/ml)	Correlation coefficient	Slope	Intercept
PGME				
Rat	2–50	0.99994	0.00754	0.00485
	50–700	0.99991	0.00755	−0.00906
Mouse	2–50	0.99993	0.00691	0.00291
	50–700	0.99983	0.00681	0.01582
Solvent	0.5–10	0.99998	0.02520	0.00217
	10–250	0.99996	0.02244	0.05289
PG				
Rat	2–50	0.9983	0.00538	0.01501
	50–700	0.99942	0.00632	−0.08018
Mouse	2–25	0.99730	0.00534	0.00543
	25–250	0.99911	0.00249	0.00166
Solvent	0.5–10	0.99960	0.02083	0.00210
	10–250	0.99995	0.02379	−0.05672

Calibration graphs of concentration versus peak area ratio of PG or PGME relative to EGME. Solvent calibration graphs were prepared in ethanol–water (2:1) and analyzed directly.

precision and accuracy were considerably better for PGME than PG at all concentrations examined.

The stability of PGME and PG in rat and mouse plasma at both 100 and 500 μg/ml was evaluated following storage under refrigeration (4–8°C) and freezing (−20°C) conditions. At refrigerator temperature, PGME was stable in rat and mouse plasma at both concentrations for at least 24 h (94–100% remaining). Although PG was also stable in rat and mouse plasma at refrigerator temperature for at least 24 h at 100 μg/ml (95–99% remaining), at high concentration (500 μg/ml) it was less stable, with only 87 and 83% remaining in rat and mouse plasma at 24 h, respectively. At −20°C, PGME was stable in rat plasma at both concentrations for at least 7 days. PG was slightly less stable in rat plasma at −20°C, 91 and 87% of the added PG remaining following storage for 7 days at 100 and 500 μg/ml, respectively. The stability of PG and PGME in frozen mouse plasma was not evaluated.

In order to evaluate if a non-refrigerated autosampler was appropriate for the analysis, the stability of PG, PGME and EGME was evaluated at room temperature in the ethanol supernatant of plasma, as samples may remain in the autosampler for up to 2 days before analysis (the total run time for one sample is approximately 27 min). The stability of the three chemicals was evaluated at 45 and 250 μg/ml in the ethanol supernatant of rat plasma. At 54 h following spiking, 100% of all three chemicals at both concentrations was recovered, indicating that PG, PGME and EGME were stable under the conditions of analysis for at least 54 h.

The method was used to investigate the oral absorption and metabolism of PGME in mice. Fig. 3 shows the plasma concentration–time curves for PGME and PG following oral gavage administration of PGME at 90 mg/kg. The results indicate that PGME was readily absorbed from the gastrointestinal tract of mice and readily metabolized to PG, as indicated by the detection of both PGME and PG in plasma as early as 2 min following dosing. The maximum concentrations (C_{max}) of PGME and PG in plasma were 76.5 ± 13.3 and 18.5 ± 14.3 μg/ml (mean ± S.D. of data from four animals), which were attained

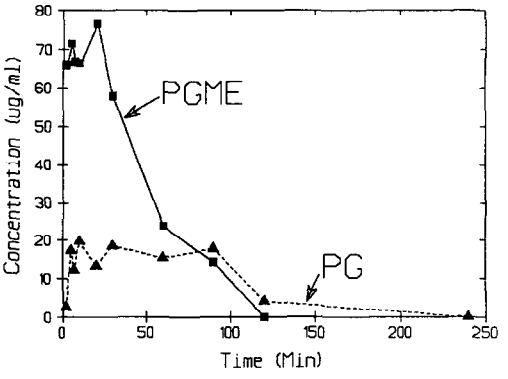

Fig. 3. Plasma concentration–time curves of PGME and PG in mice following oral gavage administration of PGME at a 90 mg/kg dose.

at times T_{max} (time after dose administration corresponding to C_{max}) of 20 and 30 min, respectively. Additional studies are in progress using this method to compare the pharmacokinetics and metabolism of PGME in rats and mice.

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