Journal of Chromatography, 391 (1987) 409–418
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 311

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TORNALATE® IN SOLUTION DOSAGE FORMS; A SPECIFICITY STUDY

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(Received November 19th, 1986)

SUMMARY

A reversed-phase high-performance liquid chromatographic method has been applied utilizing ion-pairing to measure Tornalate® in solution dosage forms. Specificity of the method was demonstrated by selectivity for Tornalate analysis, analysis of stressed samples and by peak homogeneity tests. These included the diode-array derived spectral overlay test and fraction collection with rechromatography in an alternate normal phase system. Linearity was also demonstrated in terms of recovery from synthetic samples and detector response.

INTRODUCTION

Tornalate® (bitolterol mesylate) is the di-*p*-toluate ester prodrug of the β-adrenergic N-*tert*.-butylarterenol (colterol). It is used as a bronchodilator for bronchial asthma and reversible bronchospasms. Its *Chemical Abstracts* notation is 4-{2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl}-1,2-phenylene-4-methylbenzoate (ester) methanesulfonate (salt) and it is pictured as number 4 in Fig. 1.

Bitolterol has been assayed in the past by UV spectrophotometry[1] and by phosphorimetry following thin-layer chromatography (TLC)[2]. In addition, an high-performance liquid chromatographic (HPLC) method was used to assay bitolterol in a preformulation stability study which showed the compound to be most stable at pH 3.5 in a water–ethanol (70:30) solvent mixture[3]. This method employed an ODS column with an acetonitrile–5% monosodium citrate in 5% citric acid–water (65:5:30) mobile phase.

A reversed-phase ion-pairing HPLC method has been developed to assay Tornalate in solution forms. This has supplanted a previous HPLC method which used a PAC column with a chloroform–methanol–isopropylamine (85:15:02) mobile phase for stability assay purposes. The reversed-phase method was found to be superior in terms of column stability, batch-to-batch column reproducibility, peak shape and resolution characteristics and system precision.

The present report outlines the results of a specificity study carried out in

0021-9673/87/$03.50

support of this reversed-phase method. It includes linearity of recovery, precision, detector linearity, column selectivity and peak purity data required to show the method is stability-indicating.

EXPERIMENTAL

Reagents

Water was Nanopure from a Sybron/Barnstead Nanopure II system, acetonitrile was HPLC-grade from Fisher Scientific, tetrahydrofuran was HPLC-grade from Burdick and Jackson and acetic and orthophosphoric acid were analytical-reagent grade from Mallinckrodt. Octanesulfonic acid sodium salt was from Eastman-Kodak, chloroform was HPLC-grade from J. T. Baker, methanol was analytical-reagent grade from Mallinckrodt and formic acid was analytical-reagent grade from Aldrich. Ethanol was 200 proof from U.S. Industrial Chemicals and propylene glycol was reagent grade from Fisher.

Compounds studied

The compounds utilized in this study included: bitolterol mesylate, colterol mesylate, colterol monoester mesylate, colterol triester mesylate, ketobitolterol–HCl and acetyloxybitolterol mesylate which were all from Sterling Drug. In addition 4-methylbenzoic acid (*p*-toluic acid) from Eastman-Kodak was used.

Apparatus

Several modular HPLC systems were used in this study consisting of Beckman 110A, Waters 6000A or Varian 5000 pumps; Waters 440 or 441 fixed-wavelength or Kratos SF 770 variable-wavelength UV–VIS detectors; Rheodyne 7125 manual injectors with 20-μl fixed loops and Fisher Recordall 5000 recorders. In addition, a Hewlett Packard 1040A diode array detector was used as was a Hewlett Packard 3357 laboratory automation system. Reversed-phase columns used in this study included Whatman Partisil ODS-3 10-μm, ODS-3 5-μm and IBM and Brownlee RP-18 5-μm columns. The normal phase column used was 5-μm silica gel from Alltech Assoc. All columns were 25 cm × 4.6 mm I.D. The flow-rate and detection wavelength used in all cases were 1.0 ml/min and 254 nm, respectively.

Mobile phases

A mobile phase consisting of water–acetonitrile–glacial acetic acid–sodium octanesulfonate (380:600:20:0.65, v/v/v/w) was used for the reversed-phase work. Initial variation in the water–acetonitrile ratio from 430:550 to 180:800 was examined keeping acetic acid and octanesulfonate constant with respect to column used and the capacity factor (k') obtained for bitolterol. The alternate normal phase system included tetrahydrofuran–acetonitrile–water–orthophosphoric acid (250:500:250:10) as mobile phase.

Thin-layer chromatography

A TLC system was used to provide confirmation of the stressed sample and placebo results. This consisted of 0.25-mm precoated silica gel 60 F-254 plates from Merck with a mobile phase of chloroform–methanol–formic acid (80:10:10). Follow-

ing 10-μl sample application and drying, the plates were developed to about 10 cm above the point of application, dried in warm air and viewed under both short (254 nm) and long (360 nm) wavelength UV light.

Linearity of recovery from simulated samples

Duplicate sets of simulated samples were prepared for each of the 0.083% (w/v) and 0.033% (w/v) bitolterol mesylate unit dose solutions. These contained 0%, 80%, 100% and 120% of the stated bitolterol levels in addition to the appropriate placebo and were diluted with a water–acetonitrile–acetic acid (380:600:20) dilution solvent to about 0.03 mg/ml for the 100% samples.

Detector linearity

The linearity of detector response–bitolterol mesylate concentration relationship was examined starting with an initial 0.2 mg/ml solution. This was serially diluted 1 to 10 or 1 to 5 with the above dilution solvent.

Selectivity

Solutions of bitolterol mesylate and the analogues listed in compounds studied above, potential impurities and degradation products, were chromatographed separately and together in the ion-pairing reversed-phase system.

Sample assay

Unstressed samples and samples held at 40°C for twelve months as well as a placebo solution stressed for 6 h at 70°C were chromatographed by the reversed-phase system and by the TLC system. In addition, the stressed sample was chromatographed undiluted in this LC system. The peak corresponding to bitolterol mesylate was collected and 20 μl was reinjected into the alternate normal phase HPLC system. The stressed bitolterol mesylate sample was also chromatographed using the HP 1040A detector to obtain spectral data as compared to a standard solution.

RESULTS AND DISCUSSION

The effect of mobile phase composition and reversed-phase column packings on capacity factor as well as separation of Tornalate from its monoester and triester analogues (Fig. 1 numbers 2 and 7 respectively) are shown in Fig. 2. Strongest retention in all cases was provided by the 5-μm spherical reversed-phase packings. In particular the two Brownlee and single IBM columns resulted in a k' value of 10–20 times that found for the irregular particle ODS-3 columns for colterol triester. Similarly bitolterol itself and colterol monoester were retained respectively between 3 and 9 times and between 2 and 4 times as strongly by the spherical as by the irregular bonded phases. While this powerful retention is available in these columns it is not necessarily required. In fact, a method written specifying a less retentive column could be more easily adapted to a more retentive column than the reverse. It can be noted that the differences found between irregular 5-μm and 10-μm ODS-3 columns' retention characteristics were minimal. Conversely the 10-μm ODS-3 intercolumn retention reproducibility was apparent in that the plots for these two columns are nearly coincident. It is evident that small changes in mobile phase composition have

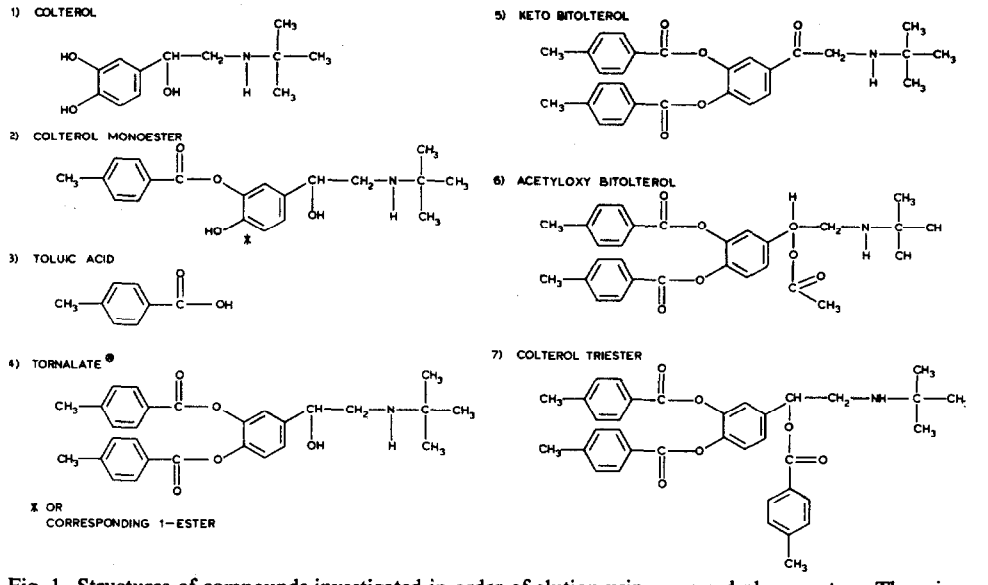

Fig. 1. Structures of compounds investigated in order of elution using reversed-phase system. These include: colterol (1), colterol monoester (2), *p*-toluic acid (3), bitolterol (4), ketobitolterol (5), acetyloxybitolterol (6) and colterol triester (7).

a larger effect on the k' of colterol triester than on either of Tornalate or colterol monoester, there being no hydroxyl groups available in the former for possible interactions with exposed silica hydroxyls.

Tornalate, as a prodrug, has the requirement that a stability-indicating assay method must separate not only potential process inpurities and degradation products

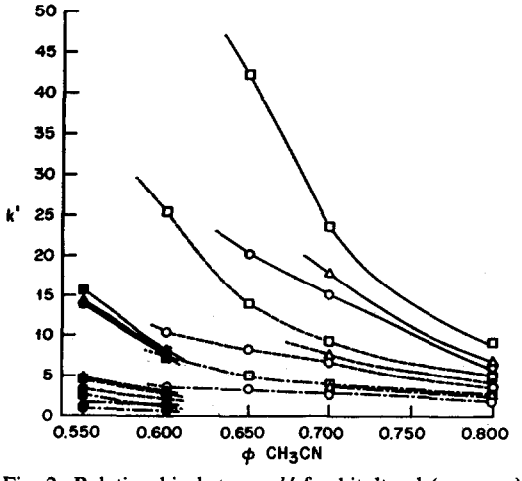

Fig. 2. Relationship between k' for bitolterol (— — —), colterol monoester (— · — · —) and colterol triester (—) and volume fraction of acetonitrile (φ) in the mobile phase showing the effect of reversed-phase column packing. 5-μm Columns, 25 cm × 4.6 mm I.D. [○ = IBM; □ = Brownlee (1); △ = Brownlee (2); ● = Partisil ODS-3]. 10-μm Columns, 25 cm × 4.0 mm I.D. [■ = Partisil ODS-3 (1); ▲ = Partisil ODS-3 (2)].

but also the intended active compound, colterol in this case. This was easily obtained with the present system owing to increased polarity of colterol over bitolterol; the former lacking the di-*p*-methylbenzoates. Overall column selectivity is shown in Fig. 3. The order of elution of bitolterol analogues corresponds to the number given compounds in Fig. 1. Good peak shape was obtained for all components on the 10-μm ODS-3 columns used. Colterol monoester, compound 2, gave two peaks in the chromatogram because of an equilibrium process that occurred in solution in which the 1- and 2-methylbenzoate esters were interconvertible.

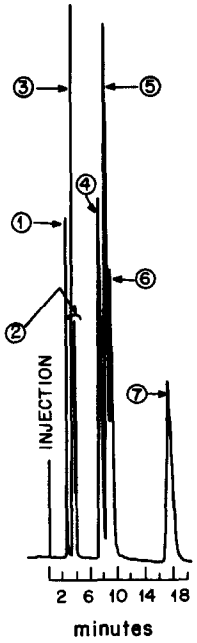

Fig. 3. Chromatogram of a mixed standard containing all compounds listed in Fig. 1 using the mobile phase water–acetonitrile–acetic acid–octanesulfonate (380:600:20:0.65, v/v/v/w). Component 4 is bitolterol.

Linearity of recovery of bitolterol from synthetic samples was obtained with results shown in Table I. Acceptable statistics were found for both the 0.033% and 0.083% (w/v) formulations with average percent recoveries of (100 ± 1)% and percent relative standard deviation (R.S.D.) recoveries of less than 1.5% by peak height or area measurement. Only slightly higher correlation coefficients were found for the 0.083% solution while the 0.033% solution gave slightly better *y*-intercepts.

An indication of system precision is given in Table II. Triplicate injections of fresh standard solutions prepared on day one to three gave the percent R.S.D. values shown, all well below 1%, by peak height or peak area measurement. In addition to a precision requirement of not more than 1.5% for replicate standards, the system suitability test consisted of a resolution factor calculation between bitolterol and colterol triester and an 80% linearity check. The minimum acceptable resolution

TABLE I

BITOLTEROL MESYLATE RECOVERY FROM SIMULATED SAMPLES

Peak height measurement						*Peak area measurement*					
0.33 mg/ml			*0.83 mg/ml*			*0.33 mg/ml*			*0.83 mg/ml*		
Added (mg)	*Found (mg)*	*Recovery (%)*	*Added (mg)*	*Found (mg)*	*Recovery (%)*	*Added (mg)*	*Found (mg)*	*Recovery (%)*	*Added (mg)*	*Found (mg)*	*Recovery (%)*
0.0	0.0	–	0.0	0.0	–	0.0	0.0	–	0.0	0.0	–
0.0	0.0	–	0.0	0.0	–	0.0	0.0	–	0.0	0.0	–
13.2	13.3	100.76	33.2	33.4	100.60	13.2	13.2	100.0	33.2	33.4	100.60
13.2	13.0	98.48	33.2	33.6	101.20	13.2	13.1	99.24	33.2	33.6	101.20
16.5	16.4	99.39	41.6	41.7	100.24	16.5	16.4	99.39	41.6	41.7	100.24
16.5	16.2	98.18	41.6	41.6	100.0	16.5	16.1	97.58	41.6	41.5	99.76
19.8	19.4	97.98	49.9	49.5	99.20	19.8	19.6	98.99	49.9	49.6	99.40
19.8	19.8	100.0	49.9	49.3	98.80	19.8	20.1	101.52	49.9	49.3	98.80
Average recovery (%)		99.13			100.01			99.45			100.00
R.S.D. recovery (%)		1.12			0.890			1.298			0.864
Slope		0.990			0.994			0.997			0.995
Intercept		0.016			0.141			−0.024			0.132
Correlation coefficient		0.99982			0.99989			0.99970			0.99990

TABLE II

SYSTEM PRECISION

	Peak height ($\mu V\ s \cdot 10^{-4}$)			*Peak area ($\mu V\ s \cdot 10^{-5}$)*		
	Day 1	*Day 2*	*Day 3*	*Day 1*	*Day 2*	*Day 3*
	7.252	7.335	7.345	8.433	8.524	8.628
	7.168	7.342	7.363	8.415	8.516	8.630
	7.183	7.332	7.394	8.479	8.486	8.645
Mean	7.201	7.337	7.367	8.442	8.509	8.634
R.S.D. (%)	0.6	0.07	0.3	0.4	0.2	0.1

TABLE III

LINEARITY OF DETECTOR RESPONSE–CONCENTRATION OF BITOLTEROL MESYLATE RELATIONSHIP

Bitolterol mesylate concentration (mg/ml)	*Peak height ($\cdot 10^{-3}$) (μV s)*	*Peak area ($\cdot 10^{-4}$) (μV s)*
0.000202	0.7	0.9
0.00202	5.2	6.5
0.00404	10.9	14.8
0.0202	51.8	64.3
0.202	476	640
Correlation coefficient	0.999968	0.999996
R.S.D. of y (%)	1.74	0.61

factor was set at 7 while the 4 in 5 diluted standard had to fall between 78 and 82% of the average standard value.

Results of the linearity of detector response–bitolterol mesylate concentration relationship are shown in Table III. Over a thousand-fold range of dilution, excellent correlation coefficients were obtained with a moderately improved %R.S.D. value found by peak area.

Duplicate HPLC analysis of stressed and unstressed bitolterol mesylate solutions gave results shown in Table IV. No degradation was seen in twelve months at 25°C while a loss of about 7% resulted from the 40°C stress for twelve months. Corroborating evidence was obtained by TLC where 6–8% estimated total inhomogeneity was determined by comparison to diluted reference standard spots. No interference resulted from stressed placebo as shown by the assay values and the blank. Chromatograms in Fig. 4D and E were unstressed and stressed placebo respectively. Fig. 4A is a bitolterol standard with a retention time of about 8 min while Fig. 4B and C represents unstressed and stressed Tornalate solutions. The stressing gave rise to additional components at about 4 and 7 min which show the method to be stability-indicating. Major degradation products have been tentatively identified based

TABLE IV

HPLC ASSAY AND ESTIMATED TOTAL INHOMOGENEITY BY TLC OF STRESSED AND UNSTRESSED BITOLTEROL MESYLATE SOLUTION AND STRESSED PLACEBO

Sample	*Conditions*	*Bitolterol mesylate assay (% claim)*	*Estimated total inhomogeneity (%)*
Solution	12 months, 25°C	100.0, 100.5	1
Solution	12 months, 40°C	92.5, 93.0	6–8
Placebo	6 h, 70°C	0.0, 0.0	–

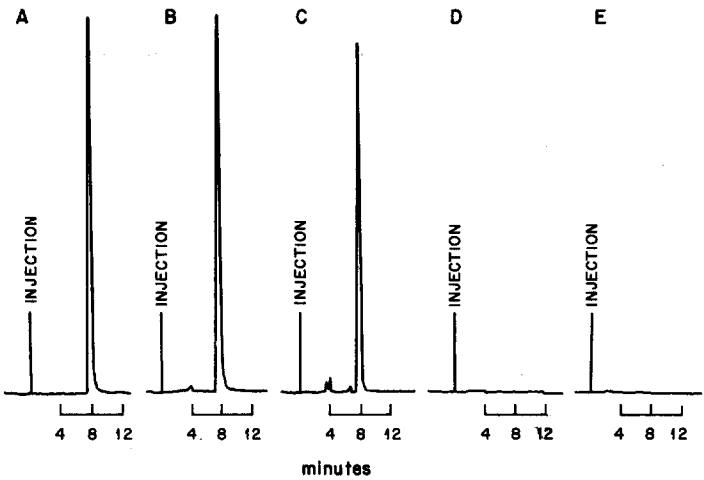

Fig. 4. Chromatograms of bitolterol mesylate reference standard (A), unstressed Tornalate solution (B), Tornalate solution stressed twelve months at 40°C (C), unstresed placebo (D) and placebo stressed 6 h at 70°C (E).

on HPLC retention time data and R_F values from TLC as *p*-toluic acid and colterol monoester. These are seen at R_F 0.62 and 0.41 respectively in Fig. 5, a drawing of the thin-layer chromatogram of standards and stressed and unstressed bitolterol solution samples and placebos.

Homogeneity of the stressed Tornalate solution bitolterol peak was indicated by chromatographing this sample using the HP 1040A diode array detector. When

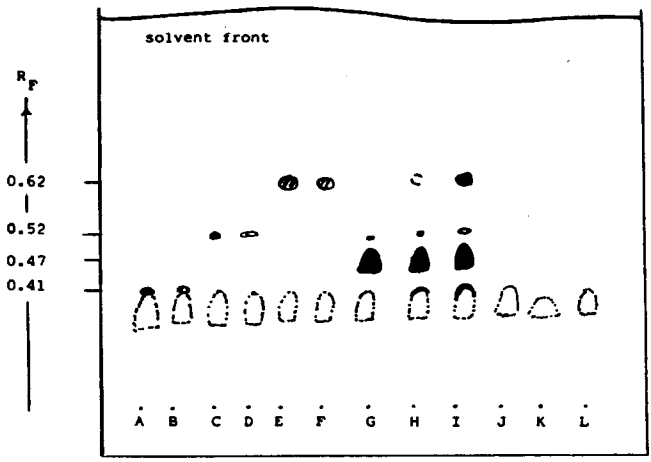

Fig. 5. Thin-layer chromatogram of standards, stressed and unstressed bitolterol samples and placebos. These are identified as: (A) colterol monoester (0.4 μg); (B) colterol monoester (0.2 μg); (C) colterol triester (0.4 μg); (D) colterol triester (0.2 μg); (E) *p*-toluic acid (0.4 μg); (F) *p*-toluic acid (0.2 μg); (G) bitolterol mesylate standard (20 μg); (H) Tornalate solution unstressed; (I) Tornalate solution stressed twelve months at 40°C; (J) unstressed placebo; (K) placebo stressed 6 h at 70°C; (L) and dilution solvent [water–propylene glycol–ethanol (50:25:25)].

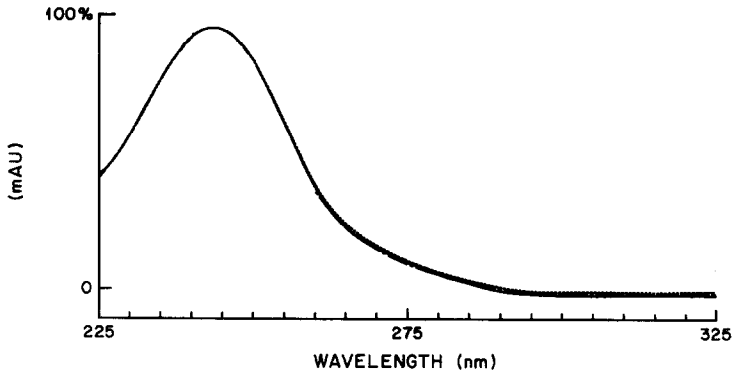

Fig. 6. UV spectral scans of the bitolterol peak in stressed Tornalate solution taken at the upslope (—), apex (----) and downslope (— — —) absorbance normalized and overlaid on a bitolterol reference standard (— · — · —) peak apex scan.

this peak was scanned at the inflection points of the upslope and downslope and at the apex from 225 to 325 nm absorbance normalized and overlaid on a bitolterol standard peak apex scan, Fig. 6 resulted. Any UV absorbing constituent coeluting with the bitolterol peak with sufficiently dissimilar spectrum, would be expected to cause a deviation from the standard spectrum. A spectral shift has been measured in this and other laboratories resulting from as little as 1–3% coeluting impurity[4,5]

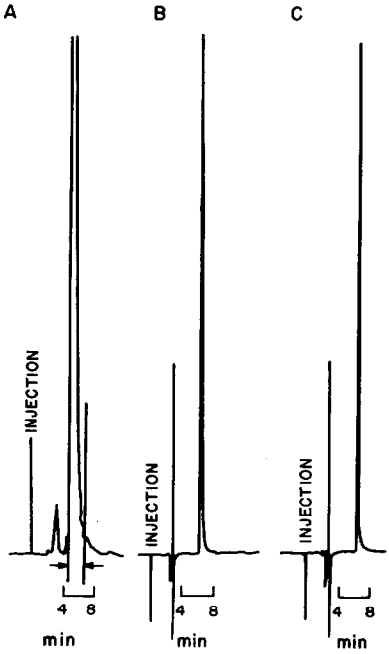

Fig. 7. Chromatogram of undiluted stressed Tornalate solution run in the reversed-phase system (A). Arrows indicate the time of fraction collection. Chromatogram of collected fraction (B) and a bitolterol mesylate standard (C) run in the alternate normal phase system.

whereas caution has been urged in the application of the spectral overlay technique especially where similar spectra are involved[6].

Further evidence for bitolterol peak purity came from the peak trapping experiment in which concentrated, stressed Tornalate solution was chromatographed and the bitolterol fraction was collected. A volume of 20 μl of this fraction shown in Fig. 7A was reinjected into the alternate normal phase system and the chromatogram in Fig. 7B was obtained. This showed only one component at a retention time of 6 min corresponding to that of a standard bitolterol peak in Fig. 7C. The normal phase system which gave capacity factors of 1.3, 1.5 and 1.7 for colterol triester, bitolterol and colterol monoester respectively, revealed by the application of a different separation mechanism that no peaks coeluted with that from bitolterol in the reversed-phase system.

The present results show that the ion-pairing reversed-phase assay method for bitolterol mesylate in Tornalate solution is stability-indicating and specific. In addition the method has the precision and accuracy required for routine analysis of stability samples. Indications of bitolterol peak purity were found using the spectral overlay method and chromatography in an alternate HPLC system. TLC of stressed samples as well, revealed no components other than those seen in the reversed-phase method.

ACKNOWLEDGEMENTS

The author wishes to thank Ms. N. L. Valcik for manuscript typing assistance. In addition, the author wishes to thank members of the Analytical Chemistry Department, especially A. V. Crain for direction and consultation, H. F. Gartelman for early work on the bitolterol TLC and HPLC systems and K. A. Shelvay for the HPLC work on alternate bitolterol formulations.

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