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SPECIFIC QUANTITATIVE GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF METHYLDOPA AND SOME FOREIGN RELATED AMINO ACIDS IN RAW MATERIAL AND COMMERCIAL TABLETS

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SUMMARY

A relatively simple gas–liquid chromatographic (GLC) procedure has been designed for the rapid detection and accurate quantitation of some theoretically possible foreign related amino acid contaminants in α-methyldopa raw material and commercial tablets. After trimethylsilylation of the drug or drug mixture with N,O-bis(trimethylsilyl)acetamide in acetonitrile at ambient temperature for 90 min, the derivatives are eluted from a methylsilicone column isothermally at 170°. Quantitation of the components is effected by simple computation relative to dibenzyl succinate as the internal standard. The results obtained by applying the GLC procedure to the analysis of a number of multicomponent synthetic mixtures are in good agreement with the theoretical values. The percentage of label claim values obtained by the GLC method for commercial tablets are compared to those measured colorimetrically by the official U.S.P. procedure. No foreign related amino acid impurities were detected in any of the three commercial dosage forms examined.

INTRODUCTION

Because of the various pathways of synthesis[1–4] available for the manufacture of the antihypertensive drug (—)-α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa), there are a number of intermediates or by-products which could be present as impurities in the end-products. Despite the relative complexity of the synthesis routes most by-products are easily removed during the purification process. However, it is more difficult to separate completely the parent drug from its foreign related amino acid contaminants which have closely similar solubility characteristics and chemical properties. There appear to be few procedures for the assay of α-methyldopa in the current literature. Chu[5] separated this drug from the other components in commercial mixtures using a cation-exchange resin and measured the eluted α-methyldopa spectrophotometrically at 280 nm. While this method would probably be suitable for the analysis of other α-methyldopa preparations, as would be, in principle, most of the published procedures for levodopa[6–10], they all would lack the required specificity in the presence of other phenylalanine amino acids to preclude the possi-

bility of overestimation of the active ingredient. This same constraint is clearly implicit in both the official U.S.P. XVIII[11] and B.P. 1973[12] procedures which utilize a non-aqueous titrimetric assay for the raw material and a colorimetric method for the tablet formulations. Although the thin-layer chromatographic (TLC) system described in the B.P. monograph is adequate for the separation of α-methyldopa from some of its related amino acid compounds, and is sufficiently sensitive to detect low levels (*ca.* 0.1%) of each of these as impurities, it is not entirely satisfactory for an unequivocal identification and quantitation of the particular contaminant(s) because of the closeness of some R_F values and the identical spot coloration after treatment with the spray reagent. This limitation could be a serious drawback in view of the fact that each amino acid can undergo highly specific enzymatic biotransformations which might give rise to toxic metabolites. It is well known, for example, that the D-enantiomer of levodopa, although therapeutically inactive, may induce several undesirable side-effects[13,14]. Whatever the pharmacological subtleties of each of the possible amino acid contaminants in α-methyldopa, the presence of these impurities even at relatively low levels (*ca.* 0.1%) could complicate the treatment of, and present a potential hazard to hypertensive patients on a high daily dosage regimen (up to 3 g) of the drug over a protracted period. It is therefore easy to recognize the importance of being able to detect, identify and quantitate accurately and rapidly each individual contaminant which might be present in the pharmaceutical raw material and finished dosage forms.

Two recent methods[15,16], in which the authors used very similar approaches to establish the levels of closely related amino acids and of the active ingredient in levodopa and levodopa products by gas–liquid chromatography (GLC), have been described. In the first of these publications, the authors successfully employed α-methyldopa as the internal standard after trimethylsilylation in pyridine. The presently proposed procedure for α-methyldopa and its preparations is an extension of these latter investigations of levodopa and its commercial formulations, and is suitable for the rapid detection and accurate quantification of the active drug, and of each of the following possible related amino acid contaminants down to about the 0.1% level: α-methylphenylalanine, α-methyl-3-methoxyphenylalanine, α-methyl-3-hydroxyphenylalanine, α-methyl-4-hydroxyphenylalanine, and α-methyl-3,4-dimethoxyphenylalanine. After silylation of the drug or drug mixture with N,O-bis(trimethylsilyl)-acetamide in acetonitrile at room temperature for 1.5 h, the derivatives are eluted from a methylsilicone column under isothermal conditions and the peak areas are measured by means of an electronic digital integrator. Dibenzyl succinate serves as the internal standard. TLC is used as an additional confirmative technique for the presence of amino acid impurities.

EXPERIMENTAL

Materials and solutions

DL-α-Methylphenylalanine and α-methyl-3,4-dimethoxyphenylalanine were received by courtesy of Merck, Sharp & Dohme (Dorval, Canada). The other compounds were obtained from the following commercial sources: DL-α-methyl-3-methoxyphenylalanine and DL-α-methyl-3-hydroxyphenylalanine from Chemical Dynamics (South Plainfield, N.J., U.S.A.); DL-α-methyl-4-hydroxyphenylalanine from Aldrich (Milwaukee, Wisc., U.S.A.); L-α-methyl-3,4-dihydroxyphenylalanine

(sesquihydrate) from U.S. Pharmacopoeial Convention (Rockville, Md., U.S.A.); acetonitrile ("Silylation grade") and N,O-bis(trimethylsilyl)acetamide (BSA) from Pierce (Rockford, Ill., U.S.A.).

The internal standard solution used was dibenzyl succinate ("Baker grade"; J. T. Baker, Phillipsburg, N.J., U.S.A.) in acetonitrile, accurately weighed to contain about 4 mg/ml.

Sample preparation

To prepare the simulated mixtures, appropriate amounts of each of the five foreign amino acid compounds and α-methyldopa were weighed into a 10-ml flask containing exactly 5.00 ml of internal standard solution.

In the case of the commercial products, ten tablets were selected at random, weighed, finely powdered, and then passed through a 60-mesh sieve. An amount of powder equivalent to about 40 mg of α-methyldopa (anhydrous basis) was accurately weighed into a 10-ml flask containing 5.00 ml of internal standard solution.

Each mixture of drug and internal standard was treated with BSA reagent (2.5 ml) and stood at room temperature for 90 min with occasional shaking. As a rule of thumb, about 0.6 ml of BSA are required per 10 mg of anhydrous α-methyldopa. At the end of this period, the heterogeneous globules had dissolved to give a clear homogeneous solution or, in the case of the tablets, a clear supernatant.

Gas chromatography

Five microliters of sample solution were injected into a Bendix Series 2500 gas chromatograph, equipped with a flame ionization detector and fitted with a 5% OV-101 (Pierce) on Chromosorb W-HP (100–120 mesh) (Chromatographic Specialties, Brockville, Canada) U-shaped glass column, (6 ft. × 6 mm O.D.) preconditioned at 265° for 18 h. The temperatures applied were: column, 170° (isothermal); injection port, 255°; detector, 260°; the following gas flow-rates were applied: nitrogen, 60 ml/min; hydrogen, 40 ml/min; air, 380 ml/min.

The detector signal was fed to a continuous-balance dual-pen, 1-mV recorder* with variable chart speed set at 0.20 in. (0.508 cm)/min and connected to an automatic printout electronic digital integrator** with a selected input signal range of 0–100 mV. The attenuation was maintained at a setting of 50×10^{-11} a.f.s.

RESULTS AND DISCUSSION

In the BSA–acetonitrile system, the difference in time required to effect maximum silylation of α-methyldopa compared to that for levodopa (1.5 h *vs.* 30 min at ambient temperature)*** suggests that steric hindrance of the α-methyl group must play a role in determining both the equilibrium point and the reaction rate. It has been reported[17] that, although there is less steric strain in the trimethylsilyl (TMS) group than within the *tert.*-butyl group, the former is larger and usually exerts appreciable steric effects. This factor is conceivably partially responsible for the for-

* Minneapolis-Honeywell, Elektronik 19 strip chart recorder.
** Kent, Model Chromalog 2.
*** These values were determined by the authors.

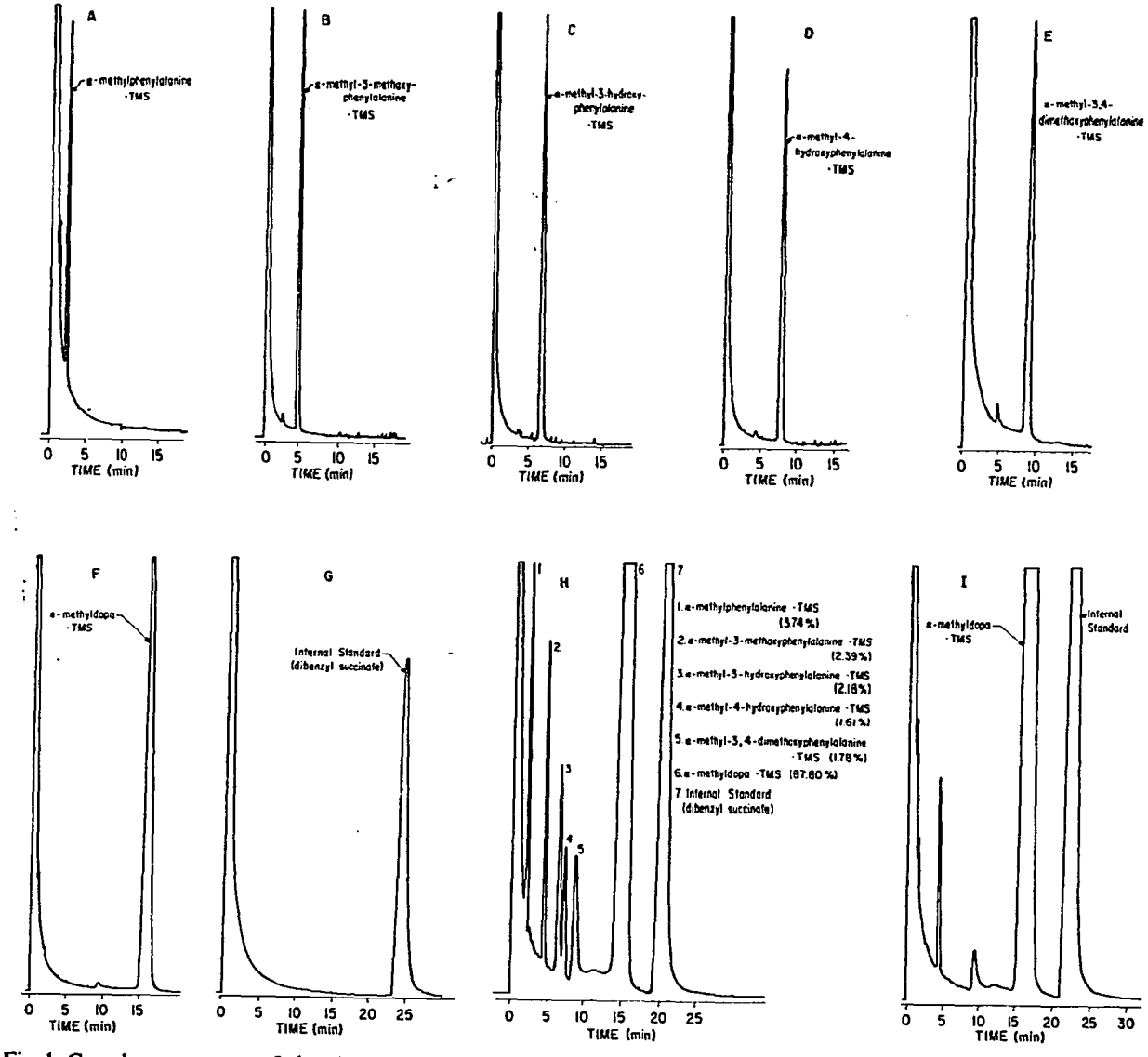

Fig. 1. Gas chromatograms of the TMS derivatives of α-methyldopa and some possible foreign related amino acids. (A) Pure DL-α-methylphenylalanine. (B) Pure DL-α-methyl-3-methoxyphenylalanine. (C) Pure DL-α-methyl-3-hydroxyphenylalanine. (D) Pure DL-α-methyl-4-hydroxyphenylalanine. (E) Pure α-methyl-3,4-dimethoxyphenylalanine. (F) Pure L-α-methyldopa (sesquihydrate). (G) Pure internal standard (dibenzyl succinate is not subject to silylation). (H) Simulated mixture of compounds A–G. (I) Commercial α-methyldopa product (125-mg tablet).

mation of a secondary product (Fig. 1F, retention time 9.06 min) which is consistently 0.5% of the major α-methyldopa·TMS peak area. Longer reaction periods, even at 75°, did not produce any change in the relative size of this secondary artifact peak. There are several compelling reasons to infer that this peak is, in fact, due to the fully O-silylated compound (with free amino group), *viz.*

(a) With the weaker silyl donors such as trimethylsilylimidazole or hexamethyldisilazane in acetonitrile under the same reaction conditions, one single sharp peak with identical relative retention time to that of the artifact peak was obtained.

(b) Upon addition of a drop of absolute ethyl alcohol to the methyldopa–BSA–acetonitrile mixture, the secondary peak became appreciably larger. Since the order of reactivity of TMS acceptor groups is known to be OH > COOH > NH_2, there seems little doubt that the hydroxyl group from the alcohol abstracts a TMS group from the hydrolytically vulnerable NH-TMS moiety resulting in partial desilylation of the persilyl compound.

Because of this high susceptibility of the NH-TMS group to moisture, it is of paramount importance that all glassware be scrupulously cleaned and thoroughly dried prior to use, otherwise one can expect significant variations in the amount of the fully O-silylated product thereby reducing the accuracy and precision of the GLC method.

Due to the closeness of retention time value with consequent overlapping between the secondary peak (retention time, 9.06 min) and that of α-methyl-3,4-dimethoxyphenylalanine (Fig. 1E, retention time 9.36 min), TLC must be viewed in some cases as an important adjunct to the GLC method in order to confirm the presence or absence of this latter amino acid as an impurity in α-methyldopa, particularly if the peak area is significantly larger than the 0.5% level.

There was no apparent difference either in reactivity or in end-silylation products between the DL and L forms of α-methyldopa so that, whatever the mechanistic implications, it can be reasonably assumed that this should be the case for other amino acids of the α-methylphenylalanine type. Several commercially available

TABLE I

GLC DATA ON α-METHYLDOPA AND POSSIBLE AMINO ACID CONTAMINANTS

Compound	*Molecular weight of fully silylated derivative*	*Retention time of derivative (min)*	*Relative* retention time of derivative*	*Relative** response factor (f) of derivative*
α-Methylphenylalanine	323.57	2.15	0.137	0.848 ± 1.2%
α-Methyl-3-methoxyphenylalanine	353.60	4.63	0.295	1.307 ± 3.5%
α-Methyl-3-hydroxyphenylalanine	411.77	6.85	0.436	1.608 ± 3.4%
α-Methyl-4-hydroxyphenylalanine	411.77	7.70	0.490	1.580 ± 3.8%
α-Methyl-3,4-dimethoxyphenylalanine	383.63	9.36	0.596	1.194 ± 5.0%
α-Methyldopa	499.95	15.70	1.000	1.745 ± 1.0%

* Relative to α-methyldopa. All amino acid compounds were dried *in vacuo* over P_2O_5 at 60° for 5 h prior to use.

** Calculated on the anhydrous basis relative to internal standard.

TABLE II

ANALYSIS OF SIMULATED MIXTURES BY GLC

Compound	*Weight (mg)*							
	1		*2*		3		*4*	
	Added	*Recovered*	*Added*	*Recovered*	*Added*	*Recovered*	*Added*	*Recovered*
α-Methylphenylalanine	0.11	0.16	0.55	0.70	1.10	1.39	2.97	3.12
α-Methyl-3-methoxyphenylalanine	0.11	0.09	0.55	0.54	1.10	1.00	3.41	3.81
α-Methyl-3-hydroxyphenylalanine	0.09	0.08	0.47	0.47	0.94	0.91	3.50	3.64
α-Methyl-4-hydroxyphenylalanine	0.10	0.07	0.50	0.45	1.01	0.97	3.37	3.27
α-Methyl-3,4-dimethoxyphenylalanine*	0.04	0.07	0.21	0.22	0.43	0.61	2.46	2.51
α-Methyldopa	33.03	32.89	33.30	33.22	20.81	21.48	35.24	34.43

* Corrected recovery values (actual area value less 0.5% of α-methyldopa peak area).

samples of α-methyldopa raw material either as the DL form or the L form (mono- or sesquihydrate) were analyzed by the GLC procedure but all were found to be impurity free.

The relatively non-polar methylsilicone OV-101 phase has already proven to be very useful for the separation of other silylated phenylalanine compounds[16] at an isothermal elution temperature of 170°. However, it should be clearly emphasized that optimum results are obtained only if the column has been well conditioned and strongly silanized in order to deactivate the highly polar silanol group sites which are demonstrably incompatible with the silylamine-silyl esters of amino acids. Under the experimental conditions, each of the silylated amino acids investigated gave one sharp peak as did the dibenzyl succinate (Fig. 1A–G), which as an inert ester is not subject to silylation or facile chemical breakdown and fulfils admirably the desirable criteria of a suitable internal standard. The relevant GLC data for α-methyldopa and some possible amino acid contaminants as their trimethylsilyl derivatives are given in Table I. The retention times are expressed both in minutes and in numerical values relative to α-methyldopa. Each relative response factor *f* given in the table represents the average of four or five determinations at varying weight ratios of compound to internal standard. The minimum weight of foreign amino acid selected would represent about an 0.3% level of impurity assuming a 40-mg sample of α-methyldopa. The precision of each quoted value is expressed in terms of its coefficient of variation which is listed beside its respective *f* value.

The results of the GLC analyses of four synthetic mixtures are given in Table II (*vide*, Fig. 1H). Each quoted experimental value represents the average of duplicate injections and is expressed as the weight in milligrams recovered. Generally, there is good agreement between amounts added and those recovered even down at the very low levels, *e.g.* 0.09 mg, which would represent an impurity level of about 0.2% assuming a 40-mg sample of α-methyldopa. Certain anomalies were observed particularly in some cases with α-methyl-3,4-dimethoxyphenylalanine. For the computation of the recoveries of this compound, the experimental peak area was corrected by subtracting a number of digital counts equivalent to 0.5% of the α-methyldopa peak area because of the previously mentioned presence of the fully O-silylated compound peak at about the same retention time.

In Table III, the percentage of label claim values obtained when the GLC technique was applied to the analysis of a commercial formulation of α-methyldopa from one manufacturer at three different dosage levels are compared to those obtained with the U.S.P. procedure. No intolerable discrepancies were noted. Fig. 1I is the chromatogram trace of an α-methyldopa film-coated tablet (125 mg). As is readily observed an unexpected sharp extraneous peak occurred at a retention time of about 4.6 min, which is virtually identical to that of α-methyl-3-methoxyphenylalanine. This same extraneous peak was seen in the 250-mg and 500-mg tablets as well. In such instances, one must necessarily take recourse to the utility of TLC[12] to confirm whether or not the artifact compound is, in fact, a related amino acid impurity. No spots other than that due to α-methyldopa were visualized on the chromatoplate, indicating that the extraneous peak was due to some excipient present in the formulation and soluble in the reaction medium.

TABLE III

GLC *vs.* U.S.P. ASSAY RESULTS FOR COMMERCIAL DOSAGE FORMS

Sample No.	*Dosage form*	*Manufacturer*	*Dosage level (mg/unit)*	*% Label claim*	
				GLC	*U.S.P.*
I	film coated tablets	A	125	99.65	101.5
II	film coated tablets	A	250	100.5	101.6
III	film coated tablets	A	500	99.47	101.9

CONCLUSIONS

The present compendial procedures for the analysis of α-methyldopa and of its commercial products are not sufficiently specific to detect any foreign related amino acids which could be associated with the parent drug and can therefore give rise to significant overestimations of the true methyldopa content. While the British Pharmacopoeia monograph incorporates a TLC check for the purity of the drug, the recommended developing system does not allow an unequivocal elucidation of the particular contaminants which might be present and the technique is, at best, only semi-quantitative. While this investigation has not uncovered any problem with regard to any such amino acid impurities in either the raw material or finished dosage forms, the presently proposed GLC procedure should prove to be a useful supplement to the official methods, particularly if a more rigorous quality control monitor should become desirable.

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