Doxorubicin Stability in Syringes and Glass Vials and Evaluation of Chemical Contamination

S. Walker, D. Lau, C. DeAngelis, J. Iazzetta and C. Coons

ABSTRACT

The concentration of reconstituted doxorubicin hydrochloride solution (2 mg/mL) was evaluated during 124 days storage in original manufacturers vials and two different brands of syringes (Terumo, Monoject) at 4°C and 23°C.

On each of 14 study days the doxorubicin hydrochloride concentration was determined using a validated, stability-indicating liquid chromatographic method. On each day pH, colour, physical inspection and the concentration of 2-mercaptobenzothiazole (MBT), 2-(2-hydroxyethylmercapto) benzothiazole (HMBT) and di-2-ethylhexylphthalate (DEHP) was also measured.

During the 124-day study period all solutions stored at 4°C and 23°C retained more than 90 percent of the initial doxorubicin hydrochloride concentration, remained clear and had no change in UV-VIS spectrum absorbance or pH. On day 124, mass balance indicated that approximately 3.5 percent of the doxorubicin had degraded at room temperature and about one percent had degraded at 4°C. The MBT, HMBT and DEHP concentrations were below detectable limits.

We conclude that doxorubicin solutions (2 mg/mL) stored at 4°C or 23°C in the original glass vial are stable for 124 days. Since investigations have failed to demonstrate contamination of these solutions stored in Terumo or Monoject syringes, we conclude that doxorubicin solutions (1 mg/ml and 2 mg/ml) are stable when stored at 4°C or 23°C in syringes, and maintain acceptable product purity.

Key Words: *doxorbucin, drug stability, leaching, plasticizers*

RÉSUMÉ

La concentration de la solution de chlorhydrate de doxorubicine reconstituée (2 mg/mL) a été évaluée pendant 124 jours d'entreposage dans les flacons originaux des fabricants et dans deux différentes marques de seringues (Terumo et Monoject) à 4°C et 23°C.

La concentration de chlorhydrate de doxorubicine a été déterminée chaque jour pendant quatorze jours d'étude en utilisant une méthode de chromatographie liquide approuvée pour déterminer la stabilité. Pendant chaque jour d'étude on a aussi mesuré le pH, la couleur, l'analyse physique ainsi que la concentration de deux produits chimiques: 2-mercaptobenzothiazole (MBT), 2-(2-hydroxyéthylmercapto) benzothiazole (HMBT) et du di-2-éthylhexylphthalate (DEHP).

Pendant la période d'étude de 124 jours, toutes les solutions entreposées à 4°C et 23°C ont retenu plus de 90 pour cent de la concentration initiale de chlorhydrate de doxorubicine; elles sont demeurées claires et sans aucun changement d'absorbance de spectre UV-VIS ou de pH. Au jour 124, la balance de masse indiquait qu'environ 3.5 pour cent de la doxorubicine s'étaient dégradés à la température de la pièce et à peu près un pour cent s'étaient dégradés à 4°C. Les concentrations de MBT, HMBT et DEHP étaient en dessous des limites qu'on peut détecter (100, 100 et 200 ng/mL respectivement) dans toutes les solutions.

Nous concluons donc que les solutions de doxorubicine (2 mg/mL) entreposées à 4°C ou 23°C en flacons de verre originaux sont stables pendant 124 jours. Puisque les expérimentations n'ont pas réussi à démontrer que les solutions (1 mg/ml and 2 mg/ml) entreposées dans des seringues Terumo ou Monoject étaient contaminées, nous concluons que les solutions de doxorubicine sont stables lorsqu'entreposées dans des seringues à 4°C ou 23°C et maintiennent une pureté acceptable du produit.

Mots clés: *doxorubicine, stabilité du médicament, filtrer, plastifiants*

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Scott E. Walker, MSc. Phm. is Coordinator, Quality Control, Department of Pharmacy, Sunnybrook Health Science Centre, and Associate Professor, Faculty of Pharmacy, University of Toronto.

Danny WC Lau, Dip. Pharm. Tech., Research Assistant, Department of Pharmacy, Sunnybrook Health Science Centre.

Carlo DeAngelis, Pharm. D., Coordinator Oncology, Department of Pharmacy, Sunnybrook Health Science Centre, and Lecturer, Faculty of Pharmacy, University of Toronto.

John Iazzetta, Pharm. D., Coordinator Drug Information, Department of Pharmacy, Sunnybrook Health Science Centre, and Assistant Professor, Faculty of Pharmacy, University of Toronto.

Carolyn E. Coons, B.Sc.Phm., was supervisor, Department of Pharmacy, Toroto Bayview Regional Cancer Centre, Sunnybrook Health Science Centre.

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Address correspondence to: Scott Walker, Pharmacy Dept., Sunnybrook Health Science Centre, 2075 Bayview Avenue, North York, Ontario M4N 3M5.

INTRODUCTION

Doxorubicin (NSC No.:123127) and epirubicin (4' epidoxorubicin; NSC No.:256942) are anthracycline derivatives used in the treatment of neoplastic diseases. Doxorubicin differs from epirubicin in the epimerization of the hydroxyl group in the 4' position of the amino sugar.[1] The stability of doxorubicin in a variety of solutions has been extensively reviewed by Bosanquet.[2] This review includes data from reports in which non-specific (not stability-indicating) biologic, ultraviolet or fluorescent methods were used to estimate the doxorubicin concentrations. In this review, even data from cited reports which used an HPLC method[3-6] give conflicting recommended expiry dates ranging from 14 hours to 180 days. Bosanquet,[2] however, does not consider any of the 14 methods used to be stability-indicating, and concludes that a "carefully designed study is urgently required".[2] A survey of 18 intravenous (IV) chemotherapy preparation sites in Ontario in July of 1989[7] revealed that expiry dates for doxorubicin ranged from 24 hours to "until used". At this same time the manufacturer recommended that doxorubicin, reconstitued with normal saline, be discarded after 24 hours storage at room temperature or 48 hours storage at 4°C.[8]

After reconstitution with normal saline, to achieve a concentration of 2 mg/mL, or sometimes 1 mg/mL, doxorubicin is generally drawn into a syringe and dispensed since this dose could be cancelled, knowledge of doxorubicin stability in a syringe would be important given the cost of doxorubicin and the relationship reported between expiry dates and wastage.[9] However, many factors, in addition to drug stability, can affect the expiry date placed on a product. While the financial incentives to extend an expiry date and reduce wastage may be great,[9] patients should not be placed at risk.

Rubber gaskets found in most syringes have been reported to leach chemicals, primarily 2-mercaptobenzothiazole-(MBT) and 2-(2-hydroxyethylmercapto) — benzothiazole (HMBT) while PVC containers have been reported to leach di-2-ethylhexyl phthalate (DEHP) into the contact solution.[10-15] These compounds are potentially harmful[16] and so evaluation of the rate and amounts of these chemicals, if present, leaching from the syringe into the solution was also thought to be necessary.

It was the aim of this investigation to establish the stability of reconstituted doxorubicin hydrochloride solution in original vials and syringes into which the solution had been transferred. To evaluate the possibility of chemicals leaching into these solutions, the concentrations of MBT, HMBT and DEHP were estimated and the solutions monitored for other contamination during storage in two different brands of syringes as well as in the original glass vials. In order to complete this study, it was necessary to have analytical methods which could separate and quantitate doxorubicin specifically in the presence of degradation products and leaching chemicals, as well as measure the leaching chemicals. This required the development and validation of liquid chromatographic methods for doxorubicin, MBT, HMBT and DEHP.

METHOD

Assay Validation

Following set-up of the chromatographic system, for doxorubicin, the suitability of this system for use as a stability indicating assay method was tested by accelerating the degradation of doxorubicin. Doxorubicin HCl, 50 mg, (Adriamycin®-Adria Laboratories; lot #9010DC) was dissolved in 125 mL of normal saline. Aliquots (25 mL) of this solution were adjusted with 1 N HCl to achieve a pH of 2.04, 2.21, 2.43 and 2.66 and 4.02. Each solution was placed in a 30 mL multidose vial (SoloPak Laboratories) and incubated in a water bath at 90°C protected from light. Samples were drawn just prior to incubation and at 30, 60, 130, 180, 240, and 300 minutes. Then studies were stopped at 300 minutes because at this point the doxorubicin hydrochloride concentration had been reduced to less than 10 percent of initial in each solution to which HCl had been added. Chromatograms were inspected for the appearance of additional peaks, and the doxorubicin peak was compared between samples for changes in concentration, retention time and peak shape. To further test if the chromatographic system could separate doxorubicin from degradation products, the purity of the peak referred to in chromatograms as doxorubicin was tested by three methods. First, using a photodiode array detector, complete UV-VIS spectra (200-800 nm, slit width 0.25 nm; resolution 1.4 nm; deuterium lamp) from the leading edge, middle and tail of the doxorubicin peak in a degraded sample were compared to UV-VIS spectra observed for the time zero sample. Once UV-VIS spectral purity had been demonstrated, mass spectroscopy (MS) was completed on degraded samples, a doxorubicin standard and doxorubicin degradation products. These standards included (i) doxorubicin (Sigma. lot #127F-0148) as well as degradation products provided by Adria Laboratories of Canada; (ii) 13(s)-dihydro-

doxorubicin hydrochloride (lot #GF6585/24); (iii) adriamycinone (lot #AB4455/63) the major degradation product and; (iv) 13-dihydro-adriamycinone (lot #EV6351/59). [Since doxorubicin and epirubicin differ only in the epimerization of the hydroxyl group in the 4' position of the amino sugar,[1] when degradation results in the loss of this sugar to form the aglycone, degradation products of doxorubicin and epirubicin become identical.] Mass spectrum analysis was completed using an LC-MS-MS system with a MS-MS detector (API III; Sciex) which monitored the LC effluent. Positive and negative orifice voltages of 60V and 90V allowed determination of parent ion mass. Mass spectrum analysis was completed at Mann Testing Laboratories Ltd., Mississaugua, Ontario. Using these standards, mass spectroscopy and photodiode array detection, most peaks in each chromatogram could be identified, the ability of the chromatographic system to separate doxorubicin from its degradation products could be tested, and loss of doxorubicin due to degradation could be accounted for by increases in degradation products (mass balance).

Following this first phase of evaluation and validation, the accuracy and reproducibility of standard curves were tested over a five-day period. On each day 120 mg of doxorubicin hydrochloride powder (Adriamycin®-Adria Laboratories - lot #9010DC - 50 mg of doxorubicin hydrochloride per 300 mg of powder weight) was dissolved in 10 mL of water; final concentration 2 mg/mL. Samples of this stock solution were then diluted to 2.0 mL to obtain standards with final concentrations of 1.4, 0.5, and 0.25 mh/mL. These standards served to construct a standard curve. Each sample was chromatographed in duplicate. As well, a 1.0 mg/mL sample of doxorubicin hydrochloride (Adriamycin®-Adria Laboratories — Lot #9010DC), prepared on each day was chromatographed and its concentration determined and compared to its known concentration (recovery).

Stability Study

On study day zero, 22 mg vials of 50 mg doxorubicin hydrochloride (Adriamycin RDF®-Adria Laboratories - lot #9010DC were reconstituted with 25 mL of normal saline each. Of these, three vials were stored at room temperature and three were stored in the refrigerator at 4°C. Eight-8 mL aliquots of 2 mg/mL solutions from a further 14 vials were transferred to separate 12 mL syringes (equal numbers of Monoject syringes Division of Sherwood Medical and Terumo Corporation syringes) and twleve-12 mL aliquots were also transferred to 20 mL syringes (equal number of Terumo and Monoject syringes). The 2 mg/mL solution from an additional two vials was further diluted with normal saline to achieve a final concentration of 1 mg/mL. Eight mL of this solution was transferred to four Monoject 12 mL syringes and six 11 mL aliquots were transferred to 20 mL Monoject syringes. These two concentrations represent the two most common concentrations encountered in our hospital. On each study day, these solutions were tested for doxorubicin, MBT, HMBT and DEHP content, UV-VIS spectra (200-800 nm), pH and observed for changes in physical appearance (colour, clarity and particulate matter). All solutions were stored at 4°C or 23°C and were not protected from light.

Doxorubicin LC Analysis

Solutions containing either 2 mg/mL or 1 mg/mL of doxorubicin hydrochloride were stored at 4°C or 23°C, and sampled on each of the 14 study days (0, 1, 2, 4, 8, 10, 14, 17, 21, 24, 28, 31, 95 and 124). Samples, 0.5 mL, were drawn from each container on each study day with a 1 mL 27-gauge tuberculin syringe. One microlitre of each of these samples was directly chromatographed in duplicate.

Standards were prepared by dissolving 120 mg of doxorubicin hydrochloride powder (Adriamycin RDF®-Adria Laboratories — lot #9006JC; 50 mg doxorubicin hydrochloride per 310 mg of powder weight, powder also contains 250 mg lactose and 10 mg methylparaben) in 10 mL of distilled water. Samples of this stock were then diluted to 2 mL to obtain standards with final concentrations of 1.5; 0.5; and 0.25 mg/mL. One microlitre of each of these standards and a blank were directly chromatographed in duplicate, and served to construct a standard curve. An additional standard of 1.0 mg/mL prepared and chromatographed on each day was used to calculate recovery.

The chromatographic system consisted of an isocratic solvent delivery pump (Waters: Model EP600) which pumped a mixture of acetonitrile (Fisher: cat. #A998), 0.2% triethylammonium phosphate (TEAP) buffer (2 mL of triethylamine (Sigma: cat. #TO886) per litre of water adjusted to pH = 2.5 with phosphoric acid) through a 25 cm x 4.2 mm reversed phase C-18, 5 μm column (Beckman Ultrasphere ODS #235329) at 1.5 mL/min. The ratio of acetonitrile to TEAP buffer was 30:70 and was held constant during a chromatographic run. On each day the strength of the mobile phase was titrated to achieve a

retention time for doxorubicin between 130 and 140 seconds and did not vary by more than four seconds during each day (mean maximal variation: three seconds). Samples were introduced into the LC system using an auto injector (712 WISP; Waters). The column eluent was monitored with a variable wavelength ultra violet detector (SF783; kratos) at 230 nm. A 10mV signal output from the detector was integrated and recorded on a chromatography integrator (SP4270; Spectra Physics) and archived on computer diskette. The area under the doxorubicin HCl peak at 230 nm was reported and used to calculate the doxorubicin concentration.

Standard curves were prepared daily, as previously described. The peak area of the doxorubicin HCl peak of known concentrations was subjected to least squares regression and the doxorubicin HCl concentration in stability study samples, was interpolated from these curves and recorded to the nearest 0.01 mg/mL.

Measurement of Degradation Products

On day zero and 124, standard curves for each of the degradation products available were prepared to allow calculation of the amount of each degradation product present. Since degradation products are present in such small concentrations, to increase the sensitivity of the method an increased volume of sample (100 μL) was injected onto the column. However, the loading capacity of the analytical column is exceeded with the injection of 200 μg of doxorubicin or more, so a semi-preparative (10 mm x 250 mm) C-18, 5 μm reverse phase column (Ultrasphere ODS #235238; Beckman) was used.

pH

On each of the 14 study days the pH of one solution of each concentration/container type was measured and recorded to the nearest 0.05 of a pH unit. The pH meter (Fisher: Accumet-model 925) was fitted with a microprobe glass body electrode (Fisher: cat #13-639-280) and was standardized each day with two commercially available buffer solutions. (pH = 7: Fisher: cat. #S108-500 and pH = 4: Fisher: cat. #SO-B-98).

The pH of each solution was obtained by withdrawing 1 mL of solution and placing it in a glass test tube. The solution was then allowed to come to room temperature, and then the pH was measured and recorded.

Physical Inspection

On each of the 14 study days, all containers were inspected visually for colour and clarity. Visual particulate matter inspection was done against a black and white background.

Colour

On each study day a complete UV-VIS spectrum (200 nm to 800 nm) was recorded using a photodiode array detector (Waters: 990: resolution 1.4 nm, Band width 1 nm). One microlitre of each solution was injected directly into the flow cell of the photodiode array detector and the UV-VIS spectrum was recorded and archived on computer diskette. This is a more extensive test than measuring the absorbance at one discrete wavelength, since the entire spectrum is evaluated. Whole spectra from each sample were then compared for maxima, minima and overall similarity using the PDA 990 software (Waters). Identical spectra have a match: 100% ± 1%.

MBT and HMBT LC Analysis

Two-(2-Hydroxyethylmercapto) benzothiazole was synthesized according to the method described by Sexton[17] from 2-mercaptobenzothiazole (Sigma) and 2-chloroethanol (Aldrich). The product, recrystallized twice from benzene, had a melting point of 56-57°C (literature: 56-58°C;[17] 56-57°C[13]) and chromatographed as a single major peak (estimated purity 98 percent). On each study day, 40 mg of 2-mercaptobenzothiazole (Sigma: M-2274), 40 mg of HMBT and 25 μL of DEHP were each dissolved in 100 mL of methanol. Aliquots of each were further diluted to 10 mL in a volumetric flask to achieve final standard concentrations of 250, 500, 1000 and 2000 ng/mL. Fifty microlitres were chromatographed in duplicate. The coefficient of variation for duplicates routinely averaged less than two percent on each day. The functional limit of sensitivity of these measurements is 100 ng/mL for both MBT, HMBT and 200 ng/mL for DEHP.

Samples of 0.5 mL drawn for doxorubicin analysis were used for MBT, HMBT and DEHP analysis. The mobile phase was a gradient that ranged from 20 percent acetonitrile and 80 percent TEAP (pH = 2.5) to 100 percent acetonitrile over 60 minutes. The mobile phase was pumped at 1.5 mL/min through a 5 μm reverse phase C-18 column (Ultrasphere ODS #235329; Beckman). The UV-VIS spectra of the effluent from 200 nm to 800 nm was monitored with a photodiode array detector (990+: Waters).

Data Reduction and Statistical Analysis

Means (+/-standard deviation) were calculated for analyses completed in duplicate or triplicate.

Reproducibility was assessed by coefficient of variation (CV — standard deviation divided by the mean). Mean results from different days of an identical test were compared statistically by least squares linear regression to determine if an association exists between the observed result and time. Log-linear and linear-linear fits for the data from the accelerated degradation study (90°C) were compared for goodness of fit by the Maximum likelihood method of Box and Cox.[18,19] Analysis of variance and the least significant difference multiple range test or Student's test (where appropriate) were used to compare differences between temperatures, and or solutions for similar analytical tests. The five percent cut-off was used as the *a priori* level for significance.

Doxorubicin concentrations were considered "acceptable", or "within acceptable limits" if the concentration on any day of analysis is not less than 90 percent of the initial (day zero) concentration. Measureable concentrations (>100, 100 and 200 ng/mL) of MBT, HMBT and DEHP, respectively, were considered unacceptable.

RESULTS

Accelerated Degradation and Assay Validation

At 90 °C, doxorubicin HCl dissolved in normal saline degraded in an apparent first order fashion. This study was stopped at 300 minutes when less than 10 percent of the initial doxorubicin concentration remained in each vial to which 1 N HCl had been added (Figure 1). The observed degradation rate could be correlated directly with the hydrogen ion concentration (r = 0.9887) and observed half-lives were 21.5 min, 37.9 min, 46.7 min, 97.1 min and 520 min for solutions of pH 2.04, 2.21, 2.43, 2.66 and 4.02, respectively. The peak corresponding to doxorubicin in each of these chromatograms was evaluated for UV-VIS spectrum similarity. UV-VIS spectra from 200-800 nm observed in the leading edge, middle, and tail of doxorubicin peaks from each sample in the accelerated study and a reference standard were all identical with respect to maxima, minima and overall shape. Maximum deviation between UV-VIS spectrum (200-800 nm) fits was less than one percent (Table I). The mass spectrum of the LC effluent fraction corresponding to the doxorubicin peak was also identical to a doxorubicin reference standard.

During the accelerated study, a single degradation product, which could be separated from the doxorubicin peak and did not interfere with quantitation of doxorubicin appeared in the chromatograms (Figure 1 and 2, Panels B and C — product labelled D1 and identified as adriamycinone). Standards of other known degradation products were also separated from doxorubicin and did not interfere with doxorubicin quantification (Figure 2, Panel B). These products were identified by mass spectroscopy and from reference compounds as 13(s)-dihydrodoxorubicin, adriamycinone — the aglycone of doxorubicin marked D1, and 13-dihydroadriamycinone marked D3 in Figures 1 and 2. Other peaks were observed, and although their mass was deter-

Table I: Concentrations of Doxorubicin and Degradation Compounds on Day Zero and 124

Retention Time (min)	Compound identification	Mass	UV-VIS Spectra Match*	Percent of mass present		
				Day 0	Day 124 4 C	Day 124 23 C
1.3	Not a doxorubicin structure	——	361	——	——	——
1.8	13(s)-dihydrodoxorubicin	545	926	.29	.78	.92
2.3	Doxorubicin	543	999	98.42	97.12	94.99
3.2	Unknown	[543]	880	.17	.21	.24
3.4	Unknown	[543]	854	.35	.32	.36
4.2	13-dihydroadriamycinone	416	974	.50	.68	.73
5.8	Methylparaben	——	468	——	——	——
6.5	Unknown	[543]	859	ND	.09	.08
8.9	Adriamycinone	414	775	.27	.80	2.68
PERCENT DEGRADATION PRODUCTS*(%)				1.58	2.88	5.01

* UV-VIS spectra determined from spectra taken from middle of the peak of interest. Match is against a 2.0 mg/mL doxorubicin standard. 1000 indicates a perfect match, compounds are considered identical if a match number greater than 990 is obtained.

Coneentrations determined for epirubicin are estimated from linear regression of data from each of the 15 study days.

Percent of degradation products calculation requires correction for changes in mass to allow mass balance. Mass determined by mass spectroscopy. Numbers in parenthesis are assumed molecular weights that were used when confident mass spectra could not be obtained.

mined, absolute identity was not (Table I).

These results (predictable degradation, observed separation of degradation products and doxorubicin, doxorubicin UV-VIS spectrum purity in each peak, mass spectral identification of the doxorubicin peak and separation of doxorubicin from known degradation products) indicated that this analytical method was stability-indicating.

Accuracy and reproducibility were then evaluated. Doxorubicin (10 mg) was added to 10 mL of water and the concentration of this sample estimated. The results of this investigation indicated that the doxorubicin concentration was measured accurately (recovery for all samples was within 97 percent to 103 percent and reproducibly (CV for all replicates averaged one percent). The reproducibility of samples run on different days averaged 3.5 percent. This indicates that differences of eight percent or more can be confidently detected with acceptable error rates.[20,21]

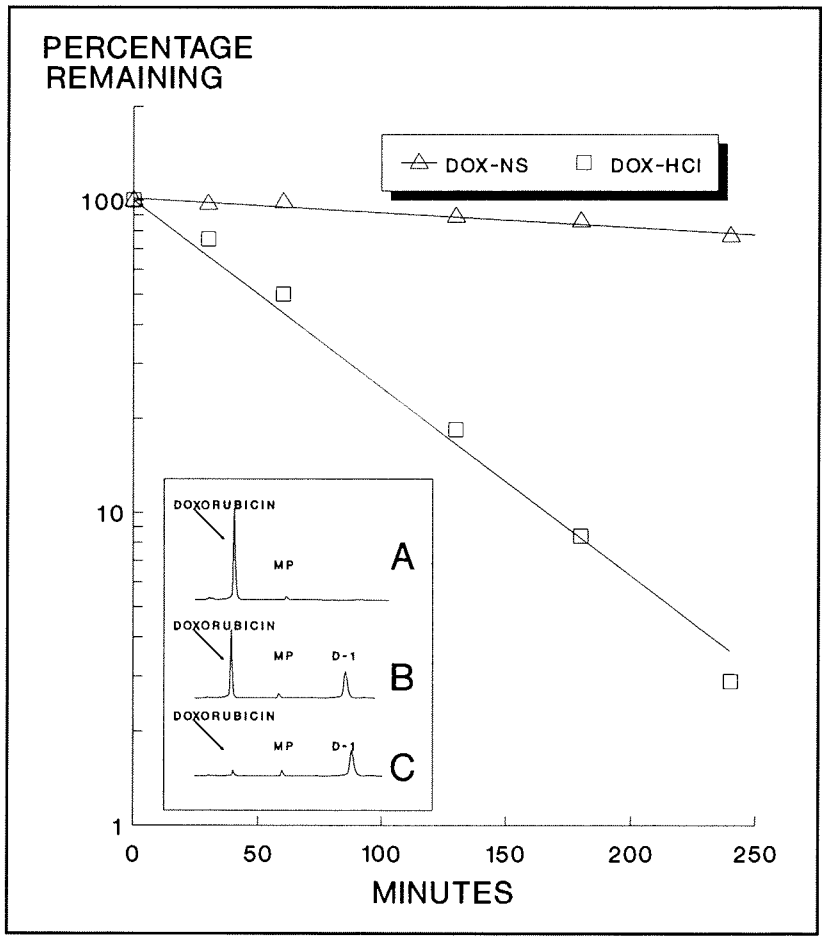

Figure 1: Rate of loss of doxorubicin during the accelerated study at 90°C over 240 minutes in normal saline and in normal saline adjusted to a pH of 2.43 with 1N HCl. During the study, as the doxorubicin concentration declined, a degradation product could be observed in chromatograms (Insert panels B and C, 60 and 240 minutes respectively compared to time zero — Panel A). The final concentration of doxorubicin is 77 percent of initial in the normal saline sample and three percent in the normal saline sample containing HCl (pH = 2.43).

Doxorubicin Stability

Over the 124-day study period there was no significant trend for the doxorubicin concentration to change in any solution stored at 4°C or 23°C. All concentrations on all study days remained greater than 90 percent of the time zero concentration. In all solutions, at both temperatures, in vials and syringes, the peak identified as doxorubicin maintained UV-VIS purity across its elution profile, (UV-VIS spectra obtained on the leading edge middle and tailing edge of the peaks were identical) as well as maintaining a UV-VIS spectrum which was identical to authentic doxorubicin, observed in standards and day zero samples.

Additional peaks, observed during assay validation accelerated degradation studies, were observed and measured on days zero and 124. As indicated in Table I, the total amount of doxorubicin mass found as degradation product on day zero was 2.88 percent. On day 124, this mass had increased to 5.01 percent, indicating that approximately two percent of the doxorubicin present on day zero had degraded by day 124. This change in doxorubicin concentration cannot be detected by measurement of doxorubicin concentrations used in this study, since only a difference of eight percent or more can be confidently reported.

No significant difference in degradation rate is apparent between concentrations of 2 mg/mL and 1 mg/mL in syringes, nor was there any significant difference in degradation rate between solutions stored in syringes compared to glass vials (since no significant degradation in any solution was observed).

During the 124-day study period pH remained unchanged (pH = 5.7-6.0) in solutions stored at 4°C and 23°C. UV-VIS absorbance

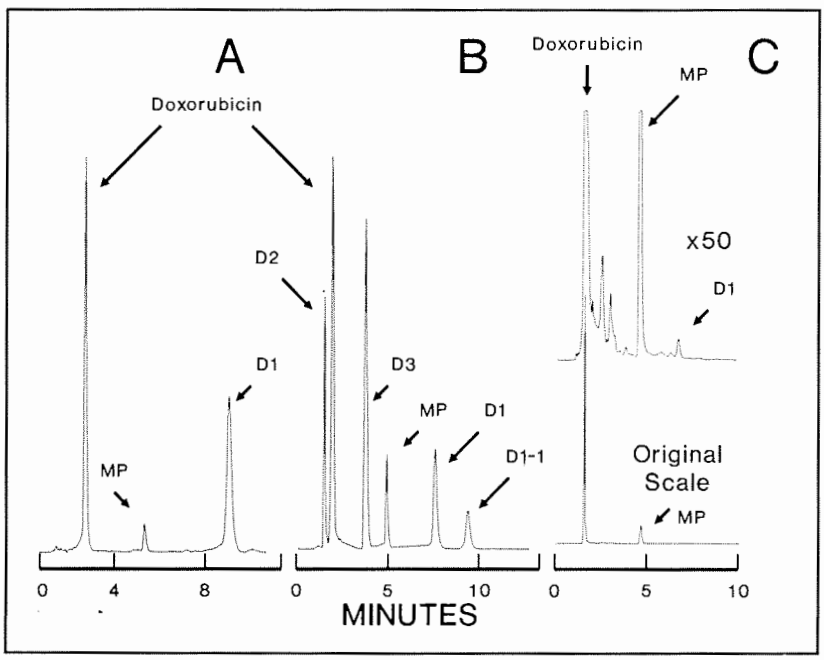

Figure 2: Chromatograms observed during the study period. Panel A is the chromatogram observed at 60 minutes in the accelerated degradation study in a normal saline solution adjusted to pH 2.43 with HCI. Panel B is a chromatogram observed when water is spiked with doxorubicin and known degradation products in concentrations of equal magnitude. Panel C (lower tracing) is typical of chromatograms observed during the stability study — shown in its original scale on day 124. The upper tracing has been magnified 50 times to show degradation products/contamination. From the original scale chromatogram it is apparent that even after 124 days, degradation products are not prominent. D1 has been identified as adriamycinone; D2 is 13(s) dihydrodoxorubicin, D3 is 13-dihydroadriamycinone, and D1-1 is an unknown degradaiton product contaminant of the adriamycinone standard (D1) provided by Adria Laboratories. MP represents methylparaben.

spectra determined for solutions stored at 4°C and 23°C on each day did not demonstrate any shift in maxima or minima throughout the study period, and spectra determined on study day zero were superimposable on study day 124 and were not significantly different (less than one percent total deviation from 200-800 nm).

MBT, HMBT and DEHP Concentrations

MBT, HMBT and DEHP concentrations in solutions stored at 4°C or 23°C in the manufacturers original glass vials and syringes (Terumo, Monoject) could not be measured because the concentrations of these three chemicals were below detectable limits in these solutions, and may have in fact been zero. Contamination by other unidentified chemicals (unknown peaks) was not observed to occur (Figure 3).

DISCUSSION

Statistical analysis of the doxorubicin HCI concentration time data in this study was limited to least squares linear regression, because demonstration of a trend for the concentration to decrease was considered more important than demonstrating a statistical difference in concentration between any two days. In fact, the random fluctuations in concentration around a line of 'best fit' are not of practical importance and should be considered 'noise' or experimental error. All conclusions and recommendations made from the data generated by this study have been based on results obtained by least squares linear regression analysis and analysis of degradation product concentration. Least squares log-linear regression indicates that less than three percent change in concentration occurred over the 124-day study period, as calculated from the line of best fit through the data. Although this represents a statistically insignificant change in concentration (within the limits of error of this measurement) it is in agreement with degradation product concentration calculations. In studies such as this, where no change in concentration of the drug of interest can be detected, assurance that the analytical method is specific for the compound of interest is important. This was demonstrated as part of the assay validation procedure. During the accelerated degradation study, we were able to degrade doxorubicin, separating the degradation products from the parent compound chromatographically. The identity of these compounds was confirmed by mass spectroscopy and comparison with reference standards. These methods are considered important and necessary to assure the validity of the analytical method used in a stability study.[22,23,24] Failure to carry-out these steps could result in the validity of the results being questioned.

Four other studies have been published concerning the stability of doxorubicin in intravenous solutions using liquid chromatographic methods.[3-6] Karlsen et al[3] and Hoffman et al[4] have completed stability studies on doxorubicin, reocnstituted with water, at final concentrations of 1.4 and 2 mg/mL. Both report less than 10 per-

cent loss after 30 days storage at -20°C and Hoffman reports less than a 10 percent loss after six months at 4°C.[4] The latter observation is in agreement with our results. However, both Poochikian et al[5] and Benvenuto et al[6] recommend short expiry dates based on extrapolation of data from their 24 and 48-hour studies. These two investigators also studied very dilute solutions (0.02 mg/mL and 0.18 mg/mL) of doxorubicin, and the photolability of doxorubicin is reported to be heavily dependant on concentration,[25] a ten-fold difference in half-life being observed between concentrations of 0.1 mg/mL and 0.01 mg/mL (the method used in this study is based on doxorubicin fluorescence and may not be stability indicating). This photolability of dilute solutions may explain the apparent discrepancies observed between studies on dilute solutions[5,6] and others[3,4] including our results.

During this stability study, solutions of doxorubicin were monitored for the presence of leaching plasticizers (MBT, HMBT and DEHP) from rubber closures and syringe tips. The analytical methods failed to demonstrate the presence of any of these or any other compounds in these solutions. Although rubber gaskets have been reported to leach both MBT and HMBT into contact solutions[12,13] many syringe manufacturers have apparently reformulated their rubber gaskets so that MBT is not used.[26,27] We observed no contamination of doxorubicin solutions which had been stored in syringes of either manufacturer (Monoject, Terumo). Although a parallel investigation has demonstrated that alcoholic solutions stored in Monject and Terumo syringes can become contaminated with a variety of chemicals other than MBT, HMBT or DEHP,[28] saline solutions were free of contamination in this study. It is apparent that the type of solvent, temperature and time also affect the amount of contamination, however, doxorubicin solutions appear to be free of this contamination over a 124-day period.

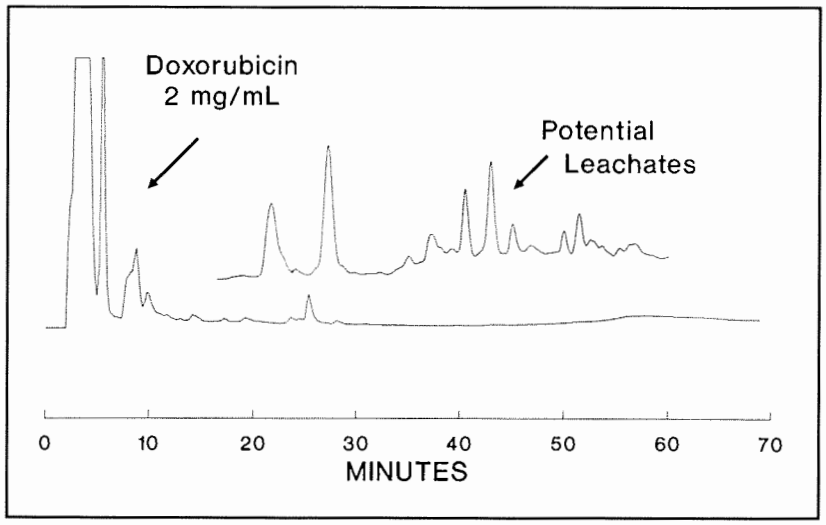

Figure 3: Tracing observed during MBT, HMBT and DEHP analysis on day 124. In this gradient system MBT elutes at 10 minutes, HMBT at 12 and DEHP at 51. The trace marked 'Potential Leachates' represents leachates contaminating a methanol solution stored in a Monoject syringe. It is apparent that none of these chemicals contaminates the doxorubicin solution even after 124 days storage at room temperature.

We conclude that doxorubicin solutions (2 mg/mL and 1 mg/mL) retain more than 90 percent of the initial doxorubicin HCl content for 124 days, when stored at 23°C or 4°C in glass vials or syringes. During this evaluation of stability, solutions were monitored for the presence of contamination by MBT, HMBT or DEHP and none was observed. Maximum recommended expiry dates are 124 days storage at 4°C or 23°C. However, this expiry date should only be used after due consideration of sterility and the contamination rate of IV admixtures in an IV additive program.

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References continued on page 88

References

DOXORUBICIN STABILITY

References continued from page 78

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