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A Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of AcetylCysteine and Taurine in API and its Pharmaceutical Dosage Forms

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Abstract

A RP-HPLC method was developed and validated for the simultaneous estimation of Acetyl cysteine and Taurine in tablets. The proposed RP HPLC method utilized a Agilent $C_{18,}$ 4.6×150 mm, 5 µ column, mobile phase comprised of 0.01N KH_2PO_4 and Methanol in the ratio 60:40 v/v and flow rate 1 ml/min. The retention time for Acetyl cysteine and Taurine were found to be 1.722 min and 2.696 min. The linearity was in the range of 300 – 900 µg/mL (r = 0.999). The percentage recovery was found to be specific, linear, accurate, precise, rugged and robust. Force degradation studies were carried out for acidic, alkaline, oxidative, reductive and photolytic exposure of the drug substance anddrug product. Hence the RP HPLC method developed and validated can be used routinely for the simultaneous estimation of Acetyl cysteine and Taurine in tablets

Key words: Acetyl cysteine and Taurine, RP - HPLC, Simultaneous estimation, Method validation and ICH guidelines

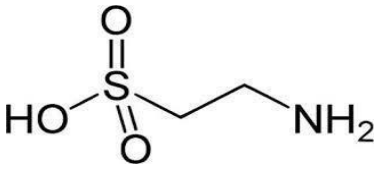
Introduction

Simultaneous estimation of drug combination[1] is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfills such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods. For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy[2].

Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures.

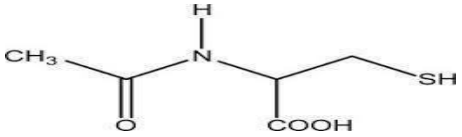
HPLC, revealed by the late Prof. Csaba Horváth for his 1970 Pittcon paper, originally presented the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the starting, pumps only

had a pressure capability of 500 psi. This is called *high pressure* liquid chromatography (HPLC)[3]. The early 1970's saw a tremendous move in technology. These new HPLC instruments could develop up to 6,000 psi (400bar) of pressure, incorporated improved injectors, detectors, and columns.
High-performance liquid chromatography (HPLC) is now one of the most important tools in analytical chemistry. It has the ability to separate, identify, and quantitate the compounds that are present in



Structure of Taurine [4,5]

Any sample that can be dissolved in a liquid. Today, compounds in few concentrations as low as *parts per trillion* (ppt)may easily be identified. HPLC can be, has been, applied to just about any sample, such as pharmaceuticals, food, nutraceutical, cosmetics, environmental matrices, forensic samples, industrial chemicals.
Taurine demonstrates multiple cellular functions including a central role as a neuro transmitter, as a trophic factor in CNS development, in maintaining the structural integrity of the membrane, in regulating calcium transport and homeostasis, asan osmolyte, as a neuro modulator and as a neuro- protectent.



Structural Features of Acetyl cysteine[6,7,8]

Acetyl cysteine serves as a pro drug to L-cysteine which is a precursor to the biologic antioxidant, glutathione and hence administration of acetyl cysteine replenishes gluta thione stores. L-cysteine also serves as a precursor to cysteine which in turn serves as a substrate for the cysteiene- glutamate anti-porter on astrocytes hence increasing glutamate release into the extracellular space. This glutamate in turn acts on mGluR2/3 receptors, and at high doses of acetyl cysteine mGluR5. Glutathione also modulates the NMDA receptor by acting at the redox site. Acetyl cysteine also possesses some anti-inflammatory effects possibly via inhibiting NF-KB and modulating cytokine synthesis. It may also facilitate dopamine release in certain brain areas.

Materials and Methodology

Chemicals and reagents: Acetyl cysteine and Taurine standard drugs, potassium dihydrogen phosphate were obtained from lara drugs, kukatpally, hyderabad. Methanol and water used were HPLC grade (QUALIGENS). Commercially available tablets NEFROSAVE are obtained from local market.

Instrument

Waters HPLC 2e2695 series consisting pump, Auto sampler, photodiode array detector, Thermostat column compartment connected with Waters (alliance) Empower-2 software.

Chromatographic Conditions

The mobile phase consisting of 0.01N potassium dihydrogen phosphate and methanol (HPLC grade) in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0 mL/min. Itwas an isocratic elution. The column used was Agilent ZORBAX C_{18}, 4.6x150 m, 5µ at 25°C. The detection was monitored at 210 nm using PDA detector and the run time was

10min.

Mobile Phase Preparation

Mix 600 ml of potassium dihydrogen phosphate and 400 ml of methanol in the ratio 60: 40 % v/v.

Standard Stock Solution Preparation

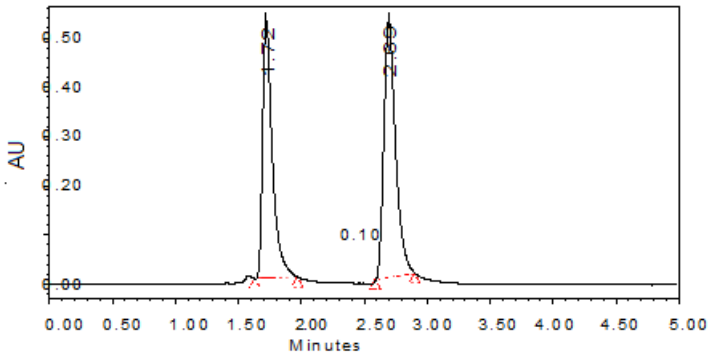
Weigh and transfer 500 mg of Taurine and 150 mg of Acetyl cysteine working standard into 50 mL volumetric flask, add 10 mL of diluent and sonicated to dissolve and dilute to volume with diluent.

Standard Preparation

Transfer 5 mL of standard stock solution into 25 mL volumetric flask and dilute to volume withdiluent.

Sample Preparation

Accurately weighed 2 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder of weight about 814.5 mg of sample into 50 ml of volumetric flask add water and sonicate for 30 mins and make up the volume with water and filtered through the 0.45 µm Millipore filter paper Transfer above solution 5 ml into 25 ml volumetric flask and make up the volume with mobile phase.



Retention Time	USP Resolution	USP Tailing	USP Plate count
1.722	-	1.79	2602
2.696	6.18	1.41	4146

Optimized method-acetyl cysteine- Taurine Optimized Chromatogram

Observation

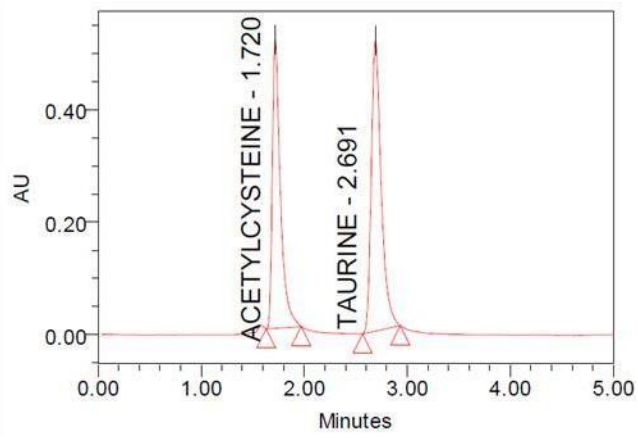
In the above method, both Acetyl cysteine and Taurine are separated well with good resolution, good symmetrical factor. The theoretical plates observed for both the peaks are also within the range and the same are eluted within a run time of 10min. This method is suitable for Validation.

Results and DiscussionPrecision

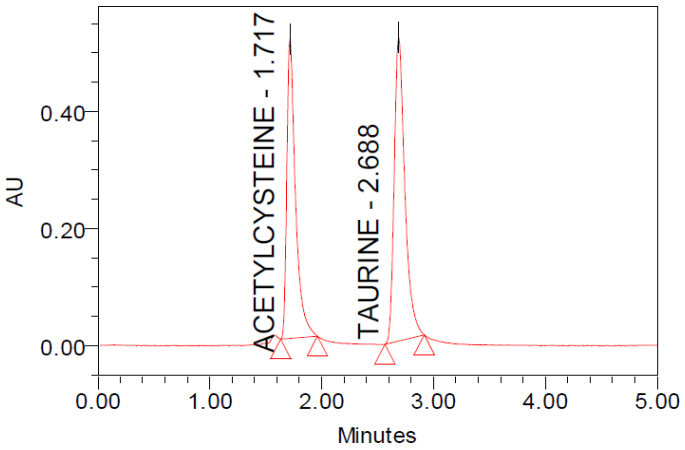
The relative standard deviation (%RSD) of the six assay preparations of Acetylcysteine and Taurine was calculated and it was found to be 0.11%

Precision- Acetylcysteine

Component Summery Table						
Name : Acetylcysteine					**Taurine**	
	Sample Name	Inj	RT (Mins)	Area	RT (Mins)	Area
1	PRECISION1	1	1.72	2747784	2.69	3427565
2	PRECISION2	1	1.717	2740623	2.688	3427438
3	PRECISION3	1	1.719	2747459	2.691	3423476
4	PRECISION4	1	1.721	2748609	2.686	3420936
5	PRECISION5	1	1.722	2746078	2.69	3420498
6	PRECISION6	1	1.721	2747062	2.691	3428783
Mean			1.72	2746269.17	2.69	3424782.67
Std. Dev			0.00179	2888.9296	0.00207	3623.7393
% RSD			0.10400	0.1052	0.07710	0.1058



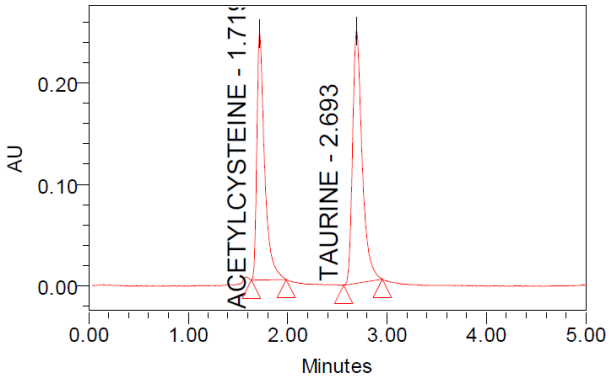
Precision-1 Chromatogram of Recommended Procedure Using Sample Drug



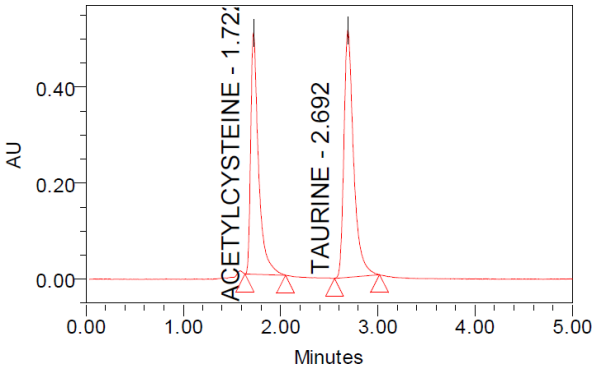
Precision-2 Chromatogram of Recommended Procedure Using Sample Drug

Accuracy

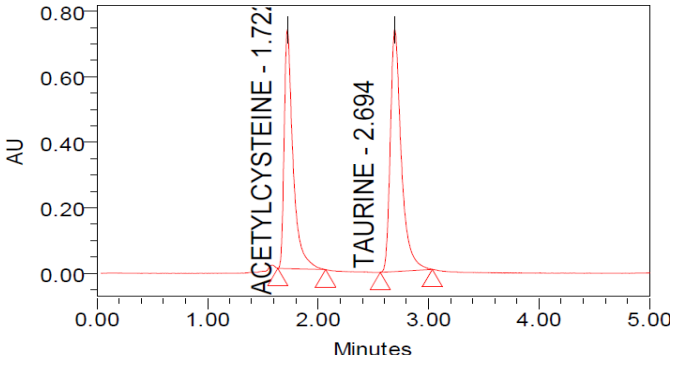
To study the accuracy of the method, recovery studies were carried out. To the formulation equivalent to 150 mg of Acetyl cysteine and 500 mg of Taurine at the levels of 50%, 100% and 150% was added to pure Acetyl cysteine and Taurine and made up to the mark with Mobile phase and filtered through Whatman filter paper and chromatograms were recorded. The concentration of drug present in resulting solution was determined using developed procedure and percentage recovery and percentage RSD were calculated.



Accuracy 50% -1 Chromatogram Using Sample Drug in 50 µg/mL

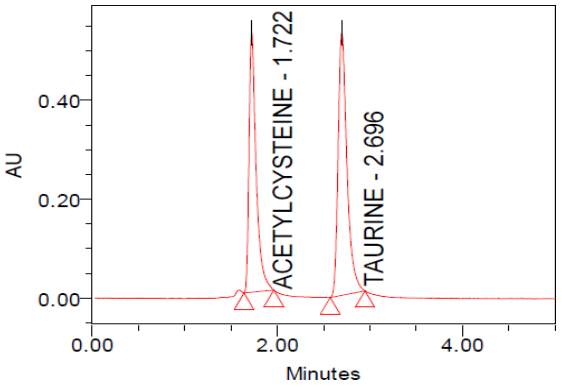


Accuracy -100% -1 Chromatogram Using Sample Drug in 100 µg/Ml

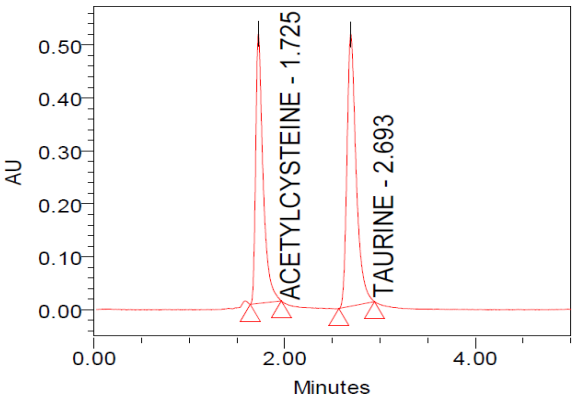


Accuracy -150% -1 Chromatogram Using Sample Drug in 100 µg/mL

Standard Drug Chromatograms



Standard chromatogram -1

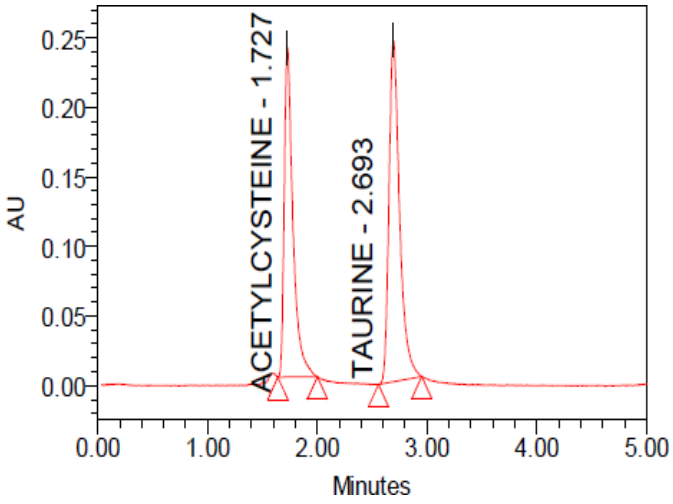


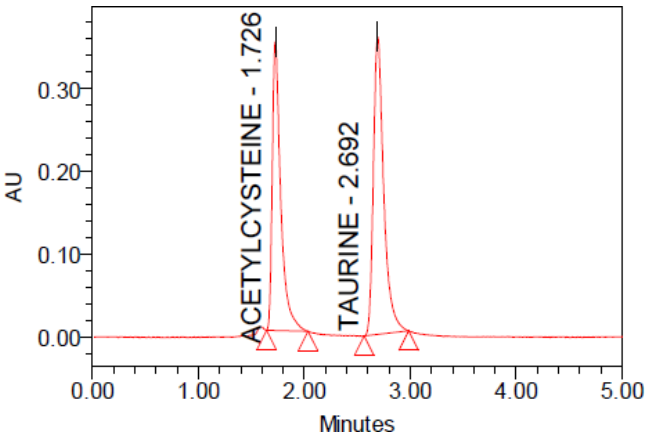
Standard chromatogram-2

Assay

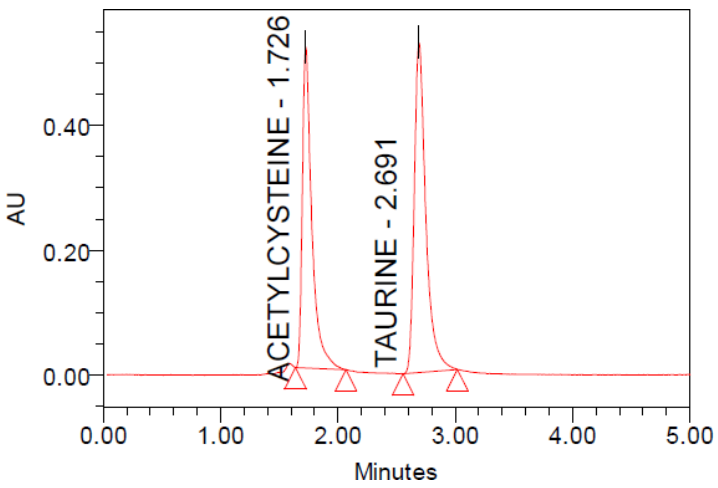
Assay Values for precision

S. No	Sample Weight	Sample Area -1	Sample Area -1	% Assay	% Assay
1	814.5	2747784	3427565	99	100
2	814.5	2740623	3427438	99	100
3	814.5	2747459	3423476	99	100
4	814.5	2748609	3420963	99	100
5	814.5	2746078	3420496	99	100
Mean				99	100

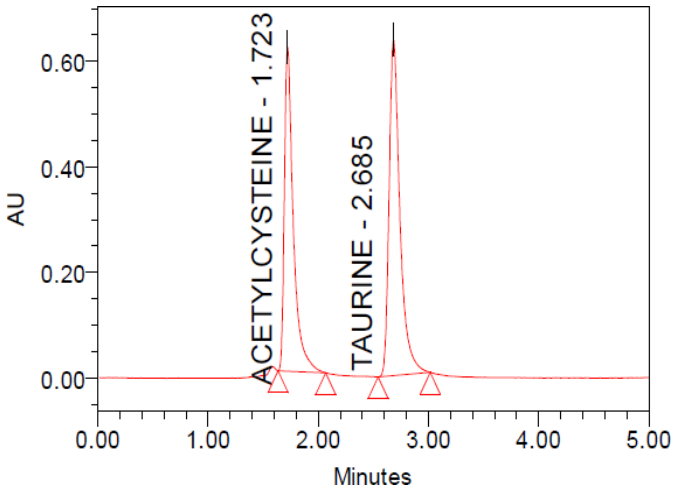




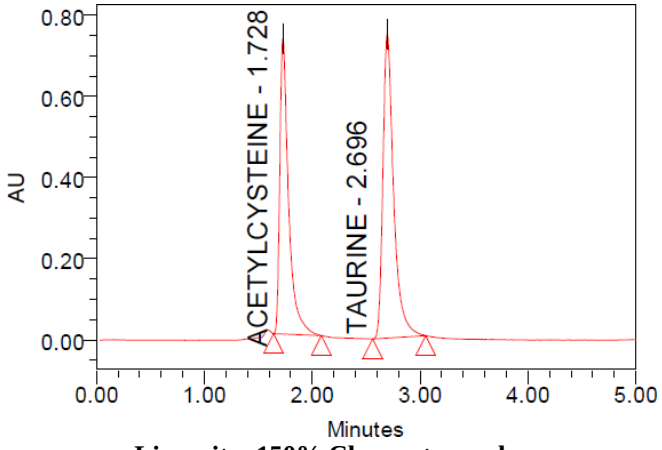
Linearity -75% Chromatographs



Linearity -100% Chromatographs



Linearity -125% Chromatographs



Linearity -150% Chromatographs

Linearity

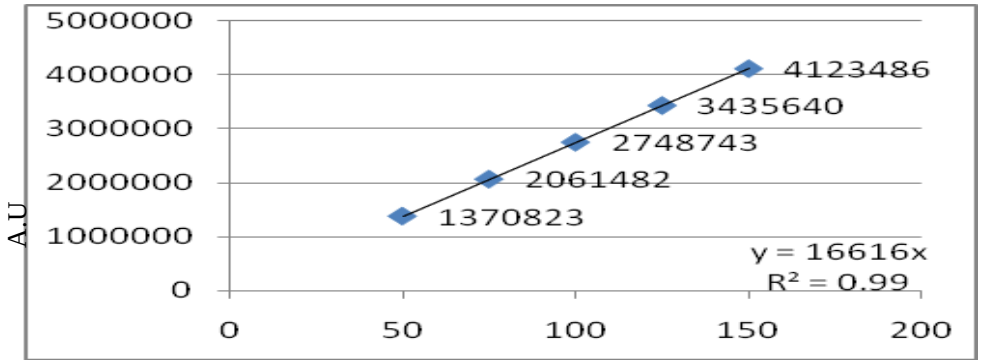
Aliquots of standard Taurine and Acetyl cysteinestock solution (0.2 ml to 0.8 ml) (1ml=1000µg/mL) were taken in different 10 ml volumetric flasks and diluted up to the mark with the diluents such that the final concentrations of Taurine and Acetyl cysteine are in the range of 300-900 µg/mL. Each of these drug solutions (10µL) was injected three times in to the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 210 nm and a calibration curve graph was obtained by plotting peak area versus concentration of Taurine and Acetyl cysteine.

Linearity Values – Acetylcysteine-Taurine

ACETYLCYSTEINE					TAURINE				
Conc %	Area	µg/ml	LOD	LOQ	CONC %	Area	µg/ml	LOD	LOQ
50	1370823	300	S.N	642	50	1711920	1000	S.N	21526
75	2061482	450	2.804	9.346	75	2561044	1500	2.7873	9.2911
100	2748743	600			100	3429290	2000		
125	3435640	750			125	4285963	2500		
150	4123486	900			150	5132946	3000		

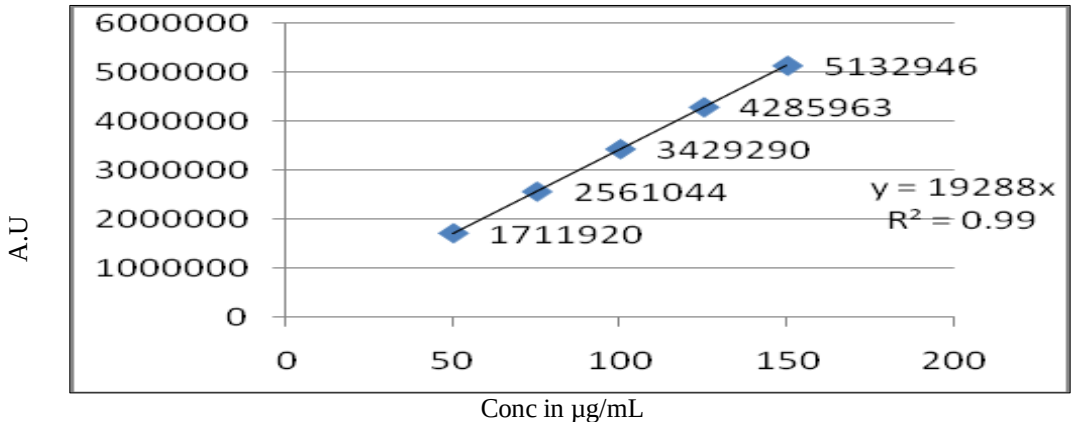
Linearity Values – Acetylcysteine-Taurine

Component Summery Table						
	Acetylcysteine				Taurine	
	Sample Name	Inj	RT (Mins)	Area	RT (Mins)	Area
1	LINEARITY-50%	1	1.727	1370823	2.693	1711920
2	LINEARITY-75%	1	1.726	2061482	2.692	2561044
3	LINEARITY-100%	1	1.726	2748743	2.691	3429290
4	LINEARITY-125%	1	1.723	3435640	2.685	4285963
5	LINEARITY-150%	1	1.728	4123486	2.696	5132946
Mean			1.73	2748034.80	2.69	3424232.60
Std. Dev			0.00187	1087742.4449	0.00404	1354567.7671
% RSD			0.10839	39.5826	0.15001	39.5583



Conc in µg/mL

STD Calibration Curve – ACETYLCYSTEINE



Conc in µg/mL

STD Calibration Curve – TAURINE

Limit of Detection (LOD)

From the linearity data calculate the limit of detection and quantitation, using the following formula.LOD= 3.3 σσ = standard deviation of the response

SS = slope of the calibration curve of the analyte.

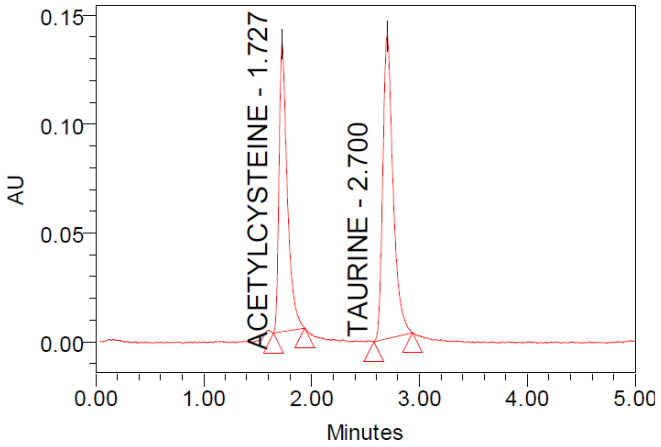
The limit of detection (LOD) and LOQ for Acetyl cysteine was found to be 2.804 and 9.346.

Limit of Quantitation (LOQ): LOQ = 10 σ σ = standard deviation of theresponseSS = slope of the calibration curve of the analyte.

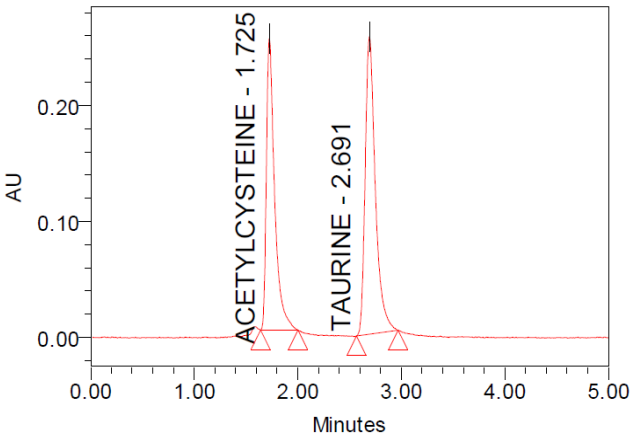
The limit of detection (LOD) &

LOQ for Taurine was found to be 2.7873 and 9.2911.

Robustness

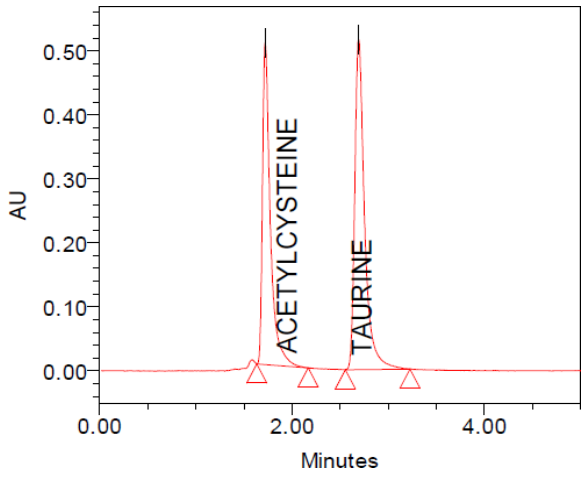


Chromatograms illustrating LOD of 0.5% Working Standards

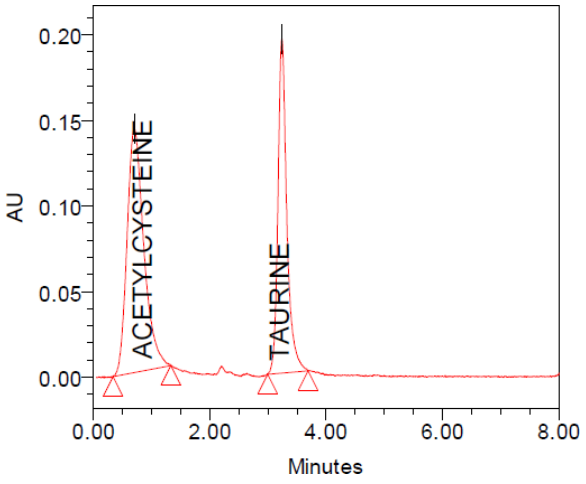


Chromatograms illustrating LOQ of 0.5% Working Standards

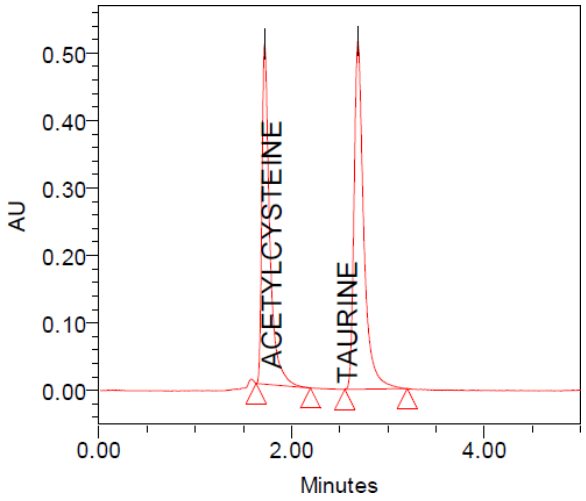
In order to prove that the method is robust, flow rate of the mobile phase (±0.2ml/min) and the column temperature (±5°c) are varied. The results showed that they have passed the system suitabilityparameters.



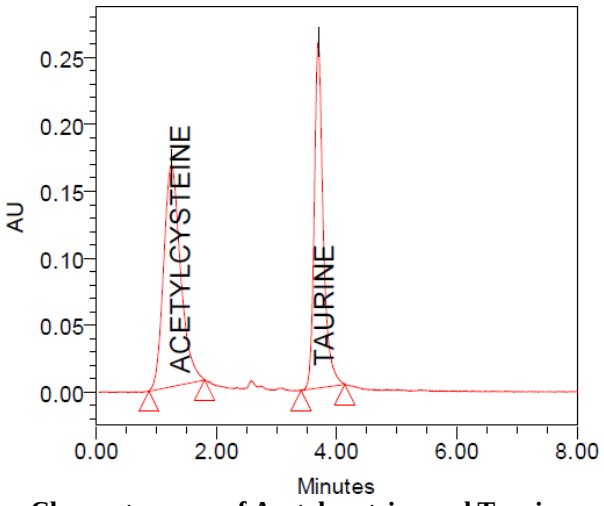
Chromatograms of Acetyl cysteine and Taurine for robustness studies – Flow change (0.8 ml/min)



Chhromatograms of Acetyl cysteine and Taurine for robustness studies–Flow change (1.2 ml/min)



Chromatograms of Acetyl cysteine and Taurine for robustness studies – Temp-25°C



Chromatograms of Acetyl cysteine and Taurine for robustness studies – Temp-35°C

Degradation Profile

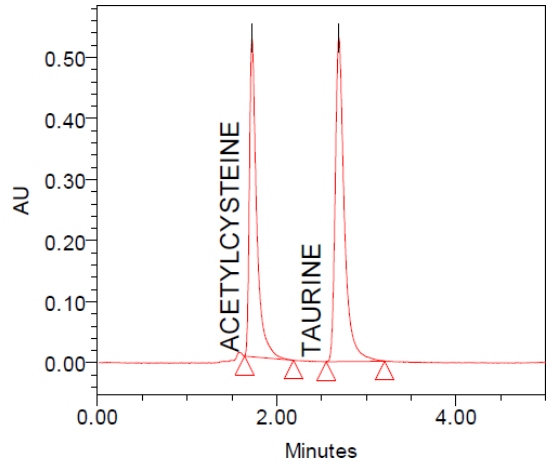
Acid: Transfer 814.50 mg weight of sample into a 50 ml of volumetric flask and add 10 ml of 0.1n HCl and sonicate30 min and add 10 ml of 0.1n NaOH make up with mobile phase. Transfer above solution 5 ml into 25 ml volumetric flask dilute to volume with mobile phase.

Base: Transfer 814.50 mg weight of sample into a 50 ml volumetric flask and add 10 ml of 0.1N NaOH and sonicate 30 min and add 10 ml of HCl make up volume with mobile phase. Tranfer above solution 5ml into 25 ml volumetric flask dilute to volume with mobile phase.

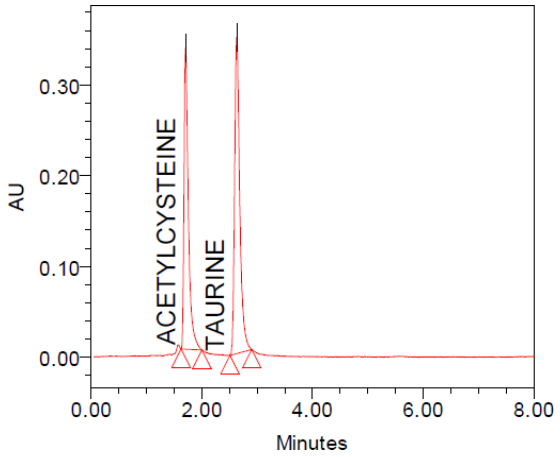
Peroxide: Transfer 814.50 mg weight of sample into a 50 ml of volumetric flask and add 10ml peroxide and sonic make up volume with mobile phase. Transfer above solution 5ml into 25 ml volumetric flask dilute to volume with mobile phase.

Heat: Before sample weighing exposes the sample at 10535°C. Transfer the 814.50 mg weight of sample into a 50ml volumetric flask and add 15ml of mobile phase and sonicate 30 min and make up with mobile phase. Transfer above 5ml into 25ml volumetric flask dilute to volume with mobile phase.

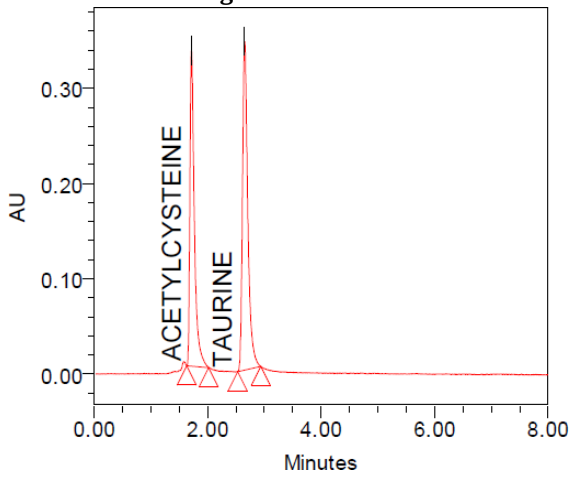
Light: Before weighing sample expose the sample in light for 24 hrs. Transfer the 814.50 mgof sample into a 50ml volumetric flask and add 15 ml of mobile phase and sonicate 30 min and make up with mobile phase. Transfer above solution 5 ml into 25 ml volumetric flask dilute to volume with mobile phase.



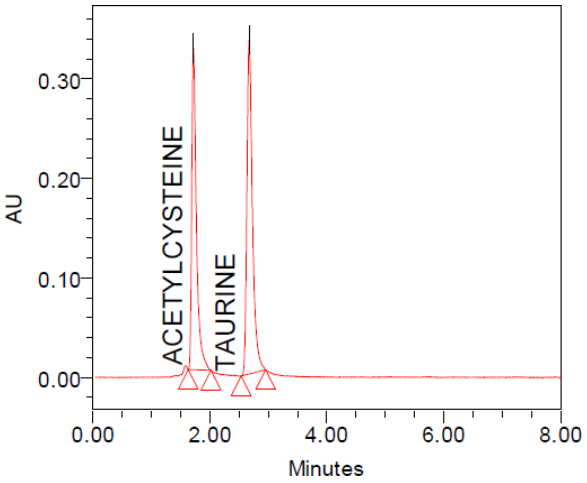
Chromatograms of Acetyl cysteine and Taurine for Degradation – ACID



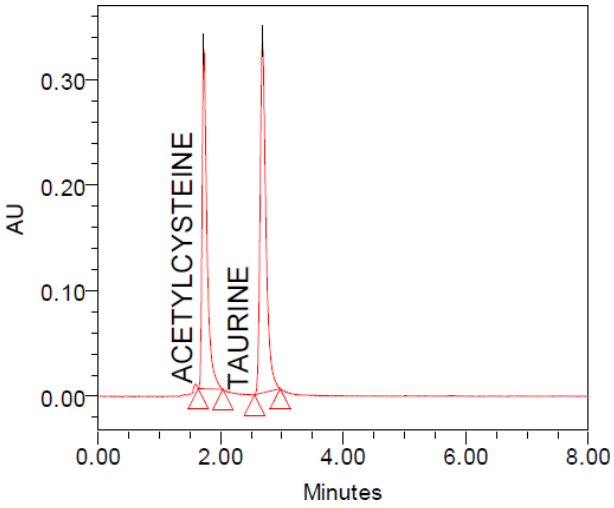
Chromatograms of Acetyl cysteine and Taurine for Degradation - Base



Chromatograms of Acetyl cysteine and Taurine for Degradation - Peroxide



Chromatograms of Acetyl cysteine and Taurine for Degradation - Heat



Chromatograms of Acetyl cysteine and Taurine forDegradation – Light

Conclusion

There are no reports on the stability indicating RP- HPLC determination of Taurine and Acetyl cysteine tablets in the literature prior to commencement of this work. The proposed method is simple, rapid, accurate, precise and specific. Reverse Phase HPLC for the estimation of Acetyl cysteine solid dosage form, from the typical chromatogram of Taurine as shown (standard & sample), it was found that the retention time for Taurine was 2.696 min and the retention time for Acetyl cysteine was 1.722 min. A mixture of potassium dihydrogen phosphate and methanol 60:40 v/v was found to be most suitable to obtaina peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (Taurine r=0.99 & Acetyl cysteine r=0.99) was observed. The assay of Taurine was found to be

100% & the assay of Acetyl cysteine was found to be 99%.

From the recovery studies it was found that about 100% of drug was recovered which indicates high accuracy of the method. It is suitable for the routine analysis of Taurine and Acetyl cysteine in pharmaceutical dosage form. The limit of detection (LOD) and limit of quantification (LOQ) for Taurine was found to be 2.7873 µg/mL and 9.2911 µg/mL. The limit of detection (LOD) and the limit of quantification (LOQ) for Acetyl cysteine was found to be 2.804 µg/mL &9.346 µg/mL. The above proposed method obviates the need for any preliminary treatment and is simple, sensitive and reliable and can be used for the routine determination of Taurine and Acetyl cysteine in bulk sample and in tablets.

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