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Validation of a Stability-Indicating Analytical Method Development for Simultaneous Estimation of Sodium Phenylbutyrate and Taurursodiol in Bulk and Formulation Using UPLC Method

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Abstract

Sodium phenylbutyrate (SPB) and taurursodiol (TRS) are commonly used in combination therapy to treat amyotrophic lateral sclerosis, popularly known as Lou Gehrig's disease. Using Ultra-Performance Liquid Chromatography, a stability-indicating analytical approach was designed and validated to evaluate SPB and TRS in bulk and dosage form simultaneously. Acquity UPLC BEH Shield RP-18 column (50 x 1.0 mm, 1.7µm) was used for the chromatographic separation. Next, a mobile phase was added at a 0.5 ml/min flow rate. The mobile phase included acetonitrile and 0.1% perchloric acid (20:80) v/v. Analytes were found at a wavelength of 287 nm with a photodiode array detector. An autosampler injected a five µl sample into the column, which was kept at 25 ^{o} C. SPB and TRS eluted values were 0.522 min and 1.311 min, respectively. For SPB and TRS, linearity was determined within the range of 75–450 µg/ml and 25–150 µg/ml, respectively. The method's robustness was evaluated by purposefully changing parameters like flow rate, detector wavelength, and column temperature. Additionally, research on forced degradation in the presence of different stress conditions, such as heat, peroxide, acid, and ultraviolet light, showed that the approach could identify stable materials. In conclusion, it was found that the developed analytical method for simultaneously determining SPB and TRS in bulk and their formulation was more precise, reliable, and specific.

Keywords: Amyotrophic lateral sclerosis; Lou Gehrig’s disease; sodium phenylbutyrate; taurursodiol; ultra-performance liquid chromatography.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurological illness that destroys nerve cells in the brain and spinal cord and progressively slows down memory, but may become fatal in later stages [1]. It results in the slow loss of motor neurons responsible for voluntary muscle

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contraction. Muscle weakening, paralysis, and, ultimately, respiratory failure are the results of ALS [2]. The symptoms progressively worsen in people with the condition and become severe when performing functions such as inhaling air, walking, speaking, eating, and performing other tasks [3]. Because ALS is a degenerative condition, symptoms progressively get worse [4].

Since glutamate transfers messages between nerve cells and motor neurons, a drug known as riluzole (Rilutek) is believed to reduce the damage that motor neurons experience [5]. Clinical trials have demonstrated that rivuzole prolongs the survival of ALS patients for several months. The drug (Exservan) that dissolves on the tongue or the thicker liquid form (Tiglutik) may be chosen if the patient has difficulty swallowing [6].

Research has shown that certain ALS patients may be able to delay their functional deterioration by using the antioxidant edaravone (Radicava), which can be given intravenously or orally. Edaravone can be taken orally or through a feeding tube; it is available as RADICAVA ORS. An oral drug called sodium phenylbutyrate/taurursodiol (Relyvrio) was developed with the goal] of preventing cell death by stopping stress cell signals. The FDA authorized Relyvrio in September 2022 based on safety and efficacy information from a smaller ALS clinical trial [7].

Figures 1 and 2 show the analytes. After a thorough analysis of the literature, it was found that only one analytical method for quantifying SPB and TRS had been previously published [8, 9, 10]. Therefore, this study's primary objective is to develop and validate a quick and simple approach for the synchronous quantification of SPB and TRS using reverse-phase ultra-performance liquid chromatography with photodiode array detector (RP-UPLC-PDA) [11, 12]. The International Conference on Harmonization's ICH (Q2) guidelines were followed to validate this newly designed approach [9, 14]. The ability of UPLC to achieve better resolution and speed in chromatographic separations is one of its most significant advantages. UPLC systems effectively separate complex analyte combinations with unparalleled precision and speed using stationary phases. This improved resolution results in increased peak capacities, reduced peak width, and sharper peak shape, making it possible to accurately identify and quantify target molecules quickly [14]. In the reported methods, retention time and flow rate were observed to be high, and organic phase composition was also found to be high. The detection and quantitation limits were high in other reported methods. Hence, the developed method can be imperative for further research studies in pharmaceutical industries.

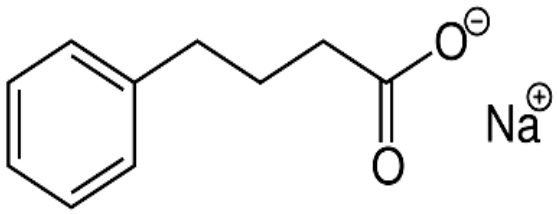

Figure 1 Molecular structure of Sodium Phenyl Butyrate

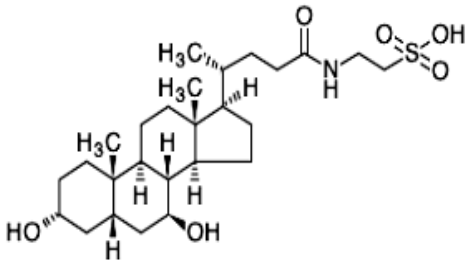

Figure 2. Molecular structure of Taurursodiol

2. Materials and Methods

2.1. Chemicals and reagents

SPB and TRS Gift samples were provided by Rankem Pharmaceuticals, located in Hyderabad, India. (HPLC) grade acetonitrile was procured from Sigma-Aldrich. Perchloric acid HPLC Analytical reagents and Water HPLC House Production (Milli Q) from Sigma-Aldrich. All chemicals used in this study were analytical grade. The analytical method was optimized using a preanalytical standard, with the mobile phase as the diluent.

2.2. Instruments and Equipment

A UPLC ACQUITY Waters - with PDA Detector, Ultra sonicator UCA 701 Unichrome, and a photostability chamber REMI SC-19 PLUS were used for the study.

2.3. Preparation of standard solution

SPB 300 mg and TRS 100 mg standards were measured exactly and placed in a 100 mL VF; 70 mL of diluent was mixed and sonicated to dissolve the sample and bring the volume to the desired level. (Stock solution) Further, 5

mL prepared stock solutions were pipetted into a 50 mL VF, and a diluent was used to make up the solution to the desired level. (300 ppm of Sodium Phenylbutyrate and 100 ppm of Taurursodiol)

2.4. Sample Solution Preparation:

Sodium Phenylbutyrate 300 mg and 100 mg of Taurursodiol sample were precisely measured and placed in a 100 mL VF, sonicate solution for 30 mins by adding diluent until the contents completely dissolve, and centrifuge for 30 min. It was dissolved completely, and the volume was adjusted with the same solvent to the desired level. Then, a 0.22-micron injection filter was used to filter the solution. (300 ppm of Sodium Phenylbutyrate and 100 ppm of Taurursodiol).

2.5. Method validation

The chromatographic method validation followed the guidelines outlined in the ICH Guidelines, specifically ICH Guidelines, Q2 (R1), 2005 [10, 11].

2.6. System suitability

By assessing the system's suitability characteristics, the system's performance was verified. Precision was measured using six consecutive injections of the same standard preparation, with special attention paid to important factors such as peak area, peak resolution, and theoretical plate count.

2.7. Accuracy (Recovery)

Recovery studies at three different concentrations—50%, 100%, and 150% of the target concentration—were used to evaluate accuracy. Chromatograms were examined in order to assess the method's accuracy.

2.8. Precision

Six repeat injections of optimal concentrations of SPB and TRS were used to determine the precision of the analytical technique, intraday, and interday. The chromatograms were used to calculate the averages and percentage RSD (Relative Standard Deviation) of the peak area and assay.

2.9. Specificity

The developed method's specificity was examined by injecting a functioning placebo solution (blank) without SPB and TRS into the UPLC system and a standard solution with 1134 µg/ml concentrations for SPB and 378 µg/ml for TRS. Chromatograms were produced, formulations were evaluated, and specificity was determined.

2.10. Linearity

Linearity was confirmed by developing and evaluating pure analytical standards at five distinct concentrations. Within the specified concentration ranges, the novel approach demonstrated exceptional linearity.

2.11. LOD and LOQ

The LOD and LOQ for SPB and TRS were determined using the response's standard deviation (SD) and slope. 3.3 times the standard deviation divided by the slope was the formula for LOD, while 10 times the standard deviation divided by the slope was the formula for LOQ.

2.12. Robustness

The robustness approach was evaluated, indicating that it could tolerate slight but intentional changes in the system settings. The influence of minute variations in the mobile phase flow rate (±0.2 units), temperature changes in the column (±5°C), and wavelength variations (±2%) were tested as part of this research.

2.13. Acid degradation:

Measure out 1 mL (1 N) of hydrochloric acid precisely, then pour into a 100 mL vacuum flask with a sample of 100 mg of taurursodiol and sodium phenylbutyrate. The vacuum flask was kept at 60°C for an hour before adding and neutralizing the diluent with 1 N NaOH. The remaining 100 ml was then diluted. After filtering, the solution was transferred into bottles using 0.22-micron syringe filters.

2.14. Alkali degradation:

A 100 ml vacuum flask was filled with a precisely weighed 100 mg sample of taurursodiol and sodium phenylbutyrate (one ml of 1N NaOH). Maintaining the vacuum flask for 1 hour at 60°C before being diluted to 100 ml with diluent. Following that, 1 N HCl was used to neutralize it. 0.22-micron syringe filters were used to transfer the filtered solution into bottles.

2.15. Thermal degradation

A mixture of sodium phenylbutyrate and taurursodiol was heated using a hot air oven set at 105°C for three hours. The mixture was dissolved and placed in a Petri dish. The next step was introducing the material into UPLC after diluting it with diluents.

2.16. Peroxide degradation

Precisely measure and transfer 100 mg of sodium phenylbutyrate and taurursodiol sample into a 100 mL vacuum flask. Further, 1 mL of 3% w/v H_2O_2 was poured into the flask, and the diluent was used to bring the volume up to the desired level. Maintaining the vacuum flask at 60oC for 1 hour and 15 minutes at room temperature. Transfer the mixture to bottles after filtering it through 0.22-micron syringe filters.

2.17. Reduction degradation

Accurately weigh 100 mg of Sodium Phenylbutyrate and Taurursodiol sample to a 100 mL vacuum flask by adding 1 1mL of 10 % Sodium bisulfate to a flask, and diluent was used for making up the volume up to the mark. Maintaining the vacuum flask at 60oC for 1 hour and 15 minutes at room temperature. Filter the solution using 0.22-micron syringe filters and transfer it to bottles.

2.18. Photolytic degradation

Sodium Phenylbutyrate and Taurursodiol samples were placed in a Photostability chamber for 3 hours. Then, the sample was taken, diluted with diluents, injected into UPLC, and analyzed.

2.19. Hydrolysis degradation

Weigh 100 mg of sodium phenylbutyrate and taurursodiol sample into a 100 mL vacuum flask. Add 1 mL of 10% sodium bisulfate to the flask, and use a diluent to adjust the volume to the required level. The vacuum flask was kept at 60ºC for an hour. After 15 minutes, the vacuum flask was kept at room temperature. Syringe filters with a 0.22-micron opening should filter the solution before transferring it to bottles.

Stress studies were carried out to understand the inherent stability features of the active compounds by the International Council for Harmonization (ICH) recommendations for stability testing for new drug substances and products. As part of this research, stress degradation experiments on SPB and TRS were carried out using the suggested method [11].

3. Results and Discussion

3.1. System suitability

System suitability was established to confirm the accuracy and validity of the analytical approach. The accuracy and validity of the analytical method were verified using a system suitability test. Some significant parameters, such as theoretical plate count (N), resolution, retention time (Rt), and tailing factor, were thoroughly examined. Every system-suitable parameter was within the permissible limits. It was noted that the system suitability parameters complied with ICH guidelines. The system suitability results are shown in **Table 1**. The PDA - Spectrum of Sodium Phenylbutyrate and Taurursodiol are represented in **Figure 3,** and the Optimized chromatogram and Standard chromatogram are shown in **Figures 4 and 5**.

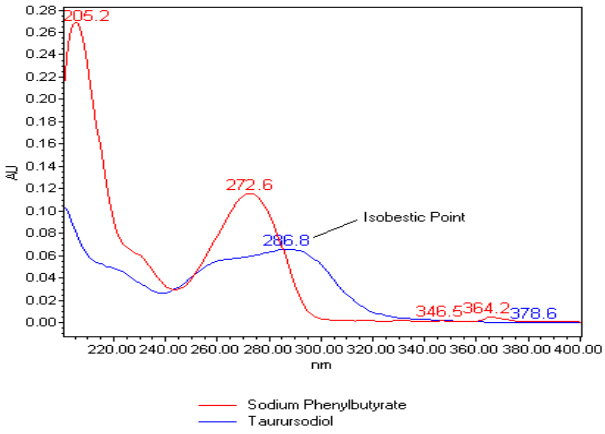

Figure 3. PDA - Spectrum of Sodium Phenylbutyrate and Taurursodiol

Table 1. System suitability parameters for Sodium Phenylbutyrate and Taurursodiol

S. No	Parameter	Sodium Phenylbutyrate	Taurursodiol
1	Retention time	0.522	1.311
2	Plate count	15364	10798
3	Tailing factor	0.89	1.00
4	Resolution	--	9.12
5	%RSD	0.23	0.57

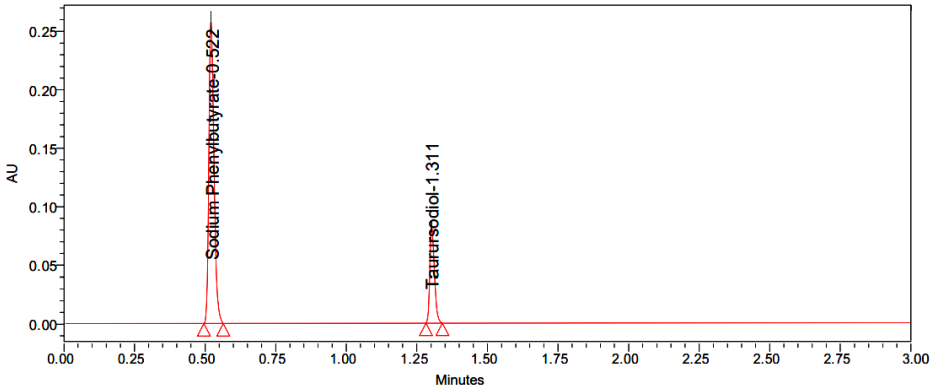

Figure 4. Optimized chromatogram

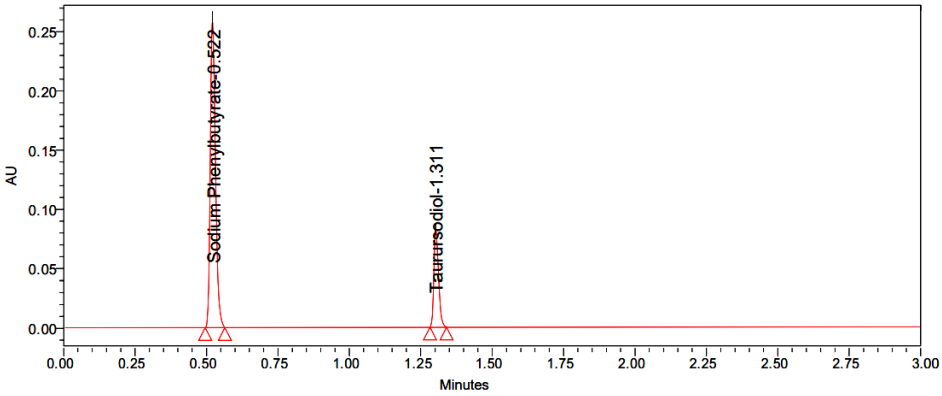

Figure 5. Chromatogram of standard

3.2. Linearity

A regression line was constructed by plotting peak areas on the X-axis against drug concentrations on the Y-axis to establish the linearity of the method. Regression equations were computed based on these curves. The linearity results for Sodium Phenylbutyrate and Taurursodiol are represented in **Table 2**. Visual representations of the linearity curves for SPB and TRS can be observed in **Figures 6 and 7,** respectively.

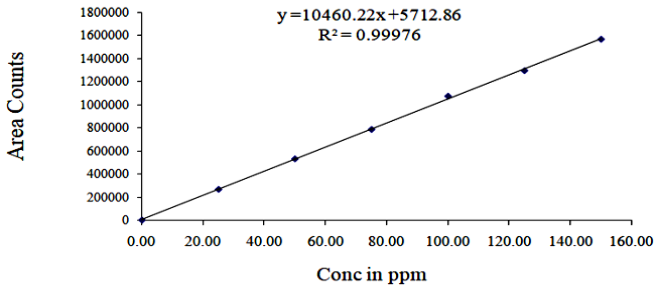

Figure 6. Calibration curve for Taurursodiol

Table 2. Results of linearity for Sodium Phenylbutyrate and Taurursodiol

S.NO	Sodium Phenylbutyrate		Taurursodiol	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	75.00	766844	25.00	266548
2	150.00	1481815	50.00	531479
3	225.00	2213037	75.00	787283
4	300.00	2976514	100.00	1076975
5	375.00	3687252	125.00	1298205
6	450.00	4368609	150.00	1571114
Regression equation	y =9733.97x + 23294.93		y = 10460.22x + 5712.86	
Slope	9733.97		10460.22	

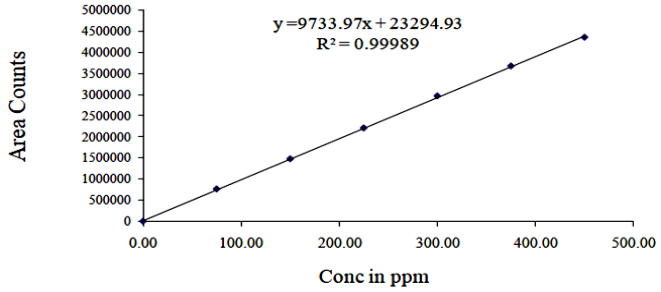

Figure 7. Calibration curve for Sodium Phenylbutyrate

3.3. Precision

The injected working standard solution was used six times for both drugs. For sodium phenylbutyrate and taurursodiol, the % RSD was 0.23% and 0.57%. As the precision limit was below 2, the system precision was high. The system precision of Sodium Phenylbutyrate and Taurursodiol is represented in **Table 3**.

The methods precision was assessed by intraday and interday variations with 1134 µg/ ml concentrations for SPB and 378 µg/ml for TRS. This assessment involved performing six replicate injections at each concentration level. Detailed results for precision are presented in **Table 3**. SPB's inter and intraday precision was found to be 0.62 % and 0.61 %, respectively, whereas for TRS, inter and intraday precision was 0.79 % and 0.19 %, respectively. Method Precision for SPB and TRS are shown in **Table 4**. Intermediate Precision for SPB and TRS are shown in **Table 5**.

Table 3. System precision table of Sodium Phenylbutyrate and Taurursodiol

S. No	Concentration Sodium Phenylbutyrate (µg/ml)	Area of Sodium Phenylbutyrate	Concentration of Taurursodiol (µg/ml)	Area of Taurursodiol
1.	300	2981428	100	1067821
2.	300	2996572	100	1059837
3.	300	2980654	100	1074679
4.	300	2982898	100	1066520
5.	300	2990947	100	1057862
6.	300	2978854	100	1066233
Mean	2985226		1065492	
S. D	6968.670		6027.060	
%RSD	0.23		0.57	

Table 4. Method Precision for Sodium Phenylbutyrate and Taurursodiol

S. No.	Area for Sodium Phenylbutyrate	Area for Taurursodiol
1	2941984	1085653
2	2995491	1048731
3	2960482	1071604
4	2953734	1080056
5	2995487	1069175
6	2977890	1063440
Average	2970845	1069777
Standard Deviation	22349.143	12996.132
%RSD	0.75	1.21

Table 5. Intermediate Precision (Day variation) for Sodium Phenylbutyrate and Taurursodiol

S. No.	Area for Sodium Phenylbutyrate		Area for Taurursodiol	
	Day-1	Day-2	Day-1	Day-2
1	2972415	2994215	1057861	1070452
2	2967881	2962418	1075836	1046921
3	2945612	2950231	1068510	1060150
4	2985206	2941724	1071649	1084627
5	2997823	2966210	1053468	1058942
6	2988679	2971203	1065423	1061045
Average	2976269	2964334	1065458	1063690
Standard Deviation	18566.187	18206.342	8447.743	12707.109
%RSD	0.62	0.61	0.79	1.19

3.4. Accuracy

Samples with three different concentrations of recovery were prepared using the standard addition method. For every recovery concentration, three injections were delivered, and the mean % recovery for taurursodiol and sodium phenylbutyrate was 100.2% and 99.9%, respectively. Accuracy results of Sodium Phenylbutyrate and Taurursodiol are shown in **Tables 6** and **7**.

3.5. Robustness

The robustness study indicated minimal chromatogram deviations compared to the optimized conditions. The findings of this study are summarized in **Tables 8** and **9**.

Table 6. Accuracy results of Sodium Phenylbutyrate

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1484840	15.00	14.92	99.5	100.3
	1506487	15.00	15.14	100.9	
	1499951	15.00	15.07	100.5	
100%	2961578	30.00	29.76	99.2	99.4
	2949854	30.00	29.64	98.8	
	2992602	30.00	30.07	100.2	
150%	4475895	45.00	44.98	100.0	99.9
	4483174	45.00	45.05	100.1	
	4456842	45.00	44.79	99.5	

Table 7. The Accuracy results for Taurursodiol

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	531142	5.00	4.98	99.6	100.5
	536234	5.00	5.03	100.6	
	539541	5.00	5.06	101.2	
100%	1076921	10.00	10.11	101.1	100.5
	1060423	10.00	9.95	99.5	
	1075084	10.00	10.09	100.9	
150%	1594817	15.00	14.97	99.8	99.6
	1591492	15.00	14.94	99.6	
	1589904	15.00	14.92	99.5	

Table 8. Robustness results of Sodium Phenylbutyrate

Parameter	Sodium Phenylbutyrate						
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count	%RSD
Flow rate Change (mL/min)	Less flow (0.45ml)	0.689	3187854	---	0.95	15427	0.72
	Actual (0.50ml)	0.522	2981428	---	0.89	15364	0.23
	More flow (0.55ml)	0.363	2871852	---	0.84	15260	0.61
Organic Phase change	Less Org (18:82)	0.842	3280612	---	0.99	15488	0.35
	Actual (20:80)	0.527	2996572	---	0.92	15351	0.23
	More Org (22:78)	0.239	2660614	---	0.87	15219	1.01

Table 9. Robustness results of Taurursodiol

Parameter	Taurursodiol						
	Condition	RT (min)	Peak area	Resolution	Tailing	Plate count	%RSD
Flow rate Change (mL/min)	Less flow (0.45ml)	1.467	994012	8.97	1.07	10815	0.17
	Actual (0.50ml)	1.311	1067821	9.12	1.00	10798	0.57
	More flow (0.55ml)	1.196	1125401	9.54	0.97	10691	1.22
Organic Phase change	Less Org (18:82)	1.575	985623	8.68	1.03	10864	0.35
	Actual (20:80)	1.314	1059837	9.15	0.96	10786	0.57
	More Org (22:78)	1.024	1352038	9.09	0.93	10637	0.64

3.6 LOD and LOQ (µg/ml)

Calculating the LOD and LOQ values allowed us to determine the method's sensitivity. The LOD and LOQ for SPB were determined to be 0.54 and 1.80 µg/ml, respectively. The LOD and LOQ, on the other hand, were observed to be 0.18 µg/ml and 0.60 µg/ ml for TRS, consecutively. The Sensitivity parameters (LOD & LOQ) by UPLC are shown in **Table 10.** The LOD and LOQ chromatograms are shown in **Figures 8** and **9**.

Table 10. Sensitivity parameters (LOD & LOQ) by UPLC

Name of drug	LOD (µg/ml)	s/n	LOQ (µg/ml)	s/n
Sodium Phenylbutyrate	0.54	3	1.80	10
Taurursodiol	0.18	3	0.60	10

3.7 Degradation Studies

Stress tests and forced degradation experiments in the presence of degradation products in bulk and solid dosage forms were used to analyze the specificity of the approach. Different stress studies were examined in these investigations. One possible explanation for the degradation could be the catalysis of ionizable groups in the analyte. A few degradation products were identified in acidic, alkaline, peroxide and reduction conditions; however, no other degrading peaks were seen within the Rt of SPB and TRS. It is important to note that both drugs degrade more noticeably in acidic, alkaline, peroxide, and reduction conditions. No degrading peaks were found in thermal, photolysis, hydrolysis, and control degradation conditions. Two degradation products were found in degradation experiments, but none in the Rt of SPB and TRS. [10, 11].

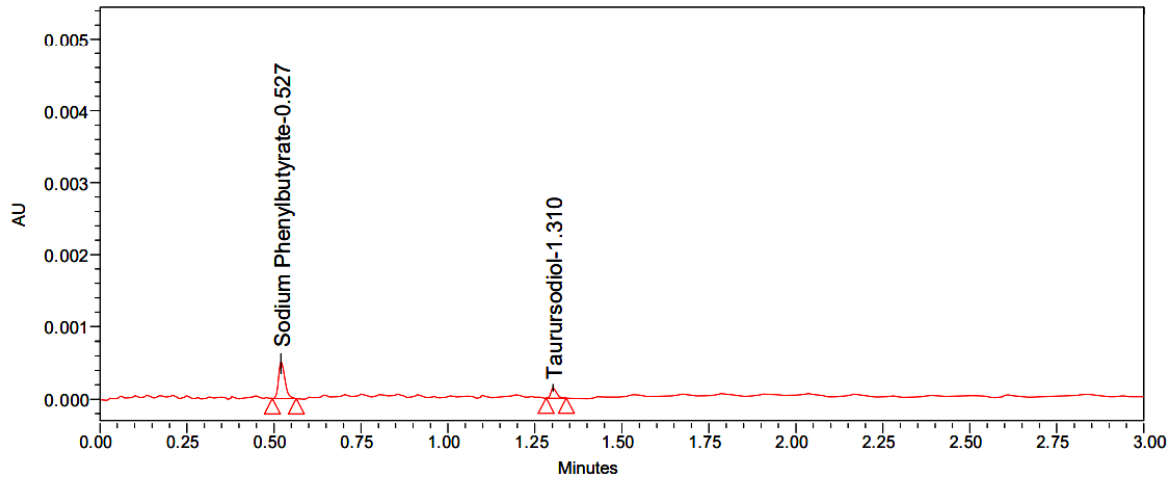

Figure 8. LOD Chromatogram

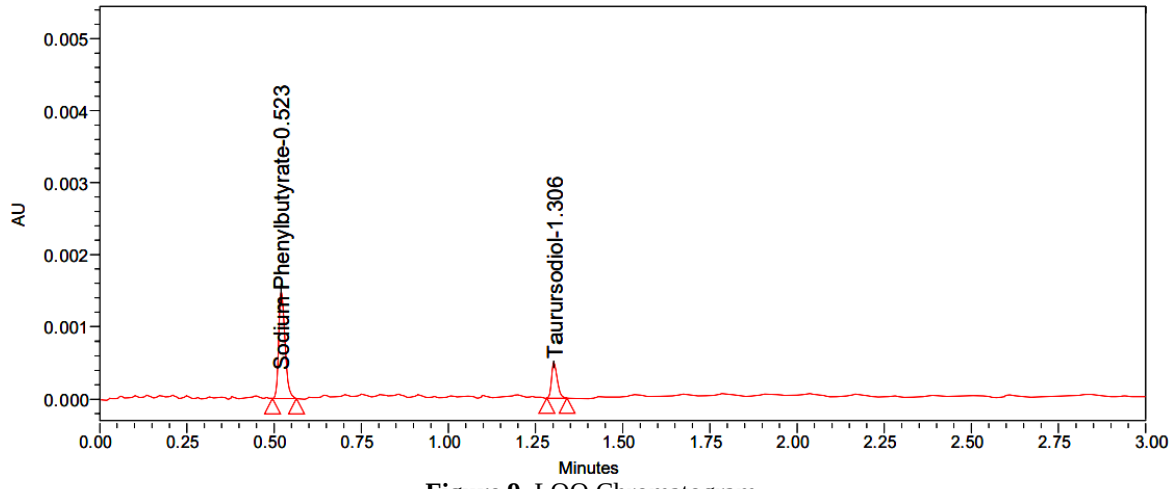

Figure 9. LOQ Chromatogram

Table 11 provides a summary of these stress study findings. This implies that SPB and TRS are vulnerable to the stress conditions indicated above but stay stable under the suggested technique even while under stress for a predetermined time (**Figure 10**) [12, 13].

Compared to traditional HPLC techniques, the use of the Acquity UPLC system in this study has shown several benefits, most notably in terms of cost savings and time efficiency. Because UPLC offers lower system back pressure and wider linear speeds, it enables analysts to perform at an advanced level. These qualities combine to make UPLC a very useful instrument for contemporary analytical chemistry. With the increasing demand for pharmaceuticals being studied worldwide, creating a new, simple, affordable, and accurate UPLC approach for simultaneous quantification in pharmaceutical formulations is imperative. This kind of approach not only makes routine medication analysis easier but it also helps pharmaceutical quality control laboratories save money and be more productive [14-16].

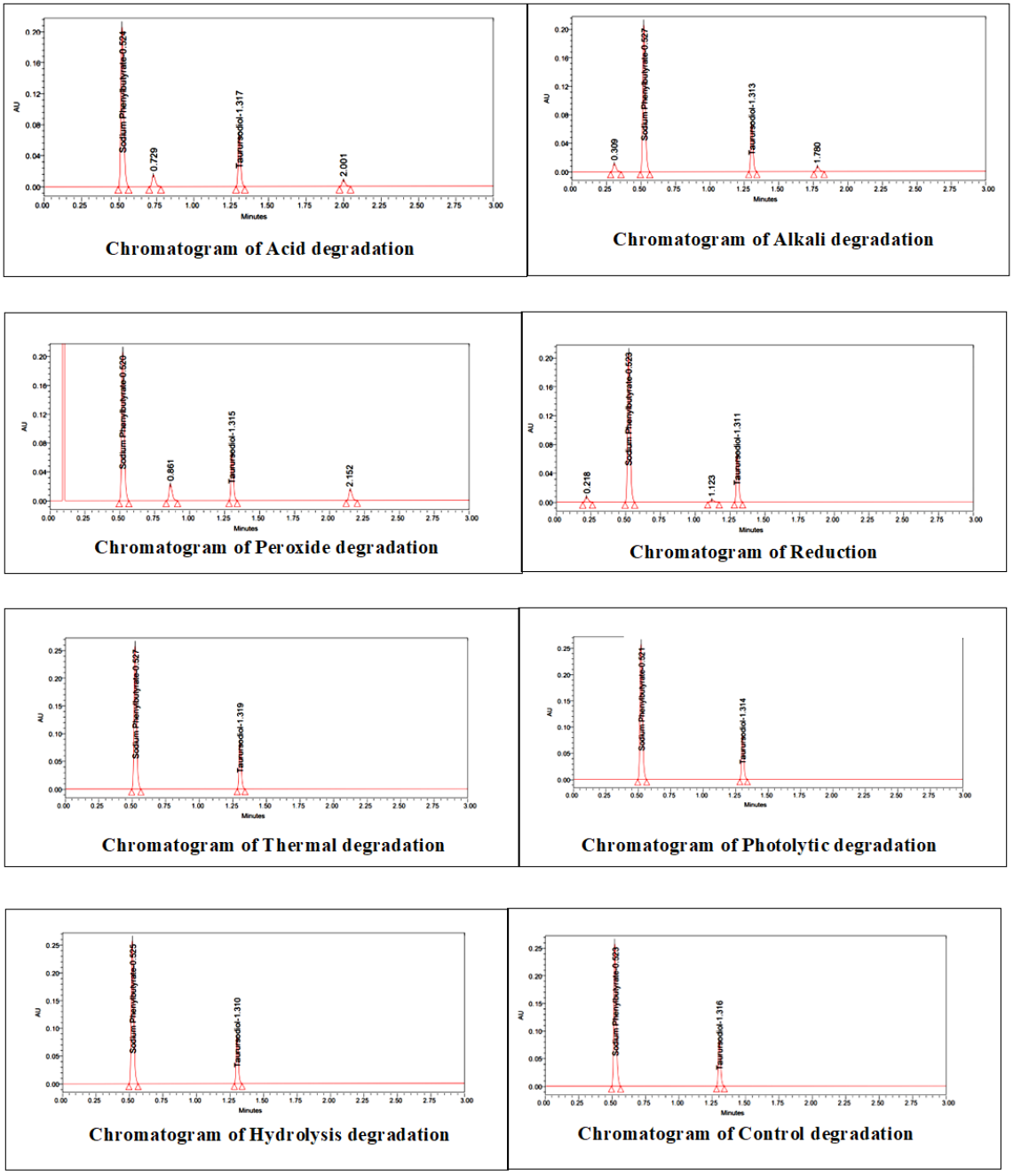

Figure 10. Chromatograms of Degradation Studies

Table 11. Forced Degradation results for Sodium Phenylbutyrate and Taurursodiol

% Deg	Sodium Phenylbutyrate					Taurursodiol				
	Response	% Assay	% Deg	Purity Angle	Purity Threshold	Response	% Assay	% Deg	Purity Angle	Purity Threshold
Control	2985672	100	0	2.435	6.214	1065569	100	0	0.134	1.228
Acid	2588241	86.7	13.3	2.486	6.269	945240	88.7	11.3	0.116	1.234
Alkali	2617147	87.6	12.4	2.471	6.263	948027	89.0	11.0	0.115	1.229
Peroxide	2527625	84.6	15.4	2.424	6.274	921129	86.5	13.5	0.147	1.233
Reduction	2656214	88.9	11.1	2.451	6.206	958129	89.9	10.1	0.158	1.226
Thermal	2951247	98.8	1.2	2.436	6.251	1051687	98.7	1.3	0.135	1.272
Photolytic	2895624	97.0	3.0	2.468	6.228	1038027	97.4	2.6	0.196	1.283
Hydrolysis	2857642	95.7	4.3	2.458	6.243	1015240	95.3	4.7	0.148	1.274

The developed UPLC method was also expanded to investigate this drug degradation behavior under various stress scenarios. [17] This thorough analysis guarantees the safety and effectiveness of pharmaceutical products by verifying the specificity of the method and providing insightful information on the drug stability profiles [18]. This raises the standard for overall quality assurance standards in the pharmaceutical industry by properly aligning with the stringent regulations and standards that control drug manufacture and quality control [19]. This project aimed to establish an analytical technique that would use the UPLC-PDA chromatography equipment to quantify SPB and TRS. Meticulous optimization of the procedure produced markedly lower Rts of 1.311 min for TRS and 0.522 min for SPB [20].

The results of this optimization method were good resolution, distinct peak forms, and remarkable sensitivity. The PDA detector, which operates at a particular wavelength of 275 nm, was added, significantly increasing the detection sensitivity for all analytes. The extremely sensitive device significantly shortened the analysis time to as little as 3.00 minutes. The validation studies then made use of these idealized settings. The method's accuracy was demonstrated by the percentage recovery of all analytes that fell within permitted limits. The method's precision was confirmed by the % RSD values in the intra- and interday precision studies, which consistently remained below 2%.

System suitability analysis (**Table 1**) demonstrated that the Ultra Performance liquid chromatography system was suitable for assessing all pharmaceuticals, with % RSD values less than 2% for all parameters. The LOD and LOQ for SPB for TRS were within the acceptable range. The robustness study showed that deliberate adjustments to several factors, such as temperature, pH, and flow rate, had no discernible impact on the outcomes. Furthermore, the marketed formulation % tests produced results of 100.00% for TRS and 99.92% for SPB, confirming the efficacy of the established test protocols. The method's uniqueness is supported by empirical evidence and offers advantages in terms of speed and cost-effectiveness [21, 22].

It is revealed to be a cost-effective option because the entire analysis time is only 3.00 minutes, and acetonitrile is used. Its exceptionally low detection and quantification limitations further highlight the method's remarkable sensitivity. The findings of the validation parameters were well within the bounds set by the ICH Q2B recommendations [9], confirming the accuracy and applicability for use in pharmaceutical analysis. In the present method, Rt was observed to be less, with a decreased flow rate. The composition of the organic phase ratio was also found to be less. The LOD and LOQ values were consecutively less compared to existing methods. The present method has a short run time, provides better resolution, and has higher sensitivity [23, 24].

Current method: The mobile phase included acetonitrile and 0.1% perchloric acid (20:80) v/v at a 0.5 ml/min flow rate. SPB and TRS eluted values were 0.522 min and 1.311 min, respectively. The LOD and LOQ for SPB were determined to be 0.54 and 1.80 µg/ml, and the LOD and LOQ for TRS were observed to be in the range of 0.18 µg/ml and 0.60 µg/ ml, respectively.

Comparison between previously published methods

S No	Mobile phase and flow rate, Rt, LOD & LOQ	Limitations	Application	Ref.
1	phosphate buffer pH 2.5 with methanol in the ratio of 45: 65 v/v was delivered at a flow rate of 1 ml/min	Expensive	Simultaneous determination of sodium phenylbutyrate and taurursodiol in bulk and formulation using RP-UPLC	[8]
2	SPB and TRS were eluted at 1.483 and 2.492 mins, respectively.	Time-consuming		
3	The LOD and LOQ for SPB were determined to be 1.56 and 5.19 µg/ml. The LOD and LOQ were 1.48 µg/ml and 4.95 µg/ml for TRS.	Less sensitive		

Conclusion

The established UPLC method is specific, accurate, reproducible, and reliable for quantifying taurursodiol and sodium phenylbutyrate in tablet and bulk formulations. As regulations require, this recently developed approach has undergone extensive validation, proving appropriate sensitivity, accuracy, and precision levels. As such, this technique can be easily used to examine these particular drugs regularly in quality control labs.

Conflict of interest

The authors declare that no conflict of interest exists.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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