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RP-HPLC Method Designed for Determining Charantin in its Capsule Dosage Form

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ABSTRACT

Charantin, a steroidal saponin found in the plant *Momordica charantia* and functions like insulin. It is combined with natural insulin, increasing insulin release and inhibiting gluconeogenesis. Charantin improves blood glucose levels by enhancing glucose absorption and glycogen synthesis in the liver, muscles, and fat cells, according to a large body of research. The great majority of the time, it is taken in the form of a capsule. Because of the increased availability of these newly released formulations, a new way of calculating the amount of medications included in the formulations is now required. The current work's RP-HPLC technique is aimed to estimate Charantin as well as confirm that Charantin is being calculated. The proposed RP-HPLC technology for assessing Charantin in capsule dosage form was found to be precise, specific, accurate, quick, and cost-effective. Label Claims (also known as labelling claims) are representations of the actual product attributes as stated on the product label. The sample recoveries from all formulations were consistent with their respective Label Claims, and this method is suitable for routine analysis. It is suitable for normal laboratory analysis and may be used to control the quality of raw materials, formulations, dissolution tests, and bioequivalence studies for the same formulation.

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INTRODUCTION

Charantin is a steroidal saponin derived from *Momordica charantia* that shows insulin-like action by boosting insulin release and inhibiting gluconeogenesis [1]. A strong basic chemical like Charantin is almost insoluble (in water) and, as a result, it is nearly impossible to dissolve in dilute acids or bases (based on its pKa). It is a whitish crystalline material, which is neutral and tasteless, melting at around 266–268 °C. As far as the water-soluble compounds are concerned, it is soluble in highly polar solvents like hexane, but is insoluble in apolar solvents like ether, ethanol, and methanol [2]. Naftidrofuryl is the IUPAC Name IUPAC name 2-(diethylamino)ethyl 3-(naphthalen-1-yl)-2-oxopropanoate. Charantin has been shown to enhance blood sugar levels by enhancing glucose uptake and glycogen synthesis in the liver, muscles, and fat cells [Figure 1]. Additionally, it improves insulin secretion from pancreatic beta cells, and aids or helps in the creation of new beta cells that secrete insulin. K-3's alcoholic extract proved to be more efficient in reducing the frequency of attacks of diabetes than did tolbutamide, which was also utilized on occasion in treating diabetes. In most cases, it is used in capsule form. HPLC

has been employed to determine various drugs. These newly released formulations necessitate a fresh analysis to estimate the medicine in the formulations. The current analysis methodologies for such medications are accessible in the scientific literature, however not all techniques are stable and cost-effective. Other procedures are frequently time-consuming. HPLC has been employed to determine various drugs [3]. There was no documentation of stability-indicating and time-consuming computational procedures [4]. In the current research, RPHPLC method was developed to estimate the Charantin and validating the procedure.

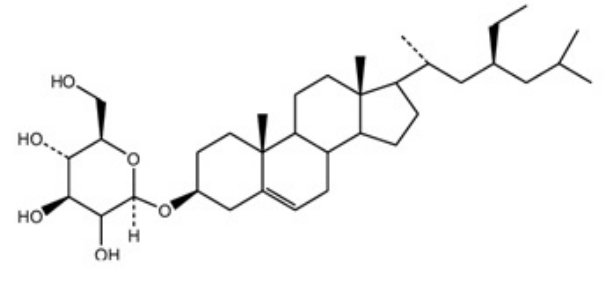

Figure 1: Structure of Charantin

MATERIALS AND METHODS

Instruments used

For the research, the Shimadzu LC-2010, CHT autosampler coupled with A PDA-SPD-M20A-Prominence-Diode Array (PDA) detector was used. A PhenomenexKromasil column with 5μ 100 RC18 (250 mm x 4.6 mm, i.e. 5 microns) dimensions was paired to the HPLC system. UV-Visible Spectrophotometer T60 developed by SHIMADZU was used. LC-solution is tools used to analyze peaks.

Mobile Phase used

A standard Charantin medication and pills were purchased from a retail vendor in Bengaluru. Using a software-controlled pump, acetonitrile, methanol, and buffer solutions are blended uniformly and balanced. The degassing process is complete, and HPLC grade solvents are employed. Various solvents were used to store solvent schemes [5].

Methodology

Optimization

After performing the trials in triplicates the optimized parameters were developed and proceeded for further experiments maintaining the same conditions.

Assay

25mg of Charantin working standard was weighed and transferred to a 25ml clean, dry volumetric flask, 5ml of diluent-1 was added, sonicated to dissolve at room temperature, and diluted to volume with diluent-2 was added and mixed. 5ml of this solution was diluted to 25ml with diluent -3 and mixed [6]. The solution was diluted using a membrane filter (milliposepvf 0.45m/ nylon 0.45m or Whatmann GF/C 1.2 m or appropriate). Along with the capsules shells, two capsules were weighed and placed to a 500ml clean and dry volumetric flask, together with roughly 50 ml of diluents-3, and sonicated for about 15 minutes at room temperature with occasional shaking to thoroughly scatter the capsule. Add about 300 ml of diluent-1 and sonicate for approximately 20 minutes at room temperature with occasional shaking, then dilute with diluent -3 up to 1cm below the mark, leave to cool at room temperature, and mix with diluent -3. Centrifuge the solutions for 5 minutes at 10,000 RPM. 5ml of this solution should be diluted to 50ml with dilutent -3 and mixed. Using a 0.45 m membrane filter, filter the solution.20 L of the aforesaid solutions were injected into the HPLC system, and the area of the peaks was measured, as well as the percentage of drug, according to the standard formula.

$$\% \; Assay = \frac{(AT \times WS \times DT \times P \times Wt)}{AS \times DS \times WT \times LC} \times 100$$

System Suitability

Three injections of standard preparation (50 mg/mL for Charantin) were used to test system appropriateness criteria such as resolution, tailing factor, peak purity, and theoretical plates [7] estimated the percent RSD of three injections. The initial mobile stage was used to calibrate the HPLC. Six tests were performed using conventional procedures to determine the device's appropriateness for the research [8].

Linearity of the estimation

Weigh and transfer around 25mg of Charantin working standards into a 25ml clean dry volumetric flask, then add about 5ml of diluents-1 and sonicate to dissolve at room temperature before topping up with diluent -2 and mixing. 1ml of each stock solution was pipetted into a 10ml volumetric flask and the full volume was made up with diluent. The peak regions were measured after injecting 50g/ml, 100g/ml, 150g/ml, 200g/ml, 250g/ml, and 300g/ml into the chromatographic apparatus [9]. The slope regression constant was calculated.

Accuracy of the estimation

Recovery tests were used to determine the method's accuracy. The drug's reference standards were added to the formulation at 50 percent, 100 percent, and 150 percent concentrations. The recovery studies were completed and the percentage recov-

ery and percentage relative standard deviation were computed [10].

Precision of the procedure

Weigh and transfer around 25mg of Charantin working standards into a 25ml clean dry volumetric flask, then add about 5ml of diluent-1 and sonicate to dissolve at room temperature before topping up with diluent -2 and mixing. 1ml of each stock solution was pipetted into a 10ml volumetric flask and the full volume was made up with diluent. The solution was injected three times, and the area of each injection was measured in HPLC [11]. The region of six duplicate injections' percent RSD was determined to be within the prescribed limits.

Robustness of the procedure

Robustness was measured by making modest changes to the flow rates, temperature, and wavelength. To determine robustness, the column temperature and flow rates were both changed to a limited extent. It was tested at three different concentrations, and the results were represented as a percentage of the total [12]. This system's identification limits and quantification limits were visually estimated by trial and error.

RESULTS & DISCUSSION

The purpose of optimizing chromatographic conditions was to take into account the many technique development goals, to appropriately weigh each goal (theoretical plates, runtime, sensitivity, peak symmetry, etc.) and to pick an acceptable technique for the estimation of Charantin in Capsules dosage form. The RP-HPLC method's sensitivity is determined by the wavelength used. 249 nm was used as the detecting wavelength. Charantin had a retention time of 2.37 minutes as measured by the chromatogram. Trial 1 has a rounded summit. The chromatogram revealed a retention period of 9.12min for Charantin. In trial 2, the wide peak failed to meet the system appropriateness requirements. The chromatogram showed that Charantin has a retention period of 3.54min. The peak is sharp, and the tailing was determined to be 1.25 within acceptable limits. The theoretical plates had a limit of 7864, which was greater than the limit of the final optimised experiment [Table 1]. In trial 3, the produced peak meets all system suitability requirements [Table 2].

The chromatogram for Charantin's blank, standard, and sample [Figure 2]. The assay limits for Charantin were 90-110 percent, whereas the findings for Charantin were 100.52 percent. As a consequence, the findings were within the parameters.

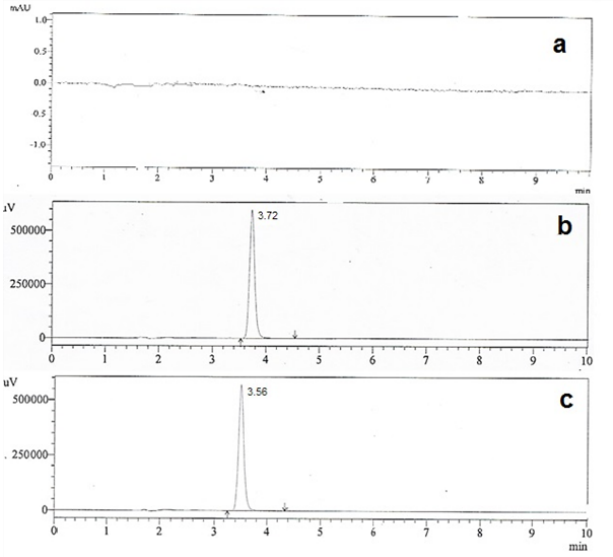

Figure 2: Assay of Chromatogram; A. Blank; B. Standard; C.Sample

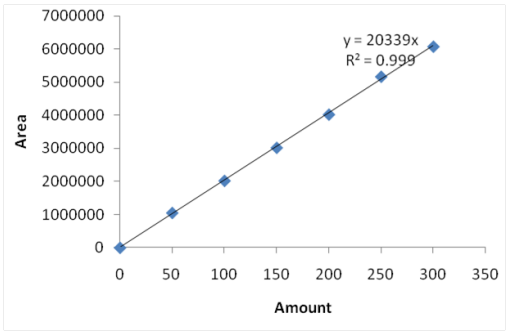

Figure 3: Calibration curve graph for Charantin

The accuracy studies were presented as percent recovery for Charantin at 50 percent, 100 percent, and 150 percent at limitations of percent recovered should be in the range of 98=102 percent the findings obtained for Charantin were determined to be within limitations. As a result, the approach was discovered to be accurate [Table 3].

The accuracy examinations revealed that the percent recovery of the Charantin is 100.36 percent. The limits of percent recovery of medicines were 98-102 percent, indicating that the procedure was accurate and that the widely used excipients found in pharmaceutical formulations did not interfere in the suggested process. Charantin's linearity range was determined to be 150-600 (g/ml). The calibration curve was drawn, and the drug's correlation coefficient was determined to be 0.999 [Figure 3].

As a consequence, the findings obtained were within the parameters. The chromatograms of linearity were recorded. The detection and quantification limits are 0.507 g/ml and 1.536 g/ml, respectively. The robustness of the experiment was determined

Table 1: Optimized chromatographic conditions

Item	Condition
Mobile Phase	MeoH:ACN: H_2O (40:40:20)
Flow rate (ml/min)	1.0ml/min
Volume of injection loop (ml)	20 (ml)
Detection wavelength (nm)	249nm
Column temperature (°C)	40°C

Table 2: Results for system suitability and Precision

Drug	RT	Area	Plate Count	USP-Tailing
System suitability	3.7±0.021	4013351±39899.57	6328±17.45	0.99±0.021
% RSD	0.583	0.99	0.275	2.182
Precision	-	4028770±30833.88	-	-
% RSD	-	0.765	-	-

Table 3: Results of Accuracy (recovery of Charantin)

Concentration	Peak Response	Average Peak area	Amount Added	Amount Found	Mean Recovery
50%	5613784	5616405	300	305.11	
100%	9928623	9924074	400	397.13	100.36%
150%	15384336	15340588	500	504.07	

Table 4: Observation of show Temperature variations

Robustness		Retention Time	Area	USP Tailing	Plate count
			Flow rate		
1	0.8 ml/min	3.62	3796285	1.02	6321
2	1.0 ml/min	3.65	4306191	0.98	6388
3	1.2 ml/min	3.51	3041944	0.98	6311
			Wave Length		
1	244nm	3.33	5555265	1.02	6322
2	249nm	3.67	4306196	0.98	6389
3	254nm	3.32	5561285	0.97	6313
			Temperature		
1	380c	3.35	5555262	1.00	6320
2	400c	3.69	4306194	0.99	6387
3	420c	3.31	5561286	0.97	6313

by adjusting specific parameters such as mobile phase, flow rate, column, and sample volume injection. The procedure was carried out at doses of 0.8, 1.0, and 1.2 g/ml [Table 4].

CONCLUSION

The suggested RP-HPLC technique for estimating Charantin in capsule dose form was found to be exact, specific, accurate, fast, and cost-effective. The sample recoveries in all formulations were consistent with their individual Label Claims, and this approach is suitable for routine analysis. It is ideal for normal laboratory analysis and may be used for quality control of raw materials, formulations, dissolution studies, and bioequivalence investigations for the same formulation.

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Conflict of Interest

The authors declare no Conflict of interest, financial or otherwise.

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