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THE DETERMINATION OF THE STABILITY OF METAPROTERENOL SULFATE INHALENT SUCCINYLCHOLINE CHLORIDE INJECTION, AND THIAMYLAL SODIUM INJECTION IN PLASTIC SYRINGES AND GLASS SYRINGES OR VIALS

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THE DETERMINATION OF THE STABILITY OF METAPROTERENOL SULFATE INHALENT, SUCCINYLCHOLINE CHLORIDE INJECTION, AND THIAMYLAL SODIUM INJECTION IN PLASTIC SYRINGES AND GLASS SYRINGES OR VIALS

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ABSTRACT

THE DETERMINATION OF THE STABILITY OF METAPROTERENOL SULFATE INHALENT, SUCCINYLCHOLINE CHLORIDE INJECTION, AND THIAMYLAL SODIUM INJECTION IN PLASTIC SYRINGES AND GLASS SYRINGES OR VIALS

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The stability of Succinylcholine Chloride, Metaproterenol Sulfate, and Thiamylal Sodium was determined. Each drug was repackaged into plastic syringes and glass vials or syringes and stored for periods from 30 to 70 days at 4°C, 22°C, and 37°C (Thiamylal was stored only at 4°C). Drug degradation was followed by monitoring active ingredient concentration, pH, clarity, color, weight change, and sterility.

It was determined that sterility, clarity, and original color were maintained throughout the study (except Metaproterenol--a yellow tinge developed within 45 days storage). Significant weight loss was seen only in drug stored in plastic syringes at 37°C. At 4°C storage, Succinylcholine maintained USP acceptable pH and concentration for at least 45 days, Metaproterenol maintained concentration within acceptable Boehringer Ingelheim limits for at least 70 days, and Thiamylal potency and pH remained within USP limits for at least 14 days.

This thesis is dedicated to my loving husband, Vincent,

in appreciation for his great patience and wisdom.

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INTRODUCTION

Unit dose pharmaceutical packaging gives the hospital pharmacist the greatest control over "in-patient" drug therapy (i.e., correct drug and dosage) and minimizes contamination risk. Pharmaceutical manufacturers, however, market a limited selection of types and concentrations of drugs in unit dose packaging; those which are available are so at an increased cost to the pharmacy. It is because of this limited availability and/or increased cost that the hospital pharmacist must often repackage certain drugs into unit dose form. After repackaging, the pharmacist does not know how the new package affects drug stability and therefore what expiration date should be assigned. A survey conducted earlier this year (Chaney and Summerfield, 1984) found that a broad assortment of arbitrarily assigned expiration dates are being used when bulk drugs are repackaged even though Current Good Manufacturing Practices (21 C.F.R. 211.137), the United States Pharmacopeia (USP, 1980), and the American Society of Hospital Pharmacists (ASHP, 1977) recommended expiration dates should ideally be supported by stability data.

The purpose of this thesis is to determine the stability of three pharmaceuticals, Succinylcholine Chloride, Metaproterenol Sulfate, and Thiamylal Sodium, in plastic unit dose syringes and to compare these results to those obtained with the dispensing unit for these drugs used presently by the hospital pharmacist. The stability

information gleaned from this study will aid the hospital pharmacist in selecting the most cost effective unit dose repackaging system which will adequately protect the product as well as in assigning accurate expiration dates.

LITERATURE REVIEW

General Background

One definition for a unit dose package is ". . . one which contains the particular dose of drug ordered for the patient" (ASHP, 1979). Innovative hospitals in the sixties initiated unit dose drug distribution in pilot programs involving one ward at a time. This system provided ". . . improved safety, control, convenience, and utilization of human resources; and more accurate dosage" (Roulette, 1972); better utilization of human resources because pharmacists would be used to their fullest capacity and nursing time, previously spent on preparing drugs for patient administration, could now be spent on delivering optimal nursing care (Latiolais, 1970; S.G.K., 1971). Some of the benefits resulting from the introduction of unit dose packaging, specifically for prefilled syringes, were cited by Elias and Apat (1965): assured accurate dosage and sterility, elimination of a source of serum hepatitis, less potential for allergic sensitization, better utilization of nursing time, and less waste of drugs due to pilferage, breakage, or incomplete use. Varnum (1974) characterizes the unit dose system as a "comprehensive, well controlled, well managed drug distribution mechanism" that does in fact reduce pharmacy costs. Costs are reduced because improved inventory control decreases acquisition costs and helps to eliminate waste or pilferage of drugs (Varnum, 1974; Hart and Marshall, 1976).

General acceptance and usage of the unit dose system in hospital pharmacies continues to increase. In 1970, a questionnaire (McDonald et al., 1972) found that 94.4% of the 144 responding hospitals were utilizing purchased prefilled syringes and 12% of the respondents were filling their own syringes. This survey also indicated that prefilled syringes for products not commercially available in unit-of-use syringes was perceived as an important function of the hospital pharmacist. This author goes on to cite the disadvantages of nurses filling syringes: particulate matter could go unnoticed due to poor lighting, contamination risk is greater due to atmospheric air entering the vial being drawn from, nurses generally fail to label syringes thereby increasing the risk of mixup with other medication, and there is greater chance of dosage calculation errors with nurses as compared to pharmacists.

Increased utilization of the unit dose system should continue, as the Minimum Standards for Pharmacies in Institutions (1977) states that all drugs should be dispensed in single-unit packages--". . . the unit dose system of preparing and distributing drugs should be used"; these Standards also say that drugs should be unit dose repackaged by the pharmacist, when feasible, in cases where an item fulfilling a need is not commercially available. Romberg (1979) states that the use of unit dose systems in hospitals is increasing, 76% of hospitals use the unit dose system to dispense more than 50% of their daily doses, but that the trend is toward purchasing supplier produced unit dose packaged drugs; these statements are based on a survey sent to 3,000 U.S. hospital chief pharmacists, with a 15% response rate.

Unit dose packaging may minimize contamination risks that are present when using multi-use vials (multidose vials or MDV's) or bottles on nursing wards, in respiratory therapy and in operating rooms (Talley et al., 1973). Microbially contaminated medications are a potential source of nosocomial infection which can at best complicate the hospitalized patients' recovery and at worst result in death. Several studies indicate that while the contamination risk in using MDV's is low, it has happened, and the potential indeed is there (Highsmith et al., 1982; Sanders et al., 1970; Moffet and Allan, 1967; Alford et al., 1966).

Unit dose packages have been shown to be a feasible alternative to MDV's with respect to maintainence of sterility, stability, and reducing costs. Talley et al. (1973) found that when seven inhalation therapy drugs were repackaged into 2 ml glass cartridges, stability and sterility were maintained throughout their six month study. Sheth et al. (1983) found that a cost savings of 46% could be realized when MDV's were replaced with unit dose alternatives; this author found that when MDV's are used on nursing wards, 25% or less of the original vial volume was used before the drug reached its expiration date.

Stability Studies

It is extremely important to determine the stability of unit dose repackaged drugs so that an expiration date can be declared which is accurate and insures that the drug is not wasted by being discarded prematurely. Current Good Manufacturing Practice Regulations state that stability testing should provide the basis for the expiration

date assigned to a packaged drug (21 CFR 211.137). The United States Pharmacopeia (1980) defines stability as ". . . the extent to which a product retains, within specified limits, and throughout its period of storage and use, i.e., its shelf-life, the same properties and characteristics that it possessed at the time of its manufacture." The USP defines five types of stability:

1. Chemical--labeled potency is maintained;
2. Physical--active ingredient maintains potency;
3. Microbiological--sterility is maintained;
4. Therapeutic--therapeutic effectiveness unchanged; and
5. Toxicological--no significant toxicity increase.

The American Society of Hospital Pharmacies (ASHP) published guidelines for unit dose drug packages in 1977 which state: "drug packages must fulfill four basic functions: (1) identify their contents completely and precisely; (2) protect their contents from deleterious environmental effects (e.g., photodecomposition); (3) protect their contents from deterioration due to handling (e.g., breakage, contamination); (4) permit their contents to be used quickly, easily, and safely." These guidelines also assert that the package material itself should not decompose over the shelf life of its contents, should not absorb or adsorb or otherwise deleteriously affect the drug they contain. The package should be easy to use and open. The package should allow direct administration of the drug to the patient or inhalation device.

In 1983 an article by Nedich described a variety of packaging materials available for injection drugs. Nedich states, "No container or closure material is totally inert"--not glass or plastic. He goes on to explain that glass is composed of a mixture of oxides, some quite loosely bound and free to migrate and leach into the preparation. These migrated or leached oxides may alter pH or act as a catalyst or reactant. Plastics may contain a variety of additives, such as lubricants and stabilizers, which can leach into the drug stored in contact with the plastic. Rubber also has a variety of components which may leach, such as reaction by-products, plastisizers, oils, and oxides.

After a thorough review of the literature it was determined that no stability studies have been conducted on the three drugs chosen for this study. As the research done on unit dose packaging of other drugs is described in the following paragraphs, one must bear in mind at all times that it can be inaccurate and misleading to equate the results obtained with a particular drug in a given package to either the same drug in a different package or a different drug in the same package. This is because each drug and package are chemically unique and the degradation of the drug in the package is affected by the interaction of the two. In 1972 Hicks et al. studied the stability of Sodium Bicarbonate injection stored in two different brands (Becton-Dickinson and Travenol) of polypropylene syringes stored at 12-14°C, 22-23°C, and 37-38°C. Hicks found that the shelf life of this drug is inversely related to storage temperature; while the rate of pH change increased as temperature increased, there was no significant difference in this rate of pH change or final pH's between the two

different syringes. Using spectrophotometric analysis, after the packaged drugs had been stored for 145 days, the drugs packaged in Becton-Dickinson syringes showed no evidence of chemical contamination but the Travenol syringes did; the rubber plunger was the suggested source of this observed contamination.

Kleinberg et al. (1980) used the Arrhenius Technique to determine the stability of five liquid drugs in four different clear or amber glass syringe-type (Hy-Pod, Ped-Pod, and Nebuject) packages. Each drug was stored in a single type of glass package--there was no attempt made to compare the stability of a given drug in different packages.

In 1982 Nolly et al. repackaged Thiamine Hydrochloride injection into glass and plastic syringes and found that the solution in both syringe types maintained at least 100% potency for 84 days at 22-24°C. It was also determined that glass syringes have a lower oxygen transmission rate than plastic syringes. Zvirblis and Ellin (1982) compared the stability of an organophosphate antidote packaged in glass and plastic cartridges (they failed to mention manufacture's names and cartridge size) and found no significant difference in pH or concentration between glass and plastic cartridges after four months storage at 5°C. These authors estimate water loss to be approximately 1% per year for glass and 2% per year for plastic at 25°C.

Valproate Sodium syrup stability, when repackaged in three different unit dose packages was compared when stored at 4, 25, and 60°C (Sartnurak and Christensen, 1982). The three packages compared

were 5 ml amber polypropylene oral syringes, 3-4 ml clear glass oral syringes and 15 ml amber glass vials. No significant difference was found between the two glass packages, but there was a significant difference in concentration between the plastic package and the glass packages--the glass vials and syringes maintained at least 95% of the label claim after 180 days storage at 4°C and 25°C while in plastic the concentration decreased to 88.5% of the label claim after 20 days at 25°C but maintained 90% potency for at least 90 days at 4°C. This increased loss of concentration seen in the plastic packages was attributed to sorbtion of the drug into the polypropylene material.

Vancomycin repackaged into amber glass unit dose vials (Mallet et al, 1982, maintained USP recommended potency for at least 90 days at 0 and 4°C; it was recommended that Vancomycin, repackaged in these vials, not be stored at 25°C because a precipitate formed within 6 days storage. A similar study was conducted to evaluate the stability of Insulin repackaged in 1 ml polypropylene syringes (Zell and Paone, 1983) with the finding that this system was stable for at least 14 days under "refrigeration."

In 1983 Christensen et al. found no significant difference in the concentration of Furosemide and Cimetidine Hydrochloride repackaged in either polypropylene oral syringes or glass vials when stored at 4°C or 25°C. At higher temperatures (i.e., 44, 60, and 76°C), the degradation rate increased for the drugs packaged in the polypropylene syringes.

To conclude, the above studies indicate what one would expect to find, that drug degradation increases with increased storage temperature and time. It can be further concluded that the stability of any drug is unique to the system comprised of that drug and the package it is stored in. Therefore, it is advisable to study the stability of Succinylcholine Chloride injection, Metaproterenol Sulfate inhalent, and Thiamylal Sodium injection in glass and polypropylene packages.

MATERIALS AND METHODS

Materials

Three pharmaceuticals were chosen for unit dose stability studies: Succinylcholine Chloride injection (a short-acting depolarizing skeletal muscle relaxant), Metaproterenol Sulfate inhalent (a bronchodilator), and Thiamylal Sodium injection (an ultrashort-acting barbiturate). These drugs were identified by Directors of Pharmacy of two Lansing hospitals as excellent candidates for this study because all three are currently unit dose repackaged and the effect the new package has on the drug's stability is unknown. The Directors expressed interest in having documentation of the stability of the pharmaceuticals in the unit dose package currently used in their respective pharmacies, glass syringes or vials, as well as in a less expensive plastic alternative. This information would provide them with bases for both selecting the most economical unit dose repackaging system and assigning accurate expiration dates.

Succinylcholine Chloride injection (Quelicin manufactured by Abbott Laboratories--lot #55-607-DK) in 20 mg/ml concentration was repackaged from the 10 ml fliptop vials, received from the manufacturer, to 5 ml BD glass (reorder number 5293) (current unit dose package used at St. Lawrence Hospital pharmacy) and polypropylene (reorder number 5603) syringes. These syringes were filled to capacity at 5 ml and stoppered with BD rubber luer tip caps (reorder number

8341). The specifics on the rubber and polypropylene formulations were unavailable from the manufacturer.

Metaproterenol Sulfate inhalent (Alupent manufactured by Boehringer Ingelheim Ltd.--lot #713013A) was repackaged from the 10 ml bottles received from the manufacturer into 10 ml Invenex glass vials (number SV-5) (current unit dose package used at Ingham Medical Center Pharmacy) and 6 ml Monoject polypropylene syringes (reorder number 8881-516911). Pharm-Aide Syringe Caps (Pharmaseal Laboratories catalog number 7820), made from plastic, were used to seal the syringes. The Metaproterenol Sulfate was diluted from 5% w/v concentration as supplied by the manufacturer to the patient dosage--.45% w/v and filled to 5 ml in each test package.

Thiamylal Sodium injection (Surital manufactured by Parke-Davis--lot #03573P) was received from the manufacturer as 10 grams of powder in a 500 ml IV style bottle (Steri-vial 123) and was diluted to a concentration of 2.5% w/v using sterile water for injection, USP (Abbott Laboratories--lot #49-505-DM-01). The solution was tested in this original vial (400 ml fill volume) as well as repackaged into Monoject 20 ml polypropylene syringes (reorder number 8881-520046) (20 ml fill volume) sealed with Pharm-Aide Syringe Caps (Pharmaseal Laboratories catalog number 7820).

Repackaging Operation

All three pharmaceutical repackaging operations were handled by the respective hospital pharmacy personnel in the manner normally used by each to unit dose repackage these pharmaceuticals. In the

case of *Succinylcholine Chloride*, a 20 ml syringe was filled from two 10 ml vials and then 5 ml transferred to each 5 ml syringe and capped. After repackaging, the glass syringes currently in use at St. Lawrence are stored at 4°C and assigned a 30 day expiration date.

Metaproterenol Sulfate was prepared by transferring the total quantity of undiluted drug needed from the manufacturer's bottles to a sterile IV style glass bottle. To this IV bottle next was added the total quantity of saline necessary to obtain patient dosage concentration (.45% w/v). Syringes and vials are filled with 5 ml of the diluted Metaproterenol Sulfate from this IV bottle. The syringes were sealed with plastic caps. The vials are the current repackaging system in use at Ingham Medical Center Pharmacy and are stored at 4°C for 30 days maximum.

Finally, *Thiamylal Sodium* is diluted in the vial, as supplied by the manufacturer, with 400 ml sterile water to 2.5% w/v. From this vial, 20 ml of the drug were drawn into each 20 ml plastic syringe. The syringes were sealed with plastic caps. Currently, Ingham Medical Center Pharmacy supplies this drug to two different sources: in-patient surgery and outpatient surgery. The pharmacy dispenses Thiamylal Sodium to in-patient surgery in the manufacturer's vial each surgical day and surgery stores the vials at 4°C. At the end of each surgical day, any leftover drug is discarded.

In the case of out-patient surgery, the exact amount of Thiamylal Sodium required for the number of patients scheduled on a given surgical day is dispensed by pharmacy in 20 ml plastic syringes. These syringes are stored by surgery, until needed, at 4°C.

Storage Treatments

The pharmacy directors specified preferred lengths of storage for each repackaged pharmaceutical on the basis that this amount of time would offset the handling costs incurred with unit dose packaging. These time lengths are given in Table 1.

Table 1. Desirable expiration dates for unit dose repackaged pharmaceuticals

Pharmaceutical	Minimum Length of Storage Preferred (Days)	Optimal Length of Storage Preferred (Days)
Succinylcholine Chloride	14	30
Metaproterenol Sulfate	30	60
Thiamylal Sodium	6	30

It is these times listed in Table 1 which determined the length of storage of each drug in the study as well as the time intervals selected for stability analysis. These storage conditions and associated lengths of storage are summarized in Tables 2, 3, and 4. Five replicates of each sample were subjected to each treatment described (storage period, package type, and temperature) with the exception of the glass vial of Thiamylal Sodium. A single glass vial of Thiamylal Sodium was subjected to each treatment. This was because of the exceptionally high cost of the drug. The repackaged pharmaceuticals were protected from direct exposure to light.

Table 2. Storage treatments--Succinylcholine Chloride

Type of package	Glass Syringe and Plastic Syringe			
Storage conditions	4°C, Ambient RH 22°C, 50% RH 37°C, 85% RH			
Storage periods (days)	0	5	30	45
Number of replicates	5	5	5	5

Table 3. Storage treatments--Metaproterenol Sulfate

Type of package	Glass Syringe and Plastic Syringe					
Storage conditions	4°C, Ambient RH 22°C, 50% RH 37°C, 85% RH					
Storage periods (days)	0	15	30	45	60	70
Number of replicates	5	5	5	5	5	5

Table 4. Storage treatments--Thiamylal Sodium

Type of package	Glass Syringe				Plastic Syringe			
Storage conditions	4°C, Ambient RH				4°C, Ambient RH			
Storage periods (days)	0	6	14	30	0	6	14	30
Number of replicates	1	1	1	1	5	5	5	5

Of the three conditions selected for storage of Succinylcholine Chloride, 4°C is of most interest because this is the manufacturer's recommended storage temperature. The 4°C and 22°C storage temperatures for Metaproterenol Sulfate are of the most interest because the manufacturer recommends storage at 25° C or below. The single storage temperature, 4°C, for Thiamylal Sodium was chosen because the manufacturer recommends the reconstituted solution be discarded after six days if "refrigerated" and 24 hours if kept at "room temperature"--indicating that the solution is quite unstable at higher than refrigeration temperatures. The temperature 37°C was included in both the Succinylcholine Chloride and Metaproterenol Sulfate studies because it is common practice to collect storage data at accelerated conditions.

Stability Analysis

Clarity and color change. The clarity of a pharmaceutical is very important because cloudiness may indicate formation of particulate matter or microbial contamination. The consequences of microbial contamination to a patient are both infection and possible shock, while particulate matter can act as emboli in the case of injectables or as an irritant in the case of inhalents.

The color change in a drug is also very important as it may indicate either a breakdown of the active ingredient or some harmless other change, such as a photochemical reaction with inert ingredients. From a physician's or nurse's point of view, however, presence of any unusual color in a pharmaceutical is undesirable. To these people, the

unusual color signifies possible loss of efficacy or source of potential harm to their patient, so such a drug would probably be discarded.

Therefore in assessing each drug's stability, a careful inspection was made to evaluate both clarity and color alteration. First, each sample was transferred directly from a syringe (those drugs stored in vials were first drawn into plastic syringes) to a glass 10 ml beaker (4.0 ml Succinylcholine Chloride and Metaproterenol Sulfate and 5.0 ml Thiamylal Sodium) and then individually inspected visually against opaque white and black backgrounds while comparing each sample to a freshly prepared control of the drug, identical to the experimental sample in concentration, lot number, and volume, also in a 10 ml beaker.

pH. Efficacy of a drug may be altered by a pH change. For this reason, pH monitoring was part of each periodic stability assessment. After evaluation for clarity and color change, the pH was measured using a digital ionanalyzer (Orion Research model 501) equipped with a combination glass pH electrode (Orion number 91-04). The pH meter was calibrated with three commercially prepared buffers: Mallinckrodt standard buffer solutions numbers 0029 and 0032 having pH's of 4.01 and 10.00 respectively; and MCB standard buffer solution number BX1635 having a pH of 7.00). The calibration was done using the two buffers whose pH's bracket the pH of the drug being measured. After rinsing the buffer from the electrode with distilled, deionized water, the electrode then was placed sequentially into each 10 ml

beaker containing drug. The meter was recalibrated after every five samples. pH was measured to two decimal places.

Measuring active ingredient concentration. Drug efficacy can be most directly related to active ingredient concentration. The active ingredient concentration of each of the three study pharmaceuticals was determined at the end of a storage period using high performance liquid chromatography (HPLC) for Succinylcholine Chloride and Metaproterenol Sulfate and gas liquid chromatography (GLC) for Thiamylal Sodium.

Succinylcholine Chloride active ingredient ($C_{14}H_{30}Cl_2N_2O_4$) concentration was assayed in accordance with the procedure published in the USP Supplement 3, found in Appendix A, with the following sections changed to read:

- Standard preparation--Initial moisture content of the USP Succinylcholine Chloride Standard (lot F) was 9% as determined by USP personnel and communicated to me by telephone. The standard was stored in its original vial within a glass weigh bottle sealed with silicone sealing lubricant; the weigh bottle was kept inside a glass desiccator filled with $CaSO_4$ desiccant and sealed with silicone sealing lubricant. To determine the water loss the standard was experiencing as the study progressed, a record was kept of the weight of the vial plus standard just before the vial was returned to storage each time and the weight of the same just before opening it again. The weight changes noted over time were assumed to be due to water loss and from this information, the percent moisture of the standard was

adjusted lower appropriately. The standard was prepared for the analysis according to the published method.

- Assay preparation--Succinylcholine Chloride samples and controls were prepared for analysis in several steps. The 4.0 ml of sample was transferred from the 10 ml glass beaker to a 10 ml glass volumetric flask following pH measurement. This transfer was accomplished by pouring the contents through a glass funnel into the flask and then rinsing the beaker three times with mobile phase (described in the published method) and pouring each rinse through the funnel. Finally, mobile phase was rinsed over the funnel into the flask. The 10 ml flask was then brought to volume with additional mobil phase.
- Chromatographic system--A Perkin-Elmer Series 3B Liquid Chromatograph was equipped with a variable wavelength Spectrophotometric Detector (LC75) set at 214 nm (UV) and a .26 cm x 25 cm stainless steel column that was packed with Silica-A (a porous silica--10 um). The flow rate was 1.0 ml per minute. A Spectra Physics SP4200 Computing Integrator was used to record the response peaks and to calculate the corresponding area ratios. To determine that the HPLC was responding in a linear and otherwise acceptable fashion, first, a standard curve was constructed by injecting in 2 µl, 4 µl, 6 µl, 8 µl, and 10 µl of standard prepared as described in the modified procedure and plotting the respective area responses against µl of injected sample. The resulting curve formed a straight line indicating that the machine was responding in a linear

fashion over the range of expected concentrations to be encountered in the study. Next, five 10 µl injections of standard were made and found to differ from each other by approximately 1.4% which is within the acceptable 1.5% limit specified in the published method. The response peaks were observed to be crisp with no discernible tailing.

- Procedure--Separate 10 µl injections of standard and assay preparations were made using a 10 µl Hamilton microsyringe. The standard was injected initially and then again after every five assay preparations were injected. The quantity of $C_{14}H_{30}Cl_2N_2O_4$ in the samples and controls was calculated using the equation (1):

$$Csam = \frac{Rsam \times Cstd \times 2.5}{Rstd} \qquad (1)$$

where:

Csam = concentration of $C_{14}H_{30}Cl_2N_2O_4$ in each assay preparation, mg/ml;

Cstd = concentration of $C_{14}H_{30}Cl_2N_2O_4$ in standard preparation, mg/ml;

Rsam = peak response of assay preparation, area units;

Rstd = average peak response of standard injections bordering the respective assay preparation injection, i.e., the average of two standard injections, area units; and

2.5 = assay preparation dilution factor.

Metaproterenol Sulfate active ingredient $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ concentration was assayed using a method provided by Boehringer

Ingelheim which is used in their quality control laboratory. This method (found in Appendix A) was used with the following changes:

- Standard preparation--The standard (BIL/USA House Reference Standard--BIL#0004 Code #400012) was initially dried, according to manufacturer's directions, at 105°C for one hour and then stored in a glass desiccator filled with $CaSO_4$ desiccant. Approximately 15 mg of standard were accurately weighed and transferred to a 25 ml volumetric flask and dissolved in and diluted to volume with the Mobile phase. Concentration of the prepared standard was expressed as anhydrous, methanol and isopropanol free metaproterenol sulfate by multiplying by a factor of 99.8%. This factor was obtained from the manufacturer's statement of potency of the powdered standard as 99.8% anhydrous by HPLC assay.
- Assay preparation--Metaproterenol samples and controls were prepared as follows: 4.0 ml of sample was transferred from the 10 ml beaker to a 50 ml glass volumetric flask following pH measurement. This transfer was accomplished as described for Succinylcholine Chloride above.
- Chromatograph conditions--

  Instrument--Perkin-Elmer Series 3B Liquid Chromatograph as described above. The Spectra-Physics Computing Integrator, also described above, was used to record peak responses as well as to calculate respective area ratios on all analysis days except for day 0 where a Perkin-Elmer model 056-3001 strip chart recorder was used and the peak

heights were measured manually in millimeters. A study was done to compare calculated concentration based on the electronic integrator response and the strip chart recorder response. The procedure used for this may be found in Appendix B. As a result of this study, a 0.116 mg/ml correction term was subtracted from all day 0 calculated concentrations.

Guard column--none used.

Column--.26 cm x 25 cm, stainless steel.

Stationary phase--HC ODS SIL-X (octadecylsilane chemically bonded to porous silica--Perkin-Elmer).

To determine that the HPLC was responding in a linear manner, a standard curve was constructed by plotting the area of the respective peak heights corresponding to 2 µl, 4 µl, 6 µl, 8 µl, and 10 µl injections of the standard preparation. The resulting curve formed a straight line indicating that the HPLC was responding linearly over the concentration range expected to be encountered in the study. The response peaks showed very slight tailing--the computing integrator does take this tailing into consideration.

- Procedure--Separate 10 µl injections of standard and assay preparations were made using a 10 µl microsyringe. The standard was injected initially and then again after every five assay preparations were injected. The quantity of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in the sample was calculated using the following formula:

$$Csam = \frac{Cstd \times Rsam}{Rstd} \times \frac{50}{4} \qquad (2)$$

where:

Csam = concentration of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in each assay preparation, mg/ml;

Cstd = concentration of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in standard preparation, mg/ml;

Rsam = peak response of assay preparation, area units;

Rstd = average peak response of standard injections bordering the respective assay preparation injection, i.e., the average of two control injections, area units; and

$\frac{50}{4}$ = assay preparation dilution factor.

Thiamylal Sodium ($C_{12}H_{17}N_2NaO_2S$) concentration was assayed in accordance with a procedure supplied by Parke-Davis (found in Appendix A), where it is used in their quality control laboratory. The following modifications were made for use in this work.

- Preparation of Phensuximide internal standard--approximately 28 mg of Phensuximide (provided by Parke-Davis Lot #583625) was accurately weighed and transferred into a 100 ml volumetric flask. The flask was then brought to volume with reagent chloroform and thoroughly mixed (concentration = .28 mg/ml).
- Preparation of Surital Acid standard solution--approximately 44 mg of Thiamylal Acid (Surital Acid provided by Parke-Davis Lot #H726803) was accurately weighed and transferred into a 100 ml volumetric flask. The flask was then brought to volume with reagent chloroform and thoroughly mixed (concentration = .44 mg/ml).

- Preparation of sample--A total volume of 19.0 ml of each reconstituted 2.5% Thiamylal Sodium sample and control was transferred to a 100 ml volumetric flask, brought to volume with distilled, deionized water, and mixed. Of this 19.0 ml, 5.0 ml were transferred from the 10 ml beaker following pH determination. This transfer was accomplished by pouring the contents of the beaker through a glass funnel into the flask and then rinsing the beaker three times with distilled, deionized water and pouring each rinse through the funnel. Distilled, deionized water was then rinsed over the funnel into the flask. The remaining 14.0 ml was transferred directly from each respective 20 ml syringe into the 100 ml flask. Continuation of the original method for sample preparation followed.
- Procedure--Initially a standard curve was constructed to insure that the machine was responding in a linear manner. Dilutions of Thiamylal Acid standard in Phensuximide were made with resulting Thiamylal Acid concentrations ranging from 9 mg/ml to 22 mg/ml giving corresponding area response ratios of Thiamylal Acid to Phensuximide of .10 to .50 respectively. The standard curve obtained formed a straight line, indicating that the machine was responding in a linear fashion over the concentration range expected to be encountered in this study. The response peaks were crisp with some slight tailing (the electronic integrator considers this tailing in its measurement). For each analysis, 2.0 ml each of prepared

sample or control or prepared Thiamylal Acid Standard and prepared Phensuximide internal standard were pipetted into a glass stoppered tube and mixed. Using a 10 µl Hamilton microsyringe, 2.0 µl of the combined internal/external standard solution were injected into the sample port of the gas chromatograph initially and then again after every five 2.0 µl prepared sample or control injections.

- Technique notes--

  Instrument--A Hewlett Packard Model 5830A Gas Chromatograph. The peak response area ratios were calculated and recorded using a Hewlett Packard Model 18850A Electronic Integrator.

  Column--3 ft x 1/4 in. O.D. x 2 mm I.D. glass column packed with 3% SP-2250 on 80/100 Supelcoport.

  Carrier Gas--Helium at 52 ml per minute.

  Temperature--(a) column--185°C isothermal
  (b) injection port--180°C
  (c) detector--350°C

  Sensitivity--Attenuation 6 millivolts on the electronic integrator.

  Retention time--Phensuximide = 2.2 minutes
  Thiamylal = 4.5 minutes

  Calculation--Quantitation of Thiamylal was calculated using the following equation:

$$Csam = \frac{B \times Cstd}{A} \times \frac{100}{10} \times \frac{100}{19} \times 1.086 \qquad (3)$$

where:

Csam = concentration of Thiamylal in each sample or control, mg/ml;

Cstd = concentration of Thiamylal Acid standard, mg/ml;

$A = \frac{\text{area ratio Thiamylal Acid standard}}{\text{area ratio Phensuximide standard}}$ averaged for two standard injections bordering the respective sample or control injection;

$B = \frac{\text{area ratio Thiamylal sample or control}}{\text{area ratio Phensuximide standard}}$;

$\frac{100}{10}$ = dilution of extracted sample;

$\frac{100}{19}$ = dilution of sample; and

1.086 = manufacturer's factor to convert Thiamylal Acid to Thiamylal Sodium.

Sterility. To determine that sterility was maintained throughout the study, 4.0 ml of sterile trypticase soy broth (TSB) (BBL Microbiology Systems #11768) in glass tubes was inoculated with a 1.0 ml aliquot directly from the respective syringe of each drug sample at each analysis interval. Each tube of TSB was then incubated at 37°C, 85% RH and observed for evidence of growth after two days. This is the standard procedure used at St. Lawrence Hospital in Lansing, Michigan.

Water loss. Water loss from water based liquid pharmaceuticals would result in an increase in concentration. If the pharmaceutical is being stored, concentration increase, due to water loss, may mask concentration decrease due to degradation. It is of interest therefore to quantitate the water lost by the three pharmaceuticals under test.

Water loss was quantitated for Succinylcholine Chloride and Metaproterenol Sulfate packages in a separate study, in which packages similar to those used in the original study were filled with distilled, deionized water and put under identical storage conditions as in the original study for 70 days. Each water filled package was weighed on a Mettler balance at various intervals. These weights were recorded to four decimal places. The change of weight seen over time was attributed to water movement into or out of the package.

In the case of Thiamylal Sodium, the packages filled with the drug were weighed on a Mettler balance concurrently with the other monitoring procedures. Weight was recorded to four decimal places. Care was taken to weigh the same packages at each period, i.e., the packages which were to be held on test for the full 30 days. The weight change observed over time was assumed to represent water movement into or out of the package.

RESULTS AND DISCUSSION

Succinylcholine Chloride

Throughout the study sterility, colorlessness, and clarity were maintained in every package. Further, there was no formation of particulate matter observed.

The changes in concentration and pH associated with glass and plastic packages over time at each storage temperature are presented respectively in Tables 5 and 6. In not every case are the presented data means based on five replicate samples; a few samples were lost due to breakage or spillage and a few replications were not included in the final analysis when they were clearly out of line with four tightly clustered values. In fact, two values out of 99 for concentration and two values out of 98 for pH were omitted in this manner. Since the ANOVA contained 70 error degrees of freedom, the statistical effect of these omissions is negligible. In these cases where replicate values were obviously out of line, it was felt that inclusion of these replicate values would have been a misrepresentation of the data. An analysis of variance (ANOVA) was done to determine if there were any statistically significant differences in pH or concentration due to storage time, storage temperature, package type (glass or plastic), or an interaction of any of these. The results of the ANOVA are presented in Table 7. Using the F values from this ANOVA it can be seen that there was a three way interaction demonstrating significance at

Table 5. Mean Succinylcholine Chloride concentration (mg/ml)

Length of Storage (Days)	Control	4°C		22°C		37°C	
		Glass Syringe	Plastic Syringe	Glass Syringe	Plastic Syringe	Glass Syringe	Plastic Syringe
0	19.8	21.0	20.9	--	--	--	--
5	20.0	20.7	20.5	20.7	20.5	20.5	20.7
30	18.8	21.1	20.6[a]	19.8	20.9	18.5	19.6
45	20.1	20.2	20.3	20.1	20.3	18.3	17.6[b]
mean	19.7						

$LSD_{(.01)} = 0.9$[c]

$LSD_{(.01)} = 1.0$[d]

[a]One replicate sample thrown out.

[b]One replicate sample lost.

[c]Used to compare treatment means; each with five replicates.

[d]Used to compare treatment means; four with five replicates.

Table 6. Mean Succinylcholine Chloride pH values

Length of Storage (Days)	Control	4°C		22°C		37°C	
		Glass Syringe	Plastic Syringe	Glass Syringe	Plastic Syringe	Glass Syringe	Plastic Syringe
0	3.65	3.67	3.67	--	--	--	--
5	3.68	3.69[a]	3.66	3.62[a]	3.65	3.55	3.57
30	3.64	3.68	3.63	3.56	3.52	3.31	3.30
45	3.62	3.63	3.61	3.48	3.45	3.20	3.21
mean	3.65						

$LSD_{(.01)} = .01$[b]

$LSD_{(.01)} = .02$[c]

[a]One replicate value thrown out.

[b]Used to compare treatment means; each with five replicates.

[c]Used to compare treatment means; four with five replicates.

Table 7. ANOVA summary--Succinylcholine Chloride

		pH		Concentration	
Source of Variation	df	ms	F	ms	F
Time	2	.287	3836.924***	8.468	26.908***
Temperature	2	.675	9017.517***	14.905	47.361***
Package	1	.006	82.287***	.457	NS
Time x temperature	4	.063	834.990***	4.593	14.595***
Time x package	2	.003	34.382***	.980	3.115+
Temp. x pkg.	2	.002	24.347***	.508	NS
Time x temp. x pkg.	4	.001	8.437***	1.025	3.256*
Error	70	.00007		.315	

Significance levels: + = .10

* = .05

*** = .001

NS = non-significant.

the 5% level for concentration and at the .1% level ($p = .001$) for pH among the treatments, i.e., time, temperature, and package. When the F values for the individual treatments are compared it can be seen that the significance of the three way interactions is due mostly to the two main effects, temperature and time. The F values for temperature and time were very large while the F value for package was non-significant in the case of concentration and much smaller than the other two for pH.

The means for each package and between each package at each temperature over time were tested for significant differences at the 1% level using the least significant difference (LSD) procedure (Steel and Torrie, 1980). The LSD's used for the comparisons are presented in Tables 5 and 6. The following conclusions were drawn:

1. At 4°C there was no significant change of concentration over time in either the glass or plastic packages; except in glass from day 30 to day 45, the concentrations were statistically significantly different. However, this difference (21.1 mg/ml to 20.2 mg/ml) is not meaningful in a practical sense, since both values are within the USP limit. There was also no significant difference in concentration between glass and plastic packaging over time. All means at 4°C remained within the USP acceptable range (18.6-21.4 mg/ml).
2. There was a significant change in concentration over time in glass at 22°C (decreased from 21.0 mg/ml on day 0 to 20.1 mg/ml on day 45) and 37°C (decreased from 21.0 mg/ml on day 0 to 18.3 mg/ml on day 45). In plastic, there was no significant

change over time at 22°C; however, there was significant degradation over time at 37°C. All concentration means at 22°C were within the USP acceptable range; at 37°C concentration remained acceptable in glass through day 5 and in plastic through day 30.

3. The LSD results for concentration showed that glass and plastic syringes were not significantly different in the concentration achieved at the various time intervals except on day 30 at both 22°C and 37°C.
4. There were statistically significant differences in pH throughout the study at all temperatures, both between means in a single package and between glass and plastic package means. While these differences are statistically significant, they are not meaningful since all pH means are within the acceptable USP range (3.0-4.5 pH units).

The relationship between storage time and concentration in each package, at each temperature is depicted in Figures 1, 2, and 3. The best straight line fit for the data was computed using regression. A summary of the significance of the correlation coefficients is presented in Table 8. With both glass and plastic the relationship between time and concentration appears to become stronger as temperature increases. In glass, there is no significant correlation between time and concentration at 4°C but the correlation is significant at 22°C and 37°C. In plastic, the correlation between time and concentration is non-significant at 4°C and 22°C, but it is significant at 37°C. The

Figure 1. Succinylcholine Chloride--4°C regression plots and USP acceptable limits for concentration (mg/ml).

Figure 2. Succinylcholine Chloride--22° regression plots and USP acceptable limits for concentration (mg/ml).

Figure 3. Succinylcholine Chloride--37°C regression plots and USP acceptable limits for concentration (mg/ml).

Fig. 1

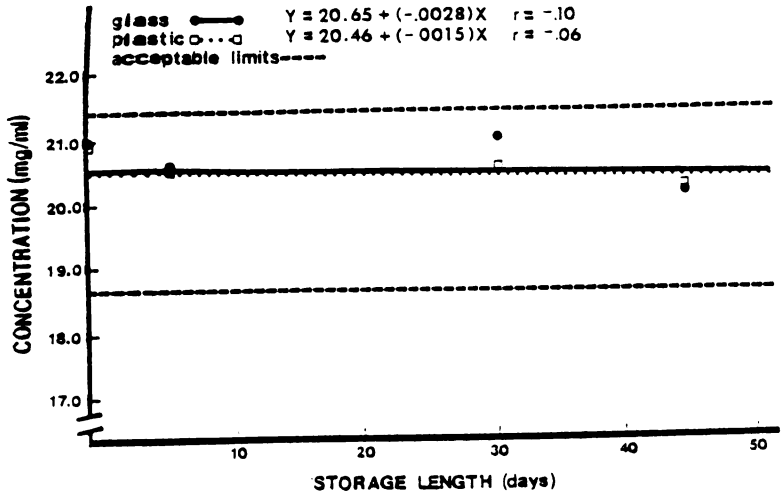


Fig. 2

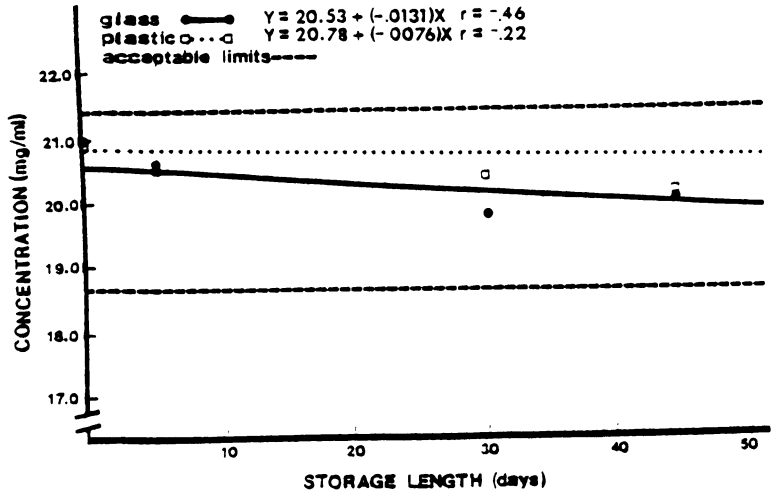


Fig. 3

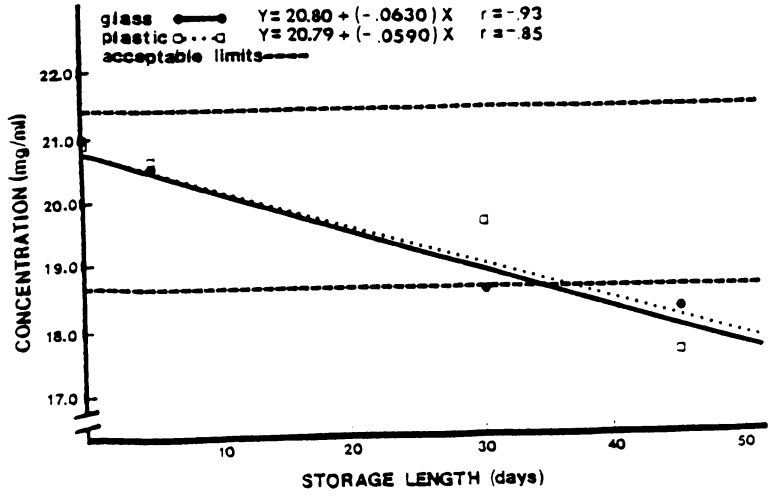


Table 8. Statistical significance of correlation coefficients (r values) for concentration vs. storage length

Package	Temperature (°C)	r Value		
		Succinylcholine	Metaproterenol	Thiamylal
Glass	4	NS	NS	NS[a]
	22	-.46*	-.54**	--[a]
	37	-.93**	-.77**	--[a]
Plastic	4	NS	-.68**	NS[a]
	22	NS	NS	--[a]
	37	-.85**	NS	--[a]

[a]Drug not tested at these temperatures.

Significance levels: * = .05

** = .01

NS = non-significant.

general conclusion here is that temperature appears to affect the concentration of Succinylcholine over time and that this effect appears at a lower temperature in glass syringes than in plastic syringes.

Also considered was the effect on concentration of water loss from the drug through each package. Theoretically, water loss could cause an increase in concentration, and this might mask degradation. The results of the study done to determine actual water loss by each package is presented in Table 9. Here also the LSD value was used to decide if a difference in the water lost/gained would significantly affect concentration. Only at 37°C in plastic syringes would water loss significantly increase concentration. However, this does not affect the final outcome of the work because Succinylcholine Chloride should never be stored at 37°C.

Metaproterenol Sulfate

All repackaged drug maintained sterility throughout this study. The drug packaged in glass vials remained colorless throughout the study at all temperatures. Metaproterenol stored in plastic syringes also remained colorless throughout the study at 4°C and 22°C, but a slight yellow tinge was noted on day 45 in drug stored at 37°C (these did remain colorless through 30 days of storage). According to Boehringer Ingelheim, this drug is very sensitive to oxidation and such oxidation is almost immediately evidenced by such a yellow hue. No particulate matter was noted in any packaged drug throughout the study.

Table 9. Theoretical effect of weight change (water loss) on concentration

Package	Storage Temperature	Days Storage: 7	14	28	42	70	Theoretical % [C] Change[a] Due to Water Loss from Day 0 to Day 70	
		Cumulative Weight Change (milligrams)						
BD glass syringe	4°C	+0.1	0.6	1.4	2.4	4.6	+.009	
	22°C	0.0	0.8	1.4	1.0	1.7	+.034	
	37°C	12.2	25.8	42.9	57.6	73.9	+1.478	LSD = .9 mg/ml
BD plastic syringe	4°C	0.8	3.3	4.8	7.7	10.1	+.202	
	22°C	3.4	5.7	8.5	10.0	14.8	+.296	
	37°C	0.7	2.5	5.2	9.9	17.1	+.342	
INVENEX glass vial	4°C	+6.2	+2.0	+3.8	+2.7	+0.7	.014	
	22°C	+1.2	1.0	+1.4	+0.2	1.2	+.024	
	37°C	+2.9	+0.7	+3.5	+1.1	+0.2	.004	LSD = .23 mg/ml
MONOJECT plastic syringe	4°C	+0.5	+1.3	0.0	0.0	0.6	+.012	
	22°C	0.4	1.3	2.5	3.6	5.7	+.114	
	37°C	1.2	2.6	5.2	8.8	14.5	+.290	

[a]Loss of weight (represented by water loss) leads to increased [C] and vice versa.

+ Indicates increase; lack of sign indicates decrease.

Concentration and pH data are presented in Tables 10 and 11 respectively. In not every case are the presented data means based on five replicate samples; a few samples were lost due to breakage or spillage and a few replications were not included in the final analysis when they were clearly out of line with four tightly clustered values. In fact, two values out of 174 for concentration and one value out of 177 for pH were omitted in this manner. Since the ANOVA contained 134 error degrees of freedom for concentration and 140 error degrees of freedom for pH, the statistical effect of these omissions is negligible. The results of an ANOVA, used to determine the significance of differences in pH or concentration due to storage time, storage temperature, or package type (glass vials or plastic syringes) can be found in Table 12. The ANOVA showed a significant three way interaction among time, temperature, and package at the .1% level (see F values).

The means for each package and between each package, at each temperature, over time were tested for significant differences at the 1% level using LSD's. The LSD's used for the comparisons are presented in Tables 10 and 11. Several conclusions drawn from the LSD comparisons follow:

1. The rate of concentration degradation at 4°C in plastic appears greater than the rate in glass; glass vials show no significant concentration loss over time, but a significant loss of concentration occurs in plastic syringes after 30 days of storage. There was no significant difference in concentration between glass and plastic packages. Every concentration

Table 10. Mean Metaproterenol Sulfate concentration (mg/ml)

Length of Storage (Days)	Control	4°C		22°C		37°C	
		Glass Vials	Plastic Syringes	Glass Vials	Plastic Syringes	Glass Vials	Plastic Syringes
0	4.54	4.51	4.71[a]	4.47	4.55	4.53	4.57[a]
15	4.66	4.39[a]	4.42	4.32	4.30	4.44	4.41
30	4.14	4.40	4.50	4.33	4.46	4.40	4.13[a]
45	3.79	4.34[b]	4.27[b]	4.19	4.38	4.33	4.41
60	4.24	4.30	4.35	4.23	4.40	4.21	4.21
70	4.58	4.46	4.31[a]	4.21[a]	4.45	4.14	4.38
mean	4.30						

$LSD_{(.01)} = .23$[c]

$LSD_{(.01)} = .24$[d]

$LSD_{(.01)} = .25$[e]

[a]One replicate sample lost.

[b]One replicate value thrown out.

[c]Used to compare treatment means; each with five replicates.

[d]Used to compare treatment means; four with five replicates.

[e]Used to compare treatment means; four with four replicates.

Table 11. Mean metaproterenol Sulfate pH measurements

Length of Storage (Days)	Control	4°C		22°C		37°C	
		Glass Vials	Plastic Syringes	Glass Vials	Plastic Syringes	Glass Vials	Plastic Syringes
0	3.83	3.85	3.76	3.85	3.74	3.85	3.74
15	3.80	3.81[a]	3.82	3.85	4.40[b]	3.87	6.51
30	3.85	3.84	3.85	3.85	5.72	3.92	6.98
45	3.79	3.84	3.89	3.87	6.44	3.89	7.08
60	3.69	3.74	3.87	3.79	6.47	3.84	7.05
70	3.74	3.82	3.94[a]	3.86[a]	6.68	3.88	7.09
mean	3.78						

$LSD_{(.01)} = .10$[c]

$LSD_{(.01)} = .11$[d]

[a]One replicate sample lost.

[b]One replicate value thrown out.

[c]Used to compare treatment means; each with five replicates.

[d]Used to compare treatment means; four with five replicates.

Table 12. ANOVA summary--Metaproterenol Sulfate

	pH			Concentration		
Source of Variation	df	ms	F	df	ms	F
Time	5	5.326	1412.556***	5	.254	7.807***
Temperature	2	26.093	6919.817***	2	.072	3.387*
Package	1	93.732	.249E+05***	1	.148	6.025*
Time x temperature	10	1.464	388.155***	10	.033	2.182*
Time x package	5	5.196	1377.885***	5	.022	NS
Temp. x package	2	23.681	6280.114***	2	.058	3.160*
Time x temp. x pkg.	10	1.393	369.327***	10	.049	3.820***
Error	140	.004		134	.015	

Significance level: * = .05

*** = .001

NS = non-significant.

mean at 4°C remained within acceptable limits (4.09-4.99 mg/ml--Boehringer Ingelheim, Personal Communication, Maureen Wilson, 1984).

2. At 22°C there was a significant loss of concentration after 30 days of storage in glass vials and no significant change in concentration throughout the study (70 days) in plastic syringes. Glass and plastic packages were not significantly different in concentration achieved at various time intervals at 22°C except at 70 days of storage. All mean concentrations remained within the acceptable range.
3. At 37°C, a significant loss of concentration occurred after 30 days of storage in glass vials and at days 30 and 60 in plastic syringes. In spite of the increased concentration loss seen at 37°C, all means were within acceptable limits.
4. At 4, 22, and 37°C there was no significant change in pH over time using glass vials--except on day 60 at 4°C. It is believed that this is an aberrant data point because it is an isolated instance of pH decrease. When this drug is stored in plastic syringes, the pH follows an upward trend; this trend becomes stronger and increases in rate as temperature increases. In plastic, there is a statistically significant increase in pH after 30 days storage at 4°C, after 15 days storage at 22°C, and at 15 days storage at 37°C. There is no clear answer as to why pH increased when temperature was increased. Consultation with Boehringer Ingelheim revealed that oxidation normally results in a drop in pH and there was no mechanism

they knew of where temperature or oxidation alone caused this drug's pH to rise. It seems that a possible explanation for this observation is that there is an interaction between this drug and the plastic syringes which is accelerated as temperature increases; this conclusion is supported by the fact that no such dramatic pH increase, associated with increasing temperature, was observed in drug packaged in glass vials.

5. At 4°C there was no significant difference in pH between glass and plastic packages until after 45 days of storage. There is a significant difference in pH between glass and plastic packages on all days at 22°C and 37°C.

The relationship between storage time and concentration of the drug in glass vials and plastic syringes at each temperature is shown in Figures 4, 5, and 6. Regression was used to find the best fit line. A summary of the significance of the correlation coefficients is presented in Table 8. In glass, the relationship between time and concentration appears to become stronger as temperature increases; plastic, however, shows no such consistent relationship, i.e., the correlation coefficient shows no consistent downward and upward trend. There does not appear to be any clear, reasonable explanation for this. The correlation between time and concentration in glass is non-significant at 4°C but is significant at 22°C and 37°C. In plastic, there is a significant correlation between time and concentration at 4°C but not at 22°C and 37°C.

Figure 4. Metaproterenol Sulfate--4°C regression plots and Boehringer Ingelheim acceptable limits for concentration (mg/ml).

Figure 5. Metaproterenol Sulfate--22°C regression plots and Boehringer Ingelheim acceptable limits for concentration (mg/ml).

Figure 6. Metaproterenol Sulfate--37°C regression plots and Boehringer Ingelheim acceptable limits for concentration (mg/ml).

Fig. 4

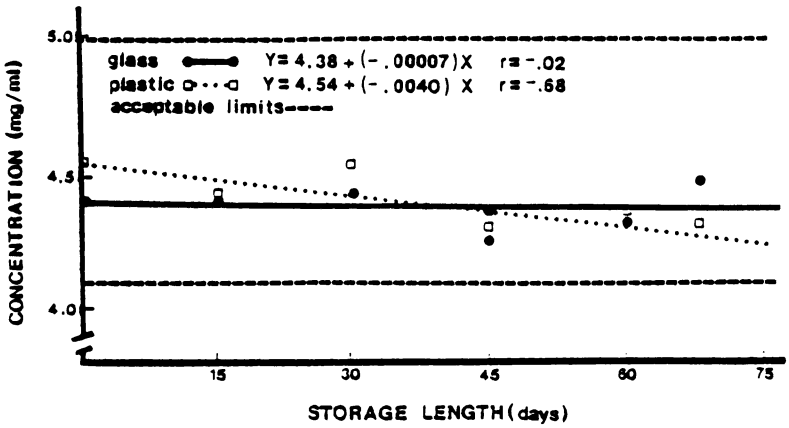


Fig. 5

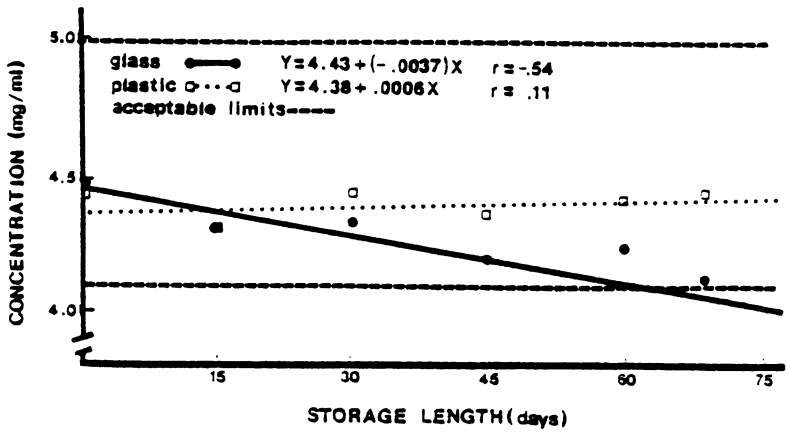
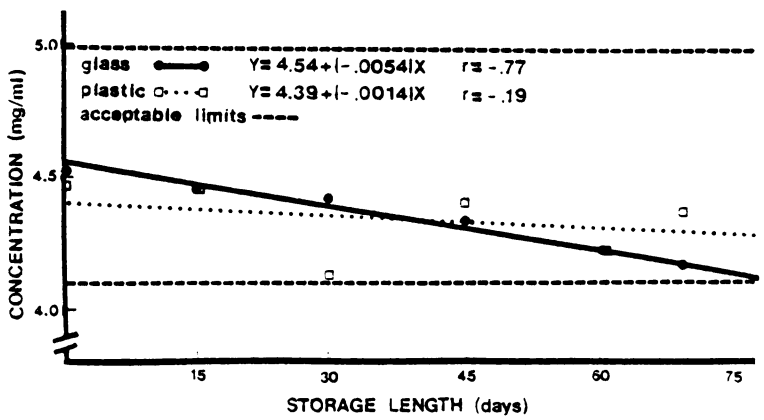


Fig. 6



The effect of water loss on concentration was studied in the same manner as for Succinylcholine Chloride (see Table 9 for data) with similar conclusions; only at 37°C in plastic syringes would water loss significantly increase concentration, and Metaproterenol Sulfate should not be stored at 37°C.

Thiamylal Sodium

Thiamylal has a characteristic pale yellow color from the moment it is reconstituted; all drug (in either glass vials or plastic syringes) showed no perceivable deviation from this initial color throughout the study. Sterility and clarity were maintained throughout the study in both packages with no sign of particulate formation.

Weight loss as well as the changes in pH and concentration over time are presented in Tables 13, 14, and 15 respectively. One way ANOVA's were used to determine if there was any significant effect of storage time on weight loss, pH, or concentration in plastic syringes. The results of these ANOVA's are presented in Table 16. Thiamylal stored in plastic syringes lost no significant amount of water over time (as demonstrated by weight loss) and there was no apparent weight loss seen over time in the glass vials. Storage time did have a significant effect on pH ($p = .001$) and on concentration ($p = .01$). An ANOVA was run a second time on the concentration data, omitting day 0 results. This second ANOVA showed no significant effect of storage time on concentration. It can be concluded that in plastic there was a significant loss of concentration from day 0

Table 13. Mean weight (grams) of Thiamylal Sodium in package

Length of Storage (Days)	Glass Vial	Plastic Syringe	
0	722.2	34.8937	
6	722.2	34.8940	$LSD_{(.01)} = .0916$[a]
14	722.2	34.8943	
30	722.2	34.8947	

[a]Used to compare plastic treatment means.

Table 14. Mean Thiamylal Sodium pH values

Length of Storage (Days)	Control	Glass Vial	Plastic Syringe	
0	10.90	10.88	10.90	
6	10.88	10.87	10.88	$LSD_{(.01)} = .003$[a]
14	10.93	10.91	10.91	$LSD_{(.01)} = .040$[b]
30	10.90	10.88	10.87	
mean	10.90			

[a]Used to compare plastic treatment means.

[b]Used to compare glass to plastic treatment means.

Table 15. Mean Thiamylal Socium concentration (mg/ml)

Length of Storage (Days)	Control	Glass Vials	Plastic Syringes	
0	24.4	23.3	24.8	
6	25.8	24.3	23.5	$LSD_{(.01)} = 1.2$[a]
14	26.2	24.3	23.4	$LSD_{(.01)} = 2.0$[b]
30	24.9	22.7	23.6	
mean	25.3			

[a]Used to compare plastic treatment means.

[b]Used to compare glass to plastic treatment means.

Table 16. ANOVA summary--Thiamylal Sodium in plastic syringes

	Source of Variation	df	ms	F
	Total	19		
pH	Days	3	13.733E-04	457.77***
	Error	16	.003E-03	
	Total	19		
Weight	Days	3	.933E-06	NS
	Error	16	.246E-02	
	Total	19		
Concentration	Days	3	2.288	5.72**
	Error	16	.400	
	Total	14		
Concentration[a]	Days	2	3.234E-02	NS
	Error	12	3727.767E-04	

[a]Values calculated after eliminating day 0 data.

Significance levels: ** = .01

*** = .001

NS = non-significant.

to day 6 but no significant loss after day 6. This conclusion was reached by using the LSD calculated to compare means for plastic syringes over time. LSD values are found in Tables 13, 14, and 15. LSD values were also used to compare plastic pH means over time as well as to compare pH and concentration means between glass and plastic packages and these additional conclusions were drawn:

1. There was a significant difference among all plastic pH means over time; however, this difference is not meaningful as the greatest difference was .03 pH units and all pH means are within USP acceptable limits.
2. Glass vials maintain an acceptable concentration (USP limits are 23.2-26.8 mg/ml) through 14 days and plastic syringes maintain acceptable potency through 30 days.
3. There was no statistically significant difference in concentration or pH over time when the drug is stored in either glass vials or plastic syringes.

The relationship between storage time and concentration in each package is depicted in Figure 7. Regression was used to calculate the best straight line. The correlation coefficients computed for both glass vials and plastic syringes were found to be non-significant (see Table 8) indicating that a consistent relationship between length of storage time and concentration degradation is not evident.

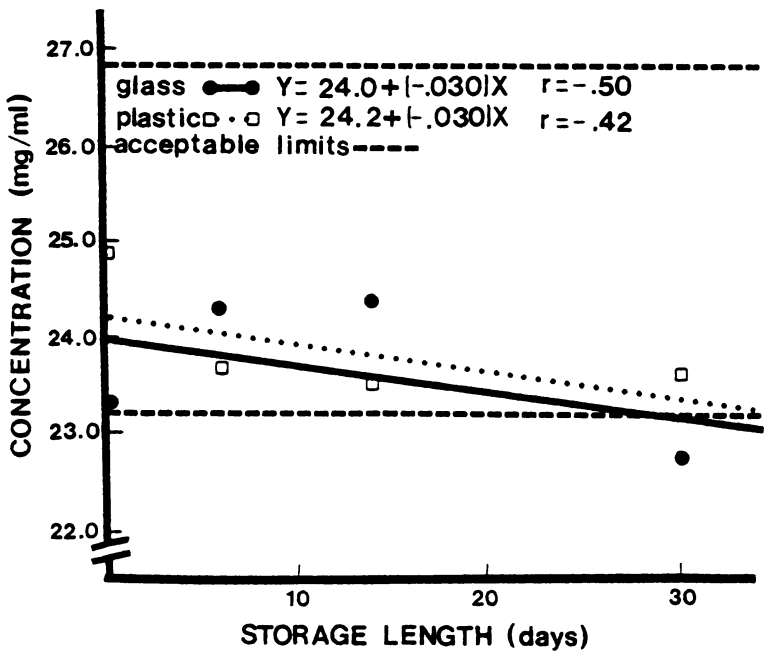


Figure 7. Thiamylal Sodium--4°C regression plots and USP acceptable limits for concentration (mg/ml).

SUMMARY AND CONCLUSIONS

This study has determined the stability of Succinylcholine Chloride, Metaproterenol Sulfate, and Thiamylal Sodium in glass and plastic unit dose packages; also, differences between glass and plastic packages in maintaining this stability have been established. Recommendations for storage of each drug at 4°C are based on the stability data collected and are presented in Tables 17 and 18. pH and concentration considerations are given in these tables so that the pharmacist can use these data to make a judgment on expiration dating of the unit dose repackaged pharmaceuticals studied. From Tables 17 and 18, it can be seen that identical recommendations are made for plastic and glass packages; this is because the data on all drugs indicated that regardless of package, the drugs maintained acceptable pH and concentration throughout the study--except in the case of Thiamylal Sodium. Thiamylal Sodium maintained concentration within the acceptable limits through 30 days storage in plastic syringes but maintained acceptable potency only through 14 days of storage in glass vials. This loss of potency on day 14 could be an aberrant data point but to be safe, a 14 day maximum storage period was recommended for storage of Thiamylal in glass and plastic. Study of the tabulated data and the graphs reveal that the storage period recommended for Thiamylal is approaching the limit. Any attempt to extend these recommendations would require considerable additional

Table 17. Recommendations for storage at 4°C based on concentration (mg/ml) data

Drug/Package	Safe Storage Length (Days)	Beginning [C] (mg/ml)	Ending [C] (mg/ml)	% Loss [C]	Nominal [C] (mg/ml)	Acceptable [C] Range (mg/ml)
S.C./glass	45	21.0	20.3	3.3	20.0	18.6-21.4[a]
S.C./plastic		20.9	20.3	2.9		
M.S./glass	70	4.51	4.46	1.1	4.54	4.09-4.99[b]
M.S./plastic		4.71	4.31	8.5		
T.S./glass	14	23.3	24.3	4.3[c]	25.0	23.2-26.8[a]
T.S./plastic		24.8	23.4	5.6		

[a]U.S.P.

[b]Personal communication with Boehringer Ingelheim.

[c]Gain.

S.C. = Succinylcholine Chloride; M.S. = Metaproterenol Sulfate; T.S. = Thiamylal Sodium.

Table 18. Recommendations for storage at 4°C based on pH data

Drug/Package	Safe Storage Length (Days)	Beginning pH	Ending pH	Nominal pH	Acceptable pH Range
S.C./glass	45	3.67	3.63	3.65	3.0-4.5[a]
S.C./plastic		3.67	3.61		
M.S./glass	70	3.85	3.82	--[b]	--[b]
M.S./plastic		3.76	3.94		
T.S./glass	30	10.88	10.88	11.1	10.7-11.5[a]
T.S./plastic		10.90	10.87		

[a]U.S.P.

[b]Not available.

S.C. = Succinylcholine Chloride; M.S. = Metaproterenol Sulfate; T.S. = Thiamylal Sodium.

laboratory evaluation. It should be noted that while 14 days is the recommended storage time for Thiamylal based on concentration, 30 days is recommended based on pH--the data are presented and the pharmacist must make his or her own decision on expiration dating.

Metaproterenol Sulfate is stable in glass vials at 22°C but not in plastic syringes at 22°C, due to the dramatic pH increase. Succinylcholine Chloride should not be stored at 22°C because the manufacturer states that the drug must be stored at 4°C to 8°C.

Suggestions for future work on these drugs includes the determination of kinetic degradation models for concentration and possibly pH for any of these three drugs, analysis for absorption of the drugs into the plastic packages, determination if migration of syringe or vial components into the drugs occurs, and possible quantitation of any migrating species. Determination of the permeability constants for water vapor and oxygen for the various plastic syringes used in this study would provide valuable information which could be applied to the packaging of many liquid pharmaceuricals. Finally, kinetic models for drug degradation can be combined with these permeability constants to develop models which can then be used to select unit dose packaging systems that have maximum probability of satisfactory results in stability testing.

APPENDICES

APPENDIX A

ANALYSIS METHODS

APPENDIX A

ANALYSIS METHODS

solution having a known concentration of about 250 µg of USP Spironolactone RS per ml.

Assay preparation—Transfer about 25 mg of Spironolactone, accurately weighed, to a 100-ml volumetric flask, add a mixture of acetonitrile and water (9:1) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 ml per minute. Chromatograph six replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µl) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention time for spironolactone is about 5 minutes. Calculate the quantity, in mg, of $C_{24}H_{32}O_4S$ in the portion of Spironolactone taken by the formula $0.1C(r_U/r_S)$, in which C is the concentration, in µg per ml, of USP Spironolactone RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained for spironolactone from the *Assay preparation* and the *Standard preparation*, respectively. •

Spironolactone Tablets

Change to read:

Identification— •Mix a quantity of finely powdered Tablets, equivalent to about 100 mg of spironolactone, with 25 ml of methanol, and filter. On a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel mixture, spot 10 µl of this solution and 10 µl of a solution of USP Spironolactone RS in methanol containing 4 mg per ml. Develop the chromatogram in a solvent system consisting of chloroform, ethyl acetate, and methanol (2:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength ultraviolet light: the R_f value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution. •

Change to read:

Assay—

•*Mobile phase, Standard preparation,* and *Chromatographic system*—Prepare as directed in the *Assay* under *Spironolactone.*

Assay preparation—Weigh and finely powder not less than 20 Spironolactone Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 25 mg of spironolactone, to a 100-ml volumetric flask, add 10.0 ml of water, and swirl gently for about 10 minutes. Add 70 ml of acetonitrile, sonicate for 30 minutes with occasional shaking to dissolve the soluble substances in the mixture, dilute with acetonitrile to volume, and mix. Centrifuge a portion of the mixture at about 3000 rpm for 10 minutes, and use the supernatant liquid.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Spironolactone.* Calculate the quantity, in mg, of $C_{24}H_{32}O_4S$ in the portion of Tablets taken by the formula $0.1C(r_U/r_S)$, in which C is the concentration, in µg per ml, of USP Spironolactone RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained for spironolactone from the *Assay preparation* and the *Standard preparation*, respectively. •

Stannous Fluoride

Change to read:

Assay for stannous ion—

•*0.1 N Potassium iodide–iodate*—In a 1000-ml volumetric flask, dissolve 3.567 g of potassium iodate, previously dried at 110° to constant weight, in 200 ml of oxygen-free water containing 1 g of sodium hydroxide and 10 g of potassium iodide, dilute with oxygen-free water to volume, and mix. Standardize this solution by titrating a solution prepared from an accurately weighed quantity of reagent tin (Sn) and hydrochloric acid. Each ml of *0.1 N Potassium iodide–iodate* is equivalent to 5.935 mg of Sn.

Procedure— •Transfer about 250 mg of Stannous Fluoride, accurately weighed, to a 500-ml conical flask, and add 300 ml of hot, recently boiled 3 *N* hydrochloric acid. While passing a stream of an oxygen-free inert gas over the surface of the liquid, swirl the flask to dissolve the Stannous Fluoride, and cool to room temperature. Add 5 ml of potassium iodide TS, and titrate in an inert atmosphere with •*0.1 N potassium iodide–iodate*• adding 3 ml of starch TS as the end-point is approached. Each ml of •*0.1 N potassium iodide–iodate*• is equivalent to 5.935 mg of Sn^{++}.

Succinylcholine Chloride

Add the following:

•**Reference standard**—*USP Succinylcholine Chloride Reference Standard*—Do not dry; determine the water content by *Method I* before using for quantitative analyses. •

Change to read:

Identification—

A: •The infrared absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Succinylcholine Chloride RS. •

B: •The retention time of the major peak in the chromatogram of the *Assay preparation* is the same as that of the *Standard preparation* obtained in the *Assay*. •

C: •Dissolve a portion in water to obtain a solution containing 1 mg per ml. Spotting 1-µl portions and using a solvent system consisting of a mixture of acetone and 1 *N* hydrochloric acid (1:1), proceed as directed under *Thin-layer Chromatographic Identification Test* ⟨201⟩. Use the following procedure to locate the spots: Heat the plate at 105° for 5 minutes, cool, and spray with potassium bismuth iodide TS, then heat again at 105° for 5 minutes. •

•

Change to read:

Assay—•[NOTE—Since the *Mobile phase* employed in this procedure has a fairly high concentration of chloride ion and a low pH, it may be advisable to rinse the entire system with water following the use of this *Mobile phase.*]

Mobile phase—Prepare a 1 in 10 solution of 1 *N* aqueous tetramethylammonium chloride in methanol. Filter this solution through a 0.45-µm membrane filter, and adjust with hydrochloric acid to a pH of about 3.0.

Standard preparation—Transfer about 88 mg of USP Succinylcholine Chloride RS, accurately weighed, to a 10-ml volumetric flask, add 4.0 ml of water, and dilute with *Mobile phase* to volume while mixing. Prepare the *Standard preparation* concurrently with the *Assay preparation.*

Assay preparation—Transfer about 88 mg of Succinylcholine Chloride, accurately weighed, to a 10-ml volumetric flask, add 4.0 ml of water, and dilute with *Mobile phase* to volume while mixing.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4-mm × 25-cm column that contains packing L3. The flow rate is about 0.75 ml per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 1.5%, and the tailing factor is not greater than 2.5.

Procedure—Separately inject equal volumes (about 10 µl) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{30}Cl_2N_2O_4$ in the Succinylcholine Chloride taken by the formula $10C(r_U/r_S)$, in which C is the concentration, in mg per ml, of anhydrous succinylcholine chloride in the *Standard preparation*, as determined from the concentration of USP Succinylcholine Chloride RS corrected for moisture content by a titrimetric water determination, r_U is the peak response of the *Assay preparation*, and r_S is the average peak response of the *Standard preparation*. •

Succinylcholine Chloride Injection

Add the following:

Reference standard—*USP Succinylcholine Chloride Reference Standard*—Do not dry; determine the water content by *Method I* before using for quantitative analyses.

Change to read:

Identification—

A: A solution (1 in 20) responds to the tests for *Chloride* ⟨191⟩.

B: It responds to *Identification tests B* and *C* under *Succinylcholine Chloride.*

Change to read:

Assay—[NOTE—Since the *Mobile phase* employed in this procedure has a fairly high concentration of chloride ion and a low pH, it may be advisable to rinse the entire system with water following the use of this *Mobile phase.*]

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay* under *Succinylcholine Chloride.*

Standard preparation—Transfer about 88 mg of USP Succinylcholine Chloride RS, accurately weighed, to a 10-ml volumetric flask, add a volume of water to correspond to the solvent composition of the *Assay preparation*, and dilute with *Mobile phase* to volume while mixing. Prepare the *Standard preparation* concurrently with the *Assay preparation.*

Assay preparation—Transfer a volume of Succinylcholine Chloride Injection, equivalent to about 80 mg of anhydrous succinylcholine chloride, to a 10-ml volumetric flask, and dilute with *Mobile phase* to volume while mixing.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Succinylcholine Chloride.* Calculate the quantity, in mg, of anhydrous succinylcholine chloride ($C_{14}H_{30}Cl_2N_2O_4$) in each ml of the Injection taken by the formula $(10C/V)(r_U/r_S)$, in which V is the volume, in ml, of Injection taken.

Sterile Succinylcholine Chloride

Add the following:

Reference standard—*USP Succinylcholine Chloride Reference Standard*—Do not dry; determine the water content by *Method I* before using for quantitative analyses.

Sulfadiazine

Change to read:

Assay—

Mobile phase—Prepare a suitable, degassed solution of water, acetonitrile, and glacial acetic acid (87:12:1).

Internal standard solution—Dissolve USP Sulfamerazine RS in methanol to obtain a solution having a concentration of about 1 mg per ml.

Standard preparation—Transfer about 100 mg of USP Sulfadiazine RS, accurately weighed, to a 100-ml volumetric flask, dilute with 0.025 *N* sodium hydroxide to volume, and mix. Mix 5.0 ml of this solution with 5.0 ml of *Internal standard solution.*

Assay preparation—Transfer about 100 mg of Sulfadiazine, accurately weighed, to a 100-ml volumetric flask, dilute with 0.025 *N* sodium hydroxide to volume, and mix. Mix 5.0 ml of this solution with 5.0 ml of *Internal standard solution.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 ml per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation is not more than 2.0%, and the resolution factor between sulfadiazine and sulfamerazine is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µl) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for sulfadiazine and 1.0 for sulfamerazine. Calculate the quantity, in mg, of $C_{10}H_{10}N_4O_2S$ in the portion of Sulfadiazine taken by the formula $200C(R_U/R_S)$, in which C is the concentration, in mg per ml, of USP Sulfadiazine RS in the *Standard preparation*, and R_U and R_S are the peak response ratios of the sulfadiazine and internal standard peaks obtained with the *Assay preparation* and the *Standard preparation*, respectively.

Sulfadiazine Tablets

Change to read:

Assay—

Mobile phase, Internal standard solution, and *Standard preparation*—Prepare as directed in the *Assay* under *Sulfadiazine.*

Assay preparation—Weigh and finely powder not less than 20 Sulfadiazine Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of sulfadiazine, to a 100-ml volumetric flask, add 75 ml of 0.025 *N* sodium hydroxide, shake for 30 minutes, dilute with 0.025 *N* sodium hydroxide to volume, and mix. Centrifuge a portion of this solution, and mix 5.0 ml of the clear supernatant layer with 5.0 ml of the *Internal standard solution.*

Chromatographic system and *Procedure*—Proceed as directed for *Chromatographic system* and for *Procedure* in the *Assay* under *Sulfadiazine.* Calculate the quantity, in mg, of $C_{10}H_{10}N_4O_2S$ in the portion of the Tablets taken by the formula $200C(R_U/R_S)$, in which the terms are as therein defined.

Sulfamerazine Tablets

Delete the following:

Disintegration ⟨701⟩: 30 minutes.

Add the following:

Dissolution ⟨711⟩—

Medium: water; 900 ml.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{11}H_{12}N_4O_2S$ dissolved from ultraviolet absorbances at the wavelength of maximum absorbance at about 243 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Sulfamerazine RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{11}H_{12}N_4O_2S$ is dissolved in 45 minutes.

Sulfamethizole

Change to read:

Heavy metals, *Method* II ⟨231⟩: 0.002%.

Sulfamethoxazole

Change to read:

Melting range, *Class I* ⟨741⟩: between 168° and 172°.

Change to read:

Selenium ⟨291⟩: 0.003%, a 200-mg test specimen being used.

Boehringer Ingelheim Ltd.

ALUPENT™
(brand of Metaproterenol Sulphate)
Solution for Inhalation

ASSAY - High Performance Liquid Chromatography

(Use special spectroquality solvents.)

Mobile phase - Dilute 10.0 ml of formic acid (reagent grade) to 1000 ml with water. Filter through a 0.45-µm membrane filter (Gelman DM-450) or equivalent.

Standard preparation - Transfer about 30 mg of Metaproterenol Sulfate Reference Standard, accurately weighed, into a 50-ml volumetric flask, dissolve in, and dilute to volume with the Mobile phase. Express the concentration as anhydrous, methanol and isopropanol-free metaproterenol sulfate.

Assay preparation - Test the solution obtained by pooling the contents of 20 units. Transfer 5.0 of the sample into a 50-ml volumetric flask, and dilute to volume with the Mobile phase.

Chromatograph conditions - May be modified as needed to achieve desired chromatographic response.

Instrument	- Waters ALC 202 Liquid Chromatograph (or equivalent)
Guard column	- Bondapak C_{18}/Corasil
Column	- 3.9 mm x 30 cm, stainless steel
Stationary phase	- µ Bondapak C_{18} (Waters')
Mobile phase	- (as defined above)
Flow rate	- 2.0 ml per minute
Detection	- UV at 278 nm

Inject 25 µl of the test solution into the chromatograph which has been suitably equilibrated. Calculate the resolution factor by the formula $2(T_2-T_1)/(W_1+W_2)$ in which T_1 and T_2 are the retention values (mm) of the peaks, and W_1 and W_2 are the widths (mm) at the baseline, obtained by extrapolation of the relatively straight sides of the peaks, for the Metaproterenol Sulfate and Metaproterenol Ketone Reference Standards, respectively. The resolution is not less than 1.5.

Procedure - Chromatograph two or more 25-µl injections each of the Standard and Assay preparations. Measure the peak heights and calculate the quantity, in mg, of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in the sample (5.0 ml) taken by the formula $5C(H_U/H_S)$ in which C is the concentration, in mg per ml, of Metaproterenol Sulfate Reference Standard, calculated as the anhydrous, solvent-free salt, in the Standard Preparation, and H_U and H_S are the averages of the peak heights for the Assay and Standard preparations, respectively.

S.V. 122 Surital 5 g.
35-122

ASSAY (SODIUM THIAMYLAL) (GAS CHROMATOGRAPHY)

Preparation of Phensuximide Internal Standard:

Transfer 300 mg. of Phensuximide accurately weighed into a 100 ml. volumetric flask, bring to volume with reagent chloroform and mix (C = 3.0 mg./ml.).

Preparation of Surital Acid Standard Solution:

Accurately weigh 460 mg. of Surital Acid into a 100 ml. volumetric flask, bring to volume with reagent chloroform and mix (C = 4.6 mg./ml.).

Preparation of Working Standard:

Pipet 5 ml. each of Phensuximide Internal Standard (C = 3.0 mg./ml.) and Surital Acid Standard Solution (C = 4.6 mg. ml.) into a glass stoppered tube and mix.

Preparation of Sample:

Carefully remove aluminum cap and rubber stopper from steri-vial. Add 25 ml. of distilled water, swirl the contents of the steri-vial until the powder is completely dissolved and transfer solution to a 100 ml. volumetric flask. Rinse steri-vial with small amounts of distilled water and transfe rinsings to the same flask. Bring to volume with distilled

S.V. 122 Surital 5 g.
15-122

ASSAY (SODIUM THIAMYLAL) (GAS CHROMATOGRAPHY)

water and mix. Pipet 10 ml. of this solution to a 125 ml. separator. Add 25 ml. of distilled water and 5 ml. 1N HCl. Mix contents of the separator and extract with 25,25,25,20 ml. of chloroform passing extracts into a 100 ml. volumetric flask Bring to volume and mix.

Procedure:

Pipet 5 ml. of prepared sample and 5 ml. of Phensuximide Internal Standard Solution (C = 3 mg./ml.) into a glass stoppered tube and mix. Inject 2 µl of sample and working standard into the chromatograph using the outlined instrument conditions. Calculate the area ratio of Phensuximide/Surital for the sample (A) and standard (B).

Calculation:

$$g \text{ Sodium Thiamylal/Vial} = \frac{A}{B} \times \frac{C}{1000} \times 100 \times \frac{100}{10} \times 1.086$$

$$= \frac{A}{B} \times C \times 1.086$$

Where:

C = concentration of Surital Acid Standard mg./ml.

100 = dilution of sample

$\frac{100}{10}$ = dilution of extracted sample

1.086 = factor to convert Surital Acid to Sodium Surital

S.V. 122 Surital 5 g.
35-122

ASSAY (SODIUM THIAMYLAL) (GAS CHROMATOGRAPHY)

Identification via Relative Retention Time:

The relative retention time of Surital/Phensuximide falls within the limits shown.

$$\frac{\text{Retention Time of Surital in Sample Preparation}}{\text{Retention Time of Phensuximide in Sample Preparation}} = R_{Std.} \pm 1.1\%$$

Where:

$R_{Std.}$ Relative Retention Time $= \frac{\text{Retention Time of Unknown}}{\text{Retention Time of Internal Std.}}$

$R_{Std.}$ is obtained from the chromatogram of the standard preparation.

35-122

TECHNIQUE NOTES

As a guideline for setting up a specific instrument, the operating conditions for the Hewlett Packard Model 5750 and H.P. Model 3370 Electronic Integrator are as follows.

Column:

4 ft. x 2 mm. I.D. packed with 3% OV-17 on Gas Chrom Q (100-120 mesh).

Sample Size:

2 ul.

Carrier Gas:

Helium at 30 ml./min.

Temperature:

a) Column - 170° isothermal

b) Injection Port - 180°. Do not exceed.

c) Detector - 190°

Sensitivity:

Range - 1000

Attenuation - 2 mv on electronic integrator

Detector:

Flame Ionization

Hydrogen at 60 ml./min.

Air at 500 ml./min.

Retention Time:

Phensuximide = 2.0 minutes

Surital = 4.3 minutes

APPENDIX B

COMPARISON OF THE RESPONSES OF THE ELECTRONIC INTEGRATOR WITH THAT OF THE STRIP CHART RECORDER IN CALCULATING METAPROTERENOL CONCENTRATION

APPENDIX B

COMPARISON OF THE RESPONSES OF THE ELECTRONIC INTEGRATOR WITH THAT OF THE STRIP CHART RECORDER IN CALCULATING METAPROTERENOL CONCENTRATION

Five successive 10.0 µl standard injections were made into the HPLC followed by five successive 10.0 µl sample injections and the response to each injection was recorded by the strip chart recorder described in the Concentration Analysis for Metaproterenol Sulfate in the Materials and Methods section. Immediately following the above injections, the HPLC was disconnected from the strip chart recorder and connected to the electronic integrater described in the same Materials and Methods section. Again, five 10.0 µl standard injections were made into the HPLC followed by five successive 10.0 µl sample injections; each response was recorded by the electronic integrator. The five standard replicate responses were averaged for each recording device. Each averaged standard response and respective sample response was used in equation 2 to calculate five concentration values for the samples run with each recording device.

A two tailed Student's t test was used to compare the concentration results obtained with the strip chart recorder with those from the electronic integrator. The calculated t statistic was -3.4501 and the critical t value was 3.355 ($p = .01$); therefore, there was a statistically significant difference between the concentrations calculated based on each of the two recording devices.

A term was calculated to adjust the concentration data collected on the strip chart recorder so that it would be comparable to the data accumulated on other days from the electronic integrator. This term, see equation 4, was subtracted from all concentrations computed from the strip chart data. These corrected concentration values appear in Table 10.

$$\frac{5.10 - 4.51}{5.10} = .116 \qquad (4)$$

where:

5.10 = the average concentration--strip chart recorder;

4.51 = the average concentration--electronic integrator; and

.116 = conversion term.

LIST OF REFERENCES

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Alford, R. H., J. A. Kasel, P. J. Gerone, and V. Knight. 1966. Human influenza resulting from aerosol inhalation. Proc. Sco. Exp. Biol. Med. 122:800-4.

American Society of Hospital Pharmacists. 1979. ASHP guidelines for repackaging oral solids and liquids in single unit dose and unit dose packages. Am. J. Hosp. Pharm. 36:223-4.

American Society of Hospital Pharmacists. 1977. ASHP guidelines for single unit and unit dose packages of drugs. Am. J. Hosp. Pharm. 34:613-4.

Anon. 1977. Minimum standards for pharmacies in institutions. Am. J. Hosp. Pharm. 34:1356-8.

Chaney, R. F., and M. R. Summerfield. 1984. Survey of expiration dating policies for repackaged pharmaceuticals. Am. J. Hosp. Pharm. 41:1150-2.

Christensen, J. M., R. Y. Lee, and K. A. Parrott. 1983. Stability of three oral liquid drug products repackaged in unit dose containers. Am. J. Hosp. Pharm. 40:612-5.

Elias, W. F., and J. A. Apat. 1965. Single dose disposable syringes--the tubex system. Bull. Parenteral Drug Assoc. 19:61.

Food and Drug Administration. Current good manufacturing practices. 21 C.F.R. 211.137.

Hart, R. J., and F. S. V. Marshall. 1976. Wastage of pharmaceuticals. Lancet 2:1239-40.

Hicks, C. I., J. P. B. Gallardo, and J. K. Guillory. 1972. Stability of sodium bicarbonate injection stored in polypropylene syringes. Am. J. Hosp. Pharm. 29:210-6.

Highsmith, A. K., G. P. Greenwood, and J. R. Allen. 1982. Microbial growth in parenteral medications. J. Clin. Microbiol. 15:1024-8.

Hubert, Barbara. 1983. Oral communication. United States Pharmacopeia--Monograph Specialist.

Kleinberg, M. L., G. L. Stauffer, and C. J. Latiolais. 1980. Stability of five liquid drug products after unit dose repackaging. Am. J. Hosp. Pharm. 37:680-2.

Latiolais, C. J. 1970. A pharmacy coordinated unit dose dispensing and drug administration system. Am. J. Hosp. Pharm. 27:886-9.

McDonald, D. E., H. M. Prisco, and R. J. Parente. 1972. Prefilling syringes in the hospital pharmacy. Am. J. Hosp. Pharm. 29:223-8.

Mallet, L., G. P. Sesin, J. Ericson, and D. G. Fraser. 1982. Storage of vancomycin oral solution. N. Eng. J. Med. 307:445.

Moffet, H. L., and D. Allan. 1967. Colonization of infants exposed to bacterially contaminated mists. Amer. J. Dis. Child 114:21-5.

Nedich, R. L. 1983. Selection of containers and closure systems for injectable products. Am. J. Hosp. Pharm. 40:1924-7.

Nolly, R. J., P. E. Stach, C. J. Latiolais, T. D. Sokoloski, and M. C. Nahata. 1982. Stability of thiamine hydrochloride repackaged in disposable syringes. Am. J. Hosp. Pharm. 39:471-4.

Romberg, M. A. 1979. More unit dose products are forecast. Am. J. Hosp. Pharm. 36:1728.

Roulette, A. W. 1972. An assessment of unit dose injectable systems. Am. J. Hosp. Pharm. 29:60-2.

Sanders, C. V., Jr., W. G. Johanson, Jr., and J. P. Sanford. 1970. Serratia marcescens infections from inhalation therapy medications: nosocomial outbreak. Ann. Intern. Med. 73:15-21.

Sartnurak, S., and J. M. Christensen. 1982. Stability of valproate sodium syrup in various unit dose containers. Am. J. Hosp. Pharm. 39:627-9.

S. G. K. 1971. Unit-dose: wonderchild comes of age. Hospitals 45:72-9.

Sheth, N. K., G. T. Post, T. R. Wisniewski, and B. V. Uttech. 1983. Multidose vials versus single-dose vials: a study in sterility and cost effectiveness. J. Clin. Microbiol. 17:377-9.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics, A Biometric Approach. McGraw-Hill Book Company, New York.

Talley, J. R., R. A. Magarian, and E. B. Sommers. 1973. Feasibility of unit dose packaging of medications for inhalation therapy. Am. J. Hosp. Pharm. 30:526-30.

United States Pharmacopeia Convention. 1980. Stability considerations in dispensing practice. United States Pharmacopeia XX. United States Pharmacopeia Convention, Maryland.

United States Pharmacopeia Convention. 1982. Succinylcholine Chloride. United States Pharmacopeia, Supplement 3. United States Pharmacopeia Convention, Maryland.

Varnum, J. W. 1974. Administrator's view of unit-dose. Hospitals 48:108-112.

Wilson, Maureen. 1984. Oral communication. Research and development--Boehringer Ingelheim.

Zell, M., and R. P. Paone. 1983. Stability of insulin in plastic syringes. Am. J. Hosp. Pharm. 40:637-8.

Zvirblis, P., and R. I. Ellin. 1982. Kinetics and stability of a multicomponent organophosphate antidote. J. Pharm. Sci. 71:321-5.