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Research Article

METHOD DEVELOPMENT AND VALIDATION OF RESIDUAL SOLVENTS BY HEXANE FRACTION IN OLAPARIB ACTIVE PHARMACEUTICAL INGREDIENT DETERMINE BY GAS CHROMATOGRAPHY

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Abstract:

In solvent extraction, n-hexane is used as a solvent for its attributes such as simple recovery, non-polar nature, low latent heat of vaporization and high selectivity to solvents. To circumvent the problem, green solvents could be a promising approach to replace solvent extraction. Solvents to be limited Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity. Solvents suspected of other significant but reversible toxicities.[1] In industry, hexanes are used in the formulation and drug substances. They are also used to extract cooking oils (such as canola oil or soy oil) from seeds, for cleansing and degreasing a variety of items, and in textile manufacturing. Hexane is a solvent widely used as an industrial cleaner and degreaser and is an ingredient in many consumer products. Easily inhaled or absorbed through the skin, hexane has been recognized for more than 40.[2]

Keywords: Hexanes, Olaparid, faractions, formulation, extraction, gas chromatography.

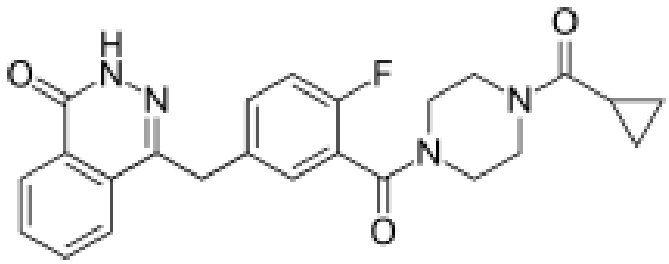


Fig-1: Olaparib Drug.

Introduction:

Hexane is an organic compound, a straight-chain alkane with six carbon atoms with the molecular formula C_6H_{14}. It is an odourless and colourless liquid, when pure, and it has a boiling point of approximately 69 °C

(156 °F). It can be used widely as a relatively safe, largely unreactive, cheap, and easily evaporated non-polar solvent.

Hexane is a significant gasoline constituent. At times, it refers to a mixture, which is composed largely (> 60%) of hexane, with differential amounts of the isomeric compounds: 2-methyl pentane, 3-methyl pentane, and, possibly, the smaller amounts of non-isomeric C_5, C_6, C_7 alkanes (cyclo). Hexane is one of the cheaper compounds and is often used in large-scale operations without requiring a single isomer (a cleaning solvent or for chromatography, as an example).

In general, hexanes are used as a non-polar solvent in chromatography. Higher alkanes exist as impurities in hexanes contain the same retention times as that of solvent, which means that the fractions containing hexane also contain these impurities. In the case of preparative chromatography, the large volume of hexane concentration can result in a sample, which is appreciably contaminated by alkanes. This can result in a solid compound being obtained as oil, and the alkanes may interfere with the analysis.[3, 4]

Materials and Methods:

The extraction was repeated seven times and the n-hexane soluble compound was collected, and then evaporated to get thick extract as n-hexane fraction.

Method Parameters:

Content Name/Code	**Limit (ppm)**
Hexanes*	: Not more than 290

*Report Hexanes as Sum of 2-Methylpentane, 3-Methylpentane, n-Hexane and Methylcyclopentane isomers.

Chemical Names / Codes:

Olaparib drug substance, 2-Methylpentane, 3-Methylpentane, n-Hexane, Methylcyclopentane, N-methyl-2-pyrrolidinone / NMP

Method of Analysis:

Chemicals: NMP: Chromatographic grade (or) equivalent

Chromatographic Conditions:

GC system	: Agilent 7890B with 7697A Headspace sampler (or) equivalent.
Column	: Rtx-624, 60 mt x 0.32 mm ID, 1.8 µm or equivalent.
Injector temperature	: 180°C

Carrier gas : Nitrogen

Column flow : 1.0 mL/min

Split ratio : 1:10

Column oven : Initially at 37°C for 25 minutes then rise to 220°C at a rate of 10°C and hold for 13.7 minutes.

Run time : 55 minutes

Detector temperature (FID) : 250°C

Hydrogen : 40 mL/minute

Air : 400 mL/minute

Make up gas (Nitrogen) : 25 mL/minute

Headspace Conditions:

Vial temperature : 90°C

Loop temperature : 100°C

Transfer line temperature : 110°C

Vial equilibration time : 30 minutes

GC cycle time : 75 minutes (May vary as needed)

Pressurize time : 0.2 minute

Loop equilibration time : 0.05 minute

Loop fill time : 0.2 minute

Inject time : 1 minute

Vial shaking mode : Off

Inject time : 1 minute

Fill mode : Fill with constant volume

Fill volume : 10 mL

Headspace vial capacity : 20 mL

Diluent : N-Methyl-2-pyrrolidinone (NMP)

Note: 1. These conditions are specific for the Agilent headspace systems (Model No.: G1888 & 7697A). Adjustments may have to be made for other headspace systems.

2. Default values are recommended for headspace conditions not specified in the method.

Preparation of Blank solution: Take 1.0 mL of diluent into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Standard stock solution: Weigh accurately about each 29 mg of 2-Methylpentane, 3-Methylpentane, n-Hexane and Methyl cyclopentane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Standard solution: Dilute 1.0 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in seven headspace vials.

Note: The weights and volumes given are the recommended amounts for routine quantitative analysis.

Alternative amounts may be used, provided that the final concentrations remain the same.

Preparation of Sample solution-1: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of diluent, immediately place the septum and crimp the vial.

Preparation of Sample solution-2: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of diluent, immediately place the septum and crimp the vial.

Procedure: Equilibrate the column at 220^{o}C for 55 minutes and then cool to initial temperature. Program the headspace to maintain the vial at 90^{o}C for 30 minutes. Inject blank solution into the system for two times and record the chromatograms. Program the data processor to inhibit the peaks due to blank. Inject standard solution into the system separately for six times and record the chromatograms. If met system suitability criteria, inject blank solution into the system and record the chromatogram. Inject sample solution-1 and sample solution-2 separately into the system and record the chromatograms. Inject standard solution (Online standard) into the system and record the chromatogram.[5]

Note: wherever required additional blank injection can be done to avoid the carryover and to obtain stable baseline.

System suitability acceptance criteria: % RSD calculated for the areas of initial six injections of standard solution should be not more than 15.0. USP Resolution between for 2-Methylpentane and 3-Methylpentane in standard solution should be not less than 3.0. Cumulative % RSD calculated for the areas of six injections of standard solution and injection of online standard solution should be not more than 15.0.[6]

Consider blank corrected peak areas if any, in calculation. Calculate 2-Methylpentane content in ppm by the following formula.

Calculations:

$$\text{2-Methylpentane content (ppm)} = \frac{AT}{AS} \times \frac{CS}{CT} \times P \times 10000$$

Where,

AT = Peak area of 2-Methylpentane in sample solution.

AS = Average peak area of 2-Methylpentane in standard solutions.

CS = Concentration of 2-Methylpentane in standard preparation (mg/mL)

CT = Concentration of sample solution (mg/mL).

P = Purity or Assay of 2-Methylpentane standard (%).

Note: Similarly calculate ppm of remaining solvents by using above formula.

Sample Information:

S. No.	Name of the content	~ RT(minutes)
1	2-Methylpentane	17.7
2	3-Methylpentane	19.5
3	n-Hexane	21.8
4	Methylcyclopentane	27.0

Experimental design

The following parameters should be considered for the validation.

System Suitability / System Precision

Preparation of Blank solution:

Take 1.0 mL of diluent into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Standard stock solution:

Weigh accurately about each 29 mg of 2-Methylpentane, 3-Methylpentane, n-Hexane and Methylcyclopentane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Standard solution: Dilute 1.0 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in seven headspace vials.

Acceptance criteria:

% RSD calculated for the areas of initial six injections of standard solution should be not more than 15.0

USP Resolution between 2-Methylpentane and 3-Methylpentane in standard solution should be not less than 3.0

Cumulative % RSD calculated for the areas of six injections of standard solution and injection of online standard solution should be not more than 15.0.[7]

Specificity

Preparation of Individual stock and standard solutions:

Preparation of 2-Methylpentane standard stock solution:

Weigh accurately about 29 mg 2-Methylpentane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of 2-Methylpentane standard solution:

Dilute 1.0 mL of 2-Methylpentane standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of 2-Methylpentane standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of 3-Methylpentane standard stock solution:

Weigh accurately about 29 mg 3-Methylpentane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of 3-Methylpentane standard solution:

Dilute 1.0 mL of 3-Methylpentane standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of 3-Methylpentane standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of n-Hexane standard stock solution:

Weigh accurately about 29 mg of n-Hexane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of n-Hexane standard solution: Dilute 1.0 mL of n-Hexane standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of n-Hexane standard solution into a headspace vial, immediately place the septum and crimp the vial.

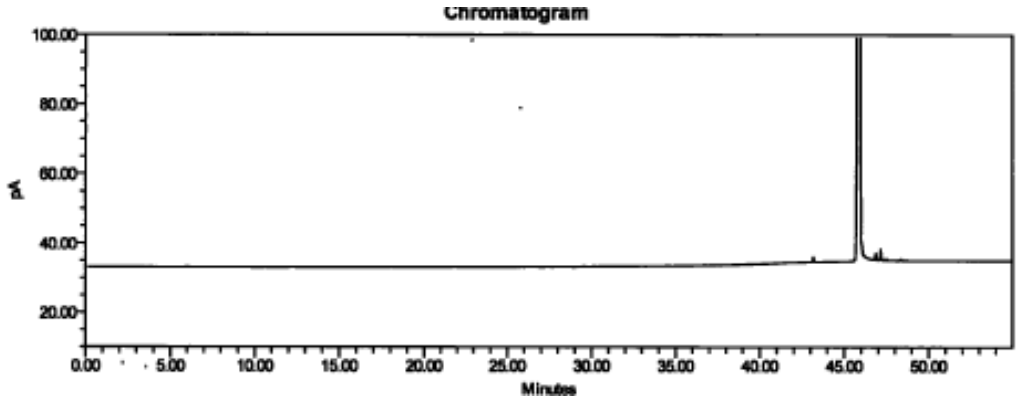


Fig-2: Blank Solution.

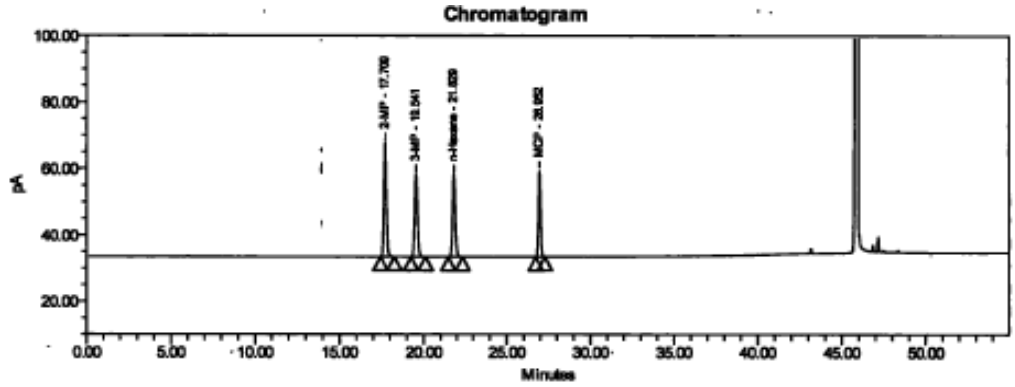
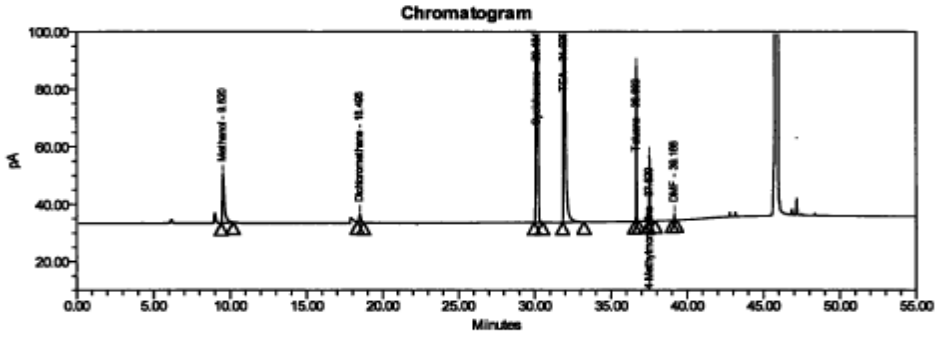


Fig-3: Blend Solution.



Peak Results

	RT (min)	Name	Area (µV*sec)	Int Type	USP Resolution
1	9.52	Methanol	173.655	BB	
2	18.50	Dichloromethane	25.955	BB	37.09
3	30.16	Cyclohexane	3178.347	BB	59.37
4	31.93	TEA	2321.326	BB	12.95
5	36.68	Toluene	154.384	BB	47.16
6	37.53	4-Methylmorpholine	79.101	BB	10.37
7	39.17	DMF	6.845	BB	19.80

Fig-4: Possibilities.

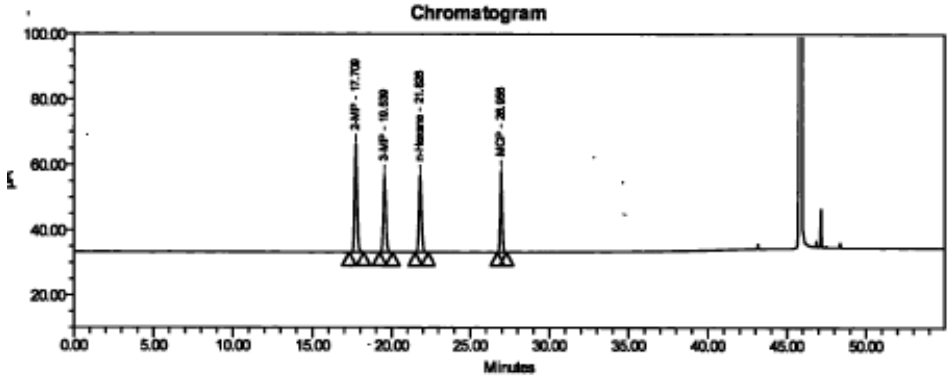


Fig-5: Std.solution(SST).

Preparation of Methylcyclopentane standard stock solution: Weigh accurately about 29 mg of Methylcyclopentane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Methylcyclopentane standard solution: Dilute 1.0 mL of Methylcyclopentane standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Methylcyclopentane standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Methanol standard stock solution: Weigh accurately about 300 mg Methanol into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Methanol standard solution: Dilute 1.0 mL of Methanol standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Methanol standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Dichloromethane standard stock solution:

Weigh accurately about 60 mg Dichloromethane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Dichloromethane standard solution:

Dilute 1.0 mL of Dichloromethane standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Dichloromethane standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Cyclohexane standard stock solution:

Weigh accurately about 388 mg of Cyclohexane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Cyclohexane standard solution:

Dilute 1.0 mL of Cyclohexane standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Cyclohexane standard solution into a headspace vial, immediately place the septum and crimp the vial.[8]

Preparation of Toluene standard stock solution: Weigh accurately about 89 mg of Toluene into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Toluene standard solution:

Dilute 1.0 mL of Toluene standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Toluene standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of n-Methyl morpholine standard stock solution:

Weigh accurately about 100 mg n-Methyl morpholine into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of n-Methyl morpholine standard solution: Dilute 1.0 mL of n-Methyl morpholine standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of n-Methyl morpholine standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Dimethylformamide standard stock solution: Weigh accurately about 88 mg Dimethylformamide into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Dimethylformamide standard solution: Dilute 1.0 mL of Dimethylformamide standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Dimethylformamide standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Triethylamine standard stock solution: Weigh accurately about 500 mg of Triethylamine into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Triethylamine standard solution: Dilute 1.0 mL of Triethylamine standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of Triethylamine standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Possibilities standard solution: Dilute each 1.0 mL of Methanol, Dichloromethane, Cyclohexane, Toluene, n-Methyl morpholine, Dimethylformamide and Triethylamine stock solutions into a 100 mL volumetric flask containing diluent and dilute to the volume with diluent. Take 1.0 mL of possibilities standard solution into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Blend solution: Dilute 1.0 mL of standard stock solution to 100 mL with possibilities solution.

Preparation of Blend spiked test solution: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of blend solution, immediately place the septum and crimp the vial.

Acceptance criteria: System suitability should comply. There should be minimum or no blank and possibilities interference at retention time corresponding to each solvent. If the interference is observed it should not be more than 5 % to the response of standard concentration. The retention time obtained from blend

spiked test solution and standard (system suitability) solution should be comparable for each solvent (i.e. ± 0.2 min)

Detection limit (DL)

Preparation of DL standard solution:

Based on the signal to noise ratio obtained from standard solution, derive DL concentration for each solvent which will yield a signal to noise ratio about 3:1. Take 1.0 mL of DL standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Acceptance criteria: The signal to noise ratio from DL standard solution should be about 3:1.

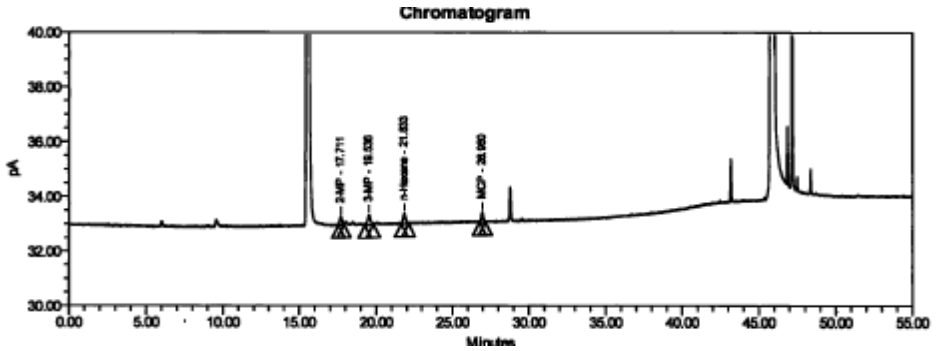


Fig-6: DL solution.

Quantitation limit (QL)

Preparation of QL standard solution: Based on the signal to noise ratio obtained from standard solution, derive QL concentration for each solvent which will yield a signal to noise ratio about 10:1.

Acceptance criteria: The signal to noise ratio from QL standard solution should be about 10:1.

Precision and Accuracy at QL

Preparations of QL standard solution procedure refer to Quantitation limit (QL) parameter.

Precision at QL: Take 1.0 mL of QL standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in six headspace vials.

Acceptance criteria: % RSD calculated for the areas of QL standard solution should be not more than 15.0

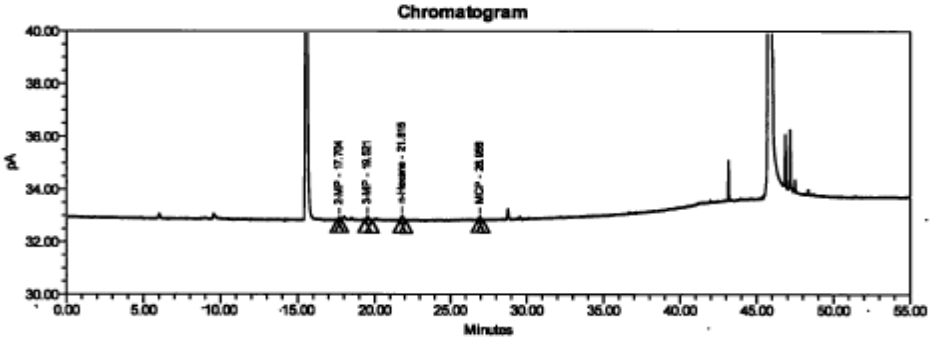


Fig-7: QL solution.

Accuracy at QL: Perform the analysis by spiking the sample with known concentration of each solvent specified in the method at QL with respect to sample concentration. Calculate the content of each solvent with corrected content of each solvent in sample and determine the % recovery.

Preparation of Sample solution: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of diluent, immediately place the septum and crimp the vial.

Preparation of Accuracy at QL solution: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of QL standard solution, immediately place the septum and crimp the vial. Prepare in triplicate.

(This solution contains sample + QL standard solution)

Calculations:

Calculate the 2-Methylpentane content in sample using the following formula:

$$\text{2-Methylpentane content (ppm)} = \frac{AT}{AS} \times \frac{CS}{CT} \times P \times 10000$$

Where,

AT = Peak area of 2-Methylpentane in sample solution.

AS = Average peak area of 2-Methylpentane in standard solutions.

CS = Concentration of 2-Methylpentane in standard preparation (mg/mL)

CT = Concentration of sample solution (mg/mL)

P = Purity or Assay of 2-Methylpentane standard (%)

Note: Similarly calculate ppm of remaining solvents by using above formula.

Calculate the % recovery of 2-Methylpentane from each preparation using the following formula:

$$\text{2-Methylpentane spiked content (ppm)} = \frac{ATs}{AS} \times \frac{CS}{CTs} \times P \times 10000$$

Where,

ATs = Peak area of 2-Methylpentane in spiked sample solution.

AS = Average peak area of 2-Methylpentane in QL standard solutions.

CS = Concentration of 2-Methylpentane in QL standard preparation (mg/mL)

CTs = Concentration of spiked sample solution (mg/mL)

P = Purity / Assay of 2-Methylpentane standard (%)

Note: Similarly calculate ppm of remaining solvents by using above formula.

Recovery calculations:

Amount recovered = Spiked sample content-sample content

$$\% \text{ Recovery} = \frac{\text{Amount recovered} \times 100}{\text{Amount added}}$$

Acceptance criteria:

Sample preparation should meet the specification.

The % recovery should be between 70 and 130 at QL level.

Linearity: Linearity is expressed in terms of variance around the slope of the regression line calculated in accordance to establish mathematical relationship between the test results obtained by the analysis of each solvent standard solution with varying concentrations. Perform the analysis with different concentrations (six levels), ranging from QL, 50 %, 75 %, 100 %, 125 % and 150 % levels respectively. Perform the Linearity Level-1, Level-2, Level-3, Level-4, Level-5 and Level-6 solution in duplicate.

Preparation of standard stock solution procedure refers to system suitability parameter.

Preparation of QL standard solution refers to Quantitation limit (QL) Parameter.

Preparation of Linearity level-1 (QL) standard solution: Take 1.0 mL of QL standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Preparation of Linearity level-2 (50 %) standard solution: Dilute 0.50 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of linearity level-2 standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Preparation of Linearity level-3 (75 %) standard solution: Dilute 0.75 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of linearity level-3 standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Preparation of Linearity level-4 (100 %) standard solution: Dilute 1.00 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of linearity level-4 standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Preparation of Linearity level-5 (125 %) standard solution: Dilute 1.25 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of linearity level-5 standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Preparation of Linearity level-6 (150 %) standard solution: Dilute 1.50 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of linearity level-6 standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in two headspace vials.

Calculation: Correlation coefficient: Calculate using microsoft excel.

Acceptance criteria: Correlation coefficient should not be less than 0.990 for each solvent.

Accuracy

Perform the analysis by spiking the sample with known concentration of each solvent specified in the method at three levels, i.e., 50, 100 and 150 % with respect to sample concentration. Calculate the content of each solvent with corrected content of each solvent in sample and determine the % recovery.

Preparation of Sample solution: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of diluent, immediately place the septum and crimp the vial.

Preparation of standard stock solution procedure refers to system suitability parameter.

Preparation of 50 % standard solution: Dilute 0.5 mL of standard stock solution to 100 mL with diluent and mix well.

Preparation of 100 % standard solution: Dilute 1.0 mL of standard stock solution to 100 mL with diluent and mix well.

Preparation of 150 % standard solution: Dilute 1.5 mL of standard stock solution to 100 mL with diluent and mix well.

Preparation of Accuracy at 50 % level solution: Weigh accurately about 50 mg of sample into a headspace vial; add 1.0 mL of 50 % standard solution, immediately place the septum and crimp the vial.

Prepare in triplicate. (This solution contains sample + 50 % standard solution)

Preparation of Accuracy at 100 % level solution: Weigh accurately about 50 mg of sample into a headspace vial; add 1.0 mL of 100 % standard solution, immediately place the septum and crimp the vial. Prepare in six replicates. (This solution contains sample + 100 % standard solution)

Preparation of Accuracy at 150 % level solution: Weigh accurately about 50 mg of sample into a headspace vial; add 1.0 mL of 150 % standard solution, immediately place the septum and crimp the vial. Prepare in triplicate. (This solution contains sample + 150 % standard solution)

Calculations:

Calculate the 2-Methylpentane content in sample using the following formula:

$$\text{2-Methylpentane content (ppm)} = \frac{AT}{AS} \times \frac{CS}{CT} \times P \times 10000$$

Where,

AT = Peak area of 2-Methylpentane in sample solution.

AS = Average peak area of 2-Methylpentane in standard solutions.

CS = Concentration of 2-Methylpentane in standard preparation (mg/mL).

CT = Concentration of sample solution (mg/mL).

P = Purity or Assay of 2-Methylpentane standard (%).

Note: Similarly calculate ppm of remaining solvents by using above formula.

Calculate the % recovery of 2-Methylpentane from each preparation using the following formula:

$$\text{2-Methylpentane spiked content (ppm)} = \frac{ATs}{AS} \times \frac{CS}{CTs} \times P \times 10000$$

Where,

ATs = Peak area of 2-Methylpentane in spiked sample solution.

AS = Average peak area of 2-Methylpentane in standard solutions.

CS = Concentration of 2-Methylpentane in standard preparation (mg/mL)

CTs = Concentration of spiked sample solution (mg/mL)

P = Purity / Assay of 2-Methylpentane standard (%)

Note: Similarly calculate ppm of remaining solvents by using above formula.

Recovery calculations: Amount recovered = Spiked sample content-sample content.

$$\text{\% Recovery} = \frac{\text{Amount recovered} \times 100}{\text{Amount added}}$$

Acceptance criteria:

Sample preparation should meet the specification.

The % recovery should be between 80 and 120 at 50, 100 & 150 % levels.

Method precision: Perform the analysis by spiking the sample with each solvent at 100 % of the specified limit with respect to the sample concentration. Prepare in six replicates as per method and calculate the content of each solvent and determine the % RSD.

Note: Consider first three injections of accuracy 100 % level solution for 100 % recovery and six injections consider for method precision.

Acceptance criteria: The % RSD for the content at 100 % level should not be more than 15.0

Intermediate precision: Perform the analysis by spiking the sample with each solvent at 100 % of the specified limit with respect to the sample concentration in six times. Calculate the content of

each solvent in spiked preparations and determine the % RSD. Carry out the intermediate precision study on a different day with different analyst, different column and different instrument with fresh preparations.

Preparation of Blank solution: Take 1.0 mL of diluent into a headspace vial, immediately place the septum and crimp the vial.

Preparation of Standard stock solution: Weigh accurately about each 29 mg of 2-Methylpentane, 3-Methylpentane, n-Hexane and Methylcyclopentane into a 20 mL volumetric flask containing diluent, dissolve and dilute to the volume with diluent and mix well.

Preparation of Standard solution: Dilute 1.0 mL of standard stock solution to 100 mL with diluent and mix well. Take 1.0 mL of standard solution into a headspace vial, immediately place the septum and crimp the vial. Prepare in seven headspace vials.

Preparation of Spiked sample solution: Weigh accurately about 50 mg of sample into a headspace vial, add 1.0 mL of standard solution, immediately place the septum and crimp the vial. Prepare in six replicates.

Calculations:

Calculate the 2-Methylpentane content in spiked sample using the following formula:

$$\text{2-Methylpentane Spiked content (ppm)} = \frac{ATs}{AS} \times \frac{CS}{CTs} \times P \times 10000$$

Where,

ATs = Peak area of 2-Methylpentane in spiked sample solution.

AS = Average peak area of 2-Methylpentane in standard solution.

CS = Concentration of 2-Methylpentane in standard preparation (mg/mL).

CTs = Concentration of spiked sample solution (mg/mL).

P = Purity or Assay of 2-Methylpentane (%).

Note: Similarly calculate ppm of remaining solvents by using above formula.

Acceptance criteria:

System suitability should comply; the % RSD for the content should not be more than 15.0. The cumulative % RSD for the content from method precision and intermediate precision should not be more than 15.0

Conclusion: Conclude from the above study that the GC method is suitable for the determination of 2-Methyl pentane, 3-Methyl pentane, n-Hexane and Methyl cyclopentane in Olaparib by GC as study.

References:

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