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METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF BENZENE AND MESITYL OXIDE IN ROSUVASTATIN CALCIUM DRUG SUBSTANCE BY GC-HS USING FID DETECTOR

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ABSTRACT

A proficient GC-HS method was developed for quantification of Benzene and Mesityl oxide in Rosuvastatin Calcium drug substance with flame ionization detector (FID) using column Rtx-35 Amine (30m length X 0.32mm diameter) 1µm film thickness, Part No: 11354, Make: Restek. Nitrogen is used as Carrier gas at with linear velocity of 19.1cm/sec. The proposed method was validated for System suitability, Specificity, Linearity, LOD and LOQ determination, Recovery, Precision, Range and Robustness. All the parameters were found within the acceptable limits. Linearity of Benzene and Ethylene Dichloride is in the range of LOQ to 150%. The established methodology was commercially useful, specific, accurate, precise and suitable for the analysis of Mesityl oxide in Rosuvastatin Calcium drug substance.

KEYWORDS

Gas Chromatography with Head space (GC-HS), Guideline for Residual Solvents Q3C(R8), Method Validation, Rosuvastatin Calcium drug substance and Benzene and Mesityl oxide.

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INTRODUCTON

Rosuvastatin Calcium is a prescription medicine used to treat the symptoms of high cholesterol (hypercholesterolemia), Homozygous Familial Hypercholesterolemia, and slowing progression of Atherosclerosis and to reduce the risk of stroke and heart attack in patient with coronary heart disease. Rosuvastatin Calcium belongs to a class of drugs called Lipid-Lowering Agents, Statins; HMG-CoA Reductase Inhibitors. Rosuvastatin Calcium is the calcium salt form of Rosuvastatin, a statin with antilipidemic activity. Rosuvastatin selectively and competitively binds to and inhibits hepatic

hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase, the enzyme which catalyzes the conversion of HMG-CoA to mevalonate, a precursor of cholesterol. This leads to a decrease in hepatic cholesterol levels and increase in uptake of LDL cholesterol.

Benzene and Mesityl oxide are organic solvents used in the synthesis of Rosuvastatin Calcium. Both Benzene and Mesityl oxide are falls under Class 1 solvent list of Guideline for Residual Solvents Q3C(R8), so need to control at 2ppm and 20ppm respectively. And these organic solvents cannot be removed completely during the synthesis and even purification. Thus, monitoring of these residual organic solvent impurities in the drug substance is mandatory according to regulatory requirements to ensure human safety.

Generally, in the pharmacopoeias like USP, BP, EP, IP etc. monographs, specific methods for residual solvents have not reported for drug substances and drug products. To determine and quantitate at such lower level of Benzene and Mesityl oxide in Rosuvastatin Calcium drug substance, highly sensitive, selective and accurate analytical methods is required. A rugged GC-HS method has been developed for quantification of Benzene and Mesityl oxide in Rosuvastatin Calcium. In this work, over eighteen other residual solvents (Methanol, Ethanol, Ethyl acetate, Methyl tert-butyl ether, Methylene Dichloride, Isopropanol, Acetone, n-Butyl acetate, Acetonitrile, n-Hexane, Tetrahydrofuran, Toluene, t-Butanol, 2, 2-Dimethoxy propane, Triethylamine, Mono methylamine, Benzene, Mesityl oxide, Acetaldehyde and Methyl isobutyryl acetate) that might be present in Rosuvastatin Calcium were separated to prove the specificity for Benzene and Mesityl oxide in Rosuvastatin Calcium.

The objective of this work was to develop a simple and rapid GC-HS method which would be accurate and robust. The method was validated according to ICH guidelines.

METHODOLOGY DEVELELOPED AND MATERIALS USED

Chemicals and Reagents

Methanol, Ethanol, Ethyl acetate, Methyl tert-butyl ether, Methylene Dichloride, Isopropanol, Acetone, n-Butyl acetate, Acetonitrile, n-Hexane, Tetrahydrofuran, Toluene, t-Butanol, 2,2-Dimethoxy propane, Triethylamine, Mono methylamine, Benzene, Mesityl oxide, Acetaldehyde, Methyl isobutyryl acetate, Dimethylsulphoxide (GC-HS grade, Make: Sigma Aldrich).

Instrumentation

Gas chromatography, model no: 2010plus with head space, Model no.: HS-20 Make: Shimadzu was utilized for this work.

Chromatographic conditions

The column Rtx amine, (30m length X 0.32mm diameter) 1µm film thickness, Part No.: 11354, Make: Restek. Column oven temperature; Initial temperature (40ºC) for 5 min; increased to 80ºC @ 5ºC/min, hold for 8 min; then increased to 210ºC @ 15ºC/min, hold for 5 min; detector temperature: 300ºC; Carrier gas: Nitrogen employed as a carrier gas. Make-up gas for FID: nitrogen gas with 40mL/min flow rate was used; Fuel gases: Used hydrogen gas and zero air with flow rate of 40 and 400mL/min. correspondingly. Split ratio: 1:1. Total run time of chromatography: 34.7 min.

Head space conditions

Oven temperature: 80ºC; Transfer line temperature: 100ºC; Loop temperature: 90ºC; Vial equilibration duration: 30 min; Vial pressurization duration: 2.0 min; Loop equilibration time: 0.1 min; Loop fill time: 0.5 min; Inject duration: 0.5 min.

Preparation of blank, standard and sample solution

The diluent used was homogeneous mixture of Dimethylsulphoxide and water in the ratio of 80:20%v/v. Residual solvents standard solution was prepared by using Benzene and Mesityl oxide reference standards to attain a concentration of about 0.0002mg/mL of Benzene and 0.002mg/mL of Mesityl oxide. Further transfer 2mL of standard solution into 20mL headspace vial. Crimp the vial with an aluminum crimp cap containing a

PTFE/Silicon septum. For sample solution preparation accurately weigh and transfer about 300.0mg of test sample into a 20mL headspace vial. Add 1.0mL of diluent and 1mL water to same vial and crimp the vial with an aluminum crimp cap containing a PTFE/Silicon septum [This standard solution concentration is equal to about 2ppm of Benzene and 20ppm of Mesityl oxide, against for 100mg/mL of test concentration as per the ICH specification limits].

Acceptance criteria for System Suitability

%RSD: The Relative Standard Deviation of peak areas of six replicate injections of standard solution and bracketing standard should not be more than 10.0.

Calculation: Calculate the residual solvents (in ppm) by using the following formula.

$$\text{Conc. in. ppm} = \frac{(AT - AB) \times WS \times DT}{(AS - AB) \times DS \times WT} \times P \times 10000$$

AB = Average peak area of respective analyte in the chromatogram obtained from blank, AT = Peak area counts of respective analyte in the chromatogram obtained from the sample solution, AS = Average peak area counts of respective analyte standard in the chromatogram of the standard solution, WS = Weight of respective analyte standard, WT= Weight of sample solution, DT= Dilution factor of sample solution, DS= Dilution factor of respective analyte standard solution, P= Purity of respective analyte standard used.

Observation: In the above method, Benzene and Mesityl oxide are separated well with good resolution to other Rosuvastatin Calcium residual solvents impurities with good symmetrical factor. Hence this method is suitable for Validation.

VALIDATION RESULTS AND DISCUSSION

Specificity

The specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as residual, degradation product and matrix components. In HPLC method, it is assured/proved by complete separation of peak of analyte from other peaks that are of other impurities that might be present in sample or blank.

Inject the Blank, Standard solution and spiked solution. Check the interference at the retention time of analyte. There should not be any interference in blank (as Diluent) and spiked sample at the retention time of analyte. If any peak is present at the retention time of analyte its response should not be more than 20% of the response at the quantification limit (LOQ).

There is no interference observed at RT of Benzene and Mesityl oxide. Hence, the method is very selective and specific for the estimation Benzene and Mesityl oxide residues in presence of eighteen other residual solvents ((Methanol, Ethanol, Ethyl acetate, Methyl tert-butyl ether, Methylene Dichloride, Isopropanol, Acetone, n-Butyl acetate, Acetonitrile, n-Hexane, Tetrahydrofuran, Toluene, t-Butanol, 2, 2-Dimethoxy propane, Triethylamine, Mono methylamine, Benzene, Mesityl oxide, Acetaldehyde and Methyl isobutyryl acetate) that might be present in Rosuvastatin Calcium were separated to prove the specificity for Benzene and Mesityl oxide in Rosuvastatin Calcium. Refer Table No.1.

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the analyte by dilution of a standard stock solution using the proposed procedure. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods by calculation of a regression line. The correlation coefficient, y-intercept, slope of the regression line should be calculated.

The test method linearity was established from, six levels of concentration over the range LOQ to 150% of, ICH limit for each residual solvent impurity. A linear correlation, and regression were determined among the concentrations, and peak area responses of each residual solvent. The correlation coefficient (r) and regression coefficient (R2) values, for both residual solvent impurities found to be higher than,

0.990. The statistical characteristics like slope, y-intercept and, % y-intercept were interpreted and found within the acceptable, limit for both solvent impurities. The data tabulated in Table No.2 demonstrate the linearity of procedure.

Limit of detection (LOD)

It is the smallest amount or concentration of an analyte that can be estimated with acceptable reliability. The detection limit is determined by the analysis of standard with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The limit of detection is determined by establishing the signal to noise ratio. Inject the blank and standard solutions at lower concentration and calculate the signal to noise ratio.

A signal-to-noise ratio between 3:1 estimating the detection limit.

The detection limit for Benzene and Mesityl oxide in Rosuvastatin Calcium is found 0.2ppm and 2.0ppm respectively. For details, refer Table No.3.

Limit of quantitation (LOQ)

The Quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The limit of quantification is determined by establishing the signal to noise ratio. Inject the blank sample and the spiked sample at LOQ level in six replicates and calculate signal to noise ratio and the % RSD at LOQ level.

A signal-to-noise ratio between 10:1 estimating the quantification limit.

The quantification limit for Benzene and Mesityl oxide in Rosuvastatin Calcium is found 0.6ppm and 6.0ppm respectively and well precise. For details, refer Table No.4.

Recovery

Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure.

Recovery assessed using a minimum of 6 determinations over a minimum of 3 concentration levels.

Acceptable limits for a recovery result during validation should be within the range of 70% - 120%.

The percentage of average recovery for Benzene and Mesityl oxide in Rosuvastatin Calcium found >95% at 2.0ppm, and 20.0ppm respectively. For details, refer Table No.5.

Precision: (Method Precision)

The precision determined under equal conditions with same homogeneous spiked sample (six different sample preparation) as per recommended test method and % RSD of the results obtained shall be calculated.

The repeatability is established by estimating the six replicates of spiked sample and calculates the % RSD of the results obtained.

The %RSD of results for the analysis of spiked sample should not be more than 10%.

The % RSD of six different sample preparation found <10% refer Table No.6.

Precision: (Intermediate Precision)

Intermediate Precision means the susceptibility of an analytical method to changes in experimental conditions which can be expressed as different columns, different analyst and different days.

Intermediate Precision of the method is established by estimating the six replicates of spiked sample by different analysts, on different days and on different columns. Calculate the % RSD of the results obtained.

The %RSD of results for the analysis of spiked sample should not be more than 10%.

The % RSD of six different sample preparation found 10%, refer Table No.7.

Robustness

The robustness of an analytical procedure is measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of the GC-HS method is established by estimating minimum six replicates of standard solution. Calculate the accuracy and % RSD of the results.

The Relative Standard Deviation (%RSD) of peak areas of six replicate injections of standard solution for each solvent peak should not be more than 10.0. The Relative Standard Deviation (%RSD) of peak areas of six replicate injections of standard solution for Benzene and Mesityl oxide peak was found below 10.0 with all the robust conditions, refer Table No.8.

DISCUSSION

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The chromatographic parameters were fixed and GC-HS with FID detector system was studied for suitability of residual solvents analysis. The developed method was performed for linearity, precision, Accuracy, specificity, LOD LOQ determination and Robustness.

Table No.1: Specificity verification: Interference study

S.No	Name of Solvent	Retention time (Minutes)	
		Blank	Spiked sample
1	Methanol	3.5	3.4
2	Ethanol	4.1	4.2
3	Ethyl acetate	8.2	8.2
4	Methyl tert-butyl ether	5.9	5.6
5	Methylene Dichloride	5.9	5.6
6	Isopropanol	4.7	4.7
7	Acetone	ND	5.1
8	n-Butyl acetate	ND	17
9	Acetonitrile	5.9	5.6
10	n-Hexane	5.5	5.6
11	Tetrahydrofuran	9	9
12	Toluene	14.8	14.8
13	t-Butanol	ND	5.1
14	2,2-Dimethoxy propane	8.5	8.7
15	Triethylamine	9	9
16	Mono methylamine	3.5	3.4
17	Benzene	ND	10.2
18	Mesityl oxide	ND	17.3
19	Acetaldehyde	3.5	3.4
20	Methyl isobutyryl acetate	ND	10.2

Table No.2: Linearity for benzene and mesityl oxide

S.No	Linearity Conc. level	Benzene		Mesityl oxide	
		Conc. (ppm)	Mean area	Conc. (ppm)	Mean area
1	LOQ level	0.07	3319	0.61	2682
2	50% level	0.11	5193	1.02	4829
3	75% level	0.17	8021	1.53	7749
4	100% level	0.22	10865	2.03	10332
5	125% level	0.28	13397	2.54	13188
6	150% level	0.33	16326	3.05	16254
7	Correlation coefficient	0.99928		0.99977	
8	Squared Correlation coefficient	0.99856		0.99954	
9	Slope	49711.4401		5536.18574	
10	Y-Intercept	-256.41655		-774.34705	
11	Residual sum of square	175996.3506		58798.81834	

Table No.3: LOD level and s/n Ratio

S.No	Residual Solvent	LOD Level (ppm)		
		Standard conc.	W.R.T test	Mean S/N ratio
1	Benzene (10.0%)	0.02	0.2	9
2	Mesityl oxide (10.0%)	0.2	2	5

Table No.4: LOQ Precision and s/n Ratio

S.No	Residual Solvent	LOQ Level (ppm)			
		Standard Conc.	W.R.T test	Mean S/N ratio	%RSD
1	Benzene (30.0%)	0.06	0.6	9	3.9
2	Mesityl oxide (30.0%)	0.6	6	5	2.1

Table No.5: Recovery results

S.No	Level (%)	Sample ID	Amount added (ppm W.R.T. Sample)	Amount recovered (ppm W.R.T. Sample)	Recovery (%)	Average Recovery (%)
1	LOQ	Injection-1	5.8634	5.4032	92.15	93.3
		Injection-2		5.5835	95.22	
		Injection-3		5.4287	92.58	
2	100	Injection-1	19.5447	18.0021	92.1	91.2
		Injection-2		17.4945	89.51	
		Injection-3		17.99	92.04	
3	150	Injection-1	29.3171	26.5547	90.57	92.7
		Injection-2		27.3219	93.19	
		Injection-3		27.6656	94.36	

Table No.6: Method Precision results

S.No	Solvent	Sample Result						
		1	2	3	4	5	6	%RSD
1	Benzene	2.13	2.16	2.19	2.17	2.22	2.17	1.4
2	Mesityl oxide	17.19	18.91	19.03	18.41	18.78	18.9	3.7

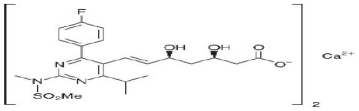
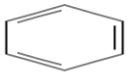
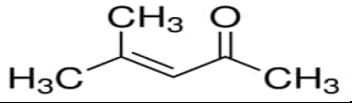
Table No.7: Intermediate Precision results

S.No	Solvent	Sample Result						
		1	2	3	4	5	6	%RSD
1	Benzene	1.83	1.82	1.82	1.88	1.85	1.86	1.3
2	Mesityl oxide	18	17.49	17.99	18.44	17.91	17.55	1.9

Table No.8: Conditions for Robustness and their observations

S.No	Conditions for Robustness	%RSD Benzene	%RSD Mesityl oxide
1	Column oven temp. Low (-2°C)	1.7	1.7
2	Column oven temp. High (+2°C)	1.1	1.3
3	Linear velocity Low (-2cm/sec)	3.2	1.6
4	Linear velocity High (+2cm/sec)	1.3	1.7
5	Column oven ramp rate Low (-2°C)	5	1.9
6	Column oven ramp rate High (+2°C)	0.9	0.9
7	HS injection time low (-0.5 min)	0.6	0.8
8	HS Injection time high (+0.5min)	0.8	1.5
9	HS oven temperature low (-5°C)	1.9	1.8
10	HS oven temperature high (-5°C)	1.2	1.4

Compound name, Chemical formula, Molar mass and Structure formula are mentioned below

S.No	Compounds Name	Chemical formula	Molar mass (g/mol)	Structure formula
1	Rosuvastatin Calcium	$C_{44}H_{54}CaF_2N_6O_{12}S_2$	1001.1	
2.	Benzene	C_6H_6	78.11	
3	Mesityl oxide	$C_6H_{10}O$	98.145	

GC-HS Chromatograms of study

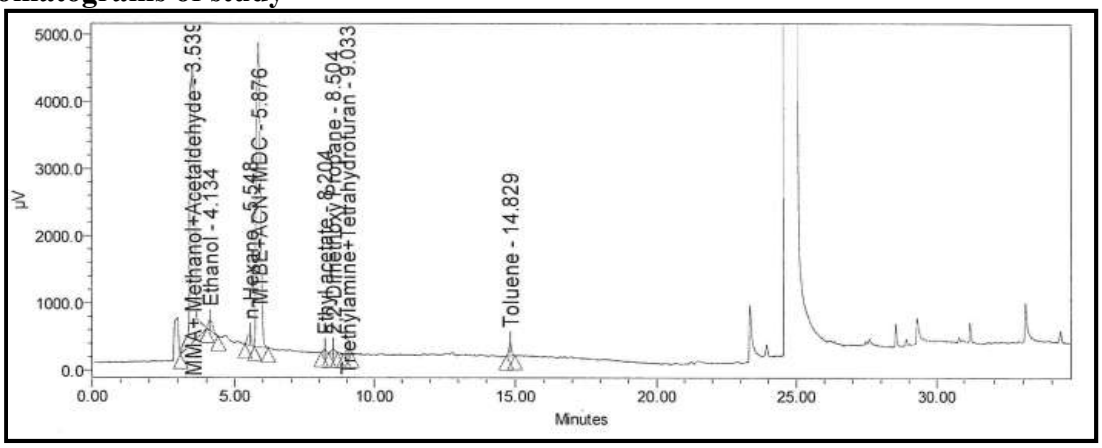


Figure No.1: Blank chromatogram

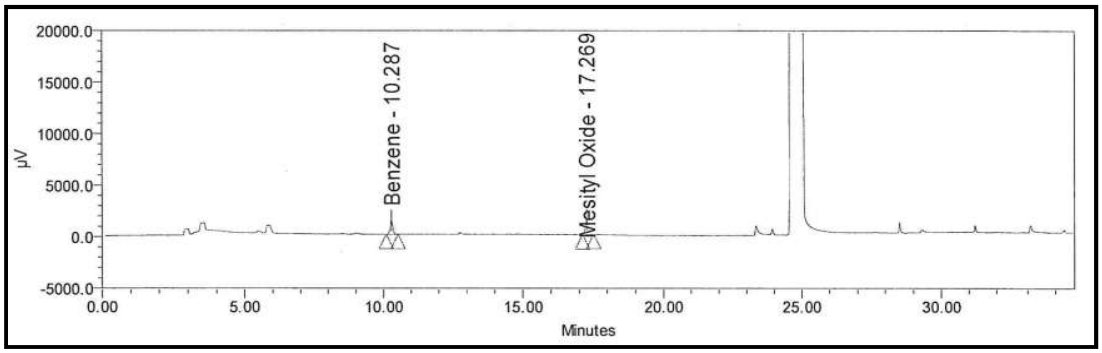


Figure No.2: Standard chromatogram

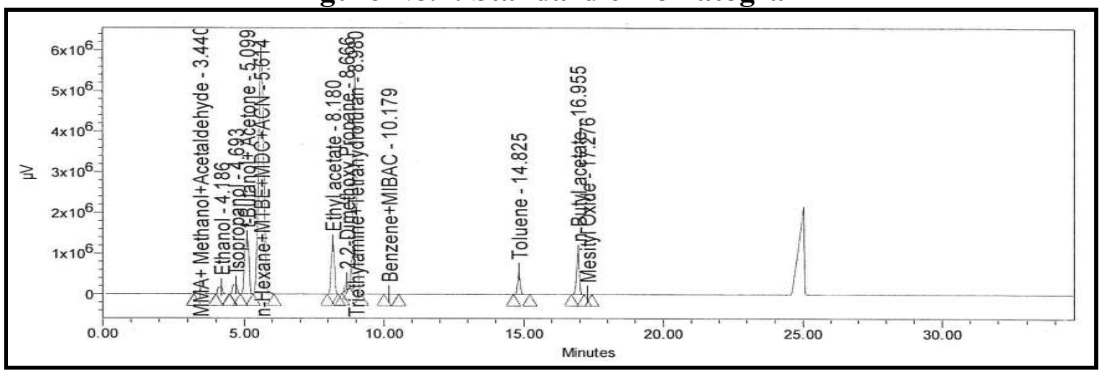


Figure No.3: Spiked sample chromatogram

CONCLUSION

A simple and sensitive method for the quantification of Benzene and Mesityl oxide in Rosuvastatin Calcium drug substance by using GC-HS with FID detector was developed, validated in accordance to ICH validation guidelines and applied for the analysis of Rosuvastatin Calcium samples. The method was validated to ensure the feasibility of the method for its application in routine analysis. The LOQs achieved through this method were very low. The methodology established was specific, robust, accurate, sensitive and linear in the range LOQ to 150% specification limit as per ICH.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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