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Note

Simultaneous determination of ephedrine sulfate, hydroxyzine hydrochloride and theophylline in tablets by reversed-phase high-performance liquid chromatography

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This laboratory received, from several manufacturers, pharmaceutical tablet samples containing ephedrine sulfate, hydroxyzine hydrochloride and theophylline. Chemical structures of the three drugs are shown in Fig. 1. Multicomponent drug preparations containing ephedrine have often been an analytical problem in this laboratory. To our knowledge there are no current methods that separate and quantitate all three components effectively, at the same time.

The United States Pharmacopeia (USP XX)[1] assay methods for tablet preparations of the single components are based on titration or ultraviolet absorption which would not be sufficiently selective for this mixture. The USP XX method for a mixture of theophylline, ephedrine hydrochloride and phenobarbital utilizes a dual gravity feed column chromatographic procedure, which is both time consuming and would not be expected to be applicable to hydroxyzine without modification.

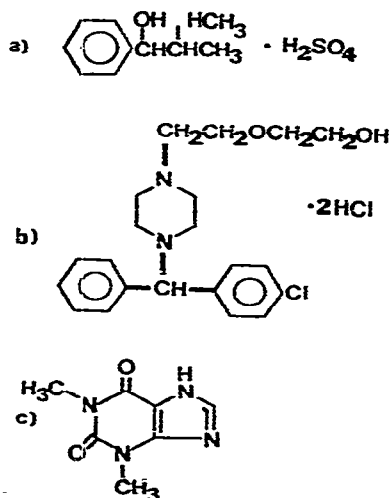High-performance liquid chromatography (HPLC) has proved to be a valuable

Fig. 1. Structures of the drug substances. (a) Ephedrine sulfate, (b) hydroxyzine hydrochloride, (c) theophylline.

tool in analyzing multicomponent drugs[2] with minimal sample preparation, high speed, and good reproducibility. This report describes a quantitative HPLC assay method for tablet formulations containing these three drugs.

EXPERIMENTAL

Materials

The water used was deionized–distilled, suitable for HPLC. The acetonitrile (J. T. Baker, Phillipsburg, NJ, U.S.A.) was HPLC grade, and the concentrated ammonium hydroxide (J. T. Baker), glacial acetic acid (J. T. Baker) and ammonium carbonate (Fisher Scientific, Fairlawn, NJ, U.S.A.) were ACS reagent grade. The drug working standard bulk powders and mixture formulations were from commercial sources. The purities of the drug standards was determined by USP methods[1], and were found to be 99.5, 99.9 and 100.0% for ephedrine sulfate, hydroxyzine hydrochloride, and theophylline, respectively. The commercial tablets were declared to contain 25, 10, and 130 mg of ephedrine sulfate, hydroxyzine hydrochloride, and theophylline, respectively.

Instrumentation

The HPLC system consisted of a dual-piston, positive-displacement pump (Model M45, Waters Assoc., Milford, MA, U.S.A.), an automatic injector (WISP, Waters Assoc.), a variable-wavelength ultraviolet absorption detector (Model LC-75, Perkin-Elmer, Norwalk, CN, U.S.A.) operated at 254 nm and an integrator–plotter (Model 3390A, Hewlett-Packard, Avondale, PA, U.S.A.). The HPLC column was a commercially packed 30 cm × 4.1 mm I.D. chemically bonded octadecylsilane reversed-phase material (μBondapak C_{18}, 10 μm; Waters Assoc.). The mobile phase was filtered through a 0.45-μm polymeric membrane filter (Nylon-66, Rainin, Woburn, MA, U.S.A.).

Standard preparation

Quantities of each drug equivalent to that in one tablet were accurately weighed and transferred to a 25-ml volumetric flask. Ten drops of concentrated ammonium hydroxide were added, then mobile phase was added, and the mixture brought up to volume. Prior to HPLC analysis, this solution was passed through a 0.45-μm membrane filter.

Sample preparation

Composite samples were prepared by grinding 20 tablets to a fine powder and passing through a 60-mesh sieve. A portion of the composite equivalent to one tablet was weighed and transferred to a 25-ml volumetric flask. Ten drops of concentrated ammonium hydroxide were added, and the sample was diluted to volume with mobile phase. Sonication was used, if necessary, to disperse the powder. Prior to HPLC analysis, the mixture was passed through a 0.45-μm membrane filter.

Mobile phase

A 0.1% (w/v) aqueous ammonium carbonate buffer solution was prepared and adjusted to pH 7.0 with acetic acid. The mobile phase was prepared by mixing equal
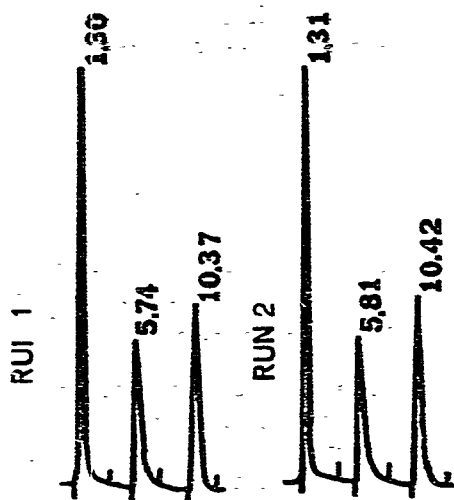
Fig. 2. Typical chromatograms. The elution order is theophylline, ephedrine, hydroxyzine.

volumes of this buffer and acetonitrile. The solution was filtered and vacuum degassed.

Procedure

The HPLC flow-rate was maintained at 2 ml/min with the mobile phase at ambient temperature. A 20-μl volume of the standard and sample solutions was injected. The precision of the system was determined by replicate injections. The coefficient of variation (C.V.) of peak height was determined for each peak and the chromatographic resolution was calculated for each pair of peaks. Recovery was determined by adding a known amount of standard to a separate sample, and taking this sample through the analysis method.

TABLE I

INTEGRATOR OUTPUT FOR THE CHROMATOGRAMS IN FIG. 2

Compound	Retentin time (min)	Height (counts)	Area/height	Height %
Run No. 1				
Theophylline	1.30	1561468	0.171	98.378
Ephedrine sulfate	5.74	11371	0.649	0.716
Hydroxyzine hydrochloride	10.37	14381	0.580	0.906
Run No. 2				
Theophylline	1.31	1562358	0.173	98.377
Ephedrine sulfate	5.81	11313	0.670	0.712
Hydroxyzine hydrochloride	10.42	14465	0.578	0.911

TABLE II

PRECISION AND RECOVERY

Drug	Precision (n = 5)			Recovery		
	Mean peak height (mm)	S.D. (mm)	C.V. (%)	Recovered (mg)	Added (mg)	% Recovered
Ephedrine sulfate	70.5	1.46	2.0	12.40	12.07	102.7
Hydroxyzine hydrochloride	134.1	0.742	0.55	5.10	4.98	102.4
Theophylline	113.8	0.274	0.24	64.6	66.4	97.3

TABLE III

RESULTS OF SAMPLE ANALYSIS

Average of six composites per sample.

Sample No.	Ephedrine sulfate			Hydroxyzine · HCl			Theophylline		
	mg/Tablet	%Decl.	Range(%)	mg/Tablet	%Decl.	Range(%)	mg/Tablet	%Decl.	Range(%)
1	24.6	98.4	92.8–102.4	10.14	101.4	95.6–107.2	130.1	100.1	94.3–105.0
2	24.4	97.6	96.0–98.4	9.96	99.6	97.7–101.5	129.9	99.9	96.4–103.8
3	23.6	94.4	91.2–97.6	9.80	98.0	96.5–99.8	126.9	97.6	93.1–101.6
4	23.7	94.8	93.2–98.4	9.88	98.8	96.8–102.2	132.5	101.9	98.7–102.8

RESULTS AND DISCUSSION

Numerous HPLC mobile phases and columns were explored during the course of this investigation. These included both reversed- and normal-phase systems, and the use of "ion-pairing" reagents. Due to the marked chemical differences between these three drugs it was difficult to meet the desired chromatographic criteria of adequate retention, suitable resolution and short analysis time. Under reversed-phase conditions theophylline tends to elute very rapidly, while hydroxyzine is strongly retained. The conditions described meet these goals. An additional important consideration was the need to add ammonia to each sample solution. Without ammonia, hydroxyzine hydrochloride does not give a peak during the time frame observed. Ammonia is apparently needed to force hydroxyzine into its free base form.

A typical chromatogram is shown in Fig. 2 with integrator output on Table I. The order of elution is theophylline (≈ 1.5 min), ephedrine sulfate (≈ 6 min), and hydroxyzine hydrochloride (≈ 10 min). Due to the large amount of theophylline in the samples, relative to the other two components, either an integrator must be used to calculate peak heights or the detector sensitivity must be increased after elution of the theophylline peak, so that the other two peaks can be quantitated.

The chromatographic resolution[2] between theophylline and ephedrine sulfate, and between ephedrine sulfate and hydroxyzine hydrochloride was observed to be greater than 3. As shown in Table II, the coefficient of variation of peak height for five replicate injections was observed to be less than 2%. The recoveries for spiked samples are observed (Table II) to range from 97.3 to 102.8%. Results from the analysis of composites of four samples from different manufacturers are shown in Table III. The procedure is seen to be simple, precise, and accurate. No interference from excipients in commercial samples was observed.

REFERENCES

1 *The United States Pharmacopeia*. Mack Publishing Co., Easton, PA, 20th rev., 1980.
2 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York; 2nd ed., 1979.