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NETHERLANDS LABORATORY for ANTICANCER DRUG FORMULATION

STANDARD OPERATING PROCEDURE

Title : Pre-formulation study of investigational cytotoxic drugs

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Approval of this page indicates approval of all pages in this procedure.

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1 Introduction

The aim of the pre-formulation study of investigational cytotoxic drugs is to obtain information about the aqueous solubility and stability, and about the solubility and stability in commonly used formulation solutions. Furthermore, the purity of the investigational cytotoxic drug is to be determined.

2 Materials and methods

2.1 Chemicals

Amount of new drug substance (NDS) obtained: 5-10 mg.

Formulation solutions:

1. Water for Injection (WFI)
2. Absolute ethanol (ethanol)
3. *N,N*-dimethylacetamide (DMA)
4. Polyethylene glycol 400/Ethanol/Tween 80 (6:3:1, v/v/v) (PET formulation)
5. 0.5% Tween 80 in 0.9% sodium chloride (saline) (g/v)
6. DMA/arachis oil (1:10, v/v)
7. Cremophor EL®/ethanol (1:1, v/v)

2.2 Visual inspection

The NDS has to be visually inspected for its appearance, color and visible signs of contamination.

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2.3 Solubility determinations

- Weigh approximately 1 mg of the NDS 7 times accurately into 7 glass test tubes.
- Add to each test tube, respectively 100 μl of each formulation solution.
- Vortex-mix the solutions for 15 seconds and inspect the solutions visually for undissolved particles.
- If the NDS did not dissolve in 100 μl formulation solution, place the test tube in an ultrasonic bath for 10 minutes.
- Inspect the solution again visually for undissolved particles. If the NDS still is not dissolved, add another 900 μl of the formulation solution.
- Vortex-mix the solution again for 15 seconds and inspect the solution visually for undissolved particles.
- If the NDS does not dissolve in 1 ml formulation solution, place the test tube in the ultrasonic bath for 10 minutes.
- Inspect the solution again visually for undissolved particles. If the NDS still is not dissolved, add another 9 ml of the formulation solution.
- Vortex-mix the solution again for 15 seconds and inspect the solution visually for undissolved particles.
- If the NDS does not dissolve in 10 ml formulation solution, place the test tube in the ultrasonic bath for 10 minutes.
- Determine the solubility range (s) of the NDS:

  $s > 10$ mg ml^{-1}: if 1 mg of the NDS dissolved after addition of 100 μl of formulation solution, the solubility is at least 10 mg ml^{-1}

  $1 \leq s < 10$ mg ml^{-1} : if the NDS dissolved after addition of another 900 μl, the solubility is at least 1 mg ml^{-1} and less than 10 mg ml^{-1}

  $0.1 \leq s < 1$ mg ml^{-1}: if the NDS dissolved after addition of another 9 ml, the solubility is at least 0.1 mg ml^{-1} and less than 1 mg ml^{-1}

  $s < 0.1$ mg ml^{-1}: if the NDS did not dissolve after addition of another 9 ml, the solubility is less than 0.1 mg ml^{-1}

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2.4 *UV/VIS spectrophotometry*

2.4.1 *Apparatus*

- Model UV/VIS 918 UV/VIS spectrophotometer (GBC)

2.4.2 *Sample preparation*

- If the NDS is soluble, to some degree, in ethanol prepare a solution with a NDS concentration of approximately 10 μg ml^{-1}. Use ethanol as the blank.
- Record an UV/VIS spectrum from 800-200 nm and determine the absorption maxima (λ_{max}) of the NDS.
- Calculate the molar extinction coefficients (ϵ) at the λ_{max} using the Lambert-Beer equation: $\epsilon = (A*MW)/(c*l)$, where A is the absorption at the λ_{max}, MW is the molecular weight, c is the concentration (in mg ml^{-1}) and l the length of the cuvette (cm).

2.5 *HPLC analysis*

2.5.1 *Apparatus*

- Model SP8800 ternary HPLC pump (Thermo Separation Products (TSP))
- Mobile phase: gradient of distilled water and methanol (HPLC grade)

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Time	% water	% methanol	flow (ml min^{-1})
0.0	90.0	10.0	1.00
25.0	0.0	100.0	1.00
30.0	0.0	100.0	1.00
35.0	90.0	10.0	1.00
37.0	90.0	10.0	1.00

- Model SP8880 autosampler (TSP)
- Injection volume: 20 μl
- RP-8 HPLC column (Apex Octyl; 15 cm x 4.6 mm, 5 μm; Jones Chromatography)
- Photodiode Array (PDA) Detector, Model Waters™ 996 (Millipore Waters)
- Model SP4270 integrator (TSP)
- Attenuation: 128
- Model Power Mate 433 Computer (NEC)

2.5.2 Sample preparation

- Dilute an aliquot of the NDS solutions (not the entire solution), which are obtained from the solubility experiments, to a final concentration of 50-100 μg ml^{-1} with methanol/water (1:1, v/v).
- Directly inject a 20 μl aliquot onto the HPLC column.
- The peak(s) in the HPLC chromatogram can be identified by comparing the UV spectrum (as determined by PDA detection) of the peak(s) in the HPLC chromatogram with the UV spectrum as determined by UV/VIS spectrophotometry.

2.5.3 Purity determination

- If the NDS can be analyzed by HPLC analysis and the peak of interest was identified, an estimate of the purity of the bulk drug can be obtained.
- The purity of the NDS can be estimated by dividing the peak area of the major

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component (component of interest) in the HPLC chromatogram by the total peak area in the HPLC chromatogram times 100 percent.

2.5.4 Stability determination

- If the NDS can be analyzed by HPLC analysis and the peak of interest was identified, an estimate of the stability in the formulation solution can be obtained.
- Stability study: at regular time intervals dilute an aliquot of the NDS solution to a final concentration of 50-100 μg ml^{-1} with methanol/water (1:1, v/v).
- Directly inject a 20 μl aliquot onto the HPLC column.
- The stability of the NDS in the formulation solution can be estimated by calculating the remaining amount of NDS relative to the amount present at t=0.