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The stability of vincristine sulphate (Teva) in concentrate and diluted with 0.9% sodium chloride

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Summary

Objective. To determine the stability of vincristine in concentrate in glass containers and diluted with 0.9% sodium chloride in polyethylene (PE) bags. ***Methods***. Solutions of vincristine 5 mg/ml and 1 mg/ml (concentrates) in glass containers were opened and stored at refrigerator temperature (2-8°C) and at room temperature (15-25°C). Another six solutions of vincristine were diluted in 0.9% NaCl and stored in PE bags in the same conditions (2-8°C and 15-25°C). Samples of each solution from the containers were analysed for vincristine concentration initially and after 1, 2, 3, 7, 14, 21, 28, and 31 days of storage. The samples were assayed with a high performance liquid chromatographic (HPLC) method with ultraviolet detection (λ = 297nm) to determine the vincristine concentration at each time of sampling. ***Results***. The concentration of vincristine at each sampling time in the analysed solutions remained within 99.18-102.76% of the initial concentration, regardless of the storage temperature and container. Conclusions. Vincristine sulphate, both undiluted in glass containers and diluted with 0.9% NaCl in PE bags, remains stable (< 10% degradation) for at least 31 days at room and refrigerator temperature. *(Farm Współ 2013; 6: 1-7)*

Keywords:vincristine, stability, concentrate, polyethylene, HPLC

Introduction

The prerequisite for effective, safe and optimal therapy is the appropriate amount of the therapeutic substance, which is strictly correlated with its stability both during storage of the preparation in its original package and after opening it or dilution. Confirmation of stability is especially important for cytostatics, as it is a more complex problem than in the case of drugs belonging to other pharmacological classes. It is related with their narrow therapeutic index, the necessity to frequently administer maximum doses tolerated by patients and the need to obtain a specific concentration. For those reasons each cytostatic to be injected or infused must retain its physiochemical properties from the moment of preparation in a hospital pharmacy to the end of its administration to the patient. Proving the stability of drug concentrates after their opening entails enormous financial benefits as it enables economical management of very expensive drugs [1].

Vincristine is a natural alkaloid isolated from *Catharanthus roseus* of the Apocynaceae family, which belongs to cytostatic drugs with antimitotic properties, inhibiting cell division at the metaphase stage [2-4]. The molecular formula of vincristine sulphate is $C_{46}H_{56}N_4O_{10,}$ H2SO4 (MW 923.0) and the structure is provided in Figure 1. Vincristine sulphate is approved for the treatment of malignant lymphomas, acute lymphocytic leukaemia, multiple myeloma and solid tumours [5-9]. The shelf life for vincristine infusions over the range of 0.01-0.1 mg/mL recommended by the manufacturer is limited to 48 hours at 2°C-8°C and 24 hours at 15°C-25°C [10].

The analytical procedures used in the stability study should be well validated. The concentration of vincristine was carried out by means of a high pressure liquid chromatographic (HPLC) method with UV detection. HPLC-UV is the most popular method in analytical laboratories due to the relative simplicity of its operation and the lower costs of its instrumentation in comparison with more sophisticated techniques.

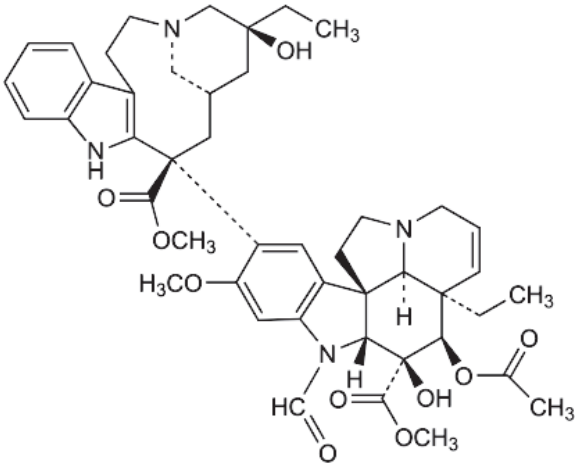

Figure 1. The chemical structure of vincristine sulphate

Material and methods

The research was done at the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań, Poland.

- **Reagents**

Vincristine sulphate standard was supplied by LGC Standards (Łomianki, Poland). Acetonitrile and methanol were liquid chromatography grade. Methanol was purchased from Merck and acetonitrile and diethylamine from Sigma-Aldrich. Water used in the mobile phase was deionised, distilled and filtered through a Millipore system before use. Vials containing 1 mg/1mL and 5 mg/5mL vincristine concentrate (batch number: 10K03KB, 09G29R) were supplied by Teva Pharmaceuticals Polska, Warsaw, Poland. The 100 mL polyethylene (Viaflo®) infusion bags, containing 0.9% sodium chloride (batch number 15DF022A1) were purchased from Baxter Polska, Warsaw, Poland. The composition of one 1 ml-vial was: 1 mg vincristine sulphate, mannitol and sodium hydroxide. The composition of one 5 ml-vial was: 5 mg vincristine sulphate, mannitol and sodium hydroxide.

- **Chromatographic assay method**

The concentration of vincristine sulphate was carried out by means of a high pressure liquid chromatographic (HPLC) method with UV detection, which was a modified version of the method developed by Embree et al [11]. An HPLC system consisting of a Waters Alliance liquid chromatograph (Milford, MA, USA; model 2695), a Waters 2487 PDA detector, an autosampler, a 100 µl syringe, degasser, column oven, and a data collection system running Waters' *Empower Pro* software was used for this analysis. The parameters of chromatographic separation: a Symmetry C18 column 250 × 4.6 mm, 5 µm particle size (Waters), mobile phase: acetonitrile – water – methanol - diethylamine (34.9 : 40 : 25 : 0.1), flow rate of the mobile phase 1 ml/min. The phase was brought to pH 7.0 by adding 80% orthophosphoric acid. The UV detector wavelength was set at 297 nm. The method used isocratic elution with a total run time of 10 min. The injection volume was 10 µl and the column was thermostated at 25°C. Under these conditions the mean retention time for vincristine was 6.2 min.

- **Standard preparation**

Stock solution was prepared by weighing 20 mg of vincristine sulphate into a 10-ml volumetric flask. The substance was dissolved and diluted to volume with water. The solution was kept at 4°C. Working standard solutions were prepared by appropriate dilutions of the stock solution in water to obtain concentrations across the range of 5.0-1200 µg/ml. Quality control (QC) samples were also freshly prepared in a similar manner by separate weighing.

- **Calibration curve**

Aliquots of the standard stock solution of vincristine were pipetted into six different 10-ml volumetric flasks and the solutions were diluted with water. The final concentrations of vincristine were 5; 10; 20; 40; 120; 240; 480; 960; 1200 µg/ml respectively. Three determinations were carried out for each concentration. Peak areas were recorded for all the solutions. The linear regression analysis was carried out by plotting the peak areas (y) of the compound against the respective concentrations (x) of vincristine. The linearity for the relationship between the peak area and concentration was demonstrated by the correlation coefficient (r).

- **Precision and accuracy**

The precision and accuracy parameters were determined from freshly made quality control standards in three different concentrations (40; 400; 960 µg/ml). Table 1 shows intra- and inter-day precision (%RSD) and accuracy of this assay method. The precision of the method at each concentration was calculated as the relative standard deviation of the mean (RSD) by means of the following equation: RSD = (SD/mean) × 100. The accuracy of the procedure was determined

as the relative mean error (RME) with the following equation: RME = [(mean – spiked concentration of the analyte)/spiked concentration of the analyte] × 100.

- **Sample preparation**

Six concentrates of Vincristine Teva® (1mg/1ml) were opened and three of them were stored at refrigerator temperature (2-8°C) and three at room temperature (15-25°C). Six fresh solutions of Vincristine Teva® 1mg/1ml were prepared by dilution of 3 ml of the concentrate with 100 ml of sodium chloride 0.9% to the final vincristine concentration of 30 µg/ml. The vincristine solutions were kept in plastic containers (PE) in triplicate in a refrigerator and also in a room. All the samples were kept away from light. An analogical procedure was applied for Vincristine Teva® 5mg/5ml. Aliquots were taken at specific time intervals and the concentration of vincristine was determined by HPLC assay, as described above. The concentrations of vincristine in the analysed samples were calculated by means of the regressed equation of the straight line $y = ax + b$.

Results and discussion

The analytical method described was developed and validated to be applied to determine the stability of vincristine solutions (concentrate and in 0.9% NaCl). For validation, a range from 5 to 1200 µg/ml of vincristine was chosen. The calibration line: $y = 8.77^{*} 10^{3} (\pm 0.006 * 10^{3}) x - 1.49 * 10^{4} (\pm 0.12 * 10^{3})$ represents the mean of the three graphs (Figure 2). The correlation coefficient (r) for each calibration graph was 0.9999 and the %RSD of each concentration studied was less than 10%. Both intra- and inter-day precisions were less than 2.0% and the intra and inter-day accuracies ranged from 99.74% to 100.58%. Figure 3 A, B and C show typical chromatograms obtained from a drug-free solution, concentrate and solution of vincristine in 0.9% NaCl, respectively. The chromatograms show that the separation from matrix constituents is sufficient for reliable quantitation and no endogenous components interfered with the analyte peak. The retention time of vincristine was 6.15 ± 0.03 min. The lower limit of quantification (LLOQ) and the limit of detection (LOD) were 5.0 and 0.05 µg/ml, respectively.

Tables II-V list the percentage of intact drug remaining at equilibrium for all cases studied. Vincristine appears to be stable for at least 31 days in concentrates and in a sodium chloride 0.9% solution at a concentration of 30 µg/ml. The stability of vincristine was similar both when the solutions were stored in plastic bags (PE) and in glass containers.

Table I. Intra- and inter-day precisions and accuracies of vincristine (n = 3 in all cases)

Concentration [µg/ml]	Intra-day			Inter-day		
	Mean ± SD	%RSD	%RME	Mean ± SD	%RSD	%RME
40	39.95±0.07	0.18	-0.18	40.17±0.46	1.14	0.43
400	398.97±0.73	0.18	-0.26	399.67±0.12	0.03	-0.08
960	960.59±0.28	0.03	0.06	965.58±0.64	0.07	0.58

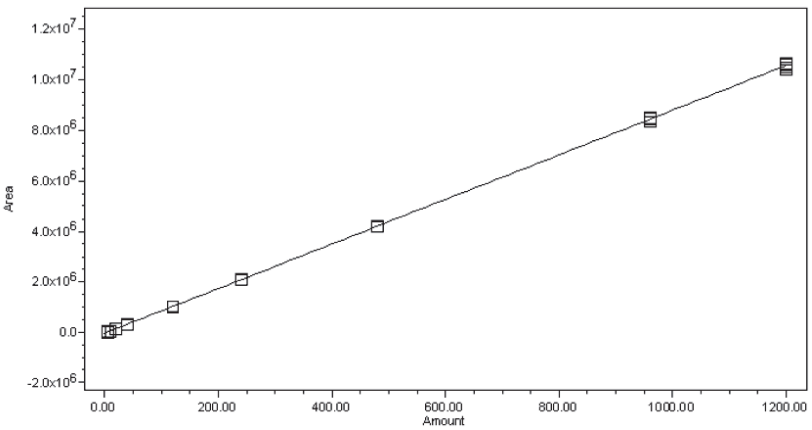

Figure 2. Standard calibration curves for vincristine (n = 3). Each curve is based on 6 calibration standards with triplicate injections

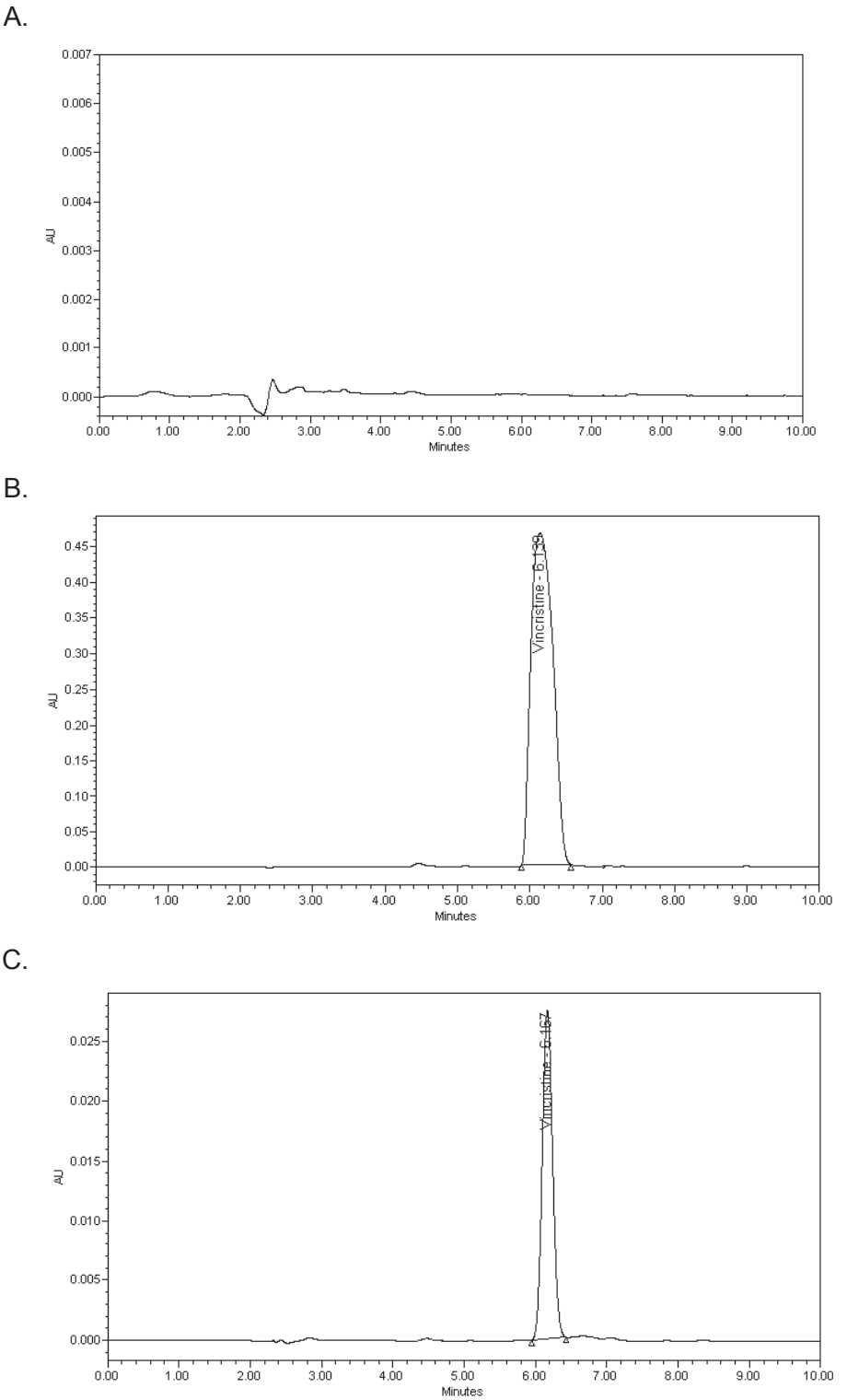

Figure 3. The chromatogram of vincristine: a drug-free solution (A), concentrate – 1120.30 µg/ml, (B) and solution of vincristine in 0.9% NaCl – 33.45 µg/ml (C)

Table II. Vincristine (%) at different sampling times in concentrate stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for Vincristine Teva® 1 mg/1 ml .

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	14	21	28	31 day
2-8°C Mean SD n	100.0 0.0 3	98.79 0.38 3	99.36 0.26 3	100.47 0.63 3	99.05 0.06 3	100.33 0.99 3	99.81 0.75 3	100.01 0.09 3	100.30 0.37 3
15-25°C Mean SD n	100.0 0.0 3	99.06 0.69 3	98.96 1.00 3	100.13 0.11 3	98.72 0.12 3	99.02 0.18 3	99.56 0.27 3	99.85 0.42 3	99.19 0.75 3

Table 3. Vincristine (%) at different sampling times in 0.9% sodium chloride stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for Vincristine Teva® 1 mg/1 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	14	21	28	31 day
2-8°C Mean SD n	100.0 0.0 3	98.75 1.22 3	98.24 2.08 3	100.02 0.63 3	98.57 2.22 3	100.0 0.0 3	99.25 0.85 3	101.42 0.51 3	102.76 2.41 3
15-25°C Mean SD n	100.0 0.0 3	100.99 0.57 3	100.54 0.37 3	102.79 1.94 3	99.61 2.00 3	100.65 0.85 3	100.77 0.71 3	101.51 1.14 3	102.62 2.37 3

Table 4. Vincristine (%) at different sampling times in concentrate stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for Vincristine Teva® 5 mg/5 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	14	21	28	31 day
2-8°C Mean SD n	100.0 0.0 3	99.98 0.01 3	99.82 0.21 3	99.39 0.51 3	100.49 0.53 3	99.83 0.23 3	99.96 0.33 3	99.98 0.50 3	99.18 0.24 3
15-25°C Mean SD n	100.0 0.0 3	99.90 0.37 3	99.92 0.08 3	99.41 0.58 3	99.51 0.18 3	99.72 0.48 3	101.19 3.09 3	99.78 0.17 3	99.86 0.27 3

Table 5. Vincristine (%) at different sampling times in 0.9% sodium chloride stored at refrigerator temperature (2-8°C) and room temperature (15-25°C) for Vincristine Teva® 5 mg/5 ml

Temp.	Percentage of initial concentration at indicated time (day)								
	0	1	2	3	7	14	21	28	31 day
2-8°C Mean SD n	100.0 0.0 3	100.58 1.62 3	99.77 0.88 3	99.68 1.56 3	99.83 1.33 3	99.95 1.36 3	100.53 1.01 3	100.15 1.32 3	100.88 1.20 3
15-25°C Mean SD n	100.0 0.0 3	97.91 2.31 3	99.74 0.36 3	99.99 0.87 3	98.96 0.34 3	98.68 2.57 3	99.84 0.66 3	100.24 0.99 3	100.31 0.67 3

The studies conducted by Trissel's team proved that vincristine solutions diluted with 0.9% sodium chloride to the concentration range of 0.01-0.12 mg/ml, protected from light and stored in polypropylene bags in a refrigerator (at a temperature of 4°C) for 7 days and then for 2 days at room temperature (25°C), retained their stability [12].

On the other hand, the results of analyses conducted by Beijnen's team proved that neither infusion solutions used to dilute the drug nor the temperature had significant influence on stability of the compound [13]. In order to investigate this correlation the scientists used the solutions of 0.9% sodium chloride, 5% glucose and lactated Ringer's fluid, diluting the drug concentrate to the concentrations of 0.02 mg/ml and placing them in polypropylene bags stored both at the temperatures of 25°C and 4°C. After 21 days the change in the concentration was only 95%.

On the basis of the latest research findings temperature and light were proved to have no influence on the rate of degradation of vincristine particles. After opening the vials with the concentrate were stored both at room temperature (25°C) and in a refrigerator (4°C). The former were exposed to light, whereas the latter were not. During the labelling of the drug concentration after 28 days no significant differences were observed in the degree of the drug loss depending on the storage conditions. On the other hand, the solutions concentrated at 0.025, 0.1 and 0.05mg/ml placed in plastic syringes and 50 ml polyolefin infusion bags, respectively, without access to light, at room temperature (25°C) and at 2-8°C were stable for 84 days [14].

The authors of this article analysed the drug concentrated at 1mg/ml, available in 1 ml and 5 ml vials, stored in a hospital pharmacy and protected from light for 31 days after opening. In order to confirm the absence of the influence of temperature on the decomposition of vincristine the vials with the concentrate were divided into two series, where one was stored at room temperature, whereas the other one was stored in a refrigerator at a temperature of 2-8°C. The concentrate was diluted to 30 µg/ml by putting 3 ml of it, concentrated at 1 mg/ml, into a 100 ml polyethylene bag filled with 0.9% NaCl solution. It was stored without access to light at temperatures of 25°C and 2-8°C.

The mean percentage change in the concentration after 31 days ranged within 99.18 ± 0.24% for *Vincristine Teva* 5 mg/5 mL concentrate at a temperature of 2-8°C, 100.30 ± 0.37% for *Vincristine Teva* 1 mg/mL concentrate at a temperature of 2-8°C, 99.86 ± 0.27% for *Vincristine Teva* 5 mg/5 mL concentrate at a temperature of 15-25°C, 99.19 ± 0.075% for *Vincristine Teva* 1 mg/mL concentrate at a temperature of 15-25°C, 100.88 ± 1.2% in 0.9 % NaCl *Vincristine Teva* 4 mg/4 mL solution at a temperature of 2-8°C, 102.76 ± 2.41% in 0.9 % NaCl *Vincristine Teva* 1 mg/mL solution at a temperature of 2-8°C, 100.31 ± 0.67% in 0.9 % NaCl *Vincristine Teva* 4 mg/4 mL solution at a temperature of 15-25°C, 102.62 ± 2.37% in 0.9% NaCl *Vincristine Teva* 1 mg/mL solution at a temperature of 15-25°C (Tables II-V).

On the basis of the results presented no influence of temperature on the decomposition of vincristine particles was observed. During the whole storage period no changes in the colour, turbidity or precipitation were observed in any of the solutions under investigation. Apart from that, during the analysis of the obtained chromatograms no extra peak indicating a vincristine decomposition product was observed.

Conclusion

Vincristine sulphate stability in Vincristine Teva® 1 mg/1 ml and Vincristine Teva® 5 mg/5 ml appears to be stable (< 10% degradation) for at least 31 days in concentrate in glass containers or diluted with 0.9% NaCl in PE bags at a concentration of 30 µg/ml, at refrigerator temperature (2-8°C) and room temperature (15-25°C).

As with all aseptically prepared medicines, it is the responsibility of the compounding unit to establish and validate aseptic procedures and to ensure the sterility and microbiological integrity of drug vials subjected to multiple piercing and of aseptically prepared infusions.

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Conflict of interest

This study was supported with an educational grant from Teva Poland.

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