SIMULTANEOUS DETERMINATION OF MULTI DRUG COMPONENTS THEOPHYLLINE, ETOFYLLINE, GUAIPHENESINE AND AMBROXOL HYDROCHLORIDE BY VALIDATED RP-HPLC METHOD IN LIQUID DOSAGE FORM

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ABSTRACT

The RP-HPLC (reverse phase high performance liquid chromatography) method was developed and validated for simultaneous determination of Multi drug components i.e., Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride in a liquid dosage form. Chromatographic separation of the four drugs was performed on a Hypersil Phenyl BDS (25cmX4.6mm, 5µm). The mobile phase constituted of triethylamine pH 3.0 buffer: methanol (85:15) v/v was delivered at the flow rate 1.5 mL/min. Detection was performed at 235 nm. The peak purity of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride were 0.99970, 0.99979, 0.99986 and 0.99949 respectively. Calibration curves were linear with correlation coefficient between 0.99995 to 0.99997 over a concentration range of 5 to 37 µg/mL for Theophylline, 19 to 140 µg/mL for Etofylline, 20 to 149 µg/mL for Guaiphenesine and 6 to 45 µg/mL for Ambroxol hydrochloride. The relative standard deviation (RSD) was found < 2.0%. The percentage recovery was found between the range of 98.6% and 100.5% at three different levels. Robustness and ruggedness were performed and result found within the RSD of 2%. All the parameters of validation were found in the acceptance range of ICH guideline.

Keywords: Theophylline; etofylline; guaiphenesine; ambroxol hydrochloride; multi drug components.

INTRODUCTION

This liquid dosage form is a fixed dose combination of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride. Airway inflammation plays a role in the pathogenesis of chronic obstructive pulmonary disease (COPD) (Tsukagoshi *et al*., 2000). Theophylline has bronchodilator/anti-inflammatory properties and is widely used in the treatment of airway inflammation in COPD (Kobayashi *et al*., 2004). Theophylline and Etofylline are xanthine derivatives with bronchodilator activity. Etofylline is a well tolerated derivative of Theophylline and acts by direct relaxation of bronchial smooth muscles. Guaiphenesine stimulate the bronchial glands lining of airway to produce a thin secretion that lubricates any thick mucous and making it easier to expel with coughing.

Guaiphenesine is known to increase the volume and reduce the viscosity of tenacious sputum (Heilborn *et al*., 1976). Ambroxol Hydrochloride is an active metabolite of bromhexine and fecilitate expectoration of excessive secretions by virtue of mucolytic and mucokinetic action via fragmentation of long mucopolysaccharide chains (Su *et al*., 2004; Hashizume, 2002). Ambroxol also acts as tissue protective due to its inhibitory effect on release of destructive mediators and free oxygen radicals by phagocytosis.

Combination of fixed dose of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride are indicated for the prophylaxis and relief of reversible bronchospasm associated with acute and chronic asthma, bronchitis and other chronic obstructive airway disease where reversible airway narrowing occurs.

Reported analytical methods for quantitative determination of Theophylline in pharmaceutical formulations are described in literature like capillary electrophoresis (Hague *et al*., 1999), UV spectroscopy (Singh and Sahu, 2006), high-performance liquid chromatography (HPLC)/ Electrospray mass spectroscopy (Hori *et al*., 2006), Thin layer chromatography (Mirfazaelian *et al*., 2002) and gas chromatography-mass spectrometry (Saka *et al*., 2007). Methods for the determination of Etofylline are UV spectroscopy and HPLC method (Nirogi *et al*., 2007). Methods for determination of Guaiphenesine includes UV spectroscopy (Abdel-Hay *et al*., 1992), HPLC method (Vasudevan *et al*., 2000), gas chromatography (Harsono

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Table 1: System suitability and system precision

Compound	Retention time (Mean ± SEM)	N	R	T	α
Thephylline	6.462 ± 0.0079	6950.50	-	1.28	-
Etofylline	8.175 ± 0.0073	6688.23	4.80	1.31	1.26
Guaiphenesine	13.506 ± 0.0092	7475.27	10.39	1.25	2.09
Ambroxol HCL	18.886 ± 0.0099	6171.93	6.80	1.31	2.92

n= Theoretical plates; R= Resolution; T= Asymetry; α = Selectivity

Table 2: Characteristics of the analytical method derived from the standard calibration curve

Compound	LOD µg/mL	LOQ µg/mL	Linearity range n=(5)	Correlation co-efficient µg/mL	Residual std. regression (σ)	Slope of regression (S)
Theophylline	0.47	1.41	5 to 37	0.99995	2231.557	5774.653
Etofylline	1.37	4.11	19 to140	0.99997	6957.156	16682.633
Guaiphenesine	1.51	4.53	20 to 149	0.99997	4849.932	10584.670
Ambroxol HCL	0.47	1.41	6 to 145	0.99997	2268.870	15672.397

LOD= Limit of detection; LOQ= Limit of quantification

Table 3: Method precision

Compound	Concentration µg/mL (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD- of assay
Theophylline	25.5	6.47 ± 0.0058	101.28 ± 0.1597	0.8
Etofylline	93.0	8.18 ± 0.0083	101.10 ± 0.0645	1.1
Guaiphenesine	100.0	13.50 ± 0.0126	102.03 ± 0.0931	0.9
Ambroxol HCL	30.0	18.85 ± 0.0112	97.72 ± 0.1906	1.3

et al., 2005), Capillary gas chromatography and electron capture detection (Sharaf and Stiff, 2004). Methods for the determination of Ambroxol Hydrochloride includes UV spectroscopy (Dincer *et al*., 2003), HPLC method (Koundourellis *et al*., 2000), Raman spectroscopy (Hwang *et al*., 2005), Capillary electrophopresis and fluorometry (Peren-Ruiz *et al*., 2000). Literature survey reveals that there is no method for the simultaneous determination of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride in pharmaceutical preparations.

The purpose of work was to develop and validate the proposed method for routine analysis in quality control labs.

MATERIALS AND METHODS

Chemicals and materials

Cores India Ltd, India supplied Theophylline, Aarti industries Ltd, India supplied Etofylline, Granules India Ltd, India supplied Guaiphenesine and Vanpetrochem, India supplied Ambroxol hydrochloride. Methanol and Triethylamine supplied by Spectrochem Pvt. Ltd. Orthophosphoric acid supplied by E-merck Ltd, India. Milli-Q water (In-house production of the company).

Instrumentation

Shimadzu 2010C integrated high performance liquid chromatographic system was used for experiment. Shimadzu 2010C system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled

Table 4: Method accuracy

Level	Drug Added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
For Theophylline				
50%	6.27	6.21	99.6 ± 0.4060	0.7
100%	12.60	12.53	100 ± 0.5298	0.9
150%	18.92	18.7	99.3 ± 0.1455	0.3
For Etophylline				
50%	22.87	22.95	100.47 ± 0.7975	1.4
100%	47.08	47.14	100.23 ± 0.2851	0.5
150%	70.18	69.95	99.63 ± 0.2188	0.4
For Guaiphenesine				
50%	24.42	24.12	99.43 ± 0.3484	0.6
100%	50.38	49.66	99.27 ± 0.0883	0.2
150%	75.15	73.78	98.87 ± 0.1858	0.3
For Ambroxol HCL				
50%	7.28	7.13	98.60 ± 0.3059	0.5
100%	14.94	14.66	98.60 ± 0.0578	0.1
150%	22.72	22.37	98.9 ± 0.1529	0.3

by CLASS-VP software. The Hypersil Phenyl BDS (250X4.6 mm), 5µm column was used as a stationary phase.

HPLC Condition

Column	Hypersil Phenyl BDS (250X4.6 mm), 5µm
Detector	235 nm
Injection volume	20 µL
Flow rate	1.5 mL/min
Temperature	30˚C
Run time	30 min
Mobile phase	Triethylamine pH 3.0 buffer: Methanol (85: 15) v/v

Buffer preparation

2 ml of Triethylamine was added in to 1000 ml of Milli-Q water and mixed, adjusted pH 3.0 with Orthophosphoric acid.

Standard preparation

Standard stock solutions were prepared in Milli-Q water and further for second dilution, diluted with mobile phase to make final concentration of Theophylline 25 µg/mL, Etofylline 93 µg/mL, Guaiphenesine 100 µg/mL, Ambroxol Hydrochloride 30 µg/mL respectively.

Sample preparation

5.0 gm of expectorant (equivalent to 12.7 mg of Theophylline, 46.5 mg of Etofylline, 50 mg of Guaiphenesine, 15 mg of Ambroxol Hydrochloride) were weighed and transferred into 100-ml volumetric flask. About 50 ml of Milli-Q water was added and sonicate it for 10 minutes. Volume was made up to the mark. Mixed and filtered the solution through 0.45 µ HVLP nylon filter. Made proper dilution with mobile phase to make final concentration 25.5 µg/mL of Theophylline, 93 µg/mL of Etofylline, 100 µg/mLof Guaiphenesine and 30 µg/mL of Ambroxol Hydrochloride and mixed.

RESULTS

The detection wavelength of 235 nm was chosen in order to achieve a good sensitivity for quantitative determination of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride in liquid dosage form. The chromatographic separation of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride in the present combination is shown in figs. 1 and 2, which illustrate the separation of four active ingredients in this system. The compounds eluted in the order of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride with retention time of 6.47 min, 8.18 min, 13.50 min and 18.90 min respectively. The isocratic program throughout HPLC method was adopted to analyze all four components in a single run.

System suitability and System precision

System suitability and system precision were performed daily during entire validation of this method. The results

Table 5: Method robustness

Compound	% RSD in Normal and Changed condition (n=5)		
Temperature	**% RSD Normal**	**% RSD (-5°C)**	**% RSD (+5°C)**
Theophylline	0.63	0.22	1.77
Etofylline	0.55	0.31	1.79
Guaiphenesine	0.56	0.38	1.78
Ambroxol HCL	0.37	0.12	1.97
Ph	**% RSD Normal**	**% RSD (-0.2 unit)**	**% RSD (+0.2 unit)**
Theophylline	0.63	0.14	0.11
Etofylline	0.55	0.08	0.05
Guaiphenesine	0.56	0.15	0.03
Ambroxol HCL	0.37	0.36	0.69
Flow Rate	**% RSD Normal**	**% RSD (-10%)**	**% RSD (+10%)**
Theophylline	0.63	0.59	0.31
Etofylline	0.55	0.52	0.22
Guaiphenesine	0.56	0.38	0.34
Ambroxol HCL	0.37	0.57	0.18
Mobile phase ratio	**% RSD Normal**	**% RSD (-2%)**	**% RSD (+2%)**
Theophylline	0.63	0.07	0.16
Etofylline	0.55	0.07	0.17
Guaiphenesine	0.56	0.10	0.17
Ambroxol HCL	0.37	0.17	0.21

Table 6: Method ruggedness

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1	**Analyst-1, Instrument-1 & Column-1**	
Theophylline	101.28 ± 0.1597	0.8
Etofylline	101.10 ± 0.0645	1.1
Guaiphenesine	102.03 ± 0.0931	0.9
Ambroxol HCL	97.72 ± 0.1906	1.3
Day 2	**Analyst-2, Instrument-2 & Column-2**	
Theophylline	100.27 ± 0.4228	1.0
Etofylline	102.00 ± 0.3835	0.9
Guaiphanesine	99.52 ± 0.4366	1.1
Ambroxol HCL	98.83 ± 0.4534	1.1

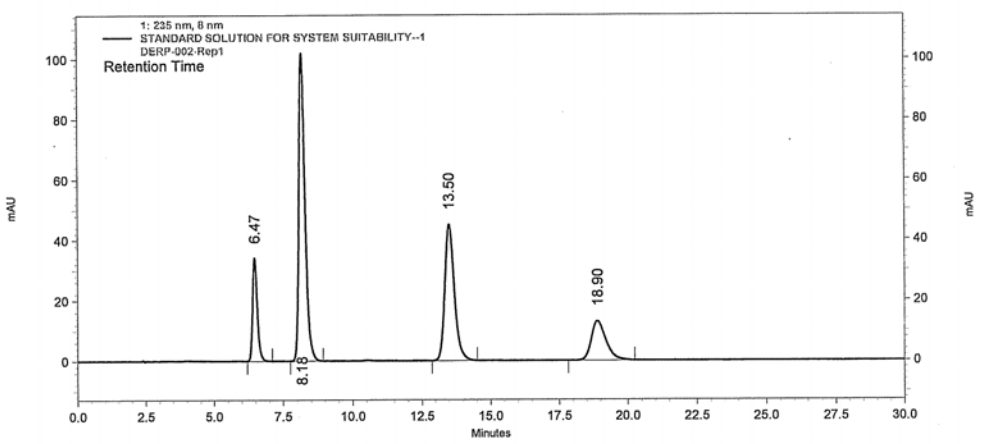
of system suitability and system precision are presented in table 1.

Linearity and calibration curve

The plot of peak area response against concentration are presented in figs. 3, 4, 5 and 6. The plot was linear over the concentration range of 5 to 37 µg/mL for Theophylline, 19 to 140 µg/mL for Etophylline, 20 to 149 µg/mL for Guaiphenesine and 6 to 45 µg/mL for Ambroxol hydrochloride. The correlation coefficient was found to be 0.99995, 0.99997, 0.99997, 0.99997 for Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochloride respectively. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification are presented in table 2.

Specificity

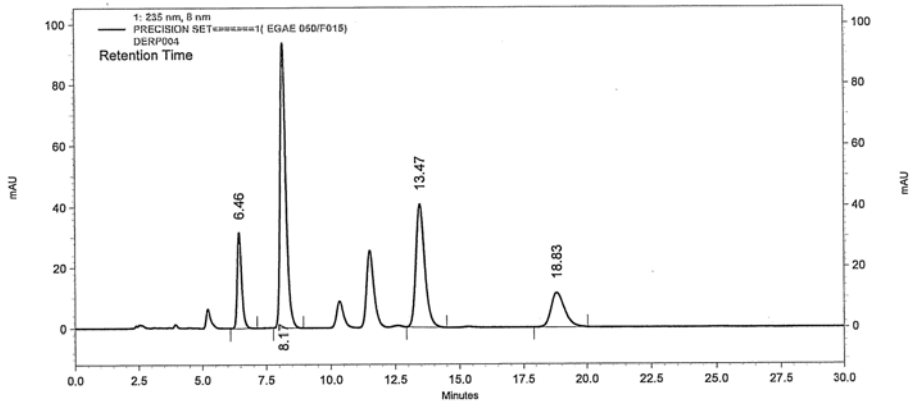
There were no interference from sample placebo and the peak purity of Theophylline, Etofylline, Guaiphenesine and Ambroxol hydrochlorides were 0.99970, 0.99979, 0.99986 and 0.99949 respectively. It shows that developed analytical method is specific for the analysis of



1: 235 nm, 8 nm

Pk #	Name	Retention Time	Area	Asymmetry	Capacity factor	Theoretical plates
1	Theophylline	6.47	405584	1.28	1.59	6950.50
2	Etofylline	8.18	1564986	1.31	2.27	6688.23
3	Guaphenesin	13.50	1081726	1.25	4.40	7475.27
4	Ambroxol HCl	18.90	479403	1.31	6.56	6171.93
Totals			3531699			

Fig. 1: Chromatogram for standard solution.



1: 235 nm, 8 nm

Pk #	Name	Retention Time	Area	Asymmetry	Capacity factor	Theoretical plates
1	Theophylline	6.46	378382	1.27	1.59	6774.47
2	Etofylline	8.17	1437059	1.26	2.27	6668.46
3	Guaphenesin	13.47	966466	1.25	4.39	7524.45
4	Ambroxol HCl	18.83	414791	1.29	6.53	6228.84
Totals			3196698			

Fig. 2: Chromatogram for sample solution.

Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochlorides in liquid dosage form.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 24 h. The relative standard deviation was found below 2.0%. It proves that both standard and sample solution are stable up to 24 h at room temperature.

Method precision

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The value of standard deviation shows that the

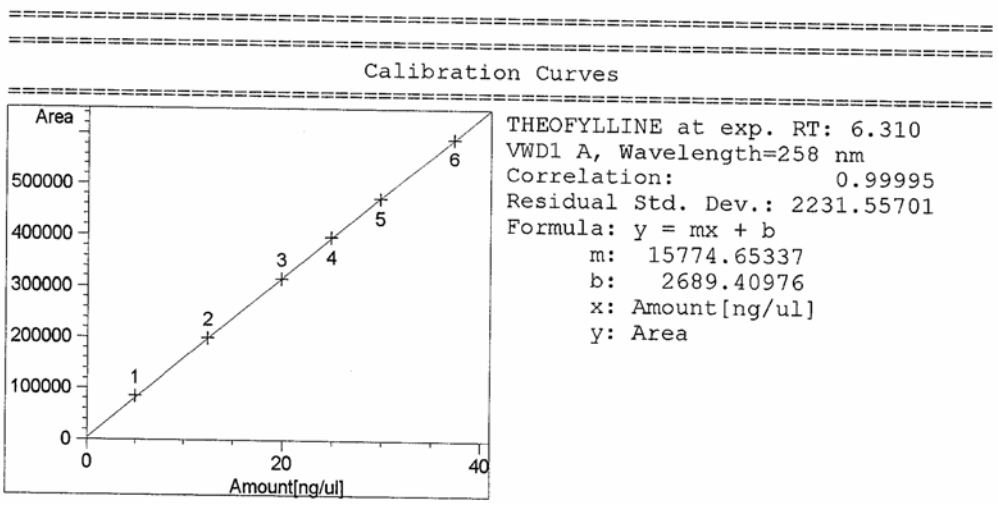


Fig. 3: Linear calibration curve for Theophylline.

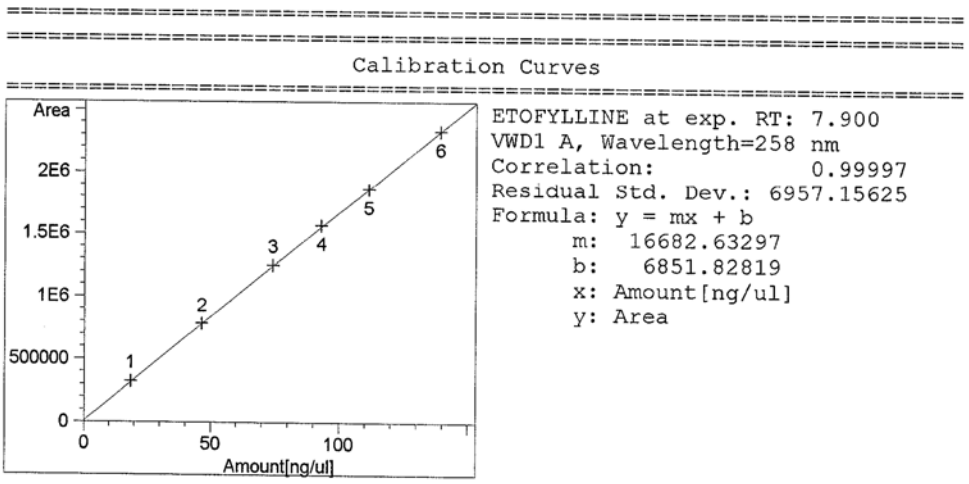


Fig. 4: Linear calibration curve for Etofylline.

method is precise. The results obtained are presented in table 3.

Method accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies are presented in table 4.

Method robustness

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio, pH and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method is robust and results are presented in table 5.

Method ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, exhibits the ruggedness of developed analytical method and results are presented in table 6.

DISCUSSION

The method described enables to the quantification of Theophylline, Etofylline, Guaiphenesine and Ambroxol Hydrochlorides. The advantages lie in the simplicity of sample preparation and the cost economic reagents were used. In addition all four compounds were eluted within 20 min. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result

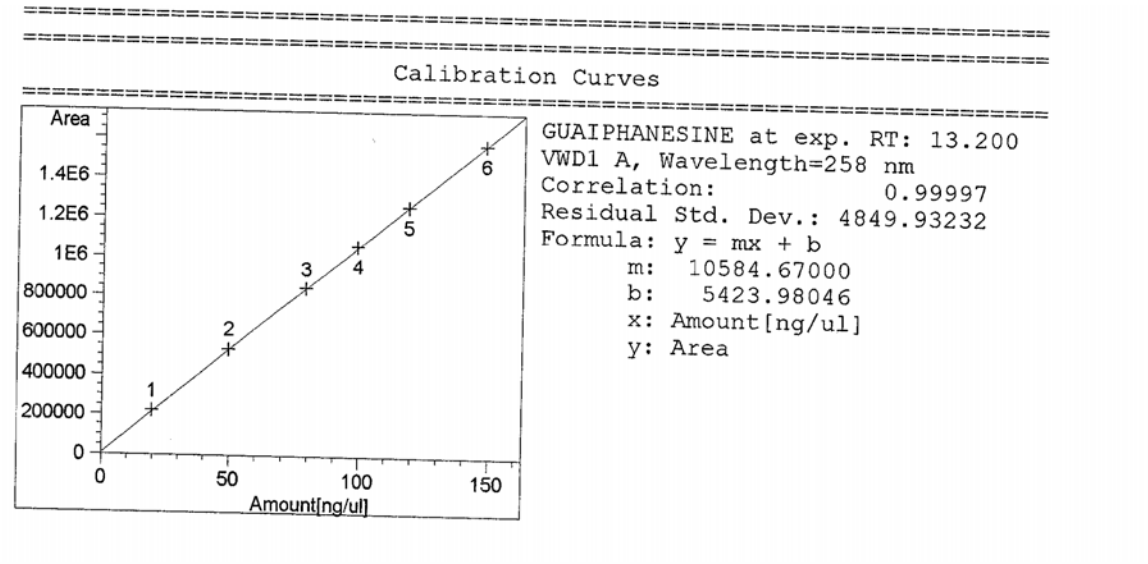


Fig. 5: Linear calibration curve for Guaiphenesine.

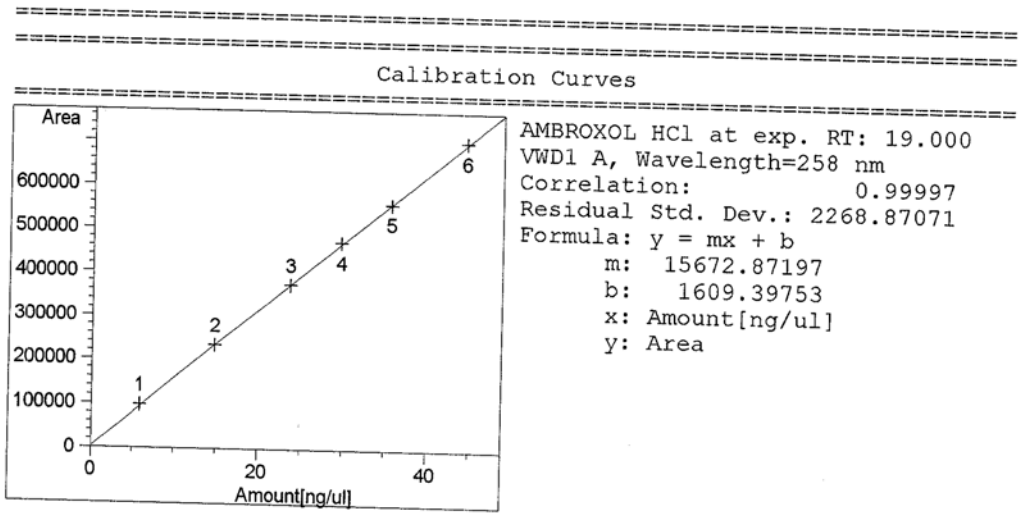


Fig. 6: Linear calibration curve for Ambroxol Hydrochloride

indicates that the precision and reproducibility data are satisfactory. Hence, this HPLC method can be used for routine analysis of these drugs in combination which are marketed in the developing country because it is simple, economic and reliable.

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