Journal of Chromatography, 549 (1991) 221–228
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 334

Stability-indicating high-performance liquid chromatographic assay for α-methyldopa in sustained-release capsules

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(First received January 9th, 1991; revised manuscript received March 19th, 1991)

ABSTRACT

A stability-indicating high-performance liquid chromatographic assay has been developed for the analysis of α-methyldopa (MD) in sustained-release capsules and in the presence of MD decomposition products and an MD industrial impurity, 3-O-methyl-methyldopa (MMD). The method utilizes reversed-phase chromatography (cyano-bonded column), an acidic mobile phase containing sodium heptanesulphonate as ion-pairing reagent and UV detection. Detector responses were linear in the ranges 0.5–200 μg/ml for MD and 0.2–100 μg/ml for MMD. The mean recoveries of MD from authentic sample and sustained-release capsules were 100.09 ± 0.38 and 100.38 ± 0.46%, respectively. The recovery of MD added to degraded MD were 99.69% by the proposed method and 153.13% by the US Pharmacopeial (USP) spectrophotometric method. The method is sensitive, accurate and rapid and can be used in routine analysis for MD.

INTRODUCTION

L-α-Methyldopa [L-3-(3,4-dihydroxyphenyl)-2-methylalanine] (MD) is a competitive inhibitor of DOPA-decarboxylase and is used in the management of hypertension [1]. Owing to the various routes used for the synthesis of MD, there are a number of by-products that might be present as impurities in the final product [2]. 3-O-Methyl-methyldopa (MMD) is one of the by-products that is difficult to separate from the parent compound owing to the similar solubility characteristics and chemical properties. In addition to the presence of impurities, MD dosage forms degrade easily under unfavourable storange conditions [3] and can undergo oxidation in alkaline media to a polymeric melanin-like pigment [4].

In spite of these inherent difficulties, there has been no reliable stability-indicating assay of MD in pharmaceutical dosage forms. Gupta and Gupta [3] used the official US Pharmacopeial (USP) method [5] to study the effect of storage of MD tablets in counting machines. MD has been determined in dosage forms by fluorimetry [6,7], ultraviolet (UV) spectrophotometry [8], spectrophotometry [9–12], proton magnetic resonance spectroscopy [13], potentiometry [14] and thin-layer chromatography [15]. These methods are not specific or they may require rigid experimental conditions such as pH adjustment and temperature control. The method described by

0021-9673/91/$03.50

Chu [16] using ion-exchange chromatography is lengthy and time consuming. The high-performance liquid chromatographic (HPLC) method described by Ting [17] was tested in our laboratory and cannot be used for the separation of MD from its degradation products. Using the ion-pair HPLC method described by Ghanekar and Das Gupta [18], one of the MD degradation products interfered seriously with MMD. A gas chromatographic method for the determination of MD in tablets and raw material was developed by Watson and Lawrence [2], but requires a lengthy derivatization step.

Several methods for the determination of MD in combination with thiazide [16,17], hydrochorothiazide [18–20] and catecholamines [21] in pharmaceutical preparations have also been reported. These methods suffer from peak tailing, multiple peaks for the same compound or incomplete separation of combined drugs [7].

This paper describes the development of an ion-pair HPLC method for the determination of MD in sustained-release capsules and in the presence of MD degradation products and the industrial impurity MMD. The proposed method is accurate, sensitive and rapid and can be easily applied for routine quality control.

EXPERIMENTAL

Chemicals

USP reference standards of MD and MMD were used. MD bulk material (checked according to the USP [22]) and MD sustained-release microcapsules were supplied by Elan Pharmaceutical Research (Gainesville, GA, USA). Methanol (HPLC grade), acetonitrile (HPLC-grade), acetic acid and sodium heptanesulphonate acid were purchased from Aldrich (Milwaukee, WI, USA). All other chemicals were of high purity and used as received.

Liquid chromatograph

A Rheodyne (Berkeley, CA, USA) Model 7125 injection system with a 20-μl loop was used. A Waters Assoc. (Milford, MA, USA) Model 590 solvent pump and a Spectroflow Model 757 variable-wavelength detector (Schoëffel Instrument, Westwood, NJ, USA) set at 280 nm were used. A Hewlett-Packard (Avondale, PA, USA) Model 3392A integrator was used for integrating the eluted peaks. A 250 mm × 4.6 mm I.D. Phenomenex (Rancho Palos Verdes, CA, USA) CN 5-μm analytical column was used.

Methanol–water (20:80, v/v) containing 2% (v/v) acetic acid and 0.005 *M* sodium 1-heptanesulphonate was used as the mobile phase. The pH of the solutions was adjusted to 2.60 ± 0.05. The mobile phase was filtered by passing it through a Millipore 0.45-μm filter and degassed before use. The mobile phase flow-rate was 1.6 ml/min. The temperature was ambient.

Determination of water content

The water content was determined in MD bulk powder and in the MD sustained-release capsules using the official USP method [22] recommended for MD. The water contents were found to be 12.3 and 13.0%, respectively.

Preparation of stock solutions

Stock solutions (0.10%) of MD and MMD were prepared in 0.1 *M* sulphuric acid.

Degradation of MD

Volumes of 5.0 ml of the MD stock solution were mixed with 5.0 ml of 0.10 or 1.0 *M* sodium hydroxide solution at 25°C. After an appropriate period, the reaction mixture was quenched by adding 5 ml of sulphuric acid of appropriate concentration (0.1 or 1 *M*). The mixture was adjusted to volume (100.0 ml) with deionized water.

Extraction of MD from sustained-release microcapsules

Beads equivalent to 500 mg of MD were ground to a fine powder and transferred to a 100-ml volumetric flask, then 50 ml of 0.05 *M* sulphuric acid were added. The flask was sonicated for 15 min and then the solution was adjusted to volume with 0.05 *M* sulphuric acid and filtered. The first 10.0 ml of the filtrate were rejected and 10 ml of the clear filtrate were diluted to 100.0 ml with 0.05 *M* sulphuric acid.

Preparation of calibration graphs

An accurately weighed 50-mg sample of USP MD or USP MMD was transferred into a 100-ml volumetric flask and 50 ml of 0.05 *M* sulphuric acid were added. The flask was sonicated for 15 min and then the solution was adjusted to volume with 0.05 *M* sulphuric acid. Serial dilutions of MD or MMD standards were made. Concentrations of MD and MMD were determined in the acidified aqueous solutions using the HPLC conditions given above, and the peak heights of the standards (calibration graphs) were recorded. The plots of the peak height *versus* concentration were linear over the range 0.5–200 μg/ml for MD with a regression coefficient of 0.999 and over the range 0.2–100 μg/ml for MMD with a regression coefficient of 0.992.

Quantification

All measurements were made using peak heights. Concentrations of sample solutions containing MD were calculated using the slope and the intercept of the calibration graph prepared under the above conditions. The slope and the intercept of the calibration graph were obtained by linear regression of peak height *vs.* concentration ($y = ax + b$), where a is the slope, b is the intercept and y is the response of the analyte.

RESULTS AND DISCUSSION

It has been reported that MD may undergo partial or complete degradation under unfavourable conditions [3]. Initial attempts to apply a C_{18} reversed-phase column with different mobile phases [17,18] failed. Ion-pair HPLC using a CN-bonded column was very useful for separating MD from its degradation products (Fig. 1), or from excepients that are present in the sustained release capsules (Fig. 2). Fig. 3 illustrates the analytical separation of a synthetic mixture of MD from MMD, together with MD decomposition products. Under the above-mentioned experimental conditions, the retention times were MD 4.5, MMD 6.03 and MD decomposition products 1.5, 1.69, 1.9, 2.28, 2.63, 2.84, 3.09 and 6.74 min. Peaks with retention times of 1.85 and 2.58 in Fig. 2 are due to excepients, namely shellac and fumaric acid, respectively.

Methanol was chosen as an organic solvent modifier where all the degradation peaks were successfully separated from MD and MMD, whereas the use of aceto-

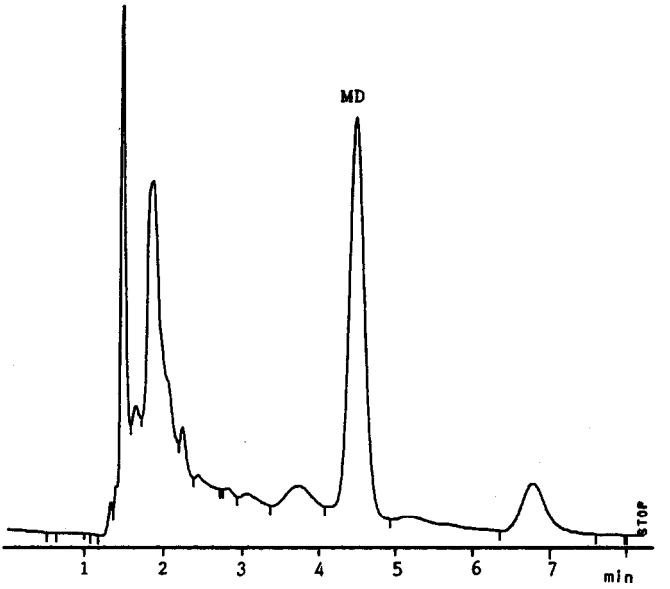

Fig. 1. Chromatogram obtained from a mixture of MD (10 μg/ml, t_R = 4.5 min) and degraded MD (100 μg/ml, 2 h degradation time in 0.1 *M* NaOH at 25°C, t_R = 1.5, 1.69, 1.9, 2.28, 2.63, 2.84, 3.09 and 6.74 min). Chromatographic conditions: column, 250 × 4.6 mm I.D. Phenomenex CN, 5 μm; mobile phase, methanol–water (20:80, v/v) containing 2% (v/v) acetic acid and 0.005 *M* sodium 1-heptanesulphonate; pH, 2.60 ± 0.05; flow-rate, 1.6 ml/min; detector wavelength, 280 nm.

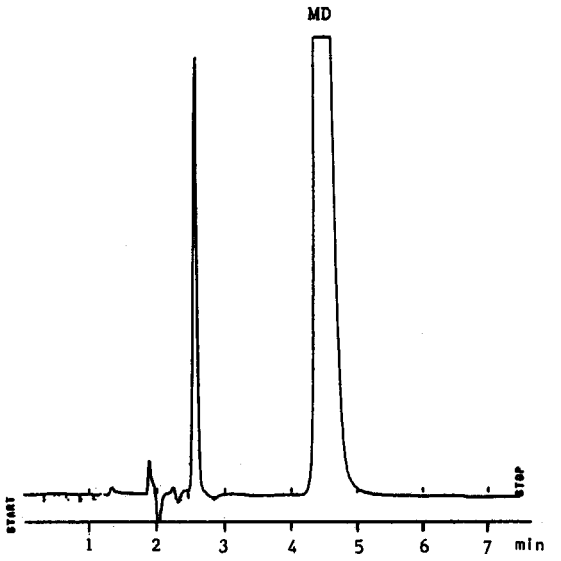

Fig. 2. Chromatogram obtained for MD sustained-release capsules (t_R = 4.5 min, 90 μg/ml). Chromatographic conditions as in Fig. 1.

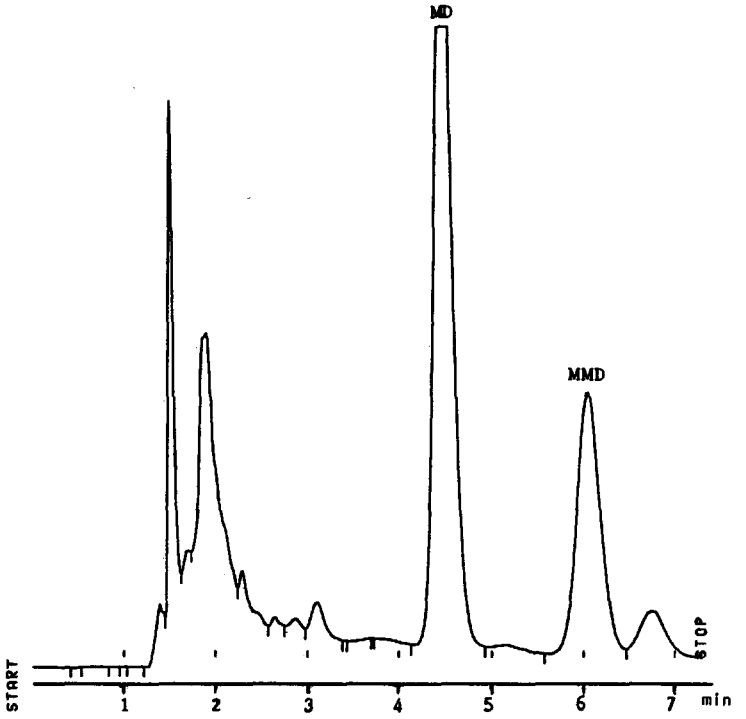

Fig. 3. Chromatogram obtained from a mixture of MD (10 μg/ml, t_R = 4.5 min), MMD (10 μg/ml, t_R = 6.03) and degraded MD (100 μg/ml, 2 h degradation time in 0.1 *M* NaOH at 25°C, t_R = 1.5, 1.69, 1.9, 2.28, 2.63, 2.84, 3.09 and 6.74 min). Chromatographic conditions as in Fig. 1.

nitrile as an organic modifier resulted in overlapping one of the peaks of degradation products of MD with that of MMD. Under identical chromatographic conditions and without using the ion-pairing reagent, separation of MD from either its degradation products or from MMD was not possible.

Conversion of MD to oxidized products [18] under alkaline conditions was followed by HPLC, and was shown to proceed via pseudo-first-order kinetics [23]. The degradation rate constant (k_{obs}) in 0.1 *M* sodium hydroxide solution at 25°C was 1.28×10^{-2} min^{-1}, with a half-life of 58.60 min. An overnight decomposition of MD gave zero recovery of MD by the proposed method and a 53.13% recovery of MD by the USP spectrophotometric method [22], an indication of the selectivity of the proposed method. These results are in good agreement with those reported by Ghanekar and Das Gupta [18].

Based on the peak-height responses of standards, the proposed method is linear in the ranges 0.5–200 μg/ml for MD and 0.2–100 μg/ml for MMD. The standard deviations based on ten injections of the standard solutions were estimated to be 0.34% for MD and 0.45% for MMD.

Accuracy was determined by recovery studies of added MD to sustained-release capsules (Table I). The average recoveries obtained were 100.38 ± 0.46% by the proposed HPLC method and 100.87 ± 0.54% by the USP spectrophotometric meth-

TABLE I

RECOVERY OF MD ADDED TO DIFFERENT BATCHES OF SUSTAINED-RELEASE CAPSULES USING THE PROPOSED HPLC METHOD AND THE USP SPECTROPHOTOMETRIC METHOD [22]

Batch[a]	Sample (μg/ml)	Claimed (μg/ml)	Added (μg/ml)	HPLC method		USP method	
				Found (μg/ml)	Recovery (%)	Found (μg/ml)	Recovery (%)
C821	13.70	8.10	13.69	21.63	98.85	21.87	100.61
	29.00	17.14	19.91	37.20	100.75	36.99	99.70
	125.50	74.17	30.43	104.29	98.97	105.32	102.35
C934	8.13	4.63	2.01	6.65	100.62	6.62	99.12
	54.23	30.87	50.10	81.92	101.90	81.92	101.90
	140.10	79.75	90.10	171.34	101.66	171.87	102.25
C1040	35.90	20.63	4.02	24.59	98.56	24.58	98.31
	75.12	43.16	40.20	84.01	101.61	84.29	102.31
	100.23	57.59	100.09	158.20	100.52	159.00	101.32
Mean					100.38		100.87
S.D.					0.46		0.54

[a] Batch C821 contained 58.710% MD, batch C934 58.75% MD and batch C1040 58.46% MD (see Table III).

od. The accuracy of the method was also tested by adding different amounts of completely degraded solution of MD to solutions containing the corersponding amount of non-degraded drug (Table II). A plot of the amount of MD added *versus* the amount recovered gave a slope of nearly unity, within experimental error (1.004), an intercept near zero (−0.037) and correlation coefficient near unity (0.999). Recov-

TABLE II

DETERMINATION OF MD IN THE PRESENCE OF ITS DEGRADATION PRODUCTS USING THE PROPOSED HPLC METHOD AND THE USP SPECTROPHOTOMETRIC METHOD [22]

MD added[a] (μg/ml)	HPLC method		USP method	
	Found	Recovery (%)[b]	Found	Recovery (%)[b]
1.500	1.467	97.800	2.243	149.500
5.000	4.990	99.800	7.635	152.700
10.000	10.010	100.100	15.250	152.500
25.010	24.860	99.400	38.340	153.300
50.210	50.612	100.800	77.775	154.900
100.030	100.260	100.230	155.917	155.870
Mean		99.688		153.128
S.D.		0.945		2.018

[a] Each solution contained an equivalent concentration of a completely degraded solution.
[b] Based on three determinations.

TABLE III

DETERMINATION OF MD IN DIFFERENT BATCHES OF SUSTAINED-RELEASE CAPSULES USING THE PROPOSED HPLC METHOD AND THE USP SPECTROPHOTOMETRIC METHOD [22]

Calculations are not based on anhydrous bases.

Batch	Sample weight (μg/ml)	HPLC method		USP method	
		Found (μg/ml)	Recovery (%)	Found (μg/ml)	Recovery (%)
C821	10.02	5.89	58.78	5.79	57.78
	40.91	24.08	58.86	23.79	58.15
	75.45	44.38	58.82	43.19	57.24
	100.31	58.47	58.29	59.29	59.11
	150.20	88.24	58.81	87.98	58.58
C934	5.98	3.45	57.69	3.54	59.20
	25.05	14.76	58.92	14.21	56.73
	40.97	23.98	58.53	23.95	58.46
	80.21	47.01	58.61	46.23	57.64
	175.23	100.76	57.50	100.21	57.19
C1040	10.29	5.90	57.34	6.00	58.31
	45.47	26.79	58.92	26.80	58.94
	50.17	29.58	58.96	29.47	58.74
	115.80	68.10	58.81	68.03	58.75
	136.40	78.50	57.55	77.20	56.60
	217.90	129.00	59.20	128.10	58.79
21B/11	20.09	11.50	57.24	11.82	58.84
	85.05	49.90	58.67	50.03	58.82
	126.60	74.10	58.53	73.42	57.99
	152.30	87.60	57.52	86.89	57.05
	205.20	117.98	57.50	119.12	58.05
Mean			58.34		58.14
S.D.			0.65		0.80

eries for the six spiked samples tested ranged from 97.80 to 100.80% (mean 99.69%, S.D. = 0.94%) These data indicate that the method is both selective and accurate. This was further confirmed by analysing the previous solutions of MD using the USP spectrophotometric method for tablets [22]. The results were overestimated and demonstrated that the USP method was not stability indicating. The observed high recovery with the USP method is due to the interference of the MD degradation products at the recommended wavelength for MD–iron(II) tartrate complex (550 nm).

Application to the determination of MD in raw samples at different concentrations covering the whole calibration graph was successfully made with a mean recovery of 100.09 ± 0.38%. The results were compared with those obtained using the USP spectrophotometric method [22] for MD tablet. Comparison with the USP non-aqueous titration method for MD raw materials was not possible owing to the high detection limit of the USP method.

In Table II, the percentage of the label claim values obtained when the pro-

posed HPLC method was applied to the determination of MD in sustained-release capsules at different dosage levels in four different batches are compared with those obtained with the USP spectrophotometric method [22]. No noticeable discrepancies were observed. No MMD was found in any analysed sustained-release batches.

The HPLC method eliminates the need to determine MD, MMD and MD degradation products by separate methods. The separation without the ion-pairing reagent was very poor. In addition, excipients (citric acid, fumaric acid, sodium lauryl sulphate, non-pariels, talc polyvinylpyrrolidone, and shellac) did not interfere in the determination of MD in the sustained release capsules. Moreover, the sensitivity of the method is such that it can be used as purity-indicating test to ascertain the presence of as little as 0.2% of MMD in MD (based on 125 mg MD per dosage form [22]), as well as a stability-indicating assay for MD.

In conclusion, the proposed method is accurate, rapid, selective and precise. It gave results for MD in sustained-release capsules which were in excellent agreement with those obtained by the compendial method. The method was superior, however, in separating MD from its degradation products. This advantage is being explored in kinetic studies on MD.

ACKNOWLEDGEMENTS

The author thanks Professor Dr. M. I. Walash, Professor Dr. M. M. Salem Rizk and Professor Dr. S. M. Hassan for their assistance during the preparation of the manuscript. The author also thanks the Quality Control Department at Elan Pharmaceutical Research (Gainesville, GA, USA) for supplying MD raw material, MMD raw material and MD sustained-release capsules.

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