tures above the T_g. Thermograms of these systems showed an exotherm followed by an endotherm, indicating crystallization followed by melting.

Citric acid and phenobarbital seem to form glass solutions rather than dispersions as in the case of citric acid and benzoic acid. Visual examination of the melts of citric acid and phenobarbital mixtures in a capillary tube, under a hot-stage microscope[11], and during bulk preparation showed them to be a single homogeneous phase.

The *in situ* procedure is useful for screening potential glass-forming materials and glass-forming drug–carrier combinations. Physical mixture and solidified melt thermograms and the T_g data indicate whether a drug–carrier mixture will form a glass solution or suspension and what optimum drug–carrier combination can be obtained. In systems that exhibit mechanical and/or thermal instability or that have a T_g below or only slightly above room temperature, the material would not be rigid enough to withstand normal handling operations and could be eliminated from further investigation.

Of particular interest would be the preparation of glass systems containing large quantities of drugs. For example, it might be possible to stabilize the glassy state of a glass-forming drug by mixing it with a small amount of a compatible inert glassy carrier. This procedure would permit the use of less carrier, thereby reducing bulk volume, and aid in the formulation of glass dispersion systems into solid dosage forms.

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Paired-Ion Reversed-Phase High-Pressure Liquid Chromatographic Assay of Pentobarbital–Pyrilamine Suppositories

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Received July 17, 1978, from the *School of Pharmacy, Oregon State University, Corvallis, OR 97331.* Accepted for publication October 23, 1978.

Abstract □ The assay of suppositories containing pentobarbital and/or pyrilamine in a water-soluble polyethylene glycol base by high-pressure liquid chromatography is described. No extraction is required. The suppository is dissolved in the mobile phase. This solution is diluted with an internal standard stock solution containing phenobarbital. Chromatographic conditions include a C_{18} bonded microporous silica column and a mobile phase of 65% 4×10^{-3} *M* *n*-butyl sodium sulfonate in 1% acetic acid and 35% acetonitrile. The procedure using commercial products gave results comparable to those obtained by GLC.

Keyphrases □ Pentobarbital–pyrilamine suppositories—analysis, paired-ion reversed-phase high-pressure liquid chromatography, compared to GLC □ Suppositories, pentobarbital–pyrilamine—analysis, paired-ion reversed-phase high-pressure liquid chromatography, compared to GLC □ High-pressure liquid chromatography—analysis, pentobarbital–pyrilamine suppositories, compared to GLC

A pharmacokinetic study involving suppositories containing pentobarbital and/or pyrilamine maleate required in-house manufacturing. This need led to the development of an assay for accurately determining the drug content and uniformity in each lot of suppositories. The rapid high-pressure liquid chromatographic (HPLC) procedure was verified by GLC using commercial products.

Several GLC assays for barbiturates (1–3) and antihistamines (4–7) have been published. In each, appropriate extraction procedures must be carried out to separate the acidic barbiturate from the basic antihistamine. Because the suppositories were made using a water-soluble polyethylene glycol base, reversed-phase HPLC seemed to be the logical approach.

EXPERIMENTAL

Reagents and Chemicals—Authentic samples of pentobarbital sodium[1], pyrilamine maleate[1], phenobarbital[2], propylparaben[1], and

[1] City Chemical.
[2] Mallinckrodt.

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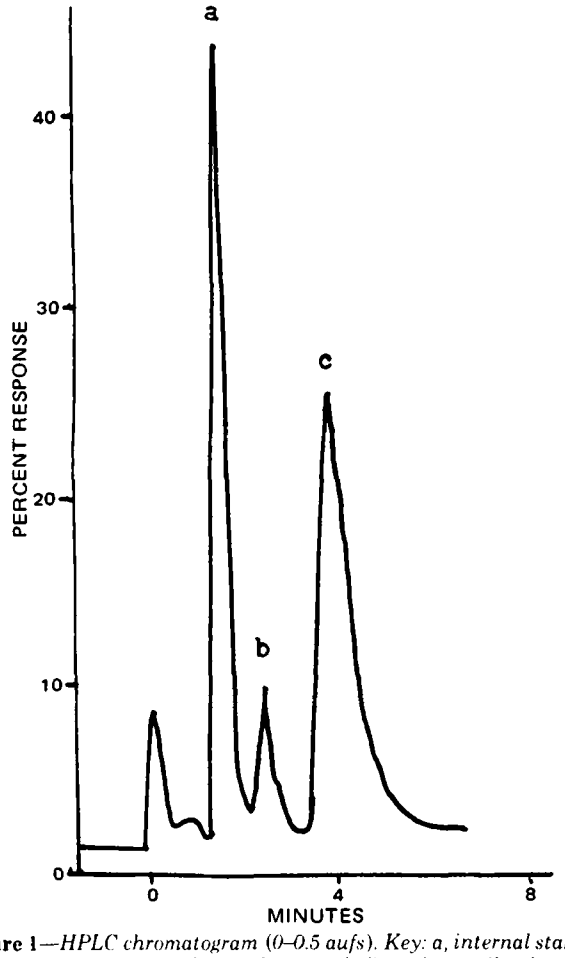

Figure 1—*HPLC chromatogram (0–0.5 aufs). Key: a, internal standard (phenobarbital); b, pentobarbital (3.5 mg/ml); and c, pyrilamine (0.972 mg/ml).*

chlorpheniramine maleate[3] were used. Reagent grade solvents were used as received. *n*-Butyl sodium sulfonate[4] was used as the anion that paired with pyrilamine. Commercial suppositories containing pentobarbital and pyrilamine maleate were purchased from local pharmacies.

HPLC Conditions—The mobile phase consisted of two parts. Solution A consisted of 1% (v/v) acetic acid and 4×10^{-3} *M* *n*-butyl sodium sulfonate (640 mg/liter). Solution B was acetonitrile. Each solution was degassed by stirring under a vacuum. They were then mixed in the ratio of 65A:35B by a solvent-flow programmer[5].

A liquid chromatograph was equipped with two constant-flow pumps[6], an on-stream injector[7], a variable-wavelength spectrophotometer[8], a 254-nm dual-pen recorder[9], and a 25-cm column packed with 10-μm porous silica chemically bonded with octadecylsilane[10]. The flow rate was 2.0 ml/min. Phenobarbital was the internal standard. An injection volume of 10 μl was used for all assays.

Each product was analyzed three times, and each analysis was done in triplicate. The spectrophotometer was set at 254 nm.

GLC Conditions—A gas chromatograph was equipped with a flame-ionization detector[11] and a 180-cm, 2-mm i.d., 6-mm o.d. U-shaped column packed with 3% phenylmethyl silicone on a silane-treated 100–120-mesh support[12]. The oven temperatures were 175 and 220° for pentobarbital and pyrilamine, respectively. For both drugs, the settings were: flash heater, 280°; detector, 310°; nitrogen carrier gas, 35 ml/min; hydrogen, 60 ml/min; and air, 400 ml/min.

[3] Amend Drug and Chemical Co.
[4] Eastman Kodak Co.
[5] Waters Associates model 660.
[6] Waters Associates model ALC 201/202 with M-6000 pumps.
[7] Waters Associates model U-6K.
[8] Varian Technicon model 635 LC.
[9] Soltec.
[10] Waters Associates μBondapak C_{18}.
[11] Hewlett-Packard model 402B.
[12] Commercially coated OV-17 on Gas Chrom Q, Applied Science Laboratories.

A 5-μl injection volume was used for all assays. All injections were made in triplicate. Each product was analyzed three times.

HPLC Stock Solutions—*Phenobarbital Internal Standard Solution*—Phenobarbital (2.200 g) was dissolved in 500 ml of warmed mobile phase. The solute remained in solution at room temperature.

Pyrilamine Stock Solution—Pyrilamine maleate, 205 mg, was dissolved in 100 ml of the mobile phase.

Pentobarbital Stock Solution—Pentobarbital sodium was dissolved in distilled water, acidified with 10% (v/v) HCl, extracted two or three times with chloroform, evaporated to dryness, and recrystallized from hot water. Then 0.700 g was dissolved in 100 ml of mobile phase.

GLC Stock Solution—Propylparaben, 400 mg, and chlorpheniramine maleate, 160 mg, were dissolved in 40 ml of ethanol and brought to 100 ml with distilled water. This stock solution was diluted 1:10 with distilled water.

GLC Standard Solutions—A separate standard solution was prepared for each suppository strength by dissolving the appropriate amount of each drug in ~10 ml of ethanol and bringing to 50 ml with distilled water. Each standard solution contained both pentobarbital and pyrilamine maleate in amounts equivalent to labeled amounts.

HPLC Procedure—*Standard Curve*—The stock solutions for each drug were diluted in the following manner. Solution 1 was prepared by diluting each stock solution 1:1 with the internal standard solution to yield a 1:2 dilution. For Solution 2, each stock solution was diluted 1:1 with mobile phase and then this new solution was diluted 1:1 with the internal standard to yield a 1:4 dilution. Solution 3 was made by diluting each stock solution 1:4 with mobile phase and then diluting this solution 1:1 with the internal standard, yielding a 1:8 dilution.

Each of the three standard solutions was prepared in triplicate, and 10 μl of each solution was injected on the column.

Suppositories—Commercially available suppositories containing 50 mg of pyrilamine maleate and 100 mg of pentobarbital were dissolved by shaking a suppository and about 30 ml of mobile phase in a 50-ml volumetric flask for 30 min. The suppositories containing lesser amounts of pentobarbital were dissolved by shaking a suppository and about 15 ml of mobile phase in a 25-ml volumetric flask for 30 min. Both solutions were brought to volume with additional mobile phase. Each resulting

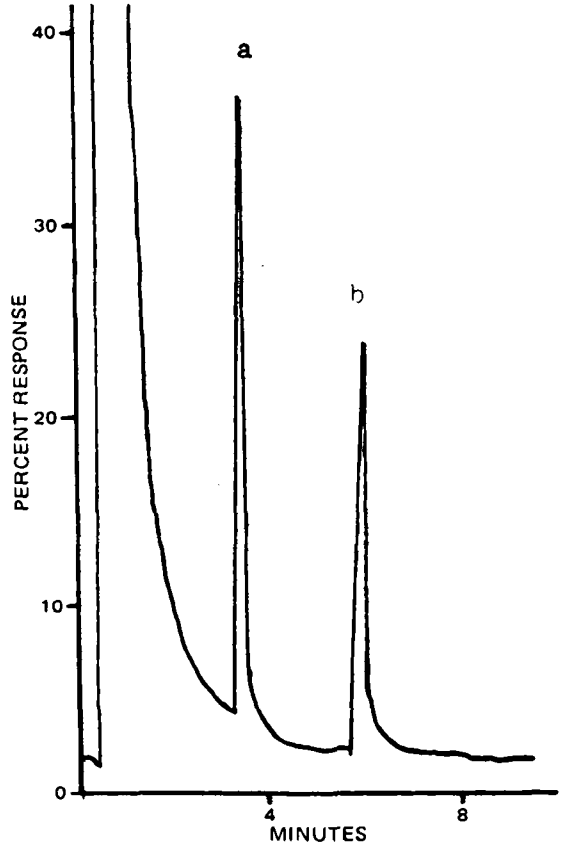

Figure 2—*GLC chromatogram (2.5×10^{-10} amps full scale). Key: a, internal standard (propylparaben); and b, pentobarbital sodium.*

Table I—GLC and HPLC Analyses of Commercial Samples

Suppository	Label, mg	Found by GLC[a], mg	Found by HPLC[a], mg	CV[b], %
Pentobarbital	30	29.21 (28.28–30.28)	28.96 (27.66–30.75)	3.04
Pyrilamine	25	24.05 (23.01–25.04)	24.10 (23.12–24.96)	2.25
Pentobarbital	45	43.79 (42.56–43.35)	44.21 (42.99–45.24)	1.81
Pyrilamine	50	48.32 (47.44–49.44)	48.38 (47.24–49.69)	1.65
Pentobarbital	100	99.47 (97.80–101.26)	96.22 (94.43–98.35)	1.64
Pyrilamine	50	47.20 (46.09–48.06)	47.48 (46.41–48.70)	1.73

[a] Average of nine injections (range). [b] Percent coefficient of variation = (*SD*/mean) × 100.

suppository stock solution was diluted 1:1 with internal standard stock solution and injected.

GLC Procedure—A suppository was placed in a 50-ml volumetric flask and dissolved in distilled water by shaking. The assay preparation for pentobarbital consisted of 2.0 ml of the suppository solution and 2.0 ml of the internal standard solution, both of which were placed in a 10-ml screw-capped vial. The pentobarbital and its propylparaben internal standard were extracted by adding 2 drops of concentrated hydrochloric acid and 2 ml of ether and shaking for 5 min. After the layers separated, 5 μl of the ether layer was injected.

The assay preparation for pyrilamine was prepared by placing a fresh 2.0-ml aliquot of the solution containing the suppository and 2.0 ml of the internal standard solution into a 10-ml screw-capped vial. A drop of phenolphthalein test solution was added, and the solution was made basic by the dropwise addition of 10 *N* NaOH. Ether, 2 ml, was added, and the mixture was shaken for 5 min. After the layers separated, 5 μl of the ether layer was injected.

These extraction procedures were repeated using 2.0-ml quantities of the stock solution corresponding to the labeled content of the suppository to be analyzed.

HPLC Calculations—A standard curve was constructed for each drug.

GLC Calculations—The following formula was used:

$$\text{mg of drug/suppository} = (0.05C)\left(\frac{b}{a}\right)\left(\frac{A}{B}\right) \qquad \text{(Eq. 1)}$$

where C = concentration (micrograms per milliliter) of drug in the standard preparation, A = peak height of the internal standard in the standard preparation, B = peak height of the drug in the standard preparation, a = peak height of the internal standard in the assay preparation, and b = peak height of the drug in the assay preparation.

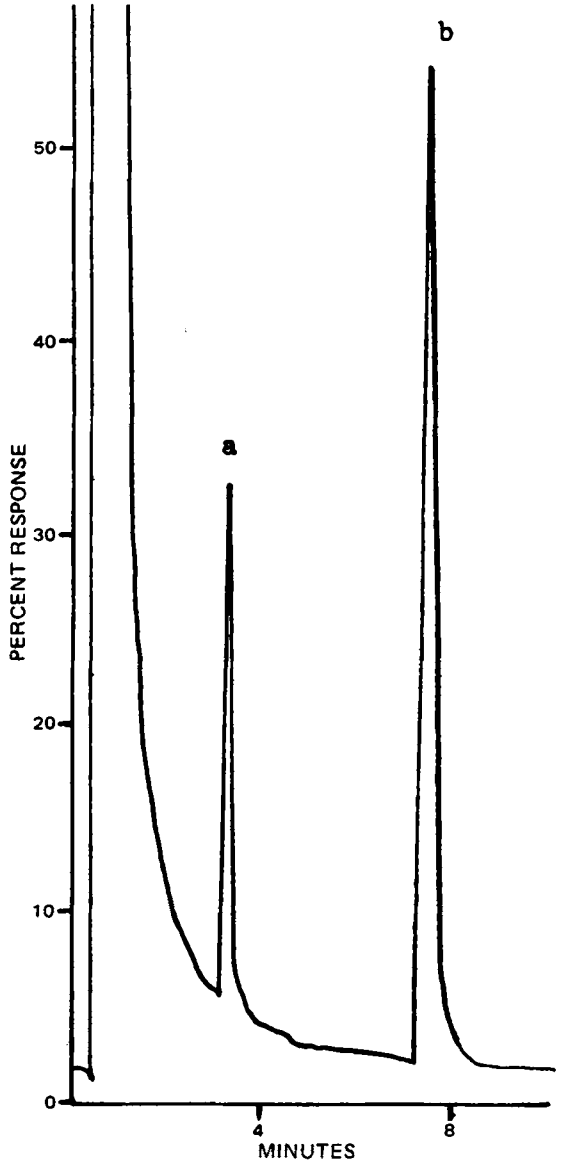

Figure 3—*GLC chromatogram (2.5 × 10⁻¹⁰ amps full scale). Key: a, internal standard (chlorpheniramine maleate); and b, pyrilamine maleate.*

RESULTS AND DISCUSSION

The objective of this study was to develop a rapid procedure for the quantitative analysis of suppositories containing pentobarbital and/or pyrilamine maleate. The described HPLC procedure eliminates the extraction steps required for GLC. The water-soluble suppository base and dyes are eluted from the reversed-phase column with the solvent front and, therefore, do not interfere with the assay. In the concentration range studied, the standard curves are straight lines: r = 1.000 for both drugs; coefficient of variation = 2.76% for pyrilamine maleate and 3.64% for pentobarbital.

Figure 1 shows a typical HPLC tracing, and Figs. 2 and 3 show GLC tracings. Table I contains a comparison of GLC and HPLC results. The only significant difference is ease of sample handling. With this particular type of suppository base, HPLC is superior since no extractions are required.

The use of the paired-ion technique produced the best separation. Initially, an acid-buffered mobile phase was tried. Good retention times were obtained for phenobarbital, but the protonated pyrilamine was eluted with the solvent front. The results were worse with a basic mobile phase. The charged barbiturate was eluted with the solvent front and pyrilamine, while having an acceptable retention time, showed considerable tailing. This procedure does not appear to affect the column since this same column was used for several months after completion of this project.

It is obvious from the chromatogram shown in Fig. 1 that pentobarbital has low sensitivity relative to the antihistamine. At the same time, pyrilamine has a very good UV-sensitive chromophore. Although a variable-wavelength detector was utilized, the 254-nm wavelength common to most fixed-wavelength detectors was selected to see if the procedure could be adapted to most instruments. Nevertheless, a wavelength closer to the end absorption of unionized pentobarbital (230 nm and lower) or perhaps a refractive index detector would produce a more esthetically satisfying chromatogram (8). An alternative procedure for obtaining a more sensitive response from pentobarbital would be to utilize the recently described postionization technique whereby pH 10 borate buffer is added to the column effluent before it enters the detector (9).

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Percutaneous Nitroglycerin Absorption in Rats

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Received June 16, 1978, from the *Department of Pharmaceutics, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication October 24, 1978. *Present address: Pharmacy Research and Development Department, Ayerst Laboratories, Rouses Point, NY 12979.

Abstract □ Percutaneous nitroglycerin absorption was studied in shaved rats by monitoring unchanged plasma drug concentrations for up to 4 hr. Drug absorption from the neat liquid state or from an alcoholic solution was considerably poorer than that from a commercial ointment. This observation was unanticipated since the driving force for percutaneous drug absorption was assumed to be drug thermodynamics. Potential artifacts such as drug volatilization from the skin, reduction of surface area through droplet formation, and vehicle occlusion were investigated, but they did not appear to be responsible for the observed results. Two experimental aqueous nitroglycerin gels were prepared with polyethylene glycol 400. One gel contained just sufficient polyethylene glycol to solubilize the nitroglycerin; the other had excess polyethylene glycol to solubilize nitroglycerin far below saturation. Both gels gave extremely low plasma nitroglycerin levels. The composite data suggested that percutaneous nitroglycerin absorption is highly vehicle dependent and that this dependency cannot be explained by simple consideration of drug thermodynamic activity.

Keyphrases □ Absorption, percutaneous—nitroglycerin, various topical dosage forms, rats □ Nitroglycerin—percutaneous absorption, various topical dosage forms, rats □ Dosage forms, topical—nitroglycerin, percutaneous absorption, rats

Topical nitroglycerin ointments produce sustained and clinically beneficial hemodynamic responses in patients with various cardiovascular ailments. Application of a 2% nitroglycerin preparation on 150–230 cm^2 of skin surface reduced the frequency and severity of exercise-induced angina attacks (1, 2) and decreased heart workload and myocardial oxygen consumption (3).

Systemic availability is the primary goal of topical nitroglycerin application. However, there is little information about the physical and physiological factors influencing its percutaneous absorption. The relatively large clinical nitroglycerin ointment doses and the extensive surface area needed for therapeutic effect suggest that transdermal delivery of nitroglycerin, at present, is inefficient and that attempts to increase percutaneous nitroglycerin absorption may be worthwhile.

The purpose of this investigation was to examine various factors that may affect the rate and extent of topical nitroglycerin absorption. *In vivo* and *in vitro* models were used to gather data to form a rational basis for improving transdermal drug delivery. The relationship between thermodynamic activity and *in vivo* nitroglycerin absorption was examined.

BACKGROUND

The skin is one of the most impermeable tissues of the body. It functions as a barrier against attack by microorganisms, viruses, and many toxic chemicals. At the same time, it limits the loss of physiologically essential components, such as water. The extensive skin barrier properties have caused it to be regarded as a poor route for systemic drug administration. Recently, serious attempts have been made to fabricate devices for controlled, sustained delivery through the dermal route. A tape capable of delivering a constant rate of transdermal scopolamine for up to 3 days was described (4).

Several factors influence percutaneous drug absorption (5, 6) including drug permeability through the stratum corneum, the drug's physical–chemical properties, the vehicle in which the drug is incorporated, the drug's ionization state, the skin's hydration state, the skin's lipid content, and regional variations in skin properties. The quantitative relationship between drug transfer and several of these factors is (7):

$$dQ/dt = KC_vDA/T \qquad \text{(Eq. 1)}$$

where dQ/dt is the steady-state drug transfer rate across a skin barrier, K is the effective skin–vehicle drug partition coefficient, C_v is the drug concentration dissolved in the vehicle, D is the drug diffusivity through the barrier, A is the surface area of application of the vehicle, and T is the effective barrier thickness.

The model assumes that the skin represents a simple diffusional barrier to drug transfer. When the surface area of application, drug diffusivity through the skin, and barrier thickness are constant, the equation addresses the role of thermodynamics in determining percutaneous absorption rates. Specifically, absorption will be enhanced from vehicles with a low affinity for the drug and in which the drug concentration approaches saturated solubility. Therefore, approaches toward optimizing drug delivery should maximize the drug's thermodynamic activity in the vehicle, as represented by the product term KC_v in Eq. 1. The validity of the thermodynamic relationship has been confirmed qualitatively (8, 9), and Eq. 1 has provided valuable guidance in the development of improved vehicles for topical application of new drug entities.

While the thermodynamic approach does include skin characteristics—*viz.*, D and T, in determining absorption rate, these parameters are usually difficult to measure as functions of vehicle changes. Therefore, they are often assumed to be independent of vehicle effects. This assumption is not always valid since topical formulations are known to affect these parameters (5, 6, 10). Vehicle-induced changes in these variables may be of sufficient magnitude to override the contribution of the thermodynamic term, KC_v. In such cases, thermodynamic considerations alone, although predictive for *in vitro* drug release, may not explain absorption differences in animals or humans.

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