DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(1), 57-66 (1987)

SIMULTANEOUS DETERMINATION OF PROBENECID AND COLCHICINE IN SOLID DOSAGE FORM BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Whe-Yong Lo* and George M. Krause

Research and Development Department

Copley Pharmaceutical Inc.,

Boston, MA 02127

ABSTRACT

A specific and precise high-performance liquid chromatographic method is described for the simultaneous analysis of probenecid and colchicine in tablet dosage form containing 500 mg probenecid and 0.5 mg colchicine. The method involved a reversed-phase chromatography using a mobile phase of 55:45, methanol-phosphate buffer (pH 3.0), delivered at 1.2 ml/minute. The detector range was 244 nm.

INTRODUCTION

Probenecid and colchicine are antigout agents. The commercial dosage form contain 500 mg probenecid and 0.5 mg colchicine are

* Author to whom corresponding should be addressed.

available in tablet formulations.The compendial methods to assay probenecid and colchicine[1] require two separate extractions followed by direct uv measurement which is not specific for the drugs with the risk of interference from formulation excipients, drug impurities or decomposition products.

Numerous chromatographic techniques have been reported for determination of probenecid and colchicine in biological fluids and dosage form[2-7]. However, none of the reports cover the assay of both ingredients in a simple determination. The purpose of the present work was to develop a HPLC method for routine assurance as well as stability study for solid dosage form containing 500 mg probenecid and 0.5 mg colchicine. The method involved one sample preparation followed by a simple chromatography. External standards were used for the assay determinations.

EXPERIMENTAL

Apparatus:

The HPLC was equipped with a pump system[1], a variable wavelength uv detector[2] at 244 nm, an automatic injector[3] with 10 microliter loop, and a chart recorder[4].

---

1. Series 3B Liquid Chromatography, Perkin-Elmer, Norwalk, CT.
2. LC-75 Spectrophotometric Detector, Perkin-Elmer, Norwalk, CT.
3. 725 Autoinjector, Micronmeritics.
4. Sigma 10B Chromatography Data Station, Perkin=Elmer, Norwalk, CT.

Reagents and Chemicals:

Probenecid[5] and colchicine[6] were USP grade quality. Phosphoric acid, sodium hydroxide, and potassium phosphate monobasic were reagent grade. Methanol was HPLC grade. Water was deionized and filtered through a 0.2 micron filter[7] prior to use for chromatography. Probenecid and Colchicine Tablets (500 mg/0.5 mg), USP, were supplied by manufacturer[8].

Chromatographic Conditions:

Phosphate buffer, 0.07 M, pH 3.0, was prepared by dissolving 5 ml of phosphoric acid and 2.7 g of sodium hydroxide in 950 ml of deionized water and the volume was brought to 1.0 liter with deionized water after adjusting pH to 3.0 $\pm$ 0.1 with 0.1 N sodium hydroxide or diluted phosphoric acid. The mobile phase was prepared by mixing 550 ml methanol with 450 ml of 0.07 M phosphate buffer, pH 3.0, and was filtered and vacuum degassed before use. The column[9] was equilibrated at ambient temperature with mobile phase delivered at 1.2 ml/min.

---

5. Plantex-Teva-Assia, Tel Aviv, Israel.
6. American Roland Corporation, New York, NY.
7. Nylon-66 Filters, Rainin Instrument Co. Inc., Woburn, MA.
8. Copley Pharmaceutical Inc., Boston, MA 02127.
9. C-18, HS-5, 12.4 cm long, 4.6 mm dia., Perkin-Elmer, Norwalk, CT.

The chromatograms were monitored at 244 nm with detector range of 0.02 aufs for colchicine and 5.12 aufs for probenecid.

Standard Solutions:

A colchicine stock solution was prepared by dissolving 26.9 mg colchicine USP in 250.0 ml deionized water. Standard solutions containing colchicine and probenecid were prepared by pipetting 0.24 ml, 0.5 ml, 1.0 ml, 1.5 ml, and 2.0 ml of colchicine stock solution into five separate 100-ml volumetric flasks containing 25.9 mg, 54 mg, 101.6 mg, 148.4 mg, and 199.3 mg probenecid USP. The volumetric flasks were then filled to volume with extraction medium of 45% methanol in 0.025 M phosphate buffer, pH 7.5[10], and stirred mechanically for one hour. Each mililiter of the standard solutions contained the following quantity of probenecid and colchicine respectively: 0.259 mg/0.258 ug, 0.54 mg/0.538 ug, 1.016 mg/1.076 ug, 1.484 mg/1.614 ug, and 1.993 mg/2.152 ug.

Preparation of Assay Sample Solution from Solid Dosage Form:

Tablets were finely powdered. Portion of the powder equivalent to 250 mg probenecid and 0.25 mg colchicine was weighed and placed in a 250-ml volumetric flask and the flask was filled with extraction medium, 45% methanol in 0.025 M phosphate buffer, pH 7.5, and was stirred mechanically for

10. To prepare, dissolve 3.4 g monobasic potassium phosphate and 190 ml 0.1 N sodium hydroxide in 1.0 liter of water. Adjust pH to 7.5 with 0.1N sodium Hydroxide.

one hour. The sample solution was then filtered through a 0.45 micron filter[11]. The assay sample solution contained approximately 1 mg/ml probenecid and 1 ug/ml colchicine.

Preparation of Thermal-Degradation Sample Solution:

In order to obtain possible thermal-degradation of the drug product, tablets were moisten with water and heated at 60°C for 7 days. The resulting tablets were powdered, and solution was prepared following procedures for the Preparation of Assay Sample Solution from Solid Dosage Form.

Chromatographing:

Standard and assay sample solutions were chromatographed with duplicate injections. Reproducibility of the system was indicated by five consecutive chromatographies of standard solution containing 1.016 mg/ml probenecid and 1.076 ug/ml colchicine. In order to observe and measure the peaks, the sensitivity of detector was set at 0.02 aufs for colchicine. After the eluation of colchicine, at approximate 3.5 minute, the sensitivity was adjusted to 5.12 for probenecid. The chromatographic responses were measured by peak heights.

RESULTS AND DISCUSSION

The assays of probenecid and colchicine in commercial available tablets containing 500 mg probenecid and 0.5 mg colchicine

11. Nylon-66, Rainin Instrument Co. Inc., Woburn, MA.

is identified in the official compendia as separate extraction procedures and followed by uv determination which is lack of specificity. This experiment was attempted to develop a HPLC method using one extraction procedure and followed by a single chromatography. Due to the large difference in the quantity of the two ingredients (500:0.5) and the low solubility of probenecid, the development of an extraction procedure which is able to dissolve both ingredients in one solution with the concentrations of both ingredients within the sensitivity range of the chromatographic detector is required.

Tenth Normal sodium hydroxide was an excellent solvent to dissolve probenecid. However, the instability of colchicine in alkali solution became a problem. A medium of 45% methanol in 0.025 M phosphate buffer, pH 7.5, by volume, was finally developped for the extraction of probenecid and colchicine from tablets. Portion of the powdered-tablet was dissolved in the extraction medium to obtain concentrations of about 1mg/ml for probenecid and 1 ug/ml for colchicine. Both probenecid and colchicine were soluble and reasonably stable in the extraction medium and both chromatograms were able to be detected when 10 micronliter of the sample solution was chromatographed.

Under the chromatographic conditions described, colchicine and probenecid were eluated from the column at approximate 2.4 and 4.3 minutes respectively (Figure 1).

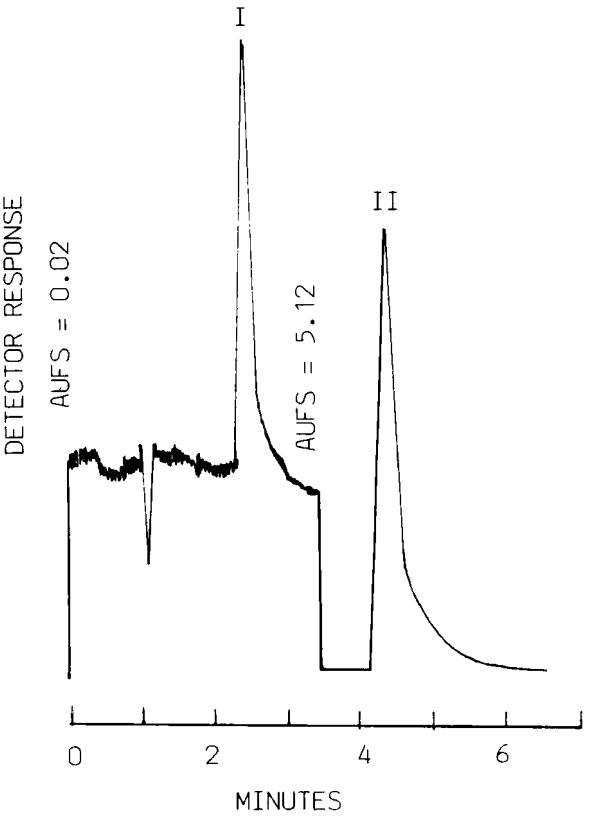

FIGURE 1

Typical Chromatogram for Colchicine (I) and Probenecid (II).

The system suitability tests were performed as following:

Reproducibility:

The reproducibility test of the system was performed on five consecutive injections of solution containing 1.016 mg/ml probenecid and 1.076 ug/ml colchicine. Peak heights of the 5 injections are listed in TABLE 1 with the mean, SD, and RSD.

TABLE 1

Peak Heights (mm) of Colchicine and Probenecid in Five Consecutive Injections.

Injection #	Peak Height (mm) Colchicine	Probenecid
1	58	61
2	57	59
3	58	59
4	58	59
5	59	60
Mean	58	59.6
SD	0.7	0.89
RSD	1.2%	1.5%

Linearity:

Standard curve of quantity chromatographed versus the corresponding peak heights were plotted for colchicine and probenecid. The correlation coefficient of the standard curves were 0.9999345 for colchicine and 0.9999573 for probenecid. The quantity chromatographed were 0.00258 ug to 0.0215 ug for colchicine and 2.5 ug to 19.93 ug for probenecid.

Resolution:

The resolution factor calculated from the chromatogram was 4.

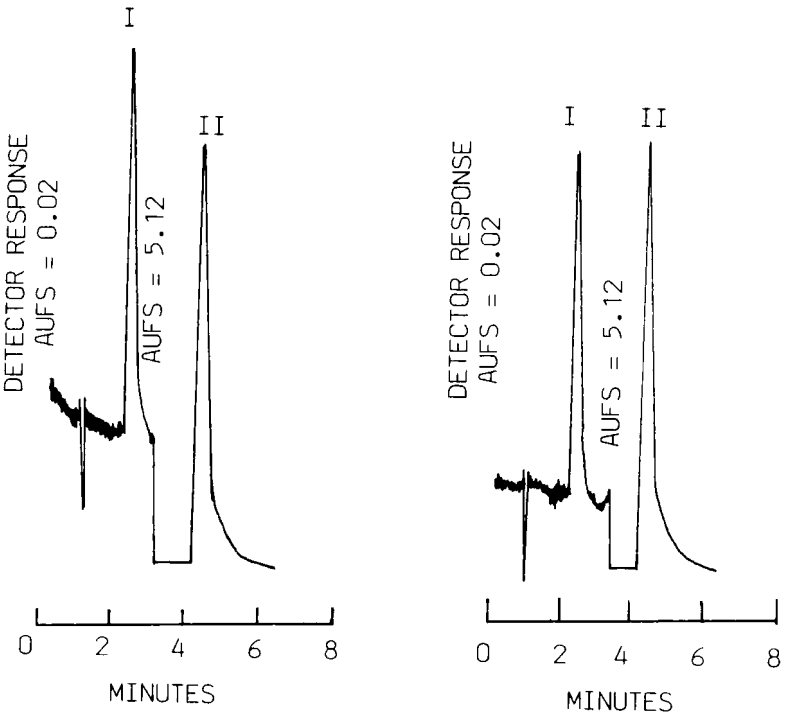

FIGURE 2

Chromatograms of Control (A) and Thermal-Degradation Sample (B). Key: Colchicine (I), Probenecid (II).

Stability-Indicating:

Tablets which were heated at 60°C for 7 days with moisture in order to obtain possible thermal degradation. Assay of such tablets were 85% for colchicine and 99.8% for probenecid by using the present HPLC method. The result indicated that colchicine decomposed during the heating process. The decreasing of peak height for colchicine was observed without the appearance of degradation product on the chromatogram (Figure 2).

Assay of Tablets:

The potency recovery from Probenecid and Colchicine Tablets was 96.8% for colchicine and 98% for probenecid by using this HPLC methodology.

In conclusion, the present assay provides a rapid, specific, and precise method for the simultaneous determination of probenecid and colchicine in commercial available tablets. This novel assay would be useful for the routine quality control as well as the stability studies.

REFERENCES

1. "The United States Pharmacopeia," 21th rev., U.S. Pharmaceutical Convention, Rockville, Md., 1985, p. 880.
2. P.J. Vollmer, T.G. Alexander and C. Haneke,J. Assoc. Off. Anal. Chem., 61, 687 (1978).
3. J.R. Veenendaal and P.J. Meffin, J. Chromatogr. Biomed. Appl., 223, 147 (1981).
4. P. Hekman, P.A.T.W. Porskamp, H.C.J. Ketelaars, C.A.M. Van Ginneken, J. Chromatogr. Biomed. Appl.,182, 252 (1980).
5. R.K. Harles and T. Cowen, Analyst.,103, 492 (1978).
6. A.E. Klein and P.J. Davis, Anal. Chem., 52, 2432 (1980).
7. D. Jarrie, J. Park, and M.J. Stewart, Clin. Toxicol., 14,375 (1979).