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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ACETYLCYSTEINE AND TAURINEIN TABLET DOSAGE FORM BY USING RP-HPLC

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Abstract:

Simple, accurate, rapid and precise reverse phase high performance liquid chromatographic method has been developed and validated for the determination of acetylcysteine and taurine in tablet dosage. The chromatographic separation was done on Isocratic elution using cosmosilC18, 250 x 4.6 mm, 5µm or equivalent. Flow rate is 1mL/min. Maintain the column temperature of acetylcysteine is 30ºC and taurine is 40ºC. Detection was carry out at acetylcysteine is 214nm and taurine is 338nm. Good linear relationship was produce acetylcysteine and taurine is 0.9998 and 0.9991. Linearity range is 0.2500mg/mL to 0.7500mg/mL solution stability acetylcysteine and taurine is 24hrs and 12hrs. The method development and validation of acetylcysteine and taurine in according to ICH guidelines.

Key words: *Acetylcysteine, RP-HPLC, Taurine, Validation*

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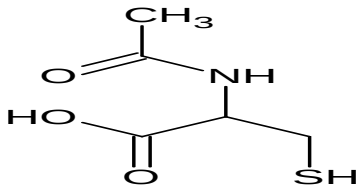
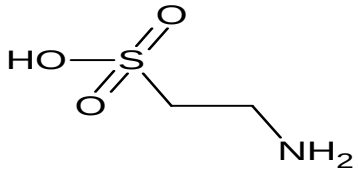
INTRODUCTION:



Structure of acetylcysteine



Structure of taurine

Acetylcysteine is used for the treatment of over dosage of acetaminophen (paracetamol) and it is good anti-oxidant. Acetylcysteine IUPAC name is a (2R)-2-acetamido-3-sulfanylpropanic acid, molecular formula is $C_5H_9NO_3S$ [1], and molecular weight 163.5. Acetylcysteine is the derivative of glutathione [2]. Soluble in water and alcohol, insoluble in chloroform and ether. In the treatment of paracetamol overdose, acetylcysteine acts to sustain or replenish depleted glutathione reserves in the hepatic and improve non-toxic metabolism of acetaminophen these actions serve to defend hepatic cells from NAPQI toxicity. It is most effective in preventing or lessening hepatic injury when administered within 8–10 hours after overdose [2]. Research recommends that the rate of hepatic toxicity is approximately 3% when acetylcysteine is administered within ten hours of overdose [2]. Equally effective oral acetylcysteine is for this indication oral administration is poorly tolerated since high oral doses are necessary due to low oral bioavailability.

Taurine is chemically 2- amino ethane-1-sulphonic acid [3]. Molecular weight of taurine is 125.14, molecular formula is $C_2H_7NO_3S$;.Taurine is vital for development and function of skeletal muscle, the retina, the central nervous system and cardiovascular function [3]. Taurine is conjugated through it is chenodeoxycholic acid with amino terminal group. Conjugation of bile acids, anti oxidation, osmoregulation, membrane stabilitilization, modulation of calcium signaling, cardiovascular function and skeletal muscle function. Shelf-life of 36 months at 25°C 100% Shelf-life was measured under accelerated conditions at 40°C and no loss was observed for six months parts of 95% alcohol dissolves 0.004 parts at 17°C; insoluble in absolute alcohol [3]. Taurine crosses the BBB (blood brain barrier) and has been implicated in a broad array of physiological phenomena together with inhibitory neurotransmission.

MATERIALS AND METHODS:

Instrumentation

Shimadzu for acetylcysteine, Agilent for taurine using cosmosil5C18-MS-11(250×4.6mm, 5µ), UV detector, Lab India- ultrasonicator, Electronic balance.

Reagents and chemicals

Acetylcysteine and taurine pure drug samples were received from fourrts India laboratories Pvt. Limited. Other reagent and chemical using Finar, Fisher and Rankem grade. Combination of tablet (Brand name: nefrosave) containing 500mg of taurine and 150mg mg of acetylcysteine.

Chromatographic conditions

S.No	Contents	acetylcysteine	taurine
1.	Flow rate	1mL/ min	1mL/ min
2.	Column	CosmosilC18MS-11 (250×4.6mm,5µ)	CosmosilC18MS-11 (250×4.6mm,5µ)
3.	Column temperature	30^0C	40^0C
4.	Injection volume	10µL	10µL(online derivatization) Buffersolution:OPA:sample/standard in the ratio of 5:3:3
5.	Mobile phase	100% buffer	Mix equal volume of mobile phase A and B
6.	Diluent	Sodium metabisulfite solution	Mix equal volume of mobile phase A and B
7.	Wave length	214nm	338nm

Acetylcysteine [6,8]

Buffer preparation

Dissolve 6.8g of monobasic potassium phosphate in 1000mL of water, filter and degas. Adjust the pH to 4.5 with phosphoric acid.

Sodium metabisulfite solution

Weigh accurately about 500 mg of sodium metabisulfite and dissolve in 1000mL of water.

Standard solution

Weigh accurately about 50 mg of acetylcysteine working standard in 100 mL volumetric flask, add 50 mL of sodium metabisulfite solution, dissolve and make up the volume with sodium metabisulfite solution.

Test solution

Weigh and powder 20 tablets. Weigh accurately about 275 mg of powdered tablets into a clean, 100mL volumetric flask. Add 50mL of sodium metabisulfite solution and sonicate for 60 minutes. Make up the volume to 100mL with sodium metabisulfite solution, mix well and filter. Use the filtrate.

Taurine

Preparation of Mobile phase

Mobile phase A

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 1000 mL of water. Add 0.18 mL of triethylamine and adjust the pH to 7.2 using dilute acetic acid and then add 3.0 mL of tetrahydrofuran. Mix well and filter and degas it.

Mobile phase B

Weigh accurately about 2.72 g of anhydrous sodium acetate and dissolve it in 200 mL of water. Adjust the pH to 7.2 using dilute acetic acid and then add 400 mL of acetonitrile and 400 mL of methanol. Mix well and filter and degas it.

Standard solution

Weigh accurately about 250 mg of taurine working standard into clean, 50 mL volumetric flask. Add 30 mL of diluents and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent and mix well. Dilute 5 mL of this solution to 50 mL with the diluent.

Test solution

Weigh and crush 20 tablets to a fine powder. Weigh accurately about 420mg of powdered tablet into clean, 50 mL volumetric flask. Add 30 mL of diluent and sonicate for 10 minutes. Make up the volume to 50 mL with the diluent, mix well and filter. Dilute 5 mL of the filtrate to 50 mL with the diluents.

Development and Validation of HPLC method[4,7]

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of acetylcysteine and taurine in tablet dosage forms. The experiment was carried out according to the official specifications of ICH- 1996, Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness. System suitability a standard solution was prepared using Saxagliptin and Metformin working standard as per the test method and was injected six times into the HPLC system. The parameters namely USP plate count, peak asymmetry factor and resolution for the standard solutions were calculated[5].

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Saxagliptin and Metformin from impurities.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of acetylcysteine and taurine at different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of acetylcysteine and taurine to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

Accuracy is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed.

Precision

Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2%.

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 mL/min, amount of diluents (10% to 15%) the temperature of the column (28°C to 32°C) and pH of the mobile phase

RESULT AND DISCUSSION:

Method development

Specificity

Placebo solution was prepared separately at a concentration of 2.25 mg/mL of expedient blend. A solution of *placebo* was spiked with the acetylcysteine and taurine at its working concentration. The solution was analyzed as per the HPLC method described. Table 1 summarizes the retention time (RT) and relative retention time (RRT) values obtained for *placebo* and acetylcysteine.

System Precision

A standard solution of 0.50 mg/mL of acetylcysteine and taurine was prepared and analyzed as per the method. Table 2: summarizes system suitability results.

Linearity and range

The linearity of the HPLC method was demonstrated for acetylcysteine and taurine ranging from 0.2500 mg/mL to 0.7500 mg/mL, which is equivalent to 50% to 150% of the acetylcysteine and taurine working strength. Five standard solutions at the concentrations within the mentioned range were prepared and analyzed as per the method. The linearity results obtained are shown in Table 3 and Figure 1 shows the line of best fit for concentration versus peak area of acetylcysteine**.**

Ruggedness (Intermediate Precision)

The ruggedness of the method was performed by analyzing a sample solution acetylcysteine and Taurine as per the test method (six replicate sample preparation) and injected each solution in duplicate using different instrument, column, reagent, and analyst on different days. The results of set I were compared with the results of set II.

Robustness

The following table shows the parameters of the method that were altered to test the robustness of the method. System suitability test was carried out to asses if these changes had a significant effect on the chromatography.

Parameter / Condition	Actual	Low	High
Flow rate	1.00 mL/min	0.90 mL/min	1.10 mL/min
Mobile phase	100% Buffer Conc.: 6.8 g / L	100% Buffer Conc.: 6.7 g / L	100% Buffer Conc.: 6.9 g / L
Buffer Ph	3.0	2.9	3.1
Column oven temperature	30°C	28°C	32°C

Solution stability

A solution of acetylcysteine and Taurine at 100% of working concentration was kept at 15°C. The solution stability was monitored at different time interval (Initial, 6 hours, and 12 hours, 24 hours, 36 hours and 48 hours).

Table 1: specificity of acetylcysteine and taurine

S.No	Parameters	acetylcysteine	Taurine
1.	Retention time	13.01	5.70
2.	Relative retention time	1.00	1.00

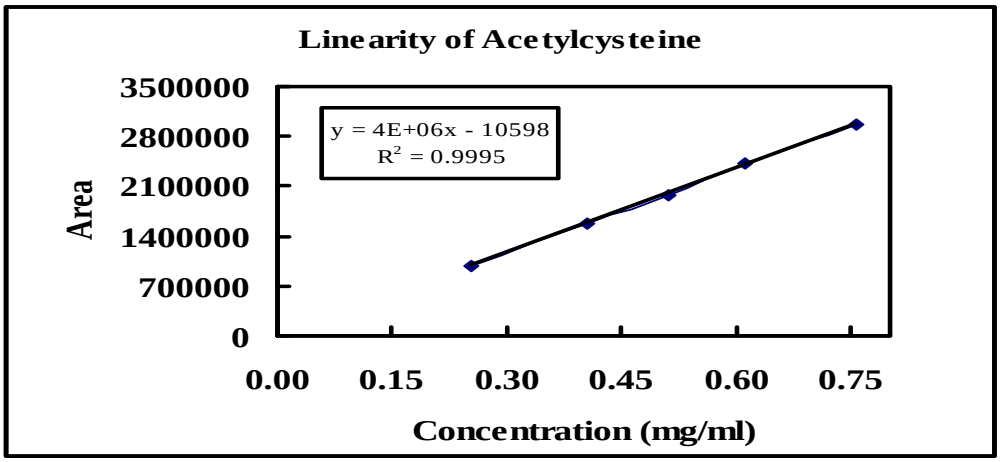
No peak was observed at the retention time of acetylcysteine and taurine in the chromatogram of *Placebo*

Table 2: Summary of retention time, % RSD of peak area, tailing factor and theoretical plates of the acetylcysteine and taurine peak

Content	acetylcysteine	taurine
Retention time(min)	13.18-13.23	5.40-5.44
Area	1884890-1900436	122758536-124554851
Tailing factor	1.29	1.56
Theoretical plates	10788	10120
Average (Area)	1891268	123552999
%RSD(Area)	0.27	0.51

Table 3: Linearity of acetylcysteine

Level	% of acetylcysteine	Concentration (mg/mL)	Peak area
50%	50.2	0.251	993424
80%	80.6	0.403	1583987
100%	101.6	0.508	1996797
120%	121.6	0.608	2436183
150%	150.8	0.754	2980211
		correlation coefficient	0.9995



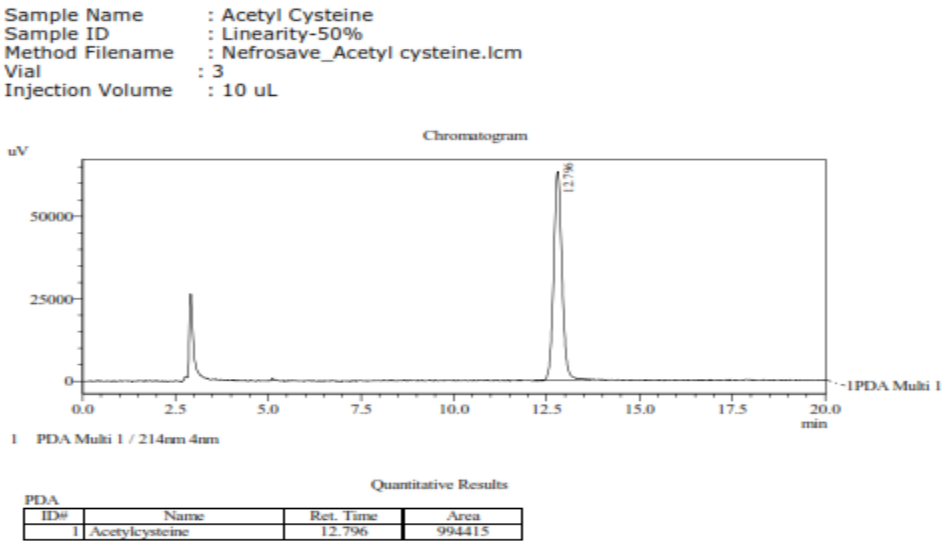
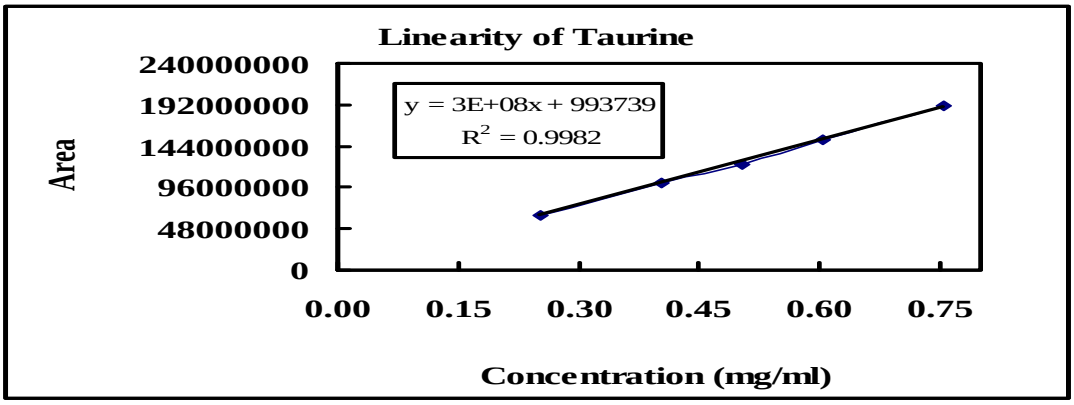


Fig. 1: Linearity of acetylcysteine

Table 3.1: Linearity of taurine

Level	% of taurine	Concentration (mg/mL)	Peak area
50%	50.12	0.25060	65341127
80%	80.19	0.40096	103727083
100%	100.24	0.50120	124124017
120%	120.29	0.60144	151062506
150%	150.36	0.75180	190862892
		correlation coefficient	0.9991



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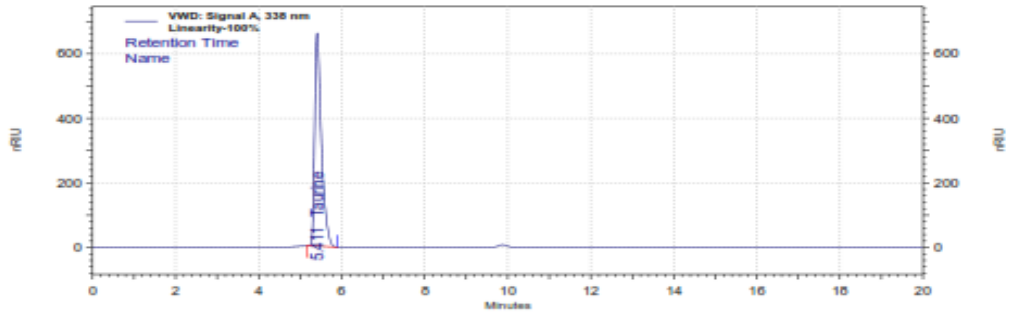
Sample Name : Linearity-100%
Injection Volume : 10 µL
Vial No : 8



VWD: Signal A, 338 nm Results

Peak Number	Name	Retention Time	Area
1	Taurine	5.41	124124017

Fig. 2: Linearity of taurine

Table 4: Accuracy of acetylcysteine

Level	% acetylcysteine working strength	Theoretical conc.(mg/mL)	Measured conc.(mg/mL)	% recovery
50%	51.0	0.25500	0.25822	101.26
	51.4	0.25700	0.25852	100.59
	50.8	0.25400	0.25772	101.46
100%	99.8	0.49900	0.50264	100.73
	100.2	0.50100	0.50134	100.07
	100.4	0.50200	0.49767	99.14
150%	149.4	0.74700	0.74422	99.63
	149.0	0.74500	0.73956	99.27
	150.2	0.75100	0.74264	98.89

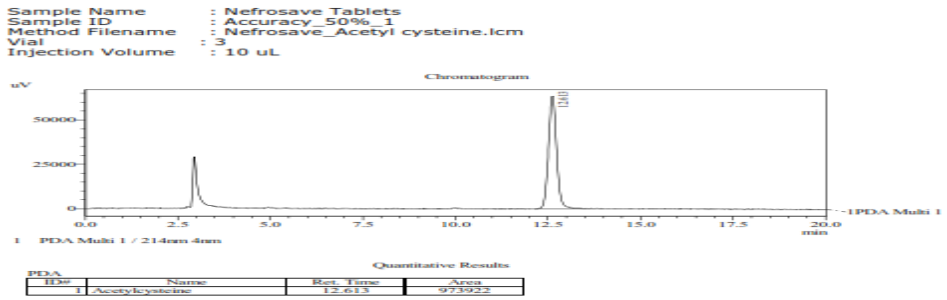


Fig.3: Accuracy of acetylcysteine

Table 4.1: Accuracy of taurine

Level	% taurine working strength	Theoretical conc. (mg/mL)	Measured conc.(mg/mL)	% recovery
50%	50.04	0.25020	0.25045	100.10
	50.24	0.25120	0.25414	101.17
	50.08	0.25040	0.25204	100.65
100%	100.04	0.50020	0.50262	100.48
	99.96	0.49980	0.49656	99.35
	100.08	0.50040	0.49598	99.12
150%	150.48	0.75240	0.76501	101.68
	150.20	0.75100	0.75375	100.37
	150.08	0.75040	0.75919	101.17

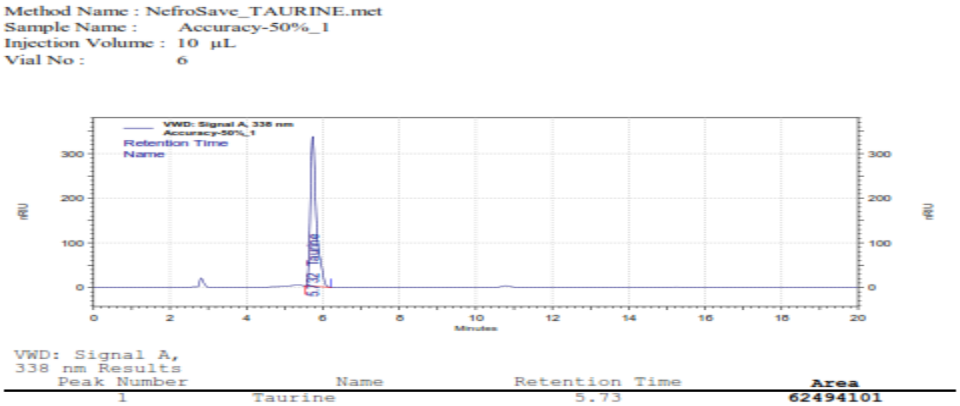


Fig. 4: Accuracy of taurine

Table 5: Summary of results for precision of the method of acetylcysteine

Sample	Level	% assay	Average	%RSD
1	100%	99.92	**99.95**	**0.67**
2	100%	100.03		
	100%	99.01		
4	100%	99.58		
5	100%	101.03		
6	100%	100.15		

Table 5.1: Summary of results for precision of the method of taurine

Sample	level	% assay	average	%RSD
1	100%	100.48	**101.32**	**0.46**
2	100%	101.63		
3	100%	101.42		
4	100%	101.09		
5	100%	101.52		
6	100%	101.75		

Table 6: Summary of results for ruggedness

Sample	% assay of acetylcysteine	
	Set – I	Set - II
1	99.92	100.85
2	100.03	99.23
3	99.01	99.95
4	99.58	99.71
5	101.03	101.19
6	100.15	99.73
Average	**99.95**	**100.11**
% RSD	**0.67**	**0.75**
Overall average	**100.03**	
Overall % RSD	**0.68**	

Table 6.1: Summary of results for ruggedness

Sample	% assay of taurine	
	Set – I	Set - II
1	100.48	100.33
2	101.63	101.40
3	101.42	99.31
4	101.09	99.18
5	101.52	100.28
6	101.75	99.77
Average	**101.32**	**100.05**
% RSD	**0.46**	**0.75**
Overall average	**100.68**	
Overall % RSD	**0.91**	

Table 7: Summary of robustness results of acetylcysteine (flow)

Flow	Actual	Low	High
Retention time(min)	13. 18-13.24	14.14-14.18	11.55-11.65
Area	1888358-1900436	2145603-2153669	1733324-1754218
Tailing factor	1.29	1.21	1.20
Theoretical plates	10788	16721	15359
Average (Area)	1891268	2152542	1740558
%RSD(Area)	0.27	0.24	0.41

Table 7.1: Summary of robustness results of acetylcysteine (mobile phase)

Mobile phase	Actual	Low	High
Retention time(min)	13.18_13.24	11.99-12.05	13.25-13.30
Area	1888358-1900436	1948798-1975414	1852245-1863360
Tailing factor	1.29	1.131	1.20
Theoretical plates	10788	15064	15359
Average (Area)	1891268	1962783	1858564
%RSD(Area)	0.27	0.57	0.24

Table 7.2: Summary of robustness results of acetylcysteine (buffer pH)

Buffer pH	Actual	Low	High
Retention time(min)	13.18-13.24	12.97-13.05	11.66-11.75
Area	1892288-1900436	1879427-1885303	2018685-2034826
Tailing factor	1.29	1.06	1.09
Theoretical plates	10788	11501	13554
Average (Area)	**1891268**	**1890268**	**2024317**
%RSD(Area)	**0.27**	**0.41**	**0.30**

Table 7.3: Summary of robustness results of acetylcysteine (column temperature)

Column temperature	Actual	Low	High
Retention time(min)	13.18-13.24	13.20-13.22	12.48-12.49
Area	1888358-1900436	1893207-1900998	1896528-1900962
Tailing factor	1.29	1.16	1.12
Theoretical plates	10788	15532	1 4663
Average (Area)	**1891268**	**1895568**	**1899345**
%RSD(Area)	**0.27**	**0.18**	**0.09**

Table 7.4: Summary of robustness results of taurine (flow)

Flow	Actual	Low	High
Retention time(min)	5.40-5.43	6.04-6.34	4.36-4.47
Area	122758536-124554851	134226070-140673310	104547629-110021178
Tailing factor	1.56	1.06	0.81
Theoretical plates	10120	9529	7530
Average (Area)	123552999	139084239	108276967
%RSD(Area)	0.51	1.75	1.89

Table 7.5: Summary of robustness results of taurine (mobile phase)

Mobile phase	Actual	Low	High
Retention time(min)	5.40-5.44	5.56-5.86	4.74-4.82
Area	123295736-124554851	119478735-123771899	115321830-121265917
Tailing factor	1.56	1.14	1.07
Theoretical plates	10120	8883	9599
Average (Area)	**123552999**	**121723251**	**118422615**
%RSD(Area)	**0.51**	**1.44**	**1.62**

Table 7.6: Summary of robustness results of taurine (buffer pH)

Buffer pH	**Actual**	**Low**	**High**
Retention time(min)	5.40-5.44	4.93-5.06	5.24-5.40
Area	122758536-124554851	136189309-141197351	117832287-122742707
Tailing factor	1.56	1.12	1.07
Theoretical plates	10120	9545	6914
Average (Area)	**123552999**	**138415423**	**119760240**
%RSD(Area)	**0.51**	**1.17**	**1.38**

Table 7.7: Summary of robustness results of taurine (column temperature)

Column temperature	**Actual**	**Low**	**High**
Retention time(min)	5.40-5.44	5.47-5.51	5.25-5.39
Area	122758536-124554851	124309655-127483018	123187016-127959978
Tailing factor	1.56	1.28	1.31
Theoretical plates	10120	11024	10701
Average (Area)	**123552999**	**125840262**	**126305901**
%RSD(Area)	**0.51**	**1.04**	**1.32**

Table 8: Summary of solution stability results

Contents	**Initial**	**6 hrs**	**12hrs**	**18hrs**	**24hrs**	**36hrs**	**48hrs**	**%RSD**
acetylcysteine	100.43	100.07	101.15	100.63	100.53	100.95	100.71	0.35
Taurine	100.17	100.49	101.18	103.06	104.89	-	-	1.25

CONCLUSION:

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of acetylcysteine and taurine using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Hence, this method can easily and conveniently adopt for routine quality control analysis of acetylcysteine and taurine in its pharmaceutical dosage forms.

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