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Method Development and Validation of Process Impurities Inritonavir Drug Substance by GCMS Technique

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Abstract: *'Quality' is the one of the important attributes, when it comes to pharmaceutical preparations. For both bulk drug industry and pharmaceutical manufactures, quality means maintaining drug standards conforming to a variety of conditions and generating profit out of it. Majority of pharmaceutical products are produced either by total synthesis approach or by altering a naturally existing product. In any case, a wide range of reactive reagents& chemicals are used. Therefore, it is accepted that trace levels of such reagents or by products are present in the final active pharmaceutical ingredient (API) or drug product as impurities. The article represents the analytical method for quantification of such process impurities from Ritonavir drug substance which is widely used as anti-retroviral agent for the treatment of HIV-AIDS. Analytical method is developed using GCMS technique. Effective validation performed as per ICH Q2 guideline suggests that the method is suitable for routine use in pharmaceutical industry.*

Keywords: Ritonavir, MTV, GCMS, Process impurities

I. INTRODUCTION

Basically, the drug products are made up of two components; The Active Pharmaceutical Ingredient (API) which is active ingredient and the excipient which is inactive ingredient. The excipient helps API to be delivered in the body. It also helps in stabilization of the pharmaceutical products throughout shelf life. It improves therapeutic activity of API by increasing their absorption or reducing viscosity etc.

According to WHO, Active Pharmaceutical Ingredient, a substance used in a finished pharmaceutical product (FPP), intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to have direct effect in restoring, correcting, or modifying physiological functions in human beings or animals.

It is important to maintain the "Quality" of all materials in finished pharmaceuticals as per the specification laid down by standards. These standards are sometimes defined in pharmacopeia and sometimes manufacturer to define following various guidelines established by health authorities. One of the important aspects to maintain the purity of all materials is adequate control of impurities.

Impurities in pharmaceuticals are undesirable chemicals that remain with the Active Pharmaceutical Ingredients (APIs) or generate during formulation or develop upon ageing of both APIs and formulated APIs to medicines. Impurities are present in trace quantity in drug product or drug substance. It is inevitable. The presence of impurities in a drug can affect its quality and consequently its efficacy, it is therefore crucial to know about impurities.

The significance of controlling the impurities in drug substance and drug product has been recognised since long by the pharmaceutical industries and the regulatory agencies. There is guidance provided by ICH (Q3A(R), Q3B(R) and Q3C(R)) to give the directives on how to control the impurities in marketed product. The impurities can be arisen from a different way, which may include starting materials, by-products, intermediates, degradation products, reagents, ligands, and catalysts(ICH Q3A; ICH Q3A (R2) and ICH Q3B (R2)).

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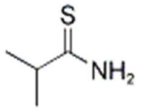
There are three main categories of impurities, which are classified based on nature of impurity: Organic impurities, Inorganic impurities, and Solvents.

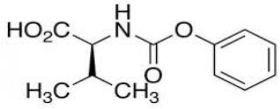
Impurities generated due to manufacturing processes are known as process impurities. Impurities that are formed during storage and transportation, because of environmental stimuli of heat, light, moisture are known as degradation impurities. These impurities are further categorised as starting materials, intermediates, by products from reactions, reagents, degradation products etc.

It is necessary to estimate the amount of these impurities in API and control as per the specified limits. Herein, I have developed a simple, sensitive, accurate, and reproducible GCMS method by direct injection for the detection of two process impurities 2-methyl-propane-thiomide (MTV-I)and N-pheoxycarbonyl-L-Valine (PCV).

2- methyl-propane-thiomide (MTV-I) structure:



N-pheoxycarbonyl-L-Valine (PCV) structure:



Limit of detection (LOD) and limit of quantification (LOQ) obtained during research work found meeting the sensitivity requirements set by standards, ICH Q3. Then, this developed method is validated according to the International Council for Harmonization (ICH) guidelines (ICH Q2) in terms of specificity, linearity, accuracy, and precision.

II. METHODOLOGY

2.1 Instrument

A Gas chromatograph Mass Spectrometer capable of temperature programming, equipped with a capillary column, split / spitless injector, a Quadrupole Mass detector, a liquid auto sampler with a suitable software.

Experiments were performed on a Shimadzu GC gas chromatography-tandem mass spectrometry with the auto sampler system. VF-624 capillary column (60 m x 0.32 mm x 1.8 µm), 6 % Cyanopropyl phenyl and 94% dimethyl polysiloxane, is used as the analytical column in this work. MS/MS detection is carried out on a Shimadzu-GC triple quadrupole mass spectrometer with electron ionization (EI) ion source. The GC oven program utilized an initial oven temperature of 40 °C, held for 5.0 min, raised at 50 °C to 1800 °C for 2.0 mins, then to 220 °C at 45 °C/min, finally held for 25 min. Helium as the carrier gas is set at a flow of 2.0 mL/min. Injector temperature is set at 180°C and interface temperature is set at 200 °C. The injection volume is1.5µL in the split mode.

2.2 Chemicals and Standards

Impurity standards of 2-methyl-propane-thiomide(MTV-I) and N-pheoxycarbonyl-L-Valine (PCV)are purchased from Sigma Aldrich. GC-grade methanol is purchased from Spectra lab.

A. Preparation of Standard and Sample Solutions:

The standard stock solutions of MTV-I and PCV impurities with each concentration of 1 mg/mL are prepared by dissolving accurately weighed impurity standards in methanol. Further the stock solutions were diluted to 0.001 mg/mL using methanol. Sample solution of Ritonavir is prepared with concentration of 1 mg/mL by dissolving in methanol.

B. System Suitability Parameters

Relative standard deviation for standard solution replicate injections of concentration 0.001 mg/mL, not more than 15.0%. The Similarity factor between two standards preparations (separately prepared and injected) is between 0.85 and 1.15.

Note: If blank interference is observed then take mean of triplicate blank injections for calculation.There should be no or not more than 10 % of blank interference.

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C. Limit

PCV : Not more than 0.10 %.

MTV-I: Not more than 0.10 %.

III. Literature Review

Ritonavir is a peptidomimetic agent that inhibits both HIV-1 and HIV-2 proteases. Ritonavir is highly inhibited by serum proteins but boosts the effect of other HIV proteases by blocking their degradation by cytochrome P450 (PubChem: Ritonavir). CAS No.- 155213-67-5

1,3-thiazol-5-ylmethylN-[(2S,3S,5S)-3-hydroxy-5-[[(2S)-3-methyl-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl) methyl] carbamoyl] amino] butanoyl] amino]-1,6-diphenylhexan-2-yl] carbamate

Ritonavir is a first-generation highly potent protease inhibitor (PI) which was approved by US FDA in 1996. It is active against HIV-1 as well as HIV-2 proteases and is currently used as a booster to optimize pharmacokinetics of other PIs and to prolong their therapeutic effects.

Rao *et al* (2010), conducted forced degradation of ritonavir, under different conditions like hydrolysis (acidic, basic, and neutral), oxidation, photolysis and thermal stress as suggested by ICH using LC–MS/MS. The method was used on LC–MS/MS for characterization of the degradation products and the pathways of decomposition were proposed. The drug was found to be degraded expansively in all conditions except oxidation and photolysis.

Kakadiya *et al* in 2011 published an article for determination of trace level impurities of methyl methane sulfonate (MMS) and ethyl methane sulfonate (EMS) in Lopinavir and Ritonavir APIs by using LC/MS/MS using electrospray ionization.

There was a short communication published in 2014 by Venugopal *et al*, Development and validation of a systematic UPLC–MS/MS method for simultaneous determination of three phenol impurities in ritonavir drug substance. The impurities separation was established by using Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 µ) using gradient mobile phase containing 0.05% ammonia in methanol and 5.0 mM ammonium acetate buffer (30:70, v/v) at a flow rate of 0.2 mL/min.

In 2015, Koppala *et al* developed a simple, sensitive, selective, and reproducible stability-indicating ultra-performance liquid chromatographic (UPLC) method for the quantitative determination of degradation products and process-related impurities of Ritonavir in a pharmaceutical dosage form.

Similar study was carried out by Mantripragada *et al*, 2018 for simultaneous estimation of impurities from Atazanavir and Ritonavir tablets. The separation of drugs and impurities were performed on Acquity BEH C18 (100mm × 2.1mm), 1.7 µ column at a flow rate of 0.4mL/min. The detector wavelength was set at 240 nm.

Estimation of impurities in Ritonavir by GC MS is almost unexplored. Two impurities are identified and considered for analytical method development and validation from Ritonavir. Both are process related impurities.

3.1 Impurities for Ritonavir

1. MTV-I: 2-methyl-propane-thiomide
2. PCV: N-pheoxycarbonyl-L-Valine

IV. Results and Discussion

4.1 Method Development

Various experiments were conducted to find out the right solubility in solvents. Methanol, acetonitrile, acetone, methylene dichloride were used for the solubility study of two impurities and Ritonavir API. The solubility of Ritonavir and impurities found higher in methanol than other solvents. For Ritonavir, API is freely soluble in methanol. For the trace level estimation of impurities, it was vital that the impurities would also be soluble in methanol and found the desired result. Hence, methanolis selected for diluent in the methodology.

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4.2 Method Validation

Method validation study is conducted referring ICH Q2 guidelines.

The specificity of the method is verified by comparison against the diluent blank, spiked sample solution with impurities and as such sample solution. No interference was observed at the retention time of the concerned impurities.

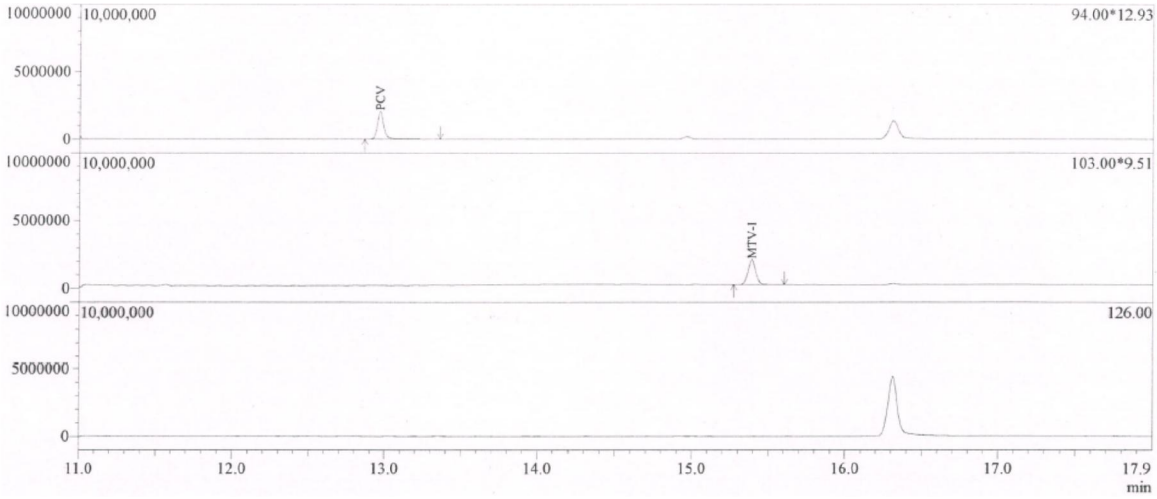

Figure 1: Spiked sample of Ritonavir with PCV and MTV-I

Precision was evaluated by injecting sample solution six times ($n = 6$). Impurities found not detected in sample solution. The values of relative standard deviation (RSD) of the area counts for both impurities. MTV-I and PCV, foundas 2.11% and 2.53% respectively at the 0.001 mg/mL concentration level (See Table I& II for results).

Sr. No	Sample name	Area	Retention time
1	Standard	475091	12.958
2	Standard	461124	12.957
3	Standard	484379	12.958
4	Standard	487575	12.958
5	Standard	484486	12.96
6	Standard	486096	12.96
Mean		479791.8	12.9585
Std. Dev		10138.9	0.00
%RSD		2.11	0.01

Table 1: Standard precision for PCV impurity

Sr. No	Sample name	Area	Retention time
1	Standard	558583	15.381
2	Standard	547218	15.381
3	Standard	573645	15.381
4	Standard	584645	15.382
5	Standard	578765	15.384
6	Standard	579578	15.383
Mean		570405.7	15.382
Std. Dev		14454.8	0.00
%RSD		2.53	0.01

Table 2: Standard precision for MTV-I impurity

The linearity of the method was evaluated between limit of quantification to 150% of limit level for each impurity separately. For both impurities, the coefficients of determination (R^2) were ≥ 0.999, showing excellent linear responses.

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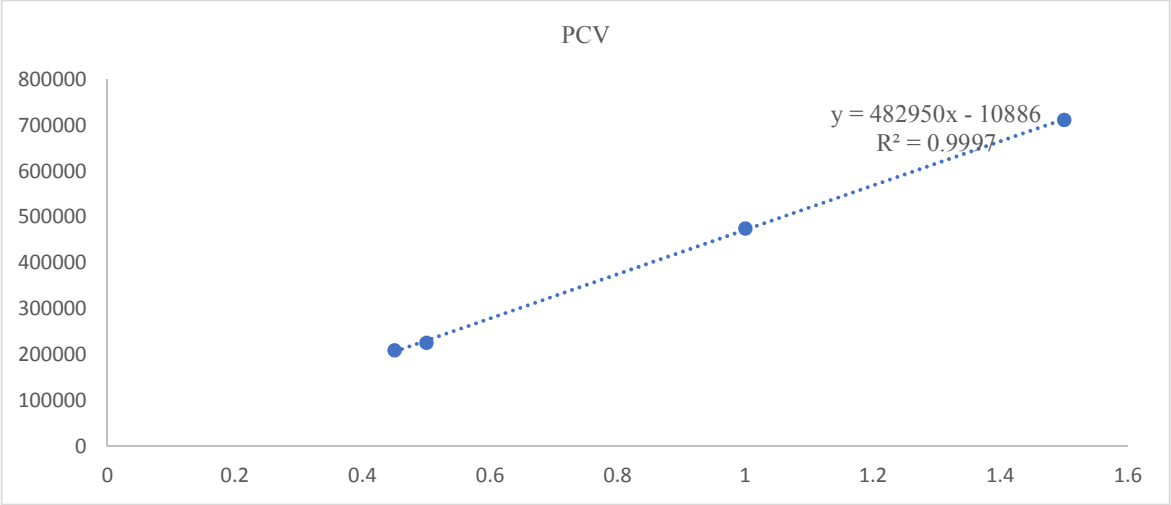

Figure 2: Linearity of PCV

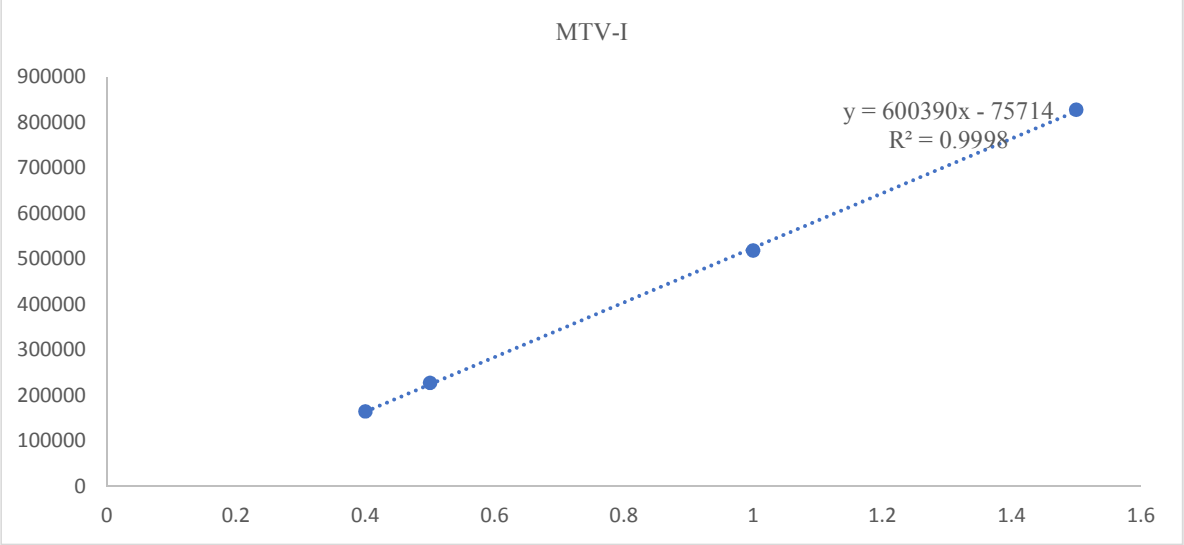

Figure 3: Linearity of MTV-I

The quantitation and detection limits (QL and DL) were calculated based on the signal-to-noise ratio (S/N)of the diluted standard solution of each impurity. Standard solution concentration is 0.001 mg/mL further diluted to obtain 0.09µg/mL and found S/N ratio as 6 for PCV impurity and 10 for MTV-I impurity. This concentration of standard is 0.01% with respect to sample concentration and considered as limit of detection.

Similarly, Standard solution concentration is 0.001 mg/mL further diluted to obtain 0.3µg/mL and found S/N ratio as 17 for PCV impurity and 24 for MTV-I impurity. This concentration of standard is 0.045% with respect to sample concentration and considered as limit of quantification. The % RSD for three replicates of both the levels found to be well within limit of not more than 15.0%.

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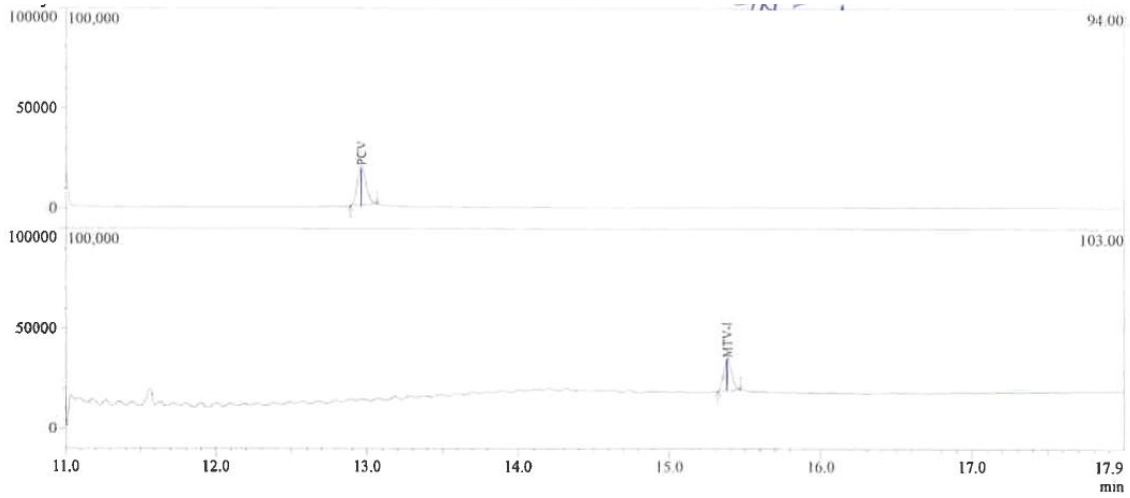

Figure 4: Limit of detection chromatogram

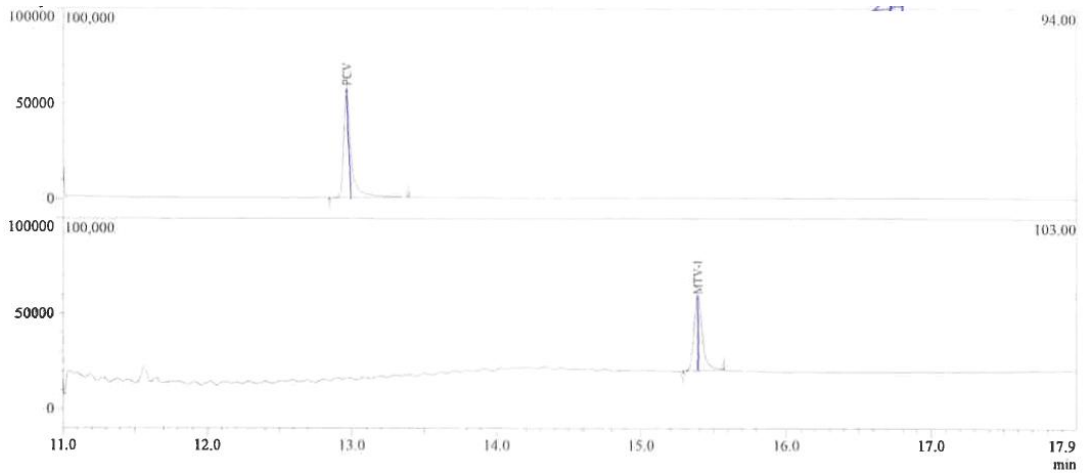

Figure 5: Limit of quantification chromatogram

The method is evaluated in the presence of MTV-I and PCV impurities in a Ritonavir API to assess the accuracy of the method by spike recovery from LOQ level to 150% limit level of each impurity. The sample is spiked with both the impurities to demonstrate the accuracy of the method (See Table III for results).

Level	Area of standard		Area in accuracy		Accuracy (%)	
	PCV	MTV-I	PCV	MTV-I	PCV	MTV-I
Level-I (LOQ)	209573	164747	188663	169314	90.02	80.79
			190987	172431	91.13	82.28
Level-II (50%)	225684	227332	235240	228765	104.23	101.37
			234015	219876	103.69	97.43
Level-III (100%)	475298	518304	486706	501234	102.40	105.46
			478976	509870	100.77	107.27
Level-IV (150%)	712078	828088	721778	838760	101.36	117.79
			712908	823456	100.12	115.64

Table 3: Accuracy results for MTV – I and PCV impurities

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V. CONCLUSION

Analytical method developed and validated for estimation of two process impurities 2-methyl-propane-thiomide (MTV-I) and N-pheoxycarbonyl-L-Valine (PCV) in Ritonavir API found to be specific, linear, precise, and accurate for the limit, not more 0.10% for both impurities respectively. Pharmaceutical industry personnel and other interested readers can use the method as it is complying the standards, ICH Q2 and ICH Q3 requirements for analysis of above-mentioned process impurities by GCMS technique. The method can be used to analyse the respective impurities in finished pharmaceutical product, such as Ritonavir tablets after specific optimisation if necessary.

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