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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SODIUM PHENYLBUTYRATE AND TAURURSODIOL

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Abstract:

Objective: A simple, Accurate, precise method was developed for the simultaneous estimation of the Sodium Phenylbutyrate and Taurursodiol in pharmaceutical dosage form.

Methods: Chromatogram was run through Phenomene x C 18 column (150x4.6mm, 5µm). Mobile phase containing Buffer: ACN:Methanol (30:5:65) pH 3.5 was pumped through column at a flow rate of 1.2 ml/min. Buffer used at pH 3.5. Temperature was maintained at Ambient. Optimized wavelength for Sodium Phenylbutyrate and Taurursodiol was 249 nm.

Results: Retention time of Sodium Phenylbutyrate and Taurursodiol were found to be 5.04 min and 9.71 min. The % purity of Sodium Phenylbutyrate and Taurursodiol was found to be 100.03 % and 99.75 % respectively. The system suitability parameters for Sodium Phenylbutyrate and Taurursodiol such as theoretical plates and tailing factor were found to be 4836,0.97 and 3568, 1.42. the resolution was found to be 8.0. The linearity study for Sodium Phenylbutyrate and Taurursodiol was found in concentration range of 5µg-15 µg and 200 µg-600 µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.19 % and 100.84 %, %RSD for repeatability was 0.75 and 0.36 %. The precision study was precise, robust and repeatable. LOD value was 0.08 and 0.25, and LOQ value was 1.08 and 0.35 respectively

Conclusion: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Sodium Phenylbutyrate and Taurursodiol in pharmaceutical dosage form.

Keywords: Sodium Phenylbutyrate, Taurursodiol, RP-HPLC, Simultaneous estimation.

INTRODUCTION:

Phenylbutyric acid is a fatty acid and a derivative of butyric acid naturally produced by colonic bacteria fermentation. It demonstrates a number of cellular and biological effects, such as relieving inflammation and acting as a chemical chaperone. It is used to treat genetic metabolic syndromes, neuropathies, and urea cycle disorders.

Tauroursodeoxycholic acid, also known as ursodoxicoltaurine, is a highly hydrophilic tertiary bile acid that is produced in humans at a [1]IUPAC name sodium 4-phenylbutanoatemolecular weight of 186low concentration. It is a taurine conjugate of ursodeoxycholic acid with comparable therapeutic efficacy and safety, but a much higher hydrophilicity.1 Normally, hydrophilic bile acids regulates hydrophobic bile acids and their cytotoxic effects. Tauroursodeoxycholic acid can reduce the absorption of

cholesterol in the small intestine, thereby reducing the body's intake of dietary cholesterol and the body cholesterol content.

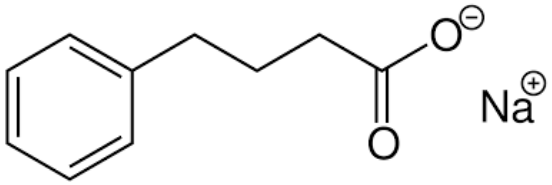

Figure 1: Structure of Sodium Phenylbutyrate

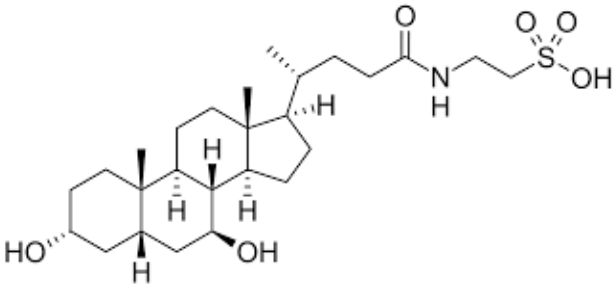

Figure 2: Structure of Taurursodiol

The literature survey revealed that There are Various analytical methods were carried out for the estimation of Sodium Phenylbutyrate and Taurursodiol as a single or combined with other drugs in pharmaceutical dosages Literature survey reveals that the retention time for the simultaneous estimation of Sodium Phenylbutyrate and Taurursodiol is more. Hence the present study, we had made an attempt to develop simple, accurate, precise, less time consuming and with less retention time using RP-HPLC for the simultaneous estimation of Sodium Phenylbutyrate and Taurursodiol in bulk and pharmaceutical dosage form by RP-HPLC.[4-8] To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

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MATERIALS AND METHODS:

Chemicals and Reagents: Taurursodiol and Sodium Phenylbutyrate were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 2 nm with Phenomene x C 18 column (150x4.6mm, 5µm). dimensions at 30 ^{0}C temperature. The optimized mobile phase consists of Buffer: ACN:Methanol (30:5:65) pH 3.5. Flow rate was maintained at 1.2 ml/min.

Preparation of solutions:

Preparation of phosphate buffer solution

.2568 gm of di-sodium hydrogen orthophosphate was gauged and adequate water (HPLC grade) was added to break up it. Then, at that point, sonicate for 10 min. Then, at that point, 1ml of tri ethanol amine was added, the last volume was made up to 1000ml with water and changed the pH to 3.5 with ortho phosphoric corrosive.

Preparation of mobile phase:

Methanol, Buffer and Acetonitrile were mixed in the ratio of 65:30:5 and sonicated for 20minutes, Filtered with 0.45 µ membrane filter.

Preparations of working standard solution:
500mg of Taurursodiol and 12.5 mg of Sodium Phenylbutyrate were exactly checked and moved in to an alternate 50 ml volumetric cup and sufficient adaptable stage was added to separate the medicine. The last volume was made up to 50 ml with flexible stage (fundamental stock course of action). Pipette out 2ml from the above stock plan into a 50ml volumetric cup and the last volume was made adequate with the convenient stage

Preparation of Sample solution
20 tablets were checked and powdered, tablets powder similar to 500mg of Taurursodiol and 12.5mg of Sodium Phenylbutyrate was moved in to a 50 ml volumetric cup, sufficient proportion of convenient stage was added and separated by 20 minutes ultrasonication. Then, made the volume adequate with the compact stage and isolated with 0.45 µ channel paper. Pipette out 2 ml from the above course of action and debilitated to 50ml with the adaptable stage.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.2 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Phenomene x C 18 column (150x4.6mm, 5µm), the mobile phase of composition Buffer: ACN: Methanol (30:5:65) was allowed to flow through the column at a flow rate of 1.2 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.
Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Taurursodiol and Sodium Phenylbutyrate in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2

Validation of Analytical method:
Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Taurursodiol and Sodium Phenylbutyrate are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 31.25ppm, 62.5ppm, 93.75ppm, 125ppm, 156.25ppm, 187.5ppm of Taurursodiol and 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Sodium Phenylbutyrate. The results are shown in figure 6 and 7.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 75%, 100%, 125% and 75%, 100%, 125 % Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Taurursodiol and Sodium Phenylbutyrate and calculate the individual recovery and mean recovery values. The results are shown in table 4.
Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.
Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day, different analyst, different instrument. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.. The results are shown in table 6.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 7.
LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION

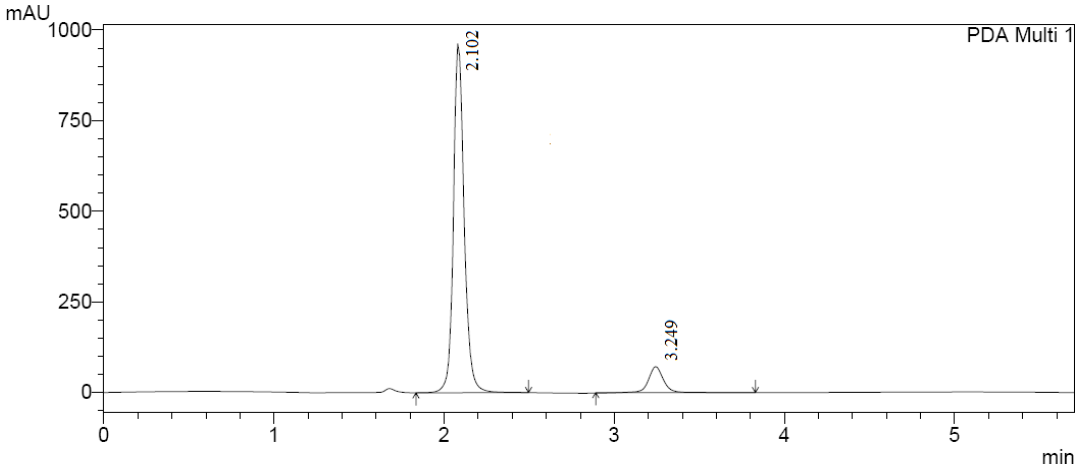

Figure 3: Standard chromatogram

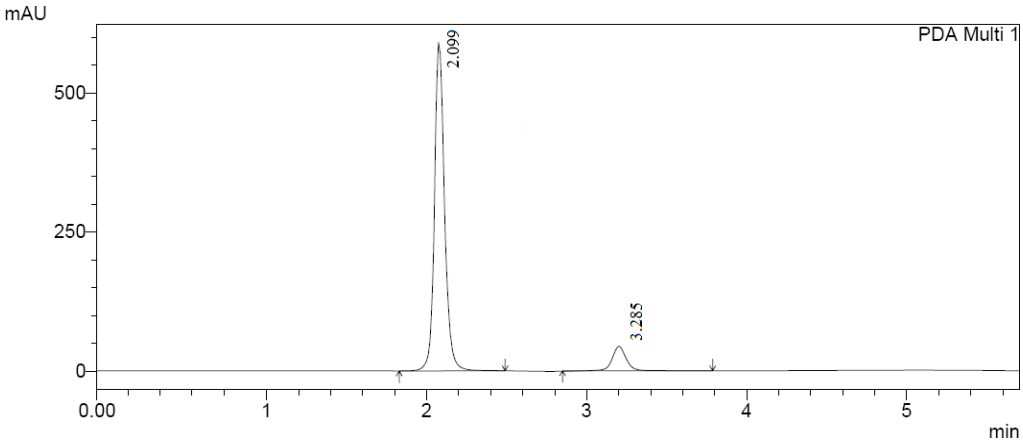

Figure 4: Sample chromatogram

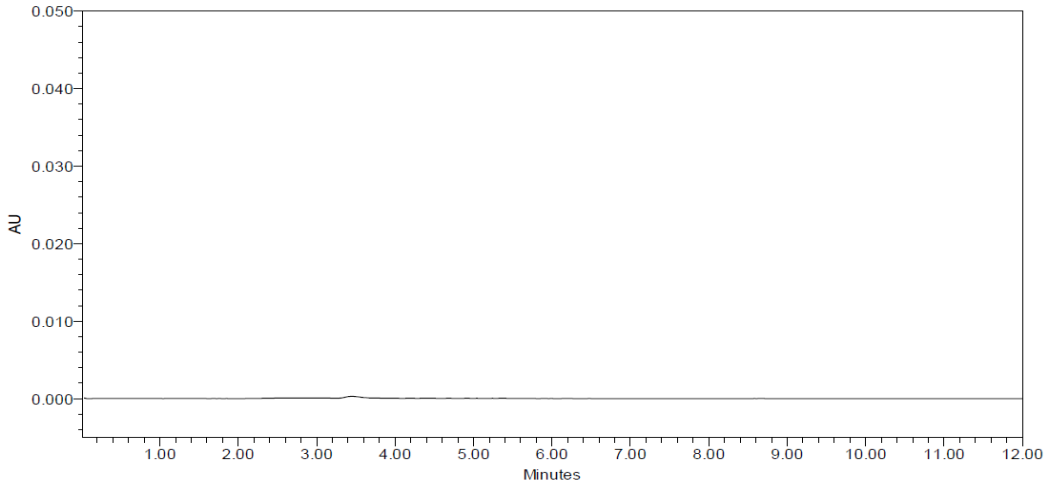

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Sodium Phenylbutyrate	Taurursodiol
Retention time	2.085	3.219
USP Plate count	3568.306	4836.128
USP Tailing	1.5	1.8

Table 2: Assay results for Sodium Phenylbutyrate and Taurursodiol

	Label Claim (mg)	% Assay
Sodium Phenylbutyrate	12.5	100.8
Taurursodiol	500	100.4

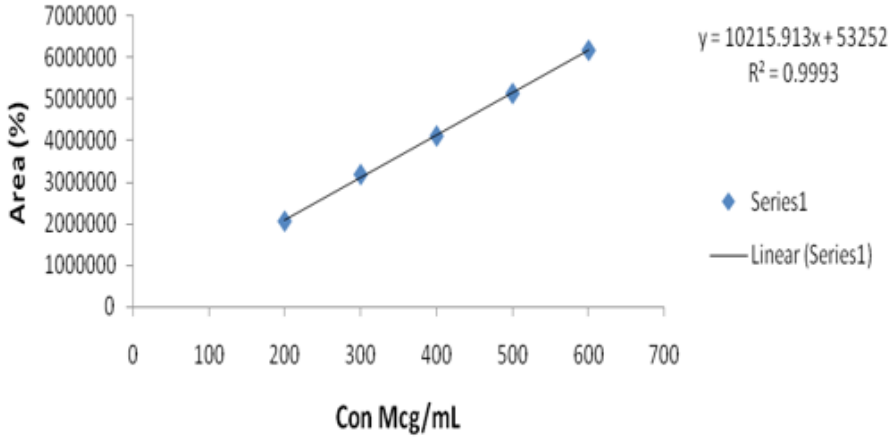

Figure 6: Linearity graph for Taurursodiol

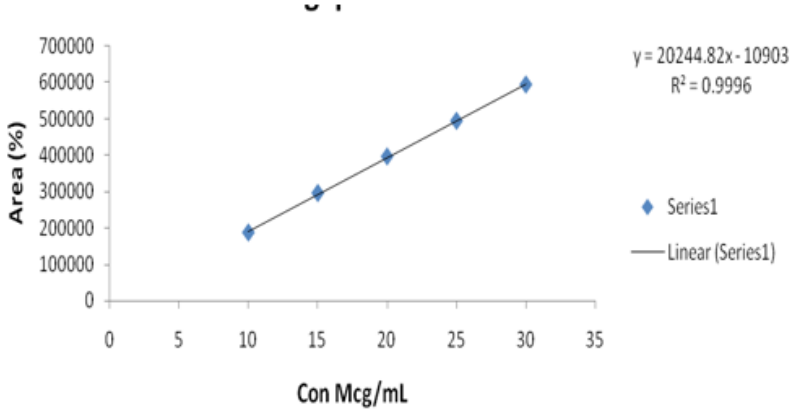

Figure 7: Linearity graph for Sodium Phenylbutyrate

Table 4: Showing accuracy results for Sodium Phenylbutyrate and Taurursodiol

Concentration (%)	Added AMT amount (mg)		Amt recovered (mg)		Amt recovered (%)	
Taurursodiol / SODIUM PHENYLBUTYRATE	Taurursodiol	Sodium Phenylbutyrate	Taurursodiol	Sodium Phenylbutyrate	Taurursodiol	Sodium Phenylbutyrate
75	376	9.385	374.66	9.395	99.91	100.30
100	501	12.6	495.18	12.659	99.04	101.29
125	622	15.725	621.84	15.497	99.59	99.12

Table 5: Precision and Intermediate precision results for Sodium Phenylbutyrate and aurursodiol

Parameters	Sampling time	Taurursodiol			Sodium Phenylbutyrate		
		Amount present (mg)	Amount present (%)	RSD (%)	Amount present (mg)	Amount present (%)	RSD %
Repeatability	0 hrs	495.21	99.04	0.0921	12.63	100.92	1.4543
	8^{th} hrs	499.79	99.92	0.9448	12.38	100.62	0.5499
	16^{th} hrs	503.58	100.19	0.3634	12.61	100.84	0.7567
Intermediate precision	I st Day	504.53	100.02	0.4994	12.56	100.43	0.7713
	2^{nd} day	503.69	100.81	0.3198	12.64	101.07	0.6142
	3^{rd} day	497.63	99.51	0.1258	12.71	101.68	0.1256
	Analyst -1	502.36	100.55	0.1908	12.64	101.09	0.8082
	Analyst -2	504.45	100.97	0.1198	12.62	100.97	0.6499
	Instrument -1	501.10	100.30	0.7277	12.67	101.31	0.1558
	Instrument -2	504.96	100.98	0.1218	12.62	100.95	0.4288

Table 6: Robustness results for Sodium Phenylbutyrate and Taurursodiol

		Taurursodiol			Sodium Phenylbutyrate		
Parameters		**Amount present (mg)**	**Amount present (%)**	**RSD %**	**Amount present (mg)**	**Amount Present (%)**	RSD %
Wavelength (nm)	249	493.05	98.61	0.1149	12.65	101.17	0.0559
	251	505.58	101.12	0.1247	12.64	101.21	0.0514
Flow Rate (mL/min)	1.4	502.87	100.58	0.3726	12.62	100.95	0.4288
	1.2	502.91	100.59	0.7916	12.66	101.24	0.0163
Mobile phase (% of (Methanol)	68	502.98	100.69	0.3908	12.66	101.28	0.1760
	64	504.87	100.98	0.09943	12.59	100.67	0.3854
pH	3.65	498.77	99.76	1.1838	12.65	101.19	0.0644
	3.55	500.31	100.07	1.3818	12.66	101.09	0.0802

Table 7: LOD, LOQ of Sodium Phenylbutyrate and Taurursodiol

Drug	LOD	LOQ
Sodium Phenylbutyrate	0.084	1.08
Taurursodiol	0.359	0.25

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Taurursodiol and Sodium Phenylbutyrate in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Taurursodiol and Sodium Phenylbutyrate in pure and its pharmaceutical dosage forms.

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