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STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ALBUTEROL SULPHATE, THEOPHYLLINE AND BROMHEXINE IN BULK AND COMBINED DOSAGE FORM

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Keywords:

UPLC, Albuterol sulfate, Theophylline, Bromohexine hydrochloride, ICH guidelines

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ABSTRACT: A new simple, precise, accurate and selective UPLC method has been developed and validated for stability indicating UPLC method for simultaneous estimation of Albuterol sulfate, Theophylline and Bromhexine HCl in the tablet dosage form. The method was carried out on a Hibra C18, 250mm x 4.6mm, 5µm. a column with a mobile phase consisting of buffer and acetonitrile and buffer in the ratio of (55: 45 v/v/v) and flow rate of 1.0 ml/ min. The detection was carried out at 260nm. The retention time for Albuterol sulfate, Theophylline, and Bromhexine HCl were found to be 5.8, 2.3 and 9.7 min respectively. The method was validated according to the ICH guidelines for specificity, LOD, LOQ, precision, accuracy, linearity and robustness. The method showed good reproducibility and recovery with %RSD less than 2. So the proposed method was found to be simple, specific, precise, accurate and linear than the methods reported earlier. Hence, the method is economical, and it can be applied for routine analysis of Albuterol sulphate, Theophylline and Bromhexine HCl in bulk drug and pharmaceutical preparations. When applied for tablet assay, drug content was within the limits of the labelled content.

INTRODUCTION: Albuterol is a beta (2)-adrenergic agonist. It stimulates beta (2)-adrenergic receptors. Binding of Albuterol to beta (2)-receptors in the lungs results in the relaxation of bronchial smooth muscles. Albuterol increases cAMP production by activating adenylate cyclase, and the actions are mediated by cAMP. Increased intracellular cyclic AMP increases the activity of cAMP-dependent protein kinase A, which inhibits the phosphorylation of myosin and lowers intracellular calcium concentrations.

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A lowered intracellular calcium concentration leads to smooth muscle relaxation. Increased intracellular cyclic AMP concentrations also cause an inhibition of the release of mediators from mast cells in the airways.

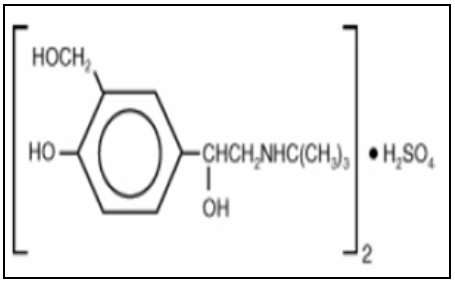

FIG. 1: ALBUTEROL SULPHATE

Theophylline, also known as 1,3-dimethylxanthine, is a methylxanthine drug used in therapy for respiratory diseases such as chronic obstructive pulmonary family, it bears structural and

pharmacological similarity to theobromine and caffeine. Disease (COPD) and asthma under a variety of brand names. As a member of the xanthine.

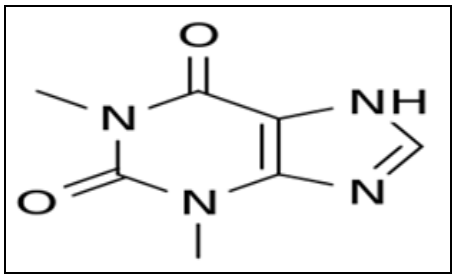

FIG. 2: THEOPHYLLINE

Bromhexine is an oral mucolytic agent with a low level of associated toxicity. Bromhexine acts on the mucus at the formative stages in the glands, within the mucus-secreting cells. Bromhexine disrupts the structure of acid mucopolysaccharide fibers in mucoid sputum and produces less viscous mucus, which is easier to expectorate.

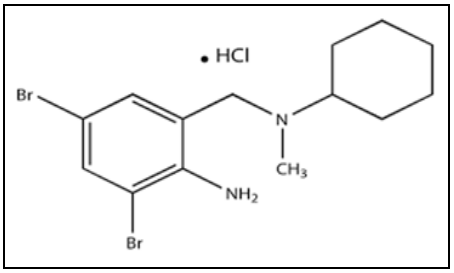

FIG. 3: BROMHEXINE HYDROCHLORIDE

According to the literature survey, few HPLC methods [1 - 8] UV methods [9, 10] and UV differential [11] method and UV-Visible spectrophotometric methods [12] are developed individually or with another combination of drugs. The proposed method aimed to developed and validate a stability indicating a method for the estimation of Albuterol sulfate, Theophylline and Bromohexine hydrochloride in pharmaceutical dosage form by UPLC.

MATERIAL AND METHODS:

Selection of Wavelength (For Detection): In setting up the conditions for the development of assay method, the choice of detection wavelength was based on the scanned absorption spectrum for Albuterol sulfate, Theophylline, and Bromhexine HCl. The UV-spectrum of Albuterol sulfate, Theophylline, and Bromhexine HCl was obtained separately by scanning the sample over the wavelength range 200 - 400 nm against blank as methanol. After a thorough examination of the spectra, the wavelength of 260 nm was selected for further analysis. The Overlay spectrum for Albuterol sulfate, Theophylline, and Bromhexine HCl was shown in **Fig. 4**.

Optimized Method:

HCl Preparation of Buffer: Taken 1 ml of triethylamine in 1L distilled water to this adjusts pH-2.5 with OPA. Filter through a 0.45µ membrane filter.

Mobile Phase: A mixture of buffer and acetonitrile in the ratio of 45:55% v/v was sonicated to degas and filtered through a 0.45µm nylon membrane filter.

Preparation of Diluent: Acetonitrile: Buffer (55: 45v/v).

Chromatographic Conditions:

Column	:	Hibra C18, 250mm × 4.6mm, 5µm
Mobile phase	:	Acetonitrile: Buffer (55:45% v/v)
Flow rate	:	1.0 ml/min
Detection Wavelength	:	260 nm
Injection Volume	:	5 µl
Temperature	:	Ambient
Run time	:	10 min

The retention time of Albuterol sulfate is about 5.8 min. The retention time of Theophylline is about 2.3 min. The retention time of Bromhexine is about 9.7 min.

Preparation of Standard Stock Solution:

Solution A:

Albuterol Sulphate: Weighed accurately about 4mg Albuterol sulfate working standard into a 100 ml volumetric flask. Added 70 ml of diluent, sonicated to dissolve and diluted to volume with diluents.

Solution B:

Theophylline: Weighed accurately about 200 mg of Theophylline working standard into a 100 ml volumetric flask. Added 70 ml of diluent, sonicated to dissolved and diluted to volume with diluent.

Solution C:

Bromhexine HCl: Weighed accurately about 8mg Bromhexine HCl working standard into a 100 ml

volumetric flask. Added 70 ml of diluent, sonicated to dissolve and diluted to volume with diluent. Further diluted every 5 ml of solution-A, B and C to 50 ml with the diluent.

Preparation of Sample Solution: Weighed accurately 10 tablets and powdered then taken 5 tablets equivalent of the sample into a 250 ml volumetric flask. Added 200 ml of diluent, sonicated to dissolve and diluted to volume diluent and further diluted 5 ml to 100 ml with the diluent and filtered through a 0.45µ nylon syringe filter.

Procedure: Injected 5µL of standard preparation five times and sample preparation in the chromatograph. Recorded the chromatograms and measured the peak responses for Albuterol sulfate, Theophylline, Bromhexine HCl. The system suitability parameters should be met. From the peak responses, calculated the content of Albuterol sulfate, Theophylline, Bromhexine HCl in the sample. The assay calculations were shown in **Table 1**. And the **Fig.** is shown in **2**, **3** and **4**.

Method Validation:

System Suitability: The UPLC system was stabilized for thirty minutes. by following the chromatographic conditions to get a stable baseline. One blank followed by six replicates of a standard solution was injected to check the system suitability. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the retention times, tailing factor, theoretical plates, and %RSD peak areas from six replicate injections. The results are shown in the below **Table 2**, **3** and **4**.

Linearity: A series of solutions were prepared using Albuterol sulfate, Theophylline and Bromhexine HCl working standard at concentration levels from 0-5µg/mL, 32-272µg/mL and 1-11 µg/mL respectively, the solutions were injected into the system as per test procedure. Measure the peak area response of the solution**.** The calibration graph was plotted with peak area in the Y-axis and concentration of standard solutions in the X-axis. The data is given in below **Table 5**, **6** and **7** and the calibration curve is shown in the below **Fig. 8**.

Accuracy: The accuracy of the developed method was determined by assay and recovery studies. Recovery studies were carried out at three different levels. The pre-analysed samples were spiked with 50%, 100% and 150% of the mixed standard solution. The mixtures were analyzed by the proposed method. The study was carried out in triplicate. The obtained recovery results are given in **Table 8**, **9** and **10**.

Precision:

System Precision (Repeatability): System precision was carried out using six replicates of the same standard concentration. The chromatograms were recorded and mean, standard deviation and % RSD was calculated. The data of the system precision is given in below **Tables 11**, **12** and **13**.

Method Precision (Intraday): Method precision was carried out using six different samples of Albuterol sulfate, Theophylline and Bromhexine HCl drug substance were prepared with a target concentration of about Albuterol sulfate 4.5 ppm, Theophylline 200.2 ppm, and Bromhexine HCl 8.3ppm. Preparations from the same homogenous blend of the marketed sample. The data of the method precision is given in below **Table 14**, **15** and **16**.

Intermediate Precision (Inter day): Six sample solutions are prepared and injected on the next day into the HPLC system as per the test procedure. The observations of Intermediate precision were given in below **Table 17**, **18** and **19**.

Robustness: The method can remain unaffected by small, deliberate variations in the method parameters. In the case of liquid chromatography examples of typical variations are: influence of variations in wavelength detectors (+/5nm), influence of variations in column temperature (+/-5nm), influence of variations in mobile phase compositions (+/-5%), influence of variations in flow rate (+/0.2%), influence of variations of p in mobile phase (+/-5%), all observed values are summarised in **Table 20**.

The Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ of the developed methods were determined by injecting progressively low concentrations of standard solutions using the developed UPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Albuterol sulfate was

found to be 0.133mg/ml, for Theophylline 0.336 mg/ml and for Bromohexine 0.18 mg/ml. The LOQ is the smallest concentration of the analyte, which gives a response that can be accurately quantified 9 signals to noise ratio of 10). The LOQ of Albuterol sulphate 0.40mg/ml, 19.20 mg/ml for Theophylline and 0.57mg/ml of Bromohexine HCl. It was concluded that the developed method is sensitive and the results are shown in **Table 21**.

Solution Stability: The solution stability of Albuterol sulfate, Theophylline, and Bromhexine HCl in diluents were determined by storing sample solution in a tightly capped volumetric flask at room temperature for 24 h. The amount of Albuterol sulfate, Theophylline, and Bromhexine HCl were measured at different time intervals like 12 and 24 h and results obtained were compared with Albuterol sulfate, Theophylline, and Bromhexine HCl freshly prepared solution. The results are shown in below **Table 22**, **23** and **24**.

RESULTS AND DISCUSSION: The main aim of the study was to develop a stability indicating UPLC method for the estimation of Albuterol sulfate, Theophylline and Bromohexine hydrochloride in bulk and tablet dosage form and to validate the method. Initially, various mobile phase compositions were tried to elute the drugs. Mobile phase ratio and flow rate were selected based on peak parameters and retention time. Standard solution of 40 µg/ml, 200 µg/ml and 8 µg/ml were prepared and scanned in the range of 200-400 nm for detecting the maximum absorption wavelength, and it was found to be 260 nm **Fig. 4**.

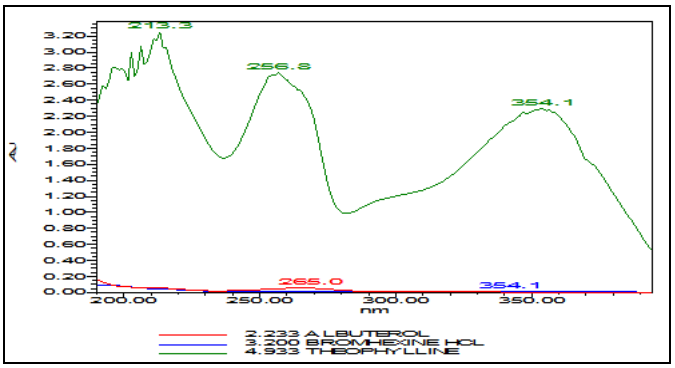

FIG. 4: SELECTION OF WAVELENGTH

TABLE 1: ASSAY CALCULATIONS

Drug	Area	Labelled amount (mg)	Amount present (mg)	% Assay
Albuterol sulphate	1805390	4	4.00	100.9
Theophylline	3243736	200	200.00	100.5
Bromhexine HCl	2986151	8	8.00	100.7

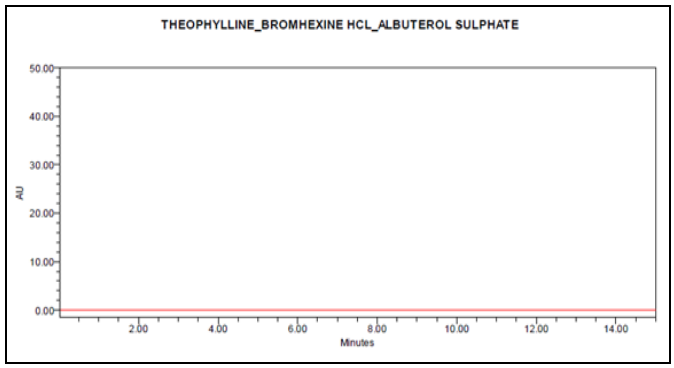

FIG. 5: A REPRESENTATIVE CHROMATOGRAM OF BLANK

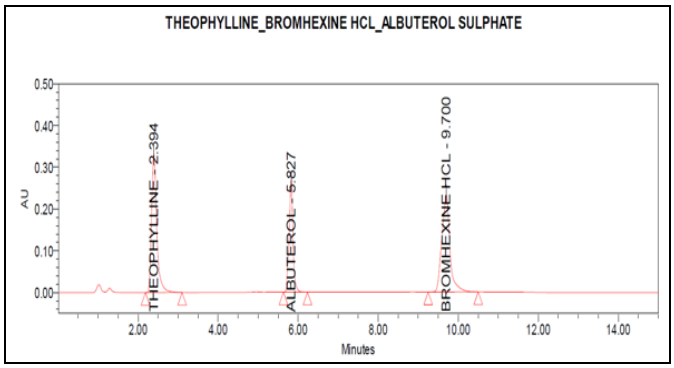

FIG 6: A REPRESENTATIVE CHROMATOGRAM OF STANDARD

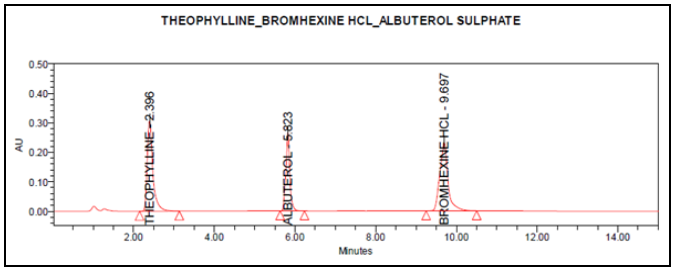

FIG 7: A REPRESENTATIVE CHROMATOGRAM OF SAMPLE

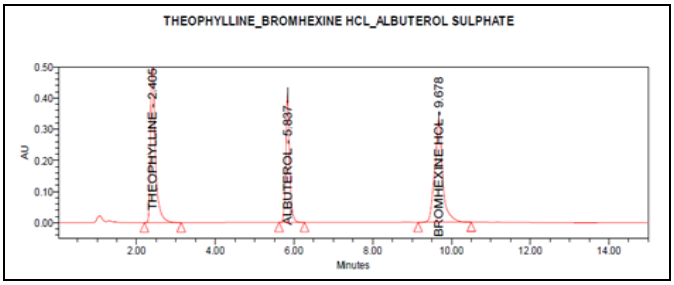

FIG. 8: CHROMATOGRAM OF LINEARITY

TABLE 2: SYSTEM SUITABILITY FOR ALBUTEROL SULPHATE

S. no.	Sample name	Name	Injection	Rt	Area	USP plate count	USP tailing
1	Std	Albuterol sulphate	1	5.845	1879279	20688	1.09
2	Std	Albuterol sulphate	2	5.840	1844526	21221	1.14
3	Std	Albuterol sulphate	3	5.845	1857798	20185	1.13
4	Std	Albuterol sulphate	4	5.846	1890599	19921	1.13
5	Std	Albuterol sulphate	5	5.838	1880684	20384	1.15
6	Std	Albuterol sulphate	6	5.838	1865163	20295	1.15
Mean					1869675		
%RSD					0.909		

TABLE 3: SYSTEM SUITABILITY FOR THEOPHYLLINE

S. no.	Sample name	Name	Injection	Rt	Area	USP Tailing	USP Plate Count
1	Std	Theophylline	1	2.404	3029729	2464	1.55
2	Std	Theophylline	2	2.404	2979135	2433	1.60
3	Std	Theophylline	3	2.404	3001398	2455	1.62
4	Std	Theophylline	4	2.402	3054458	2463	1.63
5	Std	Theophylline	5	2.403	3040229	2471	1.64
6	Std	Theophylline	6	2.403	3013715	2470	1.65
Mean					3019777		
%RSD					0.906		

TABLE 4: SYSTEM SUITABILITY FOR BROMHEXINE HCl

S. no.	Sample Name	Name	Injection	Rt	Area	USP Tailing	USP Plate Count
1	Std	Bromhexine HCl	1	9.675	3297365	10942	1.13
2	Std	Bromhexine HCl	2	9.676	3266964	11772	1.17
3	Std	Bromhexine HCl	3	9.684	3284638	11524	1.17
4	Std	Bromhexine HCl	4	9.684	3327458	11600	1.18
5	Std	Bromhexine HCl	5	9.680	3314381	11702	1.18
6	Std	Bromhexine HCl	6	9.676	3282334	11623	1.19
Mean					3295523		
%RSD					0.677		

After considering the entire system suitability parameters mobile phase buffer and acetonitrile in the ratio of (45:55% v/v) run in the isocratic mode and flow rate 1.0ml/min were selected. The retention time of Albuterol sulfate, Theophylline, and Bromohexine hydrochloride was found to be 5.7, 2.3, 9.7 min. The system suitability parameters are calculated from standard chromatograms **Fig. 6.**

TABLE 5: LINEARITY DATA OF ALBUTEROL SULPHATE

Linearity	Solution took	PPM	%W/W	Area counts
Linearity-1	0	0.00	0	0
Linearity-2	0.8	0.67	397688	380101
Linearity-3	1.7	1.43	749988	776816
Linearity-4	2.2	1.85	940260	960448
Linearity-5	2.7	2.27	1147727	1150935
Linearity_6	3.5	2.94	1468241	1507551
Linearity-7	4.4	3.70	1833042	1888109
Linearity-8	5.5	4.62	2213642	2277133
Linearity-9	6.8	5.712	2686319	2891817

TABLE 6: LINEARITY DATA OF THEOPHYLLINE

Linearity	Solution taken	PPM	%W/W	Area counts
Linearity-1	0	0.00	0	0
Linearity-2	0.8	32.06	644669	300755
Linearity-3	1.7	68.14	1216519	560960
Linearity-4	2.2	88.18	1540716	654609
Linearity-5	2.7	108.22	1856366	788671
Linearity_6	3.5	140.28	2378009	991226
Linearity-7	4.4	176.35	2964337	1245763
Linearity-8	5.5	220.44	3585385	1521448
Linearity-9	6.8	272.544	4359018	1950376

TABLE 7: LINEARITY DATA OF BROMHEXINE

Linearity	solution taken	PPM	%W/W	Area counts
Linearity-1	0	0.00	0	0
Linearity-2	0.8	1.30	749215	130987
Linearity-3	1.7	3.08	1371349	273056
Linearity-4	2.2	3.89	1708737	339988
Linearity-5	2.7	4.70	2084599	408583
Linearity_6	3.5	5.99	2634885	538022
Linearity-7	4.4	7.45	3257262	681907
Linearity-8	5.5	9.07	3882378	820950
Linearity-9	6.8	11.016	4640068	1040686

TABLE 8: ACCURACY RESULTS OF ALBUTEROLSULPHATE BY UPLC

S. no.	Accuracy	Amount added (mg)	Area	Amt recovered	% Recovery	Results
1	50%	11.25	942279	11.33	100.7	Mean=100.5
2	50%	11.3	940634	11.31	100.1	SD=0.34
3	50%	11.1	928493	11.17	100.6	%RSD=0.340
1	100%	22.5	1876041	22.57	100.3	Mean=100.3
2	100%	21.9	1821372	21.91	100.0	SD=0.25
3	100%	21.9	1830488	22.02	100.5	%RSD=0.250
1	150%	32.7	2726540	32.8	100.3	Mean=100.3
2	150%	32.5	2700649	32.48	99.9	SD=0.30
3	150%	32.3	2699856	19.14	100.5	%RSD=0.300
			Mean=100.4; SD=0.115; % RSD=0.11			

TABLE 9: ACCURACY RESULTS OF THEOPHYLLINE BY UPLC

S. no.	Accuracy	Amount added (mg)	Area	Amt recovered	% Recovery	Results
1	50%	510.2	1542700	511.17	100.2	Mean=100.4
2	50%	503.7	1534148	508.34	100.9	SD=0.50
3	50%	501.7	1513422	501.47	100.0	%RSD=0.500
1	100%	1010.4	3060683	1014.15	100.4	Mean=100.1
2	100%	980.8	2953742	978.72	99.8	SD=0.30
3	100%	981.3	2968330	983.55	100.2	%RSD=0.300
1	150%	1455.3	4410783	1461.51	100.4	Mean=100.2
2	150%	1450.8	4388689	1454.18	100.2	SD=0.32
3	150%	1455.3	4383066	1452.32	99.8	%RSD=0.320
			Mean=100.2; SD=0.153; % RSD=0.15			

TABLE 10: ACCURACY RESULTS OF BROMHEXINE HCl BY UPLC

S. no.	Accuracy	Amount Added (mg)	Area	Amt recovered	% Recovery	Results
1	50%	21.5	1717003	21.6	100.5	Mean=100.3
2	50%	21.6	1715419	21.58	99.9	SD=0.36
3	50%	21.2	1694081	21.32	100.6	%RSD=0.350
1	100%	40.8	3259072	41.01	100.5	Mean=100.4
2	100%	40.7	3231950	40.67	99.9	SD=0.37
3	100%	40.6	3246415	40.85	100.6	%RSD=0.370
1	150%	58.8	4696713	59.1	100.5	Mean=100.7
2	150%	58.3	4669160	58.75	100.8	SD=0.14
3	150%	58.4	4673845	58.81	100.7	%RSD=0.13
			Mean=100.5 SD=0.208 % RSD=0.21			

TABLE 11: SYSTEM PRECISION VALUES OF ALBUTEROL SULPHATE BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	5.845	1879279	20688	1.09
2	5.840	1844526	21221	1.14
3	5.845	1857798	20185	1.13
4	5.846	1890599	19921	1.13
5	5.838	1880684	20384	1.15
6	5.838	1865163	20295	1.15
		Mean=1869675; % RSD=0.909		

TABLE 12: SYSTEM PRECISION VALUES OF THEOPHYLLINE BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	2.404	3029729	2464	1.55
2	2.404	2979135	2433	1.60
3	2.404	3001398	2455	1.62
4	2.402	3054458	2463	1.63
5	2.403	3040229	2471	1.64
6	2.403	3013715	2470	1.65
		Mean=3019777; % RSD=0.906		

TABLE 13: SYSTEM PRECISION VALUES OF BROMHEXINE HCl BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	9.675	3297365	10942	1.13
2	9.676	3266964	11772	1.17
3	9.684	3284638	11524	1.17
4	9.684	3327458	11600	1.18
5	9.680	3314381	11702	1.18
6	9.676	3282334	11623	1.19
		Mean=3295523; % RSD=0.677		

TABLE 14: METHOD PRECISION FOR ALBUTEROL SULPHATE BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	5.849	1827803	21053	1.15
2	5.839	1887496	23100	1.15
3	5.836	1826955	22544	1.17
4	5.840	1910677	22578	1.14
5	5.841	1852287	23280	1.17
6	5.838	1836021	23554	1.1
		Mean=1856873; % RSD=1.870		

TABLE 15: METHOD PRECISION FOR THEOPHYLLINE BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	2.409	2967776	2489	1.69
2	2.408	2975437	2520	1.56
3	2.411	2962033	2531	1.67
4	2.412	2973888	2637	1.52
5	2.409	2961482	2519	1.64
6	2.409	2975765	2528	1.68
		Mean=2969397; % RSD=0.222		

TABLE 16: METHOD PRECISION FOR BROMHEXINE HCl BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	9.718	3202711	12445	1.19
2	9.703	3286920	12335	1.19
3	9.707	3262277	13511	1.21
4	9.716	3271142	11351	1.13
5	9.712	3257004	11911	1.16
6	9.714	3267645	12913	1.18
		Mean=3257950; % RSD=0.887		

TABLE 17: INTERMEDIATE PRECISION VALUES FOR ALBUTEROL SULPHATE BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	5.836	1798616	18080	1.06
2	5.831	1794579	19342	0.95
3	5.823	1784375	20022	0.99
4	5.831	1784375	18688	0.90
5	5.833	1782547	18861	1.04
6	5.833	1794919	19148	1.05
		Mean=1791097; % RSD=0.355		

TABLE 18: INTERMEDIATE PRECISION VALUES FOR THEOPHYLLINE BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	2.394	2985315	1744	1.54
2	2.398	2983757	1819	1.38
3	2.395	299209	1794	1.48
4	2.399	2979326	1844	1.26
5	2.397	2974316	1680	1.51
6	2.398	2976653	1658	1.48
		Mean=2983096; %RSD=0.299		

TABLE 19: INTERMEDIATE PRECISION VALUES FOR BROMHEXINE HCl BY UPLC

S. no.	Rt	Area	USP Plate count	USP tailing
1	9.711	3125101	9885	1.15
2	9.707	3110815	10313	1.03
3	9.703	3225915	10022	1.17
4	9.713	3234585	9683	1.02
5	9.715	3229759	10619	1.18
6	9.714	3223820	11221	1.20
		Mean=3191666 %RSD=1.798		

TABLE 20: ROBUSTNESS

S. no.	Parameters	Albuterol sulfate	Theophylline	Bromhexine	Acceptance criteria
1	Wave length +5	99.25%	99.39%	99.41%	98-102%
2	Wavelength -5	99.41%	99.35%	99.39%	98-102%
3	Column temp +5	99.24%	99.32%	99.25%	98-102%
4	Column temp -5	99.29%	99.30%	99.24%	98-102%
5	Mobile phase 60:40	99.32%	99.35%	99.30%	98-102%
6	Mobile phase 55:45	99.30%	99.39%	99.35%	98-102%
7	Flow rate +0.2	99.24%	99.24%	99.32%	98-102%
8	Flow rate -0.2	99.35%	99.25%	99.30%	98-102%

TABLE 21: LOD AND LOQ

Sample	LOD	LOQ
Albuterol sulphate	0.133	0.40
Theophylline	6.336	19.20
Bromhexine HCl	0.18	0.57

TABLE 22: SOLUTION STABILITY OF ALBUTEROL SULPHATE

S. no.	Stability (h)	Rt (min)	Peak area	USP Plate count	USP Tailing	% assay
1	0	5.835	1971291	26703	1.26	99. 9
2	12	5.806	1925425	24363	1.34	100.3
3	24	5.806	1893560	24478	1.31	100.2

TABLE 23: SOLUTION STABILITY OF THEOPHYLLINE

S. no.	Stability (h)	Rt (min)	Peak area	USP Plate count	USP Tailing	% assay
1	0	2.405	3213016	4285	1.20	100.4
2	12	2.383	3123116	3633	1.38	100.8
3	24	2.383	3065164	3650	1.36	100.5

TABLE 24: SOLUTION STABILITY OF BROMHEXINE HCl

S. no.	Stability (h)	Rt (min)	Peak area	USP Plate count	USP Tailing	% assay
1	0	9.806	2821381	23782	1.23	100.9
2	12	9.720	2848372	21191	1.42	99.9
3	24	9.720	2796055	21343	1.41	99.8

CONCLUSION: The developed and validated UPLC method was found to be rapid, accurate, precise and robust, thus can be used for routine analysis of Albuterol sulfate, Theophylline and Bromohexine HCl in the combined dosage form.

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CONFLICT OF INTEREST: Nil

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