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Note

High-performance liquid chromatographic analysis of ergonovine maleate formulations

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In a previous study designed to improve the non-specific National Formulary (NF)[1] procedures for the assay of ergonovine and related alkaloids, a gas chromatographic procedure[2] was developed. This procedure required complete removal of any aqueous solvent using mild conditions and derivatization before chromatography. The inherent instability of ergonovine, the reagents and the resultant derivative of the drug substance made a more gentle method desirable. High-performance liquid chromatography (HPLC) was investigated.

Various general HPLC procedures for the examination of ergot alkaloids have been reported[3–7]. For the present study, only those using aqueous eluting solvents[5,7] were investigated further so as to minimize sample work-up. The microparticulate columns used[7] provided the best resolution and peak shape. However, the high pH of the eluting solvent is not usually considered optimal for prolonging column life. Solvents containing acetic acid provided the desired chromatographic characteristics.

This report describes a quantitative HPLC analysis of ergonovine maleate. The method allows concomitant quantitation of the related alkaloid, ergonovinine. Other related alkaloids are also resolved.

EXPERIMENTAL

Apparatus

A Waters Assoc. M6000A high-pressure liquid chromatography capable of operating at an inlet pressure up to 6000 p.s.i.g. was used. Samples were injected through a 20-μl loop injector valve (Model 7120) onto a 4 mm I.D. × 30 cm μBondapack C_{18} column of octadecyltrichlorosilane permanently bonded to silica via silicon–carbon bonds. The emerging components were detected by a Model 770 Spectroflow monitor variable wavelength spectrophotometer set to 312 nm. The signal was fed to an LDC Model 3401M chromatographic strip chart recorder and also to a Hewlett-Packard 2100RTE computer for peak integration.

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Chromatographic conditions

The eluting solvent consisted of 20% (v/v) acetonitrile, 1% (v/v) acetic acid in water. The solvent was deaerated before use. The temperature of operation was ambient. The flow-rate was 1 ml/min (inlet pressure approximately 800 p.s.i.g.). A full scale deflection of 0.4 absorbance units and a chart speed of 2 mm/min were used.

Data treatment

Peak integration and calculation of results were performed by the computer. The peak area of ergonovine in each sample and standard was measured. Area for the standard injections were fitted to a straight line by the least-squares method. Areas for the samples were compared to the line to obtain mg equivalence. Appropriate dilution factors were applied to convert the data to mg per ml or per tablet.

Solutions

Standards were prepared by dissolving the accurately weighed alkaloid in distilled water to give a 0.2 mg/ml concentration. Ampoule and disposable syringe (0.2 mg/ml) formulations were used as is. Tablets (0.2 mg/tablet) were dissolved in distilled water to give a 0.2 mg/ml concentration and allowed to stand for 2 h. The samples must be centrifuged to obtain a clear supernatant so as not to plug the loop valve or the column.

RESULTS

Elution solvents containing acetic acid avoided the possibility of alkaline degradation of the column materials and still gave desirable chromatographic characteristics. Fig. 1 details the elution patterns of ergonovine maleate samples of differing purity. Fig. 1b shows a sample of partially degraded ergonovine maleate whereas the

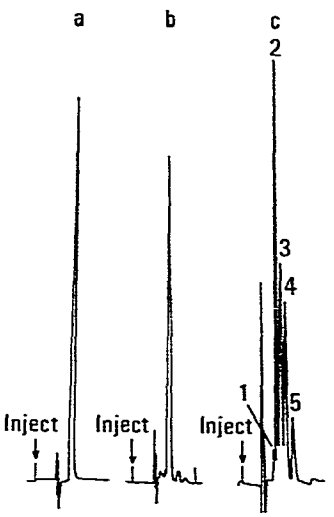

Fig. 1. (a) Pure ergonovine maleate; (b) partially degraded ergonovine maleate; (c) 1 = unknown impurity; 2 = ergonovine maleate; 3 = lysergic acid; 4 = ergonovine diastereomer (ref. 9); 5 = ergonovinine nitrate.

sample in Fig. 1c contains added lysergic acid, ergonovinine nitrate[8] and a diastereomer of ergonovine[9].

As a quantitative procedure, the method is linear to two-fold the concentration described and the extrapolated line passed through the origin. Replicate standards and samples were reproducible as shown in Table I. Since the related alkaloid, ergovinine, is resolved from the drug substance, it may be quantitated concomitantly as the stability of formulation is evaluated. Thus, the requirements of two NF assays are accomplished in a single analysis. Because no pure standard for ergonovinine was available, the response factor for ergonovine was used. The values in Table II for ergovinine therefore represent a close approximation. The values in Table II demonstrate that as tablet formulations age, the related alkaloid becomes more prominent. The ergonovine maleate content of disposable syringes also decreases with time of storage but without the appearance of the related alkaloid. Instead lysergic acid and possibly lumiergonovine are found. Contents of glass sealed ampoules are stable for more than five years and exhibit only a minimal decrease in concentration.

TABLE I

REPRODUCIBILITY OF THE METHOD

R.S.D. = relative standard deviation.

	Replicates	*R.S.D.*
Standards	10	±0.5%
Formulations		
Ampoules	6	±0.8%
Disposable syringe	10	±1.1%
Tablets	9	±1.7%

TABLE II

STABILITY OF FORMULATIONS

	Ergonovine maleate		*Ergonovinine*	*Age*
	*Theory**	*Found*		
Ampoules	0.214	0.214	none	1 year
		0.202	none	3½ years
		0.199	none	4½ years
Disposable syringe	0.214	0.202	none	¾ year
		0.191	none	1½ years
		0.182	none	
Tablets	0.200	0.198	none	1 month
		0.196	0.004	1 year
		0.193	0.008	2½ years
		0.180	0.010	3 years

* Includes excess.

This method is to be considered superior to the NF method because of its specificity and ease of sample preparation. It is superior to the gas chromatographic method[2] because of the ease of sample preparation, the elimination of the requirement for derivatizing agents and the very demanding experimental conditions.

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