ScienSage

Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN: 0976-9595
Research Article
DOI: 10.55218/JASR.202213139

HPLC BASED PROCEDURE DEVELOPMENT FOR MONITORING ACETIC ACID IN DACLATASVIR DRUG

Syed Anwar*[1], Pathan Mohd. Arif Ali Khan[2]

[1]*Department of Analytical Research and Development, Mylan Laboratories Limited, Hyderabad, India*
[2]*Department of Chemistry, Maulana Azad College of Arts Science and Commerce, Dr. Rafiq Zakaria campus, Aurangabad, India*
**Corresponding author: anwar.chem79@gmail.com*

ABSTRACT

A process for determination of acetic acid (AAD) in Daclatasvir (DAC) active medicinal ingredients applying HPLC technique was developed. The process involved separation AAD using Phenomenex made Synergi polar RP-80A column with gradient manner of elution, employing mobile solvent phase with 0.1% phosphoric acid (solvent A) and acetonitrile (solvent B), at flow velocity of 0.6 ml/min and UV sensor examination at 210 nm. AAD linearity range was 5.01289µg/ml to 30.07733µg/ml with sensitivity of 1.65425µg/ml (LOD) and 5.01289µg/ml (LOQ). Accuracy towards AAD recoveries were 111.3 to 113.0%, 86.7 to 94.4% and 90.9 to 95.7%, and precision towards AAD's RSD was 1.197% and 3.367%. The projected HPLC process could be applied to analysis of AAD in DAC active medicinal ingredients with sharp selectivity, better sensitivity, and high point accuracy.

Keywords: Daclatasvir, Acetic acid, Impurity, HPLC, Quality management.

1. INTRODUCTION

Daclatasvir (DAC) was the earliest medication to show that it was harmless and effective in managing hepatitis kind C virus genovariety without requiring the use of interferon or ribavirin [1, 2]. DAC's antiviral effect is achieved by interacting to NS5A; a non-structural phosphoprotein expressed by hepatitis kind C virus, which suppresses RNA replication then virion assembly [3]. Bestowing to "American Association for the Study of Liver Diseases", DAC (60 mg) combined with sofosbuvir (400mg) is recommended as second-line medication for genovariety 1a/b individuals even having cirrhosis [4]. Patients with HIV- type 1 coinfection, severe cirrhosis, or hepatitis kind C virus recurrence after a liver transplantation can benefit from combination treatments which included DAC [6].

Acetic acid (AAD) is oftentimes utilized in the pharmaceutical industry for making of active medicinal ingredients and their formulations. In active medicinal ingredients and their formulations manufacturing industries, AAD is engaged as intermediate chemical or used during drug purification processing. Following to ICH Q-3C (R4) recommendations, AAD is classed as a class 3 solvent [7]. Because Class 3 solvents have a minimal toxicity to humans, there is no necessity for a health-based consumption limitation. Even if, it is innocuous, any additional ingredient identified in the drug component must always be regarded an impurity [8]. Consequently, AAD is deemed an impurity. In all pharmaceutical production units, all active medicinal ingredients and their formulations must be investigated for residual AAD content.

Ajay et al., [9] and Gangrade et al., [10] devised HPLC methodologies for AAD content quantification in active medicinal ingredients. None of them, however, is particularly committed to analysing AAD in DAC active medicinal ingredient. As a result, for the assessment of AAD in DAC active medicinal ingredient with appropriate analysis time, an easy technique is typically required. The purpose of this endeavour is to establish chromatographic conditions that might well be used to determine AAD in DAC active medicinal ingredient.

2. MATERIAL AND METHODS

2.1. Instrumentation

The determinations AAD in DAC active medicinal ingredients were implemented using an "Alliance Waters HPLC device" with model no. 2695 operated with "Alliance Waters PDA detector" with model no. 2998 and "Alliance Waters UV detector" with model no. 2487. The chromatograms of AAD were integrated by exploiting Empower second version software.

2.2. Chemicals

"Qualigens fine chemicals ltd, India" supplied phosphoric acid, batch no. BCBM0926V (analytical kind), "J.T. Baker brand chemistries, India" supplied acetonitrile, batch no. 60450 (HPLC kind), "Merck India ltd, India" supplied sodium hydroxide, batch no. QD4Q640895 (analytical kind) and "Milli-Q system, USA" supplied water, "Mylan Laboratories limited, India" supplied DAC were employed in the determinations AAD in DAC active medicinal ingredients.

2.3. HPLC monitoring conditions for AAD in DAC sample

The determinations AAD in DAC active medicinal ingredients were done with following HPLC monitoring settings: Analysing column was Phenomenex made Synergi polar RP-80A, 4.0 µm, 250 x 4.6 mm, 4.0 µm, column analysing temperature with 40°C, flow rate value of 0.6 ml/min, analysing sample volume with20 µl, PDA wavelength for AAD detection at 210 nm, diluent employed included acetonitrile (50% ratio): water (50% ratio). Gradient elution manner with solvent A, comprising of 0.1% phosphoric acid, pH 3.0 attuned with adding of diluted NaOH, and solvent B, comprising of solvent acetonitrile was employed.

2.4. AAD solutions

Stock AAD solution was formulated using diluent [acetonitrile (50% ratio): water (50% ratio)] as solvent. An over-all of 125 mg of AAD was weighted sensibly and placed in 100 ml sized volumetric flask, then liquified in diluent [acetonitrile (50% ratio): water (50% ratio)] to the 100 ml spot so as to attain an AAD solution holding 1250 µg/ml. Working AAD solution was formulated through apt solubilization of stock AAD solution (1250 µg/ml) in diluent [acetonitrile (50% ratio): water (50% ratio)] so as to attain an AAD solution holding 25 µg/ml. Further, this stock AAD solution (1250 µg/ml) was aptly solubilized in diluent [acetonitrile (50% ratio): water (50% ratio)] to acquire series of AAD solutions with concentrations 5.01289 µg/ml, 10.02578 µg/ml, 15.03867 µg/ml, 20.05155 µg/ml, 25.06444 µg/ml and 30.07733 µg/ml.

2.5. DAC sample solution

DAC solution was formulated using diluent I (acetonitrile) and diluent II [acetonitrile (50% ratio): water (50% ratio)] as solvents. An 50 mg of AAD was weighed sensibly and placed in 10 ml sized volumetric flask holding 5 ml of diluent I and then liquified in diluent II [acetonitrile (50% ratio): water (50% ratio)] to the 10 ml spot so as to attain an DAC solution holding 5.0 mg/ml.

2.6. Graph of AAD linearity

Each quantity solution of AAD (5.01289µg/ml, 10.02578 µg/ml, 15.03867 µg/ml, 20.05155 µg/ml, 25.06444 µg/ml and 30.07733 µg/ml) was prepared and infused to Phenomenex made Synergi polar RP-80A column. Peak area of AAD was measured using HPLC monitoring conditions proposed at wavelength of 210 nm. For creating graph of AAD linearity, each peak area reading was graphed toward the respective known AAD concentration.

2.7. AAD analysis in DAC sample

DAC solution was evaluated for AAD content thru infusion (20 µl) to Phenomenex made Synergi polar RP-80A column and Peak area of AAD was measured using HPLC monitoring conditions proposed at wavelength of 210 nm. The DAC samples' AAD content was assessed with graph of AAD linearity.

3. RESULTS AND DISCUSSION

3.1. Developing HPLC monitoring conditions

The AAD elution in HPLC operating approach is influenced by numerous variables. On AAD elution, the influence of the static column phase, as well as the flow velocity, composition, and pH of the moving solvent phase, including elution manner were investigated. Better AAD peak shape, peak properties and sensitivity were achieved using Phenomenex made Synergi polar RP-80A column (40°C) with gradient manner of elution, employing mobile solvent phase with solvent A, comprising of 0.1% phosphoric acid, pH 3.0 attuned with adding of diluted NaOH, and solvent B, comprising of solvent acetonitrile, at flow velocity of 0.6 ml/min, UV indication examining at 210 nm, and acquisition and run times of 30 and 40 min, respectively. In optimized HPLC monitoring conditions, the AAD's retention point was 7.112 min (Fig. 1).

3.2. Validation

Validation experiments were applied on developed HPLC monitoring conditions for AAD analysis in DAC sample with respect to, selectivity, linearity, system suitability, sensitivity, accuracy, robustness and precision conferring with directives of ICH [11] as well as US Pharmacopoeia [12].

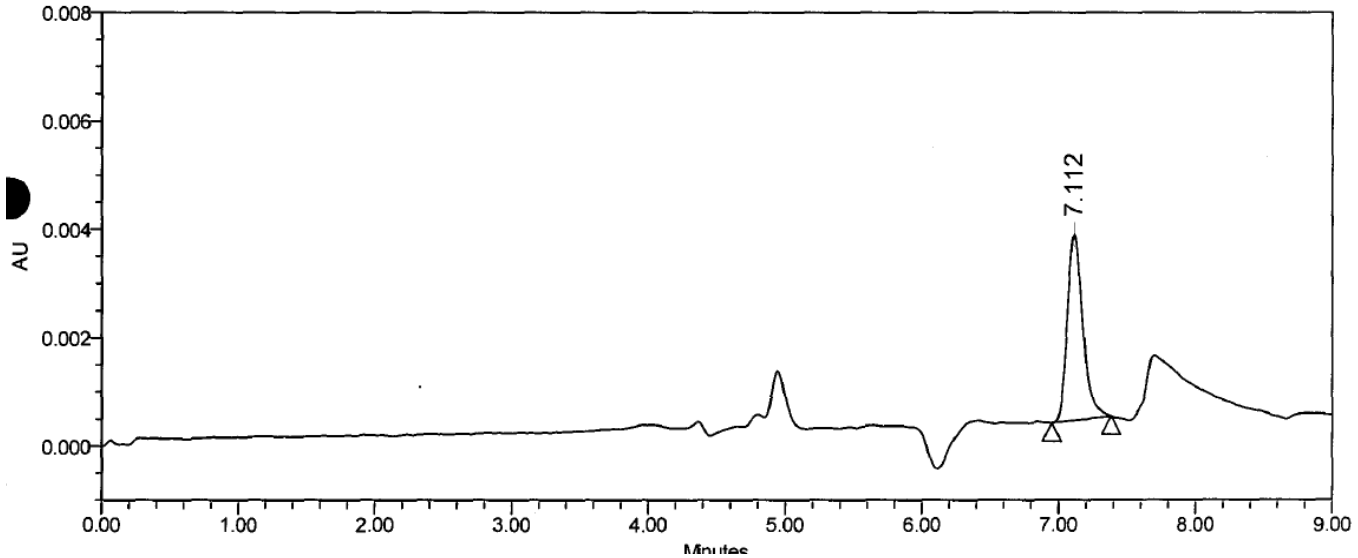

Fig. 1: AAD chromatogram with developed HPLC monitoring conditions

3.2.1. LOD and LOQ

The LOD as well as LOQ values of AAD were determined, applying signal/noise proportion approach, under the pronounced chromatographic backgrounds were 1.65425µg/ml and 5.01289µg/ml, respectively. These low assessments proved ideal sensitivity for analysing AAD in DAC active medicinal ingredients for quality monitoring.

3.2.2. Linearity

The plot of AAD peak response measured *versus* AAD concentration in diluent II solutions demonstrated a clear assured, linear correlation (Fig. 2).

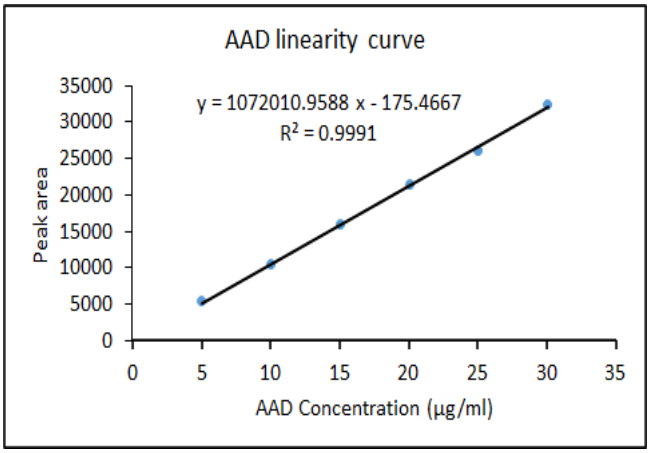

Fig. 2: Graph of AAD linearity

For diluent II dilutions of AAD, calibration curves developed demonstrated high linearity and reasonable results across the approved range of 5.01289µg/ml to 30.07733µg/ml. As a necessary consequence, the AAD peak response measured as an extent of AAD concentration was verified. Both fits had a very relatively low sum of squares in studied range (5.01289 µg/ml to 30.07733µg/ml) demonstrating a quite close fit of the linear modelling to the experimental evidence (Table 1).

Table 1: AAD linearity regression statistics information

Parameter	Measure	
Linearity		
Range	5.01289 µg/ml to 30.07733 µg/ml	
Regression statistics		
Intercept	-175.4667	
Slope	1072010.9588	
R square	0.9991	
Multiple R	0.9995	
ANOVA		
Parameter	**SS measures**	**Df mesures**
Residual	469548.4190	4
Regression	505373647.2893	1
Total	505843195.7083	5
Confidence intervals		
Parameter	**Upper 95%**	**Lower 95%**
X variable	1117372.9610	1026648.9565
Intercept	710.1083	-1061.0416

3.2.3. Selectivity

The after-effect of interferents (constituents of diluent II and DAC sample) on retention times of AAD upon

AAD analysis in diluent, working AAD solution (25µg/ml) and AAD spiked DAC sample (25 µg/ml) and DAC sample were investigated. Figs. 3 to 6 show the corresponding AAD chromatograms. The chromatogram findings demonstrate the presented approach's selectivity and the paucity of interference from diluent II and DAC sample components.

3.2.4. Precision

The method's precision was tested using six replicate batches DAC spiked with AAD using the procedure at a level concentration of 25µg/ml of AAD. The RSD for the AAD recovery measured results was very little than 4% (Table 2). The system's precision was tested using six replicate batches working AAD solution using the procedure at a level concentration of 25 µg/ml of AAD. The RSD for the AAD peak response measured results was very little than 2% (Table 2). These findings implying that the results were substantially reproducible and the technique was precise. This high precision proved ideal for analysing AAD in DAC sample for quality monitoring.

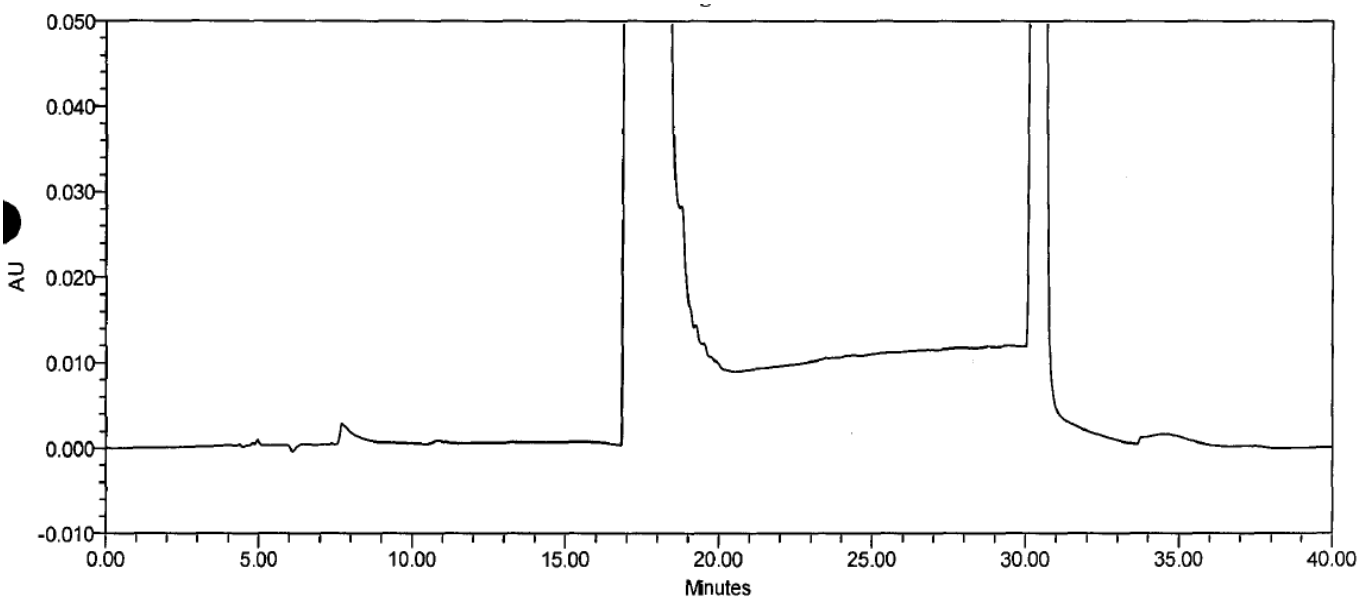

Fig. 3: Diluent II Chromatogram

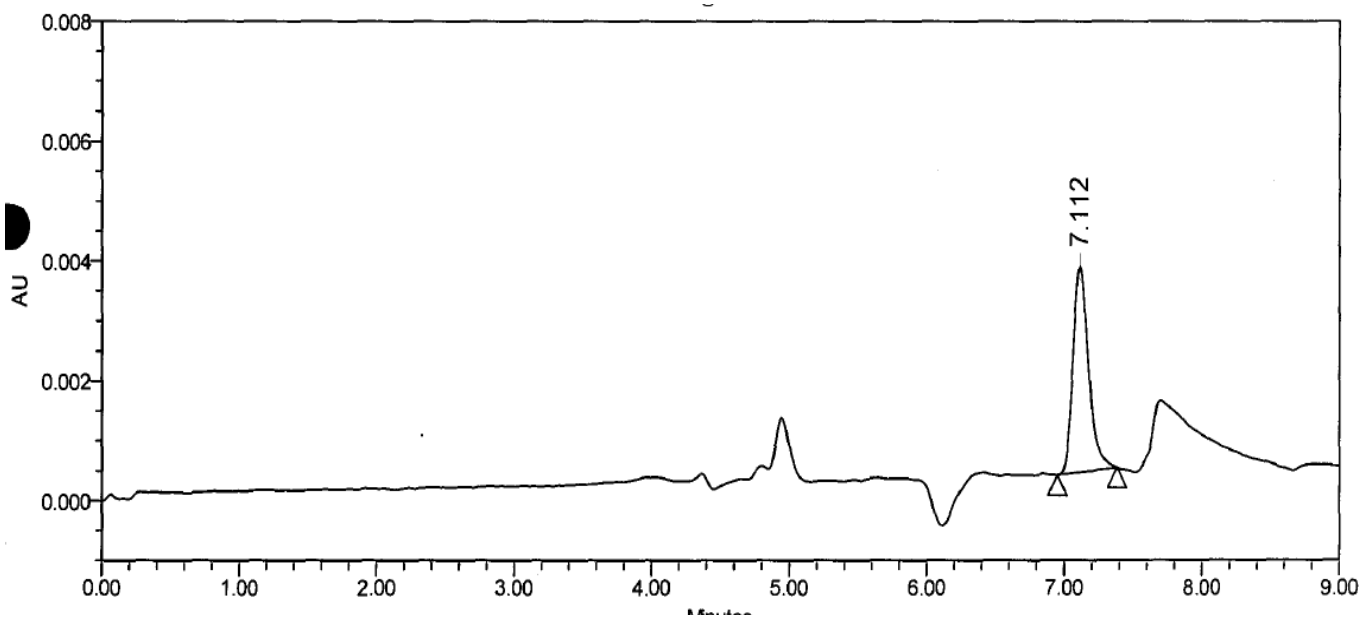

Fig. 4: Working AAD chromatogram

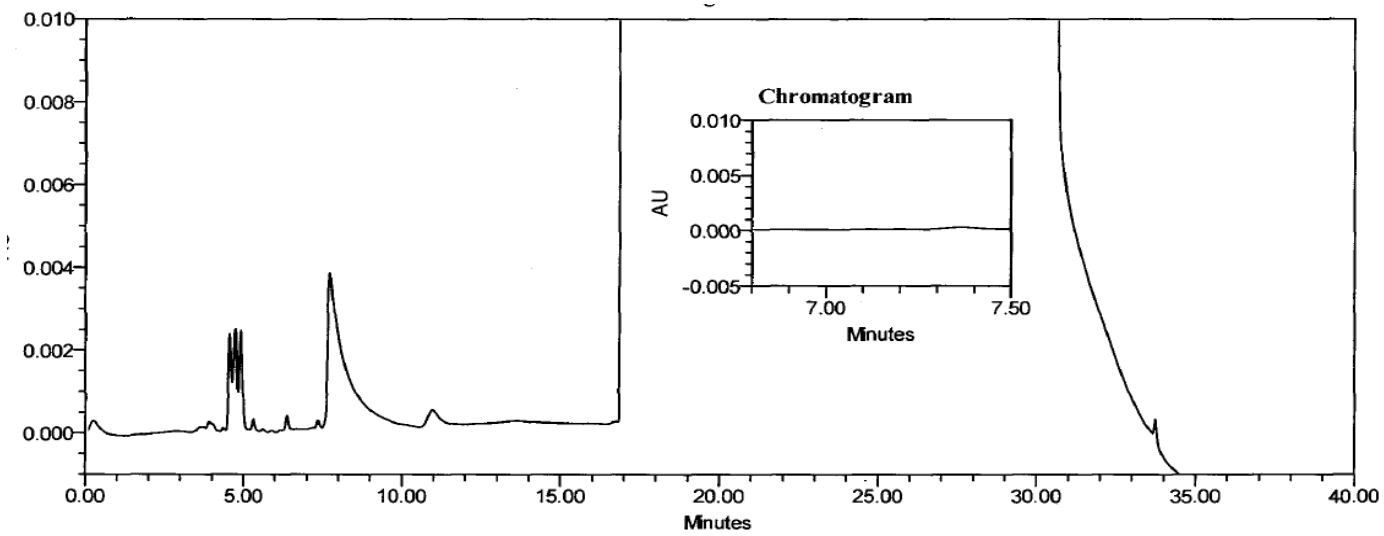

Fig. 5: DAC sample chromatogram

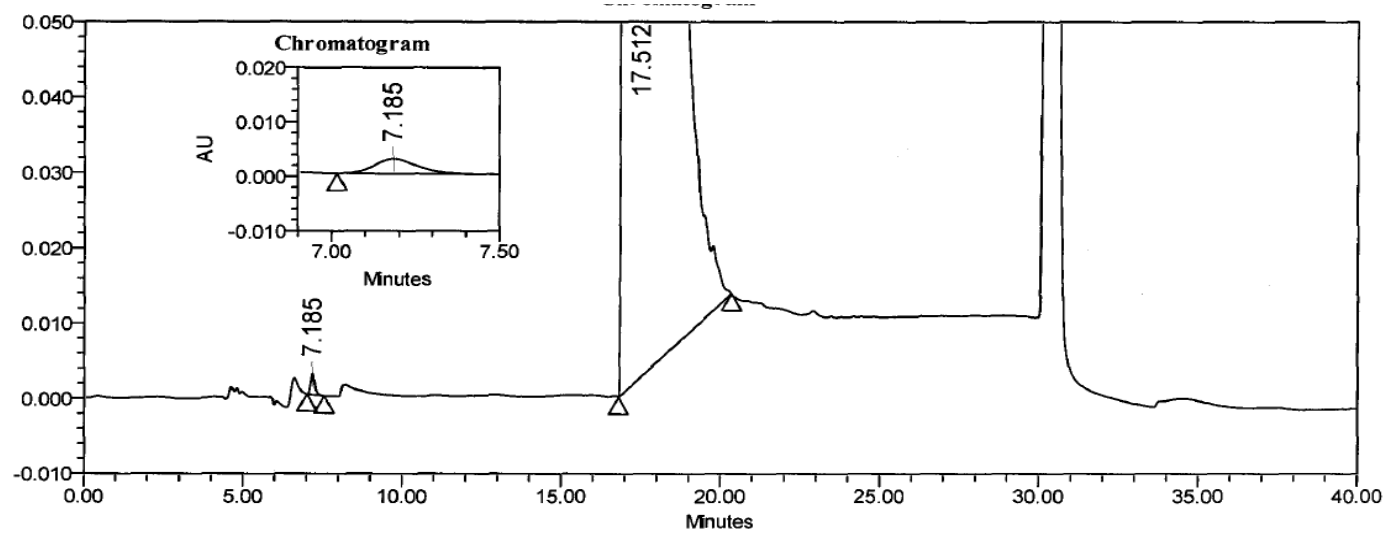

Fig. 6: AAD spiked DAC sample chromatogram

Table 2: AAD's Precision reports

AAD content (µg/ml)	AAD Peak area	AAD content (µg/ml)	AAD quantified (µg/ml)	AAD (%) recovery
25.11441	26008	24.94478	23.55819	94.4
25.11441	25711	24.98946	21.65604	86.7
25.11441	25573	25.00439	23.16876	92.7
25.11441	25401	24.96958	23.60705	94.5
25.11441	25278	24.97952	22.51548	90.1
25.11441	25178	24.94974	22.36547	89.6
Mean area value	25524.83	Mean recovery value		91.33
SD of area values	305.6085	SD of recovery values		3.0755
RSD of area values	1.197	RSD of recovery values		3.367

3.2.5. Accuracy

Revealed amounts (12.5 µg/ml -50% concentration level spiking; 25.0 µg/ml - 100% concentration level spiking; 30.0 µg/ml -1200% concentration level spiking) of the AAD were prepended to DAC sample, and then quantified by the procedure suggested. The

median recoveries achieved ranged from 111.3 to 113.0% at 50% concentration level spiking, 86.7 to 94.4% at 100% concentration level spiking and 90.9 to 95.7% at 120% concentration level spiking (Table 3). These findings support the presented method's accuracy and the paucity of influence from common excipients.

Table 3: AAD's recovery/accuracy reports

Quantity level spiking (%)	AAD added (µg/ml)	AAD quantified (µg/ml)	AAD (%) recovery
50	12.42499	14.00794	112.7
50	12.43483	14.04534	113.0
50	12.44716	13.84868	111.3
100	24.94478	23.55819	94.4
100	24.98946	21.65604	86.7
100	25.00439	23.16876	92.7
120	30.0320	28.51684	95.0
120	30.04715	28.76717	95.7
120	30.04116	27.29320	90.9

3.2.6. Robustness

The impact of differing chromatographic settings on AAD recovery measured while determining AAD in spiked DAC sample was studied. The robustness was experimented using DAC spiked with AAD using differing chromatographic settings at a level concentration of 25 µg/ml of AAD. AAD content recovery measured are comparable for each variation (Table 4). These findings support the presented method's robustness and the paucity of influence from slight distinctions in flow velocity, oven's temperature, mobile solvent phase's pH and wavelength for analysing AAD.

3.2.7. System suitability

System appropriateness tests were implemented to ensure that the resolution as well as repeatability of the system was competent for AAD analysis in DAC sample. The appropriateness of the system was measured by subjecting a freshly created working AAD sample, concentration 25 µg/ml, to the identical chromatographic settings six times, then recording the chromatograms. The measured AAD's peak areas, tailing factors and plate counts were noted for each validation criteria (Table 5). The scores meet system appropriateness criteria for analysing AAD in DAC sample for quality monitoring.

Table 4: AAD's robustness reports

Condition varied	AAD added (µg/ml)	AAD quantified (µg/ml)	AAD (%) recovery
Optimal settings	25.11441	23.55819	93.8
Flow velocity varied (ml/min)			
0.5	25.11441	25.26742	100.6
0.7	25.11441	24.79504	98.7
Temperature varied (°C)			
38	25.10841	26.75419	106.6
42	25.12040	24.10148	95.9
pH varied (units)			
2.8	25.12040	24.09497	95.9
3.2	25.13639	24.50074	97.5
Wavelength varied (nm)			
208	25.16837	25.70031	102.1
212	25.16837	25.64347	101.9

Table 5: System appropriateness reports

Parameter	AAD area RSD (%)	Plate counts	Tailing aspect
LOD, LOQ and Linearity	0.4	19438	1.1
Accuracy at 100, 120%, Method precision, System precision and Robustness actual	1.2	26334	1.2
Accuracy at 50%	0.5	25139	1.1
Robustness - Low flow	0.4	26448	1.2
Robustness - High flow	1.7	24237	1.1
Robustness - Low temperature	0.6	24759	1.2
Robustness - High temperature	0.7	25036	1.1
Robustness - Low Wavelength	0.9	24938	1.2
Robustness - High Wavelength	0.9	24018	1.2
Robustness - Low pH	2.3	23641	1.2
Robustness - High pH	1.7	24598	1.1
Selectivity	1.1	19338	1.3

4. CONCLUSION

Finally, the stated approach provides exact and reliable results for determining AAD in DAC samples. The method described here can be considered a helpful chromatographic technique for frequent quality control examination of AAD in DAC active medicinal ingredient, since it allows for easy, sensitive, and quick quantitative measurements.

5. ACKNOWLEDGEMENTS

Syed Anwar thanks the management of Mylan Laboratories Limited, Hyderabad for their support to do this work. Syed Anwar also thanks Dr. Suresh Babu Jayachandra, Dr. Arvind Gupta, Dr. Pijush Kanti Jana and Dr. Dharmendra Singh Kushwah for their genuine support to complete this work successfully.

6. REFERENCES

1. McCormack PL. *Drugs,* 2015; **75(5)**:515-524.
2. Keating GM. *Drugs,* 2016; **76(14)**:1381-1391.
3. Herbst DA, Reddy KR. *Expert Opin Investig Drugs.,* 2013; **22(10)**:1337-1346.
4. Poordad F, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, et al. *Hepatology*, 2016; **63(5)**: 1493-1505.
5. Bonora S, Puoti M. *AIDS Rev,* 2017; **19(1)**:24-34.
6. Mucenic M, Bandeira de Mello Brandao A, Marroni CA, Medeiros Fleck A Jr, Zanotelli ML, Kiss G, et al. *Transplant Proc.,* 2018; **50(3)**:769-771.
7. International Conference on Harmonization, ICH Q-3C (R4). Impurities: guideline for residual solvents, Geneva, Switzerland, 2009.
8. Noorbasha K, Shaik A. *Futur J Pharm Sci.,* 2021; **7**:40.
9. Ajay SR, Kamlesh C, Yogendrasinh P, Pooja S, Divyesh P, Chandrakant B, et al. *Chem Sci Trans.,* 2014; **3(3)**:983-988.
10. Gangrade MG, Deshpande NM, Gharat HP, More SD, Shinde SS, Raval VK, et al. *J Pharm Drug Deliv Res.,* 2018; **6(6)**:26-32.
11. International Conference on Harmonization, ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, 2005.
12. The United States Pharmacopoeia, Pharmacopeial Forum 30, 2004; 351-363.