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Design and validation of the Gingkolide estimation using RP-HPLC analytical tool

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ABSTRACT

Gingkolide is an antiseizure medicine used as an adjuvant of partial seizures and GAD to relieve neuropathic pain. It binds to the very high affinity alpha delta site in the CNS. Although the drug's mechanism remains unclear, in genetically engineered mice and other anticonvulsive models, findings showed that it binds to alpha receptors. A rapid rise in the number of drugs added to each class of drugs has been noted. Whether in a single or multi-drug delivery form, these medications are developed into newer formulations. These newest formulations put on the market need a new investigation to estimate the medication in the formulations. In the scientific literature, the current analytical procedures for such drugs are available, but not all approaches are stable and economical to use. Few other techniques are often time-consuming. The goal of this work was to develop an RP-HPLC analytical tool for Gingkolide estimation. The drug's RP-PLC study meets the drug's optimum integrity, suitability, regeneration. The drug's LOQ and LOD were reached with elevated sensitivity. Overall, the results show that the recommended analytical approach in the formulation should be used to evaluate the drug. For regular study of the medication in its dosage form, this approach may be recommended.

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INTRODUCTION

Gingkolide is an antiseizure medicine used as an adjuvant of partial seizures and GAD to relieve neuropathic pain. It binds to the very high affinity alpha delta site in the CNS [1, 2]. While the drug's function is unclear, findings showed that in genetically engineered mice and other anticonvulsive models, it binds to alpha receptors [3]. The traditional medicine used to treat neuropathic pain was designed as a possible successor [4]. A rapid growth in the number of medications added to each drug class has been observed. In either a single or multi-drug delivery type, some drugs are formulated into newer formulations.

These newest formulations put on the market need a new investigation to estimate the medication in the formulations. In the scientific literature, the current analytical procedures for such drugs are available, but not all approaches are stable and economical to use. Few other techniques are often time-consuming. HPLC, LC-MS [5–9] has been used to estimate various medications. Stability-indicating and time-consuming computational approaches were not documented [10]. The goal of the present work was to design an analytical

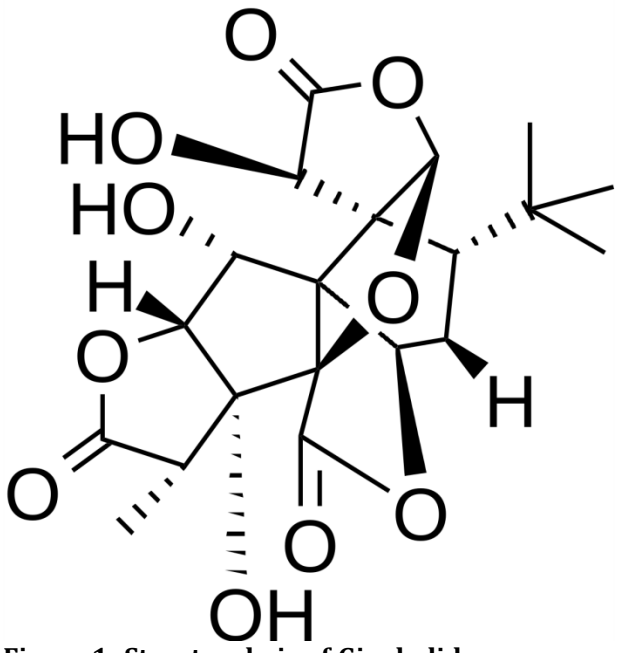

Figure 1: Structural pic of Gingkolide

method for estimating Gingkolide in bulk and tablet formulation using RP-HPLC.

MATERIALS AND METHODS

Instruments used

For the research, the Pharmaspec-1700 UV-VISIBLE Spectrophotometer developed by SHIMADZU was used. The program used for peak analysis is U.V. The 2.0.0. HPLC: LC-2010CHT, SHIMADZU with autosampler attached. A PDA-SPD-M20A-Prominence-Diode Array (PDA) detector was used as the detector. A Phenomenex Kromasil column with 5μ 100 RC18 (250 mm x 4.6 mm, i.e. 5 microns) dimensions was the column used. LC-solution is tools used to analyze peaks.

Mobile Phase used

Acetonitrile and buffer amounts are combined evenly and balanced using a software-operated pump. The degassing is finished, and HPLC grade solvents are used. To store solvent schemes, different solvents were used [2].

Standard & sample solution

Gingkolide was dissolved in 5ml of the diluent solution with 1mg of specifically measured pure medication and ultrasonicated for around 30mins. 10mg of A-Gingkolide impurity was measured in another volumetric flask and dissolved in 10ml of diluent. 0.5ml was pipetted out of this formulation and branded as a working solution. Diluents were developed for the final volumes and used for further study [11].

In 60ml of methanol, an appropriately weighed volume of oral suspension powder equal to 10mg of the substance was dissolved. In order to ensure adequate dissolution, this mixture was sonicated for 30min. The filtration of the solvent was carried out using a paper filter. The marc was washed with methanol and methanol is more diluted before it hits the level. A 100ml volumetric flask was moved with 10ml of solution (Stock). With methanol, the final volume was changed to 10μg/ml and used for further testing.

Conditional parameters

The rate of flow was set at 1ml/min. At 30°C, the temperature was preserved. The volume and runtime of the injection were held at 10 L and 10 minutes. At 262nm the detection was completed. In the HPLC and U.V., the normal stock solution consisting of pure Gingkolide was then injected. With the assistance of tools, L.C., the wavelength from 200 to 400 nm was calculated. Solution Solution

Linearity of the estimation

The calibration curve was plotted with a Gingkolide concentration range of 25 percent -125 percent. The regular Gingkolide medication was used to achieve these concentrations. These solutions have been vialized and sampled automatically. By preserving the chromatographic conditions, 20μl samples of solutions were inserted into the autosampler. By plotting the peak area against the drug concentration, the calibration curves were obtained. [12, 13] The slope regression constant was calculated.

Accuracy of the estimation

By following the traditional addition process, the opioid recovery was measured to assess the accuracy and recovery. Samples of known drug amounts have been combined with previously predicted drug samples. The Gingkolide quantity was calculated by considering the values of the calibration curve regression equations. For 6 replications, the results were replicated.

Precision of the procedure

The sample injection was repeatedly injected into the instrument 6 times. The data was viewed as the number of deviations that should be at the < 2 percent limit. The intra-day and inter-day accuracy was tested at 3 separate medication concentrations. The accuracy of the intra-day was determined by analysis of samples three times in one day, and the inter-day was calculated by analysis of samples three times in three days. The findings were reported as a percentage variance.

Robustness of the procedure

By making small improvements in the mobile phase

ratios and injection volumes, robustness was calculated. In order to find robustness, the column temperature and flow speeds were also altered to a limited degree. At three concentrations, it was measured and the findings were expressed in percentage variance. The identification limits (L.O.D) and the quantification limits (L.O.Q) of this system were visually calculated on the basis of trial and error.

Selectivity of the procedure

A resolution factor that corresponds to the drug peak inside the closest resolving peaks and also to all the other available peaks analysed the accuracy of this established process. With the aid of a PDA detector, the peak purity data confirms the selectivity. A placebo was prepared with oral suspension powder without Gingkolide to test the method selectivity, and the same is contrasted with the prepared Gingkolide standard to study the method selectivity. Normal and placebo chromatograms were planned. The distinctions were made between Rt, Purity, and Resolution factor of retention time.

Suitability of the procedure

The E.P. has recorded resolution (Rs), power factor (K '), asymmetry factor (As), retention time-(RT), theoretical plates (Tp), and tailing factor-(Tf). By using L.C. About apps. With 6 injections of the popular standard solution, HPLC was calibrated with the initial mobile stage. Six tests were tested using the standard methods to assess the suitability of the device for the study and were tested.

Stability of the procedure

In the process, the consistency of the drug solution was checked by storing the solution for 24 hours in a tightly sealed bottle. At daily intervals of 6, 18 and 24hrs, samples from this stock solution were analyzed. The findings obtained from this test were compared with solutions that were freshly prepared.

RESULTS & DISCUSSION

For accuracy, precision, linearity, LOD and LOQ, all the findings of the studies were recorded in the RP-HPLC of the drug. The above parameters were calculated using methanol as a diluent at 7 different concentrations and the prepared concentrations ranged from 25-125%. The findings revealed a perfect relationship between the peak area and the analysed sample concentration. The calibration curve was calculated, and using the detector, the AUC was estimated. The graph of the liner was plotted with r2 value of 1, giving the highest linearity. The outcomes have been demonstrated in Table 1. At many concentrations, the substance was extracted, showing the precision of this procedure. The pre-measured volume of the drug is taken and mixed with the solution which has already been analysed. Over 3 repetitive days, the same procedure was replicated and the accuracy of the inter-day validation was calculated. The corresponding percentage of the variance of this process, along with a strong % of the recovery, was calculated to be <2%.

Table 1: Linearity results of the procedure

S. No	Conc (μg/ml)	Formulation Conc (μg/ml)	Recover %	Peak
1	50	51.03	101.59	2395232
2	100	100.78	100.43	4762648
3	150	149.52	99.04	5978943

Table 2: Precision analysis result

Conc μg/ml	Peak area	% RSD
50	4791765	0.15

The accuracy of the measurement was calculated by repeated 6-fold injections of the drug solution. Table 3 provided the findings and expressed them as a % deviation that did not reach 2 %. The consistency of inter-day and intra-day differences at various concentrations was analysed and reported in Table 3. The deviation was not important, which confirms the intra-day and inter-day specificity of the system of study proposed. Measuring the suitability of the system is an integral part of the development of the HPLC approach for checking the suitability of the system for analysis. The observations were in recognition of the CDERR guidelines.

Table 3: Suitability study result

S.No.	Item	Estimate
1	Theoretical Plates	3038.921
2	LOD μg/ml	0.21876
3	LOQ μg/ml	0.65269
4	Tailing	2.32

When tested for the stability analysis, the portion of the variance of the drug solution was found to be below limits. The knowledge collected in the study was tabulated. This study showed that the drug solutions were safe, and the findings showed that the sample reacted well, and less than 2 % of the data was below limits. By changing certain parameters such as mobile phase, flow rate, column and sample volume injection, the robustness of the

experiment was determined. The method was carried out at separate 0.6, 0.8 and 1.0μg/ml concentrations.

CONCLUSION

Gingkolide, the drug, was calculated using the analytical procedure suggested in the analysis. The system has been researched for its simplicity, consistency, precision and specificity. For routine assessment of the drug in the dosage shape, the proposed analytical technique is feasible. Because of the lack of advanced instruments such as LC-MS, the simplicity of the study was stressed. The emphasis was placed on the limited study time and the method's simplicity. The drug's RP-HPLC study meets the drug's optimum integrity, suitability, regeneration. The drug's LOQ and LOD were reached with elevated sensitivity. The details indicate the method's accuracy and the method's precision. Overall, the results show that the recommended analytical approach in the formulation should be used to evaluate the drug. For regular study of the medication in its dosage form, this approach may be recommended.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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