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Invited Review

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A literature review of reports of the stability and storage of common injectable chemotherapy agents used in veterinary patients

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Abstract

Many chemotherapy drugs used in human patients are discarded after single use or within 24 h of reconstitution, as per the manufacturer's product label recommendations. This can be wasteful and costly to veterinary clients. This report reviews the published stability and storage data for 19 injectable chemotherapy drugs commonly used in veterinary medicine. Based on these data, storage procedures are presented, assuming aseptic technique and a closed system drug transfer device (CSDTD) are used for drug preparation and handling. Further studies on the risk of microbiological contamination of chemotherapeutics using a CSDTD, and validated high quality drug assays such as stability-indicating high-performance liquid chromatography, are required. The authors' intent is not to supersede product label recommendations, but to suggest that longer storage without significant loss of drug efficacy may be possible, thus reducing the costs of chemotherapeutics to some veterinary clients.

Keywords
cytotoxic, oncology, potency, purity, recommendations, small animal

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Introduction

The stability and storage of injectable chemotherapeutics for use in veterinary patients has not been extensively reviewed. Many chemotherapeutics used in human patients are discarded after single use or within 24 h of reconstitution, as per the manufacturer's product label recommendations. The main reason for this is concern about microbial contamination because the solutions usually do not include a preservative. In veterinary medicine, adhering to these recommendations can make chemotherapy costly, thus limiting therapy for many patients. In addition, single dose vials result in significant drug wastage with small patients, and drug shortages can limit consistent availability of chemotherapeutics for pets.

Therefore, veterinary practitioners commonly store chemotherapeutics for longer than the manufacturer label recommendations. The storage duration and temperature requirements for each drug are based on recommendations available in the current literature, from compounding pharmacies, manufacturer label recommendations and/or personal communications with drug companies or other veterinarians. Because there are no specific studies, consensus or gold standard documents addressing these requirements, decision-making regarding chemotherapy storage is challenging and ultimately up to the discretion of the individual veterinarian.

Several methods are used to determine drug stability. The strengths and weaknesses of these analytical methods are summarized in Table S1, Supporting Information.[1–8] The ideal assay should be validated to accurately and precisely separate, identify, and quantify the active components of

Table 1. Summary of storage suggestions for 19 common injectable chemotherapy agents used in veterinary patients

Chemotherapy[a]	Reconstitution	Storage	Expiry (days)
Actinomycin D (0.5 mg/vial)	Reconstitute with 1.1 mL SW to make 0.5 mg/mL	Refrigerated	7
Bleomycin [15 000 IU (15 mg) per vial]	Reconstitute with 3 mL 0.9% NaCl to make 5000 IU (5 mg)/mL	Refrigerated	21
Carboplatin (10 mg/mL)	Does not require reconstitution	Refrigerated	28
Cisplatin (1 mg/mL)	Does not require reconstitution	Refrigerated	28
Cyclophosphamide (500 mg/vial)	Reconstitute with 25 mL 0.9% NaCl to make 20 mg/mL	Refrigerated	28
Cytarabine (20 mg/mL)	Does not require reconstitution	Refrigerated (*If crystals seen, reheat and shake to dissolve particles*)	28
Dacarbazine (200 mg/vial)	Reconstitute with 19.7 mL SW to make 10 mg/mL	Refrigerated (*Very light sensitive; store in darkness as soon as possible*)	7
Doxorubicin (2 mg/mL)	Does not require reconstitution	Refrigerated	28
Epirubicin (2 mg/mL)	Does not require reconstitution	Refrigerated	28
5-Fluorouracil (50 mg/mL)	Does not require reconstitution	Refrigerated (*If crystals seen, reheat and shake to dissolve particles*)	28
Gemcitabine (200 mg/vial)	Reconstitute with 5 mL of 0.9% NaCl to make 38 mg/mL	Refrigerated (*If crystals or discoloration seen, discard*)	28
Ifosfamide (2 g/vial)	Reconstitute with 50 mL 0.9% NaCl to make 40 mg/mL	Refrigerated (*If crystals or discoloration seen, discard*)	14
L-Asparaginase (10 000 IU/vial)	Reconstitute slowly with 2 mL 0.9% NaCl to make 5000 IU/mL	Refrigerated	14
Mechlorethamine (10 mg/vial)	Reconstitute with 10 mL SW to make 1 mg/mL	Refrigerated (*Very light sensitive; store in darkness as soon as possible*) (*If crystals or discoloration seen, discard*)	28
Methotrexate (25 mg/mL)	Does not require reconstitution	Refrigerated	28
Mitoxantrone (2 mg/mL)	Does not require reconstitution	Refrigerated	14
Vinblastine (1 mg/mL)	Does not require reconstitution	Refrigerated	28
Vincristine (1 mg/mL)	Does not require reconstitution	Refrigerated	28
Vinorelbine (10 mg/mL)	Does not require reconstitution	Refrigerated	28

NaCl, sodium chloride; SW, sterile water.

[a] All of these chemotherapy agents do not contain an antibacterial preservative and should be protected from light. Some of these chemotherapy agents are particularly light sensitive, therefore, should return to refrigerated storage as soon as possible.

a drug, without interferences from impurities, degradation products and excipients.[1,2,6–8]

There are many factors that can potentially affect the chemical and physical stability of drugs including concentration, container material (e.g. glass versus plastic), use and type of membrane filters, storage temperature, changes in temperature, ultraviolet (UV) light exposure, type of diluent (e.g. 0.9% sodium chloride, 5% dextrose in water), chemicals added to and/or already in the mixture (e.g. preservatives, solvent, excipients), storage time, exposure to oxygen, humidity and pH of drug.[2,5,9–12] The potential impact of such factors on drug stability will be mentioned where applicable for 19 injectable chemotherapy drugs commonly used in veterinary medicine; however an extensive discussion of each factor is beyond the scope of this review (Table 1).

Readers are advised to read their own product label for each drug, as storage recommendations may vary over time and with each manufacturing company. In addition, none of the data sources evaluated in this review can be applied as documentation for extended shelf lives. Readers should review and document the extended stability data themselves.

Actinomycin D

A stability experiment performed on actinomycin D using visible absorption spectra and an

agar-diffusion microbiological assay with *Staphylococcus aureus* concluded that actinomycin D reconstituted with sterile water for injection (SW) to 0.03 mg/mL and stored refrigerated was stable (<5% degradation) for at least 5 months.[13] Actinomycin D in aqueous solution has been reported to adsorb to glass and plastic with a significant loss of the drug (although the amount of drug loss and the timing of this adsorption was not reported).[2,9,14–16] The available data justify potential alteration of actinomycin D storage procedures following reconstitution using SW, aseptic technique, and a closed system drug transfer device (CSDTD). Because of the potential for the drug to be adsorbed to glass, it may be prudent to limit storage of reconstituted actinomycin D to 7 days. Furthermore, no studies have evaluated reconstitution and storage of actinomycin D with 0.9% sodium chloride (NaCl).

Bleomycin

A radioimmunoassay stability study showed that bleomycin (1 mg/mL) frozen at −20 °C in glass was stable (>97% bleomycin) for up to 27 months, and for up to 9.5 months when stored in plastic (because of adsorption to plastic).[17] Other studies report 6–8 weeks of stability of 1% bleomycin in 0.9% NaCl at 4 °C,[18] and 4 weeks for bleomycin solution stored in glass vials at 5 °C.[2] A cell survival biological assay reported 80–95% cell kill efficacy of bleomycin (0.03 mg/mL in 0.9% NaCl) after storage in glass containers for up to 3 weeks at 4, −20 and −70 °C.[19] Unfortunately, most of these studies did not quantitatively describe stability (e.g. percentage degradation or percentage of drug remaining), and there are no existing high-performance liquid chromatography (HPLC) assays for analysing bleomycin. However, the available data justify refrigerated bleomycin storage for up to 21 days after reconstitution with 0.9% NaCl using aseptic technique and a CSDTD.

Carboplatin

There is abundant stability data on carboplatin solutions prepared in 5% dextrose in water (D5W) and 0.9% NaCl, however, only stability-indicating HPLC (SIHPLC) and liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS) studies are reported here. SIHPLC showed carboplatin in 0.9% NaCl in polyvinyl chloride (PVC) bags at 0.5, 2 and 4 mg/mL was stable (<6% degradation) for at least 7 days at 4 °C in darkness.[20] Another SIHPLC study showed carboplatin (0.70 and 2.15 mg/mL in D5W) was stable (4% degradation) for up to 84 days at 4 °C in PVC and polyolefin bags, followed by a further 24 h under 'in-use' conditions at room temperature (RT). In addition, when returned to refrigerated storage, the solution was stable (2% degradation) for at least another 7 days.[21] Two LC-ESI-MS studies showed carboplatin solution was stable (negligible <1% loss) for at least 3 months at 4 °C.[5,22] These studies justify refrigerated carboplatin storage for up to 28 days after puncture using aseptic technique and a CSDTD.

Cisplatin

Using *in vitro* cytotoxic biological assays on the native cisplatin solution (0.5 mg/mL), cisplatin showed full cytotoxic potency after storage for at least 1 month at RT, −4 and −20 °C.[23] A validated LC-ESI-MS study showed that cisplatin solution (0.1 mg/mL in 0.9% NaCl) was stable (negligible <1% loss) at 4 °C for at least 3 months.[22] The available data justify refrigerated storage of cisplatin for up to 28 days after puncture using aseptic technique and a CSDTD.

Cyclophosphamide

One SIHPLC study[24] evaluated the stability of cyclophosphamide (1 or 20 mg/mL) in SW, 0.9% NaCl or D5W at various temperatures (4, 20–22 and 37 °C), in room fluorescent light or darkness, and in glass or PVC containers. The study concluded that cyclophosphamide was stable (<5% degradation) for up to 7 days in glass or PVC containers at concentrations of 1 or 20 mg/mL at 4 °C, however, rapid degradation occurs at 37 °C with the half-life at this temperature reported to be approximately 7 days. LC-ESI-MS method showed cyclophosphamide (0.001 mg/mL in SW) was stable (negligible <1% loss) for up to 1 month at 4 °C.[5] Two historical studies reported the stability of cyclophosphamide

solution using gas chromatography[25] and infrared spectrophotometry,[26] however, these studies were not validated and are not discussed here. The validated LC-ESI-MS study,[5] support refrigerated storage of cyclophosphamide for up to 28 days after reconstitution in 0.9% NaCl; after puncture using aseptic technique and a CSDTD.

Cytarabine

UV-light spectroscopy showed cytarabine (5 mg/mL) in 0.9% NaCl or lactated Ringer's solution (LRS) in glass ampules was stable (>94% cytarabine) for 7 days at RT in fluorescent light.[25] Similarly, HPLC determined that cytarabine diluted in D5W or D5W in 0.2% NaCl to concentrations as low as 0.08 mg/mL was stable (no drug loss) in plastic syringes for 7 days at RT or refrigerated.[26] SIHPLC concluded that cytarabine (25 or 1.25 mg/mL in 0.9% NaCl or D5W) in plastic syringes was stable (no drug loss) for 28 days at 4 and 22 °C, and for 7 days at 35 °C.[27] This study[27] supports refrigerated cytarabine storage for up to 28 days after puncture using aseptic technique and a CSDTD. Furthermore, diluted cytarabine (>0.08 mg/mL) did not show significant degradation in plastic syringes for up to 7 days.[26] However, if diluting cytarabine for continuous rate infusion (CRI) it is unknown whether the drug will be equally stable in plastic bags.

Dacarbazine

Based on HPLC, dacarbazine (DTIC) (between 0.08 and 0.8 mg/mL in 0.9% NaCl or D5W), protected from light, appears to be stable. There was negligible (<1%) drug loss after 48 h at 4 °C, however 5% loss at RT, independent of the type of container material (e.g. glass and PVC bags).[10] SIHPLC showed that DTIC (10 mg/mL) in SW stored in amber glass vials or PVC bags was stable (>95% DTIC remaining) for at least 24 h at RT and exposed to light; and for at least 7 days refrigerated (2–6 °C) in darkness. Stability times were reduced after dilution (1 mg/mL) in PVC bags with D5W; to 2 h in daylight, 24 h in fluorescent light and 72 h when covered with aluminium foil. DTIC degradation was also greater when infused using a transparent infusion set (6% loss) rather than an opaque infusion set (2% loss).[28] The available data suggest refrigerated DTIC is stable for up to 7 days in darkness, after reconstitution using aseptic technique and a CSDTD. When DTIC is given as a CRI, it may be prudent to use D5W as the diluent (1 mg/mL DTIC), and protect the infusion bag and tubing from light (e.g. aluminium foil or opaque PVC infusion lines).

Doxorubicin

There are numerous studies on the stability of doxorubicin in various solutions with greatly varying results[2,29]; only SIHPLC studies are reported here. SIHPLC showed doxorubicin (0.1 mg/mL in 0.9% NaCl) was stable (<10% degradation) in PVC bags for at least 24 days at RT; and for at least 43 days refrigerated when reconstituted with SW, 0.9% NaCl, or D5W and stored in polypropylene syringes.[30] Another SIHPLC study showed doxorubicin (2 mg/mL in SW) was stable (4% degradation) for up to 6 months when stored refrigerated.[31] These studies support refrigerated doxorubicin storage for up to 28 days after puncture using aseptic technique and a CSDTD.

Epirubicin

SIHPLC showed epirubicin (2 mg/mL in 0.9% NaCl) was stable (<8% degradation) when stored in both darkness and light, in polypropylene syringes for up to 6 months refrigerated and for at least 14 days at RT.[32] Another SIHPLC study showed that epirubicin (0.1 mg/mL in 0.9% NaCl) was stable (<10% degradation) in PVC bags for at least 20 days at RT, and for at least 43 days refrigerated when reconstituted with SW, 0.9% NaCl, or D5W and stored in polypropylene syringes.[30] These studies support refrigerated epirubicin storage for up to 28 days after puncture using aseptic technique and a CSDTD.

5-Fluorouracil

SIHPLC showed 5-fluorouracil (5-FU) (10 or 50 mg/mL) was stable for up to 28 days at all temperatures tested (4, 22 and 35 °C) when stored in 0.9% NaCl or D5W solution.[27] SIHPLC also measured 5-FU under mimicked multiple in-hospital

usage, and concluded that 5-FU (8 mg/mL in 0.9% NaCl) in PVC infusion bags was stable (<6% degradation) after freezing and storage for 79 days at −20 °C, followed by thawing in a microwave oven to store in refrigerated storage (5 °C) for a further 28 days.[33] A validated LC-ESI-MS (which reported adequate controls and repeatable stability data), showed 5-FU (0.001 mg/mL in SW) was stable (negligible <1% loss) for up to 1 month at 4 °C.[5] There have been additional studies reporting stability of 5-FU in various solutions,[10,11,34,35] however the stability technique was not described or validated; therefore those studies are not discussed here. These studies support refrigerated 5-FU storage for up to 28 days after puncture using aseptic technique and a CSDTD.

Gemcitabine

HPLC showed gemcitabine (0.06–0.6 mg/mL in 0.9% NaCl or D5W) was stable (<3% degradation) for at least 7 days in darkness at RT or refrigerated.[10] SIHPLC showed gemcitabine at 0.1 or 10 mg/mL in 0.9% NaCl or D5W in PVC bags, or at 38 mg/mL in 0.9% NaCl in plastic syringes, was stable (<5% degradation) for at least 35 days at refrigerated and RT. Occasionally, gemcitabine solutions at a concentration of 38 mg/mL when stored refrigerated developed large crystals after 14 days, which did not redissolve upon warming to RT; leading to significant gemcitabine losses (20–35%).[36] Two other studies supported these stability findings. LC-ESI-MS showed gemcitabine (0.001 mg/mL in SW) was stable (negligible <1% loss) for up to 1 month refrigerated,[5] and SIHPLC showed gemcitabine (7.5 or 25 mg/mL in 0.9% NaCl) was stable (>95% gemcitabine) for 27 days at RT in the original glass vial or in PVC bags.[37] These studies justify refrigerated gemcitabine storage for up to 28 days when reconstituted with 0.9% NaCl using aseptic technique and a CSDTD. Gemcitabine appears stable at RT, but if any crystallization or discolorations are observed, the product should be discarded.

Ifosfamide

SIHPLC showed ifosfamide (10 mg/mL in 0.9% NaCl), stored in PVC portable infusion-pump cassettes at 4 °C or RT was stable (<1% degradation) for 8 days. The same study showed ifosfamide was stable (<1% degradation) at 20, 40 or 80 mg/mL in 0.9% NaCl; or at 80 mg/mL in SW, in cassettes at 35 °C for 8 days.[38] SIHPLC showed ifosfamide and mesna diluted to an ifosfamide concentration of 20 mg/mL in SW in infusion-pump cassettes, was stable for at least 14 days at 8 °C (<1% degradation), and at least 7 days at 37 °C (<3% degradation).[39] SIHPLC showed ifosfamide and mesna combination (1:1) solutions in 0.9% NaCl PVC bags (10, 20 and 30 mg/mL) was stable (<6% degradation) for at least 14 days at RT.[40] LC-ESI-MS showed ifosfamide (0.001 mg/mL in SW) was stable (<1% loss) for least 2 months refrigerated.[5] There are studies on the stability of ifosfamide in various solutions that used techniques that were not described or validated[6,9,41]; only validated SIHPLC and LC-ESI-MS assays with controls and repeatable stability data are reported here. The available data justify refrigerated ifosfamide storage for up to 14 days when reconstituted with 0.9% NaCl using aseptic technique and a CSDTD.

L-Asparaginase

A stability experiment performed on *Escherichia coli*-derived L-asparaginase using a biological assay and a conductometric method concluded that L-asparaginase (>1000 IU/mL with 0.9% NaCl or LRS) and stored at 8 °C in polyolefin and polyethylene bags was stable (<8% loss in activity) for at least 7 days.[42] *Erwinia carotovora*-derived L-asparaginase was found using UV absorption to be stable in solution (no drug loss) for at least 30 days refrigerated, and for up to 5 days at RT.[11,43] High concentration solutions (>2 mg/mL) of L-asparaginase were stable (no drug loss) for 2 days at RT but dilution to <0.001 mg/mL caused rapid deactivation,[11,29] potentially because of adsorption onto plastic vessel surfaces. Glass containers have been shown not to adsorb L-asparaginase.[9] A modified asparagine assay (colorimetric assay) of *E. coli*-derived L-asparaginase enzymatic activity, and colorimetric and fluorometric biological assays of cell proliferation and cytotoxicity of L-asparaginase, showed that L-asparaginase (2000 IU/mL in SW) stored in plastic containers refrigerated for up to 14 days (J. Wypij, personal

communication, July 21, 2016) retained enzymatic activity (<10% loss), and antineoplastic activity (>90% asparagine depletion) in two feline and two canine lymphoma cell lines *in vitro*.[44] These studies support refrigerated storage of *E. coli*-derived L-asparaginase, reconstituted with 0.9% NaCl using aseptic technique and a CSDTD, in glass or plastic containers for up to 14 days.

Mechlorethamine

Under some conditions, mechlorethamine solution may be unstable and subject to significant spontaneous decomposition,[15,45] undergoing rapid hydrolysis in aqueous solution especially at basic pH, and with increased humidity.[46] There are two published studies assessing the storage and stability of mechlorethamine in solution. The first study used a standard colorimetric assay, although the details of this assay were not reported. Mechlorethamine (0.2 mg/mL in SW) was stored refrigerated, resulting in approximately 10% drug degradation by 8 months. However, solutions kept at RT lost approximately 50% of their activity in 1–2 months.[47] HPLC showed minimal (<1%) degradation of mechlorethamine (1 mg/mL in SW) over 35 days in darkness, but less concentrated solutions showed more rapid degradation (20% degradation over 35 days at 1% concentration, and 80% degradation in 20 days at 0.02% concentration).[46] The HPLC study[46] justifies refrigerated mechlorethamine storage for up to 28 days in darkness, after reconstituted with SW to 1 mg/mL using aseptic technique and a CSDTD. Because exposure to mechlorethamine may occur through inhalation and dermal contact, causing severe toxicity, and because of potential for rapid hydrolysis in aqueous solution; some oncologists prefer not to store this drug, but rather to deactivate and dispose of any remaining drug after use. Furthermore, no studies have evaluated reconstitution and storage of mechlorethamine with 0.9% NaCl.

Methotrexate

There is conflicting data on the effect of light on stability of methotrexate; UV spectroscopy[25] showed no effect of light (<1% loss), but a SIHPLC study[48] showed 100% loss during storage in visible light for one week. HPLC showed methotrexate (0.04 or 0.4 mg/mL in 0.9% NaCl or D5W) was stable (<3% degradation) for at least 7 days in darkness at RT or refrigerated.[10] Methotrexate (0.1 mg/mL in SW) in light at RT, showed 5% degradation after 10 days and 11% degradation after 20 days using HPLC. Undiluted methotrexate (25 mg/mL) in the original vials, stored in light at RT showed negligible (<1%) degradation for >1 month.[49] These studies support light-protected, refrigerated methotrexate storage for up to 28 days after puncture using aseptic technique and a CSDTD.

Mitoxantrone

SIHPLC showed mitoxantrone (0.2 mg/mL in SW) to be stable (<1% degradation) for up to 14 days at either refrigerated or 37 °C.[50] Using a cell survival biological assay, mitoxantrone (2 mg/mL) did not lose cytotoxic efficacy following repeated freeze–thaw cycles for at least 12 months.[51] Although the biological assay results indicate that mitoxantrone can maintain its cytotoxicity against cancer cells in culture for up to 12 months,[51] SIHPLC,[50] justifies refrigerated mitoxantrone storage for up to 14 days after puncture using aseptic technique and a CSDTD.

Vinblastine

SIHPLC showed vinblastine (0.02 or 1 mg/mL in 0.9% NaCl, LRS, or D5W) was stable (<3% degradation) for at least 3 weeks refrigerated and at RT in darkness.[52] HPLC showed vinblastine (1 mg/mL in 0.9% NaCl) was stable (percentage not reported) for up to 1 month in polypropylene syringes at RT in darkness.[53] Three other studies using SIHPLC[54] or HPLC[55,56] supported the findings of the above studies; reporting stability of vinblastine in various solutions for more than 1 month at refrigerated and / or RT. These studies support refrigerated vinblastine storage for up to 28 days, after puncture using aseptic technique and a CSDTD.

Vincristine

SIHPLC showed vincristine (0.02 or 1 mg/mL in 0.9% NaCl, LRS, or D5W) was stable (<5%

degradation) when stored for at least 3 weeks refrigerated and at RT in darkness.[52] LC-ESI-MS showed vincristine (0.001 mg/mL in SW) was stable (<1% degradation) for at least 2 months refrigerated.[5] There are some vincristine stability studies where the assay and percentage degradation were not reported.[57,58] Only validated SIHPLC and LC-ESI-MS studies that include controls and repeatable stability data are discussed here. These studies support refrigerated vincristine storage for up to 28 days after puncture using aseptic technique and a CSDTD.

Vinorelbine

SIHPLC showed vinorelbine (0.5 or 2 mg/mL in D5W or 0.9% NaCl) in PVC bags, at RT and exposed to constant fluorescent lighting was stable (<6% degradation) for up to 5 days.[59] HPLC showed vinorelbine (0.05 and 0.5 mg/mL in 0.9% NaCl or D5W) in darkness, was stable (<3% degradation) for at least 7 days at RT or refrigerated.[10] Another HPLC study showed vinorelbine (0.2 mg/mL in either D5W or 0.9% NaCl) in PVC bags refrigerated in darkness was stable (<3% degradation) for 7 days in D5W, however there was >10% loss of drug after 3 days when vinorelbine was diluted in 0.9% NaCl. This study aimed to simulate CRI of vinorelbine in a hospital setting.[60] LC-ESI-MS showed vinorelbine (0.001 mg/mL in SW) was stable (negligible <1% loss) for at least 1 month refrigerated.[5] The validated LC-ESI-MS study,[5] justifies refrigerated vinorelbine for up to 28 days after puncture using aseptic technique and a CSDTD.

Discussion

This article reviews the available literature on stability and storage conditions for nineteen chemotherapeutics commonly used in veterinary medicine. While validated SIHPLC, HPLC and LC-ESI-MS assays provide the most reliable stability information, evaluation of controls and repeatability should be taken into consideration when interpreting any report. The studies reviewed here were performed over a long period of time, and therefore have not always used the most up-to-date assay techniques.

There were several limitations in performing this review. The definition of drug stability varied greatly between studies.[2,29] In the reports reviewed, investigators suggested the drug to be stable when anywhere from no drug loss to <10% drug degradation occurred by a specified time point. A stability cut-off value of >95% of the initial drug concentration remaining in solution was predominantly used in this review. When this information was lacking and publications simply stated the drug was stable, the statement was accepted at face value if the report was otherwise robust such as including the use of validated SIHPLC with adequate controls and repeatable data.[27] In addition, many studies reviewed had an arbitrary study endpoint, rather than monitoring the drug stability until a certain percentage of degradation was seen. Therefore the drug storage times reported in the literature may not be the maximum stability time. The data that was considered most reliable in this review was from validated SIHPLC, LC-ESI-MS and/or HPLC studies. When these types of studies were not available for this review, studies with adequate controls and/or repeatability stability data were selected. Despite these efforts, it was still difficult to directly compare each of the reports with one another, because of the diversity in reported stability data presented and absence of comprehensive information in the literature, such as: inconsistency or lack of reporting of controls, repeatable data, and/or validation methods[2,6,9,11,18,25,27,29,30,32,35,41,47,49,53,56–58,61]; missing information[13]; different assay techniques; lack of reporting of assay techniques[2,6,9,11,18,35,57,58]; various times for study endpoint; diverse definition of stability; variation in storage times for the same drug; etc. Future studies that address these inconsistencies would be helpful.

The authors use a CSDTD for handling all injectable chemotherapeutics. In reporting the stability and storage information provided in this review, the authors made the assumption that this system and similar ones are completely closed and that there would be minimal to non-existent microbial contamination when drugs have been reconstituted or punctured with such systems using aseptic technique. Unfortunately, there are no long-term studies assessing the microbiological

contamination rate with these systems specifically for any of these chemotherapeutics. However, there are a few short-term (<14 days) studies of the systems themselves that show low risk of contamination.[62–65] One study[62] evaluated the ability of different protective devices for reconstitution of chemotherapy agents to prevent microbial contamination, by inoculating the vial stoppers with microbial contaminants and then utilizing different mechanisms to enter the vials; then measuring the contamination in the vials. The study concluded that while a CSDTD can limit the transfer of external contamination from the vial stopper into the vial, it did not guarantee total sterility. This study had a high challenge of inoculation and it is likely the risk under best practice clinical conditions is substantially lower than this. Another study[63] aimed to test the ability of a CSDTD to prevent contamination under normal work conditions (such as use of a Class II biological safety cabinet). The study tested over 1300 CSDTDs up to 7 days and found a contamination rate of 1.8%. A further study[64] showed a similar exogenous contamination rate of 1.86% after 14 days, when using CSDTDs with 101 vials under similar conditions to those above. Fluid and bag wall contamination was assessed by bacterial culture and assays for endotoxin and ATP activity for 60 days under infrequent (once daily) and frequent (10 times daily) use conditions. The results suggest fluid bags can be used for up to 30 days when punctured 10 times daily and for up to 60 days when punctured once daily with minimal microbial contamination.[65] Based on this study, it appears likely that diluted chemotherapeutics in fluid bags have a low risk of microbial contamination. This is particularly important for chemotherapeutics that are given as a CRI, such as cytarabine. However, there are no studies to assess the rate of contamination beyond 14 days when using a CSDTD. Although some chemotherapeutics such as epirubicin[32] could potentially be stored for much longer than suggested purely based on drug stability, a maximum of 28 days of refrigerated storage was suggested until further studies become available, because of the concern about contamination and potential for development of toxic degradation products.

Reducing waste makes the option of storing partially used vials of prepared chemotherapeutics attractive. However, there are still unresolved issues including the extra-label use of drugs packaged for single use; as well as concerns for sterility and patient safety when using preservative-free solutions intended for single use, for multiple doses in client-owned pet animals with cancer. Appropriate precautions should be taken to ensure sterility when using multiple doses from the same vial.

Lastly, the biological impact of toxic degradation products that may develop in drugs over time was not evaluated in any of the reviewed studies. Although not proven, this is a potential safety concern with storing drugs for longer periods.

In conclusion, the available data may justify potential alteration of chemotherapy drug storage procedures by veterinarians. Further studies on the risk of microbiological contamination of chemotherapeutics using a CSDTD, and validated high quality drug assays such as SIHPLC, are required to make fully informed decisions about the length and conditions of storage. The storage parameters discussed in this review are not intended to supersede the product label recommendations; but to suggest that it may be possible to consider storing these drugs for longer periods without significant loss of efficacy, thus reducing the costs of chemotherapeutics for veterinary clients.

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Conflict of interest

The authors declare no conflict of interest in this research, and no external funding was sought or obtained. However, Dr Frimberger and Dr Moore hold stocks in Becton Dickinson, producer of PhaSeal™ CSDTD.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of the advantages and disadvantages of various assay techniques used to determine drug stability[1–8].

References

1. Bakshi M and Singh S. Development of validated stability-indicating assay methods – critical review. *Journal of Pharmaceutical and Biomedical Analysis* 2002; **28**: 1011–1040. doi:10.1016/S0731-7085(02)00047-X.
2. Bosanquet AG. Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays. II. Assay methods, adriamycin and the other antitumour antibiotics. *Cancer Chemotherapy and Pharmacology* 1986; **17**: 1–10. doi:10.1007/BF00299858.
3. Hofer JD, Olsen BA and Rickard EC. Is HPLC assay for drug substance a useful quality control attribute? *Journal of Pharmaceutical and Biomedical Analysis* 2007; **44**: 906–913. doi:10.1016/j.jpba.2007.04.023.
4. McMurry J. *Organic Chemistry*. 8th edn., Belmont, CA, Brooks/Cole, Cengage Learning, 2012.
5. Negreira N, Mastroianni N, Lopez de Alda M and Barcelo D. Multianalyte determination of 24 cytostatics and metabolites by liquid chromatography-electrospray-tandem mass spectrometry and study of their stability and optimum storage conditions in aqueous solution. *Talanta* 2013; **116**: 290–299. doi:10.1016/j.talanta.2013.04.070.
6. Nussbaumer S, Bonnabry P, Veuthey JL and Fleury-Souverain S. Analysis of anticancer drugs: a review. *Talanta* 2011; **85**: 2265–2289. doi:10.1016/j.talanta.2011.08.034.
7. Salman D, Barton S and Gebara SN. Improving the stability of anticancer drugs. *Journal of Oncology Pharmacy Practice* 2014; **20**: 236. doi:10.1177/1078155213491774.
8. Trissel LA. Avoiding common flaws in stability and compatability studies on injectable drugs. *American Journal of Hospital Pharmacy* 1983; **40**: 1159–1160. http://www.ashp.org/DocLibrary/AJHP/Avoidingcommonflawsinstabilityandcompatibilitystudiesofinjectabledrugs.aspx.
9. Bosanquet AG. Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays. General considerations, the nitrosoureas and alkylating agents. *Cancer Chemotherapy and Pharmacology* 1985; **14**: 83–95. doi:10.1007/BF00434343.
10. Beitz C, Bertsch T, Hannak D, Schrammel W, Einberger C and Wehling M. Compatibility of plastics with cytotoxic drug solutions-comparison of polyethylene with other container materials. *International Journal of Pharmaceutics* 1999; **185**: 113–121. doi:10.1016/S0378-5173(99)00175-1.
11. Bosanquet AG. Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays. III. Antimetabolites, tubulin-binding agents, platinum drugs, amsacrine, L-asparaginase, interferons, steroids and other miscellaneous antitumor agents. *Cancer Chemotherapy and Pharmacology* 1989; **23**: 197–207. doi:10.1007/BF00451642.
12. Benizri F, Bonan B, Ferrio AL, Brandely ML, Castagne V, Theou-Anton N, *et al.* Stability of antineoplastic agents in use for home-based intravenous chemotherapy. *Pharmacy World & Science* 2009; **31**: 1–13. doi:10.1007/s11096-008-9270-z.
13. Crevar GE and Slotnick IJ. A note on the stability of actinomycin D. *The Journal of Pharmacy and Pharmacology* 1964; **16**: 429–432. doi:10.1111/j.2042-7158.1964.tb07486.x.
14. Brothman AR, Davis TP, Duffy JJ and Lindell TJ. Development of an antibody to actinomycin D and its application for the detection of serum levels by radioimmunoassay. *Cancer Research* 1982; **42**: 1184–1187. http://cancerres.aacrjournals.org/content/canres/42/3/1184.full.pdf.
15. Rapp RP, Wermeling DP and Piecoro JJ Jr. Guidelines for the administration of commonly used intravenous drugs. *Drug Intelligence & Clinical Pharmacy* 1984; **18**: 217–232.
16. Tomlinson E and Malspeis L. Concomitant adsorption and stability of some anthracycline antibiotics. *Journal of Pharmaceutical Sciences* 1982; **71**: 1121–1125. doi:10.1002/jps.2600711011.
17. James MP, Collier PM, Aherne W, Hardcastle A and Lovegrove S. Histologic, pharmacologic, and immunocytochemical effects of injection of bleomycin into viral warts. *Journal of the American Academy of Dermatology* 1993; **28**: 933–937. doi:10.1016/0190-9622(93)70133-E.
18. Hudson AL. Letter: treatment of plantar warts with bleomycin. *Archives of Dermatology* 1976; **112**: 1179. doi:10.1001/archderm.1976.01630320077035.
19. Yang LY and Drewinko B. Cytotoxic efficacy of reconstituted and stored antitumor agents. *Cancer Research* 1985; **45**: 1511–1515. http://cancerres.aacrjournals.org/content/45/4/1511.full-text.pdf.

20. Myers AL, Zhang YP, Kawedia JD, Trinh VA, Tran H, Smith JA, *et al.* Stability study of carboplatin infusion solutions in 0.9% sodium chloride in polyvinyl chloride bags. *Journal of Oncology Pharmacy Practice* 2016; **22**: 31–36. doi:10.1177/1078155214546016.
21. Kaestner S and Sewell G. A sequential temperature cycling study for the investigation of carboplatin infusion stability to facilitate 'dose-banding'. *Journal of Oncology Pharmacy Practice* 2007; **13**: 119–126. doi:10.1177/1078155207080803.
22. Osawa T, Naito T, Suzuki N, Imai K, Nakanishi K and Kawakami J. Validated method using liquid chromatography-electrospray ionization tandem mass spectrometry for the determination of contamination of the exterior surface of vials containing platinum anticancer drugs. *Talanta* 2011; **85**: 1614–1620. doi:10.1016/j.talanta.2011.06.059.
23. Schuldes H, Bade S, Knobloch J and Jonas D. Loss of in vitro cytotoxicity of cisplatin after storage as stock solution in cell culture medium at various temperatures. *Cancer* 1997; **79**: 1723–1728. doi:10.1002/(SICI)1097-0142(19970501)79:9<1723::AID-CNCR13>3.0.CO;2-#.
24. Beijnen JH, van Gijn R, Challa EE, Kaijser GP and Underberg WJ. Chemical stability of two sterile, parenteral formulations of cyclophosphamide (Endoxan) after reconstitution and dilution in commonly used infusion fluids. *Journal of Parenteral Science and Technology* 1992; **46**: 111–116. http://journal.pda.org/content/46/4/111.full.pdf+html.
25. Cradock JC, Kleinman LM and Rahman A. Evaluation of some pharmaceutical aspects of intrathecal methotrexate sodium, cytarabine and hydrocortisone sodium succinate. *American Journal of Hospital Pharmacy* 1978; **35**: 402–406.
26. Munson JW, Kubiak EJ and Cohon MS. Cytosine arabinoside stability in intravenous admixtures with sodium bicarbonate and in plastic syringes. *Drug Intelligence & Clinical Pharmacy* 1982; **16**: 765–767.
27. Rochard EB, Barthes DM and Courtois PY. Stability of fluorouracil, cytarabine, or doxorubicin hydrochloride in ethylene vinylacetate portable infusion-pump reservoirs. *American Journal of Hospital Pharmacy* 1992; **49**: 619–623.
28. El Aatmani M, Poujol S, Astre C, Malosse F and Pinguet F. Stability of dacarbazine in amber glass vials and polyvinyl chloride bags. *American Journal of Health-System Pharmacy* 2002; **59**: 1351–1356. http://www.ajhp.org/content/59/14/1351.long?hw-tma-check=true.
29. Rosenthal RC and Michalski D. Storage of expensive anticancer drugs. *Journal of the American Veterinary Medical Association* 1991; **198**: 144–146.
30. Wood MJ, Irwin WJ and Scott DK. Stability of doxorubicin, daunorubicin and epirubicin in plastic syringes and minibags. *Journal of Clinical Pharmacy and Therapeutics* 1990; **15**: 279–289. doi:10.1111/j.1365-2710.1990.tb00386.x.
31. Hoffman DM, Grossano DD, Damin L and Woodcock TM. Stability of refrigerated and frozen solutions of doxorubicin hydrochloride. *American Journal of Hospital Pharmacy* 1979; **36**: 1536–1538.
32. Pujol M, Munoz M, Prat J, Girona V and De Bolos J. Stability study of epirubicin in NaCl 0.9% injection. *Annals of Pharmacotherapy* 1997; **31**: 992–995.
33. Galanti L, Lebitasy MP, Hecq JD, Cadrobbi J, Vanbeckbergen D and Jamart J. Long-term stability of 5-fluorouracil in 0.9% sodium chloride after freezing, microwave thawing, and refrigeration. *The Canadian Journal of Hospital Pharmacy* 2009; **62**: 34–38. http://www.ncbi.nlm.nih.gov/pmc/articles/pmid/22478863/.
34. Benaji B, Faouzi MA, Dine T, Goudaliez F, Luyckx M, Brunet C, *et al.* Compatibility study of 5-fluorouracil with PVC bags after repackaging into two types of infusion admixtures. *Thérapie* 1999; **54**: 659–663.
35. Milovanic D and Nairn JG. Stability of fluorouracil in amber glass bottles. *American Journal of Hospital Pharmacy* 1980; **37**: 164–165.
36. Xu Q, Zhang Y and Trissel LA. Physical and chemical stability of gemcitabine hydrochloride solutions. *Journal of the American Pharmaceutical Association* 1999; **39**: 509–513. doi:10.1016/S1086-5802(16)30470-3.
37. Ponton J, Munoz C, Rey M and Val M. The stability of (lyophilized) gemcitabine in 0.9% sodium chloride injection. *European Journal of Hospital Pharmacy* 2002; **1**: 23–25.
38. Munoz M, Girona V, Pujol M, Duran S, Vicente P and Sole LA. Stability of ifosfamide in 0.9% sodium chloride solution or water for injection in a portable i.v. pump cassette. *American Journal of Hospital Pharmacy* 1992; **49**: 1137–1139.
39. Priston M and Sewell G. Stability of three cytotoxic drug infusions in the Graseby 9000 ambulatory infusion pump. *Journal of Oncology Pharmacy Practice* 1998; **4**: 143–149. doi:10.1177/107815529800400302.
40. Zhang Y, Kawedia JD, Myers AL, McIntyre CM, Anderson PM, Kramer MA, *et al.* Physical and chemical stability of high-dose ifosfamide and mesna for prolonged 14-day continuous infusion.

Journal of Oncology Pharmacy Practice 2014; **20**: 51–57. doi:10.1177/1078155213478284.
41. Leone L, Comandone A, Oliva C, Bussi P, Goffredo F, Bretti S, *et al.* Stability of ifosfamide in solutions for multiday infusion by external pump. *Anti-Cancer Drugs* 1995; **6**: 604–607. http://aop.sagepub.com/content/14/5/353.full.pdf+html.
42. Stecher AL, de Deus PM, Polikarpov I and Abrahao-Neto J. Stability of L-asparaginase: an enzyme used in leukemia treatment. *Pharmaceutica Acta Helvetiae* 1999; **74**: 1–9. doi:10.1016/S0031-6865(99)00009-6.
43. Cammack KA, Marlborough DI and Miller DS. Physical properties and subunit structure of L-asparaginase isolated from *Erwinia carotovora*. *Biochemistry Journal* 1972; **126**: 361–379. doi:10.1042/bj1260361.
44. Wypij JM and Pondenis HC. *E. coli*-derived L-Asparaginase retains enzymatic and cytotoxic activity in vitro for canine and feline lymphoma after cold storage. *Veterinary Medicine International* 2013; **2013**: 786162. doi:10.1155/2013/786162.
45. de Lemos ML and Hamata L. Stability issues of parenteral chemotherapy drugs. *Journal of Oncology Pharmacy Practice* 2007; **13**: 27–31. doi:10.1177/1078155206075236.
46. Reepmeyer JC, Ye W and Ritschel WA. Modifications and insights into a method for the analysis of the nitrogen mustard mechlorethamine by high-performance liquid chromatography. *Analytica Chimica Acta* 2008; **616**: 78–84. doi:10.1016/j.aca.2008.04.020.
47. Taylor JR, Halprin KM, Levine V and Aoyagi T. Mechlorethamine hydrochloride solutions and ointment. Prolonged stability and biological activity. *Archives of Dermatology* 1980; **116**: 783–785. doi:10.1001/archderm.1980.01640310053018.
48. D'Hondt M, Vangheluwe E, Van Dorpe S, Boonen J, Bauters T, Pelfrene B, *et al.* Stability of extemporaneously prepared cytarabine, methotrexate sodium, and methylprednisolone sodium succinate. *American Journal of Health-System Pharmacy* 2012; **69**: 232–240. doi:10.2146/ajhp110208.
49. Chatterji DC and Gallelli JF. Thermal and photolytic decomposition of methotrexate in aqueous solutions. *Journal of Pharmaceutical Sciences* 1978; **67**: 526–531. doi:10.1002/jps.2600670422.
50. Northcott M, Allsopp MA, Powell H and Sewell GJ. The stability of carboplatin, diamorphine, 5-fluorouracil and mitozantrone infusions in an ambulatory pump under storage and prolonged 'in-use' conditions. *Journal of Clinical Pharmacy and Therapeutics* 1991; **16**: 123–129. doi:10.1111/j.1365-2710.1991.tb00293.x.
51. Mauldin GN and Truax R. Evaluation of the in vitro cytotoxicity of mitoxantrone following repeated freeze-thaw cycles. *Veterinary Therapeutics* 2002; **3**: 290–296. http://www.vetfolio.com/pharmacology/evaluation-of-the-in-vitro-cytotoxicity-of-mitoxantrone-following-repeated-freeze-thaw-cycles.
52. Beijnen JH, Vendrig DE and Underberg WJ. Stability of vinca alkaloid anticancer drugs in three commonly used infusion fluids. *Journal of Parenteral Science and Technology* 1989; **43**: 84–87. http://journal.pda.org/content/43/2/84.full.pdf+html?sid=2c3d0516-4e74-4d51-848b-82965ad317c2.
53. Girona V, Prat J, Pujol M and Munoz M. Stability of vinblastine sulphate in 0.9% sodium chloride in polypropylene syringes. *Bollettino Chimico Farmaceutico* 1996; **135**: 413–414.
54. Vendrig D, Smeets B, Beijnen J, van der Houwen O and Holthuis J. Degradation kinetics of vinblastine sulphate in aqueous solutions. *International Journal of Pharmaceutics* 1988; **43**: 131–138. doi:10.1016/0378-5173(88)90067-1.
55. Black J, Buechter DD, Chinn JW, Gard J and Thurston DE. Studies on the stability of vinblastine sulfate in aqueous solution. *Journal of Pharmaceutical Sciences* 1988; **77**: 630–634. doi:10.1002/jps.2600770716.
56. De Smet M, Van Belle SJ, Storme GA and Massart DL. High-performance liquid chromatographic determination of vinca-alkaloids in plasma and urine. *Journal of Chromatography* 1985; **345**: 309–321. doi:10.1016/0378-4347(85)80168-7.
57. Kirschenbaum BE and Latiolas CJ. Stability of injectable medications after reconstitution. *American Journal of Hospital Pharmacy* 1976; **33**: 767–791.
58. Wolfert RR and Cox RM. Room temperature stability of drug products labeled for refrigerated storage. *American Journal of Hospital Pharmacy* 1975; **32**: 585–587.
59. Lieu CL, Chin A and Gill MA. Five-day stability of vinorelbine in 5% dextrose injection and in 0.9% sodium chloride injection at room temperature. *International Journal of Pharmaceutical Compounding* 1999; **3**: 67–68. http://www.usc.edu/hsc/pharmacy/pd_labs/navel.pdf.
60. Dine T, Luyckx M, Cazin J, Goudaliez F and Mallevais M. Stability and compatibility studies of vinblastine, vincristine, vindesine, and vinorelbine with PVC infusion bags. *International Journal of*

Pharmaceutics 1991; **77**: 279–285. doi:10.1016/0378-5173(91)90328-L.

61. Gallelli JF. Stability studies of drugs used in intravenous solutions. I. *American Journal of Hospital Pharmacy* 1967; **24**: 425–433.
62. De Prijck K, D'Haese E, Vandenbroucke J, Coucke W, Robays H and Nelis HJ. Microbiological challenge of four protective devices for the reconstitution of cytotoxic agents. *Letters in Applied Microbiology* 2008; **47**: 543–548. doi:10.1111/j.1472-765X.2008.02463.x.
63. McMichael D, Jefferson D, Thomas Carey E, Forrey R, Spivey S, Mulvaney J, *et al.* Utility of the PhaSeal closed system drug transfer device. *The American Journal of Pharmacy Benefits* 2011; **3**: 9–16. http://www.ajpb.com/journals/ajpb/2011/ajpb_11jan/ajpb_11jan_mcmichael_9to16.
64. Rowe EC, Savage SW, Rutala WA, Weber DJ, Gergen-Teague M and Eckel SF. Economic and microbiologic evaluation of single-dose vial extension for hazardous drugs. *Journal of Oncology Practice* 2012; **8**: e45–e49. doi:10.1200/jop.2011.000488.
65. Matthews KA and Taylor DK. Assessment of sterility in fluid bags maintained for chronic use. *Journal of the American Association for Laboratory Animal Science* 2011; **50**: 708–712. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189676/pdf/jaalas2011000708.pdf.