Complete Analysis of Levodopa Capsules and Tablets by GLC

B. L. CHANG, B. F. GRABOWSKI▲, and W. G. HANEY, Jr.

Abstract □ A GLC assay was developed for the determination of levodopa in both capsule and tablet preparations. The procedure also allows for the quantification of closely related amino acids that might be present, as a consequence of either the natural or synthetic production of levodopa, in these dosage forms. The method is based on the assay of the trimethylsilyl derivatives of the constituents of the dosage form, using α-methyldopa as the internal standard for the analysis of levodopa and α-methyldopate as the internal standard for the related amino acid contaminants. This procedure has been applied to the analysis of several commercial capsule and tablet preparations containing levodopa with reproducible results ranging from 97.6 to 101.8% of the labeled amount of levodopa. In addition, several closely related amino acids in these commercial preparations were identified and quantified.

Keyphrases □ Levodopa capsules and tablets—GLC–flame-ionization analysis after silylation, quantitation of companion amino acid impurities □ Amino acids as impurities in levodopa formulations—GLC analysis after silylation □ α-Methyldopa and α-methyldopate—used as internal standards in GLC analysis of levodopa formulations containing amino acid impurities □ GLC–flame-ionization detection—analysis, levodopa and amino acid impurities in levodopa capsules and tablets

Interest in the analysis (1–3) of levodopa [(−)-3-(3,4-dihydroxyphenyl)-L-alanine] has increased considerably since the initial demonstration of its effectiveness in the treatment of some patients afflicted with Parkinson's disease (4). The development of a reliable procedure for the analysis of levodopa has been complicated because of the wide range of possible contaminants that might be present in the drug as a result of the numerous methods used for its preparation (5–7). In addition, the large daily dose of the drug suggests that a patient may be exposed to a significant daily intake of these impurities even when they are present in relatively small amounts.

DISCUSSION

Since it is the immediate precursor of dopamine, levodopa occupies an important position in the biosynthesis of bioactive aromatic amines. Its metabolism, through dopamine, results in the production of 3-methoxytyramine, norepinephrine, or dihydroxyphenylacetic acid (8), and each of these products may, in turn, be further metabolized in the body. Likewise, amino acids closely related to levodopa, such as tyrosine and 3-aminotyrosine, follow qualitatively similar routes of metabolism.

The metabolism of levodopa and of closely related amino acids, therefore, gives rise to a multiplicity of products, each with its own pharmacological activity. While the metabolism of levodopa has been implicated in its beneficial biological activity (9), the metabolism of closely related amino acids in a pharmaceutical preparation containing levodopa may introduce needless therapeutic complexities into the activity of the drug preparation. Therefore, it is important to identify and quantify these amino acids in the preparation.

While these considerations may apply to the analysis of most drugs and drug products, they are particularly important in the case of levodopa because of its large daily dose (6–8 g.). As a result of this large dose, an impurity that comprises only 1% of the preparation would result in an intake of 60–80 mg./day of extraneous material. Hence, the large therapeutic dose of levodopa increases the likelihood that impurities present in the preparation will give rise to untoward effects and makes their determination critical.

Some assay procedures proposed for levodopa do not possess sufficient specificity to differentiate levodopa from products closely related to it. For example, the spectrophotometric assay designed by Nedergaard (10) does not allow for the unequivocal determination of the drug but determines the presence of tyrosine, 3-methoxytyrosine, and 3,4,6-trihydroxyphenylalanine in addition to levodopa.

A specific assay for levodopa would, however, not be satisfactory if the procedure gave information pertaining only to the quantity of levodopa in the drug preparation. The data from an assay of levodopa must allow for the identification of related amino acids and their quantification, even when they are present in relatively small quantities (0.1–1%). Unless such data are available for the drug preparation, it is impossible to make an intelligent judgment as to its therapeutic efficacy.

While a series of different assays might give a range of information, analysis time for such a scheme would, of necessity, be substantial. Hence, a single assay procedure would be desirable. The procedure should give reliable data for substances present in concentrations ranging over several orders of magnitude. Also, it should be specific in the sense that no one component can interfere with the determination of any other component, and it should be nonselective in the sense that each component in the sample should be identified and quantified.

Currently, chromatographic procedures are the only methods capable of providing the needed information; one can isolate, identify, and quantitate many components in a single rapid procedure. In particular, the use of a flame-ionization detector in conjunction with GLC offers an appealing combination of both linear range and nonspecificity of response. Hence, a procedure for the complete characterization of levodopa-containing pharmaceutical preparations was developed based on the flame-ionization detection of levodopa and related amino acids separated by GLC.

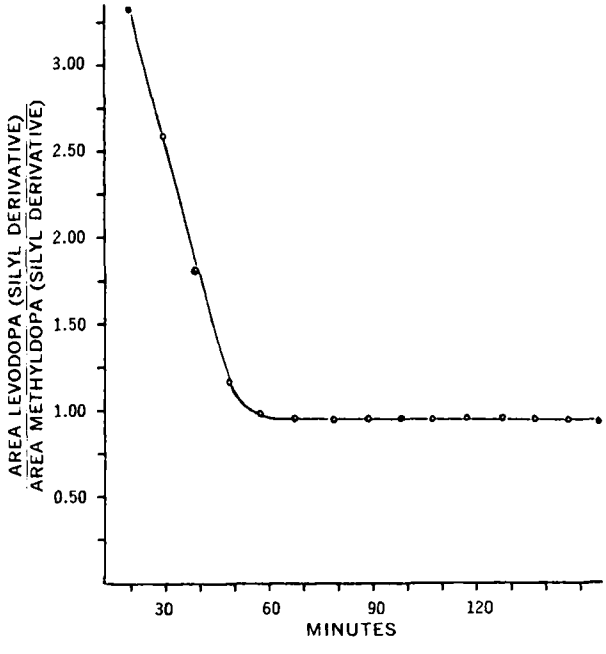

Figure 1—*Relative silylation rate of levodopa and internal standard at 80°.*

Table I—Analysis of Standard Mixtures of Levodopa and Related Amino Acids

	Tyrosine	3-Methoxytyrosine	Levodopa	3-Aminotyrosine	3,4,6-Trihydroxyphenylalanine	n
Standard mixture A:						
Calculated, %	1.60	3.40	92.30	1.50	3.20	
Found[a], %	1.54	3.31	91.74	1.51	3.22	
SD, %	4.54	3.02	1.54	5.29	2.68	9
Standard mixture B:						
Calculated, %	0.25	0.75	95.25	3.50	0.25	
Found[a], %	0.23	0.76	96.17	3.44	0.25	
SD, %	5.29	2.75	1.79	4.36	3.16	10
Standard mixture C:						
Calculated, %	3.50	0.75	92.00	0.25	3.50	
Found[a], %	3.27	0.77	90.86	0.26	3.35	
SD, %	4.89	3.22	2.33	3.47	0.017	10

[a] Mean of n determinations.

Because of their low volatility, amino acids have chromatographic characteristics unsuitable for precise quantification. Prior to analysis, therefore, it is necessary to form derivatives of the amino acids. While several procedures are available (11, 12), almost all rely on two or more reaction steps. Since the formation of trimethylsilyl derivatives is rapid and a one-step procedure, silylation was chosen for derivatization of the amino acids. In addition, the reaction process leading to these trimethylsilyl derivatives is relatively nonspecific, giving products with acids, amines, alcohols, *etc.* (13).

EXPERIMENTAL

Materials—*N,O*-Bis(trimethylsilyl)acetamide[1] was used as the silylating solvent. 3-Methoxytyrosine[2], 3,4,6-trihydroxyphenylalanine[2], levodopa[2], α-methyldopa[2], and α-methyldopate[2] were obtained. Alanine[3], phenylalanine[4], and *l*-tyrosine[4], each of which melted at literature values after recrystallization, were used. Commercial capsules and tablets containing levodopa were used in the analysis.

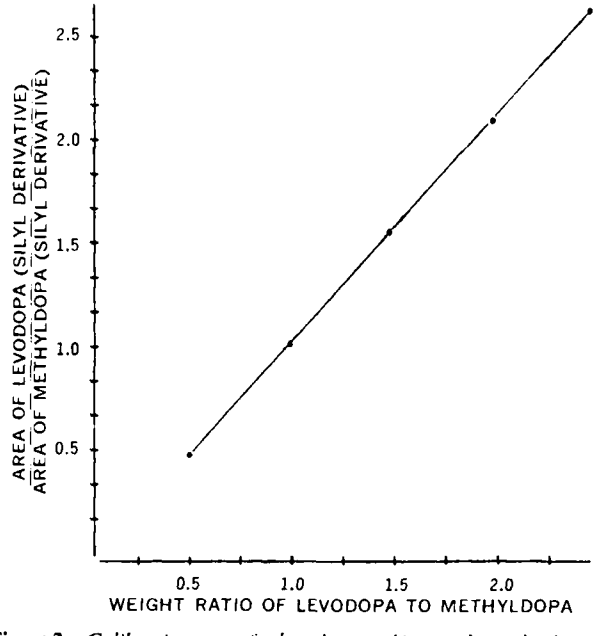

Figure 2—*Calibration curve for levodopa and internal standard.*

[1] Pierce Chemical Co., Rockford, Ill.
[2] United States Pharmacopeial Convention, Inc.
[3] Certified, Fisher Scientific Co., Fair Lawn, N. J.
[4] Standardized, Nutritional Biochemical Corp., Cleveland, Ohio.

Table II—Levodopa and Related Substances in Commercial Levodopa Capsules and Tablets

Sample Number	Dosage Form	Levodopa[a]	*SD*	Related Substances[a]	*SD*	n
I	Capsule	98.42	2.64	1.27[b]	0.04	8
II	Capsule	101.86	2.66	—	—	10
III	Capsule	97.56	1.82	1.07[c]	0.027	10
				0.57[d]	0.018	
IV	Tablet	98.24	2.37	0.27[b]	0.007	8
				0.78[e]	0.011	
V	Tablet	99.47	1.56	0.57[e]	0.022	12
VI	Tablet	98.56	1.87	1.26[c]	0.025	8
				0.54[d]	0.008	
				—[f]		

[a] Expressed as percent of labeled amount of levodopa. [b] 3-Aminotyrosine. [c] 3-Methoxytyrosine. [d] 3,4,6-Trihydroxyphenylalanine. [e] Tyrosine. [f] Phenylalanine.

Standard Solution—α-Methyldopate was used as a solution (1.0 mg./ml.) in 0.1 *N* HCl.

Apparatus—A gas chromatograph[5] with isothermal control and a flame-ionization detector was used for the analysis. The columns were 3.04 m. (10 ft.) in length, spiral-shaped, borosilicate glass tubing of 0.61 cm. (0.125 in.) inner diameter and packed with 2% methyl silicone and 6% phenyl methyl silicone on 80–100-mesh, silanized, acid-washed, flux-calcined diatomite[6]. Prior to use, the column was conditioned at 250° for 30 hr. The injector port (fitted with a glass injector sleeve), column, and detector temperatures were 225, 198, and 245°, respectively. The gas flow rates were: hydrogen, 40 ml./min.; compressed air, 226 ml./min.; and nitrogen, as the carrier gas, 40 ml./min. Column peak areas were determined using a fully automatic electronic digital integrator with printout[7].

Determination of Reaction Time and Product Stability—Ten milligrams each of 3-methoxytyrosine, levodopa, 3-aminotyrosine, 3,4,6-trihydroxyphenylalanine, and the internal standards α-methyldopa and α-methyldopate was placed in cone-shaped vials[1] fitted with Teflon septums. A 0.6-ml. sample of the silylating agent, *N,O*-bis(trimethylsilyl)acetamide, and 0.6 ml. of the silylating solvent, dry pyridine, were added to each vial. The vials were maintained in a water bath at 80°, and aliquots were taken for analysis from each vial at 10-min. intervals. After 3 hr., heating was discontinued, and the vials were protected from light and moisture. Aliquots for analysis were then taken at 4-hr. intervals for 24 hr.

Preparation of Calibration Curves—*Levodopa*—α-Methyldopa (20.0 mg.) was placed in each of six separate cone-shaped vials fitted with Teflon septums. A weight of levodopa calculated to give weight ratios of levodopa to reference standard over the range 0–2.5 at 0.5 increments was then added to the respective vials. Dry pyridine (0.6 ml.) and the silylating agent (0.6 ml.) were added to each vial, which was then heated in a water bath at 80° for 1 hr. The reaction mixture was then cooled to room temperature, and 1.0 μl. of the mixture was injected into the chromatographic system. The peak area ratios of the trimethylsilyl derivative of levodopa to the trimethylsilyl derivative of the α-methyldopa derivative were plotted *versus* the weight of levodopa, and the line of best fit was determined.

Related Amino Acids—Solutions (2.0 mg./ml. in 0.1 *N* HCl) of each amino acid included in the study were prepared. A volume of the standard solution of α-methyldopate (internal standard) equivalent to 0.2 mg. of α-methyldopate was added to each of six vials. A volume of each amino acid solution calculated to give weight ratios over the range 0–2.5 (related amino acid to internal standard) at 0.5 increments was added to the vials. Each sample was then evaporated to dryness at 60° with a stream of dry nitrogen and treated as described under *Levodopa*, beginning: "Dry pyridine . . ."

Analysis of Commercial Products—Tablets containing levodopa were sampled by selecting four tablets at random from each sample lot. These tablets were then weighed and finely powdered. In the

[5] Varian model 575.
[6] OV-1 (2%) and OV-17 (6%) on Chromosorb G, A-W, DMCS, from Varian Aerograph, Walnut Creek, Calif.
[7] Varian model 475.

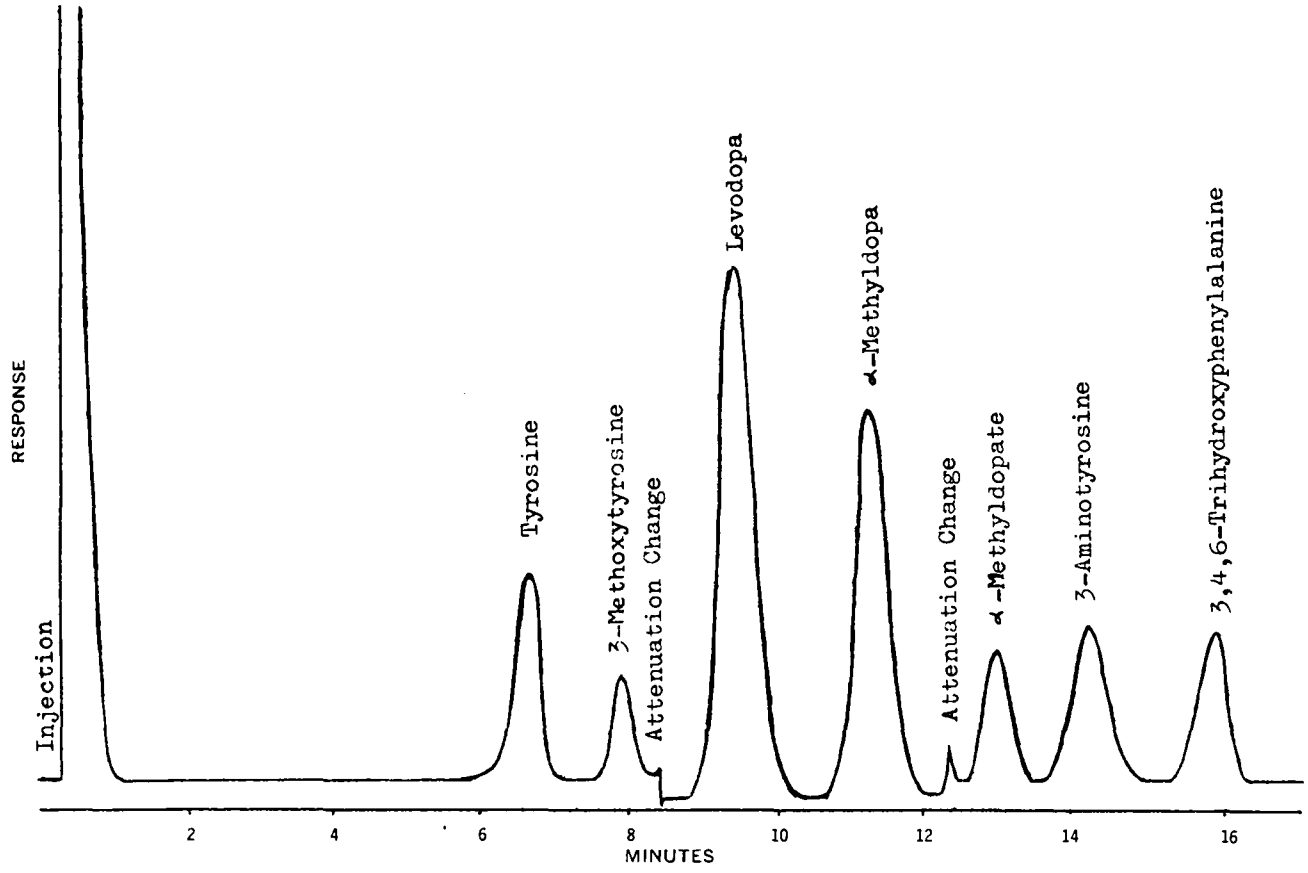

Figure 3—*Chromatographic trace of the trimethylsilyl derivatives of a synthetic mixture of levodopa, related substances, and internal standards. See text for conditions.*

case of the capsules, four capsules were weighed and emptied and their contents were weighed and then thoroughly mixed.

A volume of the α-methyldopate standard solution equivalent to 0.2 mg. of α-methyldopate was added to a vial, and the solution was evaporated to dryness at 60° with a stream of dry nitrogen. A sample of dosage form equivalent to about 25 mg. of levodopa was weighed accurately and placed in the vial along with 20.0 mg. of α-methyldopa. Dry pyridine (0.6 ml.) and the silylating agent (0.6 ml.) were added to the mixture, and the vial was capped and heated in a water bath at 80° for 1 hr. A volume of the solution (1.0–3.0 μl.) was injected into the chromatographic system, and the peak area ratio of the trimethylsilyl derivative of levodopa to the trimethylsilyl derivative of α-methyldopa was determined. The quantity of levodopa in the sample was then determined graphically from the calibration curve. Any related amino acids present in the sample were quantified, using the trimethylsilyl derivative of α-methyldopate as the internal standard. The percentage of levodopa or related amino acids in the product was calculated as the percentage of the claim of levodopa.

RESULTS AND DISCUSSION

Results of the study of the relative silylation rate of levodopa and the internal standard at 80° are illustrated in Fig. 1. From the curve, it can be seen that a stable ratio of the trimethylsilyl derivatives of levodopa to α-methyldopa is obtained after 40 min. For the related amino acids, a qualitatively similar curve showed the stable ratio of trimethylsilyl derivatives of related amino acids to α-methyldopate to occur after 30 min. However, alanine does not appear to give a response in this system. While no explanation for this anomaly is offered, a similar result was pointed out by Gehrke *et al.* (14).

Generally, trimethylsilyl derivatives are known to be sensitive to moisture (15). In these studies, however, the ratios of the trimethylsilyl derivatives of levodopa or related amino acids to their internal standards were stable for at least 24 hr. if the trimethylsilyl derivatives were protected from light and moisture. This implies that degradation of the derivatives does not occur or that it occurs at the same rate for all substances in the study.

The calibration curve for the trimethylsilyl derivatives of levodopa and α-methyldopa is shown in Fig. 2. This relationship was linear over the concentration range studied, and the slope was computed from the formula for least squares to be 1.086, with a coefficient of variation of ±1.16%. However, continued use of the chromatographic system over 2 months gave rise to a decreased slope, which was 0.31% in excess of the mean slope error. This decrease occurred despite the fact that the detector was cleaned weekly. Hence, it is necessary to prepare calibration curves periodically and to calculate the quantity of levodopa in the sample graphically.

Calibration curves for the trimethylsilyl derivatives of α-methyldopate and the related amino acids were linear and passed through the origin. One exception to this generality was *l*-tyrosine, which gave a calibration curve having negative deviation from linearity at ratios greater than 1.5. Nonlinearity might be expected in this case due to the rather large difference between the retention time of the trimethylsilyl derivatives of *l*-tyrosine and α-methyldopate. However, the precision and accuracy of the assay were not greatly affected as long as calibration curves were used.

The precision and accuracy of the assay procedure were examined by assaying prepared standard mixtures composed of levodopa and the related substances in varying ratios (Table I). Even when the ratio of levodopa to related amino acid was greater than 300:1, the method gave reliable data for both levodopa and the related amino acid. While the deviation for *l*-tyrosine was larger than that for the other substances, the deviation was still within the range required for an acceptable assay.

The selectivity of the method is exemplified in Fig. 3, which illustrates a chromatographic trace of the trimethylsilyl derivatives of levodopa, related amino acids, and the internal standards. Although levodopa and α-methyldopa are very closely related in physicochemical properties, the resolution factor for their trimethylsilyl derivatives (16) is 1.68. In addition, each peak from the trimethylsilyl derivatives of the related amino acids is relatively symmetrical and well separated. The chromatographic parameters used in the deter-

mination are necessary for the complete separation of all the components used in the study. However, once an initial determination of the constituents present in the sample has been made, the column temperature may be increased to shorten the analysis time without affecting the precision and accuracy of the results.

Table II illustrates the results of the application of this procedure to the analysis of several commercial capsule and tablet preparations. The route of preparation of levodopa (synthetic or natural) may be determined by examining the related amino acids present. TLC (17) was used to identify the amino acid phenylalanine found in Tablet VI.

Since the procedure did not employ any cleanup or extraction procedures prior to analysis, diluents and binders used to formulate the dosage forms of levodopa could offer a potential source of interference. However, since there were no extraneous peaks observed in the chromatograms of any dosage form analyzed in this study, these materials apparently do not interfere with the assay procedure.

In conclusion, a GLC determination of levodopa has been developed and is proposed as an assay for the drug contained in both capsules and tablets. Related amino acids which were present as possible contaminants were also separated, identified, and quantified. In addition, these results indicate that analysis of α-methyldopa by the same or a closely related procedure may be feasible.

This procedure was applied to several commercial tablet and capsule preparations containing levodopa, with satisfactory results.

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GLC Determination of Meclizine Hydrochloride in Tablet Formulations

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Abstract □ A method was developed for the quantitative determination of meclizine hydrochloride in tablet formulations by GLC after a suitable separation technique.

Keyphrases □ Meclizine hydrochloride tablets—GLC analysis after chloroform extraction □ GLC—analysis, meclizine hydrochloride tablets

Meclizine hydrochloride is an antihistamine which shows marked protective activity against nebulized histamine. It is indicated in the management of nausea, vomiting, and dizziness associated with motion sickness, and it has been found useful in the management of vertigo associated with diseases affecting the vestibular system. Various methods for the determination of meclizine hydrochloride have been reported, including TLC (1–4), polarography (5), NMR (6), fluorometry (7), nonaqueous titration (8), and UV spectroscopy (9–11). However, these methods do not have the rapidity, simplicity, and degree of sensitivity found in GLC methods.

Papers (12–15) published on the GLC determination of meclizine hydrochloride lack quantitative data or include chromatograms showing serious peak tailing. This paper describes a simple and direct GLC procedure for the quantitative determination of meclizine hydrochloride after extraction from a tablet formulation with chloroform.

EXPERIMENTAL

Equipment—A gas chromatograph[1], equipped with a dual flame detector and an electronic integrator[2], was used throughout this study. The column was 1.82-m. × 4-mm. (6-ft. × 0.25-in.) stainless steel tubing packed with diatomaceous earth[3], 80–100 mesh, coated with 3% OV-17. The operating temperatures were: column, 290°; de-

[1] Hewlett-Packard 5750.
[2] Hewlett-Packard 3370A.
[3] Chromosorb W, AW-DMCS, Supelco Inc., Bellefonte, Pa.