Journal of Chromatography, 455 (1988) 183–192
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 877

RESOLUTION OF DIASTEREOMERS OF N,N,N′,N′-TETRAKIS(2-HYDROXYPROPYL)ETHYLENEDIAMINE (QUADROL) BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

J. A. FULTON, H. K. POKHARNA, M. J. DUNPHY and D. J. SMITH*
Department of Chemistry, The University of Akron, Akron, OH 44325 (U.S.A.)
(Received June 15th, 1988)

SUMMARY

Quadrol, N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine, has been recently observed to display biological activity. It is an immunostimulant and has been implicated as a potentially useful agent in accelerated wound healing. Quadrol exists as a mixture of four unique diastereomers, each of which may, upon further investigation, display differences in biological activity. This paper describes an high-performance liquid chromatographic procedure (both analytical and prep) for the separation of the Quadrol diastereomers. Gas–liquid chromatograpy and NMR data are presented which corroborate the high-performance liquid chromatographic results. This procedure may be used to obtain pure Quadrol diastereomers, to monitor the progress of Quadrol synthesis from propylene oxide and ethylenediamine or to develop a quantitative assay for Quadrol diastereomers.

INTRODUCTION

Quadrol, N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine (Fig. 1), is a viscous, colorless liquid possessing four chiral carbon atoms. It has been used as a metal complexing agent[1–3], a polar base in buffers for automatic protein sequencing[4] and as a crosslinking agent and catalyst in the synthesis of urethane foams[5]. Quadrol, as a mixture of diastereomers, was recently reported to possess immunological activity. *In vitro* macrophage stimulation was observerd as an increase in spreading and

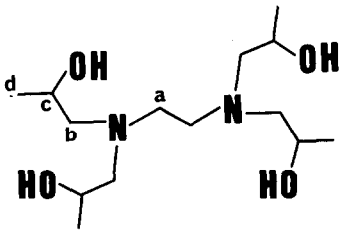

Fig. 1. Structure of Quadrol [N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine]. The carbon atoms producing unique signals in the ^{13}C NMR spectrum are labeled a, b, c, and d.

0021-9673/88/$03.50

phagocytosis [6,7]. In addition, wound healing experiments involving normal and diabetic mice[8] have demonstrated that Quadrol (as a mixture of isomers) accelerates collagen deposition in wound sites, and thus may be useful as a promoter of the wound healing process. Quadrol has also been incorporated into a hydrogel polymer[9] for potential use in wound dressings.

The development of a suitable high-performance liquid chromatographic (HPLC) procedure was initiated to monitor the progress of the reaction between propylene oxide and ethylenediamine to form Quadrol and to assess the purity of the final product. In the process, we noted that pure Quadrol consistently produced a multiplet of peaks. After verifying the absence of contaminants in our material, we hypothesized that the chromatographic system was resolving diastereomers of Quadrol. Resolution of diastereomers by HPLC has been observed for various other compounds[10–13]. The possibility exists that one of the diastereomers of Quadrol could be more biologically active than the others. Therefore, a procedure which allows one to separate and potentially assay individual isomers could be a valuable tool in the study of the biochemical mechanism of Quadrol's action. This paper describes the development of a method to separate the diastereomers of Quadrol by reversed-phase HPLC with electrochemical detection. Each of the diastereomer peaks is identified by analyzing mixtures of synthetic Quadrol diastereomers. The HPLC results are corroborated with data from ^{13}C NMR and gas–liquid chromatographic (GLC) analyses.

MATERIALS AND METHODS

Chemicals

Reagent-grade perchloric acid, acetic acid, sodium hydroxide, potassium hydroxide, sodium sulfate, methanol, ethanol, anhydrous diethyl ether, methylene chloride and HPLC-grade methanol were purchased from Fisher (Cleveland, OH, U.S.A.). N,N,N′,N′-Tetrakis(2-hydroxypropyl)ethylenediamine, ethylenediamine, propylene oxide and *S*-propylene oxide were obtained from Aldrich (Milwaukee, WI, U.S.A.). Octyl sodium sulfate was purchased from Eastman Kodak (Rochester, NY, U.S.A.).

Synthesis

Preparation of N-2-hydroxypropylethylenediamine (mono), N,N′-bis(2-hydroxypropyl)ethylenediamine (diol) and N,N,N′-tris(2-hydroxypropyl)ethylenediamine (triol). Mono, diol and triol were each prepared by appropriate stoichiometric addition of propylene oxide to ethylenediamine followed by either crystallization or fractional distillation purification according to the methods of Plucinski *et al.*[14] and Smith and Patel[9].

Preparation of N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine (Quadrol). In a typical preparation of Quadrol, propylene oxide (2.0 g, 34.4 mmol) was added to a solution of ethylenediamine (0.2 g, 3.4 mmol) in 2 ml of 50% ethanol. The reaction mixture was contained in a three-necked flask with an attached dry ice condenser and maintained at 90°C for 6 h. After concentration *in vacuo*, the resultant oil was dissolved in diethyl ether and dried over anhydrous sodium sulfate. Filtration

and concentration *in vacuo* produced a colorless oil (0.9 g) in 90% yield. Further purification, if necessary, was accomplished by fractional distillation at 175°C at 0.8 mmHg or by recrystallization of the perchlorate salt of Quadrol from methanol.

Preparation of N,N,N',N'-tetrakis([2S]-2-hydroxypropyl)ethylenediamine (SSSS-Quadrol), Syn 1. *S*-Propylene oxide (2.0 g, 34.4 mmol) was added to ethylenediamine (0.2 g, 3.4 mmol) in accordance with the procedure outlined for the synthesis of Quadrol. After the removal of the diethyl ether, a white crystalline product, *SSSS*-Quadrol (m.p. 43.5°C; $[\alpha]_D^{25} = +171$, $c = 0.035$, $CHCl_3$), was obtained (0.8 g) in 80% yield.

Preparation of a mixture of SSSS-Quadrol and N,N,N'-tris([2S]-2-hydroxypropyl -N'-([2R]-2-hydroxypropyl)ethylenediamine [SSSR-Quadrol], Syn 2. *S*-Propylene oxide (1.0 g, 17.2 mmol) was added to N-2-hydroxypropyl-ethylenediamine (mono) (0.5 g, 4.3 mmol) following the procedure outlined for the synthesis of Quadrol. After purification, a colorless oil, *SSSS*- and *SSSR*-Quadrol, was obtained (1.2 g) in 95% yield.

Preparation of a mixture of SSSS-Quadrol, SSSR-Quadrol, and N,N'-bis ([2S]-2-hydroxypropyl) -N,N'-bis(2R]-2-hydroxyproplyl)ethylenediamine (SRSR-Quadrol), Syn 3. N,N'-Bis([2*S*]-2-hydroxypropyl) ethylenediamine (*SS*-diol) was first prepared by reacting *S*-propylene oxide (1.0 g, 17.2 mmol) with ethylenediamine (0.5 g, 8.5 mmol) according to the procedure of Smith and Patel[9]. The *SS*-diol was obtained as a white crystalline solid (0.3 g, 20% yield). *SS*-Diol (0.3 g, 1.7 mmol) was added to propylene oxide (1.0 g, 17.2 mmol), and the reaction was then processed according to the procedure for the synthesis of Quadrol. The product, a colorless oil (0.4 g), *SSSS*-, *SSSR*- and *SRSR*-Quadrol, was obtained in 82% yield.

Purification and extraction of Quadrol

Quadrol perchlorate was prepared by a modification of the method of Rorabacher and co-workers[15,16]. Typically, 50 ml of a 10% (w/v) solution of Quadrol in methanol was mixed with 5 ml of 70% perchloric acid, and the salt was precipitated by adding 25 ml of anhydrous diethyl ether. The Quadrol perchlorate was purified by several recrystallizations from methanol. An aqueous solution of the salt was injected onto the HPLC column, and the typical peak pattern for the diastereomers of Quadrol was observed. During preparative HPLC experiments, Quadrol diastereomers were recovered from the mobile phase by a simple extraction procedure. After rotary evaporation to remove methanol, the mobile phase was adjusted to pH 13 with potassium hydroxide and extracted with dichloromethane. Typically, Quadrol was recovered in greater than 90% yield after evaporation of the solvent. The purity of the extracted Quadrol (as a mixture or as a pure diatereomer) was assessed by NMR and GLC analyses in addition to reinjection on the HPLC column.

Gas chromatography

GLC analyses were performed using an HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a flame ionization detector and a 6 ft. silanized glass column packed with 3% SP-2100 DB on 100–120 Supelcoport (Supel-

co, Bellefonte, PA, U.S.A.). The injector and detector were maintained at 300°C. Carrier gas (helium) flow was 30 ml/min at the analyses oven temperature of 230°C. Typically, 5 μl of an ethanolic solution of sample (0.5 mg/ml) was injected. Chromatograms were plotted on a strip-chart recorder.

Nuclear magnetic resonance spectrometry

The ^{1}H and ^{13}C NMR spectra were measured on a Varian XL-300 NMR operating at 300 MHz. The instrument was locked onto the $^{2}HCl_3$ solvent triplet as a reference for the ^{13}C NMR spectra, and the center peak was set at 77 ppm. The samples were dissolved in 1 ml of deuterated chloroform and analyzed in 5-mm high-resolution NMR tubes.

High-performance liquid chromatography

The HPLC system consisted of a Model 590 pump, a Model 710B WISP autosampler, a lambda-max 481 variable-wavelength UV detector and a Model 730 data module (Waters Assoc., Milford, MA, U.S.A.). Manual injections were done using a 50-μl Hamilton syringe and an in-line Rheodyne Model 7125 injector valve, both purchased from Supelco. Column eluent was monitored with an amperometric detector (e.c. monitor) from LDC/Milton Roy (Riviera Beach, FL, U.S.A.). As little as 2 nmol of Quadrol could be detected at a range setting of 2 nA/V, however, most analyses were performed at 10 nA/V. Quadrol was detectable at a voltage setting of +0.90 or higher, the optimum voltage being +1.00. The diastereomers of Quadrol were separated using a 15 cm × 3.9 mm I.D. Nova-Pak® C_{18} column (4 μm packing) from Waters Assoc. A precolumn (5 cm × 4.6 mm I.D.) containing 40 μm Pelligard® C_{18} packing (Supelco) was placed in-line just prior to the analytical column.

The primary mobile phase was composed of methanol–0.1 *M* acetate buffer (pH 5.5 with 3 m*M* octyl sodium sulfate) (2:8, v/v). Variations of this mixture were prepared when studying the effects of octyl sodium sulfate, methanol and pH on isomer resolution. The mobile phase was purged with helium prior to use. The mobile phase flow-rate was 1.0 ml/min which typically produced a column back-pressure of 1800 p.s.i. (124 bar). In most cases, 10 μl of aqueous solutions of Quadrol samples (typically 0.1 mg/ml) were injected.

Recovery of eluted Quadrol diastereomers

To obtain samples of each of the Quadrol diastereomers appearing in the HPLC chromatogram, the analytical column was replaced with a 25 cm × 10 mm I.D. RSil® C_{18} preparative column with 10 um packing (Alltech Assoc., Deerfield, IL, U.S.A.). The primary mobile phase was used at a flow-rate of 4.0 ml/min (back-pressure 800 p.s.i., 55 bar). The column was loaded with 25 mg of Quadrol, and fractions were collected every minute between 50 and 140 min after injection. Fractions collected from six separate injections were then pooled according to their diastereomeric content.

RESULTS AND DISCUSSION

The method used to synthesize Quadrol involves sequential attachment of 2-hydroxypropyl groups (from propylene oxide) to the two nitrogens of ethylenedi-

amine. Consequently, the diol and triol compounds, precursors to Quadrol, must typically be removed from the desired Quadrol product. We originally sought to develop an HPLC method capable of separating diol, triol and Quadrol in order to assess the purity of synthesized Quadrol. A reversed-phase column (C_{18}) and mobile phase consisting of methanol–acetate buffer (pH 5.5 with 0.5 m*M* octyl sodium sulfate (2:8 v/v) were used initially. Quadrol absorbs UV light poorly in the range of wavelengths useful with the mobile phase. Therefore, because of the presence of oxidizable groups on Quadrol, we chose to use electrochemical detection (ED). The first analyses generated interesting results. Commercially pure and our own synthesized Quadrol both produced a triplet of peaks eluting at 10.9, 11.7, and 13.3 min. Pure diol eluted as one peak at 4.8 min., and purified triol generated two peaks with retention times of 11.2 and 12.5 min. After eliminating the possibility of impurities (by several recrystallizations and GLC analysis), we hypothesized that diastereomers of triol and Quadrol were being resolved. Efforts were then focused upon verifying this assumption relative to Quadrol.

Quadrol has four chiral centers and thus may exist as a mixture of 8 diastereomeric pairs of enantiomers. However, because Quadrol has a center of symmetry, four of the isomers (*SSSR, RSSS, SSRS,* and *SRSS*) are actually identical. In addition, the *SRSR* and *SRRS* isomers are equivalent due to intramolecular rotations. Therefore, four diastereomeric structures (*SSRR, SSSR, SSSS,* and *SRSR* or the corresponding enantiomers) could possibly be resolved by a suitable chromatographic system. Assuming that a given mixture is not artificially enriched in any particular isomer, the diastereomers should exist in a 1:4:1:2 ratio respectively. A chromatogram from a typical HPLC analysis of pure Quadrol, with optimized mobile phase and ED, is shown in Fig. 2. Peak asignments are as follows: (1) *SSRR*, (2) *SSSR*, (3) *SSSS* and (4) *SRSR*. By averaging the areas of individual diastereomer peaks from numerous HPLC analyses of pure Quadrol samples, a ratio of 1.2:3.9:0.8:2 was obtained. This reasonably approximates the predicted ratios.

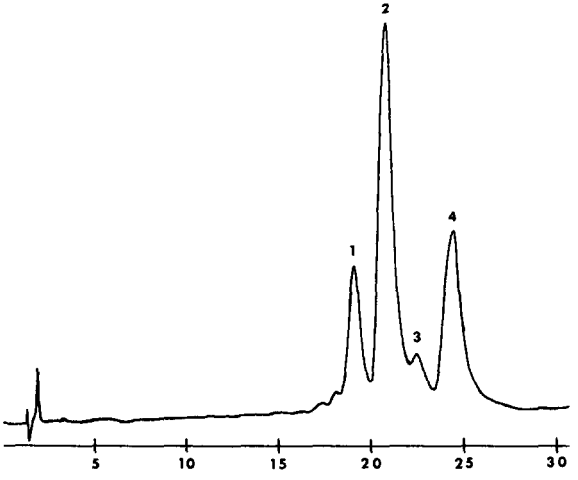

Fig. 2. HPLC chromatogram of Quadrol diastereomers separated on a 15 cm × 3.9 mm I.D. Nova-Pak® C_{18} column. The chart speed was 0.5 cm/min and the mobile phase was methanol and 0.1 *M* acetate buffer (pH 5.5) with 3m*M* octyl sodium sulfate (2:8, v/v). The mobile phase flow-rate was 1.0 ml/min.

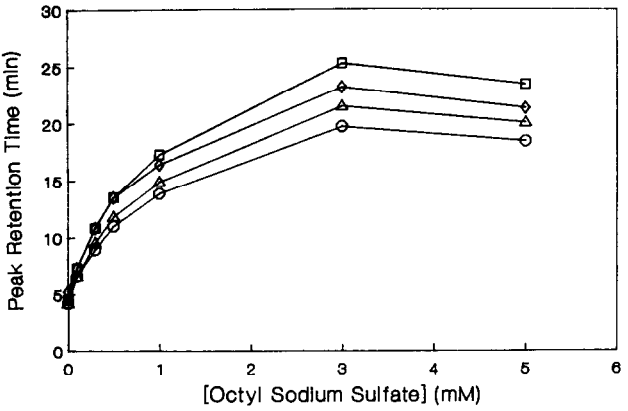

Fig. 3. Variation of Quadrol diastereomer retention times as a function of OSS concentration in the mobile phase. The Quadrol isomers are labeled as follows: (○) peak 1 (*SSRR*); (△) peak 2 (*SSSR*); (◇) peak 3 (*SSSS*); and (□) peak 4 (*SRSR*).

Because the initial mobile phase produced only free peaks for Quadrol, conditions were manipulated in order to optimize the separation of the four expected diastereomers. We investigated the effects of mobile phase pH, ion-pairing reagent, octyl sodium sulfate, concentration and methanol content on peak resolution and retention time. First, the octyl sodium sulfate concentration was varied from 0 to 5 m*M* with all other conditions as stated previously. The results, shown in Fig. 3, indicate that solute retention time increases with the concentration of octyl sodium sulfate. This was expected since Quadrol is a cation at pH 5.5 and should pair strong-

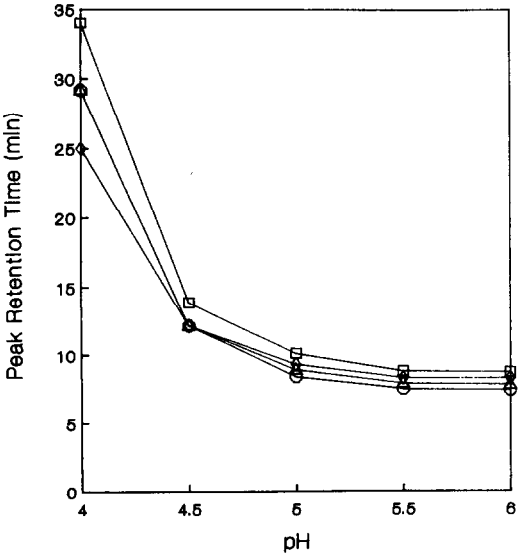

Fig. 4. Variation of Quadrol diastereomer retention times as a function of mobile phase acetate buffer pH. The Quadrol isomers are labeled as follows: (○) peak 1 (*SSRR*); (△) peak 2 (*SSSR*); (◇) peak 3 (*SSSS*); and (□) peak 4 (*SRSR*).

ly with octyl sodium sulfate. The effect on Quadrol retention time plateaus at 3 m*M* octyl sodium sulfate. The association of this type of leveling effect with ion-pair reagents has been reported[17]. Most importantly, however, a fourth peak appears in the Quadrol chromatogram when the concentration of octyl sodium sulfate is at least 1 m*M*. The best resolution of the four peaks was observed using 3 m*M* octyl sodium sulfate, and thus, this concentration was used in all subsequent analyses. The results from variation of buffer pH are shown in Fig. 4. As the mobile phase pH is increased from 4 to 6, solute retention times decrease, with peak 3 (the *SSSS* isomer) most drastically affected. The dicationic form of Quadrol, as a mixture of isomers, has two ionizable quarternary ammonium groups with pK_a values of 4.36 and 9.04 respectively[18]. The increased elution rate is probably due to diminishing Quadrol–octyl sodium sulfate interaction as the more acidic of the two ammonium groups ionizes and loses charge with the increasing pH. Apparently, intramolecular pertubations caused by individual isomer stereochemistry are affecting the pK_a values of the isomers. This might, to some extent, account for the greater sensitivity of the *SSSS* isomer to alterations in pH. The best diastereomer separation was observed at or above pH 5.5. Altering the methanol content of the mobile phase above or below 20% resulted in no particular advantage. Therefore, the mobile phase indicated in the legend of Fig. 2 was considered optimal for the separation of the diastereomers of Quadrol.

In order to verify that the peaks observed during the HPLC analysis corresponded to particular Quadrol diastereomers, preparative HPLC was employed (as indicated in the experimental section) to collect sufficient amounts of each eluting diastereomer to perform GLC and NMR analyses. Up to 20 mg of each individual eluting compound could be obtained after fractions were pooled and extracted. When any one of the four eluted compounds was re-injected onto the analytical column, the peak with the retention time corresponding to the particular diastereomer injected was observed, though minor contamination with the other diastereomers frequently occurred.

When commercial or synthesized Quadrol (as a mixture of diastereomers) is dissolved in ethanol and analyzed by isothermal GLC, one peak is observed over the temperature range from 140 to 250°C. At 230°C, the Quadrol elutes at 1.9 min, and the peak obtained is sharp and symmetrical. Each of the individual compounds obtained from the prep HPLC procedure also eluted at 1.9 min. when subjected to the same GLC analysis. Diol and triol both eluted in less than 1 min, and neither was observed in any of the purified Quadrol solutions.

The proton NMR spectrum of each diastereomer obtained from the preparative HPLC procedure matched the spectrum of pure Quadrol and thus provided additional evidence that each HPLC peak corresponds to a Quadrol structure. The ^{13}C NMR analysis of each isolated isomer displayed important chemical shift differences. The ^{13}C NMR spectrum of purified Quadrol (as a mixture of diastereomers) is shown in Fig. 5. The chemical shift regions of the spectrum are identified with letters (a,b,c,d) corresponding to the carbon atom labeling scheme in Fig. 1. When a particular isomer (1,2,3 or 4), isolated by preparative HPLC, was subjected to ^{13}C NMR analysis, a single set of absorption peaks was greatly enhanced. The peaks in each region of the spectrum are numbered (1,2,3,4) to correspond with the Quadrol diastereomers in Fig. 2.

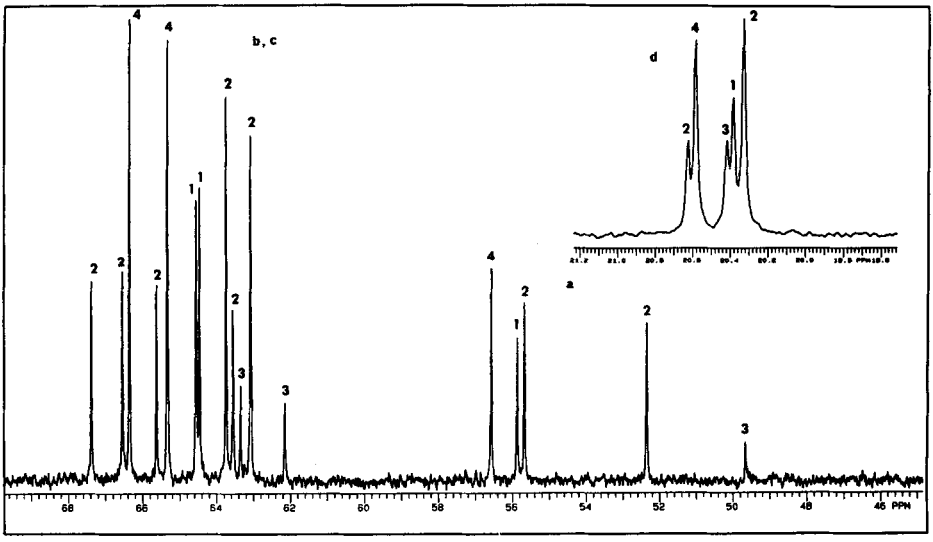

Fig. 5. ^{13}C NMR spectrum of purified Quadrol (as a mixture of diastereomers) in C^2HDCCL_3. Spectrum regions labeled a, b, c, and d correspond to the chemical shifts of the Quadrol carbon atoms as shown in Fig. 1. The individual peaks in the ^{13}C NMR spectrum are numbered to correspond to the particular Quadrol diastereomers as shown in Fig. 2.

TABLE I

CHEMICAL SHIFTS IN THE ^{13}C NMR SPECTRA OF QUADROL DIASTEREOMERS

Listed below are the 300-MHz ^{13}C NMR chemical shifts (ppm) for the synthesized diastereomers (Syn 1 = *SSSS*-Quadrol; Syn 2 = *SSSS*- and *SSSR*-Quadrol; Syn 3 = *SSSS*-, *SSSR*- and *SRSR*-Quadrol) and those obtained from preparative HPLC (peaks 1–4). The chemical shift values (δ) are listed for Quadrol carbon atoms a, and b and c as in Fig. 1.

Chemical shift	*Quadrol*	*Syn 1*	*Syn 2*	*Syn 3*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
δ_a	49.7	49.7	49.7	49.7		Trace	49.8	
(ppm)	52.4		52.4	52.4		52.5		
	55.7		55.8	55.8		55.8		
	55.9				56.1	Trace		
	56.6			56.7		Trace		56.9
$\delta_{b \text{ and } c}$	62.2	62.3	62.3	62.3		Trace	62.4	Trace
(ppm)	63.1		63.2	63.2		63.2		
	63.4	63.3	63.3	63.4		Trace	63.5	Trace
	63.6		63.6	63.6		63.7		
	63.7		63.7	63.8		63.8		
	64.5				64.6	Trace		
	64.6				64.9	Trace		
	65.3			65.4		Trace		65.5
	65.6		65.6	65.7		65.7		
	66.4			66.4		Trace		65.5
	66.5		66.6	66.6		66.6		
	67.4		67.4	67.4		67.5		

In order to provide final verification that the peaks in the HPLC chromatogram were indeed diastereomers of Quadrol, we proceeded to synthesize the isomers. The *SSSS*-Quadrol isomer was prepared and isolated as a pure substance (Syn-1), however, the other isomers were obtained in specific mixtures. Syn-2 resulted in a mixture of the *SSSS* and *SSSR* isomers, and the mixture obtained from Syn-3 included the *SSSS, SSSR* and *SRSR* Quadrol isomers. The proton NMR spectra of the *SSSS* isomer and the two mixtures matched the spectrum of Quadrol, and GLC analysis (using conditions previously described) of each produced a single peak eluting at 1.9 min. HPLC analysis of the synthesized materials generated the predicted results. The *SSSS* isomer eluted as a single peak at 22.9 min, the Syn-2 product appeared as two peaks eluting at 21.3 min (*SSSR*) and at 22.9 min (*SSSS*), and the Syn-3 product mixture produced three peaks with retention times of 21.3 min (*SSSR*), 22.9 min (*SSSS*) and 24.7 min (*SRSR*). Thus, the peak eluting at 19.1 min is apparently the *SSRR* isomer [N,N-bis([2*S*]-2-hydroxypropyl)-N′,N′-bis-([2*R*]-2-hydroxypropyl)-ethylenediamine]. The results from ^{13}C NMR analysis of these compounds, provided in Table I, correspond with those obtained by HPLC. By comparing the NMR and HPLC for Syn-1, Syn-2 and Syn-3 products, the identity of each peak in the Quadrol HPLC chromatogram (Fig. 2) was established.

This paper describes a simple HPLC procedure (analytical and preparative) for the separation of the diastereomers of Quadrol. In addition, we have provided evidence from GLC, proton NMR and ^{13}C NMR analyses that the peaks appearing in the HPLC chromatogram correspond to the four predicted Quadrol diastereomers. Recent work in our laboratory indicates that Quadrol is an immunostimulant and may be useful in some biological applications. It is possible that a particular stereoisomer may possess greater biological activity than others, and therefore this separation and isolation method can be used to provide the isomer samples needed to further our studies or any other work involving the diastereomers of Quadrol, including the development of quantitative assays for each isomer.

ACKNOWLEDGEMENT

This work was supported in part by the American Diabetes Association.

REFERENCES

1 L. Pike and J. H. Yoe, *Anal. Chim. Acta*, 31 (1964) 318.
2 J. L. Hall, F. R. Jones, C. E. Delchamps and C. W. McWilliams, *J. Am. Chem. Soc.*, 79 (1957) 3361.
3 M. Orama, H. Saarinen and L. Kaila, *Finn. Chem. Lett.*, 6 (1979) 182.
4 G. S. Begg, B. H. Leslie and F. J. Morgan, *Anal. Biochem.*, 138 (1984) 30.
5 R. B. Kennedy, R. J. Fricke and P. J. Crehan, *U.S. Pat.*, 4 515 638 (1985).
6 M. V. Bhide, S. Patel, E. C. Rowland and D. J. Smith, *J. Immunopharmacol.*, 7 (1985) 303.
7 M. V. Bhide and D. J. Smith, *Immunopharmacol. Immunotoxicol.*, 9 (1978) 129.
8 M. V. Bhide, M. J. Dunphy, N. Mirkopulos and D. J. Smith, *Immunopharmacol. Immunotoxicol.*, submitted for publication.
9 D. J. Smith and S. Patel, *J. Polym. Sci., Polym. Lett. Ed.*, 23 (1985) 633.
10 A. Van Schepdael, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.*, 370 (1986) 149.
11 S. M. Gaines and J. L. Bada, *J. Chromatogr.*, 389 (1978) 219.
12 G. W. Mihaly, K. M. Hyman, R. A. Smallwood and K. J. Hardy, *J. Chromatogr.*, 415 (1978) 177.
13 P. J. Soine and W. H. Soine, *J. Chromatogr.*, 422 (1978) 309.

14 J. Plucinski, H. Prystasz and F. Pawul, *Chem. Stosow.*, 25 (1981) 195.
15 D. B. Rorabacher, T. S. Turan, J. A. Defever and W. G. Nickels, *Inorg. Chem.*, 8 (1969) 1498.
16 D. B. Rorabacher and D. B. Moss, *Inorg. Chem.*, 9 (1970) 1314.
17 A. Bartha, H. A. H. Billiet, L. de Galan and G. Vigh, *J. Chromatogr.*, 291 (1984) 91.
18 J. L. Hall, *Proc. W. Va. Acad. Sci.*, 35 (1963) 104.