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Stability of the nitrogen mustard mechlorethamine in novel formulations for dermatological use

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ABSTRACT

Long term stability measurements were made for the nitrogen mustard mechlorethamine HCl at a concentration of 0.02% in six topical formulations: Aquaphor® ointment, Transcutol®, Labrasol®, 10% Transcutol® in Aquaphor®, 10% Transcutol® in Labrasol®, and Aquaphilic® ointment. The drug decomposed gradually in Aquaphor® ointment at room temperature, dropping to 95% in 4 weeks, 85% in 12 weeks, and 78% in 39 weeks. On the other hand, the drug decomposed rapidly in Aquaphilic® ointment, giving an assay of less than 20% of its initial concentration after 24 h at room temperature. Generally, mechlorethamine HCl was more stable in Aquaphor® ointment than in formulations containing Transcutol® or Labrasol®. However, the addition of the free radical inhibitor, BHT, significantly enhanced the stability of mechlorethamine in Transcutol® and Labrasol® formulations. Four BHT-stabilized Transcutol® and Labrasol® formulations gave assays in ranges of 92–99% at the end of 4 weeks, 77–98% at the end of 12 weeks, and 38–93% at the end of 41 weeks.

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1. Introduction

Mechlorethamine [nitrogen mustard, bis-(2-chloroethyl) methylamine, HN2] is one of the oldest synthetic anticancer drugs in clinical use today. The compound is a powerful alkylating agent and works by alkylating DNA, usually cross-linking between both strands of DNA, leading to cell death (Barrows, 1995; Chabner et al., 1996; USP DI, 1997). Unfortunately, the drug is not selective toward neoplastic cells, but will attack any rapidly growing cells producing undesirable effects, e.g., bone marrow depression. Thus, mechlorethamine is quite toxic, possessing mutagenic, carcinogenic and teratogenic properties (Barrows, 1995; Chabner et al., 1996; USP DI, 1997). The drug is used in combination chemotherapy called MOPP (mechlorethamine, Oncovin [vincristine], procarbazine, and prednisone) for the treatment of Hodgkin's disease and other lymphomas (Barrows, 1995; Chabner et al., 1996). Mechlorethamine has been particularly effective when used topically to treat cutaneous T-cell lymphoma (CTCL), and is reported to be highly effective in the treatment of mycosis fungoides, the most common type of CTCL (Van Scott and Kalmanson, 1973; du Vivier, 1979; Price et al., 1982, 1983; Vonderheid, 1984; USP DI, 1997; Estève et al., 1999; Kim et al., 2003).

Mechlorethamine is a highly reactive compound and is unstable in various media, a factor that must be considered during the formulation and administration of the drug product. It has long been recognized that mechlorethamine is unstable in water. When dissolved in water, the compound first forms an aziridinium ion, which is susceptible to nucleophilic attack, leading to a host of decomposition products (Golumbic et al., 1946a; Golumbic and Bergmann, 1946b). Because mechlorethamine is so highly reactive, pharmaceutical preparations of it are short lived. Therefore, when used topically, preparations of mechlorethamine HCl are freshly compounded by a pharmacist either as an aqueous solution or as an ointment. It is recommended that freshly prepared aqueous solutions be used once, then discarded; ointment preparations may be used over a longer period.

A problem encountered in topical mechlorethamine therapy is the high incidence of cutaneous hypersensitivity (Van Scott and Kalmanson, 1973; du Vivier, 1979; Vonderheid, 1984; Estève et al., 1999; Kim et al., 2003). This frequently observed adverse reaction may require cessation of the drug. While ointment preparations and aqueous solutions are equally effective in treating mycosis fungoides, Aquaphor® ointment preparations are reported to have a lower incidence of contact allergic dermatitis than aqueous solu-

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tions (Price et al., 1983; Kim et al., 2003). During treatment, the topical preparation may have to be applied to the whole body surface. Aquaphor® is greasy, sticky and unpleasant for the patient. Aquaphilic® ointment has been used as a more aesthetically appealing substitute for Aquaphor® ointment.

Ritschel and his group studied a number of drugs for which Transcutol® in certain topical preparations was able to form an intracutaneous depot, such as for griseofulvin (Ritschel and Hussain, 1988a,b), coumarin (Ritschel and Barkhaus, 1988c), meperidine (Ritschel and Barkhaus, 1988d) and papaverine (Shaaya et al., 1992). Using radiolabeled dexamethasone and hydrocortisone in a gel drug delivery system with Transcutol® as cosolvent, the total amounts of the corticosteroids in *ex vivo* studies permeating the skin decreased significantly, whereas the amounts of corticosteroids that collected within the skin increased significantly (Ritschel et al., 1991; Panchagnula and Ritschel, 1991). This phenomenon has since been observed for Triclosan (Lee et al., 2003) and sunscreens (Godwin et al., 2002). Labrasol® (caprylocaproyl macrogolglycerides) was included in this study because it is a high HLB nonionic amphiphilic excipient which has a similar property like Transcutol® to retain drugs studied intradermally (Ritschel and Barkhaus, 1988d).

The observation that Transcutol® is able to build up an "intracutaneous depot" of several studied drugs upon topical administration thus reducing the systemic body burden, spurred our interest in the use of Transcutol® to enhance the effect of mechlorethamine during the topical treatment of CTCL. The purpose of the present study was to first clarify the stability of mechlorethamine in transdermal drug delivery systems containing Transcutol® before clinical studies are attempted.

This study examines the stability of mechlorethamine in Aquaphor® ointment, Aquaphilic® ointment, Transcutol®, Labrasol®, and certain combinations of these vehicles.

2. Materials and methods

2.1. Materials

Mechlorethamine HCl, benzenethiol, and *tert*-butylamine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Reagent grade n-butyl phthalate (dibutyl phthalate) and ACS Certified sodium chloride were obtained from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-grade Omni-Solve acetonitrile (MeCN), methanol, isopropanol, heptane, methyl *t*-butyl ether (MTBE), *n*-butanol, dimethylformamide (DMF), and reagent grade concentrated ammonia, hydrochloric acid were from EMD Chemicals (Gibbstown, NJ, USA). Butylated hydroxytoluene (BHT, 2,6-di-*tert*-butyl-4-methylphenol) was from Matheson Coleman & Bell (Cincinnati, OH, USA). Reagent grade 50% sodium hydroxide was from Taylor Chemical (St. Louis, MO). Water was purified to a resistivity of 18 MΩ cm using a Milli-Q Water System (Millipore, Bedford, MA, USA).

Mustargen® (Mechlorethamine HCl for Injection) was from Merck & Co., Inc. (Whitehouse Station, NJ, USA) [Mustargen® has recently been sold to Ovation Pharmaceuticals (Deerfield, Illinois)]. Aquaphor® original ointment (Beiersdorf, Inc., Wilton, CT, USA) is an anhydrous, hydrophilic ointment base which contains white petrolatum (41%), mineral oil, ceresin, lanolin alcohol, panthenol, glycerin and bisabolol. Aquaphilic® ointment (Medco Labs, Inc., Sioux City, IA, USA) is a hydrous, hydrophilic ointment which contains ~50% water, ~20% white petrolatum, ~20% stearyl alcohol, and other components. Unguator jars used for storage of ointments were purchased from Total Pharmacy Supply (Arlington, TX, USA). Transcutol® HP and Labrasol® were kindly donated by Gattefossé Corporation (Paramus, NJ, USA). Transcutol® is purified 2-(2-ethoxyethoxy)ethanol [diethylene glycol monoethyl ether] and conforms to the USP monograph for that compound. Labrasol® was shown by NMR studies (Kreilgaard et al., 2000) to be a mixture of 30% mono-, di- and tri-glycerides of capric and caprylic fatty acids, 50% mono- and di-esters of polyethylene glycol (PEG 400) and 20% free PEG 400.

Table 1
Topical formulations of mechlorethamine HCl used in this study

Topical vehicle	Preparation containing mechlorethamine HCl[a]
Aquaphor® ointment	Aquaphor® Aquaphor® with BHT[b] Aquaphor® control (3 samples)
10% Transcutol® in Aquaphor®	10% Transcutol® in Aquaphor® 10% Transcutol® in Aquaphor® with BHT[b] 10% Transcutol® in Aquaphor® control (3 samples)
Transcutol®	Transcutol® Transcutol® with BHT[b] Transcutol® control (3 samples)
10% Transcutol® in Labrasol®	10% Transcutol® in Labrasol® 10% Transcutol® in Labrasol® with BHT[b] 10% Transcutol® in Labrasol® control (3 samples)
Labrasol®	Labrasol® Labrasol® with BHT[b] Labrasol® control (3 samples)
Aquaphilic® ointment	Aquaphilic® ointment

[a] Each formulation contained a theoretical concentration of 0.02% mechlorethamine HCl.
[b] Butylated hydroxytoluene (BHT) was added at a concentration of 0.1%.

The synthesis of bis(2-phenylthioethyl)methylamine picrate (PTEMAP) for use as a reference standard in the analysis of mechlorethamine HCl has been previously reported (Reepmeyer et al., 2008). Both PTEMAP and mechlorethamine HCl were used as reference standards in the assay of mechlorethamine HCl in formulations to assure standard integrity throughout the study and to serve as a check against one another. When not in use, PTEMAP and mechlorethamine HCl were stored in a desiccator over NaOH throughout the duration of the study.

2.2. Preparation of mechlorethamine HCl formulations

Mechlorethamine is a toxic nitrogen mustard and must be handled with the usual safety precautions (USP DI, 1997). The compound is also sensitive to water, so mortars, pestles, spatulas, and other utensils used in the preparation of mechlorethamine HCl formulations were washed with absolute ethanol, then acetone, and dried with a heat gun before use.

Six topical vehicles were evaluated in this study; they are listed in Table 1. During preliminary studies, particularly with Transcutol®, it was noted that the mechlorethamine concentration declined slowly initially, followed by a precipitous drop, suggesting a possible free radical chain reaction. Both Transcutol® and Labrasol® contain compounds with ether linkages capable of forming peroxides, which may serve as free radical initiators. When an aged and partially decomposed solution of mechlorethamine HCl in Transcutol® was tested, it gave a positive reaction with KI/starch, indicative of peroxides. For this reason, BHT was added to one portion of each formulation as a stabilizer for mechlorethamine HCl preparations, and used in the long term stability experiments. The concentration of BHT in FDA approved drugs normally ranges from 0.01 to 0.1% BHT, although some formulations contain levels as high as 2%. When added in this study, each formulation was prepared with 0.1% BHT. In addition, three control samples were prepared

and not opened until they were analyzed at the end of weeks 4, 8, and 12.

Mustargen® (mechlorethamine HCl for Injection), an FDA approved drug packaged in an ampoule containing 10 mg mechlorethamine HCl and 90 mg NaCl, served as the source of mechlorethamine HCl for preparation of the various formulations described below. The content of one ampoule was dissolved in 1 ml absolute ethanol and mixed with a sufficient amount of an ointment base or other topical vehicle to make 50 g, thus giving a theoretical concentration of 0.02% mechlorethamine HCl. Each 50 g preparation was proportioned into five unequal parts. One large (10–18 g) portion was left unchanged and another large portion was mixed with BHT to give a concentration of 0.1%. These two portions were analyzed at periodic intervals. Three smaller portions (5–8 g) of the topical preparation were set aside as control samples, which were opened once only on the day of analysis. The control samples contained no BHT.

Aquaphor® and 10% Transcutol® in Aquaphor® ointment preparations were stored in unguator jars, a polyethylene/polypropylene container with zero dead space. These containers have a push-up bottom to eliminate excess air space before and after sealing and a screw-on-lid with a screw cap in the center of the lid, which allow samples to be taken with minimal exposure to air. Transcutol®, Labrasol®, and 10% Transcutol® in Labrasol® preparations are liquids; these solutions were stored in glass vials filled near to the top to minimize head space and sealed with PFTE-lined screw caps. All preparations were stored in a dark cabinet at room temperature. Specific procedures for preparation of each formulation are given in the following sections.

2.2.1. Aquaphor® ointment

Aquaphor® ointment base was melted with a heat gun and cooled back to room temperature. This process made the ointment thinner and easier to mix with the drug. Absolute alcohol (1.0 ml) was injected via syringe into an ampoule of Mustargen®. The vial was swirled to dissolve the mechlorethamine HCL, leaving the insoluble NaCl as a precipitate. The liquid was withdrawn via syringe and added slowly to 5–10 g of Aquaphor® (pre-melted and cooled) in a mortar while mixing. Aquaphor® was added in portions and mixed to a total weight of 50.0 g. It was convenient to tare the mortar and pestle on a top loader balance before starting this procedure so that specific amounts of ointment could be added as needed. Three portions (5–8 g) were taken and placed in unguator jars to serve as control samples, and one portion (10–18 g) was taken as a test sample and placed in an unguator jar. The weight of the ointment remaining in the mortar was determined and an amount of BHT equivalent to 0.1% (e.g., −10 mg BHT to 10 g ointment) was added. To ensure a uniform mixture of BHT, most of the ointment was pushed to the sides of the mortar, BHT was added to a small amount of ointment in the bottom of the mortar, and the remainder of the ointment was gradually incorporated. This portion was also placed in an unguator jar.

2.2.2. 10% Transcutol® in Aquaphor®

It is difficult to obtain a uniform mixture of 10% Transcutol® in Aquaphor® by mixing the two materials together directly because the Transcutol® tends to separate from the Aquaphor®. The following procedure gave a uniform mixture. Aquaphor® ointment (49.5 g, 10% excess) was placed in a beaker and melted with a heat gun. Transcutol® (5.5 g, 10% excess) was added and mixed thoroughly. The mixture was allowed to cool to room temperature. A 5–10 g portion was transferred to a mortar, an absolute ethanol solution of mechlorethamine taken from Mustargen® as described above was added, and 10% Transcutol® in Aquaphor® was added in portions while mixing to give a total of 50.0 g. This material was divided into 5 portions, one of which was mixed with BHT, as described in Section 2.2.1 for Aquaphor® ointment.

2.2.3. Transcutol®

Transcutol® (5.0 g) was injected into a Mustargen® ampoule and the ampoule was swirled to dissolve the drug. The solution was transferred quantitatively to a tared bottle and diluted with Transcutol® to 50.0 g. A portion of this solution was dispensed into three 4-ml vials and filled close to the top; these serve as control samples. About half of the remaining solution was placed in a larger vial. The other half was weighed, and an amount of BHT equivalent to 0.1% was added and transferred to a vial.

2.2.4. 10% Transcutol® in Labrasol®

Proceed as described under Section 2.2.3, but inject the 5.0 g of Transcutol® containing mechlorethamine HCl (from Mustargen®) into Labrasol® and dilute to 50.0 g with Labrasol®.

2.2.5. Labrasol®

Proceed as described under Section 2.2.3, substituting Labrasol® for Transcutol®.

2.3. Exposure of the samples

All samples were stored at room temperature (∼23 °C) in the dark. In order to mimic the handling of the product by a patient, each preparation, except the controls, was opened and kept on the bench top exposed to the atmosphere, room light and indirect sunlight for 5 min every week day Monday through Thursday and 15 min on Friday. In the case of the ointments, the small cap in the center of the lid of the unguator jar was removed, but the larger lid remained closed. The opening under the small cap of the unguator jar was 1.1 cm in diameter and the opening of the vial containing a liquid preparation was 1.2 cm in diameter. The control samples remained closed until the day of analysis.

2.4. Sampling times and assays

Three 200 mg samples were taken from each formulation with and without BHT on days 0, 7, 14, 22, 29, 56, 84, and 188 (deviated by 1 day in two instances). A final sampling day varied arbitrarily from day 275 to 288 for various formulations. Exact days are shown later in the plotted results. One control sample for each formulation was opened for the first time and analyzed in triplicate on day 28, one on day 56 and one on day 84.

Mechlorethamine HCl standard and PTEMAP standard were analyzed in triplicate (3 weighings of each compound) on each day that a formulation was analyzed. Detailed procedures for derivative formation and HPLC assay for samples, blanks, and mechlorethamine HCl standard (Reepmeyer, 2005) and PTEMAP standard (Reepmeyer et al., 2008) were previously reported. Duplicate injections were made of each solution, and the standard assays bracketed the sample assays.

2.5. Measurement of the stability of mechlorethamine HCl in Aquaphilic® Ointment

All formulations were assayed every week for the first four weeks, then at less frequent intervals. It became apparent that mechlorethamine HCl decomposed much more rapidly in Aquaphilic® ointment than in the other formulations, and the rate of decomposition needed to be measured over periods of hours rather than weeks. Two procedures were followed to monitor the rate of decomposition of mechlorethamine HCl in Aquaphilic® ointment.

The first procedure was similar to the procedures used for the other formulations. An absolute ethanol solution of mechlorethamine HCl (~10 mg in 1 ml), prepared from Mustargen® as described in Section 2.2.1, was added to 5 g of Aquaphilic® ointment in a mortar and mixed; Aquaphilic® ointment was added in portions and mixed to give a total of 50 g. Some of this preparation was mixed in portions with BHT to give a final concentration of 0.1%. Three samples of about 200 mg ointment, with and without BHT, were accurately weighed and analyzed in the usual manner at nominal times of 0, 2, 5, 10, 24 and 49 h (exact times are shown in plotted results).

Due to the time it took to prepare the ointment and weigh samples for analysis, a second procedure was followed in order to get more accurate time measurements, especially at the shorter contact times. In this procedure, ointment-mechlorethamine preparations were prepared in individual reaction tubes and stirred continuously, and at specified times, the reagent solutions were added directly to the tubes and the derivatization was carried out in the usual manner (Reepmeyer, 2005). Aquaphilic® ointment (200 mg) was placed into 15 test tubes and a magnetic stir bar was added to each. One of these tubes served as an ointment blank in which no mechlorethamine and no internal standard was added. The other 14 tubes served as duplicate samples for measurements at 7 time intervals. A 40 μl volume of standard solution containing 40 μg mechlorethamine HCl was added to a test tube containing the 200 mg of ointment, the tube was capped and the mixture was stirred. Mixing times were 1 min, 20 min, 1 h, 3 h, 7 h, 15 h, and 24 h.

3. Results and discussion

Mechlorethamine is administered topically either as an aqueous solution or as an ointment formulation at concentrations of 0.01–0.04%. A variety of ointment bases have been used, including Aquabase®, Aquaphor®, hydrophilic petrolatum, white soft paraffin, and a 50/50 mixture of liquid paraffin-white soft paraffin (Price et al., 1983; Cummings et al., 1993; Barrows, 1995; Allen, 1997; USP DI, 1997; Zhang et al., 1998). While there are monographs for Mechlorethamine Hydrochloride and Mechlorethamine Hydrochloride for Injection in the USP, there is no monograph for a topical form of mechlorethamine. Aquaphilic® ointment has been used as a substitute for Aquaphor® ointment because the patient likes the way it feels on the skin.

3.1. Stability of mechlorethamine HCl in Aquaphor® ointment

Aquaphor® base is commonly used for topical mechlorethamine ointment preparations and has been used as the ointment base vehicle in clinical studies (Price et al., 1982, 1983; Kim et al., 2003). Procedures for preparing mechlorethamine HCl in Aquaphor® ointment have been described (Price et al., 1982; Allen, 1997; Zhang et al., 1998) and are not unlike the procedure used in this study.

The results of the stability study for mechlorethamine HCl in Aquaphor® ointment, with and without BHT added, and control samples are shown in Fig. 1. All percent concentration values are relative to a concentration of 100% on day 0. In Aquaphor® ointment, the level of mechlorethamine HCl shows a gradual decline, reaching 78% after 288 days. The addition of BHT had little effect on stability in Aquaphor®. At the end of week 12, the sample without BHT, with BHT, and the control sample gave assays of 85.0, 84.4, and 99.0%, respectively. Thus, exposure to the atmosphere and room light contributes to the decomposition of mechlorethamine in Aquaphor® ointment.

Zhang et al. (1998), studied the rate of decomposition of mechlorethamine HCl in Aquaphor® ointment at room temperature. The results reported in that study are consistently lower than the results shown in Fig. 1. For example, on days 30 and 90, assays were 90.1 and 77.2% in the Zhang study and calculated to be 94.8 and 84.6%, respectively, in our study. However, the concentration of mechlorethamine HCl in the Zhang study was 0.01%, while the concentration in this study is 0.02%, and this concentration difference may account for the differences in the rate of decomposition. Another difference between the two studies is the way in which the samples were handled. In the current study, samples were exposed to the atmosphere and light an average of 5 min per day even when not assayed; this would tend to accelerate decomposition. On the other hand, the unguator ointment jars that were used here allows one to eliminate the headspace within the jar and provides a smaller opening than conventional ointment jars when samples are taken. Zhang noted that decomposition occurred more rapidly during the first week and then proceeded more slowly. In the current study, the loss was slower for the first four weeks, and faster between weeks 4 and 8; this change may be attributed to the total exposure time (see discussion under Section 3.4).

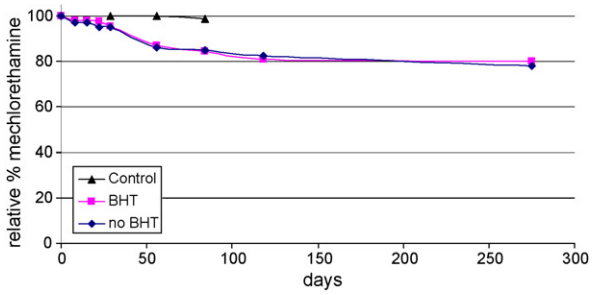

Fig. 1. Stability of 0.02% mechlorethamine HCl in Aquaphor® ointment at room temperature for (1) formulations containing BHT, (2) formulations with no BHT, and (3) control samples with no BHT. Control samples were not opened until the day of analysis; the others were opened an average of 5 min per day.

Cummings et al. (1993), studied the stability of mechlorethamine HCl in white soft paraffin ointment and observed no loss over 80 days at 4 °C and no loss over 1 month at 37 °C. White soft paraffin ointment is a water-free, hydrophobic base, while Aquaphor® is a water-free, hydrophilic base, which may account for the greater stability observed in that study. Cummings reported that the relative standard deviations (RSDs) for mechlorethamine assay in their study were less than 20% and normally less than 10%. These relatively high RSD values and the absence of tabular or graphical data in that paper, make it difficult to quantitatively compare the results from their study to the results presented here.

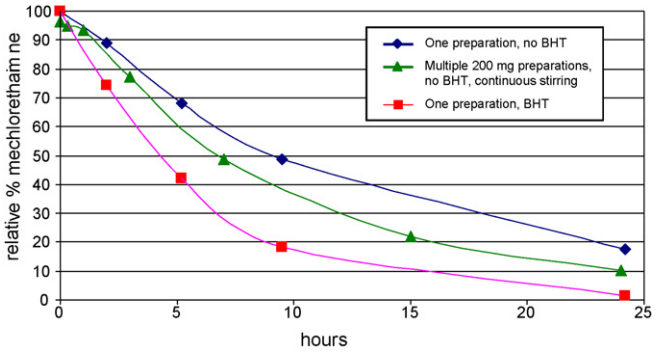

Fig. 2. Stability of 0.02% mechlorethamine HCl in Aquaphilic® ointment at room temperature.

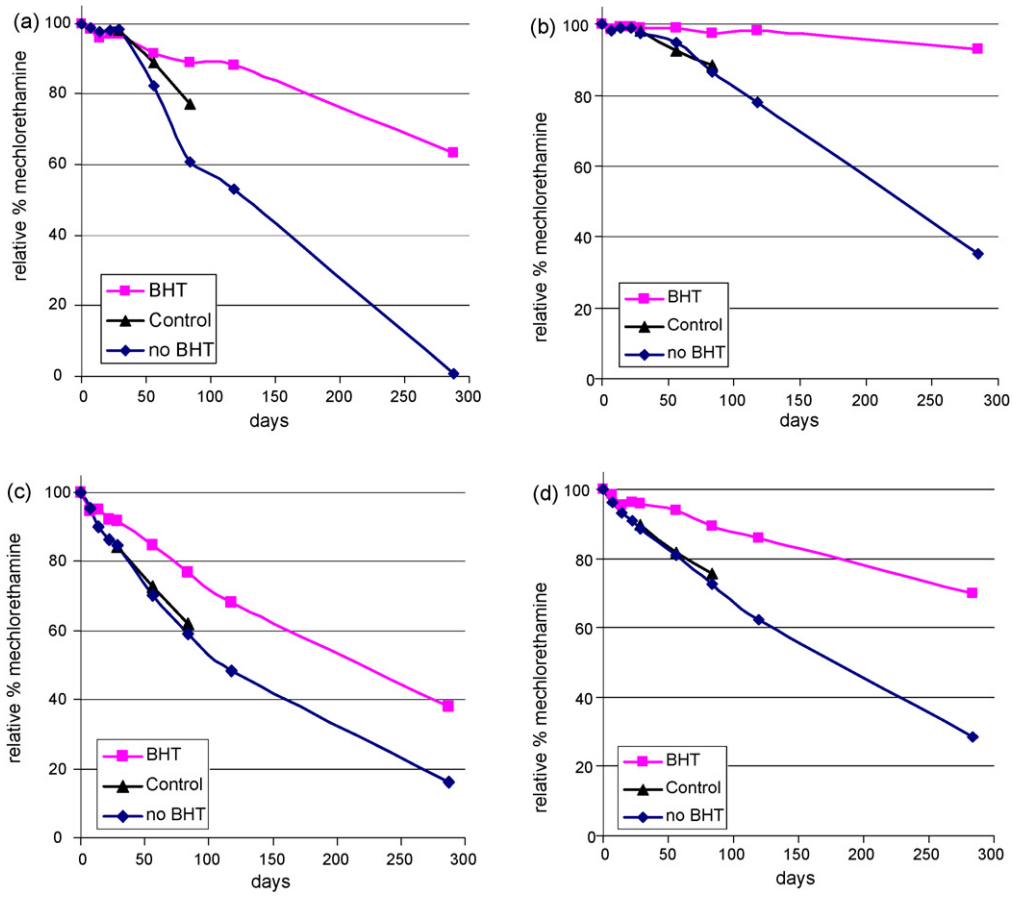

Fig. 3. Stability of 0.02% mechlorethamine HCl at room temperature in (a) 10% Transcutol® in Aquaphor® ointment, (b) Transcutol®, (c) 10% Transcutol® in Labrasol®, and (d) Labrasol®. BHT was added to one portion of each formulation. Control samples, which contained no BHT, were not opened until the day of analysis; the others were opened an average of 5 min per day.

3.2. Stability of mechlorethamine HCl in Aquaphilic® ointment

Mechlorethamine is exceptionally unstable in Aquaphilic® ointment, decomposing by more than 80% over 24 h (Fig. 2). The bulk ointment product (upper plot) was a little more stable than the individual 200 mg ointment samples (middle plot) that were mixed in the reaction tubes, probably because the latter were constantly stirred during the sampling time while the former was static. For some unknown reason, BHT hastened the rate of decomposition, although, considering the rapid rate of decomposition, this difference is inconsequential. Aquaphilic® ointment has been prescribed by physicians and used by patients as a substitute for Aquaphor® ointment, primarily because Aquaphilic® ointment feels more comfortable to the patient when applied to the skin. This study clearly demonstrates that Aquaphilic® ointment is not a suitable medium for mechlorethamine HCl.

3.3. Stability of mechlorethamine HCl in Transcutol® and Labrasol® formulations

Plots showing the stability of mechlorethamine HCl in the four formulations containing Transcutol® or Labrasol® are shown in Fig. 3. The addition of 10% Transcutol® to Aquaphor® significantly increases the rate of decomposition of mechlorethamine HCl when compared to Aquaphor® alone. However, in this formulation, BHT plays a significant role in stabilization. After 118 days in 10% Transcutol® in Aquaphor®, the preparation assays are 89.6 and 56.8%, with and without BHT, respectively. In fact, the BHT stabilized ointment gives higher assays than the control samples.

As in the case of 10% Transcutol® in Aquaphor®, BHT has a pronounced effect on the stability of mechlorethamine HCl in Transcutol® (Fig. 3b). In fact, Transcutol® with BHT was the most stable formulation of all those tested, giving an assay of 98.1% after 118 days and 92.9% after 288 days.

BHT has a pronounced effect on mechlorethamine HCl stability in all four formulations containing Transcutol® and Labrasol®. With the exception of BHT stabilized Transcutol®, all other Transcutol® and Labrasol® formulations gave lower assays than Aquaphor® ointment at the end of the test period.

3.4. Variability in sample assays

The precision of the assays for mechlorethamine HCl in the formulations are given in Table 2 (Aquaphilic® ointment was excluded because of the rapid rate of decomposition in it). Greater variability was seen in the assay of the two ointment preparations (Aquaphor® and 10% Transcutol® in Aquaphor®) than in the three liquid preparations (Transcutol®, 10% Transcutol® in Labrasol®, Labrasol®). Table 2 shows that the RSDs for the liquid preparation assays were below 1% most of the time and between 1 and 2% in a few instances; there was no case where these samples exceeded an RSD > 2%. The RSDs for the ointment assays during the early weeks of the study were also less than 2% with some exceptions, but several assay RSDs became quite high as the time of the study progressed, with a high of 19.3% RSD for 10% Transcutol® in Aquaphor® on Day 118 (day 288 for 10%

Table 2
Precision in the analysis of mechlorethamine HCl in various topical formulations

Day	Aquaphor®			10% Transcutol® in Aquaphor®			Transcutol®			10% Transcutol® in Labrasol®			Labrasol®		
	No BHT	BHT	CTRL	No BHT	BHT	CTRL	No BHT	BHT	CTRL	No BHT	BHT	CTRL	No BHT	BHT	CTRL
0	0.42	0.66		1.61	0.59		0.07	0.56		1.84	0.83		1.49	0.74	
7	1.55	1.93		0.53	0.95		0.37	1.39		0.81	0.85		0.54	0.78	
14–15	0.88	1.26		1.32	0.68		0.22	0.53		0.21	0.28		0.88	0.50	
22	2.97	2.37		1.45	1.11		0.23	0.37		0.21	0.28		0.35	0.22	
29	3.39	2.34	0.54	1.27	0.74	0.32	0.41	0.20	0.11	0.04	0.16	0.10	0.24	0.18	0.23
56	8.08	7.21	1.07	1.60	4.03	1.25	0.47	0.18	0.34	0.34	0.32	0.38	0.37	0.14	0.34
84	7.05	4.18	1.14	9.94	7.43	2.92	0.36	0.23	0.40	0.49	0.40	0.34	0.36	0.16	0.24
118–119	7.39	6.91		19.33	5.73		0.14	0.48		0.53	1.06		0.44	0.07	
275–288	2.48	2.36		56.37	11.37		0.67	0.45		0.23	0.12		0.10	0.32	
Mean	3.80	3.24	0.92	4.68[a]	3.62	1.50	0.33	0.49	0.28	0.52	0.48	0.27	0.53	0.35	0.27

Percent RSD ($n = 3$). There were 5 preparations for each topical vehicle: One with no BHT and one containing 0.1% BHT, both of which were opened an average of 5 min per day, and three control (CTRL) samples that were opened only on the day of analysis.

[a] This mean value does not include the % RSD recorded on day 288 because the assay was <1% relative concentration on that day.

Transcutol® in Aquaphor® had an RSD of 56%, but this was measured at an exceptionally low mechlorethamine concentration, <1% relative). An evaluation of the data over the 288 day period shows that there are 19 assays with RSDs > 2%; all of these are with the two ointment preparations. The raw data were evaluated to determine the cause for this variation and one striking observation was made. In all 19 cases, the first sample taken from the ointment gave the lowest assay, and in 17 of 19 cases, the second sample gave lower assays than the third sample (data not shown). We interpret these data in the following way. The first sample taken is the sample of ointment at the entrance to the unguator jar. This sample gets the most exposure to the atmosphere and to light when the sample is opened daily for 5 min. The second sample is below the surface and has less exposure, and the third sample has the least exposure. During the early stages of the study, samples were taken every week. Therefore, exposure time was 7 days for 5 min or 35 min total. During the later stages, samples were taken every 4 weeks or longer, and total exposure time was then 140 min or longer. With longer exposure time, the sample near the surface has undergone greater decomposition and there is more variability between the first, second and third samples taken from the unguator jar. This explains the high variability of the ointment assays and also demonstrates the significance of exposure of the ointment to air and light. The three liquid formulations did not show high RSDs, indicating greater sample uniformity, presumably due to disturbance during handling or simply due to Brownian motion.

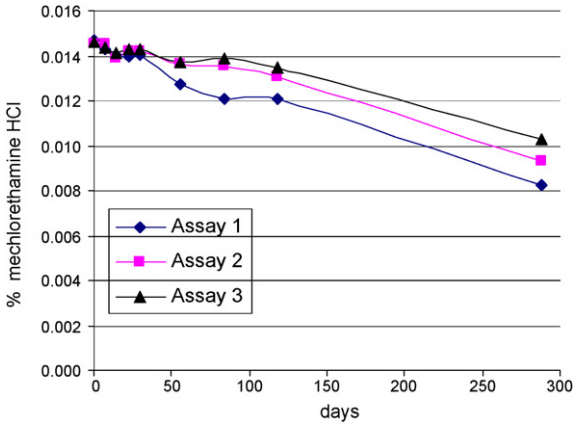

Fig. 4. Plots of individual assays of mechlorethamine HCl in 10% Transcutol® in Aquaphor® ointment containing 0.1% BHT show that for each day of analysis, beginning with day 56, the first sample taken gives a lower assay than the second, which in turn, gives a lower assay than the third.

Finally, in regard to the stability data, it is likely that the average assay determined for mechlorethamine HCl in the ointments is below the overall concentration of mechlorethamine-HCl throughout the ointment since the measurements are biased by the lower concentration at the entrance to the unguator jar. Using the data from the third sample alone would show less decomposition than the triplicate assay average. Fig. 4 shows plots of individual assays of mechlorethamine HCl in 10% Transcutol® in Aquaphor® with BHT. The ointment control samples gave higher assays than the samples that had been opened, again showing the effect of exposure of the ointment preparation to air and light.

4. Conclusions

Mechlorethamine HCl is unstable in Aquaphilic® ointment, giving assays of <20% in 24 h. This formulation has been prescribed and is preferred by patients because it feels better than the sticky, greasy Aquaphor® ointment, particularly when used in whole body applications. This study clearly shows that the stability of the drug in Aquaphilic® ointment is unsatisfactory, and this ointment base should not be used as a substitute for other ointment bases when intended for use over a period of months, weeks or even days.

The drug decomposes gradually in Aquaphor® ointment when stored in the dark at room temperature and exposed to air 5 min per day, giving assays of 95, 85, and 78% at the end of weeks 4, 12 and 39. Exposure to air accelerates decomposition.

Mechlorethamine HCl is generally less stable in formulations containing Transcutol® and Labrasol® than in Aquaphor® alone. However, the addition of BHT (0.1%) to formulations containing Transcutol® or Labrasol® significantly reduces the rate of decomposition, while it has little effect on stability in Aquaphor® ointment. Of all formulations tested, the drug was the most stable in Transcutol® containing BHT.

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