Determination of *In Vitro* Release Rates of Sustained-Action Preparations by Paper Chromatography and Electrophoresis

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Determination of *in vitro* release rates of experimental formulations requires multiple analyses by conventional methods. A general procedure is presented for determining *in vitro* release patterns using paper chromatography and electrophoresis techniques which eliminates the tedious manipulations involved with the evaluation of such formulations by conventional methods. Application of these chromatographic and electrophoretic procedures to a preparation containing phenylpropanolamine HCl, dextromethorphan HBr, and chlorpheniramine maleate is described. The procedures compare favorably in accuracy and speed with the conventional techniques. A scheme for the rapid evaluation of several experimental sustained-action formulations using the above techniques is suggested.

DRUGS that have been in general use for a long time are now becoming available in sustained-action formulations containing other active compounds. In handling the assay of these preparations, the customary methods, *i.e.*, ultraviolet spectroscopy and colorimetry, become laborious and unwieldy because additional filtering and extraction steps are generally necessary for their successful application. In addition, the difficulties are magnified by the need to assay many samples in order to determine the *in vitro* release patterns. The assays and release studies of these multicomponent preparations are considerably simplified by paper chromatographic and electrophoretic procedures. In general, separations of the various compounds are accomplished through ascending and descending paper partition chromatography by selection of appropriate solvent systems or by paper electrophoresis with selected pH buffer systems. The papergrams are sprayed with an appropriate color developing reagent and assayed quantitatively by photoelectric densitometry. These techniques have been described previously for conventional multicomponent formulations (1–3).

Application of these procedures to a sustained-action preparation containing phenylpropanolamine HCl, dextromethorphan HBr, and chlorpheniramine maleate is described. In addition, a scheme for the rapid evaluation of experimental sustained-action formulations using the above techniques is suggested.

EXPERIMENTAL

Reagents.—*n*-Butanol A.C.S., 1 *N* hydrochloric acid, absolute alcohol U.S.P., methanol A.C.S., modified Dragendorff's reagent (iodobismuthate) as described before (2, 3), ninhydrin (Fisher reagent), cadmium acetate·$2H_2O$ (Baker reagent), glacial acetic acid A.C.S., pH 4 acetate buffer (0.1 *M*), 1 *N* sodium hydroxide, petroleum ether (Fisher reagent), simulated gastric juice, and intestinal juice were employed.

Equipment.—Whatman No. 1 and 3 mm. chromatographic paper, micropipets,[1] 0.005 and 0.010 ml., photovolt densitometer, model 530 with recording and integrator attachments, pressure plate paper electrophoresis equipment, model EC-455-18 in. with power supply No. EC453 and ice water circulator No. EC322 (E. C. Apparatus Co.), rotating bottle apparatus (Smith Kline & French) as described before (4), and appropriate chromatographic containers were used.

Procedures.—Totals and release rates of a sustained-action capsule containing chlorpheniramine maleate, 4 mg., phenylpropanolamine hydrochloride, 50 mg., and dextromethorphan hydrochloride, 30 mg., are assayed as follows.

Paper Chromatography.—Powdered contents of two capsules are used directly for total assay while the residues obtained from the usual rotating bottle technique for release studies are dried and ground to a fine powder. These powders are transferred to a 25-ml. volumetric flask to which 15 ml. of absolute alcohol is added. After heating the flask on a steam bath and shaking mechanically for 15 min., the flask is cooled and diluted to mark with absolute alcohol. This mixture is well shaken and undissolved material is allowed to settle to the bottom before spotting the sample. A standard solution containing known amounts of "actives" are likewise prepared in absolute alcohol. These solutions are spotted with micropipets (0.04 ml. in 0.01-ml. increments) on Whatman No. 1 paper which was prewetted with a 1:1 mixture of butanol (saturated with 1 *N* HCl) and methanol. This moist, blotted paper is used for separating and quantitating the dextromethorphan HBr and the chlorpheniramine maleate, while another similarly prepared paper is spotted with 0.005 ml. for the assay of the phenylpropanolamine hydrochloride. A descending chromatographic technique is used with butanol saturated with 1 *N* HCl as the solvent system. An overnight migration of the solvent is sufficient for separating the chlorpheniramine maleate and dextromethorphan HBr. The air-dried chromatograms are sprayed with modified Dragendorff's reagent and the resultant orange-red spots are quantitatively evaluated and compared with a photovolt densitometer containing recording and integrating equipment (1). The other papers containing phenylpropanolamine spots are also analyzed by photoelectric densitometry after spraying with a special ninhydrin reagent (0.2% ninhydrin plus 0.5% cadmium acetate in 95% alcohol containing 2% glacial acetic acid) and heating for 10 min. at 100°. Figure 1 shows the relative migration of the active ingredients.

Paper Electrophoresis.—Again the powdered con-

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[1] Microcaps. Drummond Scientific Co., Philadelphia, Pa.

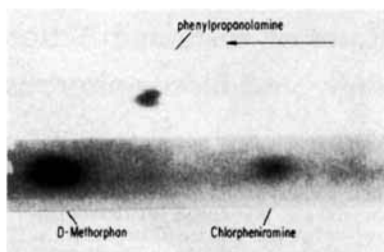

Fig. 1.—Paper chromatograms of phenylpropanolamine, dextromethorphan, and chlorpheniramine.

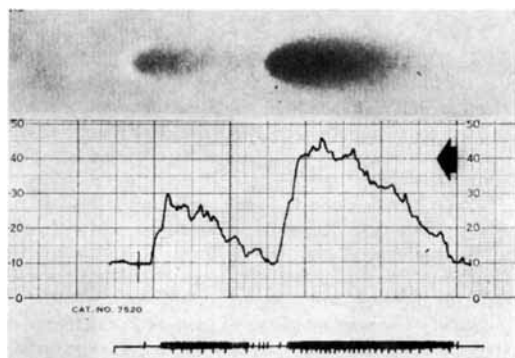

Fig. 2.—Electrophoretic separation of chlorpheniramine and dextromethorphan and their respective recorded and integrated density curves. Chlorpheniramine is the faster moving spot.

tents of two capsules and residues are dissolved and prepared as described for the paper chromatographic procedure. Whatman No. 3 mm. paper strips are prepared for electrophoresis with pH 4 acetate buffer (0.1 *M*) as described previously (1). Two strips are spotted with 0.04 ml. (in 0.01-ml. increments) of standard and sample solutions, while two other strips are spotted with 0.005 ml. of each solution. The papers are placed in an EC apparatus horizontal-type electrophoresis equipment (18 in.) for 16 hr. at 250 v. at 4° (in a walk-in refrigerator). Alternately, the migration can proceed at 600 v. for 6 hr. using salted ice water circulated by a peristaltic pump through the plates. One set of completed and dried electrophoretograms containing the 0.04-ml. spots are sprayed for the detection of the chlorpheniramine maleate and dextromethorphan HBr while the other strips are sprayed with ninhydrin reagent for phenylpropanolamine HCl as described above. Again, as described under *Paper Chromatography*, the resultant spots are analyzed and compared by photoelectric densitometry. Figures 2 and 3 show relative migration of spots as well as their typical recorded and integrated curves.

Application of Paper Chromatography and Paper Electrophoresis to Aqueous Portions of Release Studies.—It may be necessary at times (usually on initial runs) to assay the filtered aqueous portions as well as the residues from a sustained-release study in order to check out the assay and verify the release pattern. The filtered aqueous portions are placed into a 250-ml. separator and 5 ml. of 1 *N* sodium hydroxide is added. The alkaline solution is extracted with 2 × 75 ml. of petroleum ether, which is evaporated to small volume on a steam bath. Evaporation is continued to dryness at room temperature with the aid of a stream of nitrogen. The

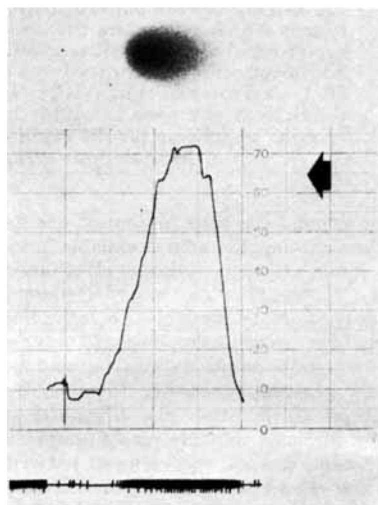

Fig. 3.—Ninhydrin sprayed pink spot of phenylpropanolamine after electrophoresis on 3 mm. paper, combined with its recorded and integrated density curve.

TABLE I.—TYPICAL DATA FOR SUSTAINED-ACTION COLD CAPSULES[a]

	% Released		
	Paper Chrom.	Paper Electr.	Conventional
		Total	
P	96	95	93
D	100	99	100
C	100	99	98
		1 hr.	
P	35	38	37
D	30	30	34
C	27	26	25
		2 hr.	
P	55	57	57
D	54	47	49
C	51	50	50
		4 hr.	
P	70	72	72
D	58	50	60
C	58	60	61
		5 hr.	
P	72	74	73
D	. . .	54	60
C	. . .	62	61
		7 hr.	
P	80	78	79
D	67	71	64
C	65	68	66

[a] Theory: each capsule = 4 mg. of chlorpheniramine maleate (C), 50 mg. of phenylpropanolamine hydrochloride (P), and 30 mg. of dextromethorphan hydrobromide (D).

TABLE II.—COMPARISON OF ASSAYS OF RESIDUES AND AQUEOUS PORTIONS

	% Released (Aqueous)		% Left in Residue	
	Chrom.	Electr.	Chrom.	Electr.
		1 hr.		
P	35	33	63	64
D	31	30	68	69
C	24	25	76	73
		2 hr.		
P	57	55	41	43
D	53	52	46	46
C	50	49	48	51
		4 hr.		
P	69	70	30	28
D	58	55	40	44
C	60	61	38	39
		5 hr.		
P	73	74	24	24
D	62	60	35	38
C	63	65	34	33
		7 hr.		
P	80	81	20	17
D	64	66	34	31
C	67	66	31	33

residue is dissolved in absolute alcohol and transferred to a 25-ml. volumetric flask and brought up to mark with alcohol. The analysis of this solution is then continued *via* paper chromatography and electrophoresis as described.

RESULTS

The results listed in Table I indicate that the procedures presented here compare favorably with a conventional method involving a periodate oxidation of phenylpropanolamine hydrochloride as reported by Chafetz (5) followed by bicomponent spectral analysis of the other two ingredients, which must include corrections for interference from base materials. Thus, in addition to eliminating the tedious manipulations involved in the assay of these preparations by the conventional methods, paper chromatography and electrophoresis have dispensed with the need for correction factors which have to be determined every time a new formulation is devised. Reliance of the proposed methods are further substantiated by data contained in Table II, which compare the assays of the residues with their corresponding aqueous portions obtained from the release studies. In general, a scan of the results obtained from the respective and combined aqueous and residue contents of "actives" approaches within 2 to 3% of the theoretical 100% combined values.

DISCUSSION

The comparative ease and success of applying these procedures to a sustained-action formulation lends credence to the following proposed scheme for the rapid evaluation of these preparations. The pharmaceutical developer could semiquantitatively compare his various formulations using the above techniques, as follows.

Large sheets of Whatman No. 1 chromatographic paper or several strips of Whatman No. 3 mm. paper are prepared by spotting known quantities of the "actives" in the release pattern that is required, *i.e.*, 50% release of active No. 1, 20% release of active No. 2, and 25% of active No. 3 for 1 hr., followed by the requirements for 2, 3, 4, 5 hr., etc. The various formulations after the various hours of the release study are then subjected to procedures as described here, and their resultant spots are compared visually with the respective reference spots. This rapid surveillance could indicate which formulation (if any) approaches the desired release pattern. In this manner, many formulations could be evaluated and compared at the same time, thus saving the experimental formulator valuable time. In addition, the analyst obtains fewer samples for final and accurate quantitation; and using the methods presented here, he saves further time which frees him for the performance of other assays.

SUMMARY

Paper chromatographic and electrophoretic procedures have been successfully applied for studying the release pattern of a multicomponent sustained-action preparation.

The procedures compare favorably in accuracy and speed with the conventional techniques.

Simplicity and ease of application of these methods have led to the suggestion that several sustained-action formulations could be rapidly evaluated by the product developer.

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