High-Pressure Liquid Chromatographic Determination of Chlorphenesin Carbamate and the β-Isomeric Carbamate

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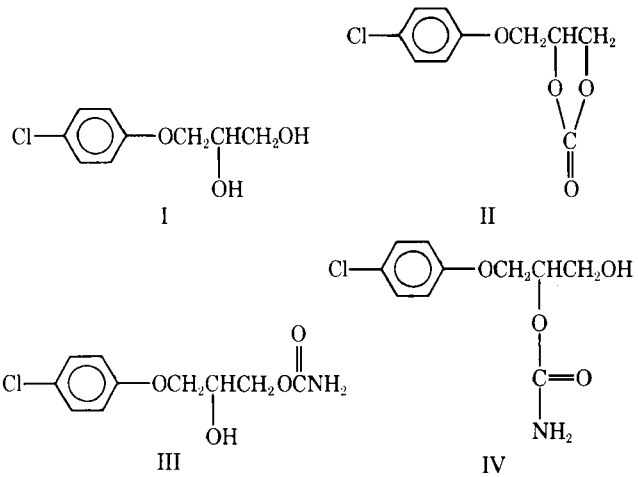
Abstract □ A high-pressure liquid chromatographic assay was developed for the determination of chlorphenesin carbamate and its β-isomeric carbamate. A single 4-mm i.d. × 30-cm column, prepacked with 10-μm fully porous silica gel particles, is used with 3% methanol in 50% water-saturated butyl chloride as the mobile phase. The procedure separates chlorphenesin carbamate from several possible impurities in addition to the β-isomeric carbamate. The assay was applied to bulk drug and compressed tablets. The relative standard deviations for the assays of chlorphenesin carbamate and the β-isomer are approximately 1 and 2%, respectively.

Keyphrases □ Chlorphenesin carbamate and β-isomer—high-pressure liquid chromatographic analysis, pharmaceutical formulations □ High-pressure liquid chromatography—analysis, chlorphenesin carbamate and β-isomer in pharmaceutical formulations □ Relaxants, skeletal muscle—chlorphenesin carbamate and β-isomer, high-pressure liquid chromatographic analysis in pharmaceutical formulations

Chlorphenesin carbamate, 3-(*p*-chlorophenoxy)-1,2-propanediol 1-carbamate (III), is commercially available in tablet form as an adjunct in short-term therapy as a skeletal muscle relaxant. The drug is produced from chlorphenesin (I) through the intermediate cyclic carbonate (II). In addition to III, the reaction can also yield the isomeric chlorphenesin 2-carbamate (IV) *in limited* quantity. Therefore, process impurities may be present in bulk chlorphenesin carbamate. Chlorphenesin also has been identified as a minor potential degradation product in the tablet formulation.

Compound III may be quantitated by spectrophotofluorometric (1), GLC (2), colorimetric (3), and IR (4) methods. Spectrophotometric (5) and GLC (6) methods are available for I. The cyclic carbonate of chlorphenesin may be quantitatively assayed using IR based on the absorption of the carbonyl at 1800 cm^{-1}. NMR spectroscopy (7) has been used in the quantitative determination of IV.



This report describes a high-pressure liquid chromatographic (HPLC) procedure for the quantitative determination of III and IV. Although I and II can be chromatographically resolved, HPLC assays for their determination were not performed due to the assay requirements.

EXPERIMENTAL

Apparatus—A commercial liquid chromatograph[1] was used at ambient temperature with UV detection at 254 nm. The column was 316 stainless steel, 4 mm i.d. × 30 cm long, prepacked with 10-μm microparticulate silica gel[2]. Chromatographic recordings were made with a standard 1-mv commercially available recorder with an integrator[3].

Reagents and Solutions—Approximately 1 part of double-distilled water is added to 3 parts of butyl chloride[4]. The mixture is shaken mechanically for approximately 3 hr and allowed to stand overnight. The upper water-saturated butyl chloride layer is removed and added to an equal volume of butyl chloride to give a 50% water-saturated butyl chloride solution. The mobile phase is 3% methanol[4] in 50% water-saturated butyl chloride.

The internal standard solution is a 0.80-mg/ml solution of 9α-fluoroprednisolone acetate[5] (V) prepared in ethanol[4]–butyl chloride (1:3). With the internal standard solution as a solvent, a 40-mg/ml reference standard[5] solution of chlorphenesin carbamate is prepared.

Sample Preparation—*Bulk Drug*—A 40-mg/ml solution of the chlorphenesin carbamate bulk drug is prepared in the internal standard solution.

Tablets—The average weight of at least 10 tablets[6] is determined, and the tablets are reduced to a fine powder. Approximately 700 mg of accurately weighed powder (equivalent to about 400 mg of chlorphenesin carbamate) is added to 10.0 ml of internal standard solution along with 10–12 6-mm glass beads. After shaking for approximately 15 min, the container is centrifuged to obtain a clear sample for injection.

Chromatography—*Bulk Drug*—Approximately 10 μl is injected; the volume should be kept constant throughout the assay. The chromatograph is operated at 1 ml/min (about 400 psig), with the UV detector sensitivity at 0.32 aufs for III and at 0.04 aufs for IV. Peak height ratios are used in the determination of III; peak heights are used in the determination of IV.

Tablets—Approximately 5 μl is injected. The chromatograph is operated at 1.8 ml/min (about 700 psig), with the UV detector sensitivity at 0.16 aufs.

Calculations—*Bulk Drug*—The amount of III is calculated by the internal standard–reference standard method, using peak height ratios. The percent of IV in the bulk drug is calculated by dividing the peak height of the isomeric carbamate (corrected for deattenuation and response factor) by the sum of the peak height of III and the corrected peak height of IV and multiplying by 100.

Tablets—The chlorphenesin carbamate content of tablets is calculated using peak height ratios and the internal standard–reference standard method. Corrections are made for the amount of powdered sample used and the average tablet weight.

[1] Model 820, du Pont, Wilmington, Del.
[2] μPorasil, Waters Associates, Milford, Mass.
[3] Electronic 19K Honeywell recorder with a Disc integrator.
[4] Burdick and Jackson, Muskegon, Mich.
[5] Control reference standards, The Upjohn Co., Kalamazoo, Mich.
[6] Maolate tablets, 400 mg of chlorphenesin carbamate, The Upjohn Co., Kalamazoo, Mich.

Table I—HPLC Linearity Data for III and IV Using 10-μm Porous Silica Gel Prepacked in a 4.0-mm i.d. × 30-cm Stainless Steel Tube[a]

Injection Number	III mg/ml	III Peak Height Ratio	III Response[b] Factor	IV mg/ml	IV Peak Height Ratio[c]	IV Response[a] Factor	Response Ratio (IV ÷ III)
			Peak Heights Used as Response Parameters				
1	2.51	0.0646	0.0257	0.1060	0.0200	0.0236	0.9183
2	4.85	0.1294	0.0267	0.3095	0.0556	0.0225	0.8427
3	9.90	0.2696	0.0272	0.6900	0.1281	0.0232	0.8529
4	20.23	0.5599	0.0277	1.2050	0.2242	0.0233	0.8412
5	39.93	1.1239	0.0281	2.5000	0.4798	0.0240	0.8541
		Average	0.0271		Average	0.0233	0.8618
		RSD	3.45%		*RSD*	2.37%	3.72%
			Peak Areas Used as Response Parameters				
1	2.51	0.1028	0.0410	0.1060	0.0335	0.0395	0.96
2	4.85	0.2072	0.0427	0.3095	0.1036	0.0418	0.98
3	9.90	0.4133	0.0417	0.6900	0.2362	0.0428	1.03
4	20.23	0.8799	0.0435	1.2050	0.4174	0.0433	1.00
5	39.93	1.7664	0.0442	2.5000	0.8890	0.0445	1.01
		Average	0.0426		Average	0.0424	1.00
		RSD	2.97%		*RSD*	4.44%	2.71%

[a]The mobile phase consisted of 3% methanol in 50% water-saturated butyl chloride. [b]Response arameters divided by milligrams per milliliter. [c]Corrected for detector sensitivity of 0.04 aufs for IV *versus* 0.32 for the internal standard.

RESULTS

A representative chromatogram of III, V (internal standard), and possible process impurities (I, II, and IV) is shown in Fig. 1. The compounds had the following relative retentions: II, 0.26; V, 0.59, I, 0.67; III, 1.0; and IV, 1.15. The number of theoretical plates for III was 4100.

Detector linearity studies for III and IV were made at five concentration levels of the compounds dissolved in the internal standard solution. Information relative to the study and calculation results based on both peak heights and peak height ratios appear in Table I. The peak area response ratio for IV:III was 0.9942, near 1.0, as would be expected for the positional isomer. When using peak heights, the ratio was 0.8618, a reflection of the later elution of IV. Correlation coefficients not significantly different from 1.0 and y-intercepts not significantly different from zero were calculated for the two compounds.

Figure 2 gives a typical chromatogram of III and IV reference standards in the internal standard solution. The peak for III was quite symmetrical for a column loading of 394 μg. The purity of III and IV reference standards was established at near 100% by differential scanning calorimetry, phase solubility, and other purity-indicating tests. A chromatogram of an impure lot of III bulk drug is shown in Fig. 3, from which 1.77% of IV was found. The relative standard deviation (*RSD*) for III obtained by replicate injections of a sample preparation of bulk drug was 0.86% when peak heights were used in calculations *versus* 2.10% for peak area ratios (Table II). Values for III were similar except for injections 1 and 6, where high and low values contributed to the high *RSD* values for peak area ratios. The content of III for this lot by replicate injection was 95.71% by peak height and 95.75% by peak area ratios. Because of better precision, ease of collecting data, and satisfactory peak symmetry, subsequent calculations were made using peak heights.

The *RSD* of the assay for IV based on repeat injections was 0.69% (Table II). The assayed value for IV was 4.43% in the bulk drug, selected for this particular study because of its unusually high IV content. The table also gives data for replicate assays for III and IV in the same bulk drug. The *RSD* for III was 1% with a purity value of 95.29%.

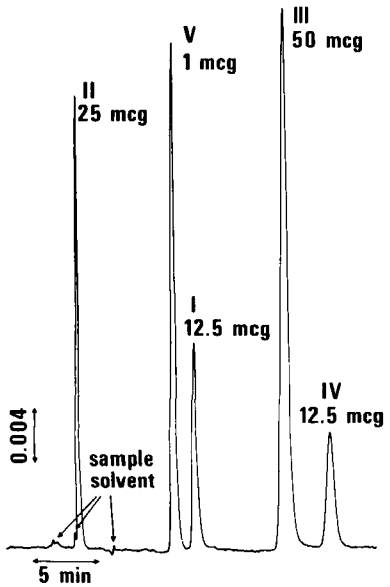


Figure 1—*Chromatogram of the cyclic carbonate of chlorphenesin (II), 9α-fluoroprednisolone acetate internal standard (V), chlorphenesin (I), chlorphenesin carbamate (III), and the β-isomeric carbamate (IV).*

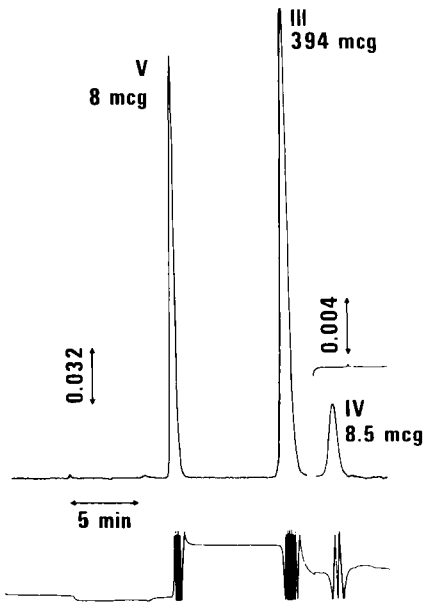


Figure 2—*Chromatogram of III, IV (2.11%), and V.*

Table II—Precision of HPLC Procedure for the Assay of III and IV in Chlorphenesin Carbamate Bulk Drug

Replicate Injections of a Single Sample Preparation for III Bulk Drug (Lot J)			
Injection	Peak Height Ratios, III, %	Peak Area Ratios, III, %	Peak Heights, IV, %
1	95.06	99.32	4.44
2	95.06	95.43	4.46
3	95.00	95.85	4.48
4	95.02	95.59	4.44
5	96.99	95.57	4.40
6	95.66	91.91	4.42
7	96.13	96.61	4.41
8	96.78	95.77	4.39
Average	95.71	95.75	4.43
RSD	0.86	2.10	0.69

Replicate Assays of Chlorphenesin Carbamate Bulk Drug (Lot J)			
Sample Weight, mg		III, %	IV, %
400.0		95.71	4.43
399.3		95.54	4.40
399.6		95.59	4.42
393.5		95.52	4.46
395.0		95.66	4.48
398.3		92.80	4.50
402.3		95.10	4.20
402.1		95.97	4.42
402.2		95.95	4.42
	Average	95.29	4.42
	RSD	1.01	1.97

For IV, the *RSD* was 1.97% and the average IV content was 4.42%. The mass balance for this lot of bulk drug was near 100% based on HPLC assays for III and IV.

Additional HPLC assays of bulk drug were conducted to verify the conditions of the assay and to ensure its manageability in a routine quality control laboratory environment. Thirty lots of the drug, manufactured over 3 years, were assayed (Table III).

HPLC conditions for the determination of III in tablets containing 400 mg of III were changed slightly from those used for the bulk drug. The injection volume was reduced from 10 to 5 μl, corresponding with a detection sensitivity setting of 0.16 aufs, and the flow rate was increased to 1.8 ml/min. These conditions increased the efficiency of the chromatographic procedure when only the assay of III was required. Representative chromatograms of sample preparations for tablet excipients and tablets are shown in Fig. 4. The excipients had no effect on the chromatogram and I and IV, if present, were below detectable limits.

The recovery of III from tablet excipients was determined by adding varying weighed amounts of III and approximately 320 mg of tablet excipients to internal standard solutions. Chromatography was then carried out on the clear extract, with an average recovery of 99.5% for the seven concentration levels of III (Table IV). The *RSD* for the determination was 0.81%, quite low considering that III was spiked at about 50–150% of the standard. Seventeen lots of tablets, varying

Table III—HPLC Assay for III and IV in Chlorphenesin Carbamate Bulk Drug

Lot	Age, months	III, %	IV, %
A	36	99.42	0.18
B	34	101.8	0.16
C	34	99.47	0.20
D	23	100.7	<0.10
E	23	99.28	<0.10
F	23	98.25	0.11
G	10	98.41	0.10
H	10	98.74	0.23
I	9	100.8	0.26
J	5	95.29	4.42[a]
K	5	100.7	0.68
L	5	99.63	0.27
M	5	102.5	0.54
N	5	100.3	0.20
O	5	102.2	<0.10
P	5	101.0	<0.10
Q	Current	98.59	1.68[a]
R	Current	100.0	0.18
S	Current	97.00	1.77[a]
T	Current	99.72	0.23
U	Current	100.7	0.20
V	Current	100.4	1.18
W	Current	99.67	0.15
X	Current	100.8	0.19
Y	Current	99.40	0.56
Z	Current	99.38	0.52
2A	Current	99.0	0.29
2B	Current	100.4	0.17
2C	Current	99.81	0.22
2D	Current	98.99	0.20

[a] Assay values confirmed by NMR.

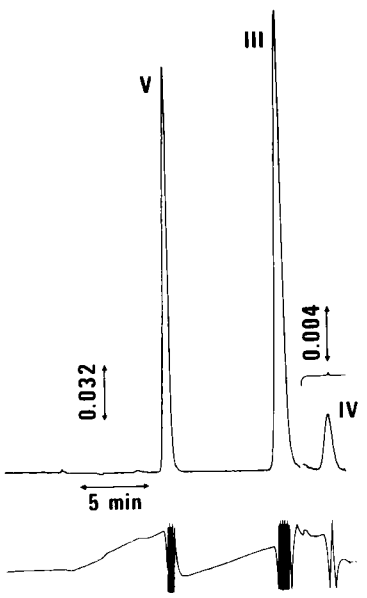


Figure 3—*Chromatogram of chlorphenesin carbamate bulk drug (Lot 5) in the internal standard solution. Compound IV assayed at 1.77%.*

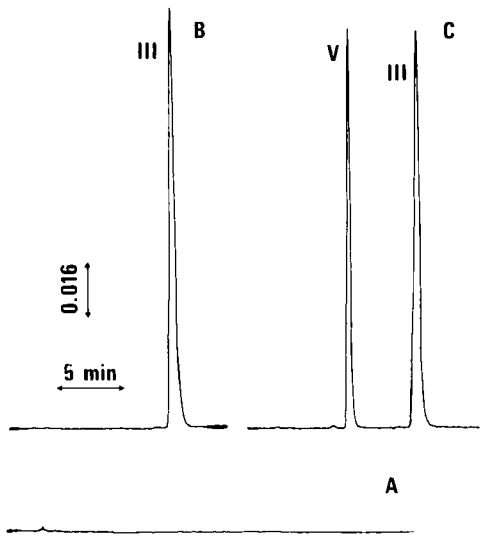


Figure 4—*Chromatograms of tablet excipients in the sample solvent (A), a sample preparation for tablets (50 months) without the internal standard (B), and a sample preparation for the same lot of tablets in the internal standard solution (C).*

Table IV—Recovery of III from Chlorphenesin Carbamate Tablet Excipients

Weight of Excipients, mg	III Added, mg	Percent of Assay Level	III Assayed, mg	Recovery, %
317.7	202.2	50.6	198.0	97.9
315.2	300.9	75.2	298.2	99.1
322.1	330.2	82.6	330.4	100.1
319.1	398.8	99.7	396.9	99.5
326.2	450.1	112.5	449.4	99.8
316.5	502.6	125.7	502.7	100.0
320.4	600.8	150.2	601.8	100.2
			Average	99.5
			RSD	0.81

in age from 7 to 62 months, were assayed by this HPLC method (Table V). Only the 62-month-old lot (Ta) gave chromatographic evidence of I, but it assayed at 99.2%.

DISCUSSION

Preliminary experiments made with HPLC to chromatograph III and possible process impurities or degradation products (I, II, and IV) using packings of 30–50-μm diameter were not considered satisfactory. Nonpolar[7] and polar[8] packings of this diameter gave less than the desired resolution for the four compounds, coupled with tailing of peaks. The properties of 10-μm fully porous silica gel[2], prepacked in a column of 4-mm i.d. dimension and modified by special drilling, were especially suited for the HPLC assay of III and IV. The application of a large amount of III to the column was required because of the relatively low degree of absorbance of the compound at 254 nm and because the determination of low levels of IV was desired.

Relatively rapid analysis was possible at low pressure requirements of the column. Reverse-flow flushing of the filter frits in the column end fittings was carried out as increased pressure was required to maintain the required flow rate. The hard surface filter paper at both ends of the column, maintained with plastic[9] washers, was replaced as needed. On occasion, small voids occurred at column ends when the end fittings were removed. Careful "troweling" with an excess of 10 μm of silica gel, wetted with a small amount of ethanol, filled the void and minimized the effect of the washer.

Water added to the mobile phase as 50% water-saturated butyl chloride provided a uniform degree of activation of the column packing for durable and uniform column performance. The addition of water also eliminated much of the peak tailing prevalent with a water-free mobile phase.

The relative insolubility of III and its possible impurities in butyl chloride required the addition of a solvent such as ethanol to the sample preparation [ethanol–butyl chloride (1:3)]. To determine IV as a minor impurity in III, a concentrated solution of III was required, *e.g.*, 40 mg/ml for a detector sensitivity of 0.32 aufs and an injection volume of 10 μl. Reasonable care was taken to keep the injection volume constant so that the ethanol added to the column from this source would contribute uniformly to the composition of the mobile phase, thereby influencing chromatography to the same degree (if at all). Controlling the injection volume was no problem with the use of a good syringe and the relatively low pressure requirements of the assay for IV (400 psig).

[7] HCP and ODS, du Pont; and C-18 reversed phase, Waters.
[8] ETH and BOP, du Pont; and Corasil, Waters.
[9] Teflon, du Pont.

Table V—HPLC Assays for III in Chlorphenesin Carbamate Tablets

Lot	Age, months	III, mg/tablet	Percent of Label
Ta	62	396.6	99.2
Tb	61	389.7	97.4
Tc	60	391.1	97.8
Td	56	395.5	98.9
Te	50	395.5	98.9
Tf	48	400.5	100.1
Tg	42	393.5	98.4
Th	42	400.2	100.0
Ti	39	398.1	99.5
Tj	39	396.5	99.1
Tk	38	388.9	97.2
Tl	27	400.8	100.2
Tm	26	392.0	98.0
Tn	26	395.3	98.8
To	9	397.4	99.4
Tp	9	394.9	98.7
Tq	7	398.7	99.7

These data for chlorphenesin carbamate bulk drug and tablets indicate that this HPLC assay is simple and rapid, with a high degree of specificity. Previous to this report, the only published procedure for the quantitation of the isomeric chlorphenesin 2-carbamate used NMR (7). The advantages of the HPLC method from standpoints of ease of operation, availability and economy of instruments, and rapidity of test results are apparent.

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