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Review

Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays

II. Assay methods, adriamycin and the other antitumour antibiotics

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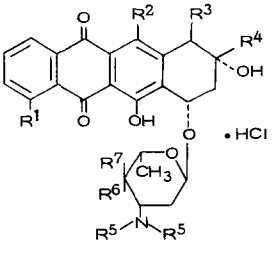
Summary. The methods used to test drug stability are discussed in the light of two recent publications using biological assays. It is concluded that, as far as possible, stability-indicating assays should be used so that possible false results do not lead to erroneous conclusions.

Many of the results of the stability studies with adriamycin were found to be at variance with each other, with a 20-fold difference in stability being reported in one case by different groups from virtually identical experiments. Definitive statements about adriamycin stability are therefore impossible, but it is clear that it is sensitive to light, adsorbs to membrane filters and containers (except polypropylene and siliconised glass), chelates metal ions and probably degrades rapidly in medium. Adriamycin's analogues may well have the same spectrum of sensitivity.

Bleomycin, actinomycin D and neocarzinostatin were found to be stable for $\geqslant 2$ weeks at room temperature. All the other antitumour antibiotics investigated (except rubidazone) are stable for $\geqslant 24$ h at room temperature and longer at 5 °C. Almost all of them are sensitive to light and are most stable in neutral or slightly acid media, and many of them adsorb to membrane filters. They can probably all be stored frozen in solution.

Introduction

In the first review in this series [7], general considerations concerning the stability of anticancer drugs in solution for in vitro chemosensitivity testing were discussed, and the alkylating agents and nitrosoureas considered in detail. In this paper the role of stability-indicating and biological assays for drug stability is discussed in the light of recently published results, and the stability of the anthracyclines (Fig. 1) and the other antitumour antibiotics (Fig. 2) investigated in detail.



Drug	NSC No.	R^1	R^2	R^3	R^4	R^5	R^6	R^7
Adriamycin	123127	$-OCH_3$	-OH	-H	$-COCH_2OH$	-H	-OH	-H
4'-Epiadriamycin	256942	$-OCH_3$	-OH	-H	$-COCH_2OH$	-H	-H	-OH
4'-Deoxyadriamycin	267469	$-OCH_3$	-OH	-H	$-COCH_2OH$	-H	-H	-H
Daunorubicin	82151	$-OCH_3$	-OH	-H	$-COCH_3$	-H	-OH	-H
Rubidazone	164011	$-OCH_3$	-OH	-H	$-C(CH_3)=N-NH-C(=O)-C_6H_5$	-H	-OH	-H
Aclacinomycin	208734	-OH	-H	$-COOCH_3$	$-CH_2CH_3$	$-CH_3$		-H

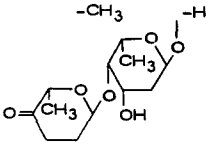


Fig. 1. Structures and NSC numbers of adriamycin and analogues

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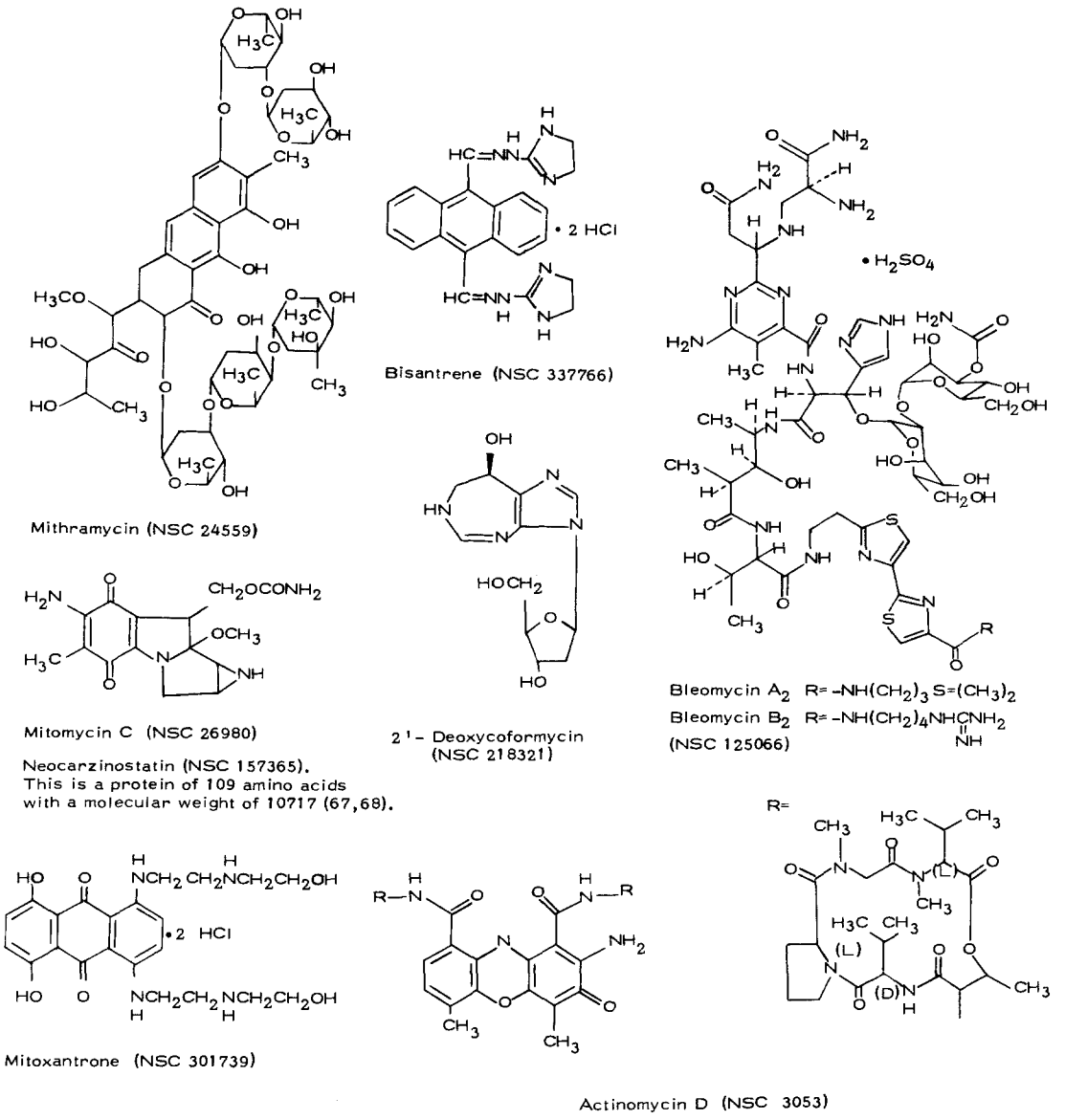

Fig. 2. Structures and NSC numbers of the other antitumour antibiotics

Table 1. Methods used to determine drug stability data[a]

Abbreviation	Assay	
SI	Stability-indicating high-performance liquid chromatography (HPLC)	
HPLC	HPLC	may or may not be stability-indicating
TLC	Thin-layer chromatography	
F	Fluorescence determination	unlikely to be stability-indicating
UV	Ultraviolet absorption	
RIA	Radioimmunoassay	
M	Information from manufacturer	
BA	Biological assay – not stability-indicating	

[a] If no information is given as to the method in text or in tables, this means it has not been possible to determine the method

In Table 1, some of the different methods of assessing drug stability are recorded along with their abbreviations. Because of the considerable discrepancies observed between results from different groups, and sometimes even within an experiment but using different methods, statements about stability will, where possible, be qualified by the method used.

Stability-indicating, biological, and other assays of drug stability

Recently two experiments on the stability of drugs frozen in solution have been published by groups in Cincinnati [35] and Houston [120]. These groups studied eight and ten drugs respectively, six of the drugs being the same. Unfortunately, out of these six, these two groups have come to different conclusions about the stability of five of them: Franco et al [35] suggest that adriamycin, bis(1-chloro-

Table 2. Problems encountered in reported versus actual drug stability

Reported drug stability	Actual drug stability	Comments
Stable	Stable	Easy to suggest, but very difficult to prove. Open to large errors in defining values such as $t_{0.95}$ (5% degraded) because the scatter on the observations of drug concentration will usually be greater than the 5% decay being looked for
Stable	Unstable	A very unsatisfactory position where people may, without realising it, use solutions of drugs that have already degraded. This situation is illustrated by adriamycin stability in medium at 37°C with one group reporting stability over 10 d (BA) [66] whereas other groups have suggested $t_{0.95}$ of about 2 h (BA) [49] and 40 min (TLC) [79]. This situation is likely to occur where the degradation products of a drug are equally (or more) cytotoxic than the parent drug, e.g. adriamycin [79] and BCNU [73, 117]
Unstable	Stable	Another very unsatisfactory position where people will be put to a great deal of extra unnecessary work, and specimens will be left longer before being set up. This is illustrated by the suggestion that melphalan was unstable frozen (BA) [120] versus the use of a SI-HPLC assay showing the opposite [8]
Unstable	Unstable	A situation which should be readily verifiable. Illustrated by the decay of melphalan in medium – found to have a $t_{1/2}$ of 1.13 h by SI-HPLC [8] and 1.8 h by BA [49]. However, once again, assays that are not stability-indicating may well overestimate the stability of a drug in this situation

ethyl)nitrosourea (BCNU), *cis*-platinum, 5-fluorouracil and melphalan are stable for between 10 and 61 weeks at −60 °C, whilst Yang and Drewinko [120] conclude that these same drugs are unstable after only 3 weeks at either −20 °C or −70 °C. These results may have been caused by the use of different drug concentrations by the two groups. However, it is more likely, as the results are so divergent, that the discrepancy came about because both groups used biological assays in their attempts to assess drug stability. It seems clear, as was stated by Trissel in 1983 [104], that stability-indicating assays (i.e. assays which have been proved to detect parent drug unequivocally and are, almost by definition, not biological) are the only way to obtain reliable data and clarify these discrepant results.

These divergent conclusions illustrate well the problems that can occur in assessing drug stability, and these are summarised in Table 2. Obviously reports of the stability of the parent drug and an accurate assessment of a value such as $t_{0.95}$ (5% degraded) are required, and this information can only be accurately gained by the use of a stability-indicating assay. Thus, all results obtained using a system which is not stability-indicating (even HPLC) will be open to criticism and should always be treated with caution.

A useful suggestion has been made that biological assays should be used as a pre-screen for drug stability until more precise methods of analysis are developed [1, 52]. In this case, the method of Hildebrand-Zanki and Kern [49], by which half-lives can be calculated, would be more useful than recording a reduction in cell kill. However, the investigation of the many new compounds that will increasingly be put through in vitro drug sensitivity assays in the search for new drugs will be severely hampered by the false-negative information which can arise (e.g. with melphalan, see Table 2). I think that it is worth the risk of freezing all drugs in solution until they are proved to be unstable, even though one may have to repeat some work when a drug is later found to be unstable when frozen.

Therefore, despite the caution about using stability-indicating assays and the distinct dearth of good drug stability data so far, we store almost all our drugs frozen in solution for use in our in vitro chemosensitivity assay [5, 6, 9].

Stability of adriamycin

Amongst the antitumour antibiotics, including mitoxantrone and bisanthrene, the most stability work has been undertaken on adriamycin. Its stability is affected by many different factors, and probably because some of these have not been controlled whilst others were under investigation, many contradictory results have been published. There have also been problems with measuring the drug, as it adsorbs to many materials, including chelating to the ferrous ion of HPLC columns. Table 3 summarises the drug stability results that have been published. Note that very different results have been recorded at virtually every temperature, and these are now discussed further.

As reconstituted for IV administration, adriamycin is reasonably stable

At 2 mg/ml, only 10.5% of adriamycin degraded when the solution was stored in the dark at 4 °C for 180 days (HPLC) [50], and it did not bind to a membrane filter (unspecified material) [50]. At 37 °C, 10% degraded in approximately 14 days (HPLC) [110]. The deep colour of the drug at this concentration may well protect it against photodegradation. However, probably due to the problems outlined below, it was originally recommended that the drug was not frozen or further diluted from 2 mg/ml [61].

Adriamycin is sensitive to light

The drug is photolabile [21, 42, 98], and this sensitivity is heavily dependent on drug concentration (Table 3), with a ten-fold reduction in half-life on ten-fold dilution of the solution (from 100 to 10 μg/ml; F) [98]. Extrapolation of these figures suggests that solutions of adriamycin in normal saline (150 m*M* NaCl; NS) at ≤5 μg/ml would have a $t_{0.95}$ in 'room light' of less than 1 h at room temperature (RT). However, Poochikian et al [84], using virtually identical conditions (RT, 10 μg/ml in NS, room light), found the drug to be about 20-fold more stable (HPLC). The free radical scavenger, butylated hydroxytoluene, was found to reduce photodegradation [20].

Table 3. Stability of solutions of adriamycin[a]

Temperature (°C)	Concentration (μg/ml)	pH	Solution	Lighting	Length of experiment/drug remaining	OR	Approximate $t_{0.95}$	Method	Reference
−70	1.5	?	NS[b]	? Dark	21 d/stable		–	BA	[120]
−70	0.6	?	NS	? Dark	21 d/unstable		–	BA	[120]
−60	100	?	NS	? Dark	70 d/stable		–	BA	[35]
−20	1.5	?	NS	? Dark	21 d/stable		–	BA	[120]
−20	0.6	?	NS	? Dark	21 d/unstable		–	BA	[120]
−20	1400	?	NS	? Dark	30 d/stable		–	HPLC	[59]
−20	2000	?	H_2O	? Dark	30 d/stable		–	HPLC	[50]
−20	?	?	plasma	? Dark	180 d/60%		–	HPLC	[33]
−20	?	?	plasma	? Dark	14 d/stable		–	HPLC	[74]
4	2000	?	H_2O	? Dark	180 d/89.5%		–	HPLC	[50]
4	1.5	?	NS	? Dark	21 d/stable		–	BA	[120]
4	0.6	?	NS	? Dark	21 d/unstable		–	BA	[120]
RT[c]	180	4.2	5% dextrose in PVC	?	48 h/stable		–	HPLC	[4]
RT	180	4.75	5% dextrose in glass	?	40 h/90%		–	HPLC	[4]
RT	100	?	NS	Room light	–		20 h	F	[98]
RT	10	?	NS	Room light	–		1.6 h	F	[98]
RT	10	?	H_2O	Room light	–		1.8 h	F	[98]
RT	10	?	Ringer's (b)	Room light	–		35 min	F	[98]
RT	290	7.0	NS + Citrate	UV light	–		20 min	UV	[21]
21	10 and 20	4.5	Dextrose	Room light	–		50 h	HPLC	[84]
21	10 and 20	6.3	Ringer's	Room light	–		14 h	HPLC	[84]
21	10 and 20	6.2	NS	Room light	–		31 h	HPLC	[84]
37	1.2	2	HCl	?	–		4.7 h	HPLC	[113]
37	0.4	?	CEM + 15% FCS	? Dark	–		2 h	BA	[49]
37	1.0	?	Enriched CRML 1066	? Dark	10 d/~90%		–	BA	[66]
37	1000	?	Various media (no serum)	Dark	–		40 min	TLC	[79]

[a] Container material has not usually been quoted. Commercial drug contains lactose
[b] NS, 150 mM NaCl
[c] RT, room temperature

Adriamycin degrades in medium to a cytostatic (but not cytotoxic) product

Pavlik et al [79] recently found that adriamycin also degrades in the dark (in various media without serum) to a form of the drug which is still cytostatic (i.e. inhibits colony formation) but is not cytotoxic (cells remain viable). This suggests that the 'stability' of the drug over 10 days found in enriched CRML 1066 medium and assessed by BA (Table 1) [1, 66] must be viewed with caution. This 'different form' of adriamycin may well have affected some of the observations on the drug's apparent cytotoxicity in colony-forming assays (e.g. [22]).

Tavoloni et al [98] and Poochikian et al [84], although reporting very different values, both showed considerably reduced stability of adriamycin in Ringer's solution compared with saline when both were exposed to light (Table 3).

Adriamycin adsorbs to containers and membrane filters

Adriamycin has been found to adsorb to polytetrafluoroethylene (PTFE), glass, polyethylene, steel and 'plastic' (most likely polystyrene) containers [43, 101], but not to siliconised glass or polypropylene [101]. The drug was also found to be more stable in polyvinylchloride (PVC) than in glass [4].

Adriamycin at 2 mg/ml was not found to adsorb to the membrane when filtered through a sterilising filter [50], but at more dilute solutions and with small volumes (a few millilitres) the problem becomes very acute, with >95% adriamycin being adsorbed to cellulose ester membranes and about 40% being bound to PTEE units [77, 78].

Adriamycin chelates or reacts with metals

Adriamycin reacts with aluminium [37], although this is probably of little consequence in practice [118]. It also flocculates with Fe^{3+} ions at adriamycin concentrations of $\geqslant 10^{-5}$ *M* (5.8 μg/ml) and Fe^{3+} concentrations over 10^{-4} *M* [3] (its reactivity with Fe $(NO_3)_3$ also being noted by Pavlik [79]) and is possibly catalytically degraded by steel [101]. This latter observation may be part of the cause of problems in the HPLC determination of the drug by some authors [66], since steel columns are most commonly used for HPLC. It has also been shown that Al^{3+}, Cu^{2+}, Fe^{2+}, Mg^{2+} and Ni^{2+} are all chelated by anthracyclines [15].

Adriamycin self-associates

The drug has been found to self-associate appreciably at concentrations over about 1 μg/ml [32, 69], although there is disagreement about the actual dimerisation constant. There is no evidence, however, that this phenomenon changes the efficacy of the drug in any way.

Effect of freezing on adriamycin

Hoffman et al [50] found that adriamycin, dissolved at 2 mg/ml in water, could be frozen at −20 °C and thawed

up to seven times over 30 days without appreciable degradation taking place (HPLC), but these authors warned against freezing in NS. Other authors found that the drug could be frozen in NS at either 1.4 mg/ml for 30 days (HPLC) [59] or 100 μg/ml for 10 weeks (BA) [35] with no detrimental effect. Yang and Drewinko [120] suggested that the stability of the drug in NS was good at 1.5 μg/ml but not at 0.6 μg/ml (BA) (but see comments above). Thus, it is most likely that the drug is stable when frozen in NS, and very many workers using in vitro chemosensitivity assays have stored it this way.

Oosterbaan et al [74] found the drug stable when stored in plasma at −20 °C for 14 days (even if frozen and thawed) (HPLC), whilst Eksborg et al. [33] came to the opposite conclusions (HPLC).

Other observations on adriamycin stability

Between pH 0.4 and 2.0, the degradation of the drug at 37 °C in HCl is proportional to the hydrogen ion concentration (HPLC) [113], whilst the drug also goes off with increasing hydroxyl ion concentration above pH 6.5 [109]. The most stable pH for the drug is said to be 3.0 to 6.5 [109] or pH 4.5 (HPLC) [84] although this latter study was not controlled. However, neither of these reports agree with the results of Kaniewska [56] who appeared to show considerably greater stability for the drug at pH 2.6 compared to pH 4.8 or 5.5.

The biological activity of adriamycin in vitro is (a) reduced to half in the presence of 25 mg/ml human albumin [97]; (b) affected by cell density in a monolayer system [17]; and (c) is very dependent on temperature [44–46, 48]. It can also exert its cytotoxicity without entering cells [13, 108].

As well as the observed problems with some metals (see above), adriamycin is incompatible with 5-fluorouracil, dexamethasone, sodium phosphate and heparin [23], although the last drug can be mixed with adriamycin as long as its concentration is kept below 1.3 units/ml [70]. Three amino acids and $NaHCO_3$ have also been implicated in causing the degradation of adriamcycin [79].

One final problem noted with adriamycin, and probably true for a number of other cytotoxic drugs, was that cells killed by adriamycin and then washed were cytotoxic to untreated cells – presumably because of the adriamycin leaching out of the dead cells as their membranes became permeable [43]. In the light of this, the effectiveness of washing drugs out of cell suspensions must be carefully considered when short-term drug incubation is employed.

In conclusion, very little can be categorically stated about the stability of adriamycin, and a very carefully designed study is urgently required to resolve these conflicting results.

Stability of the other antitumour antibiotics

An overall summary of the stability of the antitumour antibiotics is given in Table 4. This shows either how long the drug is said to be stable for (usually taken as ≤10% degradation, but where biological assays have been used the stability cannot be so clearly defined) or a value for $t_{0.95}$. Further details are given below for daunorubicin, rubidazone, mitoxantrone and bleomycin.

The stability results for the adriamycin analogues are less confusing than the results for adriamycin, presumably because either (a) not so much work has been done on them or (b) they are not so adversely affected by the experimental conditions. Until (b) is shown to be true, we must assume that (a) is correct and treat all the analogues with considerable caution. Indeed, personal communication with a number of authors at the 1985 American Association for Cancer Research Meeting in Houston showed that many of the very new adriamycin analogues are even harder to handle in solution that the parent drug.

Daunorubicin

Daunorubicin, with a very similar structure to adriamycin (Fig. 1), shows a similar spectrum of problems where the two drugs have been compared, but on the whole seems to be slightly more stable. Thus, the solution for IV injection (5 mg/ml) is stable for 7 days at RT, and less stable if the pH is raised over 8.0 [105]. In Table 4, the $t_{0.95}$ is given for a 20 μg/ml solution of the drug in NS.

The cytotoxicity of the drug is reduced 2-fold by 25 mg/ml human albumin [97], and it is incompatible with heparin [103] and dexamethasone sodium phosphate [23]. The manufacturer recommends that daunorubicin is reconstituted with preservative-free solutions [38] and that it is not mixed with any other drugs during infusion [102, 103]. Daunorubicin has also been found to self-associate [16, 32, 69].

Rubidazone

Rubidazone (zorubicin) is supplied (contaminated by 8–12% daunorubicin) with a sodium glycinate buffer. When the drug is dissolved it yields a 12.5 mg/ml solution which is stable for 8 h at RT and 24 h at 4 °C [106].

Mitoxantrone

This dark blue drug is formulated in a 2 mg/ml solution in acidified NS and as such is stable for 3 years at RT with no detrimental effect from light [107]. Its half-life in an unspecified solution at 40 μg/ml at RT was in the order of weeks (HPLC) [75]. The cytotoxicity of the drug is greatly increased by a small increase in temperature (from 37 °C to 42.4 °C) [47].

Bleomycin

Bleomycin (as the sulphate) is a mixture of at least ten components, the main ones of which are bleomycin A_2 (U. S. regulations require 60%–70%) and bleomycin B_2 (25%–32%) [2]. The powder is extremely hygroscopic [87]. As it is a mixture, any methods used to assess the drug's stability without chromatography can only produce an overall picture of stability. Having said this, it does seem to be a reasonably stable drug both in solution (Table 4) and in plasma [99].

The in vitro cytotoxicity of bleomycin is increased by both temperature [46, 48] and the presence of 25 mg/ml human serum albumin [97]. The reactivity of the drug is also increased by irradiation at 300–350 nm [27]. It is incompatible with methotroxate and mitomycin C [24] and amino acids [87].

Conclusions and recommendations

Assay methodology

1. Use stability-indicating assays to assess drug stability

Table 4. Summary of the stability of the antitumour antibiotics and similar compounds

Drug	Approximate $t_{0.95}$ in physiological solution *or* drugs quoted as stable (S) for the time given (assay method)			Stability in medium at 37°C	References to stability	Most stable pH	Membrane filtration[b]	Effect[c] of light	Drug stability reduced by	References to these effects	References to storage frozen[d]
	Frozen	5°	RT[a]								
Adriamycin[e]	See Table 3 and text for details – published results are very inconsistent				See Table 3	? ~6	+++	++	↓ drug concentration; metals; media; UV light; container material	See text	[5, 6, 18, 19, 29, 30, 39, 49, 51, 54, 55, 60, 65, 83, 89, 91–94, 111, 112, 114, 116]
4'-Epiadriamycin	. . .	S 48 h (M)[f]	S 24 h (M)[f]	. . .	[36]	. . .	. . .	. . .	. . .[g]	. . .	[6, 51]
4'-Deoxyadriamycin	. . .	. . .	S 24 h (M)[f]	. . .	[107]	. . .	. . .	. . .	. . .[g]	. . .	[92]
Daunorubicin[e]	. . .	. . .	40 h (HPLC)	. . .	[84, 105]	. . .	+	++	Metals: UV light[g]	[15, 21, 41, 77, 78]	[5, 6]
Aclacinomycin	. . .	. . .	52 h (HPLC)	. . .	[58, 72, 84, 107]	4–4.5	0	+	Preservatives[g]	[72, 107]	[6, 39]
Rubidazone[e]	. . .	S 5 h	1.3 h (HPLC)	. . .	[84, 106]	. . .	. . .	?+	UV light[g]	[21]	. . .
Mitoxantrone[e]	−20° S 2 wk (BA)[h] −70° S 2 wk (BA)[h]	S 2 wk (BA)[h]	S 48 h	$t_{1/2}$ > 14 d (BA)	[49, 107, 120]	<7	++	0	Plasma	[77, 78, 82, 88]	[6, 51, 60, 95, 111, 112]
Bisantrene	. . .	. . .	S 24 h	S 10 d (HPLC)	[66, 81, 82]	<7	0	. . .	Precipitates in media and plasma	[25, 78]	[51, 111]
Bleomycin[e]	−20° S 3 wk (BA) −70° S 2 wk (BA)	S 4 wk	S 14 d	S 10 d (RIA)	[4, 49, 66, 76, 80, 103, 120]	?4–10	0	+	UV light; metals	[12, 14, 24, 26, 27, 77, 96, 100]	[19, 60, 64, 71, 94, 95, 111, 112]
Actinomycin D	−60° S 13 wk (BA)	S 17 wk	S 21 wk	S 10 d (RIA)	[4, 28, 35, 49, 53, 66]	. . .	+++	++	Preservatives; container material	[10, 23, 38, 57, 77, 78, 90, 103, 119]	[19, 60, 95, 111, 114]
Mithramycin	. . .	S 48 h	S 24 h (HPLC)	. . .	[4, 102, 103]	⩾7	+++	. . .	Metals	[14, 40, 57, 103]	[19]
Mitomycin C[i]	−20° S 3 wk (BA) −60° S 61 wk (BA)	S 3 wk (BA)	S 24 h	$t_{1/2}$ > 14 d (BA)	[4, 31, 34, 35, 61, 85, 86, 102, 120]	⩾7	0	+	Dextrose; ? buffers	[4, 31, 34, 61, 77, 78, 85]	[19, 49, 60, 93, 94, 111, 115, 116]
Neocarzinostatin[j]	Can be frozen	16 mo (BA)	4 mo (BA)	. . .	[62, 63, 106]	5	. . .	++	UV light	[11, 62]	. . .
2'-Deoxycoformycin	. . .	S 4 d	S 3 d	. . .	[107]	. . .	. . .	. . .	. . .	. . .	. . .

[a] Usually in room light

[b] Drug binding to cellulose ester membranes: 0, negligable (<5%); +, slight (5%–15%); ++, considerable (15%–50%); +++, severe (>50%). Most drugs which bind to cellulose ester also bind to other membrane materials such as PTFE [77, 78, 90]

[c] 0, not photolabile; +, photolabile; ++, severely degraded by light

[d] References are tabulated here when the authors have reported that "all drugs were stored frozen in solution", and the drug has then been used in their work. It is conceivable, however, that this might have been an over-simplification for the purposes of writing the paper due to the large number of drugs involved, and occasionally all the drugs mentioned in a publication may not have been stored frozen

[e] Further information may be found in text

[f] Solution of IV injection (1 or 2 mg/ml)

[g] It is possible that the stability of the adriamycin analogues will show a similar spectrum of sensitivity to conditions reducing drug sensitivity that adriamycin does, but that these have not yet been documented

[h] Possibly not stable at 3 weeks at −70, −20 or 4°C [120]

[i] Mitomycin is possibly more stable when diluted in water [31, 34, 85], but its cytotoxicity is reduced in the presence of 25 mg/ml human serum albumin [97]

[j] The cytotoxicity of neocarzinostatin increases as the oxygen tension is increased [11], but is reduced in the presence of 25 mg/ml human serum albumin [97]

when at all possible. 2. Of methods that are not stability-indicating, HPLC is probably the best to use. 3. Treat the results of biological assays of assessing drug stability with caution.

Adriamycin

4. Do not sterile-filter dilute solutions. 5. Do not make the drug up in medium – use NS or water. 6. Only freeze solutions once. 7. Work in low light levels when handling dilute solutions. 8. As far as possible work with dilute solutions in polypropylene or siliconised glass, and keep them away from metals.

Observing these precautions, we have obtained very acceptable reproducibility with adriamycin in our in vitro chemosensitivity assay [5, 6].

Other antitumour antibiotics

9. Except for rubidazone, all the antitumour antibiotics are stable at room temperature for at least 24 h, and longer at 5 °C. 10. Bleomycin, actinomycin D and neocarzinostatin are stable for at least 2 weeks at room temperature. 11. No drug has consistently been found to be unstable frozen. 12. Almost all the drugs tested are sensitive to light. 13. Over half the drugs tested adsorb to filter membranes, especially adriamycin, actinomycin D and mithramycin. 14. Many of the antitumour antibiotics complex metals and can possibly be degraded by them.

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