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DEVELOPMENT OF SIMPLE GREEN SPECTROPHOTOMETRIC METHOD FOR DETERMINATION
PHARMACEUTICAL INTERACTION BETWEEN CYTOSTATIC DRUGS AND NaCl 0,9% AND DEXTROSE 5% INFUSION

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ABSTRACT

Objective. Pharmaceutical interaction between cytostatic drugs and its infusion fluids and packaging materials may cause therapy failure and reduce its cytostatic potential. Therefore, several tests on pharmaceutical interaction between several cytostatic drugs which are most commonly used in clinics and its infusion fluids need to be done.

Methods. Some cytostatic drugs (such as cyclophosphamide, 5-fluorouracil, cisplatin, and paclitaxel) are dissolved in certain concentration of NaCl 0.9% or Dextrose 5% infusion solution. Then, the solutions are incubated at room temperature and protected from sunlight. On the 0th hour (initial condition), 2nd, 4th, 8th, 12th, and 24th hour visual observations, pH measurement, and content measurement are performed.

Results. Based on visual observations, it is indicated that there is no change in color, clarity, and particles of all solutions. At pH measurement, obtained changes in pH of cytostatic solution, which is still within the tolerable range of injection preparation (pH 4-10) and on active compounds stability range. Meanwhile, based on change of cytostatic concentration in each solution, it is indicated that there is no significant difference after 24-hour observation.

Conclusion. Cytostatic drugs dissolved in NaCl 0.9% or Dextrose 5% infusion remained stable in terms of clarity, pH, and concentration level.

Keywords: cyclophosphamide, 5-fluorouracil, cisplatin, paclitaxel, pharmaceutical interaction.

INTRODUCTION

Interaction between drug compounds and its solvent and packaging materials is an important issue that needs to be solved well by pharmaceutical industries in order to produce a stable, effective, and safe product. Pharmaceutical interaction may occur between drugs active compounds and its packaging materials (1-6). Pharmaceutical interaction may also occur between active compounds and its solvent or other active compounds which are mixed before drug usage (7-10). The interaction may affect drug preparations usage in clinics or hospitals by reducing the stability of preparation or mixture, reducing solubility and contents of the drugs, and possibly resulting toxicity or unpredicted activities.

Cytostatic drugs are given on toxic dosage to growing cells; therefore any change on concentration may affect the patient. Practically, cytostatic drugs should be dissolved into infusion fluids such as NaCl 0.9% or Dextrose 5% (D5%) to obtain particular concentration. While being formulated as a solution, some drugs are very vulnerable to chemical decomposition. This may occur mainly on mixing and infusion preparation stages. Interaction between drugs and its infusion fluids and its packaging materials made from glass or plastic material such as PVC and polyethylene may occur likewise (11). In addition, the uses of continuous infusion technology will extend the contact time between active solution and its packaging materials which mostly made from polymer materials (plastic). Thus, physico-chemical interaction and incompatibility may occur before or during intravenous infusion (12,13). Incompatibility reduces the potential of the drugs and lowers its dosage into sub-therapeutic level. Besides, incompatibility also causes intolerance and toxicity, such as emboli, pH change, and irritation on injected area (14,15). Hence, it is important to determine reconstituted anti-cancer agent stability, especially during infusion.

Considering the importance of infusion fluids, especially NaCl 0.9% and D5% and its packaging materials which is commonly used in cytostatic injection for certain period, a research on cytostatic drugs preparation stability needs to be conducted.

MATERIALS AND METHODS

Materials

D5% and NaCl 0,9% (PT Widatra Bhakti). Cyclophosphamide Powder for Injection 1000 mg/vial (PT. Kalbe Farma), 5-Fluorouracil Ebeve 500 mg/10 ml vial (PT. Ferron/Ebeve), Cisplatin 50 mg/50 ml vial (Plastisin PT. Combiphar/Pharmacie), Paclitaxel 30 mg/5 ml vial (Pamup PT. Kalbe Farma). Water for Irrigation USP (PT. Osuka), Methanol HPLC Grade, Acetonitrile HPLC Grade, Kalium dihydrogen phosphate Analytical Grade (Riedel dehaen), Natrium dihydroxydialkylacetaldehyde Grade (Merck).

Preparation

All cytostatic solutions are prepared inside laminar air flow. Dosage of each cytostatic substance used in this research are: cyclophosphamide (500 and 4000 ppm), 5-Fluorouracil (1000 and 4000 ppm), cisplatin (100 and 400 ppm), and paclitaxel (120 and 300 ppm). All of these substances are dissolved into appropriate solvents namely 100 ml NaCl 0.9% and D5% inside its package. Then all concentrations are duplicated and stored in room temperature and protected from sunlight. Then the samples of all cytostatic infusion solutions are taken on 0th, 2nd, 4th, 8th, 12th, and 24th hour for visual observation, pH measurement, and concentration measurement.

Visual Observation

Visual observation is performed under laboratory standard lighting. Each solution will be observed its color change, clarity, and particle existence.

pH Measurement
pH of all solutions are measured by using Crison pH meter (Crison instruments, S. A., Barcelona) which have been calibrated on standard buffers pH 4.0, 7.0, and 9.21.

Concentration Measurement

The concentrations of each cytostatic infusion solution are measured by using HPLC Agilent 1100 series. The condition of HPLC used in this research is described on Table 1.

Statistical Analysis

Significant difference between groups of each parameter measured in this research are done using One-way ANOVA statistical analysis (SPSS 20).

RESULTS AND DISCUSSION

Interaction between drug compounds and its carrier solution or its packaging materials is an important issue in assuring stability, effectiveness, and safety of drug compound during preparation. Interaction between drug compounds and its carrier or packaging material causes decomposition of drug compounds which resulting in degradant compounds, visual and physico-chemical changes, and may harm the patients (16). This research has observed pharmaceutical interactions of several cytostatic drugs and infusion solutions produced by local pharmaceutical industries. The issue regarding interaction between active compounds and its carrier solution and packaging materials often occurs on injection preparation. During preparation process, injection preparation should be dissolved in infusion solution (such as D5% or NaCl 0.9%) in order to obtain particular concentration before being injected intravenously. Some drug compounds such as cytostatic drugs are very vulnerable to chemical decomposition during solution/injection formulation, especially during mixing stage of infusion preparation. These drugs are dripped intravenously for a certain period so that interactions between drug compounds and its infusion solution or its package material, such as glass and PVC (plastic) are likely to occur. Furthermore, the use of continuous infusion technology extends contact time between active compounds and infusion packages which are commonly made from polymer (plastic) material. Thus, physico-chemical interaction and incompatibility may occur before and during intravenous injection. Considering the vast use of the technology on hospitals and physico-chemical characteristic variability of drug compounds, it potentially causes serious problems on therapy (17). Therefore, this research is conducted to observe the interaction between cytostatic preparations, such as cyclophosphamide, 5-fluorouracil, cisplatin, and paclitaxel which are commonly injected through intravenous drips with infusion solutions Dextrone 5% and NaCl 0.9% produced by PT. Widatra Bhakti.

Interaction occurred between cytostatic drugs and its carrier or package can be observed through several evaluations. They are: visual evaluation, physico-chemical evaluation, and chemical evaluation. Visual parameter is used to observe sedimentation formed in the solution, turbidity and color changes of the cytostatic solutions which have been stored for 24 hours. Physico-chemical parameter is used to observe pH stability of the solutions when being stored for 24 hours and to assure that the pH of cytostatic solution does not exceed injection preparation range (pH 4 – 10) while chemical parameter is used to observe cytostatic solution decomposition and degradation by quantifying its drug compound contents using HPLC instrument.

Visual Observation

All of cytostatic infusion solutions dissolved in NaCl 0.9% or D5% infusions have passed visual observation in every observation period based on color parameter, clarity, and inexistence of particles. This result is not different from comparator.

Table 1: HPLC condition in the measurement of cytostatic concentration in infusion

<table>
<thead>
<tr>
<th>Detector</th>
<th>Cyclophosphamide 500 and 4000 ppm</th>
<th>5-Fluorouracil 1000 and 4000 ppm</th>
<th>Cisplatin 100 and 4000 ppm</th>
<th>Paclitaxel 120 and 300 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columnn</td>
<td>UV 195 nm e 18 (250 mm x 4.6 mm, 5um), packing L1</td>
<td>UV 204 nm e 18 (250 mm x 4.6 mm, 5um), packing L1</td>
<td>UV 204 nm e 18 (250 mm x 4.6 mm, 5um), packing L1</td>
<td>UV 226 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min Acetonitrile and water (3:7)</td>
<td>1 mL/min Acetonitrile and water (3:7)</td>
<td>0.3 mL/min Acetonitrile and water (3:7)</td>
<td>1 mL/min Acetonitrile and water (3:7)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>0.01 mol/L (5:95)</td>
<td>0.005 M phosphate buffer pH 6.5</td>
<td>0.005 M phosphate buffer pH 6.5</td>
<td>0.005 M phosphate buffer pH 6.5</td>
</tr>
<tr>
<td>Colunmn temperature</td>
<td>30 °C</td>
<td>30 °C</td>
<td>30 °C</td>
<td>30 °C</td>
</tr>
<tr>
<td>Volume injection</td>
<td>50 µL</td>
<td>1 µL</td>
<td>5 µL</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

pH Measurement

Before the addition of cytostatic, NaCl 0.9% or D5% PT Widatra Bhakti have indicated different pH (5.13 or 5.84 respectively). pH of each cytostatic infusion during 24-hour observation period is described in Table 2. Table 3, Table 4, and Table 5. Cyclophosphamide 500 ppm infusion inside D5% or NaCl 0.9% has been decreased by 0.67 units and 0.01 units respectively. Different result shown by high-concentration cyclophosphamide (4000 ppm) in which the pH decrease sharply by 1.18 units and 1.2 units respectively on NaCl 0.9% and D5%. Meanwhile, the pH of 5-fluorouracil 1000 ppm infusion shows a slight decrease by 0.27 units (on NaCl 0.9%) and 0.24 units (on D5%). This result is similar to the pH of high-concentration 5-fluorouracil (4000 ppm).

An anomaly is shown on cisplatin infusion 100 ppm and 400 ppm which are dissolved in NaCl 0.9%. pH of these infusions seem to be increased when compared to initial pH. However, this increase is only as much as 0.13 and 0.15 units. Meanwhile, the pH of paclitaxel 120 ppm and 300 ppm which are dissolved in NaCl 0.9% and D5% shows a minor change. The pH of all cytostatic infusions which are dissolved in NaCl 0.9% or D5% are on tolerable range of infusion solution pH (4 ≤ pH ≤ 10), except for cyclophosphamide 4000 ppm after 4-hour observation. This result is also shown on cyclophosphamide 4000 ppm which have been dissolved in the comparator infusion.

Concentration Measurement

Chemical stability data of cyclophosphamide, 5-fluorouracil, cisplatin, and paclitaxel in NaCl 0.9% and D5% infusions for all observation periods are presented in Table 2. An infusion is categorized as "stable" when the variation (concentration deviation) does not exceed ± 5% of initial concentration. The initial concentration (concentration at the 0th hour) is considered 100%.

Cyclophosphamide 500 ppm infusions remained stable for 24 hours either for NaCl 0.9% or D5% solutions. Similarly, cyclophosphamide 4000 ppm which has been dissolved in the two solvents remained stable for 24 hours with the lowest content as much as 98.34%. The similar result was also found on 5-fluorouracil 1000 ppm which has been dissolved in NaCl 0.9% and D5%. The solutions remained stable for 24 hours. Concentration variations of 5-fluorouracil 1000 ppm in NaCl 0.9% and D5% are 97.50% - 100.04% and 99.72% - 100.86% respectively. Meanwhile, content variations of 5-
fluorouracil 4000 ppm in NaCl 0.9% and D5% are 98.35% - 100.72% and 99.05% - 100.92%, Cisplatin 100 ppm which dissolved in NaCl 0.9% remains stable for 24 hours with content variation ranged between 99.80% - 102.30%. Meanwhile, cisplatin 400 ppm in NaCl 0.9% remains stable for 12 hours. At the 24th hour, the content was decreased by over than 5% (92.2%). The result of paclitaxel 120 ppm and 300 ppm which have been dissolved in the two infusion solutions indicated stability for 24 hours, except for paclitaxel 120 ppm in NaCl 0.9% at the 8th hour observation which indicated content decreasing over than 5% (94.42%).

Table 2: Physical and Chemical Stability of Cyclophosphamide infusion in NaCl 0.9% and D5% of PT. Widatra Bhakti over 24 hours

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>pH ± SD</th>
<th>Visual appearance</th>
<th>% concentration ± SD Cytostatics remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl 0.9%</td>
<td>D5%</td>
<td>NaCl 0.9%</td>
</tr>
<tr>
<td>Cyclophosphamide 500 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.48±0.11</td>
<td>5.11±0.15</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>5.35±0.16</td>
<td>4.95±0.03</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>5.27±0.11</td>
<td>4.83±0.02</td>
<td>Pass</td>
</tr>
<tr>
<td>6</td>
<td>5.16±0.08</td>
<td>4.78±0.00</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>5.10±0.11</td>
<td>4.74±0.04</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>4.96±0.09</td>
<td>4.65±0.05</td>
<td>Pass</td>
</tr>
<tr>
<td>24</td>
<td>4.59±0.02</td>
<td>4.44±0.01</td>
<td>Pass</td>
</tr>
<tr>
<td>Cyclophosphamide 4000 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.54±0.01</td>
<td>4.51±0.02</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>4.26±0.01</td>
<td>4.24±0.04</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>4.00±0.01</td>
<td>3.98±0.03</td>
<td>Pass</td>
</tr>
<tr>
<td>6</td>
<td>3.94±0.01</td>
<td>3.92±0.01</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>3.71±0.00</td>
<td>3.69±0.01</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>3.45±0.17</td>
<td>3.54±0.04</td>
<td>Pass</td>
</tr>
<tr>
<td>24</td>
<td>3.36±0.01</td>
<td>3.31±0.01</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Table 3: Physical and Chemical Stability of 5-FU infusion in NaCl 0.9% and D5% of PT. Widatra Bhakti over 24 hours

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>pH ± SD</th>
<th>Visual appearance</th>
<th>% concentration ± SD Cytostatics remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl 0.9%</td>
<td>D5%</td>
<td>NaCl 0.9%</td>
</tr>
<tr>
<td>5-Fluorouracil 1000 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.78±0.02</td>
<td>8.87±0.01</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>8.79±0.01</td>
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<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>8.81±0.08</td>
<td>8.83±0.02</td>
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<td>8.72±0.01</td>
<td>8.74±0.04</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>8.69±0.00</td>
<td>8.73±0.00</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>8.65±0.00</td>
<td>8.71±0.01</td>
<td>Pass</td>
</tr>
<tr>
<td>24</td>
<td>8.51±0.01</td>
<td>8.63±0.00</td>
<td>Pass</td>
</tr>
<tr>
<td>5-Fluorouracil 4000 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.80±0.04</td>
<td>8.89±0.04</td>
<td>Pass</td>
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<tr>
<td>2</td>
<td>8.80±0.01</td>
<td>8.87±0.01</td>
<td>Pass</td>
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<td>8.82±0.01</td>
<td>8.86±0.01</td>
<td>Pass</td>
</tr>
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<td>6</td>
<td>8.78±0.00</td>
<td>8.87±0.00</td>
<td>Pass</td>
</tr>
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<td>8.78±0.01</td>
<td>8.86±0.04</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>8.78±0.01</td>
<td>8.89±0.03</td>
<td>Pass</td>
</tr>
<tr>
<td>24</td>
<td>8.72±0.01</td>
<td>8.65±0.00</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Table 4: Physical and Chemical Stability of Cisplatin infusion in NaCl 0.9% of PT. Widatra Bhakti over 24 hours

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>pH ± SD</th>
<th>Visual appearance</th>
<th>% concentration ± SD Cytostatics remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl 0.9%</td>
<td>D5%</td>
<td>NaCl 0.9%</td>
</tr>
<tr>
<td>Cisplatin 100 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.67±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>5.77±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>5.69±0.08</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>6</td>
<td>5.77±0.04</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>5.75±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>5.72±0.04</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>24</td>
<td>5.01±0.04</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>Cisplatin 400 ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.36±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>4.34±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>4.40±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>6</td>
<td>4.30±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>4.43±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>12</td>
<td>4.47±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
<tr>
<td>24</td>
<td>4.51±0.01</td>
<td>-</td>
<td>Pass</td>
</tr>
</tbody>
</table>
Based on these results, it is indicated that there is no cytostatic solution which shows concentration decrease more than 5% except for cisplatin 440 ppm and paclitaxel 120 ppm which have been dissolved into NaCl 0.9% at the 24th hour observation and the 8th hour observation respectively. Compared to comparator, paclitaxel 120 ppm which has been dissolved into NaCl 0.9% also indicated more than 5% decreased concentration (93.60%) at 8th hour observation. These findings indicated that all cytostatic solution dissolved into NaCl 0.9% and D5% infusion solutions produced by PT Widatra Bhakti remained stable.

CONCLUSION

Stability research on cytostatic drugs which are dissolved into D5% or NaCl 0.9% infusions produced by PT Widatra Bhakti indicates no visual change (color, clarity, and sedimentation). Besides that, the pH and cytostatic concentration after dissolve into infusion solution also indicates insignificant changes compared to initial condition. Therefore, the data suggest that the infusions can maintain the stability of cytostatic compounds which are dissolved into it.

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