Table III—Comparison of Results by USP Manual Method and Automated Procedure with Various Vitamin Preparations

Sample	Composition	Riboflavin[a], mg/Tablet, Capsule, or ml		Coefficient of Variation	
		USP	Automated	USP	Automated
Tablets	Multivitamin	3.01	3.09	1.68	0.78
Capsules	Multivitamin	2.20	2.19	4.87	1.44
Tonic	Water-soluble vitamins plus minerals	0.12	0.12	5.69	1.57
Syrup	Water-soluble vitamins	0.16	0.16	4.17	1.30
Capsules	Multivitamin plus minerals	5.20	5.15	4.64	1.24
Tablets	Multivitamin plus minerals	5.62	5.63	3.00	1.37
Tablets	Multivitamin plus minerals	3.03	3.04	2.40	0.86
Tablets	Multivitamin	0.83	0.83	3.12	0.77

[a] Values represent the mean of eight assays on 8 different days.

through the flow cell, where the fluorescence is continuously measured and recorded on a chart. An example of such recording is shown in Fig. 2. Sample interactions were found to be effectively negligible (Table II) by analyzing levels of 0.05, 0.10, 0.15, and 0.20 μg riboflavin/ml arranged in groups of 20 containing one single concentration and four groups of 20 containing random mixtures of the four levels.

As shown in Table III, results obtained with the automated procedure compared favorably with those of the USP method in the analysis of eight different types of vitamin products. The mean values from eight assays on 8 different days were essentially the same by both procedures. The coefficient of variation with each sample was significantly smaller by the automated procedure.

In summary, results obtained in these laboratories indicate better reproducibility by the proposed automated procedure in comparison to the official USP manual procedure.

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NOTES

TLC Separation and Identification of Diastereomers of D-Ergonovine Maleate

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Abstract □ The resolution of D-lysergic acid D-2-propanolamide from D-lysergic acid L-2-propanolamide (ergonovine) by TLC and column chromatography is described. The compound was identified by comparison with authentic samples using TLC, circular dichroism, and other physical characteristics.

Keyphrases □ Ergonovine maleate—TLC separation and identification of diastereomers □ Lysergic acid derivatives—TLC separation and identification of diastereomers of D-ergonovine maleate □ TLC—separation and identification of diastereomers of D-ergonovine maleate

During TLC examination of some samples of D-lysergic acid L-2-propanolamide maleate (ergonovine maleate), in preparation for gas–liquid partition chromatography (1), a hitherto unobserved minor spot became evident. Since it had not been observed before in this laboratory, even in aged samples, it

Table I—Solvent Systems

Components	Solvent System[a]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Chloroform	114	9	—	15	5	—	5	4	10	10	—	—	—
Acetone	—	—	25	10	4	15	10	4	10	—	—	—	—
Methanol	—	—	4	5	—	—	—	2	10	5	—	3	—
Triethylamine	—	—	1	1	1	—	—	—	—	—	—	—	—
Concentrated ammonia	—	—	—	—	—	0.1	0.1	0.1	0.3	0.1	1	—	—
Ethanol	29	1	—	—	—	—	—	—	—	—	—	—	5
n-Propanol	—	—	—	—	—	—	—	—	—	—	88	—	—
n-Butanol	—	—	—	—	—	—	—	—	—	—	—	2	—
Benzene	37	—	—	—	—	—	—	—	—	—	—	—	—
Acetic acid	—	—	—	—	—	—	—	—	—	—	—	—	3
Water	—	—	—	—	—	—	—	—	—	—	12	—	2

[a] The numbers in each column refer to the parts by volume of the individual solvent making up each solvent system.

was reasoned that its presence was not due to degradation. The unknown was subsequently identified with the aid of preparative column chromatography and UV, IR, optical rotatory dispersion (ORD), circular dichroism (CD), and mass spectral analysis as D-lysergic acid D-2-propanolamide.

EXPERIMENTAL

TLC—Generally, 25 μg of each substance was applied to a 20 × 20-cm plate[1], 250 μm thick, as a solution in methanol. The plates were developed in preequilibrated paper-lined tanks containing approximately 100 ml of solvent. Each chromatogram was developed until the solvent reached 1 cm from the top of the plate. Visualization of the spots was by examination under 254 nm illumination.

Thirteen solvent systems (Table I) were tested.

Liquid Chromatography ("Dry Column")—Glass columns, 190 × 23 mm, were packed firmly with silica gel[2]. A 1-g sample of ergonovine maleate was applied to the dry column as a solution in 2 ml of methanol plus 1 ml of triethylamine. Elution was performed with chloroform–acetone–methanol–triethylamine (15:10:5:1) with a pressure head of 3 lb/in.[2] of nitrogen to produce a flow rate of approximately 20 ml/hr. Five-milliliter fractions were collected[3]. Each fraction was examined by TLC, and the appropriate tubes were pooled, concentrated, and rechromatographed on a fresh column until essentially single-peak material was obtained. The purified material was concentrated in methanol and precipitated as the maleate salt by the addition of ether (10 volumes) (2).

Spectral Studies—UV spectra were obtained[4] using methanol solutions of the various alkaloids being tested. IR spectra were obtained[5] as KBr pellets.

ORD and CD spectra were obtained from methanol solutions on a spectropolarimeter[6] equipped with a circular dichroism attachment[7]. Mass spectra were also obtained[8].

RESULTS AND DISCUSSION

Since it was reasoned that the impurity observed in the sample of ergonovine (I) was not due to degradation, because it had not been seen in aged samples, attempts to identify the impurity proceeded along two lines: isolation and comparative TLC. Preparative TLC on plates 250 μm thick provided sufficient material for UV, IR, and mass spectral analysis. The UV and IR spectra closely matched literature data (3) for ergonovine maleate, and the mass spectrum was analogous to that reported for lysergic acid 2-aminobutan-1-olamide (4). This was considered to be evidence for a stereoisomer arising from an impurity in either of the two precursors, *i.e.*, D-lysergic acid or L-2-aminopropanol.

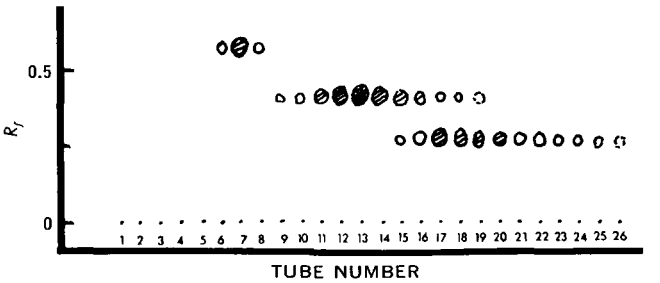
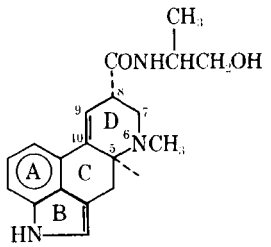


Figure 1—*TLC examination of fractions obtained from dry column chromatography. Both TLC and column chromatography were run in System 4. Key: ergometrinine, peak tube 7; ergonovine, peak tube 13; and unknown, peak tube 17.*

Authentic samples of L-lysergic acid L-2-propanolamide maleate (II), D-lysergic acid D-2-propanolamide maleate (III), and D-isolysergic acid L-2-propanolamide nitrate (IV) were obtained and subjected to comparative TLC in 13 solvent systems (Table I) along with the sample of I containing the impurity. The R_f values of II and III were identical to that of the impurity in all systems. The R_f values of the compounds tested are listed in Table II for the eight systems that gave the largest mobility. Compound IV is the result of epimerization of I at C-8 occurring readily under basic conditions.



I

System 4, which provided the best separation of the unknown from I, was used as the eluting solvent in dry column preparative chromatography. Figure 1 shows the elution pattern of the column as determined by TLC of each fraction obtained. The order of elution and relative resolution was similar to that obtained by TLC. True dry column chromatography, where the column is extruded and cut into sections, was attempted and found to be unsatisfactory. The closeness of mobility of I and the unknown required the extra running length and, indeed, rechromatography.

[1] Commercially available coated glass plates (Merck silica gel F254, Mallinckrodt Chromar 7GF and Analtech Uniplate silica gel GF) were used. The so-called preparative plates (500–2000 μm thick) afforded no resolution of the investigated minor spot.

[2] Silica gel GF254, according to Stahl, Merck.

[3] Golden Retriever Pup, model 1100, Instrumentation Specialties Co.

[4] Cary recording spectrophotometer, model 14.

[5] Beckman IR 12.

[6] Cary model 160.

[7] Cary 6001.

[8] Consolidated Electronic Corp. 21-110.

Table II—$R_f \times 100$ for Some Stereoisomers of Ergonovine

Compound	Solvent System							
	3	4	8	9	10	11	12	13
D-Lysergic acid D-2-propanolamide	37	33	44	57	62	51	40	52
L-Lysergic acid L-2-propanolamide	37	33	44	57	62	51	40	52
D-Lysergic acid L-2-propanolamide (ergonovine)	45	45	50	61	66	52	46	52
D-Isolysergic acid L-2-propanolamide(ergometrinine)	61	69	66	70	74	62	52	47

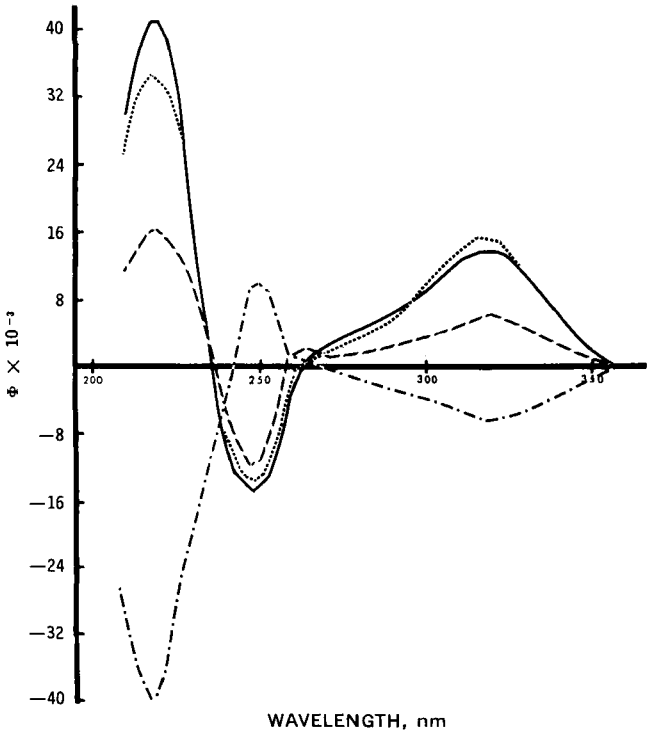


Figure 2—*CD spectra. The sample contained a drop of potassium hydroxide to aid in dissolution. Since this causes equilibration of the isolysergic and lysergic forms, the absolute values of Φ are not those of the pure compound. Key:* —, *ergonovine;* ·–· -, *II;* . . . ., *III and unknown; and* – –, *IV.*

The purified material (~5 mg), I, II, III, and IV were submitted for ORD–CD measurements and also for UV, IR, and mass spectral analyses. The latter three tests gave results unchanged from those previously obtained. CD measurements distinguished between II and III as expected (Fig. 2). The CD spectrum of the unknown resembled that of III and its identity was thereby assigned.

Unlike TLC, gas–liquid partition chromatography (1) is only capable of distinguishing derivatives of the *cis*-lysergic series from those of the diastereomeric *trans*-isolysergic series but not diastereomers within each series, *i.e.*, where a third chiral site is involved. Apparently the molecular interaction with the stationary phase in gas–liquid partition chromatography is governed by the C5–C8 relationship to the exclusion of the third chiral site in the side chain. This emphasizes the necessity for a judicious choice of derivatizing agent for gas–liquid partition chromatography resolution of enantiomers by derivatization to diastereomers, as was recently pointed out (5). Obviously, lysergic acid would not be a reagent of choice.

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