LC Determination of Propylene Glycol in Human Plasma After Pre-Column Derivatization with Benzoyl Chloride
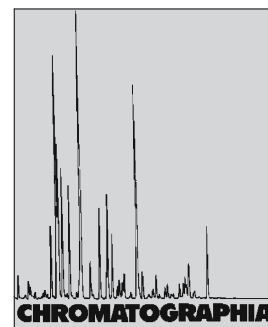
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Abstract

A simple high-performance liquid chromatographic method, using photodiode array detection was developed for the determination of propylene glycol in human plasma and in the fluid retreived after continuous veno-venous hemofiltration. The method entailed alkaline derivatization with benzoyl chloride and ethylene glycol as internal standard. The separation of the compounds, after extraction with pentane, was carried out on a Pursuit C8 column with UV-detection at 230 nm. Validation samples were analyzed with an accuracy between 95 and 105%, and intra- and inter-day coefficients of variation of less than 8%. The calibration curve was linear over a concentration range of 5–100 mg L^{-1} with a detection limit of 1 mg L^{-1}. Blood plasma samples of several patients were analysed by using the prescribed method with propylene glycol concentrations varying from 5 to 98 mg L^{-1}. Compared to previously described LC methods, this method is ten times more sensitive and thus suitable for use in pharmacokinetic studies of propylene glycol.

Keywords

Introduction

Propylene glycol (1,2-propane-diol) is a chemical agent used to increase the solubilization of non-polar pharmaceuticals in aqueous solvents. It is considered safe for use as a vehicle for IV medications by the US Food and Drug Administration (FDA) [1]. Parenteral pharmaceutical preparations containing propylene glycol include lorazepam, diazepam, nitroglycerin, trimethoprim/sulfamethoxazole, etomidate and phenytoin. The percentage of propylene glycol in these solutions varies from 35 to 80%. Although it is generally regarded as safe, potential safety concerns have been described in patients receiving these parenteral medications, especially lorazepam, which has the highest content of 80 percent of propylene glycol (PG) [2–4]. Long-term administration of high intravenous doses of lorazepam can result in propylene glycol accumulation with toxic effects. These effects include hyperosmolar metabolic acidosis, hemolysis, renal dysfunction, cardiac arrhythmias, seizures and central nervous system depression [2–4].

The commonly used analytical method to quantify glycols with a detection limit of several mg L^{-1} is based on gas chromatographic separation with flame ionization (FID) or MS detection [5–7]. However during development and validation of a gas chromatographic method, we experienced absorbance of the polar propylene glycol to glass parts of the injection system. A different method, using LC—with tandem MS detection shows a limit of detection (LOD) of 0.3 mg L^{-1} in rat plasma [8]. However in the absence of expensive LC–MS–MS equipment, a different method had to be found. The lack of UV absorption and the high polarity of PG make it almost impossible to analyse it as such with LC. Derivatization with benzoyl chloride solves both problems. A quantitative method was developed by adapting a previously described LC analysis for the confirmation of ethylene glycol poisoning [9]. The FDA recommended maximal daily amount of propylene glycol is 25 mg kg^{-1} day^{-1} [1], therefore a LOD in the range of several ppm is required for pharmacokinetic research purposes. In this paper, we report a relatively simple, fast and specific LC method for the determination of propylene glycol in hu-
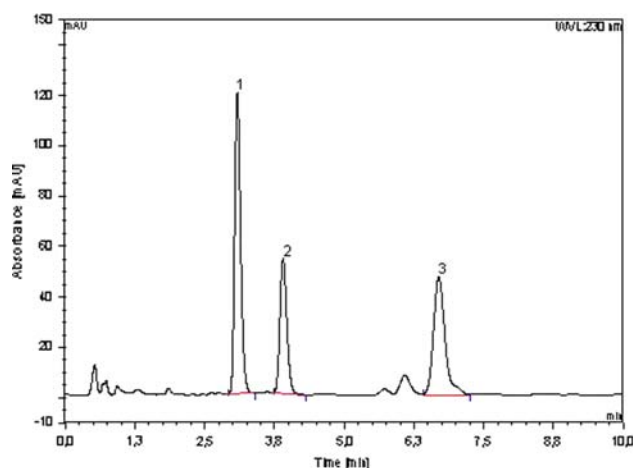
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103

Fig. 1. Chromatogram of a plasma sample with a propylene glycol content of 20 mg L^{-1} after derivatization. Peaks **1** = internal standard, **2** = PG, peak **3** = unknown

man plasma by using alkaline derivatization with benzoyl chloride and UV-diode array detection.

Experimental

Materials

Propylene glycol (2 different batches, both >99.5% pure), ethylene glycol (>99.5% pure) and benzoyl chloride (>99% pure) were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). All other solvents used were of high-performance chromatography grade, purchased from VWR (West Chester, PA, USA).

Instrumentation

The HPLC system comprised an UV 6000LP photodiode array detector (Thermo-Electron, Waltham, MA, USA), a 717Plus autosampler (Waters Corporation, Milford, MA, USA), a Dionex P680 solvent delivery pump and a personal computer with Chromeleon data-acquisition and integration software (Dionex, Breda, The Netherlands). A Varian Pursuit RP-8 column (5 μm, 100 × 3 mm ID), fitted with a disposable RP 10 × 2 mm guard column (Varian Inc, Palo Alto, CA, USA), was used for the chromatographic separation at ambient temperature. The mobile phase consisted of a mixture of 55% v/v acetonitrile and 45% v/v deionized water and was refreshed daily. The analysis was run at a flow-rate of 0.75 mL min^{-1} with the detector operating with a spectral range from 190 to 800 nm. Integration and quantitation was performed by using peak height ratios at the absorption maximum of 230 nm.

Stock Solutions, Calibrators and Control Samples

A propylene glycol standard solution of 0.10 mg mL^{-1} as prepared by diluting a 1.0 mg mL^{-1} stock standard solution 10 times with deionized water. Propylene glycol calibrators were prepared in blank plasma with a concentration of 5, 10, 20, 40, 60 and 100 mg L^{-1}. Deionized water was added to create equal volumes of 200 μL. Calibrators were also used to determine intra- and inter-day accuracy and precision (n = 6) of the method. Validation samples were prepared by spiking blank plasma with a second batch of propylene glycol at 10, 50 and 100 mg L^{-1}.

Sample Preparation

A 100 μL aliquot of plasma was added to a 1.5 mL microcentrifuge tube (Sarstedt, Nümbrecht, Germany) and 100 μL deionized water and 10 μL internal standard solution of 0.3 mg mL^{-1} ethylene glycol were added. Samples and calibrators were prepared for derivatization by adding 250 μL 4 M sodium hydroxide and 5 μL benzoyl chloride. The mixture was rotated for 10 min at 40 rpm on a rotation mixer and allowed to stand for 10 min. Hereafter 400 μL pentane were added and the mixture was rotated for 20 min on a rotation mixer at 40 rpm and centrifuged at 10,900 rpm for 5 min. The supernatant was transferred into a new microcentrifuge tube (Sarstedt), evaporated to dryness under nitrogen at ambient temperature, dissolved in 150 μL mobile phase, and 25 μL were injected into the column. All contacts with glass parts were avoided by using polypropylene tubes and flasks.

Results and Discussion

Method Development

Earlier prescribed LC methods with UV detection have insufficient LOD's to quantify low mg L^{-1} PG levels. Sensitivity was increased by optimizing derivatization by varying time duration for mixing and extraction. Optimal values were found by derivatization for 10 min, standing for 10 min, and an extraction time of 20 min.

Validation

A chromatogram obtained with plasma spiked with 20 mg L^{-1} propylene glycol and internal standard ethylene glycol after derivatization is shown in Fig. 1. Retention times for both patients and standards of propylene glycol and internal standard (ethylene glycol) after derivatization are 3.1 and 3.9 min with intra- and inter-day variations of less than 1 and 3%, respectively. Spectral analysis from 190 to 800 nm was used to confirm peak purity and to indicate the peak identity. The intra- and inter-assay accuracy and precision are presented in Table 1. Possible outliers were tested by using Dixons Q-test. The standard calibration curve (n = 6), was found to be linear over the concentration range used as confirmed by analysis of variance (ANOVA) [10]. A slope of 2.222 ± 0.0005 with an intercept of 1.0320 ± 0.024, and a correlation coefficient of 0.99994 was obtained. A detection limit of 1 mg L^{-1}, obtained at a signal-to-noise ratio of 3:1, is sufficient for pharmacokinetic studies of propylene glycol in plasma. Selectivity of the method was tested for lorazepam (test concentration 2 mg L^{-1}) and other solvents such as ethanol, benzyl alcohol and polyethylene glycol (PEG). These solvents are

frequently used as additives in injection fluids like lorazepam. No interfering peaks were found at test concentrations of 5 g L^{-1}. No interfering peaks we found in pooled human blank plasma.

Stability was tested for human plasma samples and spiked standards containing PG concentrations varying from 10 to 100 mg L^{-1}. No significant differences were found.

The method was also tested and validated for PG analysis in CVVH fluids. CVVH fluid is an aqueous substance with electrolytes and is used in continuous veno-venous hemofiltration (CVVH) as a replacement therapy in case of acute renal failure [11, 12]. With reference to the analysis in human plasma, the validation of PG analysis in CVVH fluid resulted in equal or better values for the mentioned validation parameters. No interfering peaks were found in both blank CVVH fluid and human pooled CVVH fluid.

Table 1. Intra- and inter-day assay accuracy and precision ($n = 6$)

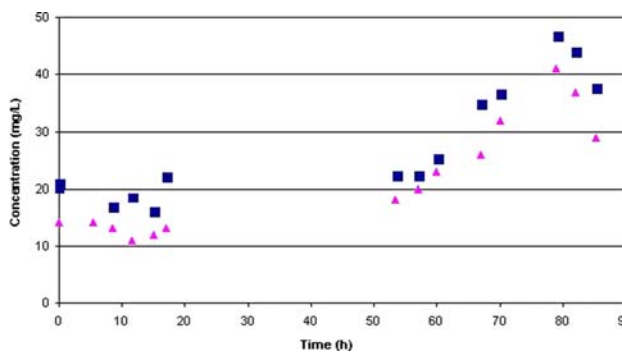| Concentration (mg L^{-1})	Intra-day		Inter-day	
	Accuracy (%)	Precision (CV) (%)	Accuracy (%)	Precision (CV)
10	98.9	5.0	101.0	4.7
50	94.6	7.8	96.7	7.3
99	104.7	6.9	104.8	6.9

Fig. 2. Concentration versus time curve of patient with renal failure. ■ marks the concentration in plasma, ▲ the concentration in CVVH fluid

Clinical Relevance

A patient with renal failure received a continuous lorazepam infusion for 98 h with a cumulative dose of 39.2 g of propylene glycol. The concentration versus time curve of this patient is shown in Fig. 2.

Conclusions

The prescribed method is shorter, uses less chemical solvents and more important is ten times more sensitive than previously described LC methods. Several patients were analysed and blood plasma concentrations of 5–98 mg L^{-1} of propylene glycol were found. The presented LC method is sensitive, simple and suit-

able to investigate propylene glycol accumulation in patients at risk and/or can be used in pharmacokinetic studies.

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