STANDARD OPERATING PROCEDURES

SOP AIG-SI-185-01-0600 Pages: Total of 10.

Monograph - Paclitaxel Injection Concentrate

ASSAY AND DETERMINATION OF IMPURITIES AND DEGRADATION PRODUCTS

Containing: 6mg/mL Paclitaxel in 30.0mg/5mL ; 100mg/16.7mL ; 300mg/50.0mL

[SI-AIG-65-0969-12/99-SP03-ED04]

ASSAY of the Month

Complete versions of key major (and minor) Pharmaceutical Development Standard Operating Procedures.

All pharmaceutical companies conducting drug research and development must have SOPs whether of a development or quality control nature. An HPLC assay is a standard operating procedure and thus a SOPs in the true sense of the word. The primary purpose of the SOP is to translate the various regulations and guidelines, which are open to interpretation, into clear and concise sets of instructions.

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Essentially generic drug development and supporting systems can be distilled into standard development procedures which any good drug developer would apply. A researcher conducts work according to a documented set of procedures - which hopefully represent the best and most current methods available i.e. drug development using “state-of-the-art” techniques. Test results are as good as the pumps columns and detectors operating at their peak designed performances.

SOPs also demonstrate that you are following a key rule of a good researcher: The procedures must be fully described as that they can be replicated by others as necessary. Testing procedures and methods should be designed in close cooperation with the plant production laboratory facilities, to insure an eventual smooth transfer of technical data (TD) and key documentation as required in future TD transfers.

" All pharmaceutical companies conducting drug research-based development or routine generic drug development must have Standard Operating Procedures. "

Not only does a drug researcher need to keep a record of every detail of the product development or assay - for both the advances and the failures of the experimental batch lots but the equipment he uses to obtain those results needs to be trusted year-in and year-out. It needs to be installed correctly, calibrated, cleaned and maintained on a routine basis - thus insuring both accurate **and** precise analytical test results all the time.

The Assays of the Month are intended for the Analytical Department or individuals or groups responsible for the management and operation of the Analytical units in either generic or innovative drug development functions. This SOP procedure may be applied to either development, scale-up, pivotal, validation or full scale commercial production units.

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ASSAY DETERMINATION

General Index:

Method is suitable for:

- ☑ Product Development
- ☑ In-process Control
- ☑ Product Release
- ☑ Stability Indicating Analysis

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RELEASE SPECIFICATIONS

PRODUCT:	**Paclitaxel Injection 6 mg/mL**
Reference: Based on: In-house	**IAGIM®**
Description Paclitaxel Injection 6 mg/mL: 30.0mg/5mL/100mg/16.7mL/300mg/50.0mL	
Identification A: **Paclitaxel 6 mg/mL**	The Chromatogram of the sample solution exhibits a peak with the same retention time as the standard solution
Individual vial Fill Weight: **30 mg**	TARGET 30 mg **Limits :** 28.5 - 31.5 mg
Individual vial Fill Weight: **100 mg**	TARGET 100 mg **Limits :** 95.0 - 105.0 mg
Assay **Paclitaxel 6 mg/mL**	**Limit: 90.0 - 110.0%** **[5.4] - [6.6] mg**
Impurity / Degradation Products	NMT 2.0 % of the labeled amount
Any Known Individual:	NMT 0.5 % of the labeled amount
Any unknown Individual: **Total**	NMT 0.1 % of the labeled amount **NMT 2.5 % of the labeled amount**
Eluting Order	RRT's are Column Specific
DAB	RRT 6.8 min. - 6.8 min
B-III	RRT 8.8 min. - 12.8 min
Unknown P1	RRT - min. - 25.3 min
DAT	RRT 19.5 min. - 28.5 min
CLM	RRT 23.0 min. - 31.30 min
Unknown P2	RRT 27.6 min. - 35.6 min
EDT	RRT min. - 6.8 min
ET	RRT 30.0 min. - 38.0 min
Unknown P3	RRT 39.0 min. - 39.8 min

The excipient COP consists of numerous FFA, Fatty Acids and esters and produces several innocuous peaks both in the middle and end of the spectrum. Check all unknown peak RRTs against the placebo stressed and unstressed peak table. (Note: Select table for column used)

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HPLC / SETUP	
Column & Packing I :	Pentafluorophenyl bonded HPLC column (Taxil column) 4.6 × 250mm, 5µm, (Metachem Technologies, Cat. No. 0335)
Column & Packing II [Alternative] :	Vydac 218MR54 4.6×250mm HPLC column (Cat. No. 218MR54).
Guard Column I :	Metachem Taxil safeguard cartridge (Cat. No. 0335-CS) and Metachem holder (cat. No. 5001-CS).
Guard Column II [Alternative] :	High performance guard column (cat. No. 218G54M) contains 218MR 5µ cartridge and GCH-4 60/cartridge holder.
Column Temperature :	30°C (± 2°C)
Pressure :	000.0 atm
Flow rate :	1.0 mL/min
Detector :	UV at 227 nm UV - 10mm flow cell path length
Sample volume/Loop :	20µL
Mobile phase :	(v/v)
Eluent A: Eluent B: Eluent C	750 : 250 (water : acetonitrile) 400 : 600 (water : acetonitrile 50 : 950 (water : acetonitrile

SPECIAL NOTE AND CAUTION:

1. **USE LOW ACTINIC GLASSWARE! USE LIGHT RESISTANT GLASSWARE! FOR GENERAL PROCEDURE FOR ALIGNMENT OF EQUIPMENT & WITHDRAWAL OF SAMPLES REFER TO USP 24, SUPPLEMENTS & IN-HOUSE SOP's**
2. Actual gradient profile may be modified in order to achieve system suitability requirements
3. All solvents used must be of HPLC grade, Reagents AR (Acetic Acid) or Ultra Pure Reagents.
4. All samples and standard solution **MUST** be protected from Light.
5. Working Standard & Sample can be used for **10 DAYS** stored at room temperature.

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GRADIENT SETUP:

Actual gradient profile may be modified in order to achieve system suitability requirements

Metachem column		Vydac column	
Time (min.)	Composition	Time (min.)	Composition
0	100% A	0	100%
35	100% B	28	45% B
40	100% B	35	100% B
40.5	100% C	38	100% C
50	100% C	52	100% C
55	100% A	55	100% A
60	100% A	60	100% A

SYSTEM SUITABILITY

The system suitability preparation is for 50 individual analysis

Weigh accurately approximately 6mg of each of the following related compounds into a 50mL volumetric flask. Add about 30mL of methanol and sonicate until compounds are completely dissolved.
Dilute to 50mL volume with methanol. This solution is the RCIRS STOCK solution.

Related Compounds Impurity Reference Stock Solution (RCIRS):

Related Compound		Weight
7-Epi-10-deacetylpaclitaxel (EDT)	-	6mg
Cephalomannine (CLM)	-	6mg
10-Deacetylpaclitaxel	-	6mg
Baccatin III (BIII)	-	6mg
10-Deacetylbaccatin III (DAB)	-	6mg
7-Epipaclitaxel (EPT)	-	6mg

Accurately weigh approximately 30mg of Paclitaxel A.S. into a 50mL volumetric flask. Add 2.5mL of RCIRS STOCK solution and 10mL of methanol sonicate until completely dissolved. Dilute to volume with methanol. ➲ This is the RCIRS solution.

Pipette 1mL of the RCIRS solution to 50 individual clear glass screw top vials. Air dry the contents of these vials in a ventilated hood. Seal each vial and store at room temperature. ➲ This is air dry RCIRS.

Reconstitute only one vial for use each analysis by adding 1mL of 0.02% (v/v) acetic acid in methanol. ➲ This is the reconstituted RCIRS.

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STANDARD PREPARATION

0.3 STANDARD SOLUTION

1. Weigh accurately about 15mg of Paclitaxel A.S. into a 25mL volumetric flask. Add about 2mL of 0.02% (v/v) acetic acid in methanol and sonicate until completely dissolved. Dilute to volume with 0.02% (v/v) acetic acid in methanol. ➲ This is Working Standard (WS) solution.
2. Pipette 1mL of the working standard solution into 100mL volumetric flask and dilute to volume with 0.02% (v/v) acetic acid in methanol.
3. Pipette 6mL of this solution into a 20mL volumetric flask and dilute to volume with 0.02% (v/v) acetic acid in methanol. ➲ This is 0.3% standard solution.

SYSTEM SUITABILITY TEST

Inject the reconstituted RCIRS solution.

The resolution between 7-epi-10-deacetylpaclitaxel peak (EDT) and paclitaxel peak should be NLT **1.5**.

The tailing of the paclitaxel peak should be NMT **1.5** and the number of plate counts per column NLT 20,000.

SAMPLE PREPARATION

1000µL of sample formulation is delivered into 10mL volumetric flask by Eppendrof pipette or other suitable equipment, weighed and diluted to volume with 0.02% acetic acid in methanol.

Use a 5mL pycnometer to determine the specific gravity for each sample.

PROCEDURE

1. Inject the 0.02% acetic acid in methanol in duplicate, reject the first injection. The system is acceptable if no significant peaks are observed with retention times corresponding to the retention time of paclitaxel or any other known impurity or degradant peak.
2. Inject the working standard solution **5** times. The relative standard deviation for five replicate injections of not more than **2.0**% should be achieved.
3. Inject the 0.3% standard solution 6 times. A relative standard deviation (RSD) of not more than 15.0 should be achieved.
4. Inject the sample solutions. Ignore all peaks due to diluent injection.

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Using a suitable integrator determine the;

CALCULATION FOR ASSAY

For PACLITAXEL as a percentage of the label claim.

Use a validated in-house program injection (w/v) assay or calculate using formula:

$$\frac{\text{Pk area smp x Wt std * (mg) x d3*** (g / mL) x 40}}{\text{Pk area std x Wt smp (mg) x Dosage (mg / mL)}} = \%\ \text{paclitaxel of label claim}$$

FOR RELATED COMPOUNDS:

For percentage of any impurity/degradant related to Paclitaxel

Use validated in-house program for injection (w/v) or calculate using formula:

$$\frac{\text{Pk area smp x Wt std * (mg) x d*** (g / mL) x F** x 3}}{\text{Pk area 0.3\% std x Wt smp (mg) x Dosage (mg / mL) x 25}} = \%\ \text{of any impurity / degradation product related to paclitaxel}$$

Related Substance		RRT (to paclitaxel)		RRF
		Vydac	Methachem	Calculated
• 10-deacetylbaccatin III	(DAB)	0.20	0.40	0.77
• Baccatin III	(BIII)	0.40	0.60	0.76
• 10-deacetylpaclitaxel	(DAT)	0.80	0.80	1.01
• Cephalomannine	(CLM)	0.90	0.95	0.83
• 10-deacetyl-7-epipaclitaxel	(EDT)	0.95	0.97	0.97
• 7-epipaclitaxel	(EPT)	1.10	1.10	0.99

* Wt std - Correct the Working Standard for percentage water and % assay

** F -1/Relative Response Factor (RRF), for unknown peaks, RRF equals 1, For known peaks refer to the above table.

*** d(g/mL) - Specific gravity in grams per mL of paclitaxel solution

Each RRF is calculated as the Slope Ratio (SR) of specific impurity to Paclitaxel, as obtained from the graph regression data.

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REPORTING

- For known impurity peak, less than 0.05%, report less than 0.05% (QL).
- For known impurity peak greater than 0.05%, report the average calculated results obtained.
- For unknown degradation product peak, greater than 0.2%, (QL for paclitaxel) report the average of two results obtained.
- Report RRT's of quantitated unknown degradation product peaks.
- Report total as sum of all quantitated impurities/ degradation products.

Compound	Retention time (min.)	Area counts (mAU)	Resolution (preceding peak)
10-deacetyl baccatin III	11.332	199.8	-
Baccatin III	18.223	191.6	22.96
10-deacetylpaclitaxel	25.520	317.6	22.60
Cephalomannine	29.098	194.5	11.23
7-Epi-10-deacetylpaclitaxel	29.743	215.7	1.99
Paclitaxel	30.758	26741.7	3.16
7-Epipaclitaxel	35.087	405.6	13.88

SYSTEM SUITABILITY CHROMATOGRAM

Use Column: Vydac.

SYSTEM SUITABILITY CHROMATOGRAM

Use Column: Methachem.

EVALUATION

Evaluate the overall impurity profile.

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Infra Red Identification of Paclitaxel

(USP or Ph. Eur. 2.2.24)

PROCEDURE

1. Prepare separately disks with dried potassium bromide containing about 1% of the Paclitaxel R.S. and the Paclitaxel Raw Material to be ID.

EQUIPMENT

1. Suitable spectrophotometer to obtain Infra Red spectra in the IR range 670cm^{-1} to 4000cm^{-1} (spectrophotometer.)

2. Place the two IR spectra side-by-side and compare the absorption maxima position and relative intensity of the IR spectra of the Standard and the Test Sample.

EVALUATION

Evaluate the overall absorption maxima and relative intensity profile.

1. The absorption maxima in the spectrum obtained with the substance to be examined correspond in position and relative intensity to those in the spectrum obtained with the Reference Standard.

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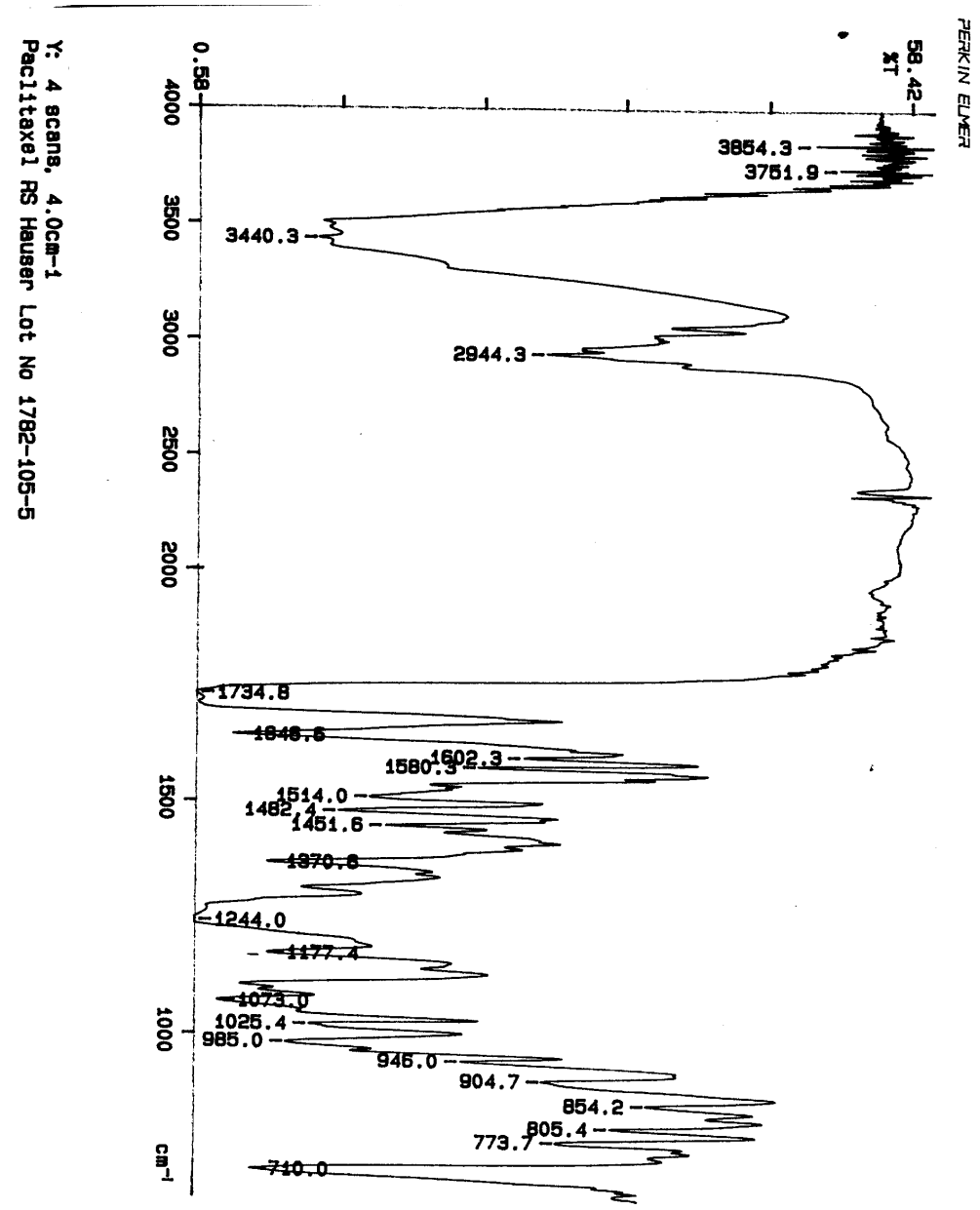
IR Scan

Spectrophotometer - Houser



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