Ind. J. Pharm. Edu. Res., 2026; 60(3s):s1253-s1260.
https://www.ijper.org

Original Article

Optimized Green Synthesis Approaches of Phenytoin Using Divalent Sulfate Salts and Chromatographic Purity Assessment via A Novel RP-HPLC Method

Arka Chakraborty[1], Rajarshi Nath[1], Biplab Debnath[1], Arijit Das[2], Anindya Bose[3], Shambo Panda[1,3,*]

[1]Department of Pharmaceutical Chemistry, Bharat Technology, Jadurberia, Uluberia, Howrah, West Bengal, INDIA.
[2]Department of Pharmacology, Bharat Technology, Jadurberia, Uluberia, Howrah, West Bengal, INDIA.
[3]School of Pharmaceutical Science, SOA University, Khandagiri, Bhubaneswar, Odisha, INDIA.

ABSTRACT

Background: Phenytoin is a first-line anti-epileptic drug. Conventionally, It is synthesized by using Benzil and urea in the presence of concentrated HCl. Our aim is to increase the yield as well as the purity of Phenytoin by applying Green approaches and developing an RP-HPLC method for purity assessment. **Materials and Methods:** 3 different reaction is carried out by using Copper sulfate, Ferrous sulphate and Aluminium sulfate as catalysts along with Benzoin, Sodium nitrate and acetic acid as solvents. Another reaction carried out changing acetic acid with ethanol and the catalyst was Ferrous sulphate. **Results and Conclusion:** Phenytoin prepared by ferrous sulphate with ethanol gives the highest yield as well as the highest purity compared to other catalysts.

Keywords: Analytical method development, Divalent sulfate salts, Green Synthesis, Phenytoin, RP-HPLC.

Correspondence:
Mr. Shambo Panda
[1]Associate Professor at Bharat Technology, Department of Pharmaceutical Chemistry, Bharat Technology, Jadurberia, Uluberia, Howrah, West Bengal, INDIA.
[2]PhD Scholar at School of Pharmaceutical Science, SOA University, Khandagiri, Bhubaneswar-751030, Odisha, INDIA.
Email: shambo8689@gmail.com

Received: 12-01-2026;
Revised: 02-02-2026;
Accepted: 27-03-2026.

INTRODUCTION

Epilepsy or convulsion is a long-term, non-communicable disease of the brain, which can be identified by a tendency to have seizures. Both males and females can suffer from epilepsy but males are more prone to epilepsy than females.[1] The development process of building an epileptical condition in a normal brain is called Epileptogenesis. It can occur due to serious hemorrhage in the head, brain stroke, and defects at the time of birth, etc. There are various anti-epileptical drugs available, like as Gabapentin, Vigabatrin, Trigabine, Phenytoin, Carbamazepine, etc.[2]

Phenytoin, a hydantoin derivative (Figure 1), was introduced as an anticonvulsant agent. It is mainly used for tonic-chronic and partial seizures.[3] It is marketed under the brand name Dilantin®. In 1938, Tracy Jackson Putnam and H. Houston Merritt introduced this as medication in clinical practices.[4] While all antiepileptic medications carry the risk of side effects, including drowsiness or sedation, phenytoin is often preferred in certain situations due to its relatively lower sedative effects.[5] The key mechanism of phenytoin's action primarily depends upon the voltage-gated sodium channel. Phenytoin blocks the sodium channel and decreases the excitability of neurons, mainly in the epileptic focus, which leads to reduced abnormal electrical activity responsible for epileptic seizures.[6]

The antiepileptic or antiseizure activity of phenytoin relies upon blocking the voltage-gated sodium channel. Specifically, it targets certain α-subtypes (1.1, 1.2, 1.3, and 1.6). And prolongs its inactivated state.[7] Phenytoin stabilizes neuronal membranes by regulating sodium and calcium ion fluxes, primarily targeting specific sodium channels. This modulation helps prevent excessive neuronal excitability, reducing the likelihood of epileptic seizures.[8] It is indicated by the electrophysiological studies that phenytoin can access its binding site within the central pore of the sodium channel via one of two pathways.[9] Phenytoin operates on multiple fronts to mitigate epileptic activity within the nervous system. By targeting voltage- and frequency-dependent sodium channels, it effectively dampens the ability of partly depolarized axons to propagate rapid bursts of action potentials, characteristic of epileptic discharges. Interestingly, this intervention seems to spare axonal traffic associated with lower impulse frequencies, suggesting a nuanced impact on neuronal firing patterns. Moreover, at elevated concentrations, phenytoin extends its influence to calcium channels present in both axonal pathways

DOI: 10.5530/ijper.20262650

and nerve terminals. This dual inhibition serves to stabilize axonal membranes and curtail the release of neurotransmitters, notably glutamate, upon the arrival of action potentials. By tempering this neurotransmitter release, phenytoin helps to mitigate the hyperexcitability often associated with epileptic activity. Furthermore, phenytoin's selectivity becomes apparent in its lack of effect on T-type calcium channels, particularly relevant in the thalamus for generating absence seizures. This specificity underscores phenytoin's tailored approach, honing in on specific neuronal pathways implicated in certain types of seizures while sparing others. Overall, phenytoin's multifaceted mechanism underscores its efficacy in modulating neuronal excitability and curtailing epileptic seizures[7]. Phenytoin inhibits calmodulin-mediated pathways, by protein mediate phosphorylation, reducing neurotransmitter release and mitigating seizures. These drugs bind to specific sites, modulating calcium-calmodulin interactions crucial for synaptic function. Changes in calmodulin-related pathways are implicated in seizure disorders, suggesting their significance in neuronal excitability and seizure development.[10] Phenytoin affects the T-type calcium channel and inhibits glutamate excitation. Phenytoin does not show GABA-mediated inhibition within its therapeutic concentrations.[11]

Green synthesis first came in the early 1990s. In the year of 1999, the first volume of the Green Chemistry Journal of the Royal Society of Chemistry was published.[12] Green synthesis is one of the newer and safer approaches to synthesizing chemical moieties. The main implementation of this approach aimed to prioritize human health and the environment. For the priorities of these objectives, chemists are focused on the use of solvents that are environmentally friendly. These strategies reduce ecological harm and increase the safety margin for human health. It accomplishes the purity level.[11] There are 12 principles of green chemistry, i.e. Prevention of Waste, Atom Economy, Less Hazardous Chemicals, Safer Chemicals Designing, Use of Safe Solvents and Auxiliaries, Energy Efficient, Renewable Feedstocks, Less Derivatives, Selective Catalyst, Degradation Design, Real-Time Analysis for Pollution Prevention, Safer Chemistry for Accident Prevention.[13] Phenytoin can be synthesized through a green approach using Benzil and Urea under basic conditions of sodium hydroxide (30%) and water as solvent.[14]

The purity of synthesized phenytoin can be measured by various methods, such as UV-vis spectroscopy, TLC, and ultimately through HPLC. In UV-vis spectroscopy, Phenytoin gives the λ_{max} at 213 nm.[15] In HPLC analysis, the mobile phase is Methanol: Phosphate buffer pH maintained at 5 using a 0.1M NaOH (50:50) ratio and the flow rate is maintained at 1.0 mL/min with 215 nm wavelength for detection (Figure 2).[16]

GAP ANALYSIS

Phenytoin can be synthesized conventionally using Benzil and urea in the presence of HCl, which gives a theoretical near about 60%.[18] But using the Green approach, alternatively, it can be synthesized by using Benzoin, Sodium nitrate, Copper sulfate (as a catalyst) in acetic acid (as a solvent), which gives a yield of about 62% and it is more acceptable than the conventional method because of uses non-hazardous substances.[19] Now also the yield is also not up to the mark, so we are trying to improve the percentage yield by changing not only the catalyst but also using a more polar solvent (Ethanol).

MATERIALS AND METHODS

Materials

Benzoin, Sodium nitrate, potassium dihydrogen phosphate, Sodium hydroxide Copper sulfate, Ferrous sulfate, and Aluminium sulfate come from Nice chemicals. Acetic acid and Methanol were received from Rankem Chemicals. For Thin-layer chromatography, The TLC plate was DC Kieselgel 60 F_{254} (Merck KGaA, Damstad, Germany). HPLC-grade water, acetonitrile, methanol, and orthophosphoric acid (Qualigens, India) were used.IR spectra were recorded using a SHIMADZU 01928 IRSpirit, Fourier Transform Infrared Spectrophotometer. The UV spectrum was recorded using a SHIMADZU UV-1780 UV-visspectrophotometer.The HPLC instrument consisted of the SHIMADZU SPD-20A UV detector, DGU-$20A_{AR}$ degasser unit, LC-20AD pump, SIL-$20AC_{HT}$ autosampler, and CIT-$10AS_{VP}$ column oven (SHIMADZU Corp, Japan). LabSolutions is the software that was used for data collection and integration of chromatographic data. A C18 column (255 mm; particle size 5 µm) (SHIMADZU, Japan) was used for the separation process.

Methods

Green Approach to prepare phenytoin: 5.0 g of benzoin, 25 mL of acetic acid, and 4 g of sodium nitrate were placed in 3 different round-bottom flasks. Then add 10 mL 2% catalyst solution, i.e., copper sulfate, ferrous sulfate and aluminum sulfate solution, in each separate reaction mixture. The mixtures were stirred on the magnetic stirrer for about 2 hr at a temperature of about 80°C. Then the reaction mixtures were cooled to (30-40)°C, and about 125 mL of ice-cold water was added to each mixture to facilitate the precipitation. The reaction mixtures were now allowed to stand for 20 min to complete the precipitation of the product. Then the products were filtered and the soluble by-products were removed using Whatman filter paper (41, Ashless). The obtained products were recrystallized by glacial acetic acid. Isolate the purified products using standard filtration techniques and dry them under mild conditions to minimize energy consumption.

A separate reaction was also started by substituting the acetic acid with ethanol. Here only ferrous sulphate was used as the catalyst (Scheme 1).

Thin Layer Chromatography

Take a Silica Gel G TLC plate and draw a thin straight line of about 1 cm from the bottom of the plate. Mark four points with equidistance on a straight line and number them. Prepare 4 different phenytoin solutions, i.e., phenytoin prepared by the conventional method, copper sulfate, ferrous sulfate, and aluminium sulfate, and place a drop of each solution on the straight line by using a capillary tube. Kept the TLC plate in a chamber containing mobile phase, i.e., Cyclohexane Acetone (6:4), and ran the chromatogram till the mobile phase travels on ¾th of the chromatographic plate. Remove the plate from the chamber, mark the solvent distance with a pencil, and dry it in the air for 15 min. Then keep the chromatogram in a UV cabinet to observe the run.

Chromatographic Condition

The mobile phase for separation was Buffer and Acetonitrile at 60:40 ratio. The buffer was prepared by using 6.8 g Potassium dihydrogen phosphate (KH_2PO_4) and 0.9 g Sodium hydroxide in 1000 mL of Mill-Q water, then adjusted the pH at 2.8(±0.05) with the help of diluted Orthophosphoric acid. The mobile phase was filtered using a 0.45 µm membrane filter and degassed by using a Sonicator. The isocratic condition was maintained throughout the experiment using a flow rate of 2 mL/min at room temperature with an injection volume of 20 µL at 210 nm wavelength. Methanol was used as a diluent to dissolve the phenytoin drug.

Preparation of Solutions

A 100 ppm stock solution was prepared by dissolving the appropriate amount of API with diluent i.e., Methanol. A 10 ppm working solution was prepared from the stock solution for the assay. Methanol was used as the blank solution.

RESULTS

Phenytoin is a white, odorless, crystalline, hygroscopic powder, soluble in water.

IR spectra

In the IR spectra of synthesized phenytoins, 3370 cm^{-1} and 3407 cm^{-1} were seen due to the stretching vibration of secondary amines. At 3060 cm^{-1} peak is seen due to the stretching vibration of =C-H present in the aromatic ring. At 1679 cm^{-1} peak was seen due to the stretching vibration of the C=O group. At 1490 cm^{-1} peak was seen due to the presence of two aromatic rings (Figure 3).

Percentage Yield

$$\text{Molecular Weight of Benzoin} = 212.24\ \text{g/M}$$

$$\text{Molecular Weight Phenytoin} = 252.26\ \text{g/M}$$

$$\text{Weight Taken of Benzoin} = 5\ \text{g}$$

$$\text{Theoretical Yield} = \frac{\text{Molecular Weight Of Phenytoin}}{\textit{MolecularWeightofBenzoin}} \times \text{Weight of benzoin}$$

$$= \frac{252.26\ \text{g/M} \times 5\ \text{g}}{212.24\ \text{g/M}} = 5.94\ \text{g}$$

$$\text{Percentage Yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

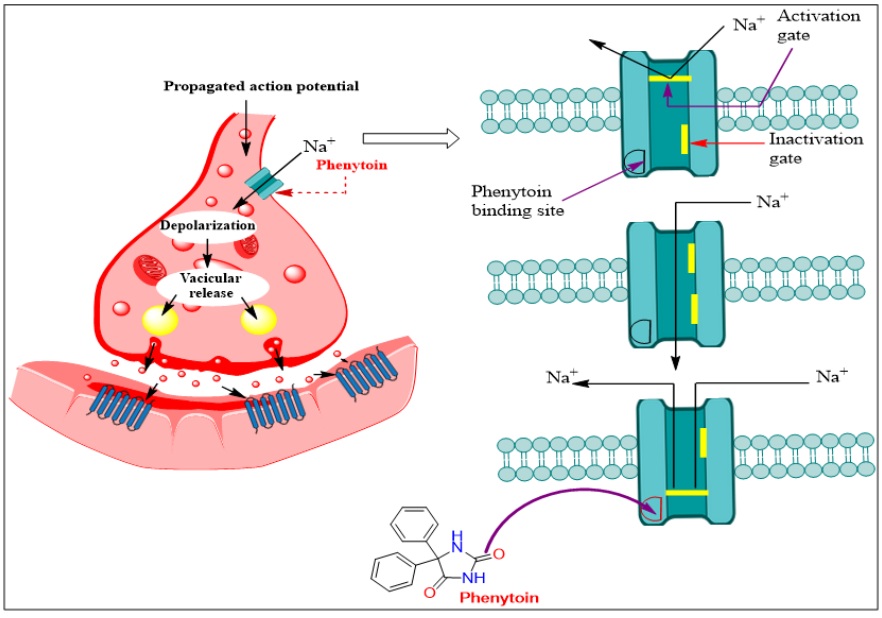


Figure 1: Structure of Phenytoin and its mechanism of action.

Table 1: Comparative study of % Yield calculation of synthesized phenytoin using divalent sulphate salts.

Sl. No.	Method	Practical yield	Percentage yield
1.	Synthesis of Phenytoin Using $CuSO_4$	5.43 g	91.41%
2.	Synthesis of Phenytoin Using $FeSO_4$ (Acetic acid as a solvent)	5.66 g	95.29%
3.	Synthesis of Phenytoin Using $Al_2(SO_4)_3$	5.54 g	93.27%
4.	Synthesis of Phenytoin Using $FeSO_4$ (Ethanol as a solvent)	5.83 g	98.2%

Table 2: Comparative study of the % Purity of synthesized phenytoin using divalent sulphate salts through a novel HPLC method.

Phenytoin by $FeSO_4$ (Using Ethanol)		Phenytoin by $FeSO_4$ (Using Acetic acid)		Phenytoin by $CuSO_4$		Phenytoin by $Al_2(SO_4)_3$	
Retention Time	% Purity	Retention Time	% Purity	Retention Time	% Purity	Retention Time	% Purity
4.556	0.015	4.556	0.007	6.043	0.519	6.069	0.975
6.039	0.431	6.039	0.392	6.496	99.086	6.528	96.087
6.492	99.482	6.492	98.552	12.571	0.012	13.159	2.260
12.190	0.017	11.828	0.064	13.730	0.022	15.837	0.052
13.726	0.020	13.040	0.982	15.693	0.046	17.001	0.047
15.686	0.035	15.686	0.033	16.848	0.032	35.811	0.579
				35.385	0.283		

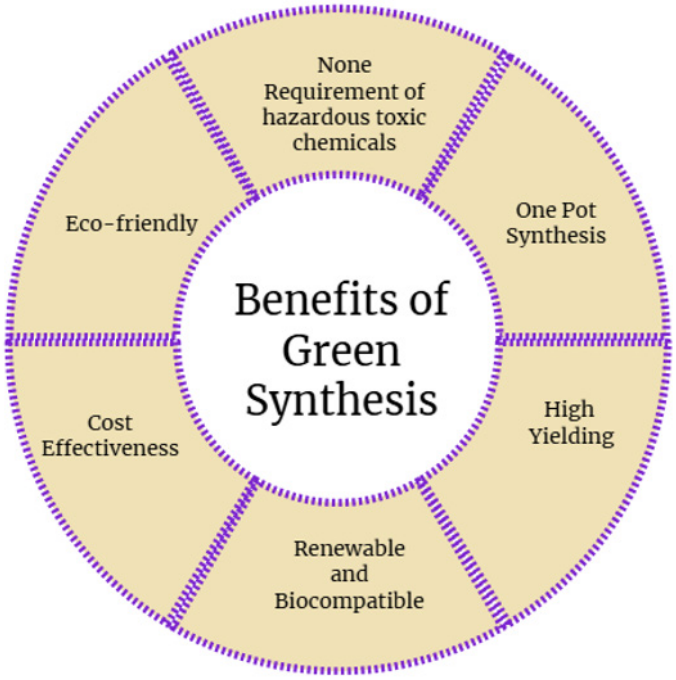


Figure 2: Benefits of Green Synthesis.[17]

UV-visible spectrum Analysis

Synthesized Phenytoins are run under a Shimadzu UV-vis 1780 Spectrophotometer at 200-400 nm to find the λ_{max}. Methanol is used as the standard solution and 5 ppm phenytoin solution is used as the test solution.[13]: The spectral analysis shows that the λ_{max} of phenytoin is 247 nm (Figure 4).

Thin Layer Chromatography

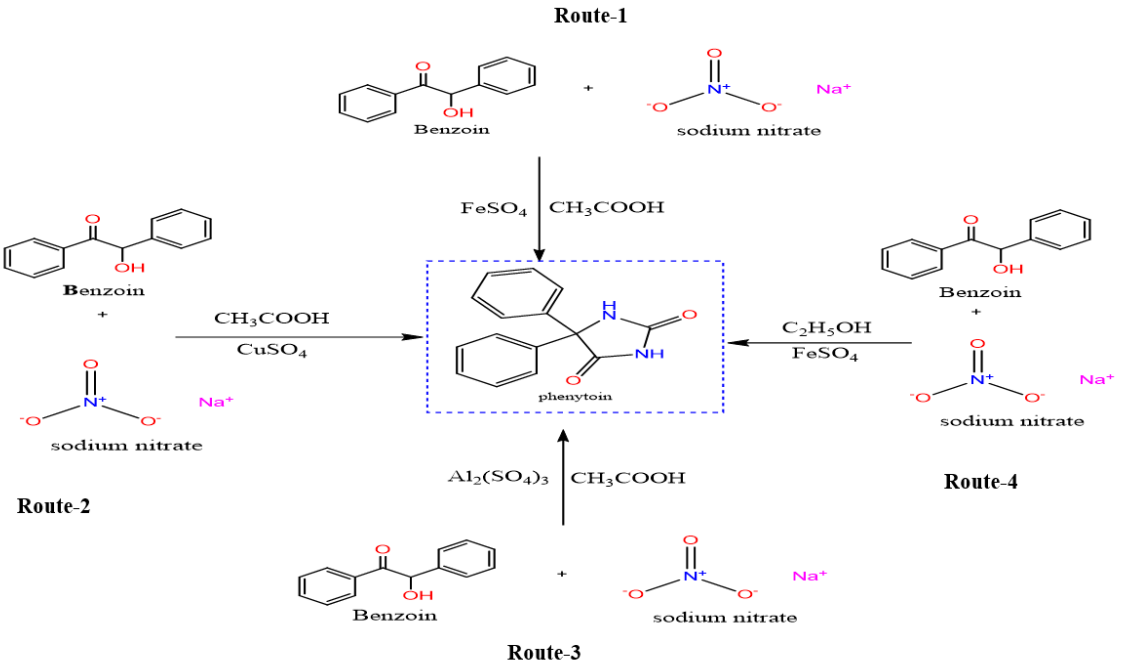
Cyclohexane-acetone (6:4) is selected as the mobile phase for TLC analysis. The obtained R_f Values are 0.936 (prepared by conventional method), 0.94 (using aluminum sulfate), 0.95 (using Ferrous Sulphate) and 0.96 (using Copper Sulphate). The result shows that 3 types of synthesized phenytoins are free from impurities (Figure 5).

Percentage Purity through HPLC

The percentage purity of Phenytoin using various divalent salts was mentioned in Tables 1, 2 and Figure 6.

DISCUSSION

In the above TLC analysis, there is no change in the R_f value (9.5) when the copper sulfate is substituted with ferrous sulfate and aluminium sulfate, In the UV-vis spectroscopy also the same λ_{max} is observed at 213 nm for all the synthesized compounds. All the products give confirmatory peaks at 3370-3407 cm^{-1} (Secondary amine group), 3060 cm^{-1} (=C-H of aromatic ring), 1679 cm^{-1} (C=O group), 1490 cm^{-1}(aromatic rings), which primarily confirms that every synthesized product is phenytoin.The retention time of every product was about 6 min using a C18 column and Phosphate buffer and Acetonitrile as a mobile phase in the RP-HPLC method. We can also see that using ferrous sulphate and Aluminium sulphate instead of copper sulphate in acetic acid gives a higher yield value. Now, we take ferrous sulphate (as it gives the highest yield) in ethanol by substituting acetic acid, which gives more yield value than any previous approaches, i.e.,



Scheme 1: Different green synthetic routes of phenytoin by using various divalent sulfate salts.

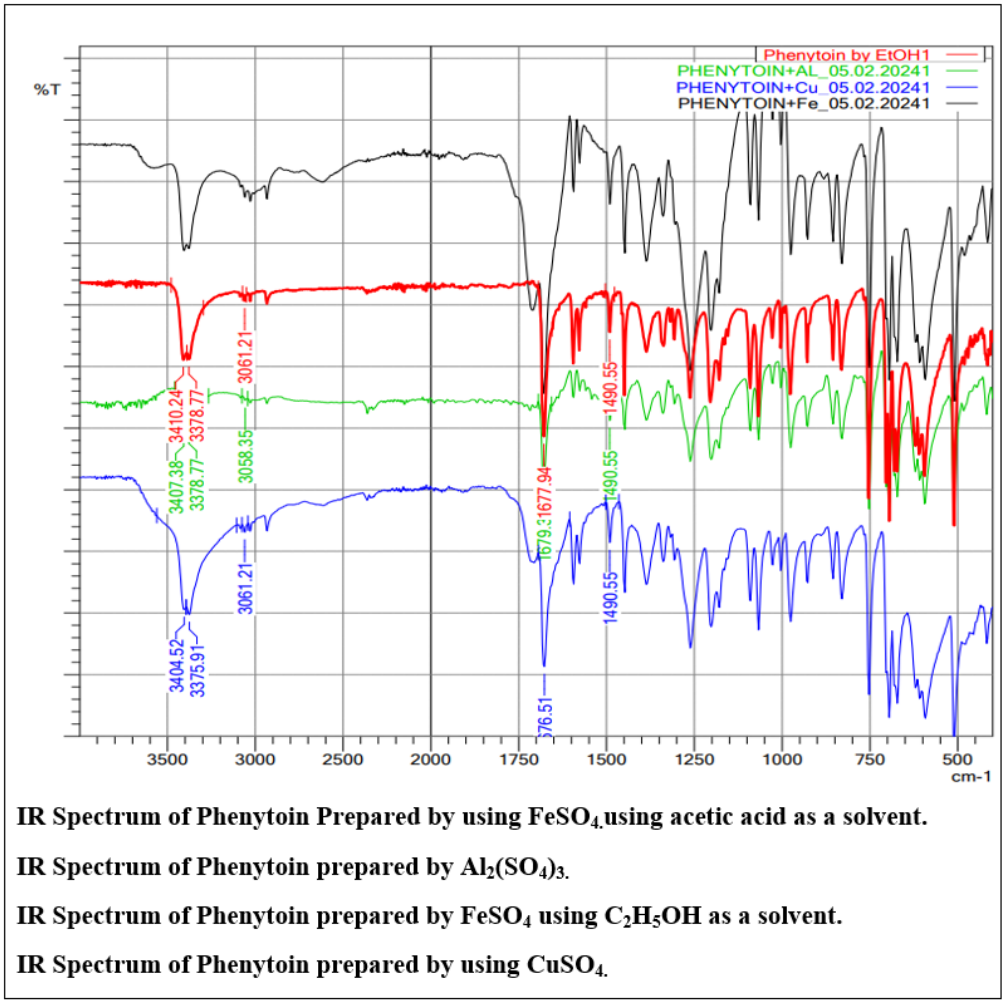
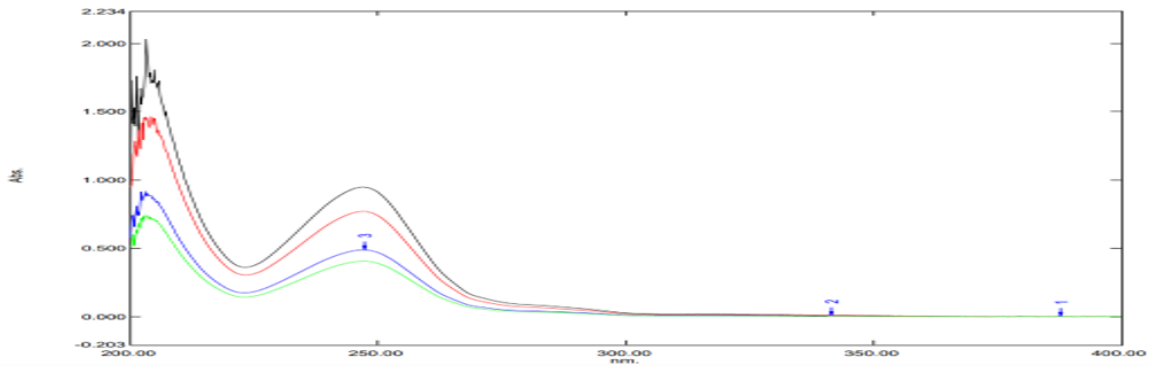


Figure 3: Comparative study of stuctural confiramtion through IR of synthesized phenytoin using divalent sulphate salts.



UV-vis Spectrum of Phenytoin Prepared by UsingFeSO4, using acetic acid as a solvent.

UV-vis Spectrum of Phenytoin prepared by $Al_2(SO_4)_3$

UV-vis spectrum of Phenytoin prepared by FeSO4 using C_2H_5OH as a solvent.

UV-vis IR Spectrum of Phenytoin prepared by using $CuSO_4$.

Figure 4: Comparative study of stuctural confiramtion through UV-vis Spectrophotometry of synthesized phenytoin using divalent sulphate salts.

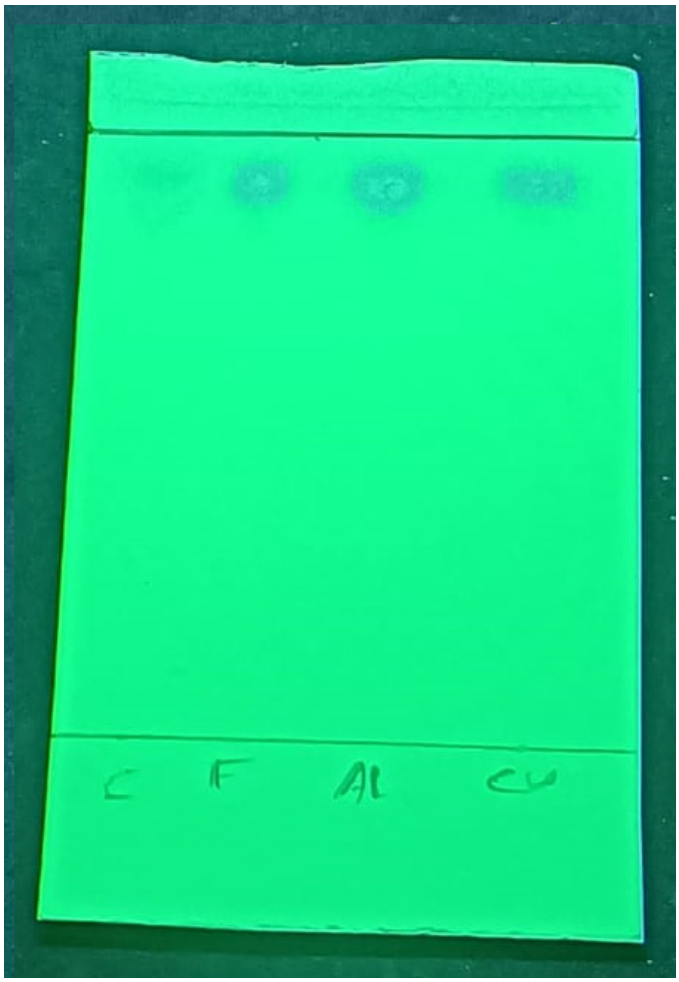


Figure 5: Comparative study of structural confirmation through TLC of synthesized phenytoin using divalent sulphate salts using Cyclohexane and acetone at a 6:4 ratio as a mobile phase.

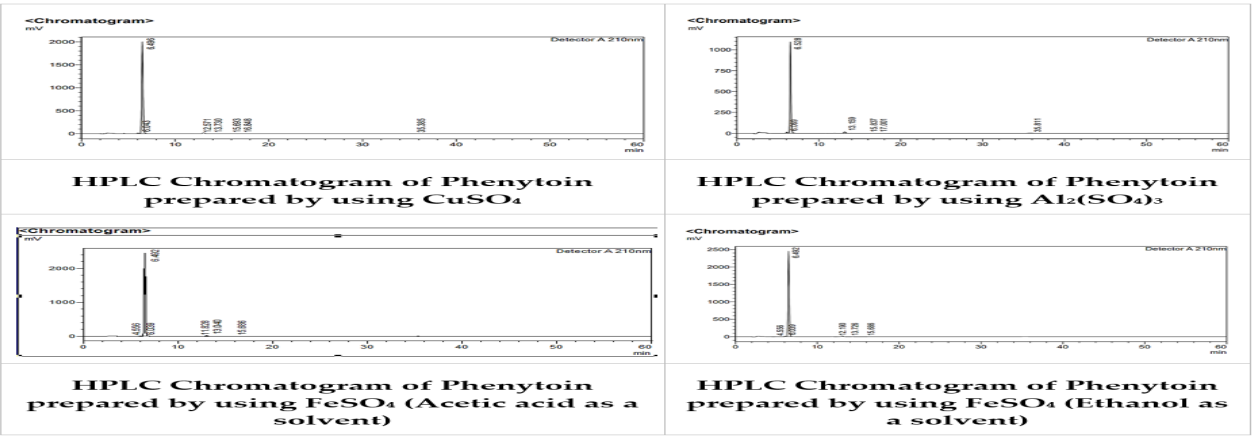


Figure 6: Comparative study of % Purity through HPLC of synthesized phenytoin using divalent sulphate salts using Phosphate buffer (pH 2.8) and Acetonitrile solution at 6:4 ratio as a mobile phase.

98.2% andnot only this, it gives the purity, 99.482%, which is the highest among other.

CONCLUSION

From the above study, it was observed that phenytoin synthesized by ferrous sulphate in the presence of ethanol solvent produces purer (99.5%) phenytoin than phenytoin produced by other metallic sulfate salts (i.e., Copper sulphate and Aluminum sulfate), which was confirmed by chromatographic purity method. It has also been observed that phenytoin prepared by ethanol instead of acetic acid shows a higher yield value than the others. The use of the Greenchemistry approach gives a higher yield value and also increases the atom economy rather than the conventional approach.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacy, Bharat Technology, Uluberia, Howrah, West Bengal support and encouragement. One of the authors Mr. Shambo Panda is thankful for the support from SOA University, Odisha, India.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Pressure Liquid Chromatography; **mm:** Mili Meter; **µm:** Micro Meter; **M:** Mole; **R_f:** Retention factor; **TLC:** Thin Layer Chromatography.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This research work was focuses on improving the synthesis of phenytoin, a widely used anti-epileptic drug, by adopting green chemistry approaches. Instead of the conventional method using hazardous chemicals, the researchers used eco-friendly solvents and divalent sulfate catalysts (Copper, Ferrous, and Aluminum sulfate). They found that using ferrous sulfate in ethanol gave the highest yield and purity of phenytoin. The synthesized products were confirmed using spectroscopic and chromatographic methods. Overall, the green synthesis approach proved to be safer, more efficient, and environmentally friendly compared to conventional methods.

REFERENCES

1. Beghi E. The epidemiology of epilepsy. Neuroepidemiology. 2020;54(2):185-91.
2. Ghosh S, Sinha JK, Khan T, Devaraju KS, Singh P, Vaibhav K, *et al.* Pharmacological and therapeutic approaches in the treatment of epilepsy. Biomedicines. 2021;9(5):470.
3. Patocka J, Wu Q, Nepovimova E, Kuca K. Phenytoin-An anti-seizure drug: Overview of its chemistry, pharmacology and toxicology. Food and Chemical Toxicology. 2020;142:111393.
4. Ferner R, Day R, Bradberry SM. Phenytoin and damage to the cerebellum-a systematic review of published cases. Expert opinion on drug safety. 2022;21(7):957-77.
5. Kolecki P, Shih RD. Adverse Effects of Anticonvulsants and Psychotropic Agents. Pediatric Emergency Medicine. 2008: 970-4.
6. Keppel Hesselink JM, Kopsky DJ. Phenytoin: neuroprotection or neurotoxicity? Neurological Sciences. 2017;38(6):1137-41.
7. Eadie MJ. Pregnancy and the control of epileptic seizures: a review. Neurology and Therapy. 2021;10(2):455-68.
8. Keppel Hesselink JM, Kopsky DJ. Phenytoin: 80 years young, from epilepsy to breast cancer, a remarkable molecule with multiple modes of action. Journal of Neurology. 2017;264(8):1617-21.
9. Martin LJ, Chao R, Corry B. Molecular dynamics simulation of the partitioning of benzocaine and phenytoin into a lipid bilayer. Biophysical chemistry. 2014;185:98-107.
10. John DeLorenzo R. Calmodulin systems in neuronal excitability: a molecular approach to epilepsy. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 1984; 16(S1):S104-S14.
11. Meurs A, Clinckers R, Ebinger G, Michotte Y, Smolders I. Substantia nigra is an anticonvulsant site of action of topiramate in the focal pilocarpine model of limbic seizures. Epilepsia. 2006;47(9):1519-35.
12. Aksoy ACA. Green Chemistry and Science Education. CURRENT STUDIES in EDUCATIONAL DISCIPLINES-2024. 2024: 1.
13. Anastas P, Eghbali N. Green chemistry: principles and practice. Chemical Society Reviews. 2010;39(1):301-12.
14. Kadam A, Jangam S, Oswal R. Application of green chemistry principle in synthesis of phenytoin and its biogical evaluation as anticonvulsant agents. Journal of Chemistry. 2011;8:S47-S52.
15. Gul S, Hameed A. UV spectroscopic method for determination of phenytoin in bulk and injection forms. Chem Int. 2018;4(3):177-82.

16. Shah R, Shah R. Development and validation of RP-HPLC method for phenytoin sodium and phenobarbitone in bulk and pharmaceutical dosage form. Int J Pharm Pharm Sci. 2017;9(10):224.
17. Wasiq M, Ahmed F, Saeed F, Raza A, Abdullah HM, Saleem S, *et al.* From waste to wellness: Green synthesis of nanoparticles from vegetable waste. Sustainable Environment. 2025;11(1):2491870.
18. Ashnagar A, Gharib Naseri N, Amini M. Synthesis of 5, 5-diphenyl-2, 4-imidazolidinedione (Phenytoin) from almond. International Journal of ChemTech Research. 2009;1(1):47-52.
19. Agrawal S, Gaikwad S, Patel R, Shinde L, Deshmukh A. Synthesis and formulation development of phenytoin by inclusion complexation. Indian J Pharm Sci. 2021;83(5):955-62.

Cite this article: Chakraborty A, Nath R, Debnath B, Das A, Bose A, Panda S. Optimized Green Synthesis Approaches of Phenytoin Using Divalent Sulfate Salts and Chromatographic Purity Assessment via A Novel RP-HPLC Method. Indian J of Pharmaceutical Education and Research. 2026;60(3s):s1253-s1260.