Analytical Method Development and Validation for the Estimation of Residual Solvents in Acyclovir by Head Space Gas Chromatography

Chandrasekar R*[1], Tharani S[2], Sivagami B[3], Niranjan Babu M[4]

[1]*Associate Professor, Faculty of Pharmacy, Department of Pharmacognosy, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.*

[2] *PG Student, Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.*

[3]*Associate Professor, Faculty of Pharmacy, Department of Pharmaceutical Analysis, SevenHills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.*

[4]*Professor, Faculty of Pharmacy, Department of Pharmacognosy, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.*

Corresponding Author Email id

chandrumnrcop@gmail.com

Abstract

Organic volatile contaminants and Residual impurities present in pharmaceutical products are estimated by using Head Space Gas Chromatography. In this study a Head Space Gas Chromatography method was used to detect residual solvents and organic volatile contaminants present in Acyclovir Dosage form. Acyclovir is an antiviral drug used to treat viral infections**.** In this study an attempt was made to analyze the residual organic solvents such as Methanol and Acetone present in Acyclovir Dosage form by headspace gas chromatography (HS-GC). The carrier gas streamed was nitrogen, the method was developed and optimized by using DB-624 (30 m × 0.25 mm × 1.4 µm) column coupled with flame ionization detector. Capillary column consisting of 6 % cyanopropylphenyl - 94 % dimethyl polysiloxane was employed as stationary phase. An injector temperature of300°C was programmed to prevent degradation. A temperature of 40°C was set as the initial oven temperature for a period of 4 min and set at a rate of 30°C min−1 and monitored at a final temperature of 200°C for 6 min**.** N,N-dimethylacetamide was selected as the sample solvent. The validation studies were performed with regard to International Council for Harmonisation (ICH) Q2 guidelines for validation of analytical experiments. All the validation parameters complied with the specification limit. Hence, the optimized methoddeveloped and validated can be utilized for the concurrent detection of residual solvents intablet formulations.

Keywords: *Acyclovir Tablets; Acetone; GC-HS; Methanol; Organic Volatile Impurities; Residual Solvents;*

Introduction

Acyclovir is a class of antiviral drug used to treat herpes viruses, such as genital herpes, cold sores, shingles, and chickenpox. The chemical entity of Acyclovir is known as 2-amino-9-(2-hydroxyethoxymethyl)-1*H*-purin-6-one. Its structural formula is depicted in (Figure 1) The antiviral agent Acyclovir is a nucleoside analogue which is used in therapy of viral infections such as herpes and varicella-zoster. [1] Acyclovir is a synthetic derivative of guanosine, a purine nucleoside that has significant antiviral action against herpes simplex virus types 1 and 2, varicella-zoster virus, and other herpesviruses. Acyclovir blocks the action of viral DNA polymerase by integrating into the expanding viral DNA chain and stopping further polymerization after conversion in vivo to the active metabolite acyclovir triphosphate by viral thymidine kinase. [2]

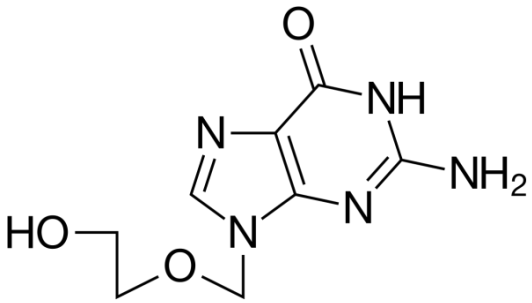

Figure 1 Structure of Acyclovir

Pharmaceutical residual contaminants and organic volatile solvents which are used or produced in the manufacture of finished products, drug excipients, in the preparation of drug substances and pharmaceutical formulations. Practical manufacturing methods do not completely eliminate residual solvents. [3] Organic solvents are entrapped within the formulation during the production of bulk drug manufacturing or during coating of solid dosage forms. These solvents are frequently used to dissolve film-coating materials so that they can be applied to formulations of tablets. [4]

Headspace Gas Chromatography (HS-GC) is an excellent technique for the qualitative or quantitative analysis of volatile contaminants and impurities in samples that can be effectively partitioned from either a matrix to be sampled into the headspace gas chromatography. It is also an excellent tool for the analysis of samples. The analysis of trace elements is also particularly amenable to Headspace gas chromatography. [5] Typical sample analysis of headspace analyses includes volatile organic compounds (VOC) from

contaminated samples and wastewater treatment, Residual solvents in pharmaceutical packaging, toxicology screening and blood alcohol, volatile components from food and alcoholic beverages and diagnostic gas analysis from oils. [6]

Hence numerous literatures were investigated few of the literatures were related to this research. A dispersive liquid–liquid microextraction (DLLME) method was developed and evaluated coupled with gas chromatography–mass spectrometry (GC-MS) for monitoring and quantification of class 1 residual impurities in pharmaceuticals formulations. [7] A highly validated RP-HPLC method was developed for the quantification of acyclovir in human plasma. [8] A cross-linked chitosan microspheres method was investigated by spray drying technique for the development and validation to estimate acyclovir (ACV) by liquid chromatographic method. [9] Another study was investigated for analysis of acyclovir in plasma samples obtained from healthy volunteers for the development and validation. [9] An Ultra Performance Liquid Chromatography (UPLC) method was evaluated for quantification of Acyclovir in lipid-based formulations. [10] A study was described to investigate the degradation pattern of acyclovir under various stress conditions (oxidation, hydrolysis, thermal decomposition and photolysis) in order to validate a stability-indicating HPLC method. [1] The study was investigated to develop and validate an analytical method for the estimation of toxic impurities present in the Acyclovir drug substance to the lowest possible level. [11] A new method was developed and validated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) micro-method to simultaneously quantify A and G from plasma and dried plasma spots (DPS). [12] A LC-MS/MS assay for the assessment of ganciclovir and acyclovir using deuterated standards of acyclovir and ganciclovir was developed. A rapid and highly validated LC-MS/MS method was developed for quantification of acyclovir and ganciclovir in human serum. [13] A liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS/MS) method was developed for the synchronous estimation of acyclovir and valacyclovir in human plasma using fluconazole as internal standard (IS). [14] A selective, precise and robust LC-MS/MS method was developed and validated for measuring of prodrug valacyclovir (VACV) and its metabolite acyclovir (ACV) in mouse and human plasma. [15] A precise, rapid and robust LC–MS–MS method was developed for the

synchronous quantification of acyclovir and valacyclovir in human plasma. [16] A simple, sensitive and precise method development and validation for the quantification of acyclovir and its evaluation towards the bioequivalence of drug formulations in human plasma was reported. [17]

Materials and Methods

Instruments used

The instruments and equipment's used for the study were

A Gas chromatograph equipped with a flame ionization detector,

A Headspace sampler Agilent – GC-HS 7890B Series with capillary column DB-624 consisting of 6 % cyanopropylphenyl and 94 % dimethyl polysiloxane stationary phase with 0.25 mm internal diameter, 30-meter length and film thickness of 1.4 µm was used.

An Analytical Balance – Radwag (semi-microbalance) or equivalent and

Micropipette – Eppendorf or equivalent were used.

Solvents and Chemicals

The chemicals used for the study were obtained from standard suppliers: Chloroform GC Grade or equivalent and N, N, Dimethylformamide GC Grade or equivalent were procured from (sigma-aldrich, Mumbai, India), Methanol and Acetone standards were procured from (E. Merck) were used. Acyclovir Dosage form 400 mg was obtained as a gift sample from Synthia Research Labs P. LTD.

Chromatographic condition:

The injection temperature was maintained at 300 °C at a split ratio of 30:1, the carrier gas streamed was nitrogen. The maintenance pressure was maintained at 14 psi with a column flow of 1 mL min-1. The detector temperature was programmed at 250 °C. Temperature gradient was maintained at 40 °C for 4 min and then increased at a rate of 30 °C min-1 up to 200 °C and maintained for 6 min. The zero-air flow was 300 mL/min, hydrogen gas flow was 30 mL/min the makeup flow was 15 mL/ min and run time was found to be 15 min.

Headspace sampler condition:

The oven temperature was maintained at 80°C, the needle temperature was 80°C and the transfer line temp was kept at 90°C. The GC cycle time was 45 minutes and thermostat time was 30 minutes. The Pressure equilibration time was 3 minutes the injection time was

set at 1.0 minute. The carrier pressure was maintained at 0.04 minutes and the withdraw time was 0.2 minutes. The headspace injector and GC conditions are provided in Table 1.

Table 1 Headspace Injector and GC conditions

Instrumentation conditions		**Head Space Gas Conditions**	
Column ID	Stationary phase: 6 % cyanopropylphenyl and 94 % dimethyl polysiloxane DB-624; 0.25 mm x 30 m; Column coated with 1.4 µm		
Carrier gas	Nitrogen	Oven Vial Incubation	80 ^{o}C
Column flow	1.0 mL/min	Needle	80 ^{o}C
Injection temperature	300 ^{o}C	Transfer Line	90 ^{o}C
Detector Temperature	250 ^{o}C	Carrier Pressure	0.04 min
Detector	FID	GC Cycle Time	45 min
Split	30:1	Thermostat Time	30 min
Hydrogen flow	30 mL/min	Pressurization Time	3 min
Zero flow	300 mL/min	Injection time	1.0 min
Make up flow	15 mL/min	Withdraw time	0.2 min

Description of Analytical Method

Method validation

The validation parameters studies were evaluated by performing accuracy, linearity, limit of detection (LOD) and limit of quantitation (LOQ), method precision, repeatability, ruggedness, specificity and system suitability of residual solvents as indicated in the ICH harmonised tripartite guidelines.

Preparation of standard stock solution:

The standard stock solution preparation was established by taking Methanol 0.06 gm and Acetone 0.1 gm was and transferred to a 50.0 mL volumetric flask and diluted to the required volume with N, N-Dimethylacetamide and mixed well. The standard chromatogram for Methanol is depicted in Figure 2

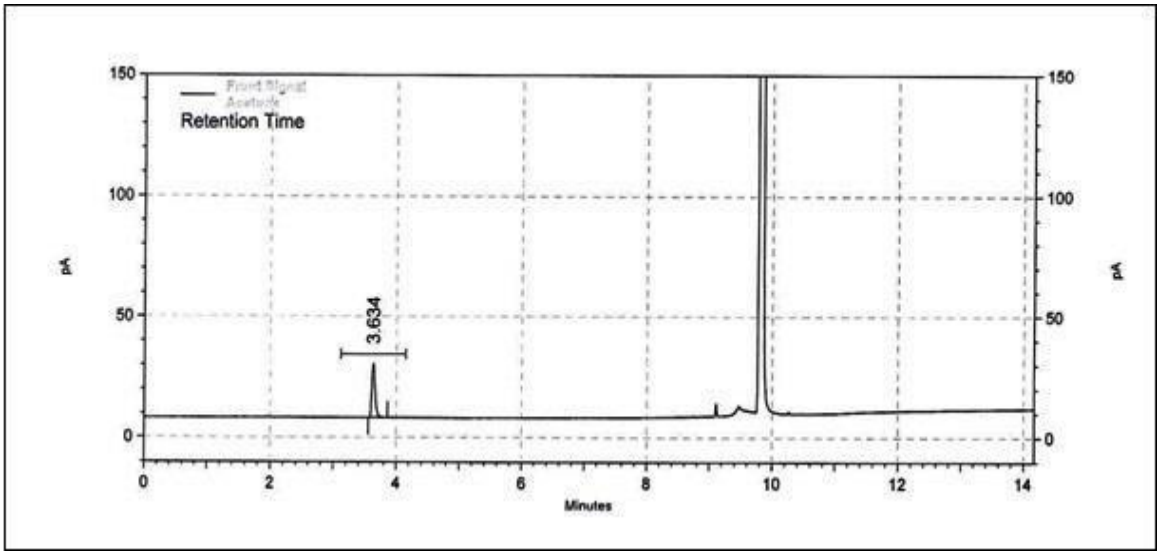

Figure 2 Standard Chromatogram of Methanol

Preparation of standard solution:

The Standard solution 1 mL was transferred to 50.0 mL volumetric flask and diluted to the volume with N, N-Dimethylacetamide, mixed well and 5 ml was transferred into 20 ml Headspace vial and sealed with crimp cap. The standard chromatogram for Acetone is depicted in Figure 3

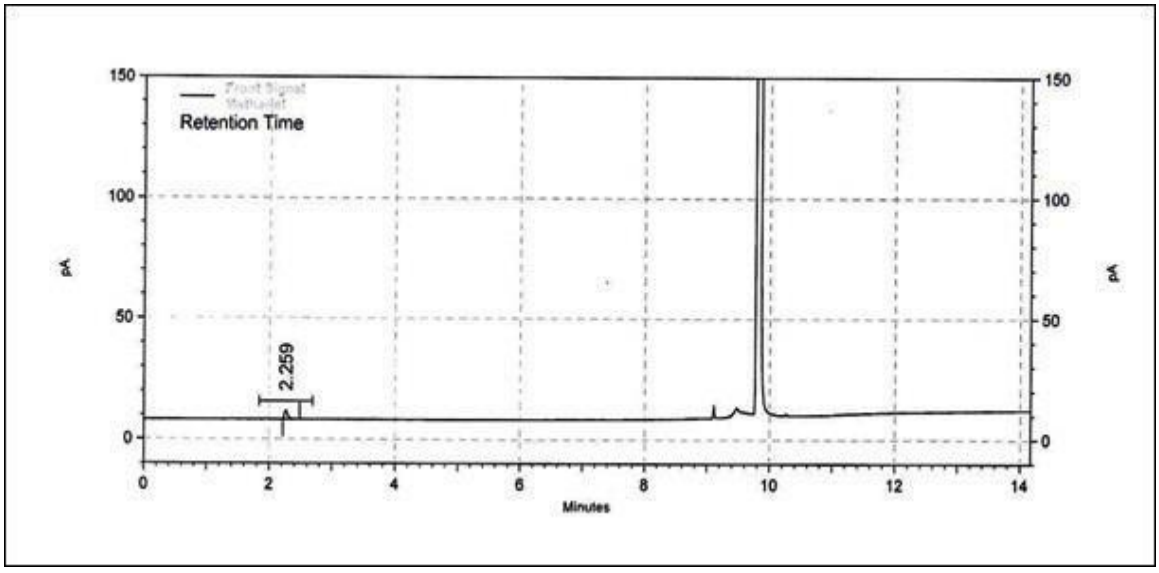

Figure 3 Standard chromatogram of Acetone

Preparation of sample solution:

The sample of 0.25 gm was weighted accurately into a tarred 20 ml Head space vial, the sample was dissolved using N, N-Dimethylacetamide, sealed with crimp cap and shaken for few seconds.

Results and Discussion

System suitability

A study was conducted to demonstrate the system suitability; standard solutions were prepared as per the test method and injected into GC/HS system. The system suitability parameters such as USP resolution and relative standard deviation for peak response of six replicate injections of standard solution was calculated and found to be within the limits. The results are summarized in Table – 2.

Table – 2 System suitability data for Methanol and Acetone

Injection No.	Peak area Methanol	Peak area Acetone
01	104096	626702
02	106696	641855
03	107493	639558
04	106970	635807
05	105947	630028
06	107191	638756
Average	106399	635451
SD	1244.71	5914.09
%RSD	1.2	0.9

Method Precision

The method precision of test method was evaluated by analyzing unspiked six samples and injected into GC/HS system. The Methanol and Acetone content in sample was calculated. The relative standard deviations of six preparation of each content (in ppm) were found to be within acceptance criteria. The results are summarized in Table – 3 & 4, Figure 4.

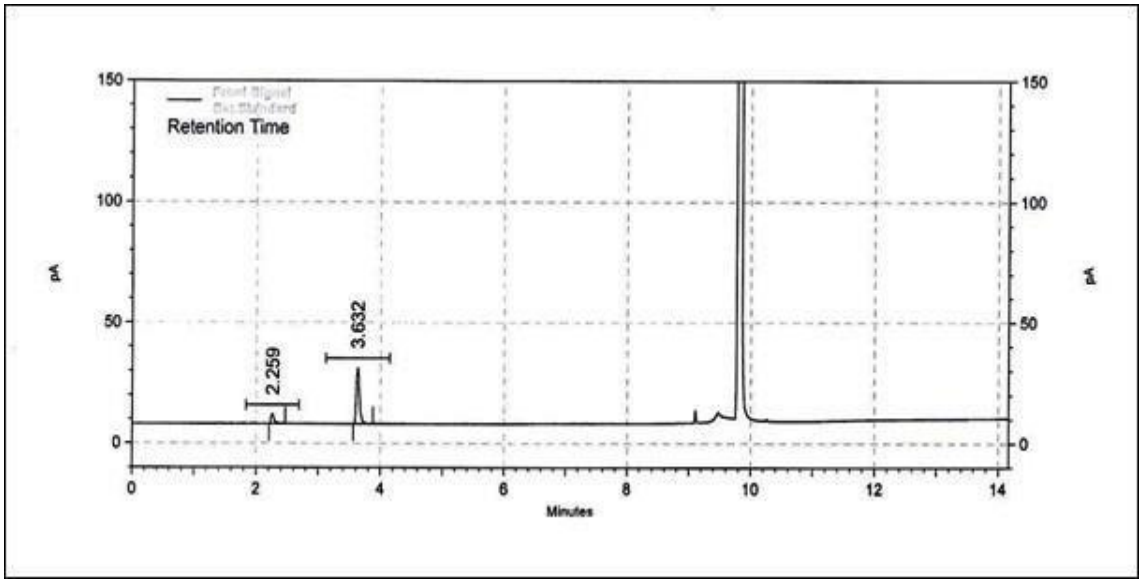

Figure 4 Chromatogram of Acetone and Methanol

Table 3 Precision data for Methanol and Acetone

Injection No.	Methanol Peak area	Acetone Peak area
Std-1	104096	626702
Std-2	106696	641855
Std-3	107493	639558
Std-4	106970	635807
Std-5	105947	630028
Std-6	107191	638756
Average	106399	635451
SD	1244.71	5914.09
% RSD	1.2	0.9

Table 4 – Precision data for Methanol and Acetone

Area of Methanol	Content of Methanol in (ppm)	Content of Acetone in (ppm)
23029	65	ND
20984	59	ND
21459	60	ND
20310	58	ND

21123	59	ND
22287	62	ND
Average	60	NA
SD	2.86	NA
% RSD	4.7	0.0

Specificity

A study was conducted to demonstrate the Blank, standard solution, test solution and individual standard solutions as per method of analysis were prepared and injected into GC/HS system. Chromatograms were evaluated for the interference of blank peaks at the retention time of known peaks in all the solutions. The specificity results are summarized in Table 5

Table 5 – Specificity data for Methanol and Acetone

Name of the peak	Retention time in Minutes
Methanol	2.26
Acetone	3.63

Determination of LOD

The LOD was determined by taking Methanol 0.06 gm and 0.1 gm Acetone were accurately weighted and transferred into a tared 50.0 ml volumetric flask and diluted to the volume with N, N-Dimethylacetamide and mixed well. Standard stock solution of 1.0 ml was transferred to 50.0 ml volumetric flask diluted to the volume with N, N-Dimethylacetamide, 2.5 ml of the above stock solution was transferred to 50.0 ml volumetric flask and diluted to the volume with N, N-Dimethylacetamide. The results are summarized in Table – 6 & 7, Figure 5.

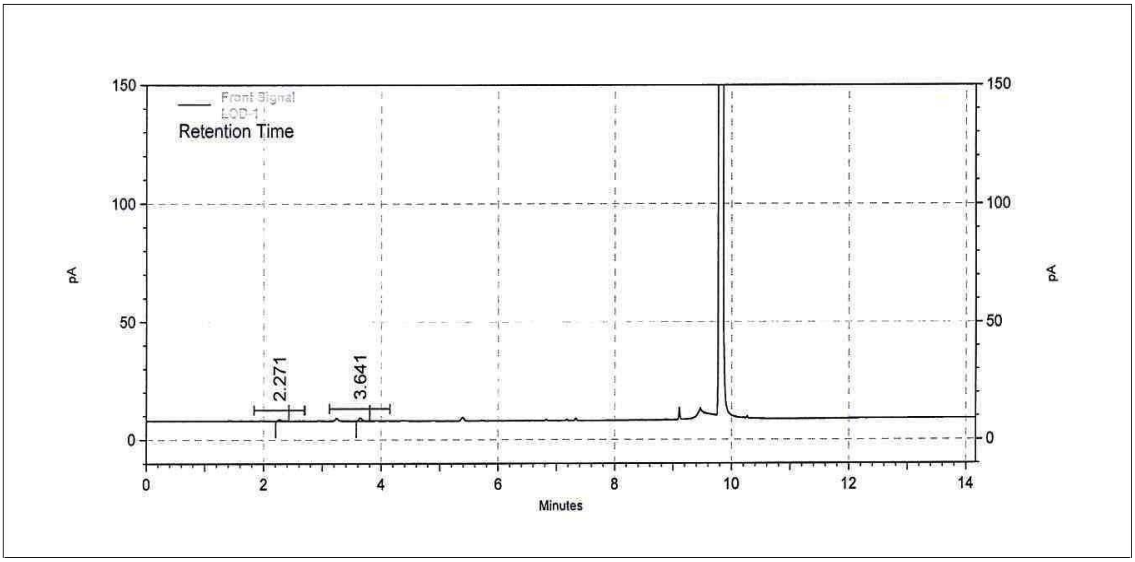

Figure 5 Chromatogram of Methanol and Acetone

Table – 6 Establishment of LOD

Injection No	Methanol	Acetone
Std-1	98715	604881
Std-2	98636	608957
Std-3	97215	597055
Std-4	97592	597075
Std-5	96456	590664
Std-6	99584	603956
Average	98033	600474
SD	1147.59	6652.99
%RSD	1.2	1.1

Table – 7 Establishment of LOD

Injection No	Methanol	Acetone
Std-1	21282	41282
Std-2	20628	40823
Std-3	20936	41662
Std-4	21462	42164
Std-5	20282	41090

Std-6	21167	40922
Average	20959.5	41323.83
SD	440.2316	507.734
%RSD	2.1	1.2

To demonstrate the linearity of test method, the standard solutions were prepared from 50% to 150% of the targeted concentration and analyzed as per the method. The correlation coefficient and Y-intercept were calculated and found to be within the acceptance criteria. The results are summarized in Table – 8.

Table 8 Linearity data for Methanol and Acetone

Methanol			**Acetone**		
Conc. ppm	**Area**	**Avg. Area**	**Conc. ppm**	**Area**	**Avg. Area**
7.27	62136	62150	20.14	314207	314205
	62164			314203	
10.91	88755	88731	30.20	480521	480167
	88706			479812	
14.54	113326	113126	40.27	624002	629669
	112936			635336	
18.17	133241	132918	50.34	768808	770930
	132595			773063	
21.81	158558	158696	60.41	923252	933280
	158843			943307	
	Slope	6528		Slope	15185
	Intercept	16207		Intercept	14096.28
	CC	0.9990		CC	0.9996
	Regression	1985.22		Regression	7405.51

Based on the linearity, precision and accuracy data, the range of the test method was from 50 % to 150% of the target concentration. The evaluated concentration for Methanol was (i.e., 7.27 ppm to 21.81 ppm) and Acetone was (i.e., 20.14 ppm to 60.41 ppm) Linearity Plot of Methanol and Linearity Plot of Acetone are depicted in Figures 6 & 7

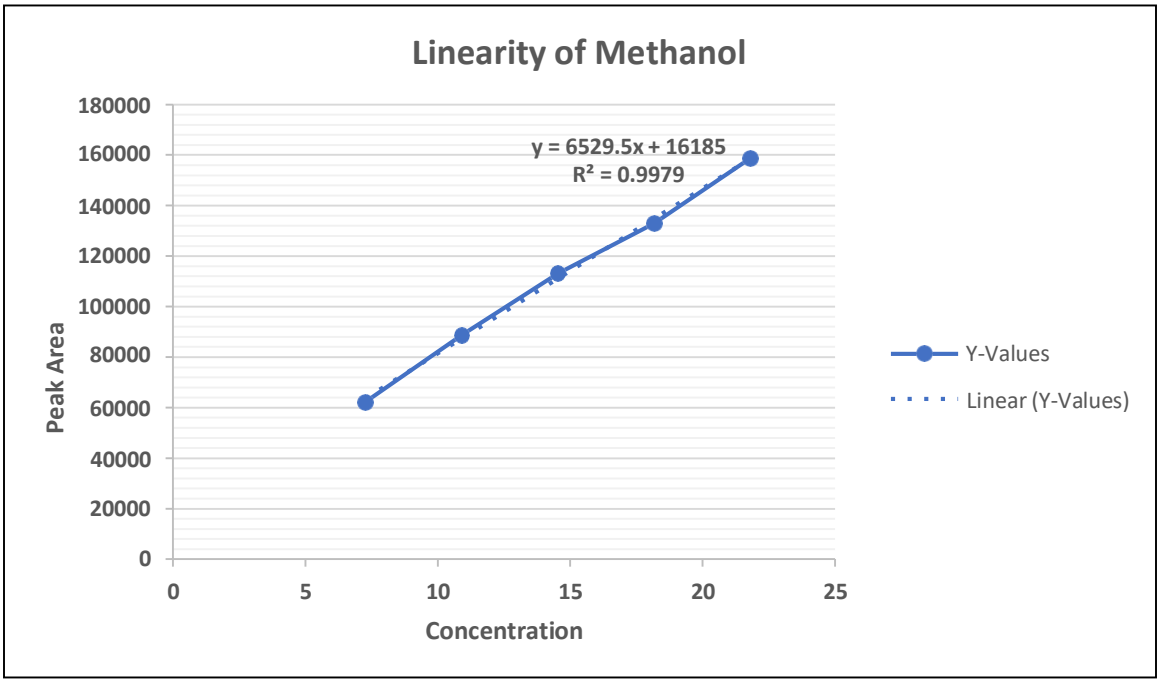

Figure 6 Calibration curve for Methanol

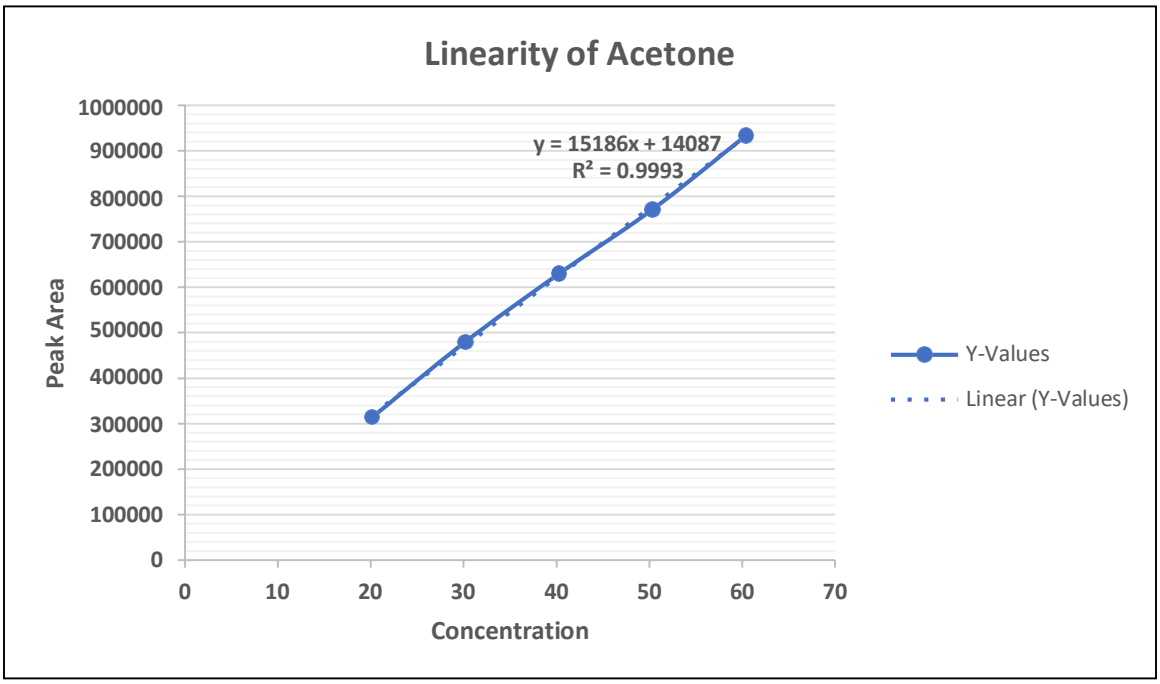

Figure 7 Calibration curve for Acetone

Ruggedness

The intermediate precision of test method was evaluated by analyzing unspiked six samples and injected into GC/HS system. The study was performed on different day and different analyst. the Methanol and Acetone content in sample were calculated. The relative standard deviations of six preparation of each content (in ppm) were found to be within acceptance criteria. The results are summarized in Table –9 & 10, Figure 8.

Table –9 Intermediate precision data

Injection No.	Methanol	Acetone
Std-1	111417	617704
Std-2	110120	611090
Std-3	109817	614144
Std-4	110700	608200
Std-5	109526	608809
Std-6	112035	621865
AVG	110603	613635
SD	973	5363.87
%RSD	0.9	0.9

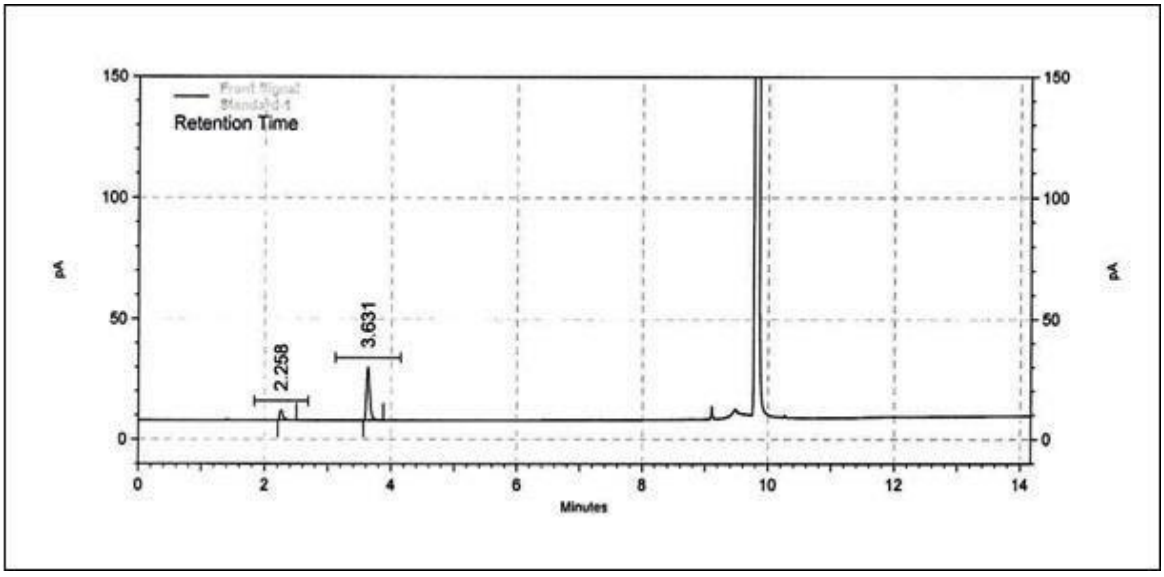

Figure 8 Precision Chromatogram

Table –10 Intermediate precision data

Area of Methanol	Content of Methanol (ppm)	Content of Acetone (ppm)
26563	72	ND
27043	73	ND
27772	75	ND
27485	74	ND
26383	71	ND
27106	73	ND
Average	73	
SD	1.46	
% RSD	2.0	

Accuracy/ Recovery

To demonstrate the accuracy of test method, recovery of Methanol and Acetone from spiked samples was evaluated. Samples were prepared by spiking Methanol and Acetone with sample at different levels ranging from 50%, 100% and 150% of the target concentration of known standards. The sample solutions were prepared in triplicate at 50%, 100% and 150% spike levels and subtract the content from the unspiked sample. The results for Accuracy are summarized in Table – 11 & 12, Figure 9.

Table – 11 Accuracy data

Recovery level	Sample No	'ppm' added	'ppm' recovered	% Recovery
50%	1	12.07	11.97	99.14
	2	12.07	11.92	98.73
	3	12.07	11.65	96.49
100%	1	24.15	24.33	100.75
	2	24.15	24.71	102.33
	3	24.15	25.20	104.36
150%	1	36.22	36.33	100.30

	2	36.22	33.40	92.21
	3	36.22	32.74	90.39
			Average	98.30
			% RSD	4.65

Table – 12 Accuracy data

Recovery level	Sample No	'ppm' added	'ppm' recovered	% Recovery
50%	1	19.95	20.18	101.15
	2		20.48	102.65
	3		20.72	103.86
100%	1	39.90	39.85	99.87
	2		40.03	100.32
	3		39.9	100.00
150%	1	59.85	60.03	100.30
	2		59.18	98.88
	3		59.54	99.48
			Average	100.72
			% RSD	1.58

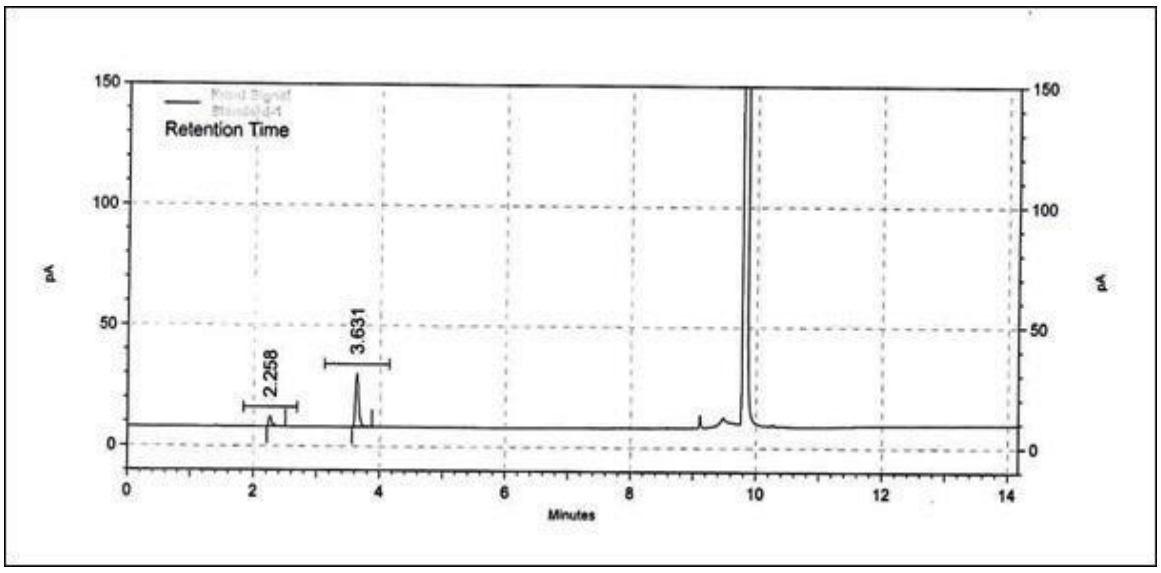

Figure 9 Accuracy Chromatogram for Methanol and Acetone

Discussion

Residual solvents are organic volatile contaminants that may be present or produced in the production of drug excipients or substances produced in the preparation of drug formulations. These solvents may not be removed completely by practical production process. The importance of quality control in identification of impurities in API and drug formulations and implementing various analytical techniques in development of an analytical method for the estimation of organic volatile contaminants like Methanol and Acetone in Acyclovir tablet in API and formulations of tablets by GC-HS with flame ionization detector. The purpose of this research was to create an effective, fast, robust and specific GC-HS method for the concurrent estimation of organic volatile contaminants such as Methanol and Acetone in Acyclovir tablet by using the most frequently used DB-624, 60 m, 0.32 mm, and 3.0 µm columns with flame ionization detector.

The validation parameters compiled with the specification limit according to ICH guidelines. The system suitability the % RSD of six replicate standard injections was NMT 15.0% and the value of Methanol and Acetone was observed to be 1.2 and 0.9. In method precision the % RSD of the content of Methanol and Acetone residual solvent in six samples should be NMT 15.0 % the precision limit for Methanol and Acetone was observed to be 1.2 and 0.9. There should be no interference at the retention time of the test solution from blank solution and standard solutions for specificity. There was no interference at the retention time of the test solution from blank solution as per the test method. The determination of LOD was observed to be in the range of 1.2 and 1.1 ppm for Methanol and Acetone. In linearity the correlation coefficient should be NLT 0.99 for Methanol it was observed to be 0.9979 and for Acetone it was observed to be 0.9993. In Ruggedness the % RSD of the content of Methanol and Acetone residual solvent in six samples should be NMT 15.0 %. Cumulative % RSD for method precision & intermediate precision should be NMT 20.0 %. It was around 0.9 for Methanol and 0.9 for Acetone. In Accuracy the % recovery of standard solution should be between 85%-115%, for Methanol and Acetone it was observed in the range of 93.24% to 102.17%, the validation parameters for Methanol and Acetone.

Conclusion

The GC-HS method developed and validated was found to be concise and consecutive for the quantification of Methanol and Acetone residual solvent in Acyclovir. The preparation procedure for samples and standard is simple, rapid and very sensitive. The proposed method is acceptable in quality or quantity for the purpose of concurrent investigation of residual impurities and organic volatile solvents in the pharmaceutical dosage forms. This method can be consecutively applied for the estimation in marketed formulations.

List of Abbreviations

GC-HS : Gas chromatography with Headspace; RSD: Relative Standard Deviation; LOD: Limit of Detection; LOQ: Limit of Quantitation; mL: milliliter; %: Percentage; ppm: Parts per million; g: Gram; mg: milligram; min: minutes; Std: Standard; Conc.: Concentration; IPA: Isopropyl alcohol; DCM: Dichloromethane; NLT: Not less than; NMT: Not more than

Acknowledgements

Authors express their sincere gratitude to Seven Hills College of Pharmacy, Tirupati, for continuous motivation, support, and guidance for research activity and for providing all required facilities to accomplish the entitled work

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data and material are available upon request.

Competing interests

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST.

Funding

Not applicable

Authors' contributions

All the authors have equally contributed to the article.

References

[1] V. R. Sinha, A. Trehan, M. Kumar, S. Singh, and J. R. Bhinge, "Stress studies on acyclovir.," *J. Chromatogr. Sci.*, vol. 45, no. 6, pp. 319–324, Jul. 2007, doi: 10.1093/chromsci/45.6.319.

[2] A. Ärlemalm, A. Helldén, L. Karlsson, and B. Carlsson, "Rapid determination of acyclovir, its main metabolite 9-carboxymethoxymethylguanine, ganciclovir, and penciclovir in human serum using LC-MS/MS.," *Biomed. Chromatogr.*, vol. 36, no. 4, p. e5315, Apr. 2022, doi: 10.1002/bmc.5315.

[3] Y. Liu and C.-Q. Hu, "Preliminary identification and quantification of residual solvents in pharmaceuticals using the parallel dual-column system.," *J. Chromatogr. A*, vol. 1175, no. 2, pp. 259–266, Dec. 2007, doi: 10.1016/j.chroma.2007.10.042.

[4] K. Grodowska and A. Parczewski, "Analytical methods for residual solvents determination in pharmaceutical products.," *Acta Pol. Pharm.*, vol. 67, no. 1, pp. 13–26, 2010.

[5] L. S. Ettre, "Headspace-gas chromatography: an ideal technique for sampling volatiles present in non-volatile matrices.," *Adv. Exp. Med. Biol.*, vol. 488, pp. 9–32, 2001.

[6] K. Karthikeyan, G. T. Arularasu, P. Devaraj, and K. C. Pillai, "Determination of residual epichlorohydrin in sevelamer hydrochloride by static headspace gas chromatography with flame ionization detection.," *Sci. Pharm.*, vol. 78, no. 4, pp. 835–846, 2010, doi: 10.3797/scipharm.1007-20.

[7] R. Heydari and S. Azizi, "Rapid Monitoring and Determination of Class 1 Residual Solvents in Pharmaceuticals Using Dispersive Liquid–Liquid Microextraction and Gas Chromatography–Mass Spectrometry," *J. Chromatogr. Sci.*, vol. 53, no. 6, pp. 1020–1025, 2014, doi: 10.1093/chromsci/bmu147.

[8] S. Muralidharan, J. Kalaimani, S. Parasuraman, and S. A. Dhanaraj, "Development and Validation of Acyclovir HPLC External Standard Method in Human Plasma: Application to Pharmacokinetic Studies," *Adv. Pharm.*, vol. 2014, p. 284652, 2014, doi: 10.1155/2014/284652.

[9] H. K. Stulzer, M. P. Tagliari, F. S. Murakami, M. A. S. Silva, and M. C. M.

Laranjeira, "Development and validation of an RP-HPLC method to quantitate acyclovir in cross-linked chitosan microspheres produced by spray drying.," *J. Chromatogr. Sci.*, vol. 46, no. 6, pp. 496–500, Jul. 2008, doi: 10.1093/chromsci/46.6.496.

[10] K. A. Al-Amri, K. Mohsin, and F. K. Alanazi, "Development and validation of a UPLC method for quantification of antiviral agent, Acyclovir in lipid-based formulations," *Arab. J. Chem.*, pp. 1707–1714, 2019, doi: 10.1016/j.arabjc.2014.08.024.

[11] N. Sivanandham and B. K. Kempegowda, "Identification and Determination of Potential Impurities Present in the Acyclovir Drug Substance by GC-MS," *Current Pharmaceutical Analysis*, vol. 13, no. 5. pp. 473–480, 2017. doi: http://dx.doi.org/10.2174/1573412913666170321110612.

[12] F. Pigliasco *et al.*, "A UHPLC-MS/MS Method for Therapeutic Drug Monitoring of Aciclovir and Ganciclovir in Plasma and Dried Plasma Spots.," *Biomedicines*, vol. 9, no. 10, Oct. 2021, doi: 10.3390/biomedicines9101379.

[13] A.-G. Märtson, K. van Hateren, G. van den Bosch, T. S. van der Werf, D. J. Touw, and J. W. C. Alffenaar, "Determination of Ganciclovir and Acyclovir in Human Serum using Liquid Chromatography-Tandem Mass Spectrometry," *J. Appl. Bioanal.*, 2018.

[14] M. Yadav, V. Upadhyay, P. Singhal, S. Goswami, and P. S. Shrivastav, "Stability evaluation and sensitive determination of antiviral drug, valacyclovir and its metabolite acyclovir in human plasma by a rapid liquid chromatography-tandem mass spectrometry method.," *J. Chromatogr. B, Anal. Technol. Biomed. life Sci.*, vol. 877, no. 8–9, pp. 680–688, Mar. 2009, doi: 10.1016/j.jchromb.2009.01.042.

[15] J. Shi, Y. Hu, D. E. Smith, and H.-J. Zhu, "A sensitive liquid chromatography-tandem mass spectrometry method for the quantification of valacyclovir and its metabolite acyclovir in mouse and human plasma.," *J. Chromatogr. B, Anal. Technol. Biomed. life Sci.*, vol. 1092, pp. 447–452, Aug. 2018, doi: 10.1016/j.jchromb.2018.06.040.

[16] R. Kanneti, R. Rajesh, J. R. Aravinda Raj, and P. A. Bhatt, "An LC–MS–MS Method for the Simultaneous Quantitation of Acyclovir and Valacyclovir in Human

Plasma," *Chromatographia*, vol. 70, no. 3, pp. 407–414, 2009, doi: 10.1365/s10337-009-1171-3.

[17] D. Zendelovska *et al.*, "Determination of Acyclovir in Human Plasma Samples by HPLC Method with UV Detection: Application to Single-Dose Pharmacokinetic Study.," *Open access Maced. J. Med. Sci.*, vol. 3, no. 1, pp. 32–36, Mar. 2015, doi: 10.3889/oamjms.2015.011.

Author Information

First Author: Chandrasekar Raju, Associate Professor, Faculty of Pharmacy, Department of Pharmacognosy, Pharmaceutical Sciences, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.

Second Author: Tharani Sathyanarayana, Post Graduate Student, Department of Pharmaceutical Analysis, Pharmaceutical Sciences, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.

Third Author: Sivagami B, Associate Professor, Faculty of Pharmacy, Department of Pharmaceutical Analysis, Pharmaceutical Sciences, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.

Fourth Author: Dr. Niranjan Babu M, Professor, Faculty of Pharmacy, Department of Pharmacognosy, Pharmaceutical Sciences, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Chittoor, Andhrapradesh, India.

Corresponding Author **Email id chandrumnrcop@gmail.com**