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The Long Term Stability of Mechlorethamine Hydrochloride (Nitrogen Mustard) Ointment Measured by HPLC

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Abstract—HPLC has been applied to determine the stability of mechlorethamine hydrochloride (nitrogen mustard) formulated as an ointment in white soft paraffin (10 mg drug, 50 g paraffin and 1 mL acetone). A new solubilization technique is described for extraction of the drug from the ointment for HPLC analysis which has an extraction efficiency of 76·1% with a coefficient of variation of 10·4%. Stored at 4°C the drug content of the ointment remained stable for at least 84 days and stored at 37°C the drug content remained stable for at least 40 days. In comparison aqueous solutions of mechlorethamine at the same concentration, fully degraded after 4 days. These data will aid hospital pharmacists decide on a standardized protocol for the controlled usage of the ointment which is both safe and cost-effective.

Mechlorethamine hydrochloride (nitrogen mustard, HN2, NSC-762) has been used systemically as an anticancer drug since the early 1940s (Jacobson et al 1946; Rhoads 1946). Due to its high chemical reactivity as a bifunctional alkylating agent with a wide variety of nucleophiles, including DNA, analysis of the drug in complex matrices has proved difficult (Skibba & Collins 1980). Consequently, there is only limited data on the drug's stability in solution and its pharmacokinetics and biological fate after administration (Ausman et al 1961). Most published work on mechlorethamine has used a colorimetric assay employing nitrobenzyl pyridine (NBP) which measures alkylating activity rather than the intact drug (Epstein et al 1955; Friedman & Boger 1961). Several derivatizing reagents have been tried unsuccessfully to trap mechlorethamine in a form which can then be subjected to chromatographic evaluation (White et al 1989). There have also been reports of HPLC methods for direct determination of the parent compound (Kirk 1987).

As an anticancer drug, mechlorethamine has been largely replaced in combination chemotherapy regimes by newer, more stable, bifunctional nitrogen mustards such as melphalan and cyclophosphamide, which have a better therapeutic index. However, another role has emerged for the drug in the treatment of the skin conditions, mycosis fungoides and psoriasis (Van Scott & Kalmanson 1973; Breza et al 1975). This involves topical application to the affected parts of the skin with either a drug solution, which is normally prepared by dissolving the contents of a 10 mg vial in 50 mL of water, or the same dose of drug formulated in a white soft paraffin (BP) ointment. Manufacturers advice for drug solutions is that they should be used immediately following preparation and then discarded. In the case of the ointment, which can be given on an outpatients basis in the UK, no firm data exist on shelf-life, safe handling and disposal. Mechlorethamine is a strong vesicant and very harmful to mucous membranes; it can also cause secondary skin cancers. Therefore, there is a need for a set of recommendations regarding the usage of this hazardous ointment. We have recently published the first fully validated HPLC assay for the determination of mechlorethamine in complex specimens such as human whole blood and plasma (Cummings et al 1991). In this report we have applied this method, with a new sample preparation technique to perform long-term stability studies on mechlorethamine in ointment form.

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Materials and Methods

Chemicals

Diethyldithiocarbamic acid (sodium salt, DDTC) was from Sigma Chemical Co. (Poole, UK); acetonitrile, chloroform, propan-2-ol and methanol were HPLC reagent grade from Rathburn Chemicals (Walkerburn, UK); acetone, hydrochloric acid (HCl) and sodium hydroxide (NaOH) were analytical reagent grade (AnalaR, BDH, Poole, UK). White soft paraffin (BP) was obtained from Pearce Laboratories (Leeds, UK). Mechlorethamine hydrochloride salt used as an analytical standard was from the Aldrich Chemical Co. (Poole, UK). Mechlorethamine hydrochloride used in the ointment for stability studies was obtained from Boots Company plc (Beeston, UK) as Mustine Hydrochloride for Injection BP in 20 mL vials containing 10 mg drug. Water was deionized and double-distilled in a quartz glass still.

Drug analysis techniques

Mechlorethamine was determined by HPLC using derivatization with DDTC as described in detail (Cummings et al 1991). The chemistry of the derivatization reaction is illustrated in Fig. 1. The procedure for preparing ointment samples for HPLC is shown schematically in Fig. 2. The initial stage was solubilization of 0·1 g of ointment into a homogeneous solution with 8 mL chloroform, 1·9 mL propan-2-ol and 0·1 mL water. For control extractions 0·1 mL water was replaced with 0·1 mL mechlorethamine standard at the same concentration as the ointment and 0·1 g of a blank ointment base not containing drug was substituted. Mechlorethamine was then extracted into 10 mL of 10 mM HCl using a separatory funnel by inverting for 2–3 min twice with a 10 min interval. The lower organic phase was

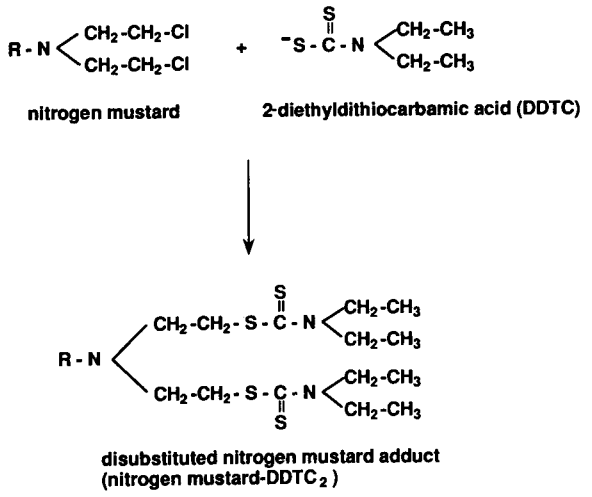

FIG. 1. Reaction mechanism for derivatization of mechlorethamine hydrochloride with diethyldithiocarbamic acid. R=CH_3. Taken from Cummings et al (1991).

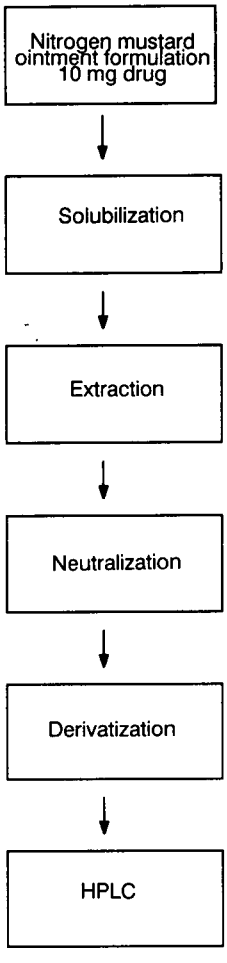

FIG. 2. Schematic representation of the sample preparation technique for determination of mechlorethamine hydrochloride in white soft paraffin ointment (10 mg drug: 50 g ointment).

discarded and the aqueous phase was neutralized with 1 mL 0·1 M NaOH. To this solution was then added 1 mL 100 mg mL^{-1} DDTC in 0·1 M NaOH as a derivatizing agent which was incubated for 1 h at 37°C. A 1 mL sample was then withdrawn from the solution and mixed with 1 mL methanol and 100 μL was subjected to HPLC.

Stability studies

Samples of formulated mechlorethamine ointment were prepared freshly as follows and stored in clear glass jars: 10 mg mechlorethamine was dissolved in 1 mL acetone which was then worked into 50 g white soft paraffin to produce an even consistency. Jars of ointment were stored either at 4 or 37°C in a water bath with a lid. At the various times indicated in the results section a minimum of 10 samples were taken per jar and analysed as described above. For comparative studies, aqueous solutions of mechlorethamine at the same concentration as the ointment were kept at room temperature (21°C) and analysed for stability.

Results

Drug analysis of mechlorethamine hydrochloride ointment

The HPLC retention time (t_R) of mechlorethamine as the DDTC derivative (see Fig. 1) is 13·1 min ± 1·5% (Cummings et al 1991). Fig. 3 shows HPLC analysis of mechlorethamine ointment showing a peak at 13·1 min which was absent in control analyses of blank base without drug. By use of a multi-diode array detector it was shown that this peak had a UV spectrum identical to the disubstituted DDTC-adduct of mechlorethamine with absorbance maxima at 276 and 248 nm. Several peaks were assignable to the ointment itself with retention times of 7·5, 10·7 and 15·3 min. The extraction efficiency of the sample preparation technique was 76·1% with a coefficient of variation of 10·4%. HPLC analysis of mechlorethamine in aqueous solution is illustrated in Fig. 4. and analysis of the same solution after 24 h at room temperature is illustrated in Fig. 5. Here considerable degradation had occurred (approx. 50% reduction in peak height/area compared with time zero) and a series of peaks corresponding to degradation products was evident with retention times of 4·9 and 5·2 min.

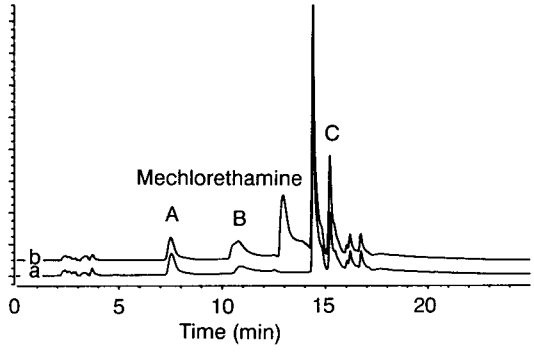

FIG. 3. HPLC analysis of mechlorethamine hydrochloride ointment formulation. Chromatogram a, blank ointment base not containing drug. Peaks A (t_R, 7·5 min), B (t_R, 10·7 min) and C (t_R, 15·3 min) are identified as being due to the white soft paraffin/acetone formulation. Chromatogram b, ointment base plus the drug. The mechlorethamine derivative (t_R, 13·1 min) is identified distinctly from the components formulation vehicle.

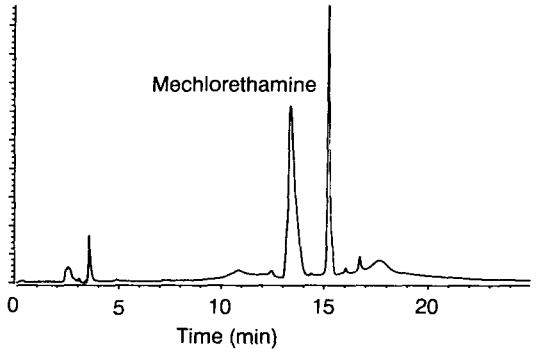

FIG. 4. HPLC analysis of a freshly made up aqueous solution of mechlorethamine hydrochloride.

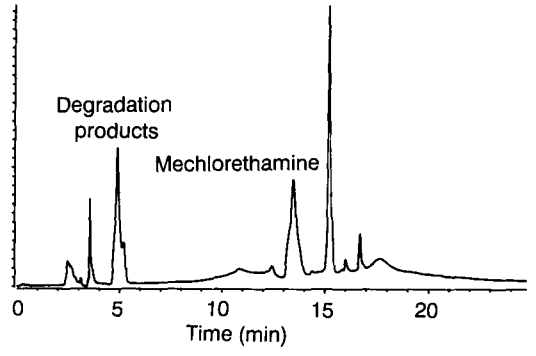

FIG. 5. HPLC analysis of an aqueous solution of mechlorethamine hydrochloride incubated at room temperature for 24 h. The mechlorethamine-derived peak had reduced by approx. 50% and degradation products were evident at t_R 4·9 and 5·1 min.

Stability studies

The long-term stability profile of 10 mg mechlorethamine formulated in 50 g white soft paraffin ointment stored at 4°C in glass jars was followed over 84 days. Drug analyses were performed at 13 points during this period: 3 during the first day, 6 during the first week and the remaining 7 spread throughout the next 77 days. Within experimental error, over the entire period, no significant reduction in the content of active drug occurred. Coefficients of variation in drug determinations did not exceed 20% and were normally less than 10%. Long term stability of the ointment incubated at 37°C was followed over 40 days. Drug analyses were performed at 8 different time points throughout this period: 3 during the first day, 4 during the first week and the remaining 4 spread throughout the next 33 days. Here again, no significant reduction in drug content occurred over the entire study period. Similar coefficients of variation in drug determinations to the 4°C studies were also recorded. This data should be compared with the stability of an equivalent concentration of mechlorethamine made up as an aqueous solution and kept at room temperature. In this case, drug analyses were performed at 6 points over a 4-day period including 4 during the first day. Here, the drug completely degraded after 4 days with an approximate terminal half-life of 24 h.

Discussion

In this work HPLC has been employed to determine the stability of mechlorethamine in ointment. The degradation (hydrolysis) of mechlorethamine is well characterized giving rise to *N*-methyl ethanolamine (compound V) and is detailed in Fig. 6 (Kirk 1987). Alternatively, the drug can degrade by reacting with a wide variety of nucleophiles to form covalent adducts. Several intermediates in the degradation of mechlorethamine (compounds II–IV, Fig. 6) retain alkylating activity and would be detected by NBP colorimetric assays. However, only the species which have the potential to form DNA cross-links, i.e. bifunctional species (I and II, Fig. 6) are believed to be the active forms of the drug; the monofunctional intermediates have been related to carcino-

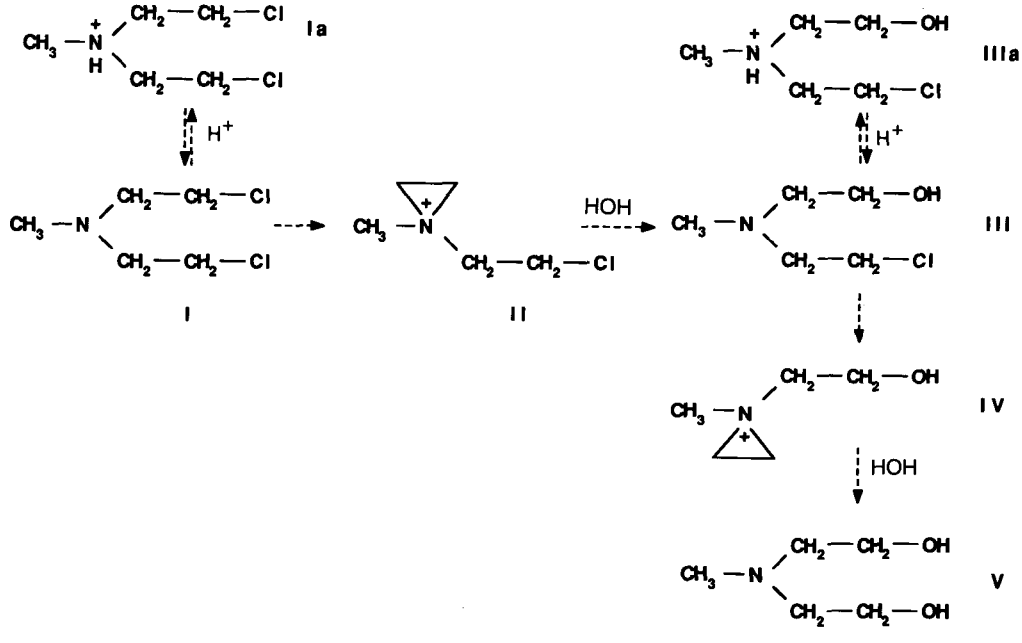

FIG. 6. Chemical pathway of hydrolysis of mechlorethamine hydrochloride. Taken from Kirk (1987).

genicity (Jeffrey 1985). Therefore NBP assays are likely to underestimate the degree of drug degradation, especially since the chlorohydrin intermediate (III, Fig. 6) has been shown to be ten times more stable than the parent compound (Cohen et al 1952), which has been confirmed by recent studies by Kirk (1987) with an HPLC method which depends on electrochemical detection of mechlorethamine and its byproducts. In that work the NBP assay suggested only 2·7% breakdown of mechlorethamine in aqueous solution after 24 h whereas the HPLC method recorded 15% breakdown after only 6 h, in agreement with our observation of 50% degradation after 24 h.

The HPLC method featured in this present work relies on derivatization of both functionalities of the mustard and whilst DDTC will form monosubstituted adducts, these are clearly resolved during chromatography (di-DDTC, t_R 13·1 min; mono-DDTC, 5–6 min) (Cummings et al 1991). Thus, the assay will distinguish between the active forms of the drug (I and II) with bifunctional alkylating activity and the toxic forms of the drug with monofunctional alkylating activity (III and IV). Indeed, in the aqueous stability studies, degradation products with t_R values of 5–6 min were identified.

Few studies have been performed on the stability of mechlorethamine ointments and these have used an NBP assay, which must be considered suspect. In the study of Taylor et al (1980), little breakdown appeared to occur over a 50-day period. The data presented in this paper show that mechlorethamine remains stable, formulated as an ointment for at least 80 days stored refrigerated at 4°C, and at least 1 month stored at 37°C. These results will aid hospital pharmacists to decide on a standardized protocol for the controlled preparation and storage of this formulation, which is both safe and cost effective.

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