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RESEARCH ARTICLE

Synthesis and Preclinical Evaluation of Indole Triazole Conjugates as Microtubule Targeting Agents that are Effective against MCF-7 Breast Cancer Cell Lines

Vidyasrilekha Yele[1,2,*], Sai Kiran S.S. Pindiprolu[3], Sravani Sana[4], D.S.V.N.M. Ramamurty[5], Jayanthi R.K. Madasi[1] and Swapna Vadlamani[1,6,*]

[1]*Department of Pharmaceutical Technology and Process Chemistry, National Institute of Pharmaceutical Education and Research, Hyderabad, Telangana, India;* [2]*Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Tamilnadu, India;* [3]*Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Tamilnadu, India;* [4]*Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Hyderabad, Telangana, India;* [5]*Department of Chemistry, Government Degree College (A), Tuni, Andhra Pradesh, India;* [6]*Department of Chemical Engineering, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India*

Abstract: ***Background*:** Microtubules are considered to be an important therapeutic target for most of the anticancer drugs. These are highly dynamic structures comprising of α-tubulin and β-tubulin which are usually heterodimers and found to be involved in cell movement, intracellular trafficking, and mitosis inhibition of which might kill the tumour cells or inhibit the abnormal proliferation of cells. Most of the tubulin polymerization inhibitors, such as Vinca alkaloids, consist of Indole as the main scaffold. The literature also suggests using triazole moiety in the chemical entities, potentiating the inhibitory activity against cell proliferation. So, in our study, we used indole triazole scaffolds to synthesize the derivatives against tubulin polymerization.

***Objective*:** The main objective of this study to synthesize indole triazole conjugates by using environmentally friendly solvents (green chemistry) and click chemistry. To carry out the MTT assay and tubulin polymerization assay for the synthesized indole triazole conjugates.

***Methods*:** All the synthesized molecules were subjected to molecular docking studies using Schrodinger suite and the structural confirmation was performed by Mass, proton-NMR and carbon-NMR, documented in DMSO and CDCL3. Biological studies were performed using DU145 (prostate cancer), A-549 (lung cancer) and, MCF-7 (breast cancer), cell lines obtained from ATCC were maintained as a continuous culture. MTT assay was performed for the analogues using standard protocol. Cell cycle analysis was carried out using flow cytometry.

***Results*:** The Indole triazole scaffolds were synthesized using the principles of Green chemistry. The triazole formation is mainly achieved by using the Click chemistry approach. Structural elucidation of synthesized compounds was performed using Mass spectroscopy (HR-MS), Proton-Nuclear Magnetic Spectroscopy (^{1}H-NMR) and Carbon-Nuclear Magnetic Spectroscopy (^{13}C-NMR). The XP-docked poses and free energy binding calculations revealed that **2c** and **2g** molecules exhibited the highest docking affinity against the tubulin-colchicine domain (PDB:1SA0). *In vitro* cytotoxic assessment revealed that **2c** and **2g** displayed promising cytotoxicity in MTT assay (with CTC50 values 3.52 μM and 2.37 μM) which are in good agreement with the computational results. **2c** and **2g** also arrested 63 and 66% of cells in the G2/M phase, respectively, in comparison to control cells (10%) and tubulin polymerization inhibition assay revealed that **2c** and **2g** exhibited significant inhibition of tubulin polymerization with IC_{50} values of 2.31μM, and 2.62 μM, respectively in comparison to Nocodazole, a positive control, resulted in an IC_{50} value of 2.51 μM.

***Conclusion*:** Indole triazole hybrids were synthesized using click chemistry, and docking studies were carried out using Schrodinger for the designed molecules. Process Optimization has been done for both the schemes. Twelve compounds (**2a-2l**) have been successfully synthesized and analytical evaluation was performed using NMR and HR-MS. *In vitro* evaluation was for the synthesized molecules to check tubulin polymerization inhibition for antiproliferative action. Among the synthesized compounds, **2c** and **2g** have potent anticancer activities by inhibiting tubulin polymerization.

Keywords: Indole triazole conjugates, tubulin polymerization, antiproliferation, Click chemistry, molecular modeling studies, breast cancer.

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1. INTRODUCTION

Indole is an excellent pharmacophore moiety present in numerous natural products such as alkaloids, peptides, neurotransmitters, and various synthetic compounds. Owing to its biodynamic properties, Indole and its derivatives occupied a unique platform in nitrogen heterocyclic chemistry and are imperious heterocycles in drug discovery and development studies. Fischer indole synthesis, the oldest method used for the synthesis of indole and its derivatives [1, 2]. This scaffold contributes a crucial role in cell physiology, and regulation and acts as a probable intermediate for various bioreactions [3]. Indole and its derivatives correspond to scores of relevant modules of therapeutic agents in medicinal chemistry such

*Address correspondence to these authors at the Department of Pharmaceutical Technology and Process Chemistry, National Institute of Pharmaceutical Education and Research, Hyderabad, Telangana, India;
E-mails: vidyasrilekha16@gmail.com, swapnavad2003@googlemail.com

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Original Article

Development and Validation of Stability Indicating RP-HPLC Method for Quantitative Estimation of Enzalutamide in Enzalutamide Capsules Dosage Form

DSVN Sitamahalakshmi[1,*], P Bharath[1], D Ramachandran[1], DSVNM Ramamurty[2]

[1]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, INDIA.
[2]Department of Chemistry, Government Degree College (A), Tuni, Andhra Pradesh, INDIA.

ABSTRACT

Objectives: A precise, accurate and selective stability-indicating reverse phase high performance liquid chromatographic assay method has been developed for the quantitative estimation of Enzalutamide in Enzalutamide capsules dosage form. **Materials and Methods:** The separation was achieved by using a stationary phase Waters X-Bridge Shield RP18 (150 x 4.6 mm, 3.5µ) and the mobile phase consisted of perchloric acid buffer and acetonitrile in the proportion of (20:80 volume/volume). The run velocity was 1.2 mL/min. Enzalutamide was identified using UV detector at the wavelength of 210 nm. Column oven temperature 25°C and sample cooler temperature 25°C and infused quantity 20 µL, run time was 15 min. **Results:** As there is no meddling flanked by blank and placebo at the retention time of Enzalutamide. Degradation study results were shown significant degradation was observed in acid and oxidation (peroxide) stress condition. Hence it can be concluded that Enzalutamide is sensitive to acid and oxidation. To obtain system precision, a study was conducted with six replicate injections. %RSD was estimated from the peak areas of Enzalutamide establish to be 0.55% correspondingly. The relative standard deviation for method exactitude was establish to be 0.55%. The suggested HPLC technique was linear over the range of 100.6-301.8 µg/mL, the correlation coefficient was establish to be 0.9999. The accuracy studies were shown as % recovery for Enzalutamide 50% to150% level. The limit of % recovered revealed is in the assortment of 98 and 102% and the consequences obtained were establish to be within the limits. Hence the technique was establish to be accurate. The solution steadiness of the standard and samples are stable upto 48 hr on a bench top and refrigerator (2-8°C). The method is robust for changes like flow rate and column oven temperature. Performed the filter validation for sample solution 0.45 µm PVDF and 0.45 µm Nylon filterers are suitable for filtration. The method has validated as per ICH guidelines and all the validation parameters are satisfy the ICH Q2 specification acceptance limits. **Conclusion:** The developed method was validated for an assortment of parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability and robustness. The consequences obtained were within the acceptance criteria. So, it can be concluded that the urbanized technique is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Enzalutamide in bulk and pharmaceutical dosage forms.

Keywords: Enzalutamide, Liquid chromatography, Forced degradation, Validation.

Correspondence:
Dr. DSVN Sitamahalakshmi
Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur- 522510, Andhra Pradesh, INDIA.
Email id: dittakavirc@gmail.com

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INTRODUCTION

Enzalutamide a androgen receptor antagonist suitable for the treatment of adult men with metastatic castration resistant prostate cancer. It is 4-{3-[4-cyano-3- (trifluoromethyl) phenyl]-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamide. Enzalutamide is indicated for the treatment of adult men with metastatic castration-resistant prostate cancer who have received docetaxel therapy, compared with other anti-androgen, it shows reduced expression of androgen receptor dependent genes, decreased growth of prostate cancer cells, induction of cancer cell death and tumor regression. Molecular formula and molecular weight of Enzalutamide are $C_{21}H_{16}F_4N_4O_2S$ and 464.44 g/mol, respectively.[1-3] Enzalutamide is freely soluble in acetonitrile and absolute ethanol and practically insoluble in water.[4-13] The chemical structure of Enzalutamide was shown in Figure 1.

The fiction review discloses that there are no HPLC methods were statemented in major pharmacopoeias like USP, EP, JP and BP. Only a few methods were reported to date for the estimation of

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Evaluating the Effectiveness of Online Learning in Private College Education

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G. P. Raja, Aangothu Sreenivasa Rao, Vikas Prajapati, A. Jayapradha, Meenakshi Sharma

Abstract

As online learning has become more popular in private colleges, its usefulness has been debated. This study discusses private college online learning efficiency evaluation factors and methods. Online learning has grown in popularity, allowing students flexibility and accessibility while also bringing new difficulties and opportunities for private colleges. Its efficacy must be assessed across student results, engagement, institutional resources, and teaching techniques. Online learning evaluation requires measuring student results. Online versus face-to-face learning can be contrasted by course completion, grade, and retention rates. Assessing whether online learners obtain the same learning outcomes as traditional students is crucial. Student performance and learning gains studies illuminate online learning's efficacy. Another important factor is student engagement. Online learning is flexible but difficult to interest students. Online students' participation in discussions, peer collaboration, and course materials must be assessed. New digital technologies and learning analytics can analyze and improve engagement. Online learning should also be assessed for its impact on teaching. Effective online instruction requires faculty training and support. This examination examines how educators use technology, modify their teaching approaches, and support online students. Online learning in private colleges works beyond academics. Institutions must evaluate financial and resource impacts. Online program infrastructure, course development, and maintenance should be assessed for cost-effectiveness. Assessing online products' scalability and impact on private universities' finances is crucial. Consider students, professors, and administrators' perspectives. Surveys, interviews, and focus groups can reveal these groups' perspectives and satisfaction, revealing non-academic efficacy. The main aim of the study is to examine the effectiveness of online learning in private college education.

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Section

Articles

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Computational biology approaches in drug discovery against hepatitis-B

A. Venkateswararao[a], D.S.V.N.M. Ramamurty[b], Slesha Kumar Kosuru[c], Ramakrishna Gummadi[d*]

[a]School of Pharmaceutical Sciences and Technologies, JNTUK, Kakinada, AP, India

[b] Department of Chemistry, Government Degree College (A), Tuni

[c] Lydia College of Pharmacy, Ethakota, Ravulapalem, Andhra Pradesh, India

[d] Aditya Pharmacy College, Surampalem, Andhra Pradesh, India

* Corresponding author: Assistant Professor, Aditya Pharmacy College, Surampalem, AP, India. Email: g.ramakrishnampharm@gmail.com

Abstract

Hepatitis B virus (HBV) infects 250 million people worldwide, resulting in nearly one million deaths annually. HBV reactivation due to persistence of HBV genomic reservoirs is the major clinical limitation for currently available anti-HBV agents. There is a need, therefore, for understanding persistence mechanisms of HBV and to develop novel antiviral agents against HBV. Computational biology approaches like homology modeling, molecular dynamics and bioinformatics are being employed in recent years to understand the mutations of drug resistance and persistence in HBV and to study the DNA and RNA protein sequences of viruses. In the present review we discuss recent studies on discovery of anti-HBV agents using these computational approaches.

Original Article

Synthesis and Characterization of Some Novel N-Phenylpyrazole Analogues for the Evaluation of their Potentials as Anticancer, Antimicrobial and Antioxidant Agents

Vijai Varma Pothuri[1,2], Venkata Satya Machiraju Palagummi[3,*], Samba Siva Rao Vasa[4]

[1]Department of Chemistry, Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, INDIA.
[2]R&D Centre, Vasudha Pharma Chem Ltd, J N Pharma City, Visakhapatnam, Andhra Pradesh, INDIA.
[3]Department of Chemistry, Pragati Engineering College (Autonomous) Surampalem, Andhra Pradesh, INDIA.
[4]Department of Chemistry, Government Degree College, Tuni, Andhra Pradesh, INDIA.

ABSTRACT

Objectives: With a view to invent new anticancer agents, the authors proposed to prepare a series of N-phenylpyrazole derivatives by introducing biologically active pharmacophores viz., Fluoro/Fluoromehtyl benzamide and 2, 3-dihydrobenzo[b][1,4] dioxin at 3-, 5- positions of N-phenyl pyrazole motif respectively. **Methods:** The newly formed products are characterized by ^{1}H NMR, ^{13}C NMR, Mass and FTIR spectroscopic techniques and are subjected to screened for anticancer activity against human liver cancer cell line (Hep G2), antimicrobial and antioxidant activities. Further, Molecular docking study has also been applied on the newly synthesized compounds to study the binding efficiencies with protein BCL2 using GOLD docking software. **Results:** Among all the newly synthesized compounds, three compounds 8(d), 8(e), 8(h) exhibited higher potentials of anticancer activity compared to the rest of the compounds. All the newly synthesized compounds exhibited antimicrobial and antioxidant activities. Further study of molecular docking with protein BCL2 revealed that three title compounds 7, 8(f) and 8(h) exhibited very good binding efficiencies.

Key words: Anticancer, BCL2, Chalcone, Hep G2, Molecular docking, Pyrazole.

INTRODUCTION

In the present scenario, Cancer becoming a biggest threat at global level and is the second most leading cause for the deaths. Nearly 70% of mortality from cancer occurs in low income of nations. About 33.33% of cancer mortalities are due to the five major behavioural and dietary risks: i). Obesity ii). Less fruit consumption iii). Lack of physical exercise iv). Usage of tobacco and consumption of alcohol.[1]

Since last two decades, heterocyclic rings bearing pyrazole, especially N-Phenyl pyrazoles, attracts the scientists to invent a new type of anticancer agents in the area of drug discovery, medicinal and pharmaceutical chemistry. Recent literature also revealed that N-phenylpyrazoles were found with very good biological activities viz., antifungal, anti-bacterial and anticancer activities.[2] In addition, some of the pyrazole derivatives are also exhibiting versatile inhibitory activities including, c-Jun N-terminal kinase,[3] CGT1 inhibitors,[4] $BRAFV^{600E4}$ inhibitors,[5] CDKs inhibitors,[6] BACE1 inhibitors,[7] telomerase inhibitors,[8] xanthine oxidoreductase inhibitors,[9] COX-2 inhibitors[10] etc., Besides the synthetic pyrazoles, natural products bearing pyrazole motif are also of great value in our daily life.[11]

Keeping in view, the recent research work and the innovations in the discovery of

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Correspondence:
Dr. Venkata Satya Machiraju Palagummi
Department of Chemistry, Pragati Engineering College (Autonomous) Surampalem, Andhra Pradesh, INDIA.
Phone: +91 08852-252233
E-mail: drpvsm.res@gmail.com

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Original Article

Development And Validation Of Stability Indicating Rp-Hplc Method For The Quantification Of Amine Impurity In Tofacitinib Tablets Dosage Form

D.S.V.N. Sitamahalakshmi*[1], P. Bharath[1], D.Ramachandran[1] and D.S.V.N.M. Ramamurthy[2]

[1]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Andhra Pradesh, India.
[2]Department of Chemistry, Government Degree College, Tuni, Andhra Pradesh, India.

Corresponding author E-mail: dittakavirc@gmail.com
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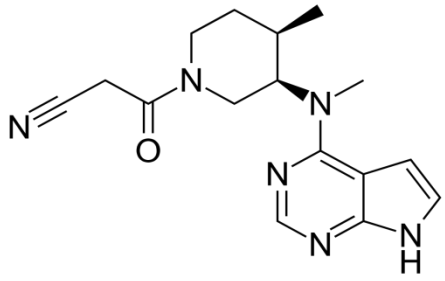
Abstract

Highly sensitive method for the determination of degradation impurity such as Methyl-[(3R,4R)-4-methyl-piperidin-3-yl]-(7H-pyrrolo[2,3-d]pyrimidin-4yl)-amine(Amine impurity) in Tofacitinib solid dosage form by using RP-HPLC method. Samples are analysed by reverse phase (RP-HPLC) using stationary phase Inert Clone ODS(3) (250 x 4.6mm, 5µm) column and the mobile phase-A consisted of pH 3.0 phosphate buffer and the mobile phase-B consisted of Acetonitrile in the proportion of gradient elution. The flow rate is 1.0 mL/min, the column oven was preserved at 40°C and sampler cooler oven was preserved 5°C, injection volume 25 µL and wavelength fixed at 210nm. The established HPLC method was validated with admiration to specificity, precision, linearity, accuracy, LOD, LOQ and solution stability. Validation study compared as stated by ICH instruction.

Key words: Tofacitinib, Amine impurity, Forced degradation, and liquid chromatography.

1.0 Introduction

Tofacitinib chemically known as 3-[(3R, 4R) - 4 -methyl-3-[methyl (7H-Pyrrolo [2, pyrimidine-4yl) amino] piperidin-1-yl]-3- oxopropanenitrile. It is an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis [1]. Cytokines work within a complex regulatory network in RA, signaling through different intracellular kinase pathways to modulate the recruitment, activation, and function of immune cells and other leukocytes [2-6]. Several research works elucidated the safety and efficacy of Tofacitinib drug [7-14]. The chemical structure of Tofacitinib [15-16] was shown in **Figure 1.**



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Stability Indicating RP-HPLC Method for Quantitative Estimation of Lenalidomide and Its Impurities in Solid Dosage Form

D.S.V.N. Sitamahalakshmi*[1], P. Bharath[1], D. Ramachandran[1] and D.S.V.N.M. Ramamurty[2]

[1]*Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.Andhra Pradesh, India.*

[2]*Department of Chemistry, Government Degree College (A), Tuni, Andhra Pradesh, India.*

Abstract: The main aim and objectives of the research are to develop an effective, sensitive, economical, and simple reverse-phase HPLC method developed for determining and quantifying Lenalidomide impurities in Lenalidomide solid dosage formulations. The lack of research work and no compendial methods available for estimating this drug influenced the current research investigation to give a simple, sensitive, rapid, precise, accurate and robust gradient high-performance liquid chromatographic method for the determination and quantification of Lenalidomide and its impurities. Samples are analyzed using reverse phase (RP-HPLC) using stationary phase an Inertsil ODS-3V (150 x 4.6 mm, 3µm), and the mobile phase consists of two channels A and B. channel-A: pH 3.0 phosphate buffer and Channel-B: Acetonitrile: water (90:10 v/v) in the proportion of gradient elution. The flow rate is 1.0 mL/min. The column temperature was maintained at 40°C, and the sample cooler temperature was maintained at 5°C, injection volume of 20 µL, and wavelength of 210 nm. The developed HPLC method was validated concerning specificity, and the chromatograms were recorded for blank, placebo, standard, sample, and spiked sample solutions of Lenalidomide and its related substances. Specificity studies reveal that the peaks are well separated from each other. For precision, the results were found to be within acceptable limits. The limit of detection (LOD) and limit of quantitation (LOQ) for impurity-A 0.1124 µg/mL and 0.0371µg/mL, Impurity-B 0.2247µg/mL and 0.0742µg/mL, respectively. The linearity results for Lenalidomide and all the impurities in the specified concentration range are satisfactory, with a correlation coefficient greater than 0.99. The accuracy studies were shown as % recovery for Lenalidomide and its impurities at the specification level; the results obtained were within limits. Solution stability parameter was established; standard, sample, and spiked sample solutions are stable up to 48 hrs on a bench top at the refrigerator. Filter validation parameters were established, and the filtered spiked sample solutions are compatible with both 0.45 µm PVDF & 0.45 µm Nylon filters.

Keywords: Lenalidomide, determination of related substances, Forced degradation, LOD and LOQ, liquid chromatography.

***Corresponding Author**

D.S.V.N. Sitamahalakshmi , Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.Andhra Pradesh, India.

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Synthesis and Biological Activity of Some Novel Derivatives of 4-[5-(2,3-Dihydrobenzo[*b*][1,4]dioxin-7-yl)isoxazole-3-yl]benzoic Acid

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Aims and scope

Submit manuscript

Vijai Varma Pothuri, P. V. S. Machiraju & V. Samba Siva Rao

Abstract

Some novel isoxazole derivatives of 4-[5-(2,3-dihydrobenzo[*b*][1,4]dioxin-7-yl)isoxazole-3-yl]benzoic acid bearing biologically active pharmacophores like benzodioxane and peptide bond have been synthesized, and their structures are supported by FTIR, ^{1}H and ^{13}C NMR, and mass spectra. The title compounds have been tested for antimicrobial and antioxidant activities. Molecular docking of the structures has also been carried out with protein *Sortase A*, according to which some compounds are characterized as potentially antimicrobial agents. Four products demonstrate strong antioxidant activity.

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applied sciences

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Article

Long Term Household Electricity Demand Forecasting Based on RNN-GBRT Model and a Novel Energy Theft Detection Method

Santanu Kumar Dash [1], Michele Roccotelli [2,*], Rasmi Ranjan Khansama [3], Maria Pia Fanti [2] and Agostino Marcello Mangini [2]

[1] TIFAC-CORE, Vellore Institute of Technology (VIT), Vellore 632014, India; santanu4129@gmail.com or santanukumar.dash@vit.ac.in
[2] Department of Electrical and Information Engineering, Politecnico di Bari, 70126 Bari, Italy; mariapia.fanti@poliba.it (M.P.F.); agostinomarcello.mangini@poliba.it (A.M.M.)
[3] Department of Computer Science, C. V. Raman Global University, Bhubaneswar 752054, India; rasmiranjankhansama@gmail.com
* Correspondence: michele.roccotelli@poliba.it

Abstract: The long-term electricity demand forecast of the consumer utilization is essential for the energy provider to analyze the future demand and for the accurate management of demand response. Forecasting the consumer electricity demand with efficient and accurate strategies will help the energy provider to optimally plan generation points, such as solar and wind, and produce energy accordingly to reduce the rate of depletion. Various demand forecasting models have been developed and implemented in the literature. However, an efficient and accurate forecasting model is required to study the daily consumption of the consumers from their historical data and forecast the necessary energy demand from the consumer's side. The proposed recurrent neural network gradient boosting regression tree (RNN-GBRT) forecasting technique allows one to reduce the demand for electricity by studying the daily usage pattern of consumers, which would significantly help to cope with the accurate evaluation. The efficiency of the proposed forecasting model is compared with various conventional models. In addition, by the utilization of power consumption data, power theft detection in the distribution line is monitored to avoid financial losses by the utility provider. This paper also deals with the consumer's energy analysis, useful in tracking the data consistency to detect any kind of abnormal and sudden change in the meter reading, thereby distinguishing the tampering of meters and power theft. Indeed, power theft is an important issue to be addressed particularly in developing and economically lagging countries, such as India. The results obtained by the proposed methodology have been analyzed and discussed to validate their efficacy.

Keywords: time series analysis; energy demand forecast; ARIMA; hybrid model; power theft

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1. Introduction

With the current increase in global warming, the focus of energy dependency has moved towards renewable energy sources (RESs), which seemingly have zero emission of greenhouse gases. As the percentage of carbon footprint rises with the use of traditional sources of energy, such as coal, the utilization of solar or hydro energy helps in reducing carbon footprints, providing a green energy alternative [1,2]. In order to ensure a pollution free ecosystem, we must move towards the utilization of RESs. Hence, a proper investment in RESs is essential [1–3].

RESs are integrated to the existing grid infrastructure to satisfy the energy demand of consumers, reducing the need for power from the main grid [1]. At the same time, storage devices are essential to optimize the use of renewable energy by storing energy when available and supplying it to consumers according to their requirement. Hence, for an optimal use of the energy from RESs, it is important to predict the energy demand of customers based on historical measurements. However, the evaluation of energy demand from the