flavin and niacinamide. The high degree of interaction between formulations and methods probably accounts for the inability to detect any significant differences between the method means of riboflavin and niacinamide. However, the thiamine method means were significantly different ($p < 0.01$) from the chemical method, giving a consistently higher result. These higher results may be due to a reagent or background blank not detected by the polarographic procedure, as indicated in Sample F where no thiamine was found by the polarographic method but 0.4 mg. was found by the chemical procedure. By assuming that the 0.4 mg. was a background blank and subtracting this value from the chemical result obtained on all samples, good agreement with the polarographic data is obtained.

CONCLUSIONS

It was established that it is possible to detect and analyze most multivitamin pharmaceutical preparations for their riboflavin, thiamine hydrochloride, and niacinamide content by cathode ray fast sweep polarography. Preliminary results indicate that the cathode ray fast sweep polarographic instrumentation is adaptable and has several readily apparent advantages for the analysis of multivitamin products containing riboflavin, thiamine hydrochloride, and niacinamide. Some problems and their subsequent solution associated with the utilization of this type of analytical instrumentation are provided. The method appears to have an excellent potential for becoming a practical analytical routine with further refinements.

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GLC Analysis of Multicomponent Suppository Formulations

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Abstract □ A GLC procedure for drug assay in commercial suppositories is described. Aminophenazone, *d*-propoxyphene hydrochloride, caffeine, chlorpheniramine maleate, lidocaine, phenacetin, fenalamide, and sodium benzoate were determined, in various associations, in three types of suppositories. Simultaneous determinations of up to five components were performed on the following columns: Column A, a mixed column of 2% polyethylene glycol (20,000) + 2% methyl silicone gum rubber on silanized diatomite impregnated with 5% KOH; Column B, a column of methylvinyl silicone gum rubber on silanized diatomite; and Column C, a mixed column of 0.4% isophthalic acid + 0.25% polyethylene glycol (20,000) on glass beads. The sample preparation procedure involves only a dissolution step. Precision and accuracy, calculated on simulated suppository preparations, were satisfactory.

Keyphrases □ Suppositories, commercial—GLC drug assay □ Antipyretic drug assay, suppositories—GLC □ Drug assay, multicomponent suppositories—GLC method □ GLC—analysis

It is well known that drug assay in suppositories following the classical techniques (*e.g.*, spectrophotometric and colorimetric methods) is cumbersome and often leaves much to be desired, both in accuracy and precision, due to interference of the excipients and other additives. It seemed particularly desirable to have a rapid, specific, and precise technique, such as GLC, for the assay of some suppository formulations marketed for the symptomatic treatment of the common cold and related diseases. According to the different formulations, the main antipyretic drug is either alone or, more often, associated with antihistamines and/or analgesics and other components (*e.g.*, sulfa drugs and caffeine).

Few papers have been published concerning the determination of active ingredients in suppositories by GLC (1–4). Reportedly, only single-drug (chlordantoin, 7-chlor-4-hydroxyindan, vitamin K_3, and prenylamine) determinations in single- (1, 2) and dual- (3, 4) component suppositories are performed. In the present report, data are presented regarding the GLC assay of suppositories containing: (Type I) aminophenazone in association with sodium sulfadimethoxine (not determined); (Type II) chlorpheniramine maleate, *d*-propoxyphene hydrochloride, lidocaine, and caffeine in association with paracetamol and calcium urea acetylsalicylate (not determined); and (Type III) aminophenazone, phenacetin, chlorpheniramine maleate,

Table I—Composition of Formulations of Suppositories Examined

Suppository Type	Active Ingredients	Amount Declared, mg.	Additives and Excipients
I	Aminophenazone	300	Butylhydroxytoluene
	Sodium sulfadimethoxine	250	Butylhydroxyanisole
			Saturated fatty acids glycerides
			Polysorbate 61
II	*d*-Propoxyphene hydrochloride	60	Butylhydroxytoluene
			Butylhydroxyanisole
	Caffeine	50	Saturated fatty acids glycerides
	Chlorpheniramine maleate	3	Aluminum hydroxide gel
	Lidocaine	20	
	Paracetamol	250	
	Calcium urea acetylsalicylate	650	
III	Phenacetin	300	Saturated fatty acids glycerides
	Aminophenazone	400	
	Caffeine	60	
	Chlorpheniramine maleate	4	
	Fenalamide	30	
	Sodium benzoate	60	

fenalamide[1], caffeine, and sodium benzoate. Although GLC determination of these compounds in other pharmaceutical forms as components of different associations (5–15) and/or as single drugs (16–26) was already reported, the procedures described could not be utilized in the present case.

The procedure adopted for sample preparation is very simple, involving only a dissolution step, with consequent time saving and a better warranty of quantitative recovery. The choice of columns and preparation were accomplished on the basis of the literature indications. Due to the presence of very polar groups in the compounds studied (basic nitrogenous and amidic functions and, in one case, a carboxylic group), problems were encountered in finding suitable inert solid supports. Concerning basic nitrogenous compounds, three suggestions came from the literature: (*a*) use of support-coating with alkali to neutralize the "acid sites" and thus prevent adsorption (8, 21, 27–33) [actually, the amount of alkali used is far greater than the stoichiometric amount necessary for the neutralization process and, therefore, it modifies the retention characteristics of the stationary phases (28)]; (*b*) use of suitably silanized supports (12–18, 34, 35); and (*c*) use of inert supports such as glass beads or Teflon (5, 7, 22, 36, 37).

Two columns were prepared following suggestions (*a*) and (*b*). One, Column A, was a mixture of methyl silicone gum rubber[2] and polyethylene glycol[3] (20,000) on silanized diatomite[4] impregnated with KOH instead of acid- and base-washed silanized diatomite[5] (12) because of its failure in terms of column efficiency. No difference was observed either by varying the KOH concentration (from 2 to 10%) or by treating the support with KOH before or after coating with the stationary phase (29). No appreciable troubles, as cited by some authors (38–40), were met. Aminophenazone alone (Type I) and chlorpheniramine maleate, *d*-propoxyphene hydrochloride, lidocaine, and caffeine (Type II) were determined simultaneously on this column.

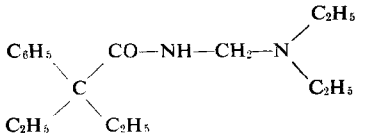
The other column (B) was prepared using silanized diatomite[6] coated with methylvinyl silicone gum rubber[7]. This column was found suitable both for basic and amidic compounds and was consequently used for simultaneous determination of aminophenazone, phenacetin, chlorpheniramine maleate, and fenalamide (III). For the carboxylic compound, benzoic acid in III, Nikelly's work (26) was taken as a basis for preparing an adequate column (C). Accordingly, carboxylic acids (also the aromatic ones) are chromatographed without preliminary esterification. This column, prepared by coating glass beads with polyethylene glycol (20,000) and isophthalic acid, was found to be somewhat cumbersome to reproduce.

All the columns used were glass, and the on-column injection technique was adopted; however, metallic columns and the metallic injector were also found suitable. Injector port temperature was maintained at 230–250°, sufficient to dissociate the salts (19).

EXPERIMENTAL

Column Preparation—Three columns (2-m. Pyrex glass U-tubes 4-mm. i.d.) were prepared: (Column A) a mixed column of 2%[8] polyethylene glycol (20,000) + 2% methyl silicone gum rubber on 100–120-mesh silanized diatomite impregnated with 5% KOH, (Column B) a column of 10% methylvinyl silicone gum rubber on 80–100-mesh silanized diatomite, and (Column C) a mixed column of 0.4% isophthalic acid[9] +0.25% polyethylene glycol (20 *M*) on 80–120-mesh glass beads[9] (26). The column packings were prepared by the standard procedure by dissolving a weighed amount of

[1] Fenalamide is the common name of *N*-(2-diethylaminoethyl)-2-phenyl-2-carbethoxybutyramide [A. Buttini, M. M. Melandri, and P. Galimberti, *Boll. Chim. Farm.*, **107**, 362(1968)]:



[2] SE 30; Carlo Erba, Milan, Italy.
[3] Carbowax (20,000); Carlo Erba, Milan, Italy.
[4] Gas Chrom P; Carlo Erba, Milan, Italy.
[5] Anakrom ABS.
[6] Diatoport S; Hewlett Packard, Avondale, Pa.
[7] UCC W 98; Hewlett Packard, Avondale, Pa.
[8] Percentage of stationary phase or KOH refers to the weight of support.
[9] Applied Science Laboratories, Inc., State College, Pa.

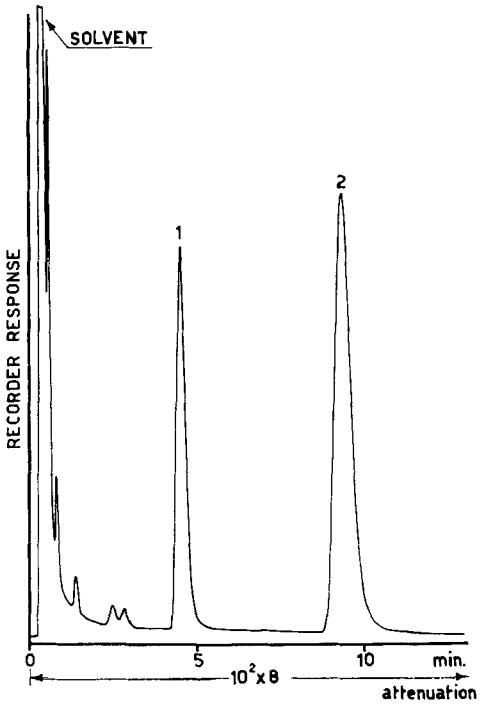


Figure 1—*Representative chromatogram for suppository Type I (Column A; temperature, 200°; flow, 100 ml./min.). Key: 1, lidocaine (internal standard); and 2, aminophenazone.*

stationary phase in a volatile solvent, adding a weighed amount of solid support, and evaporating the solvent to dryness with frequent manual stirring and mild heating. In the case of Column A, the support was impregnated beforehand with KOH, applied by means of a methanol solution, and rescreened to remove crushed particles (19). The columns were filled in the conventional manner using vacuum and tapping until no more packing was accepted (10, 8, and 35 g. for Columns A, B, and C, respectively) and conditioned for 24 hr. with a nitrogen flow rate of about 80 ml./min. at 210, 250, and 180° for Columns A, B, and C, respectively.

Apparatus and Operating Conditions—A Carlo Erba model Fractovap GV 200 gas chromatograph, equipped with a flame-ionization detector and a 2.5-mv. Leeds & Northrup Speedomax model W recorder, was used.

In all cases, hydrogen and air flow rates were 50 and 300 ml./min., respectively. The on-column injection technique was used. Column conditions were as follows:

1. *Column A;* oven, injector, and detector temperatures were 200, 210, and 210°, respectively; nitrogen flow rate was 100 ml./min.
2. *Column A;* oven, injector, and detector temperatures were

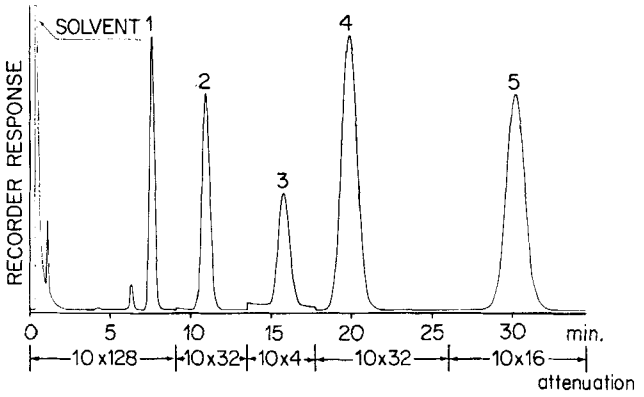


Figure 2—*Representative chromatogram for suppository Type II (Column A; temperature, 195°; flow, 75 ml./min.). Key: 1, diphenylamine (internal standard); 2, lidocaine; 3, chlorpheniramine; 4, d-propoxyphene; and 5, caffeine.*

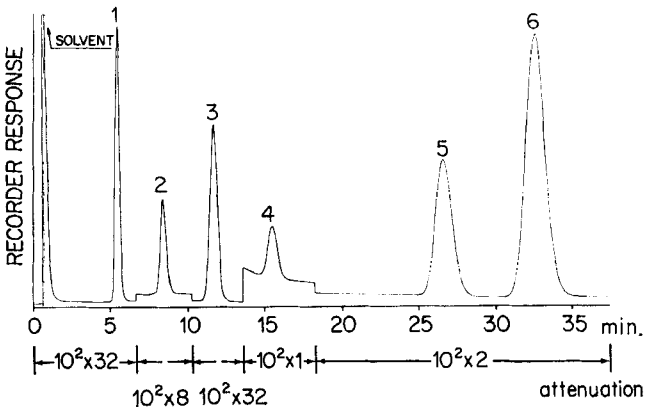


Figure 3—*Representative chromatogram for suppository Type III (Sample IIIa) (Column B; temperature, 205°; flow, 60 ml./min.). Key: 1, phenacetin; 2, caffeine; 3, aminophenazone; 4, chlorpheniramine; 5, fenalamide; and 6, 2-chlorothioxantone (internal standard).*

195, 210, and 210°, respectively; nitrogen flow rate was 75 ml./min.

3. *Column B;* oven, injector, and detector temperatures were 205, 250, and 250°, respectively; nitrogen flow rate was 60 ml./min.
4. *Column C;* oven, injector, and detector temperatures were 165, 190, and 190°, respectively; nitrogen flow rate was 60 ml./min.

PROCEDURE

Three types (I, II, and III) of commercially available suppositories, whose compositions are shown in Table I, were examined.

Internal Standard Solutions—Lidocaine hydrochloride and diphenylamine were used as internal standards for Types I and II, respectively; 2-chlorothioxantone and *p*-toluic acid were used for Type III. Internal standard solutions, I, II, III*a*, and III*b*, were prepared by dissolving about 600, 200, 350, and 240 mg., exactly weighed, of lidocaine hydrochloride, diphenylamine, 2-chlorothioxantone, and *p*-toluic acid in 250 ml. of absolute ethanol, 100 ml. of chloroform, 50 ml. of chloroform, and 100 ml. of 95% ethanol, respectively.

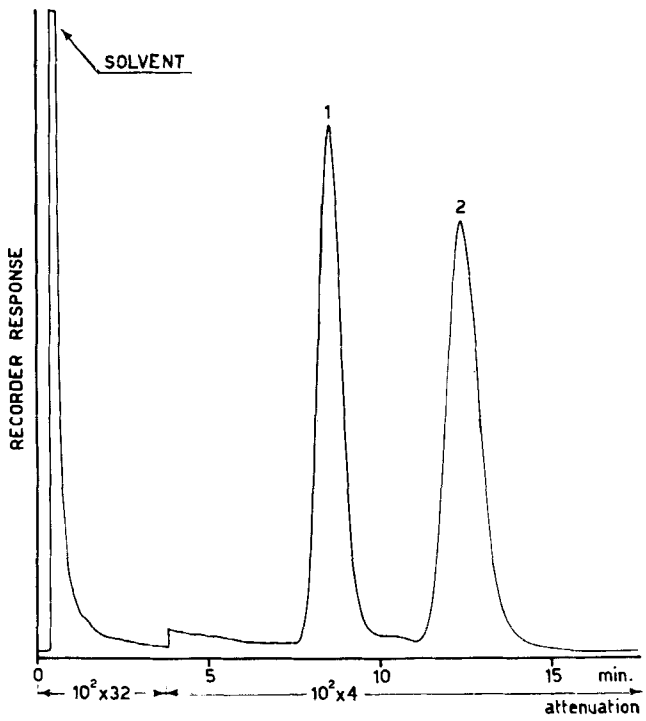


Figure 4—*Representative chromatogram for suppository Type III (Sample IIIb) (Column C; temperature, 165°; flow, 60 ml./min.). Key: 1, benzoic acid; and 2, p-toluic acid (internal standard).*

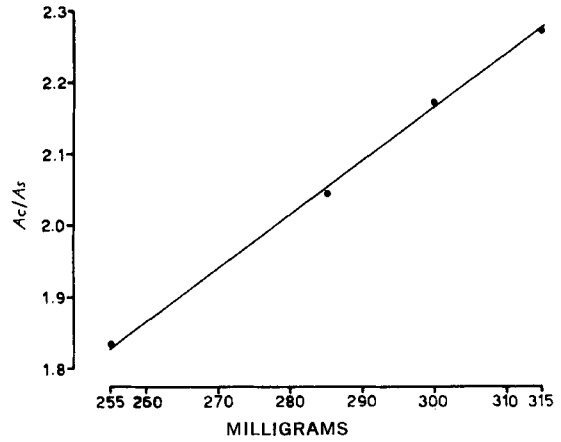


Figure 5—*Calibration curve for aminophenazone in suppository Type I.* (Ac *and* As *equal component peak area and internal standard peak area, respectively.*)

Sample Preparation—One suppository for Types I and II, and two suppositories, separately treated, for Type III were used for the analysis. The suppositories were melted in a suitable flask by heating in a water bath at 50–60° and treated as described here.

Sample I—Internal Standard Solution I (50 ml.) was added to one melted suppository of Type I. Heating was continued with shaking until complete dissolution. About 2.5 μl. of the clear solution, still hot, was injected into the gas chromatograph under operating conditions No. 1. Aminophenazone was the active ingredient determined (Fig. 1).

Sample II—Internal Standard Solution II (25 ml.) was added to one melted suppository of Type II. Heating was continued with shaking for a few minutes. The suspension was filtered, and about 4 μl. of the filtrate was injected into the gas chromatograph under operating conditions No. 2. *d*-Propoxyphene hydrochloride, caffeine, chlorpheniramine maleate, and lidocaine were the active ingredients determined (Fig. 2).

Sample IIIa—Internal Standard Solution III*a* (10 ml.) and about 40 ml. of chloroform were added to one melted suppository of Type III. Heating was continued until complete dissolution. About 4 μl. of the clear solution, still hot, was injected into the gas chromatograph under operating conditions No. 3. Phenacetin, aminophenazone, caffeine, chlorpheniramine maleate, and fenalamide were the active ingredients determined (Fig. 3).

Sample IIIb—Internal Standard Solution III*b* (25 ml.) and 0.5 ml. of concentrated hydrochloric acid were added to one melted suppository of Type III. The mixture was completely dissolved by heating in a water bath at 50–60° with shaking. About 4 μl. of the still hot solution was injected into the gas chromatograph under operating conditions No. 4. Sodium benzoate was the active ingredient determined (Fig. 4). After every four consecutive sample injections, a break of about 90 min. must be made for the elution of the sample components not determined.

Standard Solutions—Quantities of the sample components to be determined, corresponding to about 85, 95, 100, and 105% of the declared amount, were weighed for each of the four samples. Corresponding internal standard solution and solvent, in the same amount used for the sample preparation, were added; shaking and heating was performed as for the sample. The resulting solutions were then analyzed under the same operating conditions as for the corresponding samples. The data obtained were used to set up calibration curves for each component in each type of suppository. These curves were obtained by plotting the ratio of the component peak area to the corresponding internal standard peak area against the amount of the components. Each point represents the average of three determinations.

Simulated Suppository Preparations—To simulate the suppositories, known mixtures were prepared by mixing all the active ingredients, additives, and excipients in quantities corresponding to the declared amounts. These mixtures were then treated and analyzed exactly as the corresponding samples.

RESULTS AND DISCUSSION

Typical chromatograms for the three types of suppositories are shown in Figs. 1–4. All peaks are well resolved and symmetrical. The technique used for the quantitative analysis was "internal standardization." Quantitation of the peaks was based on peak area, obtained by multiplying the peak height by width at half-height. The calibration curves shown in Figs. 5–7 were utilized to calculate the amount of each ingredient; the range examined was limited to 85–105% of the declared amount as a reasonable range for covering the expected deviations for commercial preparations. These curves appear to be linear for the examined interval. Frequent check of the curves is, however, necessary if the highest accuracy is required.

The major problem encountered in obtaining and evaluating quantitative data was due to the possible interferences. Three types of interference were considered: (*a*) chromatographic interferences due to overlapping between extraneous peaks and those to be measured; (*b*) incomplete availability of the active ingredients in the solution to be chromatographed, owing to physicomechanical hindrance exerted from the undissolved portion of the sample (*e.g.*, excipients); and (*c*) interferences due to physicochemical interaction between dissolved components under the GLC operating conditions. The (*a*) and (*c*) types of interference were checked by analyzing the simulated preparations and were ruled out on the basis of the accuracy data obtained by these analyses. Interference of type (*b*) was to be considered only in the case of suppository Type II, where some

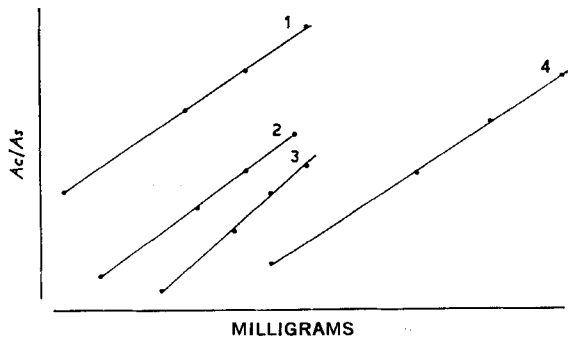


Figure 6—*Calibration curves for suppository Type II components. Key: 1, caffeine (for milligram range of 52.5–42.5,* Ac/As *range of 0.357–0.289); 2, lidocaine (for milligram range of 21–17,* Ac/As *range of 0.313–0.254); 3, chlorpheniramine maleate (for milligram range of 3.15–2.55,* Ac/As *range of 0.0299–0.0248); and 4,* d-*propoxyphene hydrochloride (for milligram range of 63–51,* Ac/As *range of 0.823–0.668).* (Ac *and* As *equal component peak area and internal standard peak area, respectively.*)

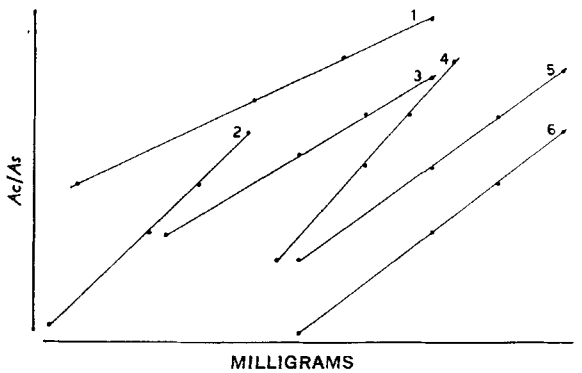


Figure 7—*Calibration curve for suppository Type III components. Key: 1, aminophenazone (for milligram range of 420–340,* Ac/As *range of 4.070–3.315); 2, fenalamide (for milligram range of 31.5–25.5,* Ac/As *range of 0.435–0.347); 3, phenacetin (for milligram range of 315–255,* Ac/As *range of 3.801–3.083); 4, chlorpheniramine maleate (for milligram range of 4.2–3.4,* Ac/As *range of 0.0467–0.0377); 5, sodium benzoate (for milligram range of 63–51,* Ac/As *range of 0.887–0.714); and 6, caffeine (for milligram range of 63–51,* Ac/As *range of 0.832–0.648).* (Ac *and* As *equal component peak area and internal standard peak area, respectively.*)

Table II—Analysis of Simulated and Commercial Suppository Preparations

Suppository Type	Weight Declared and Range Found	Component Determined	Simulated Preparation: Weighed[a] or Declared[b], mg.	Simulated Preparation: Found, Average, mg.	Simulated Preparation: Number of Replicate Analyses	Simulated Preparation: Relative *SD* (p = 0.05)	Simulated Preparation: Relative Error	Commercial Suppository: Found, Average, mg.	Commercial Suppository: Number of Lots Examined	Commercial Suppository: Relative *SD* (p = 0.05)	Commercial Suppository: Relative Error
I	1.65 ± 0.02	Aminophenazone	300	299.8	6	1.01	−0.07	304.2	29	2.38	+1.40
II	3.30 ± 0.03	*d*-Propoxyphene hydrochloride	60	60.4	6	0.67	+0.67	61.3	25	2.56	+2.17
		Caffeine	50	50.5		1.62	+1.00	51.9		3.33	+3.80
		Chlorpheniramine maleate	3	3.04		2.58	+1.33	3.08		5.16	+2.67
		Lidocaine	20	20.2		2.40	+1.00	20.6		2.98	+3.00
III	2.65 ± 0.02	Phenacetin	300	298.8	6	0.97	−0.40	299.3	20	2.20	−0.23
		Aminophenazone	400	399.4		1.25	−0.15	397.7		2.61	−0.58
		Caffeine	60	59.9		1.18	−0.17	59.5		3.53	−0.83
		Chlorpheniramine maleate	4	3.99		2.72	−0.25	3.94		5.90	−1.50
		Fenalamide	30	29.9		1.45	−0.33	29.6		3.46	−1.33
		Sodium benzoate	60	59.6		1.32	−0.67	59.0		6.18	−1.67

[a] For simulated preparation. [b] For commercial suppository.

of the components were not soluble under the operating conditions and some trapping action on the active components to be determined might have been suspected. In this case the absence of the interference in the sample cannot be directly inferred from the ascertained absence in the simulated preparation, because it was practically impossible to reproduce exactly in the latter the dissolution process of the suppository components concerned. Therefore, the accuracy estimated by analysis of the simulated preparation, although it proved acceptable, cannot be strictly considered true. Accordingly, Table II shows precision and accuracy data, both obtained on the simulated preparations, for each component, as well as the results on several lots of each commercial preparation examined. As is evident, both precision and accuracy also are excellent for components (chlorpheniramine maleate and lidocaine) present in very small quantities. However, this precision and accuracy require optimum experimental conditions, particularly a frequent check of the calibration curves. For the commercial samples, as expected, the relative standard deviations are higher, especially for chlorpheniramine maleate and sodium benzoate, due to the added error associated with the manufacturing process.

In conclusion, it can be stated that the GLC analysis appears to be particularly suitable for the routine control of preparations of the types examined. In fact, this technique provides a simple way of obtaining precise and specific results with noticeable time and manpower savings.

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