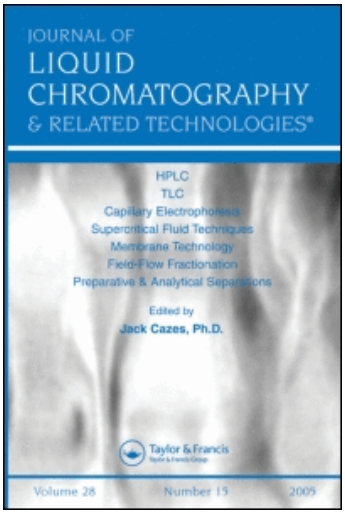
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Improvement of Sensitivity for the Determination of Propylene Glycol in Rat Plasma and Lung Tissue Using HPLC/Tandem MS and Derivatization with Benzoyl Chloride

Songmei Gao[a]; David M. Wilson[a]; Leslie E. Edinboro[a]; Gerard M. McGuire[b]; Stephen G. P. Williams[b]; H. Thomas Karnes[a]

[a] Department of Pharmaceutics, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, USA [b] Inveresk Research, Tranent, Scotland

Online publication date: 12 February 2003

To cite this Article Gao, Songmei , Wilson, David M. , Edinboro, Leslie E. , McGuire, Gerard M. , Williams, Stephen G. P. and Karnes, H. Thomas(2003) 'Improvement of Sensitivity for the Determination of Propylene Glycol in Rat Plasma and Lung Tissue Using HPLC/Tandem MS and Derivatization with Benzoyl Chloride', Journal of Liquid Chromatography & Related Technologies, 26: 20, 3413 — 3431

To link to this Article: DOI: 10.1081/JLC-120025599

URL: http://dx.doi.org/10.1081/JLC-120025599

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 20, pp. 3413–3431, 2003

Improvement of Sensitivity for the Determination of Propylene Glycol in Rat Plasma and Lung Tissue Using HPLC/Tandem MS and Derivatization with Benzoyl Chloride

Songmei Gao,[1] David M. Wilson,[1] Leslie E. Edinboro,[1] Gerard M. McGuire,[2] Stephen G. P. Williams,[2] and H. Thomas Karnes[1,*]

[1]Department of Pharmaceutics, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, USA
[2]Inveresk Research, Tranent, Scotland

Downloaded At: 19:40 23 January 2011

ABSTRACT

Propylene glycol (PG), a simple alcohol, is a commonly used vehicle for aerosol dosage formulations. Quantification of PG in plasma and lung tissue is, therefore, important for new drug development. We describe a highly sensitive and selective method for the quantitative determination of PG in rat plasma and lung tissue, using liquid chromatography (LC) with positive atmospheric pressure chemical ionization (APCI) tandem mass

*Correspondence: H. Thomas Karnes, Department of Pharmaceutics, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980533, Richmond, VA 23298-0533, USA; E-mail: htkarnes@vcu.edu.

DOI: 10.1081/JLC-120025599
1082-6076 (Print); 1520-572X (Online)
Copyright © 2003 by Marcel Dekker, Inc.
www.dekker.com

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

spectrometry (MS) detection. Propylene glycol and the internal standard (IS) 1,4-butanediol were derivatized with benzoyl chloride under alkaline conditions to enhance the sensitivity of detection. Ionization efficiency was improved following derivatization. The limits of detection (LOD) for rat plasma and rat lung tissue were 0.269 μg/mL and 1.12 μg/g, respectively. The LOQs for rat plasma and lung tissue were 0.448 μg/mL and 1.62 μg/g, respectively. Calibration curves were linear from 2 to 4000 μg/mL for rat plasma ($r = 0.9990$) and from 2 to 2400 μg/g for rat lung tissue ($r = 0.9985$). The assay showed intra-assay and inter-assay precision (%RSD) of 3.8–4.8% ($n = 6$) and 4.2–6.6% ($n = 18$) for rat plasma, 3.8–4.0% ($n = 6$) and 6.5–7.4% ($n = 18$) for rat lung tissue, respectively. The percent inaccuracy for inter-assay results in rat plasma were 2.5–4.7% and in rat lung tissue were 1.3–6.8% for different concentrations. All plasma and lung tissue samples demonstrated acceptable freeze/thaw stability, bench stability, and prepared sample stability over a 24 hours period. An alternative derivatizing method using perfluorooctanoyl chloride with negative APCI/MS detection was investigated. Although strong fragment ions of the derivatives could be detected, the feasibility of this method was limited by sample preparation. Additionally, fragment ions produced from the perfluorooctanoyl moiety lacked selectivity. The benzoyl chloride method is proved to be more sensitive, selective, and robust.

Key Words: LC/MS/MS; Derivatization; Benzoyl chloride; Aerosol; Perfluorooetanoyl chloride; APCI.

INTRODUCTION

Propylene glycol (PG), is a commonly used vehicle in many modern pharmaceutical formulations to enhance the absorption of drugs. The degree of enhancement appears to be related to the percentage of PG in the dosing solution.[1] Lakind reviewed the mammalian toxicity of PG and ethylene glycol using oral, inhalation, and dermal routes of exposure[2] and concluded that PG is safer than ethylene glycol. Even though PG is generally considered safe for use in medication, patients with renal failure may suffer PG intoxication, with symptoms of lactic acidosis and severe nervous system depression.[3,4] Consequently, accurate and precise quantification of PG in plasma and tissues is important for new drug development. Generally, the quantitative tests required for human plasma have been in the μg/mL to mg/mL range. A more sensitive method may be needed, however, to detect PG in lung tissue and plasma. To our knowledge, no extensive study about PG absorption in lung tissue following inhalation has been reported.[2] Because of the lack of information about PG absorption and kinetics in the lung, a sensitive method

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

needed to be established. This work is, therefore, intended to provide a more sensitive method for determination of PG in plasma and lung tissue at the sub-ng level following exposure to PG via inhalation.

Few methods have been developed for the determination of PG in plasma and serum. In most methods, PG was described as an interference in the analysis of other glycols.[4–6] Although, ion-exclusion chromatography with refractive index detection,[7] gas-chromatography (GC) with flame ionization detection,[8,9] have been reported for analysis of PG in pharmaceutical intravenous solutions or plasma sample, the limits of quantification generally were at concentrations of 2 to 10 μg/mL. Moreover, no methods have been established for determination of PG in lung tissue.

In recent years, the powerful usefulness of liquid chromatography (LC)/mass spectrometry (MS) has been demonstrated in determination of many compounds in biological matrices. However, poor atmospheric pressure ionization (API) using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) has been noted for the analysis of polar and nonpolar compounds with relatively low molecular weights[10,11] and were continued for PG in preliminary experiments. Sensitivity may be low for PG using ESI because of the absence of a readily ionizable functional group, and limited sensitivity with APCI may be due to poor proton transfer. We established an approach for the analysis of PG using pre-column derivatization with benzoyl chloride to perhaps improve proton transfer for PG. In order to facilitate extraction, the derivatizing reagent and extraction solvent were added to the sample together. A benzoyl ester derivative was formed directly in plasma or lung tissue via the Schotten-Baumann reaction[12] and was detected by LC/APCI/tandem MS with positive detection mode.

An alternative APCI/MS, using negative mode based on an electron capture mechanism, was also investigated following derivatization of PG with perfluorooctanoyl chloride.

EXPERIMENTAL

Chemicals

Rat plasma and rat lung tissue were obtained from Charles Rivers UK Limited (Margate, Kent, CT9 4LT, UK). Propylene glycol USP/FCC was obtained from Chrysalis Technologies Incorporated (Richmond, VA). 1,4 butanediol internal standard (IS), Glycine (sodium salt), the two derivatizing reagents–benzoyl chloride and perfluorooctanoyl chloride, were purchased from Sigma Aldrich Chemical Co. (Milwaukee WI 53233). Methanol was HPLC grade and was purchased from Rathburn Chemicals Limited (Walkerburn,

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Peebleshire, EH43 6AU, UK). Pentane (HiperSolv Grade) and Sodium hydroxide pellets (AnalaR Grade) were purchased from BDH Chemical Limited (Poole, Dorset, BH15 1TD, UK). Formic Acid (Certified Grade) was obtained from Fisher Chemicals (Loughborough, Leicestershire, LE11 0RG, UK). Water was purified by a Milli-Q system (18.2 MΩ cm).

The benzoyl chloride and pentane mixture was prepared by adding 125 μL benzoyl chloride to 10 mL of pentane.

LC/MS Instrumentation and Conditions for Derivatization of PG with Benzoyl Chloride

The liquid chromatography-mass spectrometry (LC/MS) system consisted of a Perkin Elmer Series 200 HPLC pump (Beaconsfield, Buckinghamshire, UK), a Perkin Elmer Series 200 autosampler (Beaconsfield, Buckinghamshire, UK), an Applied Biosystems API365 tandem mass spectrometer (Warrington, Cheshire, UK), and an Applied Biosystems data handing system Analyst Version 1.1 (Warrington, Cheshire, UK). All separations for the derivative of PG and benzoyl chloride were performed using a 2.1 × 100 mm Waters Symmetry Shield RP-18, 3.5 μm column (Watford, Hertfordshire, UK). The flow rate was 0.25 mL/min. The mobile phase consisted of methanol : water 78 : 22, both with 0.1% formic acid (v/v). The injection volume was 10 μL. The parameters for the MS/MS system were set as follows: Ion Spray Voltage (IS) 4500 Volts; Declustering Potential (DP) 1 V; Focusing Potential (FP) 120 V; Entrance Potential (EP) −2.5 V; Collision Entrance Potential (CEP) 8.57 V; Collision Energy (CE) 13 V; and Collision Exit Potential (CXP) 16 V. APCI probe temperature was set at 450°C. Turbo gas flow rate was 7000 mL/min. The ions at m/z 285.1 and 299.0 were used as precursors and the ions at m/z 163.2 and 177.2 as product ions for PG and the IS, respectively, in the MS/MS mode.

LC/MS Instrumentation and Conditions for Derivatization of PG with Perfluorooctanoyl Chloride

The liquid chromatography-mass spectrometry system consisted of two Shimadzu LC10ADvp high pressure pumps (Columbia, MD), a Shimadzu DGU-14A solvent degasser (Columbia, MD), a Shimadzu SCL10Avp system controller (Columbia, MD), a Shimadzu SIL10Advp autosampler (Columbia, MD), a Micromass Quattro Triple mass spectrometer (Manchester, UK), and a MassLynx 3.4 Micromass data acquisition system. Separations for the derivative of PG and perfluorooctanoyl chloride were performed using a 2.1 × 50 mm Waters Symmetry Shield RP-18, 3.5 μm column (Milford, MA

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

01757). The flow rate was 0.5 mL/min. The mobile phase consisted of methanol : water 80 : 20. The injection volume was 1 μL. The parameters for the MS/MS system were set as follows: corona 3.50 kV; cone 12 V; extractor 2 V; source temperature 120°C; APCI probe temperature 350°C. The desolvation gas flow was 150 L/hr. Ions were scanned in negative APCI mode.

GC/MS Apparatus and Conditions for Derivatizing Efficiency of Perfluorooctanoyl Chloride

The gas chromatograph-mass spectrometric (GC-MS) analysis was carried out using a Model 5890 Hewlett-Packard (Palo Alto, CA) GC coupled with a 5971A mass-selective detector. The column used for PG and the derivative of PG and perfluorooctanoyl chloride was a DBWAX, 15 × 0.25 mm I.D. capillary column (Agilent Technologies). The initial oven temperature for the perfluorooctanoyl chloride derivative was 60°C, the temperature was then increased to 180°C at 15°C/min, and subsequently increased to 240°C at 40°C/min and kept for 1 min. Injector temperature and detector temperature were 230°C and 290°C. The mass spectrum was scanned from m/z 50 to m/z 650. The solvent delay was set to 2 min.

Standards and Controls

Rat plasma was spiked with PG to obtain standards of 2.0, 4.0, 20.0, 40.0, 200.0, 400.1, 1000, 2000, 4001 μg/mL and controls of 8.0, 160.5, 3210 μg/mL. Standard and control samples of lung homogenate were prepared as μg PG/g lung tissue. Rat lung was spiked with PG to obtain standards of 2.0, 4.0, 5.0, 10.0, 40.0, 100.0, 400.0, 1000, 2000, 2400 μg/g and controls of 8.0, 160.0, 1900 μg/g. When PG standards or controls were prepared in lung tissue, lungs were weighed (about 0.6–0.8 g) and placed in a 20 × 150 mm-culture tube. The final volume to be added was approximately four times the weight of the tissue. To achieve the final volume, PG stock solution and additional water were added. After homogenization for 45–60 seconds on high speed, samples were extracted. Working standards and controls were stored at −80°C.

Quality control samples of 12,000 μg/mL and 4000 μg/g were prepared as dilution controls for rat plasma and rat lung tissue sample, respectively. After dilution control samples were diluted 10 fold with aliquots of the matrix to bring the determined concentration of PG within the linear range, they were processed as quality controls were.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Extraction and Derivatization of PG with Benzoyl Chloride in Rat Plasma

A 50 μL volume of rat plasma sample was mixed with 10 μL of 50 μg/mL IS and 50 μL of 4 M NaOH. After 1 mL of the benzoyl chloride/pentane mixture was added, each specimen was vortexed for 30 min. A 25 μL volume of 1% glycine solution was then added to stop the reaction and the solution was vortexed for 15 min followed by standing at room temperature for 45 min. The mixture was centrifuged at 4000 rpm for 5 min and 100 μL of the pentane layer was transferred to a disposable conical screw cap tube. The sample was dried under dry nitrogen at 40°C and the residue was reconstituted with 1000 μL of methanol.

Extraction and Derivatization of PG with Benzoyl Chloride in Rat Lung Tissue

A 100 μL volume of rat lung homogenate was thoroughly mixed with 10 μL of 100 μg/mL IS. All samples were equilibrated for 30 min at room temperature, vortexed thoroughly, then centrifuged at 4000 rpm for 10 min. 50 μL of the supernatant of each sample was then transferred to a clean eppendorf tube, and 50 μL of 4 M NaOH was added to the sample. After the 1 mL benzoyl chloride and pentane mixture was added, each specimen was vortexed for 30 min. A 25 μL volume of 1% glycine solution was then added and all tubes were vortexed for 15 min, followed by standing at room temperature for 45 min. The mixture was centrifuged at 4000 rpm for 5 min and 100 μL of the pentane layer was transferred to a clean test tube. The samples were dried under dry nitrogen in a Turbovap® evaporator at 40°C for ~5 min. The residue was reconstituted with 200 μL of methanol.

Assay Validation

The calibration curve for rat plasma ranged from 2.0 to 4000.0 μg/mL and was constructed using least-squares regression of the ratios of the PG peak area to that of the IS plotted versus PG concentration. The calibration curve for rat lung tissue ranged from 2.0 to 2400.0 μg/g and was constructed similarly to the plasma curve. The intra-assay precision and accuracy in rat plasma or rat lung tissue were studied by determining three quality control and dilution control samples within the same day ($n = 6$). The inter-assay precision and accuracy for the samples were detected on three different days ($n = 18$). Analytical relative recovery was determined by comparison of the peak area of PG extracted from water versus the areas of PG controls in the same analytical

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

run. The same procedure was used to establish the relative recovery of the IS (1,4-Butanediol). Freeze/Thaw stability was evaluated over three freeze/thaw cycles (from −80°C to room temperature). Bench stability was also determined by removing samples from −80°C storage, thawing to room temperature, and incubating on the benchtop 4 hours prior to starting analysis. After prepared samples were stored 24 hours on the autosampler at ambient temperature, stability was tested by comparison with fresh samples.

Extraction and Derivatization of PG with Perfluorooctanoyl Chloride in Rat Plasma

A 50 μL sample of rat plasma (1 mg/mL PG) was mixed with 50 μL of acetone. The sample was vortex-mixed and then centrifuged at 13000*g* for 5 min. The supernatant was evaporated to dryness under dry nitrogen with the temperature not more than 45°C. 50 μL of perfluorooctanoyl chloride was added to the residue. After incubation at 60°C for 25 min in a heating block, the reaction mixture was evaporated under dry nitrogen and the dry residue reconstituted with 50 μL of methanol for LC/MS analysis.

Derivatization Efficiency Experiments

A 50 μL perfluorooctanoyl chloride was added to 1 mg PG. After incubation at 60°C for 25 min in a heating block, 1 mL of methanol was added to the mixture to quench the reaction by reacting with the excess derivatizing reagent. The reaction mixture was incubated at 60°C for 10 min, then injected into GC system. Derivatization efficiency was estimated by comparing the peak area of PG after the derivatization reaction versus PG peak area before derivatizing reaction.

RESULTS AND DISCUSSION

Generally, electrospray ionization is used for compounds with high polarity, while APCI is applicable to compounds with low polarity.[13] Direct analysis of PG using ESI or APCI lacked sensitivity due to poor ionization efficiency. Introduction of proton-affinitive atoms, such as oxygen and nitrogen, has been shown to improve the capability of transferring protons for several compounds.[13,14] Benzoyl chloride, a derivatization reagent for hydroxyl groups and amine groups in HPLC/UV detection,[15,16] can offer a proton-affinitive group from its benzoyl moiety. Sunner has discussed the relationship of detection sensitivity and gas-phase basicity of compounds

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

using APCI.[17] The detection sensitivity for most of oxygen bases increased with gas-phase basicity. After introduction of proton-affinitive atoms to the structure, it is very possible that the gas-phase basicity of a compound is increased. Although the gas-phase basicity value for PG and its benzyol chloride derivative couldn't be obtained directly, we expected that the introduction of the benzoyl moiety also would improve the gas-phase basicity and proton transfer for PG when considering the gas-phase basicities of methyl benzylketone (PhCOMe) and methanol, which are 195 and 172 Kcal/mol,[18] respectively. The formation of derivatives can also effectively shift the analytical mass range to a high mass field, therefore decreasing the noise in the background and improving the detection sensitivity.

The molecular ion of the PG derivative at m/z $(M + H)^+$ 285 was detected in the positive ion mode (Fig. 1). A base peak was observed at m/z 163, and represented the ion formed by loss of a benzoyl acid group from the molecular ion. Similarly, a molecular ion $(M + H)^+$ at m/z 299 and an abundant fragmental ion at m/z 177 could be observed for the IS due to loss of benzoyl acid.

Tandem MS/MS is useful in the detection of known or targeted compounds to give a combination of high sensitivity and high selectivity.[19] Based on the intensity of ions and fragmentation pathways of the derivative of PG and the IS, the best precursor and product ion pairs for quantitation could be defined in the MRM mode. The ions at m/z 285.00 and 299.00 were used as precursor ions and the ions at m/z 163.00 and 177.00 as product ions for PG and the IS,

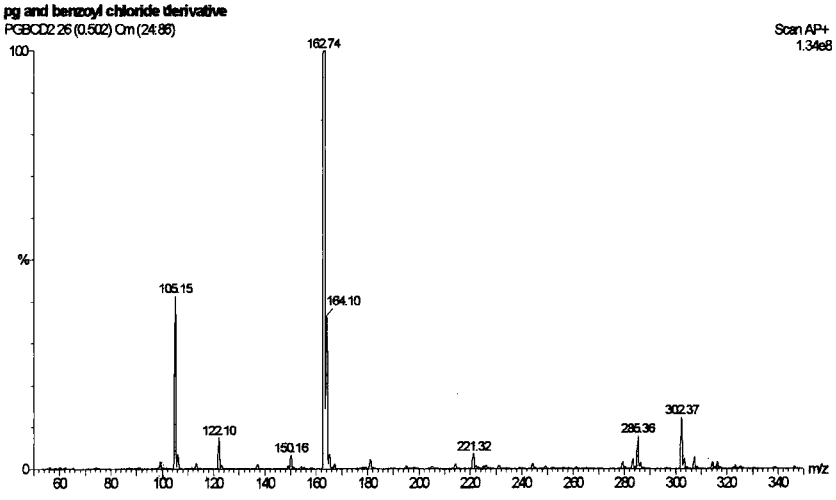


Figure 1. Atmospheric pressure chemical ionization mass spectrum of the benzoyl chloride derivative of PG.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

respectively. Chromatograms for PG and the IS are shown in Fig. 2. The retention time for PG was 3.5 min; and the retention time for the IS was 4.4 min.

The assay in plasma was linear over the concentration range of 2.0 μg–4000 μg/mL with a correlation coefficient of 0.9990, when weighted

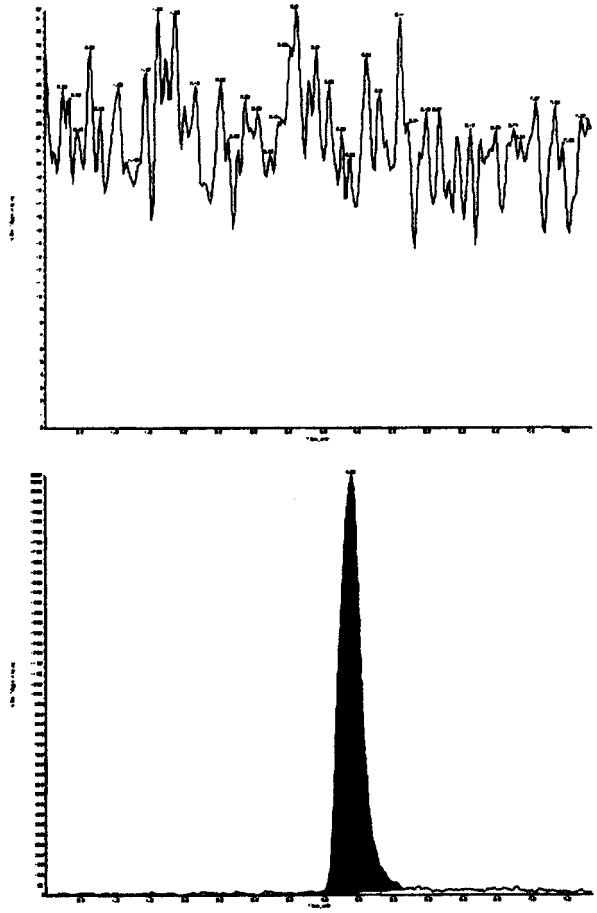


Figure 2. Chromatogram of PG sample in rat plasma using the multiple reaction monitoring detection mode. (a) Blank with IS; (b) Blank with PG. For each pair of pictures, the upper one shows the chromatogram of PG, the bottom one shows the chromatogram of IS.

(continued)

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

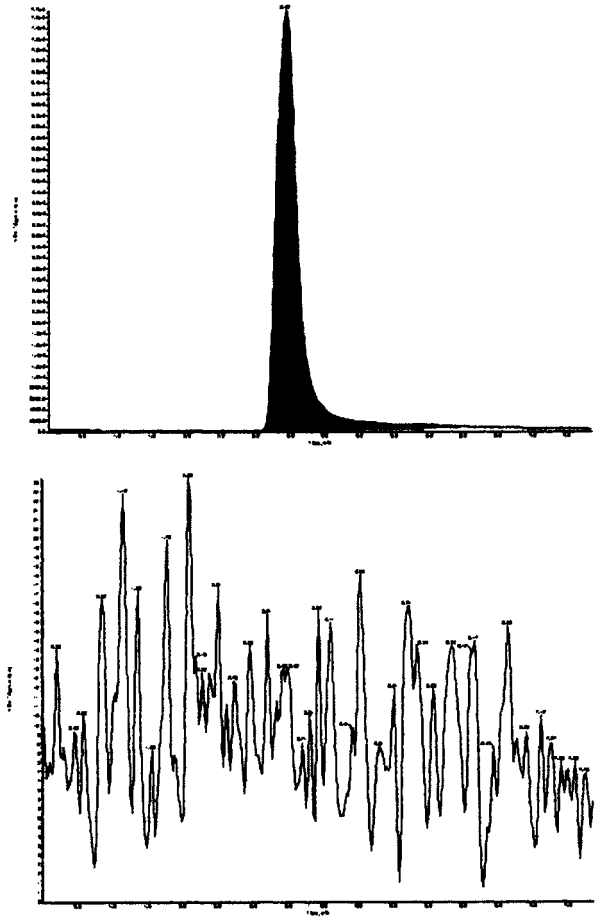


Figure 2. Continued.

1/concentration^2. The linear equation was produced as: peak ratio = $-0.000965 + 0.0222 \times$ concentration; and the concentration residuals were within 9.0%. The precision and accuracy of the method were calculated as the relative standard deviation (%RSD) and the percent difference from nominal (%DFN), respectively. Table 1 summarizes the precision and accuracy of the quality control samples. The assay demonstrated inter-assay precision (%RSD) in rat plasma between 4.2 and 6.6% ($n = 18$), and the percent inaccuracies of inter-assay data in rat plasma was between 2.5 and 4.7%. The precision

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Downloaded At: 19:40 23 January 2011

Table 1. Precision and accuracy of quality control samples.

		Intra-assay Precision ($n = 6$)			Inter-assay Precision ($n = 18$)		
	Nominal concentration	MEAN	%RSD	% DFN[a]	MEAN	%RSD	% DFN[a]
Rat Plasma	8.0 (μg/mL)	8.1	3.8	1.3	8.2	5.7	2.5
	160.5 (μg/mL)	159.0	4.0	−0.9	168.0	6.6	4.7
	3210 (μg/mL)	3310	4.8	3.1	3300	4.2	2.8
Rat Lung Tissue	8.0 (μg/g)	8.23	3.8	2.5	8.4	6.5	5.0
	160.0 (μg/g)	161.0	4.0	0.6	162.0	7.4	1.3
	1900 (μg/g)	1880	4.0	−1.1	2030	6.7	6.8

[a]%DFN percentage of difference from nominal value.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

(%RSD) and the accuracy of the dilution control were 5.8% and 2.0%, respectively ($n = 6$).

The assay for lung tissue was linear over the range 2.0 μg–2400 μg/g, with correlation coefficient $r = 0.9985$. The linear equation was: peak ratio $= 0.000159 + 0.000624 \times$ concentration; and the concentration residuals were within 10.3%. The inter-assay precision for PG in rat lung tissue was 6.5–7.4% ($n = 18$) and the percent inaccuracies of inter-assay data in rat lung were between 1.3 and 6.8%. The precision and accuracy of the dilution controls were 3.7% (%RSD) and 5.0% (% DFN) ($n = 6$). These data show that this method is reliable for quantitation in the concentration range studied.

The detection limits of the assay for rat plasma and rat lung tissue were 0.269 μg/mL and 1.12 μg/g, respectively, based on three times the standard deviation of the blank. The limits of quantitation were 0.448 μg/mL for rat plasma and 1.62 μg/g for lung tissue based on five times the standard deviation of the blank. When the dry residue after derivatization was reconstituted in 50 μL of methanol instead of 1000 μL, the limit of quantitation for rat plasma was lowered to 0.25 μg/mL with the %DFN at 4.1%.

Samples were considered to be stable if the mean difference between initial to final result was less than 15%. All samples demonstrated acceptable three times freeze/thaw stability, bench stability, and prepared sample stability.

Derivatization with benzoyl chloride is a Schotten-Baumann reaction, which is known as acylation of alcohols or amines with acyl halides in aqueous. In the published methods,[12,15,16] a small amount of benzoyl chloride (20–50 μL) was added to the sample directly, followed by vortex and extraction. Since benzoyl chloride is insoluble in water, the reaction takes place only at the phase boundary, therefore, the speed of reaction and derivatization efficiency is lowered. We modified this method by combination of the derivatization step and extraction step together. Adding benzoyl chloride with solvent pentane to the sample, not only made the reactants mixed well, but also reduced the de-esterification of the derivative in aqueous alkali by transferring the derivative to pentane. The extraction/reaction efficiency, therefore, was increased 16 times based on increasing of peak area of PG.

Extraction solvents like pentane, hexane, and *n*-butyl chloride were studied to optimize extraction recovery and selectivity. Pentane was chosen as the extraction solvent in this experiment, because pentane did not extract the amides formed with benzyl chloride and the biological matrix as readily.[15] The derivatization and extraction were processed simultaneously, moreover, no primary standard of the derivative of PG was available; therefore, only relative recovery could be determined. Recovery was determined by comparing the peak height of PG extracted from water versus that of PG controls in the same analytical run. The same procedure was used to establish relative recovery of the IS. Relative recovery in these experiments could be regarded as to the

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

derivatization efficiency and extraction efficiency combined. Relative recovery of both PG and the IS for rat plasma were 27.8% and 47.1%, respectively. The relative recovery of PG in rat lung tissue was 31.3%, while the relative recovery of the IS was high, 355.0%. This is probably due to the increased solubility of the IS in the lung matrix versus water. Both PG and the IS were recovered consistently, as demonstrated by calibration and control results.

Ferral et al. reported the carry-over of PG in a GC method.[8] We, therefore, investigated carry-over in this method. Although there was no carry-over for rat lung tissue, a slight carry-over for rat plasma samples was observed. We detected blank samples in matrices following the injection of samples at the upper limit of linearity. The mean detector response for PG in a rat plasma sample was 35.7% of the detector response for an LLOQ standard. Therefore, running a blank matrix sample after a high concentration sample, was carried out to prevent misquantification of subsequent low concentration samples.

After electron capture negative chemical ionization (ECNCI)/MS was introduced into gas chromatographic detection by Hunt and co-workers in 1976,[20] it became a method of choice for the analysis of many drugs and biomolecules at trace concentration. G. Singh applied electron capture APCI/MS with a LC interface to establish an attomole sensitivity for steroids, prostaglandins, and DNA-adducts. He postulated that the low-energy electrons generated in the APCI source could potentially ionize suitable analytes through dissociative electron capture. The sensitivity of detection was enhanced by tagging analytes with an electron-capturing group.[21] Therefore, in addition to the benzoyl chloride method, the possibility of analyzing PG by ECAPCI after derivatization with perfluorooctanoyl chloride, an electron capturing derivatizing reagent,[22] was investigated.

Gas-chromatography/mass spectrometric was used to evaluate derivative formation, and two new peaks were observed in a GC/MS total ion chromatogram after derivatization of PG with perfluorooctanoyl chloride (Fig. 3). A very small peak corresponding to PG could be observed with a retention time at 5.2 min. The peak at 4.3 min is likely to be mono-derivatized PG, because the ion with the highest mass to charge detected was at m/z 457. This could be the fragment ion produced after loss of a methyl group from the molecular ion. The strong fragment ions at m/z 31, 45, and 59 could be explained as the $CH_2CH(CH_3)OH$ group in mono-derivatized PG. Moreover, the retention time of mono-derivatized PG became shorter and could be due to the derivatization of the hydroxyl group and the decrease of its interaction with the polar column. We speculate that the peak at 6.2 min is PG with both hydroxyl groups derivatized, unfortunately, we could not observe this molecular ion at m/z 868 because the scan range of the HP 5971 GC/MS is limited to m/z 650 da. The proposed mass spectral fragmentation pattern, which is consistent with the di-derivatized product of EG,[22] is shown in Fig. 4.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

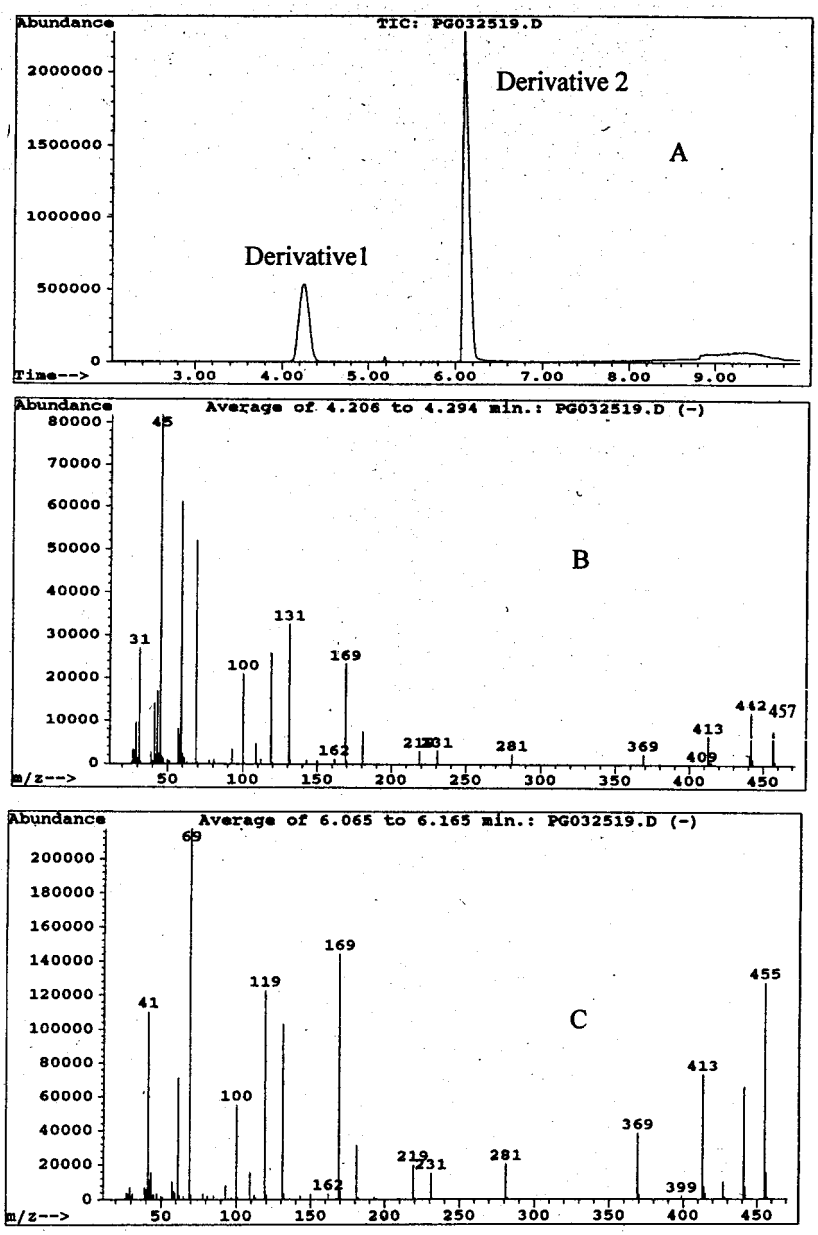


Figure 3. Total ion chromatogram and mass spectra of the perfluorooctanoyl chloride derivatives of PG using GC/MS. (A) Total ion chromatogram of 1 : 1000 dilution of derivatives.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

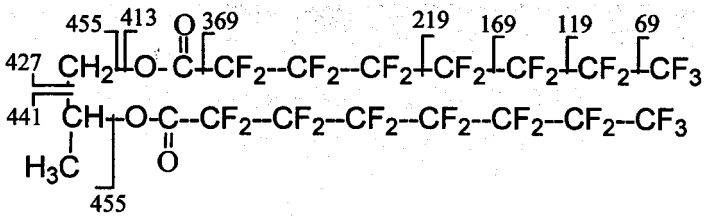


Figure 4. Proposed fragmentation pattern of the perfluorooctanoyl derivative of PG.

Although the formation of the derivatives was demonstrated, no signal was detected by LC/MS following the rat plasma sample preparation step described previously in the section of extraction and derivatization of PG with perfluorooctanoyl chloride in rat plasma. It was hypothesized, that there were two possible reasons for this result, poor recovery efficiency or poor ionization efficiency.

Poor recovery efficiency was demonstrated after the remaining PG was detected for each sample preparation step. About 40% of PG was lost in the protein precipitation step, and less than 10% (about 8.65%) of PG remained after the evaporation of the supernatant. Derivatization efficiency was estimated using the peak area of PG before the derivatization reaction to that of peak area after the dervatization. The derivatization efficiency was 94.5%, although the derivatization efficiency decreased significantly with the addition of water (Fig. 5). While the evaporation step was necessary for sample preparation prior to derivatization, it became the major limitation of this

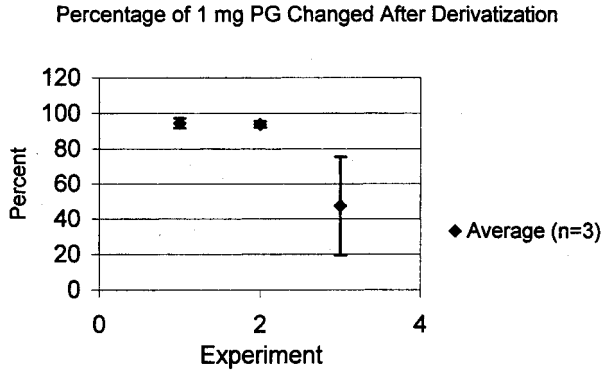


Figure 5. Derivatizing efficiency of PG with PFOCL. Experiment 1: 1 mg PG; Experiment 2: 1 mg PG with 1 mg H_2O; Experiment 3: 1 mg PG with 25 mg H_2O.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

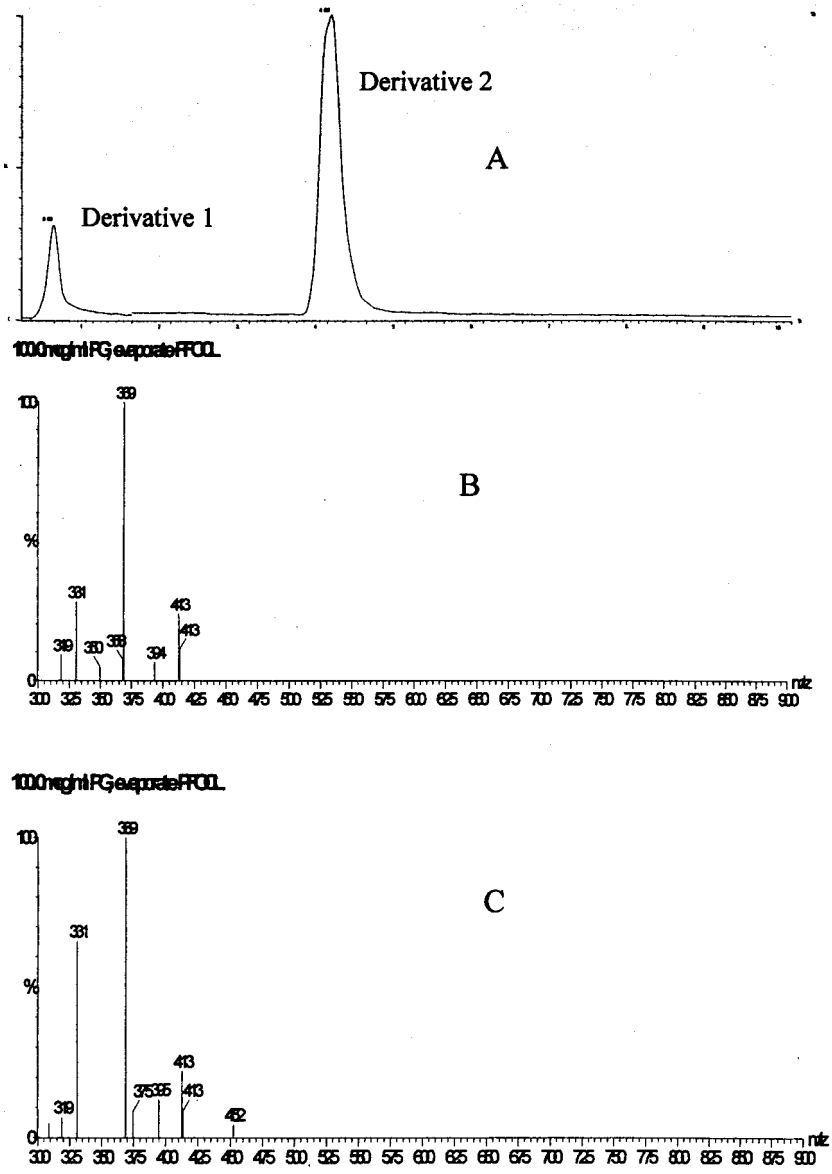


Figure 6. Chromatography and mass spectra of derivatives of PG and perfluorooctanoyl chloride using LC/MS. Excessive perfluorooctanoyl chloride was evaporated after the derivatization reaction. (A) Total ion chromatography; (B) Mass spectrum of derivative 1 (Rt = 0.48 min); (C) Mass spectrum of derivative 2 of PG (Rt = 4.01 min).

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

analytical method. When pure PG was derivatized and analyzed by LC/MS, two derivative peaks could be observed with the current conditions (Fig. 6). No molecular ions were detected, but two intense ions were observed at m/z 369($^{-}CF_2(CF_2)_5CF_3$) and m/z 413 ($^{-}OCOCF_2(CF_2)_5CF_3$) for each peak. The fragmentation pathway is different from the proposed mechanism of electron capture APCI, in which the molecular ion is formed by the loss of the electron capturing group radical.[21] Fluorine atoms in the compound may have made the negative fragment ions stable, resulting in two intense peaks in the negative ion detect mode. This, however, also made this method not very selective, since other intense ions came from perflurooctanoyl moiety.

CONCLUSION

A short-chain alcohol with poor intrinsic MS ionization efficiency in the API mode, PG, was subjected to the Schotten-Baumann reaction with benzoyl chloride, resulting in a derivative having high APCI detection sensitivity. The ionization efficiency of the benzoic acid derivative was increased due to the introduction of the benzoyl moiety, which may have resulted in improved proton transfer for the PG derivitive. Validation for the method was established in a wide linear range for rat plasma and lung tissue with an LOQ suitable for analysis in rat plasma and rat lung tissue. This method may be adoptable to detect other alcohols in complex matrices.

Derivatization with PFOCL could improve detection sensitivity for PG using the negative ion MS detect mode, however, the sample preparation step was a critical weakness of that method. About 90% of PG was lost during the evaporation step, which was necessary for sample preparation prior to derivatization. Additionally, intense ions resulting from the derivatization reagent resulted in a lack of selectivity.

ACKNOWLEDGMENTS

These studies were financially supported by Chrysalis Technologies Inc. The authors would like to thank Dr. Zong-Ping Zhang for helpful suggestions and discussions during these experiments.

REFERENCES

1. Bailey, D.N. Propylene glycol as a vehicle for percutaneous absorption of therapeutic agents. J. Anal. Toxicol. **1992**, *16*, 97–98.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

2. LaKind, J.S.; McKenna, E.A.; Hubner, R.P.; Tardiff, R.G. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit. Rev. Toxicol. **1999**, *29* (4), 331–365.
3. Demey, H.E.; Daelemans, R.A.; Veropooten, G.A.; De Broe, M.E.; Van Campenhout, Ch.M.; Lakiere, F.V.; Schepens, P.J.; Bossaert, L.L. Propylene glycol-induced side effects during intravenous nitroglycerin therapy. Intens. Care. Med. **1988**, *14*, 221–226.
4. Dasgupta, A.; Macaulay, R. A novel derivatization of ethylene glycol from human serum using 4-carbethoxyhexafluorobutyryl chloride for unambiguous gas chromatography-chemical ionization mass spectrometric identification and quantification. Am. J. Clin. Pathol. **1995**, *104* (3), 283–288.
5. Williams, R.H.; Shah, S.M.; Maggiore, J.A.; Erickson, T.B. Simultaneous detection and quantitation of diethylene glycol, ethylene glycol, and the toxic alcohols in serum using capillary column gas chromatography. J. Anal. Toxicol. **2000**, *24*, 621–626.
6. Baffi, P.; Elneser, S.; Baffi, M.; De Melin, M. Quantitative determination of diethylene glycol contamination in pharmaceutical products. J. AOAC **2000**, *83*, 793–801.
7. Iwinski, G.; Jenke, D.R. Determination of alcohols in pharmaceuticals by ion-exclusion chromatography. J. Chromatogr. **1987**, *392*, 397–405.
8. Ferrala, N.F.; Ghanayem, B.I.; Nomeir, A.A. Determination of 1-methoxy-2-propanol and its metabolite 1,2-propanediol in rat and mouse plasma by gas chromatography. J. Chromatogr. B **1994**, *660*, 291–296.
9. Yu, D.K.; Sawchuk, R.J. Gas–Liquid chromatogrphic determination of propylene glycol in plasma and urine. Clin. Chem. **1983**, *29*, 2088–2090.
10. Bayer, E.; Gfrorer, P.; Rentel, C. Coordination-ionspray-MS (CIS-MS), a universal detection and characterization method for direct coupling with separation techniques. Angew. Chem. Int. Ed. **1999**, *38*, 992–995.
11. Van Berkel, G.J.; Quirke, J.M.E.; Tigani, R.A.; Dilley A.S.; Covey, T.R. Derivatization for electrospray ionization mass spectrometry. 3. Electrochemically ionizable derivatives. Anal. Chem. **1998**, *70*, 1544–1554.
12. Vollmer, P.A.; Harty, D.C.; Erickson, N.B.; Balhon, A.C.; Dean, R.A. Serum ethylene glycol by high-performance liquid chromatography. J. Chromatogr. B **1996**, *685*, 370–374.
13. Higashi, T.; Awade, D.; Shimada, K. Determination of 24, 25-dihydroxyvitamin D3 in human plasma using liquid chromatography-mass spectrometry after derivatization with a cookson-type reagent. Biomed. Chromatogr. **2001**, *15*, 133–140.
14. Novak, T.J.; Yuan, H. The determination of a chlorinated benzofuran pharmaceutical intermediate by HPLC-MS with on-line derivatization. J. Pharm. Biomed. Anal. **2000**, *23*, 705–713.

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

15. Gupta, R.N. Determination of trichloroethanol, the active metabolite of chloral hydrate, in plasma by liquid chromatography. J. Chromatogr. **1990**, *500*, 655–659.
16. Mei, Y.H. A sensitive and fast method for the determination of polyamines in biological samples. Benzoyl chloride pre-column derivatization high-performance liquid chromatography. J. Liq. Chromatogr. **1994**, *17*, 2413–2418.
17. Sunner, J.; Nicol, G.; Kebarle, P. Factors determining relative sensitive of analytes in positive mode atmospheric pressure ionization mass spectrometry. Anal. Chem. **1988**, *60*, 1300–1307.
18. Sunner, J.; Ikonomou, M.G.; Kebarle, P. Sensitivity enhancements obtained at high temperatures in atmospheric pressure ionization mass spectrometry. Anal. Chem. **1988**, *60*, 1308–1313.
19. Reemtsma, T. Analysis of sulfophthalimide and some of its derivatives by liquid chromatographyelectrospray ionization tandem mass spectrometry. J. Chromatogr. A **2001**, *919*, 289–297.
20. Hunt, D.F.; Stafford, G.C., Jr.; Crow, F.W.; Russell, J.W. Pulsed positive negative ion chemical ionization mass spectrometry. Anal. Chem. **1976**, *48*, 2098–2104.
21. Singh, G.; Gutierrex, A.; Xu, K.; Blair, I.A. Liquid chromatography/electron capture atmospheric pressure chemical ionization/mass spectrometry: analysis of pentafluorobenzyl derivatives of biomolecules and drugs in the attomole range. Anal. Chem. **2000**, *72*, 3007–3013.
22. Dasgupta, A.; Blackwell, W.; Griego, J.; Malik, S. Gas chromatographic-mass spectrometric identification and quantitation of ethylene glycol in serum after derivatization with perfluorooctanoyl chloride: a novel derivative. J. Chromatogr. B **1995**, *666*, 63–70.

Received January 14, 2003
Accepted February 20, 2003
Manuscript 6063

Downloaded At: 19:40 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016