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Analytical Method Development by RP-HPLC for the Quantification of Sodium Phenylbutyrate in Solid Oral Formulation

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Abstract: *A simple, precise, accurate, and robust reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantification of Sodium Phenylbutyrate in solid oral pharmaceutical formulations. Chromatographic separation was achieved using a C18 column with a suitable mobile phase consisting of buffer and organic solvent in optimized proportions under isocratic conditions. The flow rate, detection wavelength, injection volume, and column temperature were optimized to obtain satisfactory peak symmetry and resolution. The analyte was detected using a UV detector, and the retention time for sodium phenylbutyrate was found to be reproducible with good peak characteristics.*

The developed method was validated according to International Council for Harmonisation guidelines for system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve exhibited good linearity over the selected concentration range with a correlation coefficient (R^2) greater than 0.999. The percentage recovery values indicated high accuracy of the method, while low relative standard deviation (%RSD) values confirmed precision and repeatability. The method demonstrated adequate robustness against small deliberate variations in chromatographic conditions.

The validated RP-HPLC method was successfully applied for routine quantitative analysis of sodium phenylbutyrate in solid oral dosage forms and was found to be suitable for quality control and stability studies in pharmaceutical analysis.

Keywords: *RP-HPLC*

I. INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.[1-6]

HISTORY OF ANALYTICAL CHEMISTRY

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860. Most of the major developments in analytical chemistry took place after 1900. During late 20th century analytical chemistry found wide application in forensic, environmental, industrial and medical field.

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Importance of Analytical Chemistry:

- It finds numerous applications in various disciplines of chemistry.
- It finds wide applications in other fields of related sciences.
- Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.

Qualitative analysis deals with the identification of elements, ions or compounds present in the sample.

Quantitative analysis

Quantitative analytical measurement plays a vital role in many research areas in chemistry, biochemistry, biology, geology and other sciences. It deals with the determination of how much amount of one or more constituents are present in the sample.[1-6]

METHOD DEVELOPMENT

Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still very much a trial-and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance.[7]

EXPERIMENTAL WORK

Material and instruments:

Materials:

The drugs used for the present investigation were obtained from Arrow chem Mumbai.

Details of Pure drug:

Table No. 8: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Sodium Phenylbutyrate	Arrow Chem Mumbai.	10 g	99.6 % w/w

Marketed Preparation:

Table No. 9: Details of marketed Preparation

Brand Name	Mfd by	Content	Quantity
AMMBULA® Sachets (Oral Powder)	LAURUS Lab	Oral powder for reconstitution (0.94 g/g)	Sachet

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade.

Methanol (HPLC grade).
Acetonitrile (HPLC grade)
Disodium hydrogen phosphate (AR grade).
Distilled Water (HPLC grade).
Triethylamine (HPLC grade).
Ortho Phosphoric Acid (HPLC grade).

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Instruments:

Table No. 10: Instruments Used

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50

Study of Functional Group by Using Infrared Spectroscopy:

Sodium Phenylbutyrate API: - Accurately weighed 3 mg of **Sodium Phenylbutyrate** API was mixed properly with 300 mg of dried KBr, then carefully triturated in a mortar pestle. Keep this mixture in a die and IR spectrum was taken using the Diffused Attenuated reflectance mode.

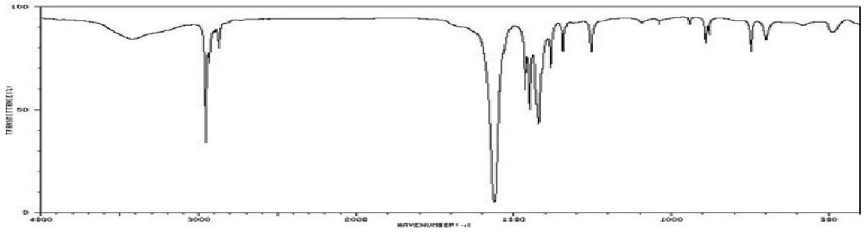

Fig. No. 8: IR Spectra of Sodium Phenylbutyrate

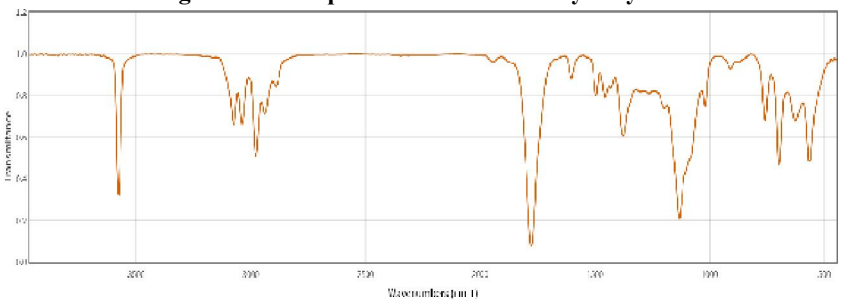

Fig. No. 9: Reference IR Spectra of Sodium Phenylbutyrate

Conclusion:

The IR spectra of the given test drugs is matches with the IR spectra of standard drugs.

Determination of wavelength maxima

Sodium Phenylbutyrate standard stock solution:

An accurately weighed quantity of **Sodium Phenylbutyrate** (SPG) 5 mg was transferred to the 10 ml volumetric flask and dissolved in HPLC grade ACN. The volume was made g/ml). up to the mark with the same to make (500

The aliquot portions of stock standard solutions were g/ml of diluted appropriately with HPLC grade ACN to obtain concentration 5 SPG. The solutions were scanned in the range of 400–200 nm in 1 cm cell against blank. The UV absorbance spectrum of SPG were recorded and found to be 260 nm.

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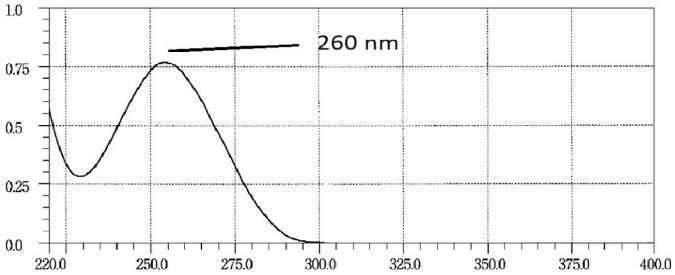

Fig. No. 10: Wavelength Maxima for Sodium Phenylbutyrate

Development of HPLC method for estimation of Sodium Phenylbutyrate

Method Development Strategy:

Selection of Common Solvent (Diluents):

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in mobile phase. The selection was made after assessing the solubility of SPG in different solvents i.e. Acetonitrile and water.

Preparation of standard stock solution:

Accurately weighted SPG 100 mg was dissolved in 100 ml ACN. This solution was used as standard stock solution.

Preparation of diluent:

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing SPG was injected in different combinations of solvents, to get a stable peak with good peak characters. Each solution was filtered through membrane filter (size 0.15µ). To achieve peaks with good symmetry various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Chromatographic Parameters:

Column: C_{18} (Thermo Hypersil gold) /4.6 x 250 mm, 5µ particle size
Flow Rate: 1.0ml/min
Wavelength: 260 nm
Injection volume: 20µl
Column oven Temperature: Ambient (25^0C)
Run Time: 10 minutes
Mobile Phase: 10mM Phosphate buffer (pH 3.5) and ACN **(**70:30)

Preparation of 10mM phosphate buffer: Weigh accurately 1.36 g of potassium dihydrogen phosphate in dissolve it in 1000 ml of HPLC grade water. The pH was modified to 3.5 using 1 M OPA solution.

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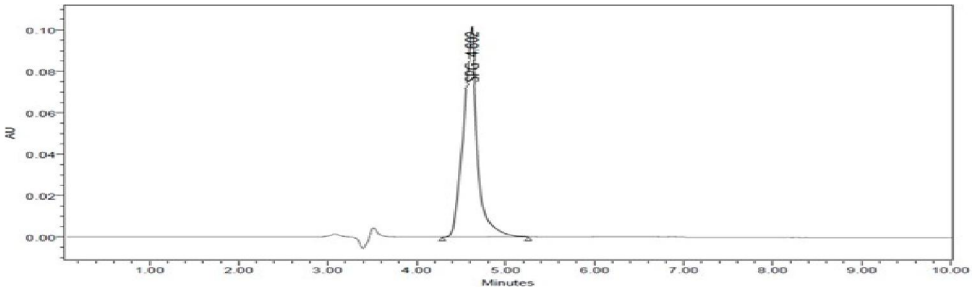

Fig. No. 11: Separation of SPG in selected mobile phase showing retention time at 4.602 min.

System suitability studies

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from 5 replicate injections of standard solutions.

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. Standard working solution of SPG was injected five times under optimized chromatographic conditions. System suitability parameters were recorded and reported.

B) Procedure:

Filtered mobile phase was allowed to equilibrate with L std. drug solution□stationary phase until steady baseline was obtained. A 20 was injected which was made in five replicates and the system suitability parameters were recorded.

Table No. 11: Result of System suitability test

Sr. No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	SPG	SPG	SPG	SPG
1	540280	4.609	1.10	8526
2	548970	4.608	1.20	8688
3	545895	4.720	1.10	8545
4	542670	4.620	1.15	8500
5	547870	4.650	1.10	8550
Mean	545137	4.641	1.13	8561
S.D	2620	0.04	0.04	63.22
%R.S.D	0.81	1.01	2.45	0.96

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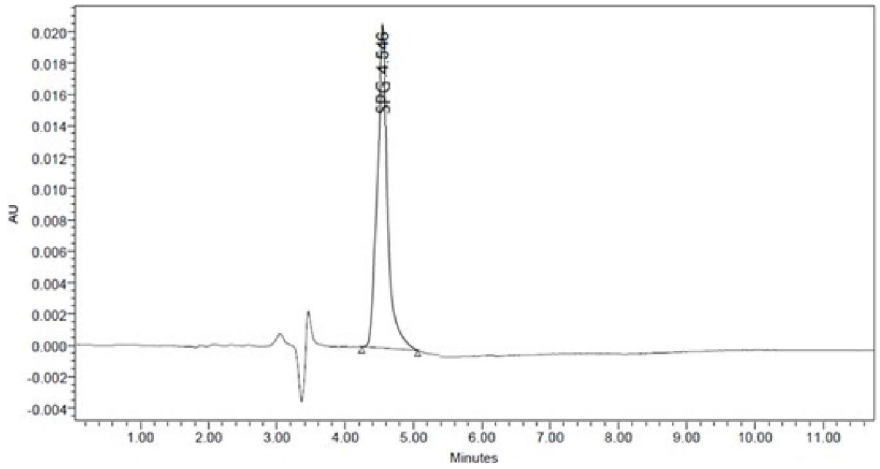

Fig. No 12: Separation of SPG in selected mobile phase showing retention time at 4.546 min.

Application of proposed method for estimation of SPG in powder formulation:

Standard stock solution:

a) Preparation of standard solutions:

Sodium Phenylbutyrate standard stock solution: Accurately weighed quantity of 100 mg SPG was dissolved in ACN and volume was made up to 100 ml mark by same to obtain 1000 µg/ml stock solution.

Sodium Phenylbutyrate standard working solution: Pipette out 1 ml from standard stock solution and dilute it with 10 ml ACN to obtain 100 µg/ml of SPG.

Sample solution preparation:

Entire content of **AMMBULA® Sachets** (Oral powder for reconstitution (0.94 g/g) was transferred to a 100 ml volumetric flask, the volume was made upto the mark with ACN, the resultant concentration was 940 µg/ml. The whole content was centrifuged at 5000 rpm for 10 min followed by passing through 0.45 µ membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with ACN, the concentration of working sample solution was 94 µg/ml.

Procedure:

Equal volume L)□(20 of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response

i.e. peak area of major peaks were measured. The content of SPG was calculated by comparing a sample peak with that of standard.

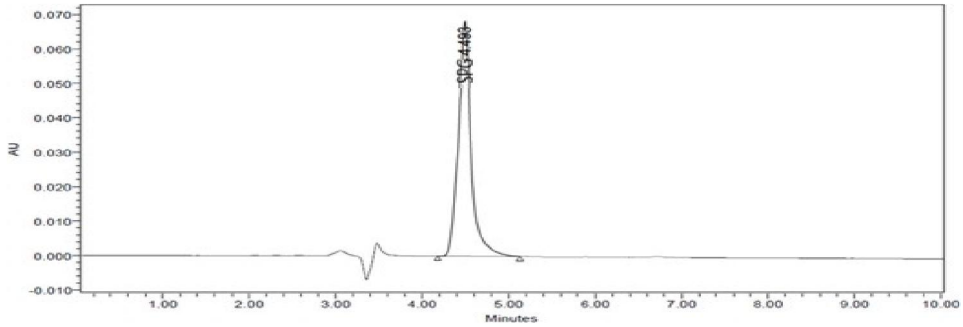

Fig. No.13: Chromatogram of marketed formulation showing retention time 4.493 min.

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Brand name : **AMMBULA® Sachets**

Sr.No.		
	Assay (mg)	**% Purity**
1	93.98	99.97872
2	93.99	99.98936
3	93.93	99.92553
4	93.95	99.94681
5	93.98	99.97872
Average	93.96	99.96
SD	0.025	0.025
% RSD	0.025	0.025

Table No. 12: Results and statistical data for estimation of SPG in marketed formulation

Validation parameters:
Accuracy
Precision
Ruggedness
Robustness
Linearity and range
Specificity
Placebo Interference study
Accuracy:

The accuracy samples were prepared by spiking the standard into the pre- analyzed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated.

The % Recovery was then calculated by using formula

% Recovery = A-B x 100/ C

Where- A = Total amount of drug estimated.
B = Amount of drug found on pre analyzed basis.
C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

	SPG		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120

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	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.99	99.99	119.95
	79.95	99.98	119.98
	79.98	99.97	119.98
% Recovery	99.98	99.98	99.95
	99.93	99.98	99.98
	99.97	99.97	99.98
Mean % recovery	99.90	99.98	99.93
% RSD	0.15	0.28	0.01

Table No 13: Accuracy studies by standard addition method Acceptance criteria:

The % RSD for the triplicate at each spike level shall be NMT 2.0.
The overall % RSD for % recovery for all spike levels shall be NMT 2.0.
The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.

Precision:

System precision

Prepared the standard solution as per test method and inject into the HPLC system in three replicates. Calculate the % RSD for the area responses and record the observations into the following table

Sr. No.	**Parameter**	**Observations**	**Limits**
1	The % RSD of peak area response for three replicate injections of standard	1.45	NMT 2.0
2	Theoretical plates	8560	NLT 2000
3	Tailing factor	1.36	NMT 2.0

Table No. 14: Results for System Precision showing system suitability

Where, NMT - Not More Than NLT – Not Less Than

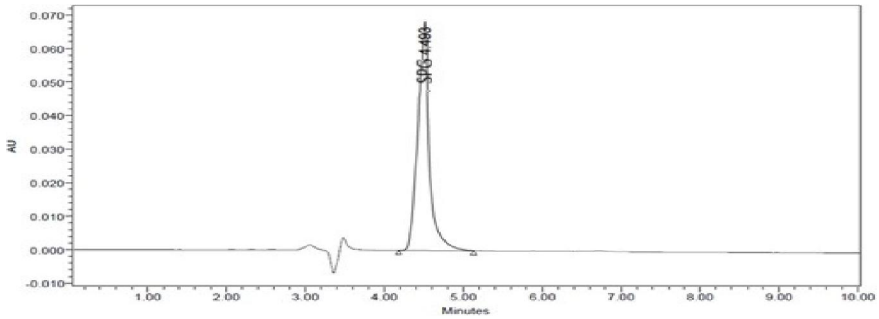

Fig.no 14: Chromatogram System precision Showing Repeatability

Injection No.	**Area Response**
	SPG
1	546520
2	545780
3	543250

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Average	545183
SD	2714
% RSD	0.39

Table No. 15: System precision Showing Repeatability Acceptance criteria:

% RSD for replicate injections shall be NMT 2.0

Method precision:

Prepared three sample solutions as per the test method and injected into the HPLC system by following the conditions prescribed in the Test method.

Procedure:

Sample solution was prepared and injected into the HPLC system, the chromatograms were recorded for peak area response for the SPG. The assay and % label claim for SPG was calculated.

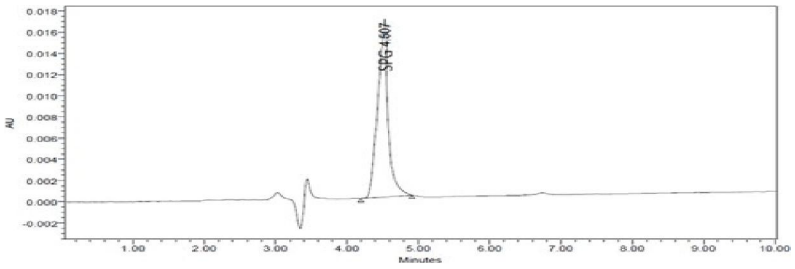

Fig.no 15: Chromatogram of Method precision

Sr.no.	**SPG**	
	Assay (mg)	**Assay % of LC**
1	99.98	99.98
2	99.99	99.99
3	99.97	98.97
Average	99.98	99.97
SD	0.02	0.02
% RSD	0.03	0.03

Table No.16: Method Precision Studies Set – I

Acceptance criteria: The% RSD for the three determinations shall be NMT 2.0

Ruggedness: Intermediate precision

Prepared three sample solutions as per the test method. Injected into the different HPLC system (preferably with different manufacturer or same manufacturer with different configuration) by using the different column and by the different analyst at different date.

Sr.No.	**SPG**	
	Assay (mg)	**Assay % of LC**
1	99.97	99.97
2	99.97	99.97
3	99.96	99.96
Average	99.65	99.65
SD	0.03	0.03

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% RSD	0.04	0.04

Table No. 17: Intermediate precision Studies (Ruggedness)

Set – II Acceptance criteria: The % RSD for the three determinations shall be NMT 2.0

Data analysis between method precision and Intermediate precision:

Compared the data obtained in this section verses the data obtained in method precision and evaluate the overall average, overall SD and overall % RSD and recorded the observation into the following table

Sr.no.	**% Assay of LC**	
	SPG	
	Set – I	**Set - II**
1	99.98	99.97
2	99.99	99.97
3	98.97	99.96
Average	99.98	
SD	0.55	
% RSD	0.55	

Table No. 18: Intermediate precision (Ruggedness) evaluation of data

SET – I: Method Precision data **, SET – II:** Intermediate Precision data

Acceptance criteria: The overall % RSD for the twelve determinations shall be NMT 2.0

Robustness:

Effect of Variation in flow rate of mobile phase by ± 10%:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system at –10% flow rate (0.9mL/min) and +10% flow rate (1.1mL/min) when compared with the Test method flow rate.

Procedure: Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measured the peak response for the major peaks.

0.9 ml/min

1.1 ml/min

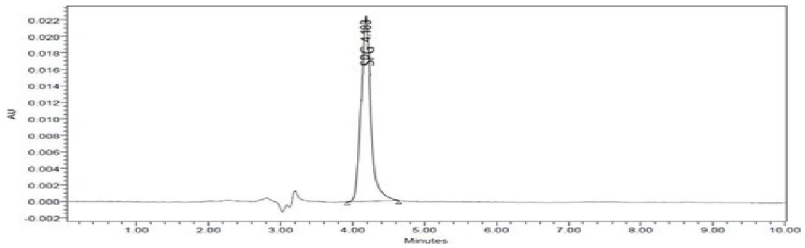

Fig. No. 16: Chromatograms of Change in Flow Rate

System suitability parameters were recorded and the results are presented in the table below.

Sr. No.	**System Suitability parameter**		**Observations for flow rate**			**Limits**
			Unchanged	**0.9 ml**	**1.1 ml**	
1	The % RSD of peak area response for five replicate injections	SPG	1.45	1.86	1.15	NMT 2.0
2	Theoretical plates	SPG	8597	8538	8457	NLT 2000

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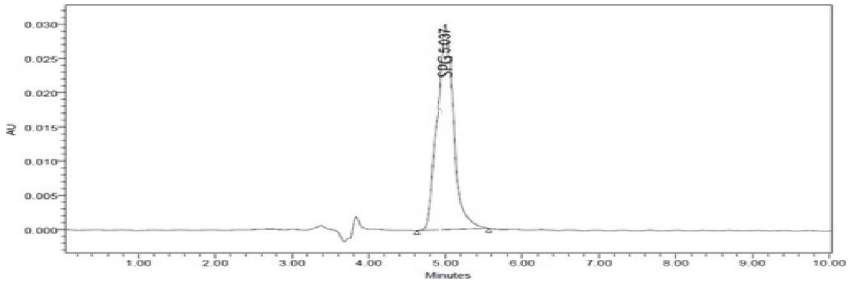
3	Tailing factor	SPG	1.58	1.81	1.16	NMT 2.0
4	Retention Time (Min)	SPG	4.50	5.04	4.16	

Table No. 19: System suitability of change in Flow Rate

Observation: The allowable variation in flow rate of the method is from 0.9ml/min to 1.1 ml/min

Acceptance criteria: All the system suitability parameters shall pass 10mM Phosphate buffer (pH 3.5) and ACN (70:30)

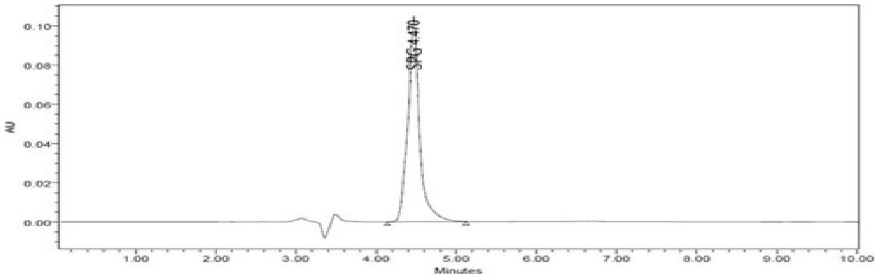


Change in organic composition±10%

System suitability dilution was prepared and injected into the HPLC system at –10% and + 10 % ACN (Organic phase) compared with the optimized method mobile phase concentration.

Procedure: Injected standard solution into the HPLC system in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks. Check the system suitability and record the results in the table.

-10% ACN:- (Phosphate buffer pH 3.5: ACN 73:27 % v/v)



+10% ACN: (Phosphate buffer pH 3.5: ACN 67:33)

Fig. No. 17: Chromatograms of Change Organic Composition of mobile Phase

Sr. No.	**System Suitability parameter**		**Observations**			**Limits**
			Unchanged	**- 10%**	**+ 10%**	
1	The % RSD of peak area response for five replicate injections	SPG	1.15	1.49	1.41	NMT 2.0
2	Theoretical plates	SPG	8497	8516	8447	NLT 2000

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3	Tailing factor	SPG	1.15	1.40	1.45	NMT 2.0
4	Retention Time (Min)	SPG	4.52	4.470	5.037	

Table No. 20: System suitability of change in Organic Composition Observation:

The allowable variation in ACN composition of method is from 90% to 110%.

Acceptance criteria: 1. All the system suitability parameters shall pass.

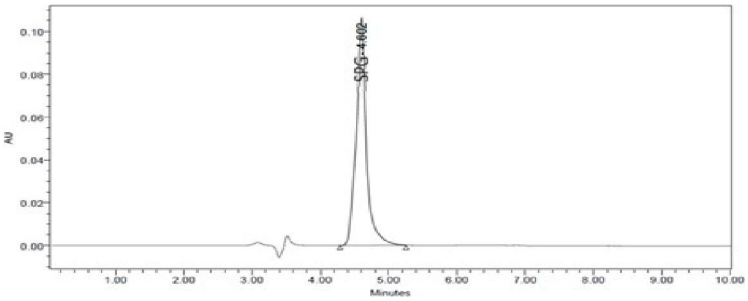
Effect of Variation in Wavelength by ± 2 units:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system. Measure the peak area response at different wavelengths at flow rate 1 ml/min.

Procedure:

Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks.

At 258nm wavelength



At 262 nm wavelength

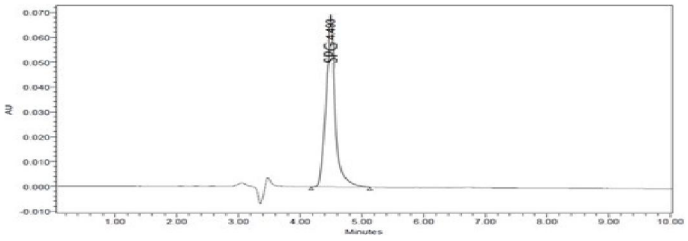


Fig. No. 18: Chromatograms of Change in wavelength.

Sr. No.	**System Suitability parameter**		**Observations for wavelength**			**Limits**
			Unchanged	**248nm**	**252nm**	
1	The % RSD of peak area response for five replicate injections	SPG	1.33	1.41	1.44	NMT 2.0
2	Theoretical plates	SPG	8457	8498	8478	NLT 2000
3	Tailing factor	SPG	1.65	1.45	1.41	NMT 2.0

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4	Retention Time (Min)	SPG	4.65	4.602	4.493	

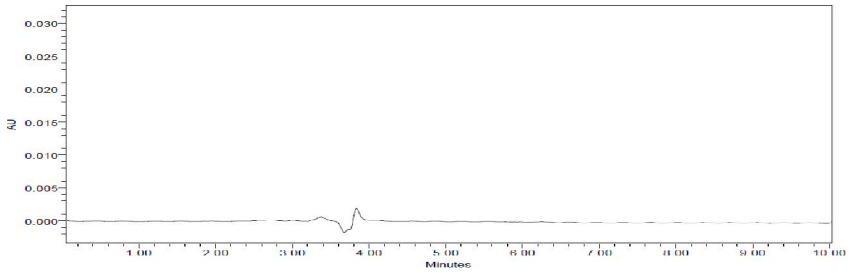
Table No.21: System suitability of change in wavelength

Specificity:

5.4.5.1. Placebo Interference study:

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

Sample matrix



Placebo Preparation

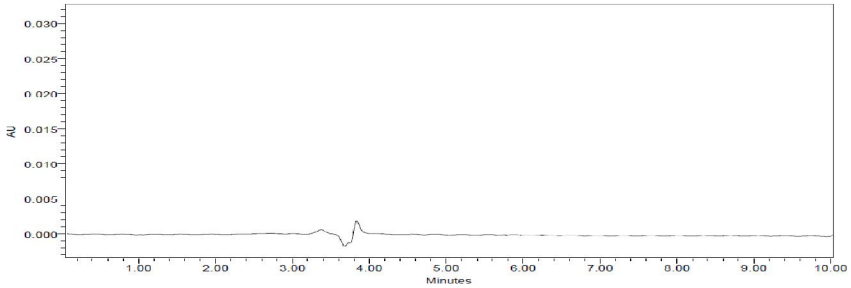


Fig. No. 19: Chromatograms of placebo interference study

Observation	Placebo prep.1	Placebo prep.2	Placebo prep.3
% Interference	No Interference	No Interference	No Interference

Table No. 22: Placebo Interference Acceptance criteria:

No interference should observe from placebo at the retention time of SPG.

Linearity and range:

Prepared the series of standard concentrations ranging from 50 % to 150 % of the targeted concentration of SPG. Each of the linearity dilution was injected into the HPLC system with optimized chromatographic parameters.

Procedure:

Separately inject standard preparation and linearity preparations into the HPLC system, record the chromatograms and measure the peak responses for SPG peaks.

The details of mean peak areas for linearity concentrations are presented in following table and plot the graph of concentration verses average area response for SPG, the correlation coefficient and equation of regression were recorded.

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Sr. No.	% Level	SPG	
		Conc. (µg/ml)	Mean peak area
1	50	50	327220
2	80	80	455200
3	100	100	555325
4	120	120	665300
5	150	150	804052

Table No.23 : Observations of Linearity and range study for SPG Acceptance criteria: The correlation coefficient shall be NLT 0.99

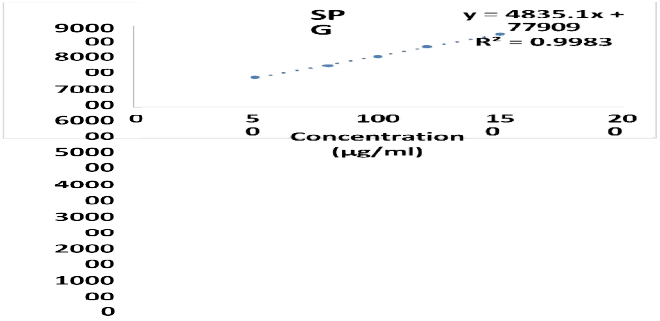

Fig. No. 20: Plot of linearity and range study for SPG

II. RESULT AND DISCUSSION

High Performance Liquid Chromatography which is a highly sophisticated technique, it is used for the determination of active molecules from their formulations. In the present study a HPLC method was developed for analysis of SPG from its tablet formulation.

Sodium phenylbutyrate is an important therapeutic agent used as a nitrogen- scavenging drug in the management of urea cycle disorders and is also explored for other metabolic and neurological conditions. Several analytical techniques have been reported in literature for the estimation of sodium phenylbutyrate and its metabolites, including **spectrophotometry, LC–MS/MS, and UPLC** methods. There is a relative paucity of simple, validated, and stability-indicating RP-HPLC methods specifically targeting the assay of sodium phenylbutyrate in solid oral formulation in form of powder for reconstitution.

In the present investigation an attempt has been made to develop a simple HPLC method for estimation of sodium phenylbutyrate from its formulation. Pure standards of sodium phenylbutyrate were procured from Arrow chem Mumbai. Percent purity of above-mentioned drug was reported by Supplier Company as follows-

Table No. 24: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Sodium phenylbutyrate	Arrow Chem Mumbai.	10 g	99.6 % w/w

The standard was not pre-analyzed and the % purity stated by the suppliers was taken as standard for comparison studies.

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system. This technique was

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employed in the present investigation for estimation of sodium phenylbutyrate in oral powder formulation. Careful evaluation
of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

HPLC Column Selected:

HPLC Waters 600 system with C18 (Thermo Hypersil gold) /4.6 x 250mm, 5µ particle size column and PDA detector were used for the study. The standard and sample solution of sodium phenylbutyrate were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

Mobile Phase selected:

Mobile phase composed of 10mM Phosphate buffer (pH 3.5) and ACN (70:30% v/v). An isocratic program was developed contributing a total run time of 10 min. The wavelength 260 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table below. The following chromatographic conditions were established by trial and error and were keptconstant throughout the method.

Table No. 25: Chromatographic Parameters:

Column	C18 (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	260 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	10mM Phosphate buffer (pH 3.5) and ACN (70:30 % v/v)

Mobile phase-preparation

Weigh accurately 1.36 g of potassium dihydrogen phosphate in dissolve it in 1000 ml of HPLC grade water. The pH was modified to 3.5 using 1 M OPA solution.

Preparation of diluent:

ACN of HPLC grade was selected as common solvent for preparation ofstock solution and developing spectral characteristics of drugs, further dilutions from stock
solutions were made in the Mobile phase.

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	SPG	SPG	SPG	SPG
1	540280	4.609	1.10	8526
2	548970	4.608	1.20	8688
3	545895	4.720	1.10	8545
4	542670	4.620	1.15	8500
5	547870	4.650	1.10	8550
Mean	545137	4.641	1.13	8561
S.D	2620	0.04	0.04	63.22
%R.S.D	0.81	1.01	2.45	0.96

Table No. 26: Summary of system suitability of Test results

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After establishing the chromatographic conditions, Mix standard and marketed preparation solutions were prepared and analyzed by procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Amount of drug in tablet was calculated using following formula:

$$\text{Assay (mg/ml)} = \frac{A_t}{A_S} \times \frac{D_S}{D_t} \times \frac{W_S}{W_t} \times \frac{p}{100} \times \text{Wt mg/ml of test sample} \quad \text{——}$$

$$\% \text{ Label claim} = \frac{\text{Assay (mg/ml)} \times 100}{\text{Label claim in mg/ml}}$$

Where,

At = Area count for sample solution. As = Area count for standard solution. Ds = Dilution factor for standard.
Dt = Dilution factor for sample. P = Potency of drug

VALIDATION

Validation of these methods was performed as per the USP guidelines for these following parameters:

Precision:

System Precision

Prepared the standard solution as per test method and injected into the HPLC system in three replicates. It was found that all system suitability parameters are well within the limits.

Method Precision

Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed R.S.D. less than 2.

Table No. 27: Data showing system Precision

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.45	NMT 2.0
2	Theoretical plates	8560	NLT 2000
3	Tailing factor	1.36	NMT 2.0

Table No.28: Method Precision Studies Set – I

Sr.no.	SPG	
	Assay (mg)	Assay (mg)
1	99.98	99.98
2	99.99	99.99
3	99.97	98.97
Average	99.98	99.97
SD	0.02	0.02
% RSD	0.03	0.03

Linearity & Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was carried out for five levels in the range of 50% to 150%. A graph was plotted with concentration on X axis and mean peak areas on Y- axis. The R^2value

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was found to be 0.999 for SPG. The result show that an excellent correlation exists between concentration and mean peak areas within the concentration range. Thus the method developed is accurate, precise, specific, & linear. Hence it can be said that, RP-HPLC is the most accurate, precise and reproducible among all methods.

Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results werewell within the range **99.90-99.98%.** Thus the method was found to be accurate.

Table No. 29: Result of Accuracy Studies

	SPG		
	Levels		
	80%	80%	80%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.99	99.99	119.95
	79.95	99.98	119.98
	79.98	99.97	119.98
% Recovery	99.98	99.98	99.95
	99.93	99.98	99.98
	99.97	99.97	99.98
Mean % recovery	99.90	99.98	99.93
% RSD	0.15	0.28	0.01

Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

Following Parameters were covered under robustness parameter.

Effect of variation in flow rate of mobile phase by ± 10%

Organic phase composition (± 10%)

Change in Wavelength by ± 2 units

The results suggested all the system suitability parameters were within limits.

Specificity:

Is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of SPG. Thus, no interference was foundat the Retention time of SPG.

III. SUMMARY AND CONCLUSION

SUMMARY

Powder formulation in sachet containing **sodium phenylbutyrate** is introduced in market, the drug is used as a nitrogen-scavenging drug in the management of urea cycle disorders and is also explored for other metabolic and neurological conditions treat and manage pain. Literature survey revealed very few analytical methods for the estimation of **sodium phenylbutyrate**.

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The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for estimation of **sodium phenylbutyrate** in solid oral powder formulation.

In the developed RP-HPLC method the analyte was resolved using Mobile phase composed of 10mM Phosphate buffer (pH 3.5) and ACN **(**70:30 %v/v). An isocratic program was developed contributinga total run time of 10 min. using HPLC auto-sampler system containing PDA detector with EMPOWER Software and C_{18} (Thermo Hypersil gold) /4.6 x 250 mm, 5µ particle size column, the detection wavelength was 260 nm. The method gave the good resolution and suitable retention time.

The results of analysis in all the method were validated in terms of accuracy, precision, ruggedness, linearity and range. The methods were found to be sensitive, reliable, reproducible, rapid and economic also.

CONCLUSION

From the results of the study it can be concluded that the present RP-HPLC technique was successfully used for the estimation of the **sodium phenylbutyrate** in the solid powder formulation.

The method showed good reproducibility, it was accurate, precise, specific, reproducible and sensitive. The analysis of powder formulation of **sodium phenylbutyrate** was done by the developed and validated RP-HPLC method

The RP-HPLC method was also simple, accurate, precise, reproducible and economical too. It may be adopted for routine control analysis of **sodium phenylbutyrate** alone in powder formulation.

No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. Suitability of these methods on biological samples needs to be studied.

REFERENCES

[1]. Khopkar S. M. Basic concepts of analytical chemistry, New Age International *Ltd. Publishers, New Delhi (1998); 2:178-179.
[2]. Settle F. Handbook of Instrumental techniques for analytical chemistry, Prentice Hall PTR, NJ (1997); 17(19): 56-57.
[3]. Skoog D. A. Holler F. J, Crouch S. R. Principle of Instrumental Analysis, Thomson Publications, India (2007); 6: 1-3, 145-147, 180.
[4]. Mendham J, Denney R. C, Barnes J. D, Thomas M. Vogel's Textbook of Quantitative Analysis, Pearson Education, Singapore (2003); 8-9.
[5]. Sharma B. K. Instrumental Methods of Chemical Analysis, Goel Publication, Meerut (1983); 25, 3, 6.
[6]. Christian G. D. Analytical Chemistry, John Wiley and Sons (2003); 5: 35-42, 131-132.
[7]. Beckett A. H, Stenlake J. B. Practical Pharmaceutical chemistry, CBS Publisher and Distributor, New Delhi (1997); 2:1-85.
[8]. Christianah M. A, Pui-Kai L. Analytical Profile of Drug Substances. Edi. By Klaus Florey, 124-141.
[9]. Dong M. W. Modern HPLC for Practicing Scientist. John Wiley and sons, (2006).
[10]. Willard H. H, Merritt L. L, Dean J. A, Settle F. A. Instrumental Methods of Analysis. Seventh ed., CBS Publishers and Distributors, New Delhi, (2001).
[11]. Synder L. R, Kirkaland J. T, Glajch J. L. Practical HPLC Method Development. Second ed, John Wiley and Sons Publication, Inc, New York, (1997).
[12]. Kasture A. V, Mahadik K. R, Wadodkar S. G, More H. N. Pharmaceutical Analysis, Nirali Prakashan, (1999); 2: 6-7, 28-30, 49, 64, 67.
[13]. Scott R. P. W. Technique and Practice of chromatography. Marcel Dekker, New York, (2003); 70:1-12.
[14]. Brown P. R. Advances in Chromatography. Marcel Dekker, New York, (2001); 41.
[15]. Sethi P D. HPLC-Quantitative analysis of pharmaceutical formulations. CBS publishers and distributors, New Delhi, (2001); 1: 1-5, 58-67, 116-120.

[16]. Pattan S, Jamdar S. RP-HPLC Method for Simultaneous Estimation Of Paracetamol and Etoricoxib from bulk and Tablet, Journal of Chemical and Pharmaceutical Research(2009);1(1): 329-335.

[17]. Gowramma B, Rajan S. A Validated RP-HPLC Method for simultaneous estimation of Paracetamol and Diclofenac pottasium in pharmaceutical formulation, International journal of chemtech research(jan-mar 2010);2(1):676- 680.

[18]. Reddy P, Battu. The Simultaneous RP-HPLC determination of Nimesulide and Paracetamol in tablet, International journal of Pharmtech Research (july- sept2009);1(3):514-516.

[19]. Gopinath R, Rajan S, Meyyanathan S. N, Krishnaveni N, Suresh B. (RP- HPLC) method was developed for Paracetamol, Aceclofenac and Etoricoxibin pharmaceutical dosage forms, Indian Journal of pharmaceutical sciences, 2007; 69 (1): 137-140.

[20]. Beludari MI, Chedela HK, Lingareddy SSR, Karimulla S. Development and validation of a stability-indicating analytical method for simultaneous estimation of sodium phenylbutyrate and taurursodiol in bulk and formulation using UPLC method. *Iran J Pharm Sci*. 2025;21(1):70–81.

[21]. Laryea MD, Herebian D, Meissner T, Mayatepek M. Simultaneous LC-MS/MS determination of phenylbutyrate, phenylacetate, benzoate, and their metabolites in plasma and urine. *J Inherit Metab Dis*. 2010;33(6):759–64.

[22]. Jiang Y, Almannai M, Sutton VR, Sun Q, Elsea SH. Rapid UPLC-MS/MS quantification of phenylbutyrate and its metabolites in biological samples. *Mol Genet Metab*. 2017;122(3):39–45.

[23]. Ebbel EN, Leymarie N, Schiavo S, Sharma S, Gevorkian S, Hersch S, Matson WR, Costello CE. Identification of phenylbutyrate-generated metabolites in Huntington disease patients using parallel liquid chromatography/electrochemical array/mass spectrometry and off-line tandem mass spectrometry. Anal Biochem. 2010;399(2):152-61.

[24]. SK Karishma, B Anusha, Swetha Vegensa. Stability Indicating Method Devleopment and Validation for The Estimation of Sodium Phenyl Butyrate and Taurursodilol by Using Rp-HPLC. (2024). *African Journal of Biomedical Research*, *27*(4S), 15422-15432.

[25]. Raju, C., B, S., M, N. B., & Minchala, N. (2025). Validation of a Stability- Indicating Analytical Method Development for Simultaneous Estimation of Sodium Phenylbutyrate and Taurursodiol in Bulk and Formulation Using UPLC Method. *Iranian Journal of Pharmaceutical Sciences*, *21*(1), 70–81.

[26]. Serkan Levent, Abeer Elriş, Hazal Avcı, Ülfet Erdoğan Uzunoğlu, Saniye Özcan, and Nafiz Öncü Can. LC-MS/MS and LC-PDA Methods for Robust Determination of Glycerol Phenylbutyrate in Biological Fluids and High- Resolution Mass Spectrometric Identification of Forced Degradation Product and Its Whiteness. *ACS Omega* 2025 *10* (17), 17836-17846.

[27]. Rayudu, S, Manoranjani, M, & Anitha, D. (2026). analytical method development and validation of sodium phenylbutyrate and taurursodiol by using rp-hplc in bulk and pharmaceutical dosage form. International Journal of Applied Pharmaceutics, 18(1), 380–388.

[28]. SK Karishma, B Anusha, Swetha Vegesna. Stability Indicating Method Development and Validation for The Estimation of Sodium Phenyl Butyrate and Taurursodilol by Using Rp-HPLC. (2024). African Journal of Biomedical Research, 27(4S), 15422-15432.

[29]. Chaudhari B, Patel N, Shah P. Stability indicating RP-HPLC method for simultaneous determination of atorvastatin and amlodipine from their combination drug products. Chem Pharm Bull. 2007;55:241–246.

[30]. Dewani M, Bothara K, Madgulkar A, Damle M. Simultaneous estimation of perindopril erbumine and indapamide in bulk drug and tablet dosage form by HPTLC. Pharmacie Globale, International Journal of Comprehensive Pharmacy. 2011;2(1)

[31]. Patel P, Patel H. Two spectroscopic methods for the simultaneous estimation of indapamide and nebivolol hydrochloride in combined dosage form. Int J Pharm Res. 2011;3:55–57.

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International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
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[32]. Sharma V., and Mehta P. "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Tablet Dosage Form." International Journal of Pharmaceutical Sciences and Research, 2023, 14(5): 2150-2157.

[33]. https://pubchem.ncbi.nlm.nih.gov/compound/5258