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QUANTIFICATION OF HAZARDOUS HIGH BOILING SOLVENTS IMPURITIES IN ANTI-DIABETIC DRUG LINAGLIPTIN BY NOVEL GC-ALS METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT
A new precise and accurate GC-ALS method was developed and validated for quantitative determination of hazardous high boiling solvents impurities i.e. acetic acid and dimethyl sulphoxide in Linagliptin drug molecule using direct injection technique by GC-ALS with the help of Flame ionization detector (FID). The column used was Agilent J & W DB-FFAP, 30M X 0.530 mm X 1µm capillary column. The stationary phase of the column was nitro terephthalic acid-modified polyethylene glycol (PEG). The method was validated for specificity, precision, LOD, LOQ, accuracy, linearity, range, solution stability, and robustness. The detection limit and quantitation limit obtained for acetic acid was 83.3µg/g and 252.4 µg/g respectively. For Dimethyl sulphoxide detection limit and quantitation limit obtained were 83.7 µg/g and 253.7 µg/g respectively. The method was found to be linear in the range between 253.9 µg/g to 7616.9 µg/g for acetic acid and 253.5 µg/g to 7605.8 µg/g for dimethyl sulphoxide. The average recoveries obtained were 85.20% and 89.69% at LOQ level for acetic acid and dimethyl sulphoxide respectively, and for 150% level recovery were 94.26% and 100.43% for acetic acid and dimethyl sulphoxide respectively. The developed method was found to be robust, specificity was also demonstrated, and standard and the spiked solution was stable for up 20 Hrs. Thus, the method is used for routine analysis of their intended use. For quantification of Acetic acid and Dimethyl sulphoxide in Linagliptin, no pharmacopeial method is available so developed this simple method for laboratory use.

Keywords: Gas Chromatography, AMD, AMV, High Boiling Solvent, ICH, APIs, and OVIs.
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INTRODUCTION

Linagliptin is used widely as an anti-diabetic drug. During the synthesis of Linagliptin Dimethyl, sulphoxide, and acetic acid have been used as residual solvents.[1-3] The detection and quantification of residual solvents present in active pharmaceutical ingredients (APIs) is necessary from both patient safety and regulatory perspectives. Head-space gas chromatography is routinely used for the quantitation of residual solvents for small molecule APIs produced through synthetic processes.[4] In Linagliptin dimethyl sulphoxide and acetic acid are hazardous to human health if their permeable daily dose exceeds 50 mg per day or 5000 µg/g as per the International Council for Harmonization (ICH) guideline. The objective of this guideline is to recommend acceptable amounts of residual solvents in pharmaceuticals for the safety of the patient. The guideline recommends the use of less toxic solvents and describes levels considered toxicologically acceptable for some residual solvents.[5] According to this guideline acetic acid and dimethyl sulphoxide is hazardous materials to human health if both solvent dose is exceeding than 50 mg per day or 5000 µg/g. Therefore, it is necessary to control this solvent in the linagliptin drug molecule. Control by means of quantifying acetic acid and dimethyl sulphoxide to restrict limit level to serve good health to the diabetic patient of our society. Generally, the low boiling solvent is quantified easily by means of GC-HS (Gas Chromatography-Head Space) or GC-ALS (Gas Chromatography-Auto sampler) technique in different drug molecules. However, in linagliptin, high boiling solvents are used acetic acid (118ºC) and

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dimethyl sulphoxide (189ºC) which are difficult to quantify by conventional gas chromatography methods. Therefore, it is very challenging to develop a quantification method for high boiling solvents impurities i.e. acetic acid and dimethyl sulphoxide in linagliptin drug molecule. No simultaneous determination of acetic acid and dimethyl sulphoxide method is available in any pharmacopeia or literature to quantify acetic acid and dimethyl sulphoxide in linagliptin drug molecule by simple analytical technique. Therefore, in the present study, we developed a novel gas chromatography-auto sampler (GC-ALS) analytical method for the quantification of acetic acid and dimethyl sulphoxide in the linagliptin drug molecule.

EXPERIMENTAL

Material and Methods

Linagliptin working standard having purity ≥ 99% was gifted by MAPI Pvt. Ltd, Tarapur. All used solvents (Acetic acid, Dimethyl sulphoxide, Dichloromethane) are GC grade having purity ≥ 99.0%, and obtained from Spectrochem Pvt. Ltd. Mumbai.

General Procedure

5000 µg/g acetic acid and dimethyl sulphoxide standard in dichloromethane (MDC) with respect to the test sample was prepared as a standard solution and 50000 µg/g sample of linagliptin in dichloromethane was prepared as a test sample.

Detection Methods

A Perkin Elmer Clarus 580 gas chromatography system with an autosampler tray was used for the present research work. The gas chromatography set as Injector temperature 220°C; split ratio 10:1; column flow 2.8 mL/min Nitrogen; run time 22 min; Flame Ionization detector temperature 240°C; detector gas flow Hydrogen at 40 mL/min, air at 400 mL/min; purge flow 3 mL/min; Injector volume 0.1 µL/min; Range 1; Attenuation 2; software used for chameleon 6.8.

RESULTS AND DISCUSSION

Analytical Method Development

With a predefined mix standard of acetic acid and dimethyl sulphoxide 5000 µg/g, test sample 50000 µg/g. Direct, injection method on Gas chromatography instrument was adopted for good separation and detection of acetic acid and dimethyl sulphoxide. Different 30-meter GC capillary columns with different stationary phases i.e. an Agilent DB-WAX and an Agilent DB-FFAP were used for the primary trial for column selection. After that, it is observed that an Agilent DB-FFAP column is suitable for analysis because it gives better resolution in acetic acid and dimethyl sulphoxide with excellent peak shapes and there is no interference due to diluent at retention time of acetic acid and dimethyl sulphoxide as shown in Fig.-1, Fig.-2. So, the DB-FFAP column is used for further analysis with the following column oven temperature program Initially 80°C hold for 1 min; then increase @ 5.0°C/min to 155°C hold for 2 min, then increase @25.0°C/min to 230°C hold for 1 min.

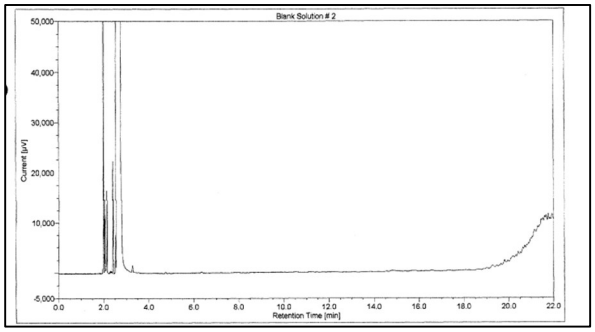

Fig.-1: Diluent Peak (Blank) i.e., Dichloromethane

Analytical Method Validation

After Analytical method development for routine use of this method analytical method, validation is a must as per regulatory compliance.[6] Following parameters were used to validate analytical method specificity, system precision, method precision, intermediate precision, the limit of

detection (LOD), the limit of quantitation (LOQ), linearity and range, accuracy by recovery, robustness, and solution stability.[7] Analytical method validation starts with specificity in which no interference due to diluent at Retention time (RT) of acetic acid and dimethyl sulphoxide was observed then system precision was performed with six replicates of standard (5000 µg/g) and method precision performed with six replicates of samples spiked with acetic acid and dimethyl sulphoxide at the specification level. (5000 µg/g). Intermediate precision achieved by reputability of analysis by method precision on different days, different columns.

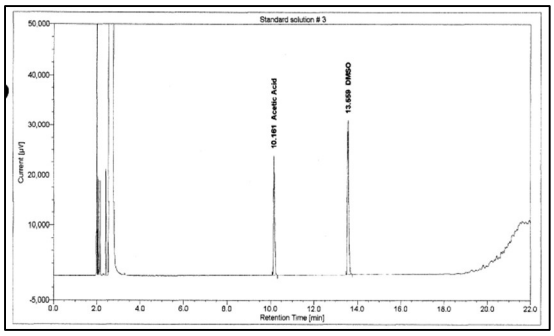

Fig.-2: Chromatogram of Mix Standard Acetic Acid and Dimethyl Sulphoxide Showing Better Resolution, Excellent Peak Shapes

Table-1: Result of System, Method, and Intermediate Precision Based on Peak Area for Acetic Acid, Dimethyl Sulphoxide

Replicates	System Precision		Method Precision		Inter. Precision	
	Acetic Acid	DMSO	Acetic Acid	DMSO	Acetic Acid	DMSO
1	77494.7	135518.1	76240.9	136670.0	4244.1	4316.0
2	85225.8	148134.8	75078.5	135573.9	4649.5	4614.0
3	76287.7	134031.9	73911.8	132246.0	4247.4	4254.7
4	80156.5	141000.0	72880.8	129892.7	4279.5	4351.0
5	79674.7	137465.0	75096.5	132997.6	4270.9	4240.6
6	91049.6	162071.5	73458.1	130848.4	4294.3	4297.0
Average	81648.2	143036.9	74444.4	133038.1	4447.4	4507.7
Std. Dev.	5537.0	10593.6	1245.5	2643.4	169.1	202.7
% RSD	6.8	7.41	1.7	2.0	3.8*	4.5*

* Cumulative %RSDs method precision + Intermediate precision

As data shown in Table-1, the system precision for acetic acid and dimethyl sulphoxides was in the limit as both had % RSDs of less than 10% Also, the method precocious % RSDs of ≤ 2% for Acetic acid and dimethyl sulphoxide. Intermediate precision gated was again excellent cumulative % RSD sis < 5% for Acetic acid and dimethyl sulphoxide. The next parameter encountered was LOD and LOQ determination. To demonstrate the limit of detection and limit of quantification of the analytical method, prepared the series of solutions having the lowest concentration with respect to the limit level concentration and injected each solution once. The limit of Detection and the limit of quantitation level of the method were predicted based on the S/N ratio of analytes,[8] assuming S/N ratio NLT 3 for LOD and NLT 10 for LOQ. Obtaining LOD and LOQ results were as shown in Table-2 and Table-3.

Table-2: LOD Determination Results

S. No.	Analyte	Conc. (µg/g)	S/N Ratio
1	Acetic Acid	83.3	10.7
2	DMSO	83.7	15.1

Table-3: LOQ Determination Results

S. No.	Analyte	Conc. (µg/g)	S/N Ratio
1	Acetic Acid	252.4	43.6
2	DMSO	253.7	62.3

Linearity solutions for known impurities were injected in the concentration range of LOQ to 150% (LOQ, 50%, 80%, 100%, 120%, 150%) with respect to the specification limit. Both acetic acid and dimethyl sulphoxide impurities are linear in response to the concentration. For acetic acid concentration was 253.9 µg/g to 7616.9 µg/g and the correlation coefficient was 0.999 as shown in Fig.-3. For dimethyl sulphoxide concentration was 253.5 µg/g to 7605.8 µg/g and the correlation coefficient was 0.998 as shown in Fig.-4.

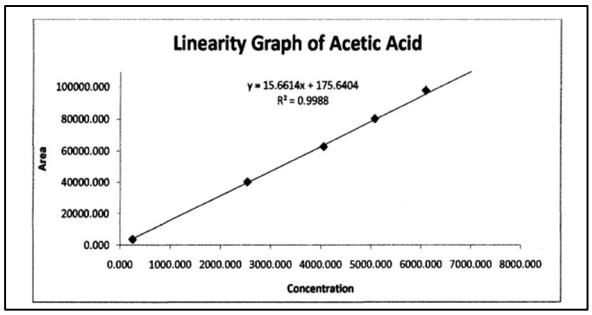

Fig.-3: Linearity Graph for Acetic Acid

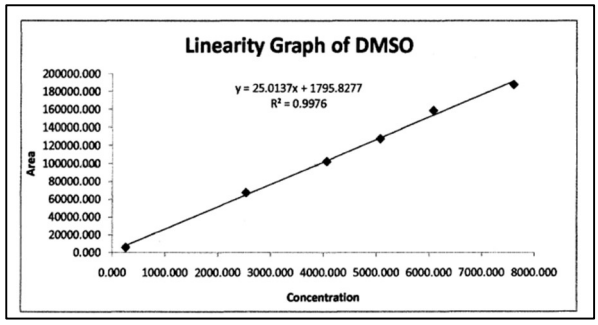

Fig.-4: Linearity Graph for Dimethyl Sulphoxide

The accuracy of an analytical method is studied by spiking acetic acid and dimethyl sulphoxide in linagliptin in the LOQ to 150 range and recoveries were obtained for acetic acid and DMSO as shown in Table-4. This result shows that all % recovery is well within the ICH limit and the method deliver accurate result and is used for their intended use. The method is robust. Standard and spiked test sample solution stable for up to 20 hours.

Table-4: Recovery Obtained by Spike Study

	% Recovery	
Recovery level	Acetic acid	DMSO
LOQ	85.20	89.69
50 %	94.12	99.43
100 %	93.43	97.68
150 %	94.26	100.43

CONCLUSION

Quantifying hazardous high boiling impurities i.e. acetic acid and dimethyl sulphoxide in the anti-diabetic drug Linagliptin is critical to ensure patient health safety. It's a very difficult task to quantify these impurities by the conventional GC-HS method due to the high boiling nature of these impurities to overcome these challenges we have developed and validated the Novel GC-ALS method which precisely quantifies these impurities so this analytical method is used for their intended use.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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