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Alternative liquid chromatographic method to gas chromatography for the determination of acetic acid in pharmaceuticals

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ABSTRACT

A simple, accurate and precise validated method has been developed for determination of acetic acid fro pharmaceuticals. 0.1% Ortho phosphoric acid is used as a Mobile phase A with Acetonitrile (Mobile phase B). Separation of Acetic acid achieved using L1 column of 150x4.6mm, 5µ dimension. Flow rate was 1.0mL/minute and 210 nm was the detector wavelength. Method has been extensively validated as per ICH guideline for parameters like Specificity, Linearity, Precision, Accuracy, solution stability, filter compatibility and robustness. Accuracy for Acetic acid is 100.8%. Correlation coefficient for Acetic acid is found 0.99, all other validation parameters found ok. Literature survey revealed that, there is no method reported for the determination of Acetic acid in pharmaceuticals by using liquid chromatography. The proposed method is very simple, accurate, precise and alternative option to Gas chromatography. Hence, this validated novel liquid chromatographic method can be easily and conveniently adopted for routine analysis of Acetic acid content from pharmaceuticals.

Keywords: Reverse phase, development, validation, Acetic acid, Chromatography

INTRODUCTION

Residual solvents are organic volatile chemicals which are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. In practical manufacturing techniques, it is very difficult to remove completely. The residual solvent plays very vital role and a critical parameter in the synthesis of drug substance. Solvents have been using to enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, determination and justification of the content of solvents in such products are mandatory. To determine

residual solvents from pharmaceuticals, accurate analytical methods are required.

High performance liquid chromatography (HPLC) is a powerful analytical tool and most preferable analytical technique used in pharmaceutical industries [1-5].

In pharmaceutical and chemical industries, there are number of analytical techniques have been used for analysis and development of method of analysis for pharmaceuticals and chemicals. These techniques include Liquid chromatography or High performance liquid chromatography (HPLC), Gas chromatography (GC), Ultraviolet spectroscopy (UV), Titrations etc. Among these techniques Liquid chromatography or in pharmaceutical drugs,

Residual solvents have no any therapeutic advantage that's why it is necessary to remove all residual solvents to the extent possible to meet product specifications [6], good manufacturing practices, or other quality.

Acetic acid is a widely used residual solvent in pharmaceuticals. Acetic acid helps to facilitates reactions that follow polar mechanisms. Acetic acid is a hydrophilic solvent with a high boiling point with the formula CH_3COOH [7]. Structure of Acetic acid is shown in figure 1.

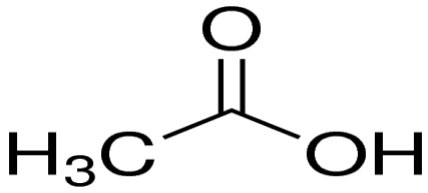

Fig 1: Structure of Acetic acid

Proposed method is alternative liquid chromatographic method to Gas chromatography for the determination of Acetic acid from the pharmaceuticals. Our extensive Literature survey revealed that there is no method reported for determination of Acetic acid in pharmaceuticals by using HPLC. This proposed method is novel work and will give alternative option to Gas chromatographic method for determination of Acetic acid from pharmaceuticals. Method has been developed and validated as per ICH guideline [8]. All parameters meet specifications . Therefore this method can be easily adopted for routine quality analysis for determination of Acetic acid.

MATERIALS AND METHODS

Chemicals and reagents

Acetic acid and pharmaceutical samples to be tested were procured from market. Milli Q grade water, ortho phosphoric acid (GR grade) and acetonitrile (HPLC grade) were used for method development. Auto sampler high performance liquid Chromatograph Dionex along with UV and PDA detector was used.

Chromatographic Conditions for developed method

0.1% Ortho-phosphoric acid is used as a mobile phase (A) and Methanol is mobile phase (B) for separation by using gradient programme as follows

Time	Mobile phase	Ratio
0	A→B	100→0
14	A→B	100→0
20	A→B	30→80
30	A→B	30→80
32	A→B	100→0
40	A→B	100→0

Good peak symmetry and resolution was achieved on L1 250x4.6mm, 5 µ.

Column with 1.0mL/minute flow rate at 25° column oven temperature. 210nm was the UV wavelength selected for the detection of Acetic acid peak. the injection volume was 20µL. Under these chromatographic conditions, Acetic acid peak eluted at about 5 minutes.

Preparation of Diluent

Diluent was prepared by adding 2 mL of ortho phosphoric acid in 1 liter of water i.e. 0.2% orthophosphoric acid in water.

Preparation of Acetic Acid standard solutions

Accurately weighed 50 mg of Acetic acid standard was taken in a 100 mL volumetric flask. About 60 mL diluent was added and mixture was dissolved by sonication and it was diluted up to mark with diluent.
10 mL of this solution was transferred to 50 mL volumetric flask and diluted up to mark with diluents. This final standard solution contains about 100 ppm of Acetic acid.

Preparation of sample solution

Accurately weighed 100 mg of sample to be analysed into 5 mL volumetric flask to which 3 mL of diluent was added and the mixture was sonicated for about 45 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with diluent up to the mark. Filtered sample solution through 0.45 µm Nylon syringe filter.

RESULTS AND DISCUSSION

Method development

For effective method development for determination of Acetic acid, lot of developmental parameters were studied which includes but not limited to Solubility, Selection of diluents, UV Detection Wavelength, Selection of working pH range for Mobile phase, Choice of Buffer, Buffer Concentration, Selection of Columns and final method optimization.

To select proper diluents for standard and sample solution, it's very important to know the solubility or miscibility of compound/s to be used for method development. Therefore solubility of compound/s to be used in method development was checked in some HPLC compatible solvents like Methanol, Acetonitrile, Tetrahydrfuran, Isopropyl alcohol, water well as mixture of solvents and water. Solubility in acidic and basic media was also checked. On the basis of solubility study and some initial trials 0.2% orthophosphoric acid was selected as diluents for this method development. Both the compounds showing comparatively better response at 210nm UV wavelength, therefore 210nm wavelength was selected for this method after comparative study.

Initialy, trials were conducted by using acidic and basic pH of mobile phase and after comparative study it was observed that, acidic pH is favorable for separation of these compounds. Therefore 0.1% Orthophosphoric acid was selected for the mobile phase (A) and methanol as mobile phase (B).

Column selection is the major part in the reverse phase chromatography for method development, column acts as stationary phase. In reverse phase chromatography, very wide range of columns can be used as a stationary phase depending on column chemistry. These columns include C-18, C-8, Cyano, phenyl, amide etc. On the basis of literature survey, number of runs was taken on 25 and 15cm columns of different make having 4.6 mm diameter and 5µ particle size. After very extensive study and comparisons, Inertsil ODS 150 x 4.6 mm, 5.0 µ particle size column was selected for method development.

Finally, Method was optimized by making required changes in mobile phase composition, flow rate, column oven temperature, standard and sample concentration. Effect of each individual parameter was studied on separation.
After several trials and optimization, final parameters selected which gives better separation between Acetic acidwith good peak shapes and resolution between both peaks.

Representative chromatograms of standard solution and sample solution are shown in figure 2a and 2b respectively.

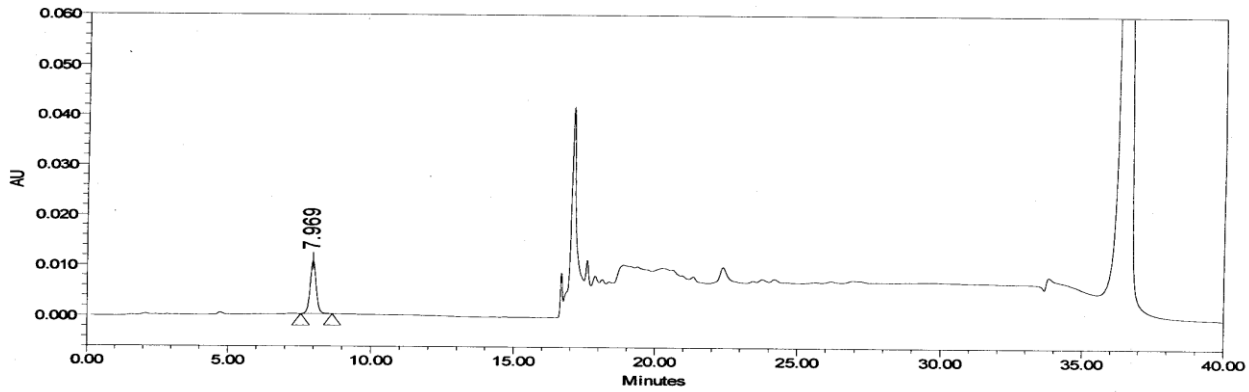

Fig 2a: Chromatogram – Standard solution

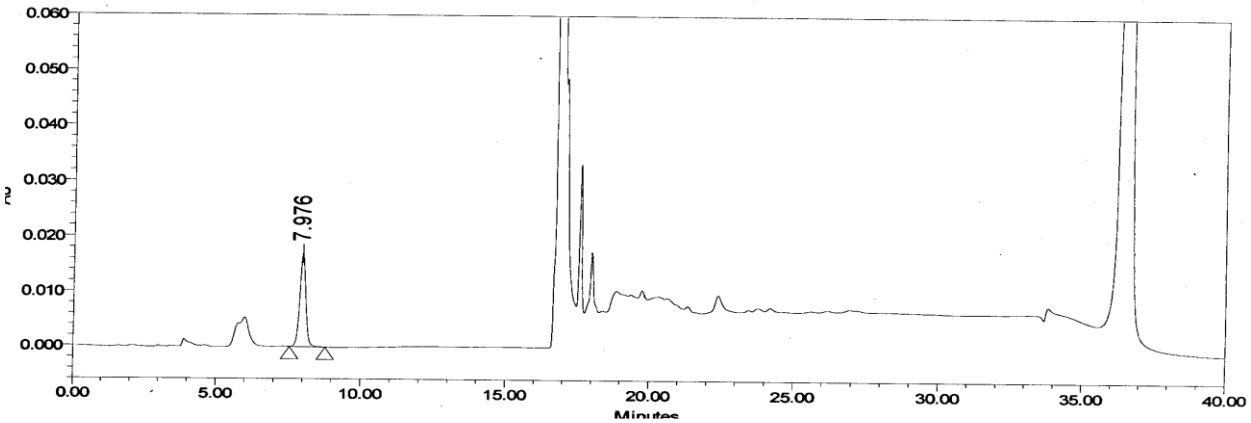

Fig 2b: Chromatogram – Sample solution

Method validation

Method validation of developed method was extensively performed as per International Conference on Harmonization (ICH) guideline. To validate the method parameters like specificity, Linearity, Precision, Accuracy, Robustness, Solution stability, Filter compatibility and System suitability were performed as per ICH guideline.

Specificity

Interference from diluents was checked at the retention time of Acetic acid peaks. It was observed that at the retention time of Acetic acid, no any interference was there from the diluent. For identification purpose, Retention time of Acetic acid peak in sample solution matches the retention time of Acetic acid peak in standard solution. This result shows that method is specific enough for determination of Acetic acid without any interference.

Linearity

Linearity was performed by preparing five solutions of different concentrations in the range of 50% to 150% of working concentration of Acetic acidi.e. 50%, 80%, 100%, 120% and 150%. Correlation coefficient obtained from graph was 0.999. Linearity curves of Acetic acid are shown in figure 3.

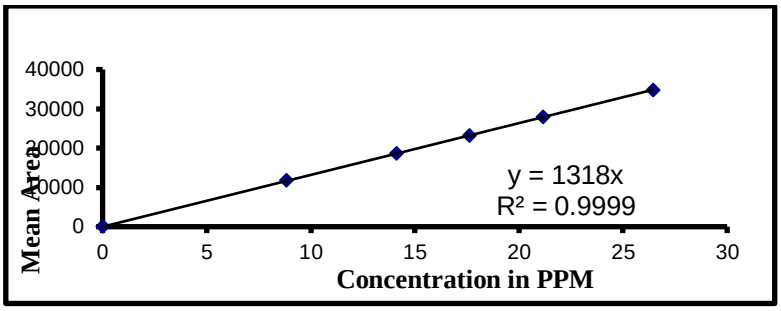

Fig 3: Linearity curve of Acetic acid

Precision

Precision of method was demonstrated by preparing six different samples of same concentration and calculated for content of Acetic acid. Relative Standard Deviation (RSD) calculated for all results and found 0.48%. Results of Repeatability study are shown in table 1.

Table 1: Repeatability results for Acetic acid

Precision samples	Aceic Acid Content(ppm)
Precision_1	5044
Precision_2	4998
Precision_3	4985
Precision_4	5006
Precision_5	4973
Precision_6	5013
Mean	**5003**
SD	**24.7**
%RSD	**0.48**

Accuracy

Accuracy of method was performed by recovery study using standard addition method and recovery values for Acetic acid found between 99% and 103% for 50%, 100% and 150% levels. Mean recovery (n=9) for Acetic acid found to be 100.8%. Results of recovery study presented in Table 2.

Table 2: Accuracy results for Acetic Acid

Accuracy Level	Sample preparations	% Recovery for Acetic acid
Accuracy_50%	Preparation_1	102.6
	Preparation_2	99.7
	Preparation_3	102.8
Accuracy_100%	Preparation_1	100.6
	Preparation_2	99.6
	Preparation_3	101.3
Accuracy_150%	Preparation_1	100.2
	Preparation_2	100.1
	Preparation_3	100.6
Mean Accuracy (n=9)		**100.8**

Robustness of method was performed by altering chromatographic condition i.e. flow rate and column oven temperature and analyzed standard solutions and system suitability parameter (Relative standard deviation) was evaluated. Percent relative standard deviation (RSD) of area response and retention time was below 2%.

Filter compatibility of method was confirmed by comparing centrifuged sample with filtered samples and observed difference between centrifuged sample and filtered samples was not more than 2.0%. It was observed that filter paper does not adsorb compound of interest during filtration of sample solutions.

Solution Stability was performed by comparing results of freshly prepared sample solution and stored sample solution at room temperature for 24 hours. Absolute difference observed between results of freshly prepared and stored sample solutions was not more than 2.0%.This study confirm that sample solution was stable up to at least 24 hours.

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CONCLUSION

Proposed work is alternative HPLC method to Gas chromatography for determination of Acetic acid content from pharmaceuticals. Our extensive Literature survey reveals that there is no Reverse phase liquid chromatographic method alternative to Gas chromatography reported for determination of Acetic acid content from pharmaceuticals. The proposed method is very simple, accurate, precise and economical. Hence, this novel and validated method can be easily and conveniently adopted for routine analysis of Acetic acid content from pharmaceuticals.

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