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Stability-indicating method for the determination of levodopa, levodopa–carbidopa and related impurities

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Abstract

A simple and sensitive high-performance liquid chromatography method was developed for the determination of levodopa and levodopa–carbidopa formulations. In the combined formulation, the method separates these drugs from their potential impurities. A C_{18} column with a 0.05 *M* acetate buffer as mobile phase was utilized for this separation. The components in the column effluent were monitored with a coulometric detector. The method is simple, precise, stability indicating and represents an improvement over the currently available methods of analysis. The method was utilized to investigate the stability of the analyte solution. Upon standing at room temperature, the analyte solution developed several peaks which correspond primarily to the degradation of 6-hydroxydopa.

1. Introduction

Levodopa is indicated in the treatment of the prominent symptoms of Parkinson's disease. Levodopa is formulated with carbidopa to prevent its decarboxylation in the extracerebral tissues [1]. The *United State Pharmacopeia National Formulary* (USP XXII) specifies two impurities in each of the two separate bulk substances (Fig. 1). Levodopa impurities, 6-hydroxydopa and 3-methoxytyrosine are quantitated by a TLC method [2]. This method is known to suffer from lengthy analysis time which results in degradation of levodopa on the TLC plate. Furthermore, 6-hydroxydopa is extremely unstable and is not easily visualized. For levodopa–carbidopa formulations the USP XXII requires that the major components be assayed by HPLC. The USP HPLC method is not developed for concurrent testing of impurities.

The chromatographic separation and amperometric detection of the combination dosage form was first reported by Rihbany and Delaney [3]. While the sensitivity and selectivity of the detector is ideal, chromatography is not optimized. Ting [4] has reported an HPLC method with UV detection. The analysis time is shorter and has a better separation. Ting's method requires an internal standard and the detector sensitivity must be changed when going from the levodopa peak to the carbidopa peak. In this paper, we report an HPLC procedure with coulometric detection. A simple mobile phase that permits the simultaneous isocratic separa-

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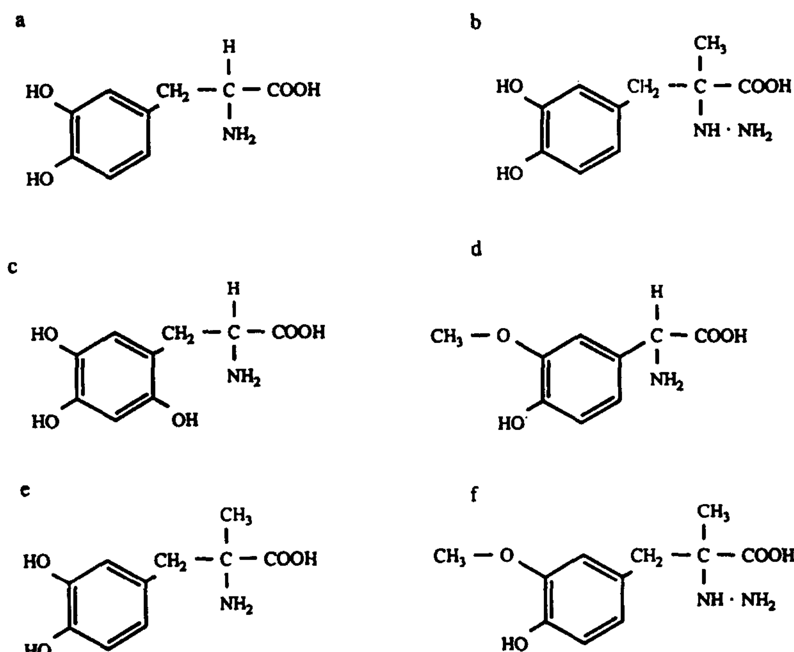

Fig. 1. Molecular structures of (a) levodopa, (b) carbidopa, (c) 6-hydroxydopa, (d) methoxytyrosine, (e) methyldopa and (f) 3-O-methylcarbidopa.

tion of carbidopa, levodopa and their potential impurities is used.

2. Experimental

2.1. HPLC apparatus

Chromatography was carried out with a Model 510 pump (Waters, Division of Millipore, Milford, MA, USA). Sample was injected via a WISP Model 710B automatic injector (Waters). Separation was achieved on a 250 × 4.6 mm I.D. μBondapak C_{18} reversed-phase column (10-μm porous support particles, Waters). The column effluent was monitored simultaneously with two detectors in series. The first detector was an ultraviolet absorption detector set at 280 nm (Model 783; Applied Biosystems, CA, USA). The second detector was a dual-electrode coulimetric detector (Model 5100A Coulochem; Environmental Sciences Assoc., Bedford, MA, USA). The detector was protected with a guard cartridge containing a 0.2-μm filter. The applied cell potential of the screen electrode was set at +0.3 V and the sample electrode +0.6 V. Technical details of this detector were first reported by Matson [5].

2.2. Chemical and reagents

Carbidopa, levodopa, methyldopa and 3-O-methylcarbidopa were USP reference standards (US Pharmacopeial Convention, Rockville, MD, USA). All other reagents were analytical-reagent grade and were purchased from Sigma (St. Louis, MO, USA).

2.3. Mobile phase preparation

The HPLC mobile phase used in the present study was 0.05 *M* ammonium acetate with 0 to 2% methanol in which the pH of acetate buffer was adjusted to 4.1 with 0.6 *M* acetic acid. A proper pH of the acetate buffer was essential to the peak resolution. The mobile phase was filtered and degassed before use.

2.4. Sample preparation

Standard and sample solutions were prepared by dissolving the appropriate amount of each compound in the mobile phase. Samples were filtered before injection into the chromatograph.

3. Results and discussion

3.1. Optimization of mobile phase

The general controlling factors for the separation of catecholamines by HPLC are well understood [6–10]. An optimum mobile phase containing sodium acetate, citric acid, sodium octyl sulfate, tetrasodium EDTA, sodium chloride and methanol was reported for the simultaneous determination of 24 neurochemicals [6]. Several mobile phases including the one proposed by Ting [4] were investigated for the present study. The mobile phase we selected for the analysis is a 0.05 *M* ammonium acetate containing up to 2% of methanol [11]. Increasing the amount of methanol in the mobile phase resulted in an overall decrease in the retention times of levodopa, carbidopa and their potential impurities. At 1 ml/min flow-rate, a composition of 1% methanol gave baseline separation for all components. For shorter analysis times, the amount of methanol in the mobile phase can be adjusted. The pH of the mobile phase was found to have a profound effect on both of the peaks. In general, the lower the pH the longer the retention time, and thus the lower the response. However, when the pH of the acetate buffer in the mobile phase was higher than 4.1 a shoulder peak next to levodopa would appear; and in addition, the 6-hydroxydopa would not be resolved from the levodopa peak. To maximize the performance of the column, therefore, a pH of 4.1 of the buffer was adopted in this study.

3.2. Optimization of the detector

The electrochemical detector used in the present study is a coulochem detector with dual electrodes in series which can be set in screen mode of operation. In this mode, the first electrode was at a potential lower than the second electrode. The coulometric efficiency of the detector thus decreased background currents and eliminated undesirable components at the first electrode while quantitating at the second electrode. In the assay development, the optimal cell potential was first explored using the pure components dissolved in mobile phase. A standard solution of the six compendial substances was chromatographed repetitively. The detector was operated in an identical manner for all injections. A series of 0.05-V step potentials was applied and the response was recorded at each potential. For each potential setting, the peak area for each component in the chromatogram was obtained. While at potential over 0.3 V all six components showed response; optimum potential was about 0.6 V. Based on these results, 0.3 and 0.6 V were then chosen for the first and second electrode, respectively.

Fig. 2 illustrates the high sensitivity of the present HPLC method. Chromatographic parameters for this separation are listed in Table 1. The peak shapes for most of the individual components of the mixture appear to be symmetrical. In spite of the use of filter element installed before the detector cell, there is no significant peak broadening.

The coulochem response for both levodopa and carbidopa was linear from 0.1 to 250 nmol. The relative standard deviation (three determinations) at the 10-nmol level for levodopa and carbidopa were 0.6 and 1.3%, respectively. For the 0.1–1 nmol range, levodopa gave a slope of 0.11 μA/nmol, and intercept of 0.05 μA and a standard error of estimate of 0.002 μA. Over the same range carbidopa exhibits a slope of 0.06 μA/nmol, an intercept of 0.02 μA and a standard error of estimate of 0.003 μA. For comparison Table 2 shows the minimum amount of each compound detected as compared to other reported methods. The current USP limits for these impurities are also listed. Fig. 3 shows a chromatogram corresponding to the determination of the six compendial compounds near the detection limit.

Excellent reproducibility was obtained for the

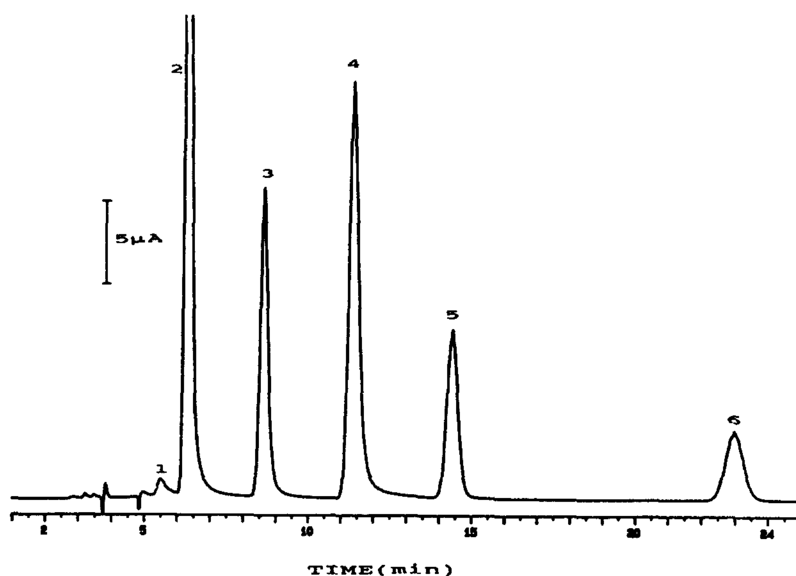

Fig. 2. Chromatogram of a mixture of 0.02 mg/ml of (1) 6-hydroxydopa, (2) levodopa, (3) methyldopa, (4) carbidopa, (5) methoxytyrosine and (6) 3-O-methylcarbidopa. Conditions: flow-rate 0.9 ml/min; applied potential, first electrode 0.3 V, second electrode 0.6 V.

Table 1
Chromatographic parameters for levodopa, carbidopa and their potential impurities

Component	Retention time (min)	Capacity factor	Tailing factor	Resolution factor
6-Hydroxydopa	5.49	0.37	1.36	2.24
Levodopa	6.35	0.59	1	5.4
Methyldopa	8.59	1.15	1.52	4.74
Carbidopa	11.41	1.85	1.22	4.25
3-Methoxytyrosine	14.31	2.58	1.02	10.42
3-O-Methylcarbidopa	22.68	4.67	0.50	

For conditions, see text. As defined in the USP XXII, under system suitability tests.

Table 2
HPLC detection limit comparison

Component	UV (280 nm) (μg/ml) (3[a])	Amperometric (ng/ml) (1[a])	Coulometric (ng/ml)	USP limit (μg/ml)
6-Hydroxydopa	1.8	20	2	2
Levodopa	1.0	–	20	–
Methyldopa	1.4	80	20	1
Carbidopa	0.2	–	2	–
3-Methoxytyrosine	100	40	40	10
3-O-Methylcarbidopa	10	60	50	1

[a] USP XXII acceptability limits for carbidopa and levodopa bulk.

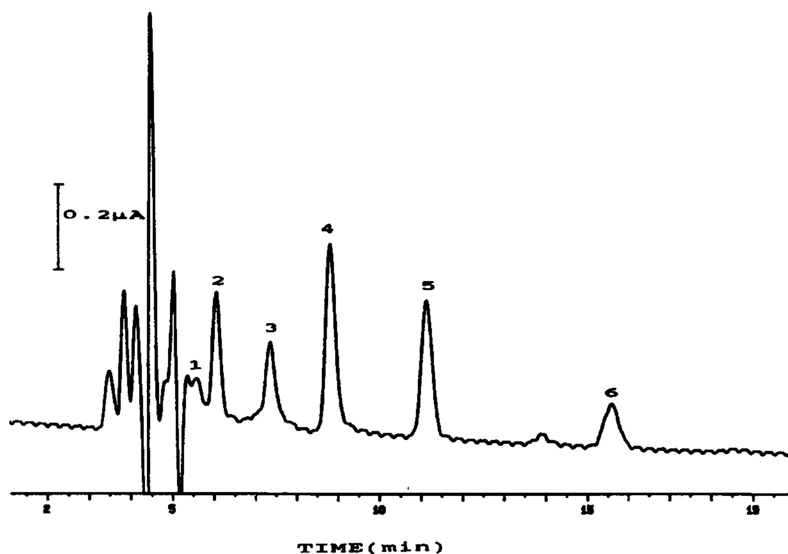

Fig. 3. Chromatogram representing the determination of the six compendial compounds near the detection limit. Peaks: 1 = 6-hydroxydopa (0.2 ng); 2 = levodopa (2.0 ng); 3 = methyldopa (2.0 ng); 4 = carbidopa (0.2 ng); 5 = methoxytyrosine (5.0 ng); 6 = 3-O-methylcarbidopa (5.0 ng). Conditions: flow-rate 1.1 ml/min; applied potential; first electrode 0.3 V, second electrode 0.6 V.

present HPLC method; relative standard deviation (R.S.D.) for six injections of carbidopa/levodopa (0.02 mg/ml) mixture was normally less than 1.0%. The intra-day precision was usually between 1.5 and 2.0%. Six replicate analyses on three different days gave inter-day precision of 2.0% for levodopa and 1.6% for carbidopa.

3.3. Stability of the analyte solution

Fig. 4 shows chromatograms a–f for a standard solution of the five compendial compounds (methyldopa was not included in this solution). Upon standing at room temperature, two major impurity peaks appeared. Peak eluting after levodopa had a retention time identical to that of methyldopa. The other peak, eluting later had a retention time different from those tested; its identity is presently under investigation. Solutions containing each individual compounds were prepared. Upon standing, 6-hydroxydopa solution developed an impurity peak corresponding to peak 4 in the standard mixture. The peak area for levodopa had fallen slightly (< 0.01%) after 8 h; however, no significant impurity was found. For carbidopa and levodopa, the R.S.D. for six injections with 1 h time interval were 0.8 and 1.2%, respectively.

The coulometric detector performed without particular incident for the duration of the study; however, the background noise was noticeable after about two months of continuous operation. When the manufacturer-suggested procedure was followed for cleaning the detector, signal-to-noise ratio was returned to its original level.

A 0.1 mg/ml USP primary standard solution corresponding to the analysis of a 100 mg levodopa and 25 mg carbidopa was monitored simultaneously with a UV and coulometric detector. With UV detection, change in sensitivity of the detector is required when going from the levodopa peak to the carbidopa peak because of the large difference in their respective concentrations. With coulometric detector the relative response for carbidopa is almost twice the response for levodopa. Fig. 5 shows the chromatographic separation of a Sinemet tablet containing 100 mg levodopa and 25 mg carbidopa. With coulometric detector, both actives are on scale and sensitivity change is not required.

In summary, a simple and sensitive HPLC

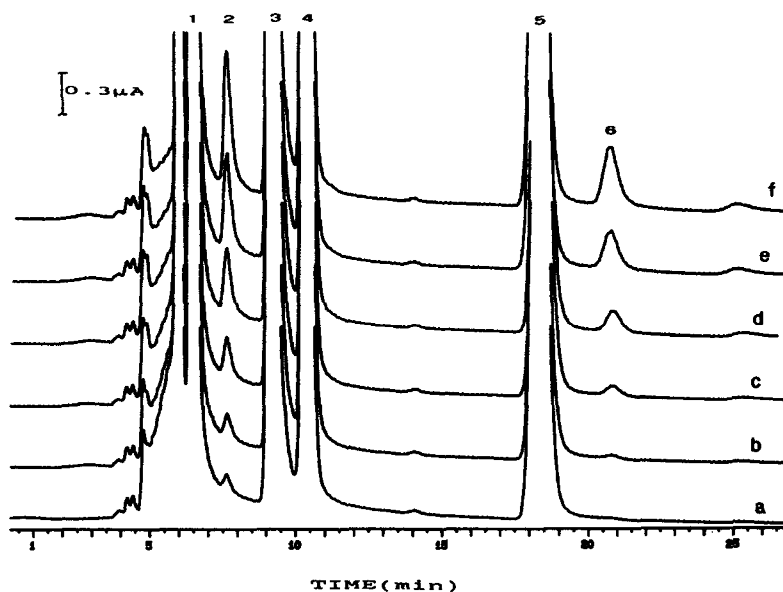

Fig. 4. Chromatograms representing the stability of the analyte solution at room temperature: Chromatograms a–f are from the same solution injected at 4 h time interval. Peaks: 1 = levodopa; 3 = carbidopa; 4 = methoxytyrosine; 5 = 3-O-methylcarbidopa. Peaks 2 and 6 were not present in the fresh standard mixture. Conditions: flow-rate 1.2 ml/min; applied potential, first electrode 0.3 V, second electrode 0.6 V.

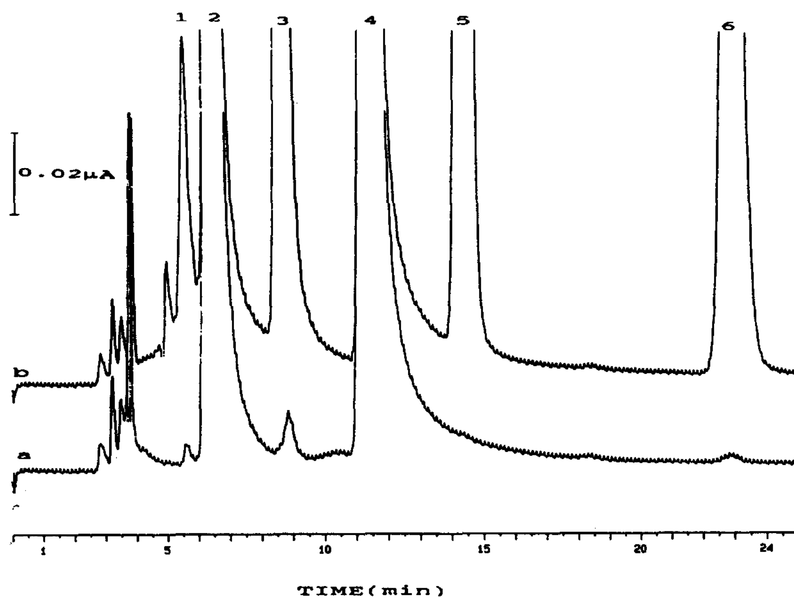

Fig. 5. Chromatograms for (a) levodopa–carbidopa tablet and (b) standard solution of (1) 6-hydroxydopa, (2) levodopa, (3) methyldopa, (4) carbidopa, (5) methoxytyrosine and (6) 3-O-methylcarbidopa. Conditions: flow-rate 0.9 ml/min; applied potential, first electrode 0.3 V, second electrode 0.6 V.

method was developed for the determination of levodopa and levodopa–carbidopa formulations. In the combined formulation, the method separates these drugs from their potential impurities and their excipients. The proposed method is stability indicating.

4. References

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