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Stability of the principal cytostatic agents during storage at unusual temperatures

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Abstract

The aim of our study was to determine the stability of ten antineoplastic drugs stored in their initial package, in conditions of temperature which may be encountered during transport to hospital pharmacy or storage. Stability was determined by high-performance liquid chromatography after storage at cold temperature (+4°C) and warm temperature (+33°C) for 6 or 12 months comparatively to room temperature (+21°/+23°C) as recommended by the manufacturers. No change in color was observed in any sample throughout the study except for a yellow hue in fluorouracil vials stored at +33°C. With the exception of melphalan, all the drugs were stable when stored at both temperatures for 6 and 12 months. Melphalan concentration decreased to less than 90% of theoretical concentration from 6 months storage at +4°C. In spite of the yellow hue which developed in the fluorouracil vials stored at +33°C, this drug was found at greater than 90% of the theoretical concentration. Among the ten drugs assayed for stability at unusual temperatures, eight were found to be stable. Two displayed temperature sensitivity as shown by change of color or loss of drug. Temperature should be controlled during storage and transport of drugs, particularly for fluorouracil and melphalan. © 1997 Elsevier Science B.V.

Keywords: Antineoplastic agents; Stability; Storage; Temperature; High-performance liquid chromatography

1. Introduction

Stability is defined as the time during which an antineoplastic drug retains its integrity in terms of quantity and chemical identity. It can be affected by environmental factors such as temperature, pH, light, and air.

The single most important factor is pH, which can have a dramatic effect on the stability of labile drugs (Williams, 1990).

The second most important factor that can influence the drug rate of degradation or stability

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Table 1
Drugs used and reconstitution conditions

Drug	Manufacturer	Lot number	Amount	Reconstitution solvent	Reconstitution volume
Cisplatin	Qualimed	BA07/BA12	10 mg	0.9% Sodium chloride[a]	10 ml
Cyclophosphamide	ASTA Medica	547/563	500 mg	Sterile water[b]	25 ml
Dacarbazine	Roger Bellon	105/118	100 mg	Sterile water	10 ml
Doxorubicine	Farmitalia	3018A/SA003	10 mg	Sterile water	5 ml
Epirubicine	Farmitalia	2036A/3045A	10 mg	Sterile water	5 ml
Etoposide	Sandoz	048/052	100 mg	None	—
Fluorouracil	Roche	F020W/B005V	250 mg	None	—
Ifosfamide	ASTA Medica	146/150	1 g	Sterile water	10 ml
Melphalan	Wellcome	A2713A/B2852A	50 mg	Solvent[c]	10 ml
Methotrexate	Lederle	9/151	500 mg	Sterile water	10 ml

[a]Lot number 93J04G50, Baxter Laboratories, Maurepas, France.
[b]Lot number 404687B, Aguettant Laboratories, Lyon, France.
[c]Lot number A2739A/B2938A, Wellcome Laboratories, Issy les Moulineaux, France.

is temperature. A 10°C increase of the storage temperature can enhance chemical degradation by a factor 2–5. Most water-insoluble drugs are less soluble at refrigerated temperatures than at room temperatures (Williams and Lokich, 1992).

Some data on drug stability, compatibility as well as on factors affecting the stability during storage of chemotherapeutic agents are available in the package insert, and in the Handbook on Injectable Drugs (Trissel, 1988), but stability studies under exceedingly high or low temperatures of storage have not been performed or are not recorded.

Temperature is a factor difficult to control during transport, particularly in winter and summer, and during storage in hospital pharmacies without proper air-conditioning. The study reported here was designed to evaluate the stability of ten cytostatic drugs: cisplatin, cyclophosphamide, dacarbazine, doxorubicine, epirubicine, etoposide, fluorouracil, ifosfamide, melphalan, and methotrexate—when stored as received, in their initial package, at refrigerated temperature (+4°C) and at +33°C (temperature occasionally encountered in summer during transport or storage) compared to room temperature (+21°/+23°C).

According to the manufacturer's guidelines for quality assurance, all these drugs should have been stored at room temperature (+15°/+25°C). Little or no data about stability at +4°C or at +33°C of these ten drugs can be found in the literature.

2. Methods

2.1. Stability study

Cisplatin, cyclophosphamide, dacarbazine, doxorubicine, epirubicine, ifosfamide, melphalan, methotrexate lyophilized powder vials and etoposide, fluorouracil solutions were obtained from commercial sources (Table 1).

All substances and solvents were of analytical reagents grade or high-performance liquid chromatography grade.

Three vials of each drug were stored, protected from light, for 6 and 12 months, under three different temperature conditions: (i) refrigeration (+4°C); (ii) at room temperature (+21°C/+23°C); and (iii) at +33°C in an oven.

Just before analysis, lyophilized powder vials were reconstituted according to the manufacturer's instructions, under sterile conditions (class 100 laminar airflow hood), and samples were diluted 100-fold with sterile water.

Duplicate HPLC determinations were performed on each sample.

2.2. Analysis per visual examination

Visual examination of the samples was performed with the unaided eye, in normal laboratory light. All lyophilized powder vials were examined for color and clarity before and after reconstitution. The etoposide and fluorouracil solutions were examined for color, clarity and evidence of precipitation.

Compatibility was defined as no evidence of precipitation or change in physical appearance was found to occur.

2.3. Analysis by high-performance liquid chromatography

Stability-indicating high-performance liquid chromatographic (HPLC) methods, modified to achieve satisfactory determination in our laboratory, were used to measure the drug concentrations.

Analyses were performed with an HPLC equipped with a reciprocating piston pump with flow feed-back control (LC-9A, Shimadzu, Kyoto, Japan), which delivered mobile phase at a constant flow rate to a reverse-phase column 5 μm Kromasil C18 column (150 × 4.6 mm) (Touzart et Matignon, Vitry/Seine, France). Samples were introduced to the column using a manual injection valve equipped with a 20-μl sample loop. The column effluent was monitored either with a variable wavelength ultraviolet detector (SPD-6A, Variable wavelength UV detector, Shimadzu, Kyoto, Japan) or with a fluorescence HPLC monitor (RF 535, Fluorescence HPLC detector, Shimadzu, Kyoto, Japan). Integration of the peaks was performed with an integrator (C-R5A Chromatopac, Shimadzu, Kyoto, Japan).

All separations were performed isocratically at ambient temperature.

HPLC conditions for each drug are listed in Table 2.

Concentrations of intact drug were determined for each sample and are reported as the percentage of the theoretical concentration. Quantitation was by peak areas and standard curves. All six-point calibration curves were linear ($r > 0.999$) in the concentration range of interest. The intra- and inter-day coefficients of variation for all the assays were $< 4\%$ and $< 5\%$, respectively.

2.4. Analysis of urea

Urea analysis was performed using an enzymatic method (kinetic UV test) (Boehringer Mannheim Laboratories, 1995).

2.5. Analysis of data

The theoretical concentration of intact drug was the drug concentration indicated on the package label (as reconstituted if necessary) and was designated as 100%. All subsequent concentrations were expressed as percentages of the theoretical concentration.

Stability was defined as a concentration 90–105% of the theoretical concentration, in accordance with Health Registration of France, the French Regulatory Agency for drug and drug-related products.

3. Results and discussion

At each sampling time, there was no evidence of precipitation. Visual examination of all tested vials showed them to be clear and without any change of color, except for the fuorouracil vials.

At +33°C, a yellow hue appeared in all the fuorouracil vials. These results did not differ from what has been reported previously (Trissel, 1988). In therapeutic conditions, Pinguet et al. (1990) recommend that the yellow vials should not be used. At +4°C there was no evidence of precipitation or change in the clarity or color of any fluorouracil vials during the study period. These results are not in agreement with the manufacturer's recommendations (Roche Laboratories, 1994) according to which during storage at < 15°C temperature a precipitate might appear.

There was no difference between the time necessary for complete dissolution of the lyophilized powder vials at the three storage temperatures, except for cyclophosphamide. Brooke et al. (1975) showed that cyclophosphamide is quickly reconstituted when powder is stored at high tempera-

Table 2
HPLC conditions for the analysis of antitumor agents[a]

Drug analysed	Composition of mobile phase	Flow rate (ml/min)	Detector setting (nm)	Retention time (min)	References
Cisplatin	Tetrabutylammonium hydroxyde 0.1 M/H_2O (8:92%, v/v) pH 6 (orthophosphoric acid)	1	313	2.3	Cheung et al., 1987
Cyclophosphamide	Acetonitrile/H_2O (25:75%—v/v)	1.5	195	5.2	Zheng et al., 1994
Dacarbazine	Sodium acetate 0.15 mol/l pH 6/acetonitrile (75:25%, v/v)	0.75	280	2.2	Bienvenuto et al., 1981
Doxorubicine	Sodium acetate buffer pH 4 (sodium acetate 0.1 M 16.6%–acetic acid 0.1 M 83.4%)/acetonitrile (2.5:1, v/v)	1	Exc = 480 Em = 580	5	Oliary, 1989
Epirubicine	Sodium acetate buffer pH 4 (sodium acetate 0.1 M 16.6%–acetic acid 0.1 M 83.4%)/acetonitrile (2.5:1, v/v)	1	Exc = 480 Em = 580	6	Oliary, 1989
Etoposide	Methanol/H_2O (60:40%, v/v)	1.2	Exc = 255 Em = 320	2.2	Jardine et al., 1982
Fluorouracil	KH_2PO_4 buffer (50 mM, pH 3)	1.3	254	3	Barberi-Heyob et al., 1992
Ifosfamide	Acetonitrile/H_2O (25:75%, v/v)	1.5	195	4.7	Zheng et al., 1994
Melphalan	Methanol/H_2O (50:50%, v/v) 1% acetic acid	2	254	3.2	Chang et al., 1978
Methotrexate	Sodium dihydrogen phosphate 0.1 M pH 6.7 (NaOH 4 N)/methanol (80:20%, v/v)	2	260	4.9	Dyvik et al., 1986

[a]All separations were achieved on 5μ C18 reverse-phase column.

ture. In our study, the reconstitution time of the cyclophosphamide lyophilized powder contained in vials stored at +33°C was shorter ($\approx$30%) than that of the vials stored at room temperature.

The results of the stability assay at the three storage temperatures (+4°C, +21°/+23°C, +33°C) have been summarized in Table 3.

At +4°C, all the drugs except melphalan were stable throughout the study. Melphalan concentrations after 6 and 12 months were below 90%. However, no evidence of decomposition products (monohydroxy and dihydroxy-melphalan) appeared in the chromatograms (Pinguet et al., 1994). These findings are consistent with those of the manufacturer who explained that melphalan reconstitution was incomplete after storage at +4°C (Wellcome Laboratories, 1992).

We have also studied melphalan concentrations after storage at +4°C during only 1, 3 and 7 days, conditions occasionally encountered in winter during transport. We obtained, respectively, 98.37%, 95.08% and 95.11% of the theoretical concentration. Melphalan stability is not affected by a short storage at cold temperature.

At +21°/+23°C, all concentrations remained above 90% of the theoretical value and most were near 100%.

At +33°C, no significant degradation of all the drugs occurred within the storage period. This was found for the drugs which appeared normal at visual examination as well as for the fluorouracil yellow solutions.

We left fluorouracil vials at these three temperatures for more than 18 months. No change of color appeared except at +33°C which resulted

Table 3
Stability of cisplatin, cyclophosphamide, dacarbazine, doxorubicine, epirubicin, etoposide, 5-fluorouracil, ifosfamide, melphalan, and methotrexate stored at different temperatures for 6 and 12 months

Drug analysed	Time (months)	% Theoretical drug concentration remaining[a]		
		Storage temperature		
		4°C	21°/23°C	33°C
Cisplatin	6	96.2 ± 3.9	101.9 ± 0.3	99.1 ± 5.8
	12	101.5 ± 0.5	100.3 ± 2.4	99.8 ± 3.7
Cyclophosphamide	6	95.7 ± 1.7	95.4 ± 2.1	101.7 ± 3.6
	12	96.3 ± 3.0	100.8 ± 2.4	99.8 ± 3.7
Dacarbazine	6	98.8 ± 1.0	95.7 ± 2.7	101.6 ± 1.0
	12	101.9 ± 1.3	99.8 ± 1.3	101.4 ± 2.8
Doxorubicine	6	101.5 ± 1.3	101.7 ± 1.8	98.0 ± 3.5
	12	100.7 ± 1.3	100.1 ± 2.5	98.8 ± 2.2
Epirubicine	6	95.1 ± 1.9	98.9 ± 1.7	99.7 ± 4.9
	12	101.4 ± 3.3	100.7 ± 2.2	96.8 ± 3.4
Etoposide	6	100.0 ± 4.6	100.5 ± 4.0	99.7 ± 1.5
	12	101.6 ± 2.2	100.8 ± 2.4	97.7 ± 2.2
Fluorouracil	6	99.7 ± 2.0	100.1 ± 0.4	98.0 ± 1.5
	12	101.8 ± 1.1	97.6 ± 2.0	95.6 ± 0.4
Ifosfamide	6	98.1 ± 0.9	100.5 ± 1.9	100.3 ± 1.4
	12	99.4 ± 1.4	98.4 ± 1.0	101.4 ± 3.1
Melphalan	6	89.7 ± 0.6	96.7 ± 1.3	100.4 ± 3.1
	12	89.8 ± 0.9	99.9 ± 1.1	101.2 ± 0.2
Methotrexate	6	99.5 ± 0.5	100.0 ± 0.5	100.5 ± 0.2
	12	99.9 ± 0.3	100.3 ± 0.9	95.3 ± 1.9

[a]Mean ± S.D. of six determinations.

in a darker yellow hue. After 18 months, fluorouracil concentration at +4°, +21°/23° and +33°C was, respectively, 95.8%, 95.9% and 80.4% of the theoretical one, which means a fluorouracil loss of 16.2, 15.8 and 75.4 mmol/l, respectively. We therefore assayed urea which is a degradation product of fluorouracil (Rochard et al., 1992). In recently arrived vials, we found a mean urea concentration of 5.1 mmol/l. After 18 months of storage at +4°C, +21°/23°C and +33°C, urea concentrations were 16.8, 15.2 and 75.9 mmol/l, respectively. These findings confirm fluorouracil degradation when stored at higher than ambient (+21/23°C) temperature.

4. Conclusion

The various drugs tested (cisplatin, cyclophosphamide, dacarbazine, doxorubicine, epirubicine, etoposide, fluorouracil, ifosfamide, melphalan, and methotrexate) are stable when stored for a long period of time as received from laboratories, at the two temperatures studied (+4°C and +33°C) compared to the temperature recommended by manufacturers (+23–25°C), except for melphalan when stored at +4°C and fluorouracil when stored at +33°C. Melphalan stability was not modified by a short period of storage at +4°C. Fluorouracil degradation was not negligi-

ble when stored at +33°C and increased with time.

Temperature should be carefully controlled during storage and transport of melphalan and fluorouracil.

References

Barberi-Heyob, M., Merlin, J.L. and Weber, B., Determination of 5-fluorouracil and its main metabolites in plasma by high-performance liquid chromatography. *J. Chromatogr.*, 573 (1992) 247–252.

Bienvenuto, J.A., Anderson, R.W., Kerkof, K., Smith, R.G. and Loo, T.L., Stability and compatibility of antitumor agents in glass and plastic containers. *Am. J. Hosp. Pharm.*, 38 (1981) 1914–1918.

Boehringer Mannheim Laboratories, *Determination of urea package insert*. Meylan, France, 1995.

Brooke, D., Scott, J.A. and Bequette, R.J., Effect of briefly heating cyclophosphamide solutions. *Am. J. Hosp. Pharm.*, 32 (1975) 44–45.

Chang, S.Y., Alberts, D.S., Farquhar, D.S., Melnick, L.R., Walson, P.D. and Salmon, S.E., Hydrolysis and protein binding of melphalan. *J. Pharm. Sci.*, 67 (1978) 526–531.

Cheung, H.W., Cradock, J.C., Vishnuvajjala, B.R. and Flora, K.P., Stability of cisplatine, iproplatin, carboplatin, and tetraplatin in commonly used intravenous solutions. *Am. J. Hosp. Pharm.*, 41 (1987) 124–130.

Dyvik, O., Grislingass, A.L., Hjorth Tonnesen, H. and Karlsen, J., Methotrexate in infusion solutions. A stability test for the hospital pharmacy. *J. Clin. Hosp. Pharm.*, 11 (1986) 343–348.

Jardine, I., Strife, R.J. and Colvin, M., Analysis of VP 16-213 and VM 26 in plasma by high-performance liquid chromatography with UV and or fluorescence detection. *Cancer Chemother. Pharmacol.*, 7 (1982) 237.

Oliary, J., *Deux techniques simples et rapides de dosage plasmatique d'anticancéreux par CLHP: doxorubicine-épidoxorubicine, mitoxantrone*. Opito-Laboratoire Lederle, 1989, pp. 5.

Pinguet, F., Fabre, G., Canal, P., Pujol, A. and Soula, G., Stabilité des solutions de 5-fluorouracile à la chaleur et à la lumière. *J. Pharm. Clin.*, 9 (1990) 155–158.

Pinguet, F., Martel, P., Rouanet, P., Fabbro, M. and Astre, C., Effect of sodium chloride concentration and temperature on melphalan stability during storage and use. *Am. J. Hosp. Pharm.*, 51 (1994) 2701–2704.

Rochard, E., Barthes, D. and Courtois, P., Stability of fluorouracil, cytarabine, or doxorubicin hydrochloride in ethylene vinylacetate portable infusion-pump reservoirs. *Am. J. Hosp. Pharm.*, 49 (1992) 619–623.

Roche Laboratories, *5-Fluorouracil package insert*. Neuilly/Seine, France, 1994.

Trissel, L.A., *Handbook on Injectable Drugs*, 5th Edn, American Society of Hospital Pharmacist, Washington, 1988.

Wellcome Laboratories, *Melphalan package insert*, Issy les Moulineaux, France, 1992.

Williams, D.A. and Lokich, J., A review of the stability and compatibility of antineoplastic drugs for multiple-drug infusions. *Cancer Chemother. Pharmacol.*, 31 (1992) 171–181.

Williams, D.A., Stability and compatibility of admixtures of antineoplasic drugs. In Lokich, J. (Ed.), *Cancer Chemotherapy by infusion*, 2nd Edn, Precept, Chicago, 1990.

Zheng, J.J., Chan, K.K. and Muggia, F., Preclinical pharmacokinetics and stability of isophosphoramide mustard. *Cancer Chemother. Pharmacol.*, 33 (1994) 391–398.