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USE OF FLOW PROGRAMMING IN PAIRED-ION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF DOSAGE FORMS CONTAINING DYPHYLLINE

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SUMMARY

The use of paired-ion high-performance liquid chromatography (HPLC) for analyzing dosage forms containing dyphylline alone and in combination with other products is described. Liquid dosage forms were diluted and then injected without further treatment. Tablets required a simple extraction using the mobile phase or, in some cases, a modified mobile phase. Flow programming was utilized to hasten the analysis time for those products containing ephedrine and phenobarbital. Chromatographic conditions included a mobile phase consisting of 20 parts acetonitrile and 80 parts water which contained $4 \cdot 10^{-3}$ *M* octyl sodium sulfate and 1% acetic acid. The flow-rate was 2 ml/min for the first 4.5 min and then 5 ml/min for the remaining 5 min. Sodium barbital was the internal standard. This procedure permitted the analysis of dyphylline, guaifenesin, ephedrine and phenobarbital. The HPLC results for dyphylline were compared with results obtained by ultraviolet spectrophotometry. There was no interference from dyes or preservatives.

INTRODUCTION

Dyphylline [7-(2,3-dihydroxypropyl)-theophylline] is a member of the methylated xanthine family and is marketed for the treatment of bronchial asthma. It is water soluble (1 g/5 ml) and does not lend itself to gas chromatographic assay without some type of derivatization[1,2].

Dyphylline has an excellent ultraviolet maximum at 274 nm. While dyphylline, by itself, could be analyzed spectrophotometrically, there are too many interfering substances such as dyes, preservatives and other drugs in commercial products that would have to be removed prior to the analysis. The good water solubility of dyphylline makes extraction procedures difficult, but, at the same time, makes reversed-phase high-performance liquid chromatography (HPLC) a good possibility. Two HPLC procedures for analyzing dyphylline levels in serum have been reported. One

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utilized adsorption chromatography and the other used a reversed-phase column and mobile phase consisting of aqueous sodium acetate and acetonitrile[3,4]. The adsorption chromatographic technique requires extraction and evaporation steps. The weakly alkaline mobile phase described in the reversed-phase procedure did not lend itself to analyzing the other drugs commonly found in dyphylline-containing products.

EXPERIMENTAL

Reagents and chemicals

Authentic samples of dyphylline (Lemmon Pharmacal, Sellersville, PA, U.S.A.), guaifenesin (Matheson, Coleman & Bell, East Rutherford, NJ, U.S.A.), phenobarbital (Mallinckrodt, St. Louis, MO, U.S.A.), ephedrine sulfate (Mallinckrodt), and sodium barbital (Mallinckrodt), were used. Reagent-grade solvents were used as received. Octyl sodium sulfate (Eastman-Kodak, Rochester, NY, U.S.A.), was used as the anion which paired with ephedrine. All water was distilled.

HPLC assay

Chromatographic conditions

Solution A was acetonitrile and solution B consisted of 1% (v/v) acetic acid and $4 \cdot 10^{-3}$ *M* octyl sodium sulfate (0.928 g/l). The actual mobile phase was mixed using a solvent programmer (Waters Assoc., Model 660) in the proportion of 20 parts A and 80 parts B (both degassed). A liquid chromatograph (Waters Assoc., Model 630), a variable-wavelength detector (Varian Model 635LC) set at 254 nm, a column containing a microporous silica packing bonded with octadecylsilane (Waters Assoc., μBondapak C_{18}), a 25-cm strip chart recorder (Soltec) and an integrator with a digital printout (Hewlett-Packard, Model 3373B), were utilized. The flow-rate was 2.0 ml/min for the first 4.5 min. It was increased to 5.0 ml/min for the remaining 5 min. Sodium barbital was used as the internal standard. The sensitivity settings on the ultraviolet detector were as follows: dyphylline and sodium barbital, 0–2.0 a.u.f.s., quaifenesin, and phenobarbital, 0–0.5 a.u.f.s., and ephedrine, 0–0.1 a.u.f.s. A 10-μl injection was used for the dyphylline and guaifenesin analyses and 100-μl for the phenobarbital and ephedrine determinations. Even though the sodium barbital peak would go off scale for the latter injections, the integrator accurately measured the total signal. Three separate dilutions were made for each product, and each dilution was injected in triplicate. All concentrations were determined by peak areas. Standard curves were obtained for each ingredient over the required concentration range.

Internal standard solution. Powdered sodium barbital (100 g) was dissoved in 1.0 l acidic mobile phase. A 5.0-ml volume of internal standard stock solution was added to 5.0 ml of each solution being analyzed. Even though the resulting mixture was acidic, the sodium barbiral concentration was low enough so that, along with the presence of the acetonitrile, there was no precipitation of the free barbital.

Standard solutions for calibration curves

Dyphylline. A stock solution containing 19.948 mg/ml in the mobile phase was prepared. This was diluted 1:1 (9.974 mg/ml), 3:5 (7.481 mg/ml) and 1:3 (4.987 mg/ml) with mobile phase.

Four test solutions were prepared by diluting each of the above with an equal volume of internal standard stock solution.

Guaifenesin. The stock solution contained 15.998 mg/ml. Serial dilutions and test solutions were prepared in the same manner as that of the dyphylline.

Phenobarbital. The stock solution contained 11.632 mg/ml. Serial dilutions and test solutions were prepared as described for dyphylline.

Ephedrine sulfate. The stock solution contained 10.800 mg/ml. Serial dilutions and test solutions were done as described for dyphylline.

Sample preparation

Elixirs. All elixirs were diluted 1:1 with barbital internal standard and injected.

Injectables. Dilutions were made mixing 0.125 ml of the contents of the vial with mobile phase and bringing to a volume of 10.0 ml. This solution was diluted 1:1 with internal standard stock solution.

Tablets. One tablet was powdered, extracted with 10 or 20 ml of mobile phase by shaking for 20 min on a mechanical shaker and filtering through a membrane filter (Millipore, No. HATF 01300). The degree of dilution with mobile phase varied with content of the tablets. The dilution was then mixed 1:1 with the internal standard. Tablets containing ephedrine and phenobarbital were extracted with acetonitrile–solution B (40:60).

UV Assays

Reference solutions. Dual sets of solutions were prepared for each of the possible concentrations of dyphylline and guaifenesin by accurately weighing the two drugs and dissolving in water to 100.0 ml total volume.

Sample solutions. Four tablets or capsules were each dissolved in water to 100.00 ml total volume and filtered.

Assay procedure. Of the reference and sample solution 20-ml volumes were treated alike with three 20-ml extractions of dichloromethane. The aqueous layer was centrifuged and 1.0 ml was removed and diluted to 50 ml with water. The absorbance of this solution was determined at 274 nm on a spectrophotometer (Beckman Model, DB-GT), with 1.0-cm cuvets using water as the reference.

The concentration of dyphylline in the solid dosage forms was calculated from

$$\text{mg Dyphylline} = \frac{A_x^{274}}{A_s^{274}} (\text{mg Dyphylline}_s)$$

where A_x = absorbance of dosage form at 274 nm, A_s = absorbance of standard at 274 nm, mg Dyphylline_s = quantity (mg) of dyphylline in the standard.

Dyes. FD & C Blue No. 1, FD & C Yellow No. 5, FD & C Red No. 2 and D & C Yellow No. 10 were dissolved in water to produce an absorbance in the visual spectrum equivalent to that of the freshly dissolved appropriate solid dosage form. These solutions were then diluted following the procedures for HPLC and ultraviolet spectroscopy.

RESULTS AND DISCUSSION

The assay described in this paper posed several problems. One is the strong chromophore of dyphylline. Fortunately, this is at 274 nm and is separate from maxima of the other ingredients. At the other extreme is ephedrine which was present in small amounts and has a weak chromophore at 256 nm ($\varepsilon = 387$). Ephedrine also proved to be a problem in separating from the dyphylline and graifenesin peaks. Varying the ratios of acetonitrile to water and changing the pH did not prove helpful. Replacing acetonitrile with methanol caused increased peak tailing. Paired-ion chromatography did prove workable.

Ephedrine proved quite sensitive to the lipophilicity of the complementary ion. Neither butyl sodium sulfate nor hexyl sodium sulfate caused ephedrine to elute far enough away from guaifenesin. Octyl sodium sulfate gave ephedrine an excessive retention time of 13.3 min at a flow-rate of 2 ml/min. At first solvent programming seemed to be the way to solve this problem. However, it required a longer time period to reestablish the inital column condition when the mobile phase contained a complimentary ion. Flow programming was used with good results. Flow programming also reduced the retention time for phenobarbital from 7.82 to 5.76 min.

Good standard curves were obtained. The pertinent statistics are summarized in Table I. The dyes and preservatives used in the elixirs do not interfere. The dyes either eluted with the solvent front or, as with the parabens, are not seen at their low

TABLE I

STATISTICAL INFORMATION FOR EACH STANDARD CURVE

Product	r^2	*C.V. (%)**	s**	*Intercept(a)***	*Slope(b)***
Dyphylline	0.9968	3.60	0.1695	0.0597	0.7532
Guaifenesin	0.9999	0.85	0.00489	−0.00985	0.1386
Phenobarbital	0.9987	3.20	0.00896	0.0231	0.0778
Ephedrine	0.9965	4.68	0.00123	0.0036	0.00708

* Coefficient of variation of the quantity (calculated concentration/known concentration) at each point along the standard curve.

** Standard deviation of the regression equation defined as (mean square error)$^{\frac{1}{2}}$.

*** Peak area ratio $= a + b$ (concentration mg/ml).

concentration and at the wavelength that was used. Fig. 1 shows a sample chromatogram obtained using this procedure. The results utilizing the commercial products are shown in Table II.

In order to check further the accuracy of the chromatography procedures in terms of the dyphylline determination, an alternative ultraviolet procedure was developed, which involved extraction with organic solvent. It was found that dichloromethane, compared to chloroform, extracted a larger amount of the guaifenesin while not removing a larger proportion of the dyphylline. The dyes did not interfere with the assay of dyphylline at 274 nm at the dilution and with the extraction procedure used in spectrophotometric assay. The results shown in Table III generally parallel those in Table II.

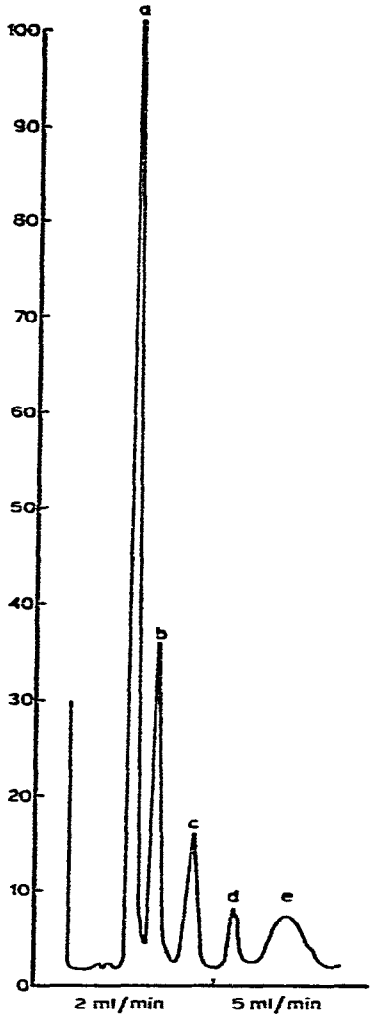

Fig. 1. Sample chromatogram. (a) Dyphylline 132 sec, (b) sodium barbital 177 sec, (c) guaifenesin 258 sec, (d) phenobarbital 345 sec, (e) ephedrine (octyl sulfate) 443 sec. See Experimental for sensitivity settings in an actual assay.

TABLE II

ASSAY RESULTS USING COMMERCIAL SAMPLES

Product	*Label claim*	*Found**	*Range*	*Label claim (%)*	*C.V. (%)***
*Tablets****					
1	200 mg Dyphylline	220.6 mg	(201.8–216.2)	103.5	0.86
	200 mg Guaifenesin	204.8 mg	(199.2–213.0)	102.4	2.86
2	100 mg Dyphylline	109.2 mg	(100.2–105.1)	102.6	1.20
	200 mg Guaifenesin	192.0 mg	(188.5–194.0)	96.0	1.09
	16 mg Phenobarbital	16.5 mg	(16.0– 17.0)	103.0	1.89
	16 mg Ephedrine	16.1 mg	(15.9– 16.6)	100.6	1.52
3	100 mg Dyphylline	110.0 mg	(98.0–105.2)	101.4	1.87
	50 mg Guaifenesin	53.7 mg	(47.8– 52.9)	98.7	1.6
4	200 mg Dyphylline	204.4 mg	(199.6–207.4)	102.2	1.12
	100 mg Guaifenesin	93.9 mg	(92.9– 95.0)	93.9	0.66
5	200 mg Dyphylline	203.3 mg	(195.8–209.0)	101.7	0.27
6	200 mg Dyphylline	208.6 mg	(207.0–213.3)	104.3	0.88
7	200 mg Dyphylline	203.5 mg	(198.0–209.8)	101.8	1.60
8	400 mg Dyphylline	394.6 mg	(386.0–405.2)	98.7	1.89
9	200 mg Dyphylline	200.0 mg	(196.0–205.0)	100.0	1.42
	200 mg Guaifenesin	198.7 mg	(194.8–203.8)	99.4	1.72
*Injectables****					
1	250 mg/ml Dyphylline	243.1 mg/ml	(237.0–249.0)	97.2	1.72
2	250 mg/ml Dyphylline	234.4 mg/ml	(230.8–238.8)	93.8	1.17
Elixirs					
1	160 mg/15 ml Dyphylline	167.4 mg/15 ml	(165.6–169.7)	104.3	0.75
2	160 mg/15 ml Dyphylline	156.9 mg/15 ml	(154.2–160.1)	98.1	1.20
3	100 mg/15 ml Dyphylline	97.5 mg/15 ml	(95.4– 99.3)	97.5	1.30
4	100 mg/ 5 ml Dyphylline	93.0 mg/ 5 ml	(92.1– 94.7)	93.0	0.94
	100 mg/ 5 ml Guaifenesin	85.3 mg/ 5 ml	(83.9– 87.7)	85.3	1.52
5	100 mg/15 ml Dyphylline	96.0 mg/15 ml	(95.2– 97.4)	96.0	0.75
	100 mg/15 ml Guaifenesin	98.4 mg/15 ml	(94.7–100.9)	98.4	2.30
6	100 mg/ 5 ml Dyphylline	90.6 mg/ 5 ml	(88.8– 92.7)	90.6	1.39
	50 mg/ 5 ml Guaifenesin	45.2 mg/ 5 ml	(44.0– 46.0)	90.4	1.28
7	100 mg/15 ml Dyphylline	105.0 mg/15 ml	(102.5–108)	105.0	1.74
	50 mg/15 ml Guaifenesin	51.9 mg/15 ml	(51.0– 53.1)	103.8	1.49
8	100 mg/10 ml Dyphylline	104.2 mg/10 ml	(102.2–106.1)	104.2	1.21
	200 mg/10 ml Guaifenesin	190.8 mg/10 ml	(186.9–194.9)	95.4	1.26
	16 mg/10 ml Phenobarbital	14.8 mg/10 ml	(14.1– 15.3)	92.3	3.08
	16 mg/10 ml Ephedrine	16.0 mg/10 ml	(15.8– 16.3)	100.0	1.04

* Average of nine injections.

** Percent coefficient of variation = S.D./Mean × 100%.

*** Three injections for each of three different tablets or ampules.

In conclusion, a rapid procedure for analyzing dyphylline, guaifenesin, ephedrine and phenobarbital in commercially available pharmaceutical dosage forms has been described. It is obvious that flow programming and the use of a complementary ion are unnecessary for those products not containing ephedrine.

TABLE III

RESULTS OF UV ANALYSIS OF DYPHYLLINE IN PRODUCTS CONTAINING DYPHYLLINE AND GUAIFENESIN

Each value represents an average of four assays; analyses were performed at 274 nm.

Product	*Label claim (%)*	*C.V. (%)**
1	105.2	0.30
3	101.2	0.50
4	101.7	1.20
5	100.8	0.30
6	101.3	0.70
7	99.6	1.30
8	100.5	0.90
9	101.8	1.80

* Percent coefficient of variation $= \frac{\text{S.D.}}{\text{mean}} \times 100\%$.

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REFERENCES

1 Z. K. Shihabi and R. P. Dave, *Clin. Chem.*, 23 (1977) 942.
2 W. C. Butts, V. A. Raisys, M. A. Kenny and C. W. Bierman, *J. Lab. Clin. Med.*, 84 (1974) 451.
3 A. G. Maijub and D. T. Stafford, *J. Chromatogr. Sci.*, 14 (1976) 521.
4 *Bulletin H72*, Waters Assoc., Milford, Ma, October 1976.