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ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ETOPHYLLINE AND THEOPHYLLINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, accurate and validated reverse phase UPLC method has been developed for the simultaneous estimation of Etophylline and Theophylline in injectables. The quantification was carried out using Silica gel column, packed with octadecylsilane , 2.1 mm × 100 mm, i.d, 1.7 μm particle size and separation was carried out in an isocratic mode, having a mixture of 0.05 M Sodium Acetate and Acetonitrile (90:10), pH 4.5 and mobile phase at a flow rate of 0.3 ml / min. The detection wavelength was 270 nm at ambient temperature. The retention time was 2.368 min and 3.129 min for Theophylline and Etophylline respectively. The results obtained showed a good agreement with the declared content. Recovery values for Theophylline and Etophylline were 99.84 - 100.89 %. The proposed method is reliable, rapid, precise, selective and may be used for the quantitative analysis of Theophylline and Etophylline in injectables.

Key words: Theophylline, Etophylline, UPLC, method development, validation.

1. INTRODUCTION

Theophylline and Etophylline is used to prevent and treat wheezing, shortness of breath, and difficulty in breathing caused by asthma, chronic bronchitis, emphysema, and other lung diseases. However the use of theophylline is often restricted by its narrow therapeutic range. Etofylline is a bronchodilator and normally applied in combination with theophylline. It relaxes and opens air passages in the lungs, making it easier to breathe. The pharmacological actions of etofylline are generally considered like those of theophylline. Unlike other xanthine derivatives, etofylline does not convert into theophylline in the body. This offers a wide therapeutic window and combination of etofylline and theophylline exhibits less frequent adverse side effects than an equivalent dose of theophylline alone. (Pather SI et al. 1998; Amol P et al.2008;

Lorenzo R et al.1998; Mutasem G et al.2000; Sanghavi NM et al. 1990; Evelyn O et al. 2005; Meshali MM et al. 1996; Nakano M et al. 1983)

UPLC refers to Ultra Performance Liquid Chromatography. It improve in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption.(Jerkovich AD et al. 2003; Wu N et al. 2001; Unger K K et al. 2000; Swartz M E et al. 2004).

UPLC comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also

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increases. As particle size decreases to less than 2.5μm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation (Van Deemter JJ .1956) . By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance.

The classic separation method is of HPLC (High Performance Liquid Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. It's main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis (Zhang YH et al. 2000; Zhou C et al. 2005) due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary phase. The Van Deemter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can operate only up to 400 bar. That is why short columns filled with particles of about 2μm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load.

To improve the efficiency of HPLC separations, the following can be done :-

a. work at higher temperatures
b. use of monolithic columns

1.1 Use of the UPLC system.

Elevated-temperature chromatography also allows for high flow rates by lowering the viscosity of the mobile phase, which significantly reduces the column backpressure (Zhu J et al. 2005; Greibrokk T et al. 2003).

Monolithic columns contain a polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns (Gerber F et al. 2004; Tanaka N et al. 2001; Wu N et al. 2004).

Simultaneous estimation of Etofylline and Theophylline is not official in any Pharmacopoeia. Literature study reveals that a UV and HPLC method and individual are available for estimation of Etofylline and Theophylline. Moreover there is

no UPLC method reported for Simultaneous estimation of Etofylline and Theophylline in injectable formulations.

The goal of this study was to develop a rapid, more accurate, precisely reliable, less expensive and least time consuming UPLC method for the analysis of Etofylline and Theophylline in injectables.

2. MATERIALS AND METHODS

All the chemicals and reagents used were of AR/HPLC grade from Merck. Pure standard and samples of Etophylline and Theophylline were obtained from Nirlife Healthcare Division, NIRMA LTD.

A reverse phase UPLC system (WATERS, ACQUITY UPLC), consisting of Binary Solvent Manager along with TUV detector and Sample Manager, having Empower2 software was used for analysis. UPLC Column, Acquity UPLC BEH, consisting of silica Bonded, packed with octadecylsilane (2.1 mm X 100 mm), 1.7 μm was used for analysis.

2.1 Preparation of Mobile Phase

A degassed mixture of 0.05 M Sodium Acetate and Acetonitrile in the ratio of 90:10 (v/v) was prepared and the pH adjusted to 4.5 with glacial acetic acid. The mixture was filtered through 0.22 μ membrane filters.

2.2 Standard Preparation

Weigh 169.4 mg of Etophylline and 50.6 mg of Theophylline working standard and transferred accurately into a 50 ml clean and dry volumetric flasks. It was further diluted to volume with HPLC grade water. Resulting into final concentration of Etofylline (338.8 mcg) & Theophylline (101.2 mcg).

2.3 Sample Preparation

A 5 ml of sample solution from Etophylline and Theophylline injection was taken and transferred accurately to 50 ml clean and dry volumetric flask. It was further diluted to volume with HPLC grade water. Resulting into final concentration of Etofylline

(338.8 mcg) & Theophylline (101.2 mcg). The solution was filtered through 0.22 μ membrane filters and it was degassed.

2.4 Chromatographic Conditions

Freshly prepared Buffer and Acetonitrile 90:10(v/v) mobile phase and adjust pH to 4.5, were filtered through 0.22 μ membrane filter and sonicated before use.

Flow rate of Mobile phase was maintained at 0.3 ml/min. The column temperature was maintained at ambient condition. The detection was carried out at 270 nm, Injection volume was 0.5 μl and total run time was 4 min. Column used was WATERS Acquity UPLC BEH, consisting of silica Bonded, packed with octadecylsilane (2.1 mm X 100 mm), 1.7 μm.

2.5 Assay Procedure

A 0.5 μl of placebo, standard preparation (6 times) and sample preparation (3 times) were separately injected into the chromatographic systems. Then the chromatograms and the peak responses were measured. The placebo chromatogram was examined for any extraneous peaks that were observed in the chromatograms of sample and standard preparations. Chromatogram of the standard preparation was recorded and the peak responses were measured. The tailing factor for the principal peak should not be more than 2.0 and the number of the theoretical plates should not be less

than 5000. The % RSD (Relative Standard Deviation) should not be more than 2.0.
A 0.5 μl of standard preparation and assay preparation was separately injected, and the chromatograms were recorded and the responses for the major peaks were measured.

3. RESULTS AND DISCUSSION

Chemical structure and chemical properties are the most important facts that predict chromatographic behavior. In the present investigation the best resolution was achieved using WATERS Acquity UPLC BEH, consisting of silica Bonded, packed with octadecylsilane (2.1 mm X 100 mm), 1.7 μm and mobile phase Buffer and Acetonitrile 90:10 (v/v). The lower percentage of acetonitrile in mobile phase resulted in peak broadening of the component and long analysis duration, while higher percentage of acetonitrile in mobile phase resulted peak splitting. Optimal retention time, 2.368 min and 3.129 min for Theophylline and Etophylline was achieved when the pH of mobile phase was adjusted to 4.5 with Glacial Acetic Acid. Small changes in pH of the mobile phase had a great influence to the chromatographic behavior of these substances. Higher pH of the mobile phase resulted in peak tailing, and lower pH resulted in broadening of peak.

3.1 Analytical Separation performances

A typical chromatogram of Etophylline and Theophylline injection is shown in Fig .1.
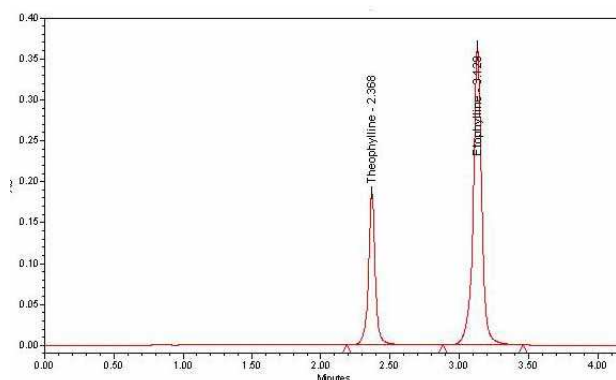
The retention times were 2.368 min and 3.129 min for Theophylline and Etophylline respectively. These retention times did not vary to any considerable degree during and in between analyses (% R.S.D. less than 2% for the retention time of each peak). System suitability test are the integral part of chromatographic method. To ascertain its effectiveness , system suitability test were carried out on freshly prepared standard stock solution of Etophylline and Theophylline. All the above parameters are shown in Table-1.

Table -1
System suitability Parameters

Sr.No	Parameters	Etophylline	Theophylline
1	Tailing Factor	0.94	0.98
2	Resolution	3.45	8.4
3	Theoretical Plates	14943.84	13463.55
4	% RSD	0.12	1.09

3.2 Precision
Method precision was investigated by the analysis of six separately prepared samples of the same batch. From this six, separate sample solutions were injected and the peak areas obtained were used to calculate mean and percentage R.S.D. values. The results obtained are shown in Table 2. In all instances the accepted criteria of % R.S.D. of less than 2% was met.

Table -2
Method precision results

Sr.No	Analyte (n=6)	Amount Percent (Mean)	% RSD of Assay
1	Etophylline	84.7 mg	0.15
2	Theophylline	25.3 mg	0.39

Precision of the system was evaluated by injecting a freshly prepared standard solution for six times. The % RSD results obtained, were all well below the accepted maximum of   1 %.

3.3 Accuracy
Accuracy of the method was studied by recovery investigation. The results of this investigation are shown in table 3. For both the analytes, at the different concentration levels evaluated the recovery values meet the acceptance criteria of 100 ±2%. In addition, these results provide the working range for the method.

Table -3
Accuracy (recovery) study results

Sr.No	Percentage of target concentration	Etophylline	Theophylline
1	80	99.96	99.86
2	100	99.99	100.89
3	120	100.71	99.84

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3.4 Ruggedness

Ruggedness of the method was studied and showed that chromatographic patters did not significantly change when different UPLC system, analyst, column were employed. The value of percentage R.S.D. was below 1%, exhibits the ruggedness of developed analytical method.

3.5 Robustness

Robustness of the method was determined by small deliberate change in flow rate, pH, and temperature. The content of the analytes were not adversely affected by these changes as evident from the low

value of relative standard deviation indicating that the method is robust.

3.6 Stability

In case of an unexpected delay during analysis, it is important to have information about the stability of all solution. In this study the stability of Etophylline and Theophylline in the working standard solution and sample preparation were studied.Both of these two analytes did not show evidence of significant degradation for at least 24 hr, when kept in release medium (HPLC grade water). During these periods, results do not decrease below 98 %.

The proposed method was found to be simple, specific and highly accurate, required less time consumption for analysis and this can be employed for the routine analysis.

4. CONCLUSION

The method described, enable to quantification of Etophylline and Theophylline. The advantages lies in the simplicity of sample preparation and in the cost economic reagents. In addition, both the compounds were eluted within 3.5 min. The proposed UPLC condition ensures sufficient resolution and precise quantification of the compounds. Statistical analysis of the experiment indicates that the precision and reproducibility rate are satisfactory.

5. ACKNOWLEDGEMENTS

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