ANALYTICAL METHOD DEVELOPMENT ANDVALIDATION OF DOXOFYLLINE

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ABSTRACT:
Doxofylline (DXE) is a novel methylxanthine derivative used in the treatment of asthma and Chronic Obstructive Pulmonary Diseases (COPD). Therapeutic Drug Monitoring (TDM) has been proposed in adults, while the adapted analytical method and TDM data are still missing in children.

KEY WORD:
Doxofylline
Chronic Obstructive Pulmonary Disease

INTRODUCTION

Doxofylline is a xanthine that is structurally different from theophylline by having adioxalane group at position 7 of the xanthine ring [1]. Consequently, it has mechanisms of action distinct from those of theophylline [2-4] in that lacks adenosine receptor antagonism or the ability to inhibit any of the known PDE isoforms, which may contribute to the better safety role.Furthermore, unlike theophylline, doxofylline does not interact with histone deacetylases [3], butis able to positively interact with f2-adrenoceptors [5]. The narrative analysis of literature has suggested that doxofylline is an effective bronchodilator for relieving airway obstruction in patients with asthma or chronic obstructive pulmonary disease (COPD) and displays a better safety profile with respect to theophylline, having a favourable risk-to-benefit ratio [2,4,6].

II.MATERIALS AND METHODS

HPLC System and Instruments: -
The details of the analytical HPLC system and instruments used are as below:
System: HPLC Binary Gradient System
Model No.: HPLC 3000 Series
Company: Analytical Technologies Ltd.
Detector: UV-3000-M

Pump: P-3000-M Reciprocating (40MPa)
Column: Cosmosil C18(250mm × 4.6ID, Particle Size: 5 micron)
Software: HPLC Workstation

Balance:
Wenser High Precision Balance
Model: PGB 100
Max: 100gm
Min: 0.001gm

Sonicator:
Wenser Ultra Sonicator
Model: WUC- 4L
Capacity: 4 L

Preparation of Mobile Phase:
The mobile phase used for the analysis by HPLC was prepared by the composition of methanol and water in 80: 20 ratios respectively.

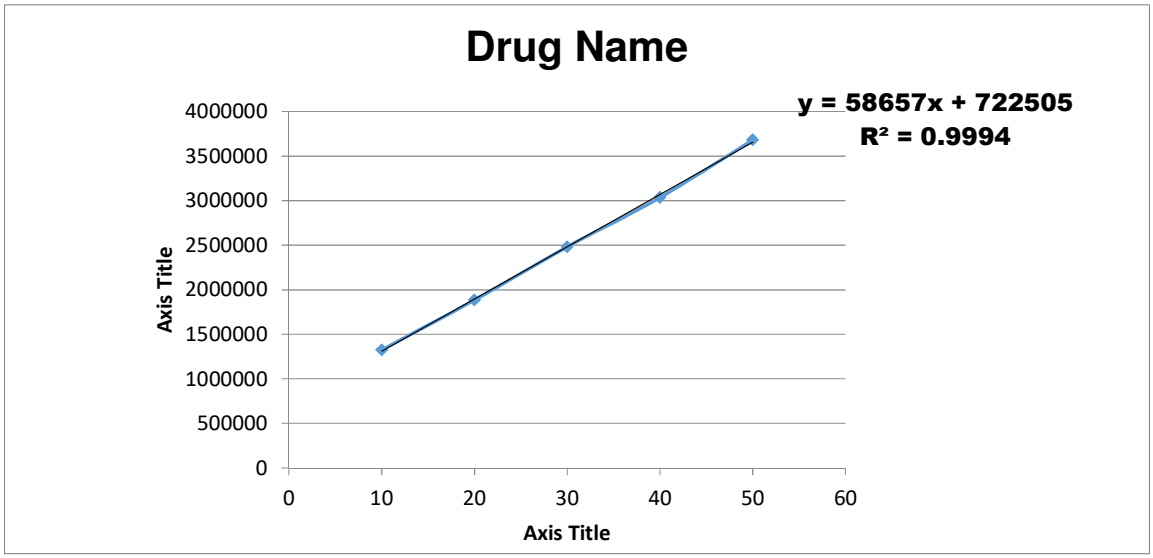
Preparation of Standard Stock Solution:
1000 ppm of individual drug:

10 mg of pure drug was dissolved in a 10 ml of solvent (mobile phase) for the preparation of 1000 ppm solution.

Sr. No.	Composition	Name of Drugs (conc.)		Final Volume
		Doxofylline(ml)	(ppm)	(ml)
1.	Composition 1	0.1	10ppm	10
2.	Composition 2	0.2	20ppm	10
3.	Composition 3	0.3	30ppm	10
4.	Composition 4	0.4	40ppm	10
5.	Composition 5	0.5	50ppm	10

Method Validation:
Linearity: -
Linearity of the calibration curve was determined at five different concentrations(10, 20, 30, 40 & 50). Aliquots of these solutions were injected into HPLC system and the detector response (peak area) was plotted against the concentrations to generate calibration curve and the method was found linear.

Conc.	Area
10	1324829
20	1885412
30	2482271
40	3036374
50	3682202



Accuracy

			Standard Deviation		Accuracy	Precision
Conc.	Conc.	Area	Mean	SD	%SD	%RSD
1	10 10 10	1324829 1320400 1319671	1321633.333	2791.428726	0.21121053	
2	30 30 30	2482271 2474509 2472674	2476484.667	5094.413247	0.20571148	0.05167718
3	50 50 50	3682202 3667224 3688570	3679332	10958.58586	0.29784172	

Acceptance Criteria for %SD and %RSD: - It must be less than 2%
Results: %SD and %RSD found to be less than 2%.

Precision: -

Reproducibility

Interday	Day 1			Day 2			Mean	%RSD
	2482271	2474509	2462674	2481807	2479999	2479088	2479088	0.30%

Intraday	Morning			Evening				%RSD
	2482271	2474509	2462674	2487566	2478566	2480887	2477746	0.34%

Acceptance Criteria for %RSD: - It should be less than 2%
Results: %RSD found to be less than 2%.

% Recovery:

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	2482271	2467223	99.39378094
2	100% Recovery	3066374	3060554	99.81019928
3	150% Recovery	3682202	3675630	99.82151984

Assay:

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	2479446	2482271	100.114

Limit of Detection: - LOD was calculated by using following formula.

$$LOD = \frac{3.3 \times \text{Standard Deviation}}{\text{Slope}}$$

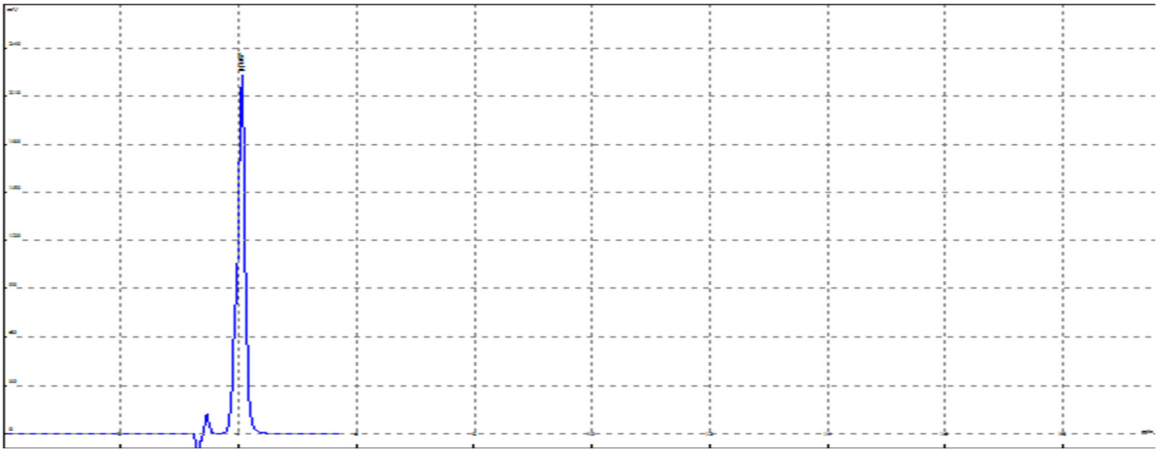
$$= \frac{3.3 \times 6281.4759}{58657}$$

$$= 0.3533912486$$

Limit of Quantitation: - LOQ was calculated by using following formula.

$$LOQ = \frac{10 \times \text{Standard Deviation}}{\text{Slope}}$$
$$= \frac{10 \times 6281.4759}{58657}$$
$$= 1.0708825716$$

Development And Optimization Of HPLC Method:
Sample Name: Doxofylline Trial 01
Wavelength: 273nm
Mobile Phase: Methanol:Water (80:20)
Sample volume: 20µl
Flow rate: 0.8 ml/min
Pressure:9-10MPa
Run time: 5.72min



Rank	Time	Area	Resolut.	T.PlateNum	Asymmetry
1	4.037	2627331	0.00	8984	1.07

Methods: A highly sensitive and stability indicating High-Performance Liquid Chromatography (HPLC) method of DXE with caffeine as the internal standard, was developed and validated by separating its metabolites,Hydroxyethyltheophylline (HPE) and Theophylline (TPE). HPLC separation is achieved on C18 column connected to an ultraviolet detector (276 nm), using acetonitrile and ultra-pure water in a gradient mode of elution at a flow rate of 0.9 mL/min at 25°C. A liquid-liquid extraction method using ethyl acetate was developed with a small sample volume of plasma of 50 L. Trough concentration was monitored in children receiving DXE therapy.

CONCLUSION

An RP-HPLC method was developed and validated for Doxofylline determination in bulk drug and dosage form. The mobile phase selected for chromatographic analysis was 80:20 Methanol: Water, cosmosil C18 (250mm × 4.6ID, Particle Size: 5 micron) and 20µl injection volume with the flow rate of 0.9 ml/min was applied and wavelength kept was 204 nm. Further the retention time was found to be 4.037 minutes. Also, the linearity and precision were found good. The pharmaceutical dosage form i.e. tablets by proposed method gave 99.94 % recovery and the method is rapid, economic and simple for analysis of the dosage forms on industrial scale.

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