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Quantitative estimation of negundoside in *Nirgundi taila* by HPLC

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ABSTRACT

By optimizing the extraction, separation and analytical conditions, a reliable, rapid, simple and accurate liquid chromatography method with UV detection was developed for the quantitative determination of negundoside in an ayurvedic preparation Nirgundi taila. The estimation of negundoside was achieved on an reversed phase C_{18} column (250 mm X 4.6 mm ID, 5 µm particle size), with isocratic elution using a mixture of acetonitrile–methanol (70: 30, v/v) at a flow rate of 1 mL min^{-1} with UV detection at 254 nm, the sample injection volume was 10µL. The calibration curve was linear with correlation coefficients of 0.9996 for negundoside. The % Relative standard deviation (%RSD) value was less than 2% in the concentration range of 10–100 µg mL^{-1}. Intra-day assay and inter-day assay precision of the analyte was less than 2%, and the average recovery rate obtained was in the range of 98–102% with %RSD below 2%. This method can provide a scientific and technical platform to the product manufacturers for setting up a quality control standard as well as to the public for quality and safety assurance of the proprietary ayurvedic formulations.

Key words: Column liquid chromatography, UV detection, Negundoside, Nirgundi Taila,, Ayurvedic formulation

INTRODUCTION

The quality control of herbal crude drug and their bio-constituents is of paramount importance in justifying their acceptability in modern system of medicine. One of the major problems faced by users in industry is non availability of rigid quality control profiles for herbal raw materials and their formulation. With the advent of new analytical techniques and sophisticated instrumental technology, it is possible to suggest a practicable quality assurance profile for a crude drug or its bioactive constituents[1].

According to American Herbal Product Association, "Standardization refers to the body of information and controls necessary to produce material of reasonable consistency. This is achieved through minimizing inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes[2]." Directives on the analytical control of a vegitable drug must take account of the fact that the material to be examined has complex and inconsistent composition. Therefore the analytical limits can not be so precise as for the pure chemical compound[3].

One of the best methods of standardizing herbs and herbal formulations based on the modern scientific tools is chromatography[4]. It not only helps in establishing the correct botanical identity but also helps in regulating the chemical sanctity of the herbs. One such technique is marker compound testing and fingerprint analysis. Different chromatographic methods are used to analyze the marker compounds in herbs with the help of modern sophisticated tools. HPTLC is most frequently used where only finger printing of the herbs is required without quantifying the compound though the same

can also be quantified with the help of a densitometer[5].

EXPERIMENTAL

Chemicals and Reagents

The standard substance of negundoside as a marker was purchased from Regional Research Laboratories, Jammu, India. The proprietary ayurvedic medicine of reputed manufacturer was purchased from a local drug store. Acetonitrile, methanol was of LC grade (Rankem, Delhi, India). All the solvents were filtered through a 0.2 µm Nylon 66 membrane filter prior to use.

Instrumentation

The chromatographic system (Analytical, India) consisted of a solvent delivery module (ALC), a manual injector 2010 fitted with a 10 µL fixed loop and an ASPD UV–visible detector. The separation was performed on a Grace Smart C_{18} column (particle size 5 µm; 250 mm X 4.6 mm ID) at an ambient temperature. Chromatographic data were recorded and processed using Analchrom software.

Analytical Conditions

Analysis was isocratic at 1.0 mL min^{-1} flow rate with acetonitrile–methanol (70:30, v/v) as mobile phase[6]. The mobile phase was prepared freshly every day. The mobile phase was filtered through a 0.2 µm membrane filter to remove any particulate matter, mixed and degassed by sonication before use and found to be stable with no precipitation with time or decrease in temperature. The absorbance of negundoside was good at 254 nm and further it was free from any interference. Hence, the eluted peak was detected at 254 nm. Prior to injecting solutions, the column was equilibrated for at least 60 min with the mobile phase flowing through the system. Each solution was injected in triplicate, and the relative standard deviation (RSD) was required to remain below 1.0% on peak area basis.

Standard Solution Preparation

The standard solution was prepared by accurately weighing 5 mg of negundoside standard dissolving in 5 mL of acetonitrile obtaining the stock concentrations of 1000 µg mL^{-1}. Different aliquot were prepared to obtain solutions in the range of 10–100 µg mL^{-1} in acetonitrile. The stock solutions were refrigerated and were found to be stable for 2 weeks.

Preparation of Sample

About 10 mL of the formulations was extracted in 15 mL of butanol by sonication for 10 min at room temperature which was then fractionated with 10 mL acetonitrile thrice to dissolve the desired component. Thus the sample solution of strength of 100 µg mL^{-1} was obtained. Further dilutions were made in acetonitrile to give the solution of strength 20, 40 and 80 µg mL^{-1}.

Optimization of Chromatographic Conditions

Chromatographic separations are significantly affected by the mobile phase conditions, such as the type and composition of the organic modifiers. Therefore, before selecting the conditions for the optimization, a number of preliminary trials were conducted with different combinations of various organic solvents and buffers at various pH values, mobile phase compositions, and flow rate to determine the retention time, shape, resolution, and other system suitability parameters of all the peaks.

Validation

In order to verify that the proposed method is applicable to formulation analysis, validation was performed as per the ICH Guidelines Q2B (7).

Calibration Curve

Seven different concentrations of negundoside were analyzed and their calibration curve was constructed in the specified concentration range (10–100 µg mL^{-1}). The calibration plots were generated by replicate analysis (n = 3) at all concentration levels and the linear relationship was evaluated using the least square method (8) within Microsoft Excel Program.

Repeatability, Precision and Stability

The injection repeatability was determined by the analysis of six consecutive injections using the same sample, while the analysis repeatability was examined by the injection of six different samples prepared by the same procedure. The prepared standard solution (40 µg mL^{-1}) was used for the test of injection repeatability and analysis

repeatability. The instrument precision was examined by performing the intra-day and inter-day assays of six replicate injections of the mixture of standard solutions at three concentration levels (20, 40 and 80 μg mL^{-1}). The intra-day assay precision was performed with the interval of 4 h in 1 day, while the inter-day assay precision was performed over 14 days.

Limit of Detection and Limit of Quantification

LOD and LOQ were determined by k.SD/s where k is a constant (3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of the concentration versus response graph[8].

Specificity

Specificity is the ability of the analytical method to measure analyte response in the presence of interferences present in the sample matrix. It was checked by detecting the analyte of interest in synthetic laboratory formulation. The resolution of the intented peak was determined. Furthermore, the proposed method was also applied to other proprietary formulation.

Accuracy

The result of three concentrations from standard dilutions (20, 40 and 80 μg mL^{-1}) was used for determining the recovery of negundoside. The amount of the standard present in the solutions was estimated by measuring the peak areas and fitting these values to the straight-line equation of the calibration curve.

RESULTS AND DISCUSSION

Validation

Calibration Curve (Linearity)

The calibration curves (n = 3) constructed for the marker was linear over the concentration range of 10–100 μg mL^{-1} for negundoside marker. Peak areas of the marker was plotted versus the concentration and linear regression analysis performed on the resultant curve. The regression equation was found to be y = 16.013x + 7.8206 and the coefficient of correlation was found to be 0.9996 with %RSD values were less than 1.5% across the concentration range studied.

Table 1: Intra-day and inter-day accuracy and precision data of *Nigundoside marker*

Spiked	Intra-day			Inter-day		
Conc. (μg mL^{-1})	Found[a] (μg mL^{-1})	Precision %CV	Accuracy[b] %Bias	Found[a] (μg mL^{-1})	Precision %CV	Accuracy %Bias
20	19.824	0.094	-0.88	19.63	1.447	-1.83
40	40.149	0.426	+0.37	40.212	0.563	+0.53
80	81.006	0.668	+1.26	80.184	0.367	+0.23

a – Mean value of triplicate reading b - Bias % = [(found-spiked)/spiked] x 100

Table 2: Assay data of *Nirgundi Taila*

Quantity Claimed (μg. mL^{-1})	Quantity Found (μg. mL^{-1})	% Quantity Found (± SD)
20	19.46	97.30 ± 0.029
40	40.18	100.44 ± 0.083
80	80.63	100.79 ± 0.095

* Values are mean ± SD, n=3 SD = Standard Deviation

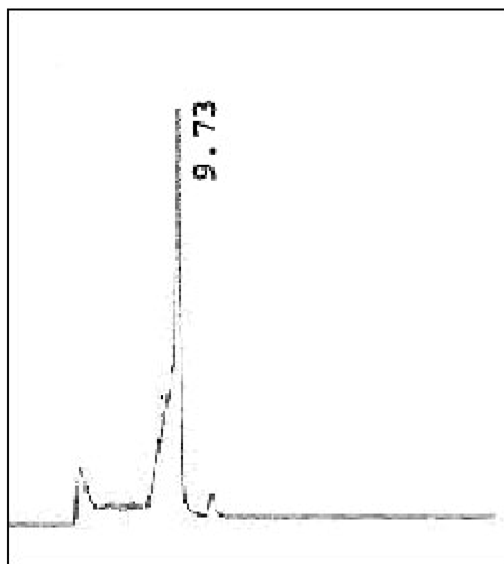

Fig 1: Chromatogram of Negundoside (Nirgundi Marker)

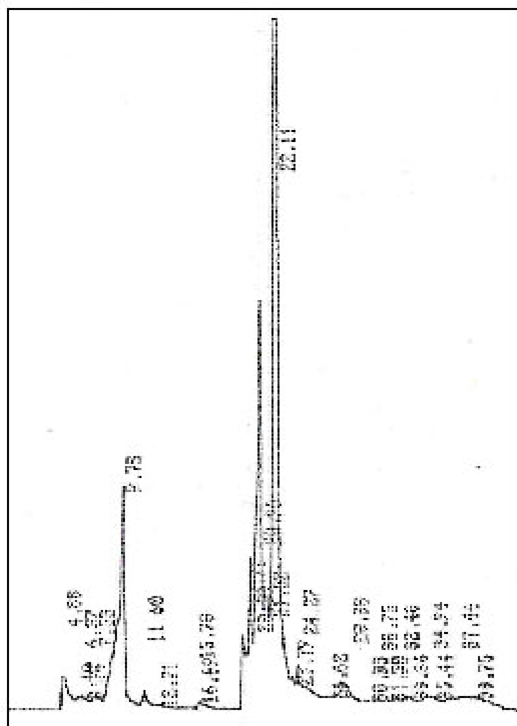

Fig. 2: Chromatogram of Negundoside in Ayurvedic preparation (Nirgundi Taila)

Repeatability, Precision and Stability

Injection repeatability

The calculated %RSD of the peak areas for negundoside was less than 2% at each of the three concentration levels.

Analysis repeatability

The RSD value for analysis repeatability was less than 2.0% both for retention time and peak area.

Limit of Detection and Limit of Quantification

The LOD and LOQ were found to be 29.44 and 98 ng mL^{-1}, respectively for negundoside.

Specificity

Satisfactory results were obtained, indicating the high specificity of the proposed method for the determination of marker in formulations containing the markers. Good resolution and absence of interferences between the drugs determined is shown in Fig 1.

Accuracy

As shown in Table I, the recovery of the investigated component ranged from 99.12 to 101.26%, and their %RSD values were all less than 2%. It was known from recovery tests that the developed method manifested the reliability and accuracy for the measurement of these components.

Applicability of the Developed Method in Formulations

The developed LC method was applied to the determination of negundoside in the Ayurvedic formulations. The percent found was ranging from 97.3 to 100.79 %. The result is presented in Table II and chromatogram was observed as shown in fig. 2.

CONCLUSION

Method validation data indicates that the present method is a reliable, reproducible and accurate LC method for the determination of the negundoside in the proprietary ayurvedic medicines when using the optimized extraction and separation conditions. Thus, the rationale and judicious use of modern scientific methods pertain to the development of ayurveda.

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