HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD
for the simultaneous assay of reserpine, clopamide, and
dihydroergocristine mesylate in tablets

RESERPINE (RP), a Rauwolfia alkaloid, has been used extensively in the treatment of hypertension and mental disorders due to its remarkable physiological properties. Its combination with clopamide (CP), a diuretic of the thiazide type that increases water and sodium excretion, increases the antihypertensive effects. Dihydroergocristine mesylate (DM), a component of co-dergocrine mesylate, inhibits the hypertensive responses to stress. A combination of RP, CP, and DM was used for the treatment of hypertension with different severity and was recommended as a favorable combination for the treatment of hypertension in the elderly and in individuals with concomitant cerebral sclerosis. The pharmaceutical combination of these three components is available in tablets containing 0.1 mg of RP, 5.0 mg of CP, and 0.5 mg of DM.

Various methods have been reported for measuring RP, CP, or DM in pharmaceutical preparations or in biological samples. However, no HPLC analytical method has been reported for the simultaneous determination of RP, CP, and DM in pharmaceutical preparations containing these three components.

This paper describes a reversed-phase HPLC method for the simultaneous quantitation of RP, CP, and DM in tablet formulations.

Experimental

Materials

HPLC-grade methanol and acetonitrile were purchased from Labscan Ltd. (Dublin, Ireland) and used without further purification. Triethylamine (TEA) was obtained from Riedel-de Haen (Hannover, Germany), and glacial acetic acid was from E. Merck (Darmstadt, Germany). 1-Heptanesulfonic acid sodium salt was purchased from the Tokyo Chemical Industry (Tokyo, Japan). Ultrapure water was drawn from a Milli-Q water purification system (Millipore Corp., Bedford, MA). CP and DM were kindly donated by Sandoz Pharma AG (Basel, Switzerland), while RP and betamethasone (used as HPLC internal standard [IS]) were purchased from U.S.P.C., Inc. (Rockville, MD).

Standard materials of CP, DM, RP, and IS for the preparation of reproducibility, linearity, and recovery studies were all used as received.

Chromatography

The LC was composed of a model 510 HPLC pump (Waters, Milford, MA), which was connected to a model 991 UV-VIS diode-array spectrophotometer (Waters) with a wavelength range of 190–800 nm, and a model 5200 printer-plotter (Waters). The HPLC system was monitored by a PowerMate SX/16 computer (NEC, Japan) with PDA software (Waters). An Inertil ODS-2 5-μm column (150 × 4.6 mm i.d.) (GL Sciences Inc., Tokyo, Japan) was used at ambient temperature. The elution was achieved isocratically (flow rate, 1.0 mL/min) with a mobile phase (1 mL/min) of aqueous solution (pH 4.4):acetonitrile:methanol (45:35:20, vol/vol/vol) containing acetic acid (2 mL/L), 1-heptanesulfonate (1 g/L), and TEA (2 mL/L). Prior to analysis, this mobile phase was degassed and filtered using a Milli-Q system and a 25 mm × 0.45 μm nylon filter unit (Sun Brokers, Inc., Wilmington, NC). The system was equilibrated for 30 min before making an injection.

Stock standard solutions

Standard materials of RP USP (obtained from the United States Pharmacopoeia), CP, DM, and IS were accurately weighed, and all stock standard solutions were prepared in methanol.

Reproducibility and linearity

Five standard solutions containing different concentrations of active ingredients were prepared from the stock standard solutions, and 20 μL of each standard solution was injected into the LC six times.
Assay methods

At least 10 tablets were weighed and an average tablet weight was determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was weighed and quantitatively transferred into a 50-mL volumetric flask. Forty milliliters of methanol and 1 mL of IS stock solution were added. The mixture was sonicated for 15 min in an ultrasonic bath, then diluted to volume with methanol, mixed, and filtered. The sample solutions (20 μL) were injected into the LC.

Recovery study

Percent recovery of active components was performed by adding the known levels of RP, CP, and DM in an amount corresponding to 50, 75, 100, 125, and 150% of labeled claim to accurately weighed portions of pulverized placebo mixtures, and assaying the spiked mixtures using the procedure similar to that used in the assay methods.

Calculation

An in-house developed computer program coded in BASIC language was used for the calculations of reproducibility and linearity, and the quantitation of model mixtures was prepared in the recovery study and commercial samples. The slope and the intercept of the related equations were calculated by least-squares regression analysis.15

Results and discussion

Initial work was directed toward the development of a simple, rapid, and accurate HPLC procedure for the routine determination of CP, DM, and RP in combination tablets. To facilitate the method development activities, such as mobile phase selection, an HPLC connected to a photodiode array detector was used. The effect of 1-heptanesulfonate and TEA in mobile phase on the relative retention and separation of RP, CP, DM, and IS betamethasone was also investigated for the selection of mobile phase.

Figure 1 shows a typical chromatogram obtained from a standard solution for a system on a reversed-phase ODS-2 column using the selected mobile phase. It can be seen that all of the peaks in the chromatogram were well resolved and baseline separation was achieved. The retention times for the peaks corresponding to CP, IS, DM, and RP were 3.0, 4.5, 5.3, and 7.7 min, respectively. The percent relative standard deviation (% RSD) calculated from the ratio of each peak area versus IS peak area for each component was found to be not more than 1.0%.

Straight lines (Figures 2–4) were obtained by plotting the peak area ratios of CP, DM, and RP to IS against the respective weight ratios of these drugs. The correlation coefficients for the three components were as follows: CP, 0.9998; DM, 0.9997; and RP, 0.9998, which demonstrated that the method is linear.

The method precision was tested on nine different lots of commercial samples, including eight lots of tablets from a domestic manufacturer and one lot from a foreign manufacturer. No interference from excipients was observed in the sample chromatograms. The peaks were identified by spectral analysis, comparison of retention times with those of a standard mixture, and spiking the sample with standard components. As shown in Table 1, the results obtained are in good agreement with the label claim amount.

The calculated percent recoveries for the different components from the placebo mixtures for the recovery study in this study are: CP, 97.4%; DM, 100.8%, and RP, 97.8%.

Conclusions

The HPLC procedure described provides an easy and accurate technique for the simultaneous separation and quantitation of CP, DM, and RP in marketed tablets. This method can be used routinely in the QC laboratory.
References


