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HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF FORMALDEHYDE AND ACETALDEHYDE TRACES IN DRUG SUBSTANCE

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ABSTRACT

A simple rugged and user-friendly and cost effective method to quantify formaldehyde and acetaldehyde in the drug substance, Rasagline by derivatizing it with 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH) is presented using High Pressure Chromatography (HPLC). By employing HPLC-UV, the cost of analysis is reduced dramatically as compared to the traditional methods. Additionally, the use of 2, 4-DNPH ensures that all the probable carbonyl compounds in the drug substance, can be monitored using the same method. The separation were accomplished on an Ascentis Express C18 (100 mm x 4.6 mm x 2.7µ) using Water: Acetonitrile (70: 30 v/v) with a flow rate of 2.0 ml /min. The Method was validated for the analytical parameters viz., Specificity, Limit of Detection (LOD), Limit of Quantitation (LOQ), Linearity and Range, Precision – System, Method and Spiked and Accuracy. The limit of detection and quantitation was found to be 10 PPM and 30 PPM for formaldehyde w.r.t sample concentration 10 mg/ ml. The limit of detection and quantitation was found to be 20 PPM and 60 PPM for acetaldehyde w.r.t sample concentration 10 mg/ ml.

Keywords *– HPLC, 2, 4-DNPH, Formaldehyde, Acetaldehyde, Drug Substance.*

1. INTRODUCTION

Formaldehyde is a naturally-occurring organic compound with the formula CH2O. It is the simplest aldehyde and is also known by its systematic name methanal. The common name of this substance comes from its similarity and relation to formic acid[1].

Formaldehyde has been produced commercially since 1889 by the catalytic oxidation of methanol. Formaldehyde (CAS No. 50-0-0) is a colourless, highly flammable gas that is sold commercially as 30–50% (by weight) aqueous solutions[2].

Acetaldehyde (systematic name ethanal) is an organic chemical compound with the formula CH3CHO, sometimes abbreviated by chemists as MeCHO (Me = methyl). It is one of the most important aldehydes, occurring widely in nature and being produced on a large scale in industry. Acetaldehyde occurs naturally in coffee, bread, and ripe fruit, and is produced by plants. It is also produced by the partial oxidation of ethanol by the liver enzyme alcohol dehydrogenase and may be a contributing factor to hangovers from alcohol consumption[3].

Exact quantification of acetaldehyde in food products has become of increased interest as the International Agency for Research on Cancer (IARC) has changed the cancer risk classification of AA from an agent possibly carcinogenic to humans (Group 2B) to an agent

carcinogenic to humans (Group 1) International Agency for Research on Cancer (IARC) has changed the cancer risk classification from an agent possibly carcinogenic to humans (Group 2B) to an agent carcinogenic to humans (Group 1) [4].

During manufacturing of drug substance catalytic oxidation reaction, Oxidation of Methanol leads to formation of formaldehyde as by product. Methanol always contains trace level of ethanol. Oxidation of ethanol leads to formation of Acetaldehyde as by product.

Formaldehyde and acetaldehyde are not ICH listed solvents. Therefore its control limit is not available in ICH and USP. Drug regulations have specific and stringent requirements for its registration and usage. There are stringent requirements specifically impurities[5, 6].

IARC has classified formaldehyde as a known human carcinogen (Group 1) while in United States, it (specifically formaldehyde gas) is classified as reasonably anticipated to be carcinogen by NTP[7].

Acetaldehyde is an exogenous chemical substance to which humans are exposed as well as an endogenous substance that is internally generated within humans and animals. Acetaldehyde is generated from ethanol in the liver and finally degraded to carbon dioxide and water through acetic acid. Acetaldehyde is absorbed through the lung and gastrointestinal tract. Absorbed acetaldehyde is distributed in the blood, liver, kidney, spleen, heart and muscle. Regarding repeated dose toxicity of acetaldehyde, oral administration to rats for 4 weeks caused slight hyperkeratosis of the fore stomach at a dose of 675 mg/kg/day. The NOAEL is 125 mg/kg/day. [8]

Formaldehyde is a small molecule and has one carbon and one heterogeneous oxygen atom. This molecule is not readily amenable to gas chromatographic (GC) with flame ionization detection (FID). Also, formaldehyde is not easily ionizable and cannot be easily analysed by mass spectrometry (MS). The analysis of formaldehyde is commonly achieved by a high-performance liquid chromatography (HPLC) method following a derivatization reaction with 2, 4-dinitrophenylhydrazine [9, 10].

DNPH derivatization improves chromatographic properties and increases UV absorptivity for analysis by high-performance liquid chromatography (HPLC –UV), in addition to increased thermal stability. However, most methods for the quantitation of multiple carbonyls – DNPH derivatives require long analysis times and have limited selectivity, especially when the sample matrices of the drug substance comes into effect. For instance, lengthy chromatographic separations using HPLC–UV, which can be as long as 60 minutes, are described for the separation of DNPH derivatives of commonly occurring carbonyls (especially aldehydes and ketones) in air and water[11-16].

The present work demonstrates a simple, sensitive and accurate HPLC method for analysis of formaldehyde and acetaldehyde in Pharmaceutical drug substance, Rasagline by Derivatizing the aldehydes with 2, 4-DNPH. The formation of the derivative was confirmed using HPLC. The chromatographic separation and detection techniques employed could also be applied to the analysis of carbonyls from pharmaceutical products / substances to environmental samples.

2. MATERIALS AND METHODS

2.1 Drug and Reagents

Pure Rasagline sample was obtained as gratis sample from Microlabs. Acetonitrile Grade: HPLC Grade was purchased from Merck. Sulphuric Acid: AR grade was purchased from Sigma Aldrich 2, 4 Dinitro Phenyl hydrazine: AR grade was purchased from Sigma Aldrich. Formaldehyde: 40% in Water was procured from Merck. Acetaldehyde 100% was procured from Merck. Milique HPLC grade water was used in experiment.

2.2 Apparatus and equipment

HPLC analysis was carried out on HPLC Instrument: Agilent 1100 The output signal was processed and monitored using suitable integration software. All studies and separation were achieved on HPLC column, Ascentis Express C18 100 mm x 4.6 mm x 2.7µ

2.3 Preparation of mobile phase

The mobile phase was prepared by mixing HPLC grade Acetonitrile from Merck and Milique HPLC grade water. In Mobile phase was sonicated in ultra-sonication bath in (Acetonitrile: Water (80:20) v/v) and filtered through suitable filter paper.

2.4 Derivatization experiment

Preparation of 2,4Dintrophenyl hydrazine (DNPH) – Weigh 100 mg of 2,4 DNPH in 200 mL volumetric flask. Add 150 mL of Acetonitrile, sonicate to dissolve. Cool and dilute up to mark with Acetonitrile.

Preparation of 2% Sulphuric acid – In 200 mL of volumetric flask take about 50 mL of water and slowly add 4 mL of Sulphuric acid with intermittent cooling. Further dilute up to mark with water.

2.5 Chromatographic condition

Separation was achieved on HPLC column, Ascentis Express C18 100 mm x 4.6 mm x 2.7µ, Mobile phase Water: Acetonitrile (70: 30) v/v with flow rate 2.0ml / min was used. UV detector wavelength was adjusted to 360 nm. Column oven temperature was adjusted to 30 C.

3. RESULTS AND DISCUSSION

3.1 Method development and Column Selection

Characterization sample Rasagiline API selected for method development of formaldehyde and acetaldehyde. Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of formaldehyde and acetaldehyde impurities in Rasagiline. A number of column chemistries supplied by different manufacturers and different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Rasagiline. Poor peak shape and resolution was observed when hypersil BDS C18 (250mm x 2.1mm, 5µm) and mobile phase consisting of mixture of 0.1% triethylamine in water: Acetonitrile and Methanol(70:15:15 v/v) formaldehyde and acetaldehyde peak eluted at 12 and 13 minutes good separation, however peak shape of formaldehyde and acetaldehyde peak was not good. By using another attempt with mixture of mobile phase 0.1% Triethylamine, Acetonitrile and methanol (60:20:20 v/v) and column Luna C18 (250mm x 2.1mm, 5µm), peak of formaldehyde and acetaldehyde merged and no separation achieved. Separation was achieved on HPLC column, Ascentis Express C18 100 mm x 4.6 mm x 2.7µ Mobile phase Water: Acetonitrile (70: 30) v/v with flow rate 2.0ml / min was used. UV detector wavelength was adjusted to 360 nm. Column oven temperature was adjusted to 30 C.

3.2 Accuracy

The accuracy procedure is shown with the recovery studies. Content of Formaldehyde was not detected in Rasagline Sample. Content of Acetaldehyde was found below detection level (refer Table I). The recovery studies were carried out by spiking aliquots of API solution with 50, 100 and 150 PPM of formaldehyde and 100, 200 and 300 PPM of acetaldehyde. Average recovery for formaldehyde was between 94.9 and 102.9% and Average recovery for acetaldehyde was between 101.1 and 102.2%(Specimen chromatogram figure 1,2,3& 4 are given).

3.3 Linearity

Linearity standard solutions of formaldehyde derivative (formaldehyde 2, 4 DinitroPhenylhydrazone) from 30, 50, 100 and 150 ppm. 10 µl solution was injected. The calibration curve derived out of chromatograms for peak area vs concentration of formaldehyde derivative (formaldehyde 2, 4 DinitroPhenylhydrazone) found to be linear (Refer Graph I).

[% Y intercept is (0.078 x 100/ 7.7 (mean) is 1.017546 (Calculation for % Y intercept)].

Linearity standard solution of Acetaldehyde derivative (Acetaldehyde 2, 4 DinitroPhenylhydrazone) from 60, 100, 200 and 300 ppm were injected (For details refer - Table II) 10 µl solution was injected. The calibration curve derived out of chromatograms for peak area vs concentration of formaldehyde derivative (Acetaldehyde 2, 4 DinitroPhenylhydrazone) found to be linear (Refer Graph II).

[% Y intercept is (0.3867 x 100/ 38.4 (mean) is 1.00589 (Calculation for % Y intercept)].

3.4 Limit of Detection (LOD) and limit of Quantification (LOQ)

The limit of detection and quantitation was found to be 10 PPM and 30 PPM for formaldehyde w.r.t sample concentration 10 mg/ ml. The limit of detection and quantitation was found to be 20 PPM and 60 PPM for acetaldehyde w.r.t sample concentration 10 mg/ ml. (Refer Table II).

3.4.1 Formaldehyde

"As noted in ICH M7 Section 7.5, "Higher acceptable intakes may be justified when human 225 exposure to the impurity will be much greater from other sources e.g., food, or endogenous 226 metabolism (e.g., formaldehyde)." For example, formaldehyde is not a carcinogen orally, so 227 that regulatory limits have been based on non-cancer endpoints. Health Canada, IPCS and US."

EPA (Integrated Risk Information System [IRIS]) 228 recommend an oral limit of 0.2 mg/kg/day, or 10 mg/day for a 50 kg person. 10 mg / day is equivalent to 10000 PPM. LOD of the method is 10 PPM which less than EPA and ICH M7 expectation.[17]

3.4.2 Acetaldehyde

Detection limit for acetaldehyde is 20 PPM. Limit of detection for Acetaldehyde by this method is 20 PPM which is far less than current OSHA Permissible exposure level 200 PPM. [18]

Table 1: Content of Formaldehyde and Acetaldehyde in Rasagline Samples:

Rasagline	Formaldehyde	Acetaldehyde
Sample 1	Not detected	Below detection.
Sample 2	Not detected	Below detection.

Table 2: LOQ & LOD

	Formaldehyde (in PPM)	**Acetaldehyde (in PPM)**
LOQ	30	60
LOD	10	20

Figure 1: Blank Chromatogram

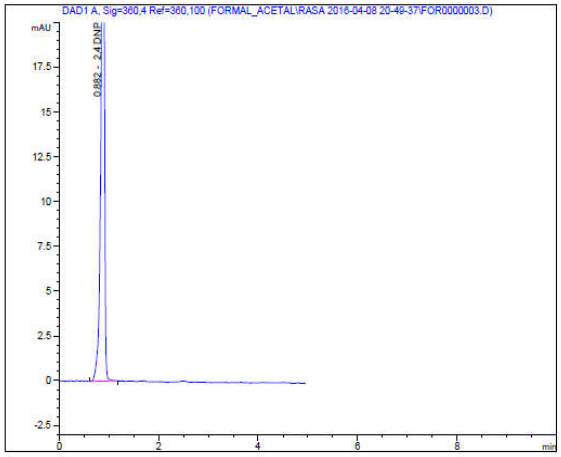


Figure 2: Standard Chromatogram

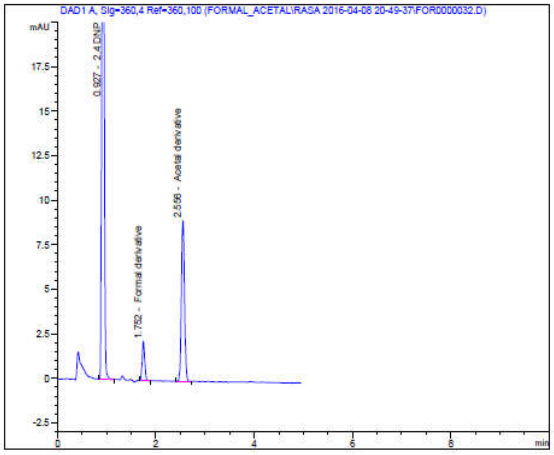


Figure 3: Sample Chromatogram

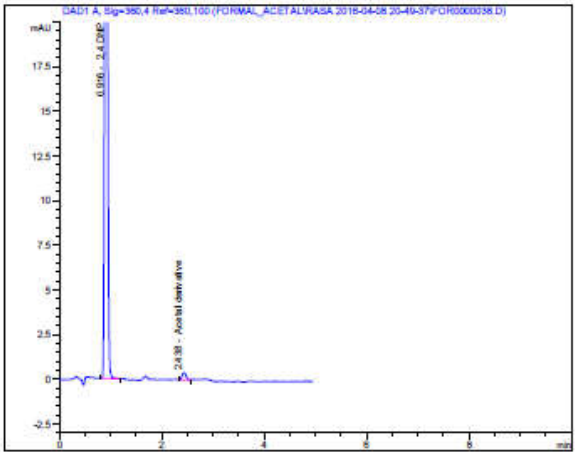
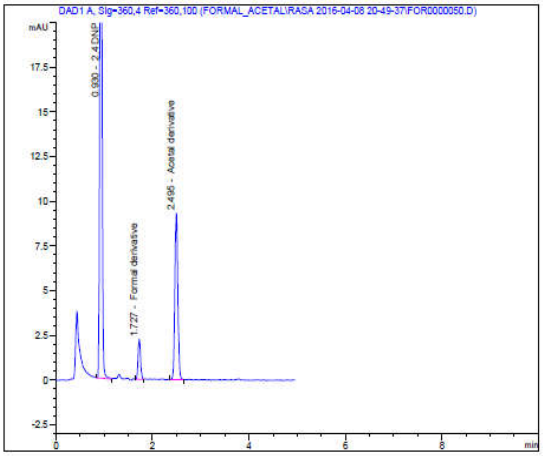
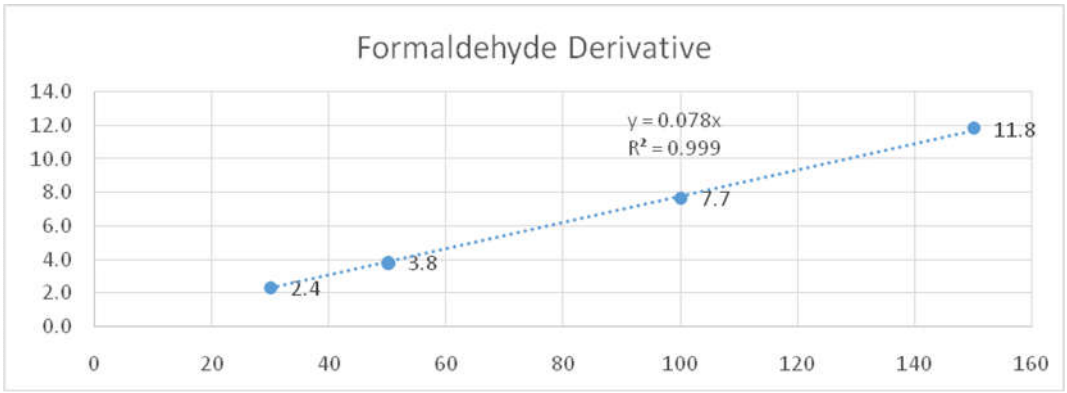


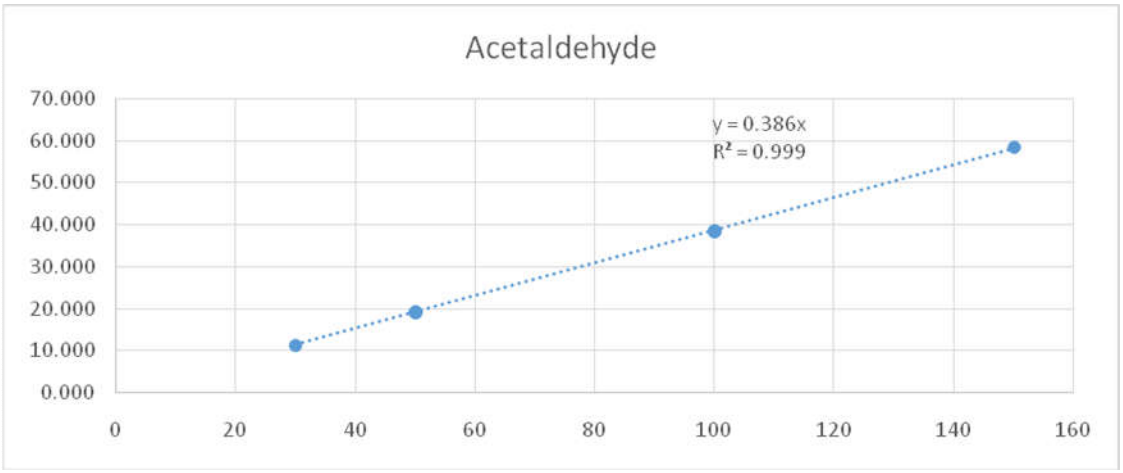
Figure 4: Accuracy (limit 100% level) Chromatogram



Graph : I



Graph : **II**



4. CONCLUSION

The proposed LC method is selective for the detection and quantification of Formaldehyde and Acetaldehyde in Rasagline drug substance. The method is capable of detecting Formaldehyde and Acetaldehyde impurities. Hence this method is useful for detection and quantification of Formaldehyde and Acetaldehyde present in Drug Substance.

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