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## Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Theophylline and Bambuterol in Bulk and Synthetic Mixture

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### ABSTRACT:

A simple, precise, accurate and reproducible Spectrophotometric method has been developed for Simultaneous Estimation of Theophylline and Bambuterol by employing first order derivative method in methanol and water. The first order derivative absorption at 273 nm (zero cross point of Theophylline) was used for quantification of Bambuterol and 264 nm (zero cross point of Bambuterol) for quantification of Theophylline. The linearity was established over the concentration range of 20-180 $\mu$ g/ml for Theophylline and 10-50 $\mu$ g/ml for Bambuterol with correlation coefficient  $R^2$  0.999 and 0.998, respectively. The mean % recoveries were found to be in the range of 99.49 – 100.70 % and 98.91 – 100.37 % for Theophylline and Bambuterol, respectively. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Theophylline and Bambuterol in their Synthetic Mixture.

**Keywords:** Theophylline, Bambuterol, First order derivative, Synthetic mixture, Method validation.

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### INTRODUCTION:

Theophylline is Chemically 3, 7-Dihydro-1, 3-dimethyl-1H-purine-2, 6-Dione<sup>[1]</sup>. Theophylline is vasodilator agents, phosphodiesterase inhibitors, bronchodilator agents, respiratory smooth muscle relaxant. Theophylline competitively inhibits type III and type IV phosphodiesterase (PDE), the enzyme responsible for breaking down cyclic AMP in smooth muscle cells, possibly resulting in bronchodilation<sup>[2-4]</sup>. Bambuterol is chemically, 5-(2-tert-butylamino- 1-hydroxyethyl)-m-phenylene bis (dimethylcarbamate)<sup>[1]</sup> is a direct acting sympathomimetic with predominantly active precursor of the Selective B<sub>2</sub> adrenergic agonist. Bambuterol is an active precursor of the selective B<sub>2</sub> adrenergic agonist, terbutaline. It is an ester prodrug of terbutaline which is a B<sub>2</sub> adrenergic agonist. It is the first once daily oral B<sub>2</sub> agonist with 24-hour duration for the treatment of asthma<sup>[2,5]</sup>. Theophylline and bambuterol are commercially available in various dosage forms an individual formulation. Combination of Theophylline and Bambuterol are study under Clinical Trial phase. Identifier No: NCT01566565 by Norwegian Armed Forces Medical Service<sup>[6]</sup> (Oslo University Hospital). Combination of Theophylline and Bambuterol are useful in High Altitude Pulmonary Hypertension. Theophylline is official in IP<sup>[3]</sup>, BP and USP<sup>[4]</sup>. Bambuterol is official in BP<sup>[5]</sup>. From Literature survey, various methods (UV<sup>[7-9]</sup>, HPLC<sup>[10-17]</sup>, HPTLC, GC and Colorimetric) were reported for the analysis of individual drug and in combination with other drug but no method were

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reported for simultaneous estimation of Theophylline and Bambuterol. Hence, the purpose of the present work was to develop and validate first order derivative spectrophotometric method for simultaneous estimation of Theophylline and Bambuterol in synthetic mixture.

## MATERIAL AND METHODS

### Instruments

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

### Chemicals and Reagents

The bulk drug, Theophylline was obtain from Modi pharmaceutical Pvt. Ltd. Ahmedabad and Bambuterol was obtain from Sun pharmaceutical Ltd. Vadodara. Fixed dose of synthetic mixture of Theophylline 150 mg and Bambuterol 10 mg were prepared in laboratory scale as pilot batch. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

### Selection of a Solvent

Methanol: Water (1:1) was selected as solvent for studying spectral characteristic of drugs.

### Preparation of Standard Stock Solution

Accurately weighed 100 mg of Theophylline and 10 mg of Bambuterol standard were transferred to separate 100 ml volumetric flask and dissolved 50 ml methanol:water (1:1). The flasks were shaken and volume was made up to the mark with methanol:water (1:1) to give solution containing 1000 $\mu$ g/ml Theophylline and 100 $\mu$ g/ml Bambuterol.

### Preparation of Working Standard Solution of Theophylline and Bambuterol

From above solution of Theophylline pipette out 0.2, 0.6, 1.0, 1.4, 1.8 ml of the stock solution were further diluted to five 10 ml volumetric flasks individually with methanol:water (1:1) to get concentrations 20, 60, 100, 140, 180 $\mu$ g/ml. From above solution of Bambuterol pipette out 1.0, 2.0, 3.0, 4.0, 5.0 ml of the stock solution were further diluted to five 10 ml volumetric flasks individually with methanol:water (1:1) to get concentrations 10, 20, 30, 40, 50 $\mu$ g/ml.

### Selection of Analytical Wavelength

Standard 20-180 $\mu$ g/ml solutions of Theophylline and 10-50 $\mu$ g/ml solutions of Bambuterol were prepared in methanol:water (1:1) by appropriate dilution and spectrum

was recorded between 200-400 nm. All zero order spectrum ( $D^0$ ) were converted to first derivative spectrum ( $D^1$ ) using delta lambda 2.0 and scaling factor 10. The overlain first derivative spectrums of Theophylline and Bambuterol at different concentration were recorded. The zero crossing point (ZCP) of Theophylline was found to be 273.00 nm and ZCP of Bambuterol was found to be 264.00 nm.

### Assay of Synthetic Mixture

The quantity of synthetic mixture powder equivalent to 150 mg of Theophylline and 10 mg of Bambuterol was transferred in to 100 ml volumetric flask, containing methanol:water (1:1). The volume was made up to the mark with methanol:water (1:1) and the solution filtered through 0.45 $\mu$ m Whatman filter paper. An aliquot of this solution (1.0 ml) was transferred in to 10 ml volumetric flask and volume was made up to the mark with methanol:water (1:1) to obtain final concentration of 150 $\mu$ g/ml Theophylline and 10 $\mu$ g/ml Bambuterol. Absorbance of a sample solution recorded using first order derivative spectroscopy at 273 nm (ZCP of Theophylline) and 264 nm (ZCP of Bambuterol) for determination of Bambuterol and Theophylline, respectively. The analysis procedure was repeated three times with synthetic mixture.

### Method Validation

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

### Linearity (Calibration curve)

Appropriate volume of aliquot from Theophylline and Bambuterol standard stock solution was transferred to 10 ml volumetric flask. The volume was made up to the mark with methanol:water (1:1) to give solution containing 20-180 $\mu$ g/ml Theophylline and 10-50 $\mu$ g/ml Bambuterol. All  $D^1$  spectrums were recorded using above spectrophotometric condition.  $D^1$  absorbance at 273 nm and 264 nm were recorded for Bambuterol and Theophylline, respectively (n=6). Calibration curve were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Theophylline was  $y = 0.0042x + 0.0931$  ( $R^2 = 0.999$ ) and Bambuterol  $y = -0.0037x - 0.0182$  ( $R^2 = 0.998$ ).

### Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration levels 80 %, 100 % and 120 %, taking in to consideration percentage

purity of added drug sample. The amounts of Theophylline and Bambuterol were estimated by applying obtained values to the respective regression line equations. Each concentration was analysed 3 times and average recovery were measured.

#### Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

The repeatability was evaluated by assaying 6 times of sample solution of 100 µg/ml Theophylline and 30 µg/ml Bambuterol prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Theophylline and Bambuterol was carried out by estimating different concentration of Theophylline (20, 60, 100µg/ml) and Bambuterol (20, 30, 40µg/ml), 3 times on same day and on 3 different day (first, second and third). The reproducibility was carried out by estimating Theophylline and Bambuterol on changing of instrument and analyst. (Standardization of methodology) and the results are reported in terms of % RSD.

#### Limit of Detection and Limit of Quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the  $3.3 \times (SD/Slope)$  and  $10 \times (SD/Slope)$  criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slope of the calibration curve.

#### RESULT AND DISCUSSION

A reliable first order derivative spectrophotometric method was developed for simultaneous estimation of Theophylline and Bambuterol in synthetic mixture by UV Spectrophotometry. Beers law was obeyed in concentration range of 20-180µg/ml for Theophylline and 10-50µg/ml for Bambuterol at 273 nm and 264 nm wavelengths, respectively. The correlation coefficient Theophylline and Bambuterol was found to be  $R^2 = 0.999$  and  $0.998$ . The mean % recoveries were found to be in the range of 99.49-100.70 % and 98.91-100.37 %, respectively. Precision (% RSD) of Theophylline and Bambuterol was found to be 0.360-1.399 % and 0.972-1.907 %, respectively. The LOD and LOQ were 1.006µg/ml and

3.050µg/ml of Theophylline and 1.077µg/ml and 3.264µg/ml of Bambuterol, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analytes, which can be applied for the analysis of Theophylline and Bambuterol in synthetic mixture.

#### CONCLUSION

The results of our study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate. The developed UV spectroscopic method was found suitable for determination of Theophylline and Bambuterol in bulk and synthetic mixture without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of Theophylline and Bambuterol. It can therefore be conclude that use of the method can save time and money and it can be used in small laboratories with accurate and wide linear range.

#### ACKNOWLEDGEMENT:

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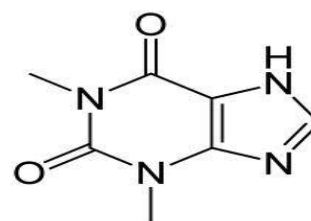


Figure 1: Structure of Theophylline

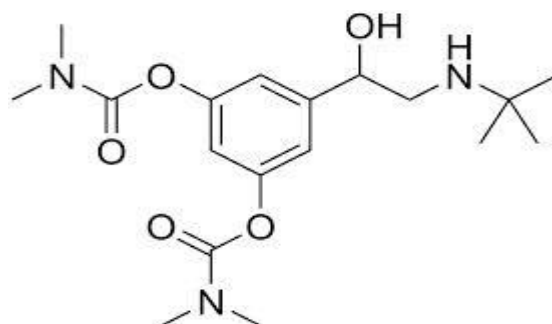


Figure 2: Structure of Bambuterol

**Table 1: Regression analysis data and summary of validation parameters for the proposed method**

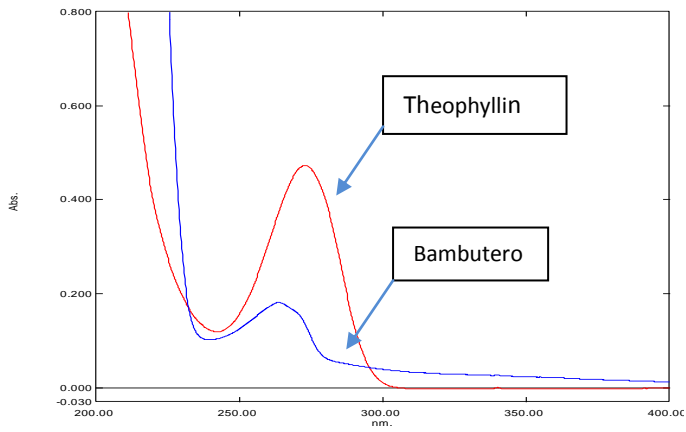
Parameter	First-Derivative UV Spectrophotometry	
	Theophylline	Bambuterol
Concentration range ( $\mu\text{g/ml}$ )	20-180	10-50
Regression equation	$y = 0.0042x + 0.0931$	$y = -0.0037x - 0.0182$
Slope	0.0042	-0.0037
Intercept	0.0931	-0.0182
Correlation Coefficient ( $r^2$ )	0.999	0.998
Accuracy (% recovery, n=3)	99.49-100.70	98.91-100.37
Repeatability (%RSD, n = 6)	0.159-0.781	1.025-1.907
Intraday (%RSD, n=3)	0.360-0.974	0.972-1.277
Interday (%RSD, n=3)	0.490-1.399	1.301-1.711
Reproducibility (% RSD, n=3)	0.567-1.246	1.213-1.740
LOD ( $\mu\text{g/ml}$ )	1.006	1.077
LOQ ( $\mu\text{g/ml}$ )	3.050	3.246

**Table 2: Recovery data of proposed method**

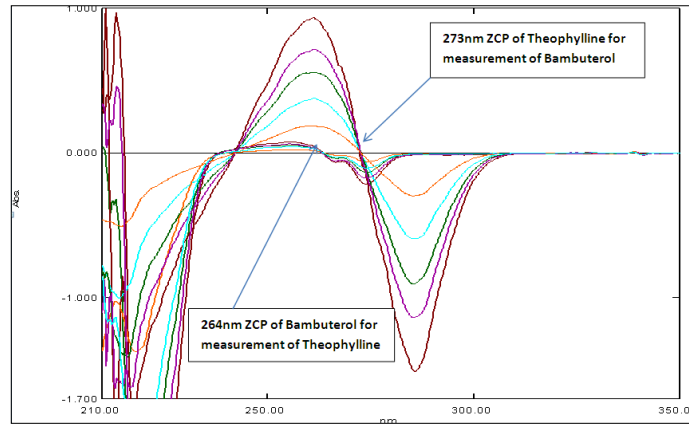
Drug	Level (%)	Test amount ( $\mu\text{g/ml}$ )	Spiked STD amount ( $\mu\text{g/ml}$ )	Total amount recovered ( $\mu\text{g/ml}$ )	% Mean recovery $\pm$ SD. (n=3)
Theophylline	80	15	12	26.88	99.55 $\pm$ 0.722
	100	15	15	30.21	100.70 $\pm$ 1.065
	120	15	18	32.83	99.49 $\pm$ 0.768
Bambuterol	80	15	12	27.10	100.37 $\pm$ 0.961
	100	15	15	29.67	98.91 $\pm$ 1.094
	120	15	18	33.45	99.72 $\pm$ 1.058

**Table 3: Analysis of Theophylline and Bambuterol by proposed method**

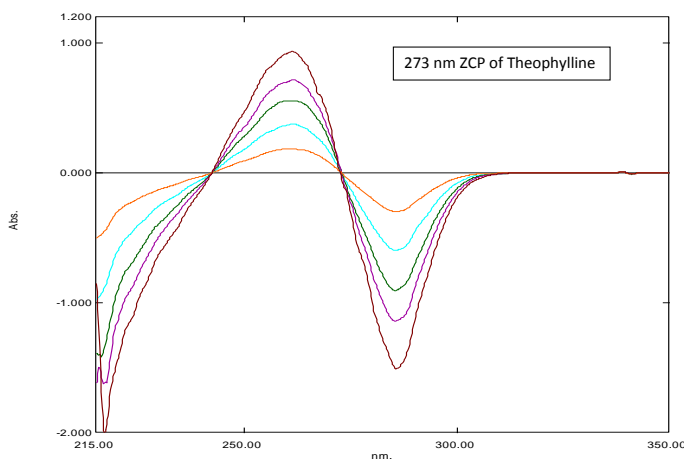
Synthetic mixture	Label claim (mg)		Mean amount found (mg)		% Label claim $\pm$ SD (n=3)	
	Theophylline	Bambuterol	Theophylline	Bambuterol	Theophylline	Bambuterol
	150	10	148.54	9.94	99.03 $\pm$ 0.663	99.45 $\pm$ 0.427



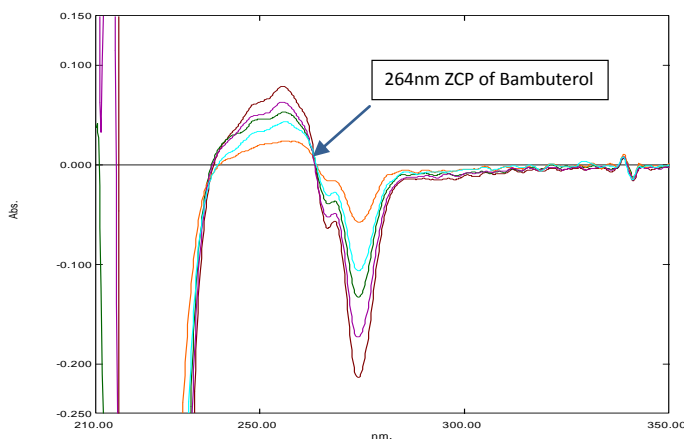
**Figure 3: UV Spectrum for Theophylline (20 µg/ml) at 273 nm and Bambuterol (50 µg/ml) at 264 nm in methanol:water (1:1).**



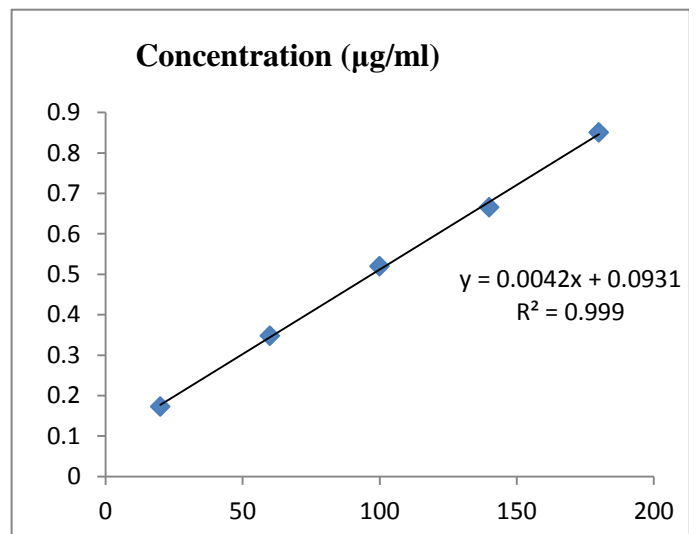
**Figure 6: Overlain D<sup>1</sup> spectrum of Theophylline (20-180 µg/ml) and Bambuterol (10-50 µg/ml) in methanol:water (1:1).**



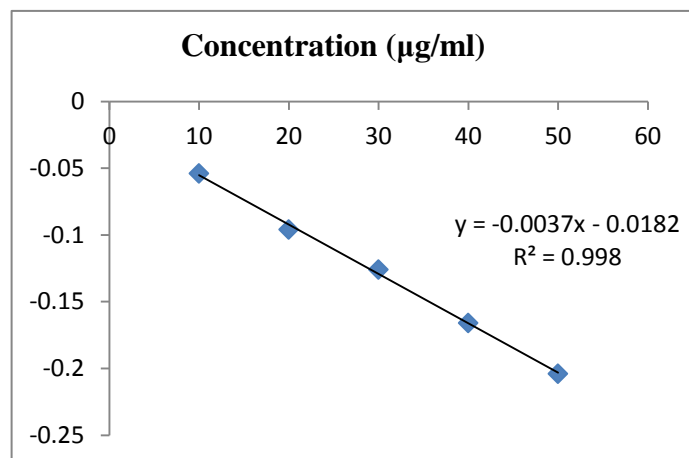
**Figure 4: Overlain D<sup>1</sup> spectrum of Theophylline (20-180 µg/ml) in methanol:water (1:1).**



**Figure 5: Overlain D<sup>1</sup> spectrum of Bambuterol (10-50 µg/ml) in methanol:water (1:1).**



**Figure 7: Calibration curve of Theophylline at 264 nm in methanol:water (1:1).**



**Figure 8: Calibration curve of Bambuterol at 273nm in methanol:water (1:1).**

## REFERENCES

1. Budavari S, The Merck Index, An encyclopedia of chemicals, drugs and biological, Merck Research Laboratories, UK, Fourteen Edition (2004):953, 9285.
2. Tripathi KD, Essential Medical pharmacology, Jaypee Brother Medical Publisher, Six Edition:216-222,225.
3. Indian Pharmacopoeia, Ministry of Health and Family welfare, The Controller of Publication, Ghaziabad, Govt. of India, Volume 3(2007):386.
4. United State Pharmacopoeia and National Formulary, USP-24 NF-19, The official compendia of standards Inc. Rockville, Asian Edition (2004):1811-1812.
5. British Pharmacopoeia, Department of Pharmaceutical Science, London, The Pharmaceutical Press, (2009):580.
6. A service of the U.S. National Institutes of Health, Bambuterol Combination Therapy with Theophylline in Patients with High Altitude Pulmonary Hypertension, (<http://clinicaltrials.gov/show/NCT01566565>). Accessed 3 September 2013.
7. Rowe RC, Sheskey P and Owen S, Hand book of Pharmaceutical Excipients, Pharmaceutical Press, London, Fifth Edition (2006):430, 611, 725, 767.
8. Patel SK, Patel DJ and Patel NJ, Simultaneous Spectrophotometric Determination of Montelukast Na and Bambuterol HCL in Tablets, *International Research Journal of Pharmacy*, 2011; 2(8):154-158.
9. Latha PE and Abiram L, Simultaneous Determination of Montelukast Sodium and Bambuterol Hydrochloride In Tablet Dosage Form By Ultraviolet Spectrophotometry, *International Research Journal of Pharmaceutical and Applied Sciences*, 2011; 1(1): 1-8.
10. Patel RB, Parmar RR, Patel VM and Shah DA, Simultaneous estimation of Montelukast sodium and Theophylline in pharmaceutical dosage form by UV Method, *International Journal of Institutional Pharmacy and Life Sciences*, March-April 2012; 2(2):150-160.
11. Patel SA, Patel SK , Patel DJ and Patel NJ, Analytical Method Development and Validation of Montelukast Sodium and Bambuterol Hydrochloride in Combined Dosage Form by RP-HPLC, *International Journal of Pharm Tech Research*, 2(3):1767-1771.
12. Gupta AVN, Bhargavi CH, Runa YA, Jhansi MR, Anusha PD, Lavanya G, Beulah TJ and Parvathi P, RP HPLC method development and validation of bambuterol in formulation, *International Journal of Research and Review in Pharmacy and Applied Sciences*, 2011; 1(3):186-191.
13. Jain N, Jain H, Jain A and Jain P, Development and Validation of RP- HPLC Simultaneous Estimation Method of Montelukast and Bambuterol for Stability Indicating Assay, *Journal of Drug Discovery and Therapeutics*, 2013;1(9):6-12
14. Branislava S, Vukosava D, Nevena G, Rade I and Zika L, Simultaneous HPLC Determination of Caffeine and Theophylline in Food, Drinks, and Herbal Products, *Journal of Chromatographic Science*, February 2008; (46):144-149.
15. Mand NP and Kaushal KC, Method development, validation and stability study for simultaneous estimation of Etofylline and Theophylline by RP-HPLC chromatography in marketed formulation, *Journal of Chemical and Pharmaceutical Research*, 2011; 3(3):597-609.
16. Sundan S, Muralidharan S, Mohammed S, Rajendran SD and Sundaram S, A Reliable reverse phase high performance liquid chromatography method for Theophylline, *Asian Journal of Pharmaceutical and Clinical Research*, 2013; 6(2):214-217.
17. Venkatesh V, Elphine PA, Venkata SP, Umamaheswari C and Rama Rao N, A New RP-HPLC Method for Simultaneous Estimation of Etophylline and Theophylline in Tablets, *Research Journal of Pharmaceutical and Technology*, January 2011; 4(1):128-131.
18. Maithani M, Ranjit Singh, Development and Validation of a Stability-Indicating HPLC Method for the Simultaneous Determination of Salbutamol Sulphate and Theophylline in Pharmaceutical Dosage Forms, *Journal of Analytical and Bioanalytical Technique*, 2011; 2(1):1-5.

