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Novel Validated Ultra Violet Spectroscopic Methods for the Determination Of Nordihydroguaiaretic Acid in Dietary Supplements.

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Research Article

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Nordihydroguaiaretic acid, UV determination, Method validation.

ABSTRACT

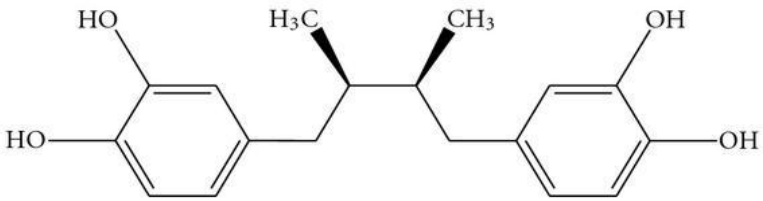
A simple, rapid, accurate and precise, Spectrophotometric method was developed for determination of Nordihydroguaiaretic acid. In this method Nordihydroguaiaretic acid shows absorbance maximum at different wavelength in different solvents, so absorbance was measured and the same wavelengths used for the estimation of Nordihydroguaiaretic acid in respective solvents. Nordihydroguaiaretic acid obeys the beer lamberts' law. Method is validated according to ICH guideline and carried out for analysis of Nordihydroguaiaretic acid.

INTRODUCTION

Nordihydroguaiaretic acid (NDGA) is a phenolic antioxidant found in the leaves and twigs of the evergreen desert shrub, *Larrea tridentata* ,Coville (creosote bush) family Zygophyllaceae. It has a long history of traditional medicinal use by the Native Americans and Mexicans. Creosote bush dominates areas of the desert southwest in the USA and Northern Mexico, as well as some areas of Argentina. It is also known as chaparral and greasewood in the USA and gobernadora (governess) and hediondilla (little smelly one) in Mexico [1-8].

Larrea tridentata has been introduced as a dietary supplement, mainly due to its antioxidant activity. Nordihydroguaiaretic acid (NDGA) which possesses antioxidant and inhibitory activity on inflammation mediators. Also, NDGA was used as an antioxidant food preservative for fats and butter. NDGA is a recognized inhibitor of lipoxygenase (LOX) and has antioxidant and free radical scavenging properties[9-12].

Figure 1: Structure of NDGA



4, 4'-(2, 3-dimethylbutane-1, 4-diyl) dibenzene-1, 2-diol

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MATERIALS AND METHODS

A double beam UV spectrophotometer (ELICO SL 210) with a pair of matched quartz cell of 1cm width was used for measuring absorbance. Elder digital balance was used for weighing and ultra sonicator used for sonicating the drug and sample solution. NDGA was purchaged from Sigma Aldrich, Banglore used for analysis. All other chemicals used for method development are analytical grade.

TLC analysis of NDGA

Standard solution of NDGA was prepared by dissolving 20 mg of drug in 10 ml. of methanol. A mixture of methanol, ethyl acetate (7:3) was used as mobile phase. 10×15cm Pre coated silica gel TLC plate used as stationary phase.1µl of standard solution was applied along a line 1.5 cm above the bottom edge .The solvent was allowed to run a distance of 12 cm in 30 mins and aqueous 5% silver nitrate solution was used as visualizing agent. As above by using methonolic Chaparral tablets was also analysed[13-14].

Determination of [λmax]

Selection of analytical wavelength[15-17]

NDGA dissolved in different solvents and scanned for absorption maxima, the observed wavelengths used for analysis. In methanol absorbs at 283.6 nm and in Acetonitrile at 282 nm.

Preparation of standard stock solution

About 10mg.of Nordihydroguaiaretic acid pure drug was weighted accurately and transferred into 10ml, volumetric flask. The volume was made upto 10ml.using methanol to obtain a solution that has a concentration1000µg/ml.1ml.of this stock solution was taken and diluted upto 10ml to get a concentration of 100µg/ml.

Selection of analytical concentration range

From the above stock solution take 10 ml and scanned by UV-VIS Spectrophotometer in the range of 200- 400 nm, using respective solvent as a blank .The wavelength corresponding to the maximum absorbance (λmax) was found.

Calibration curve for the LH (2 – 10 µg/ml)

Aliquots of 0.5-2.5 ml Stock solution were transferred to a series of 10 ml volumetric flasks with subsequent volume is adjust by respective solvent up to 10 ml .the solutions scanned in UV-VIS Spectrophotometer. The samples analyzed for their absorbance at respective λ_{max} .The calibration curve plotted and is shown in Fig 2 and Fig 3. The regression equation and correlation coefficient were determined which are presented in Table 1.

Validation of UV Spectroscopic method:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of detection and Limit of quantification.

Linearity

For each solvent, various aliquots were prepared from the stock solution ranging from 5-25 µg/ml .The samples analyzed with the help of UV-VIS Spectrophotometer, using respective solvent as a blank. The linearity of the above mentioned sample can be observed in table no.1.

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Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of drug was kept constant i.e. 5 µg and amount of pure drug was varied , that is 10 µg, 15 µg ,20 µg for 80%, 100% and 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery. Data obtained from accuracy study are given in Table no.4.

Precision

The precision of method was demonstrated by interday and intraday variations study, the solutions of same concentration were prepared and analyzed thrice for three consecutive days, and the absorbance were recorded. In the intra – day variations study, nine different solutions of the same concentration were prepared and analyzed thrice a day i.e. morning, afternoon and evening .The result was indicated by % RSD. Data obtained are given in the Table 3.

Limit of Detection

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$LOD = 3.3 (SD / S)$$

Where,

SD = the standard deviation of the response
S = the slope of the calibration curve

Limit of Quantitation

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precise.

$$LOQ = 10 (SD / S)$$

Where, SD = the standard deviation of the response
S = the slope of the calibration curve

Estimation of NDGA in Chaparral tablets

2-5 tablets are weighed and average weight was calculated. From that 1g of powder is weighed into a 100ml volumetric flask and made upto the volume with respective solvents. It is then sonicated for 15 min, filter the extract. Calculate the concentration of extract by measuring the absorbance at 282nm (Acetonitrile) and 283.6nm (Methanol) by using respective solvents as blank. The amount of drug present in extract solution can be determined by performing assay. Results obtained are shown in Table no.5.

Robustness study

The Robustness of the method was determined by carrying out the analysis using two different analytical equipments and the respective absorbances were noted. Robustness of the methods was assessed by carrying out assay with same analyst by using different equipment.Data obtained is shown in Table no.7.

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Figure 2: Absorption spectra of Nordihydroguaiaretic acid (Methanol)

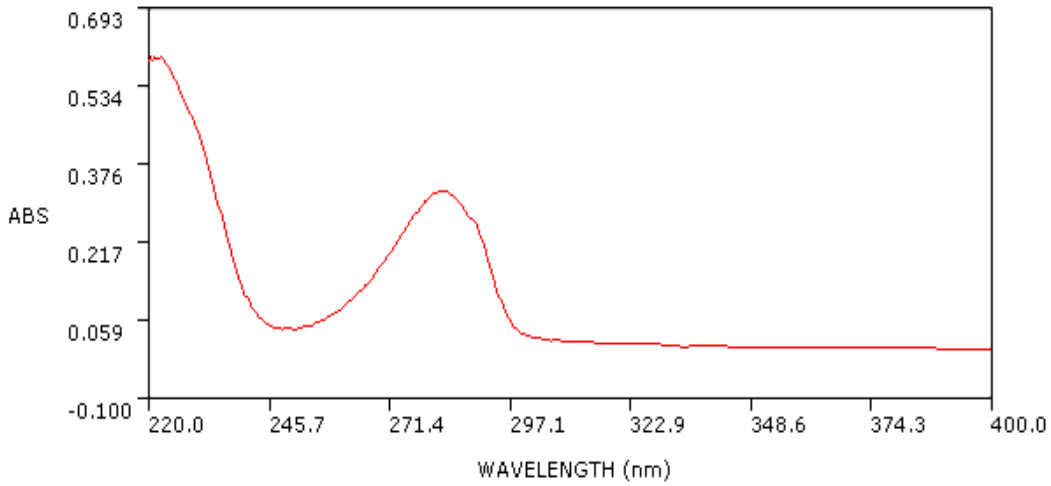

Figure 3: Calibration curve of Nordihydroguaiaretic acid (Methanol)

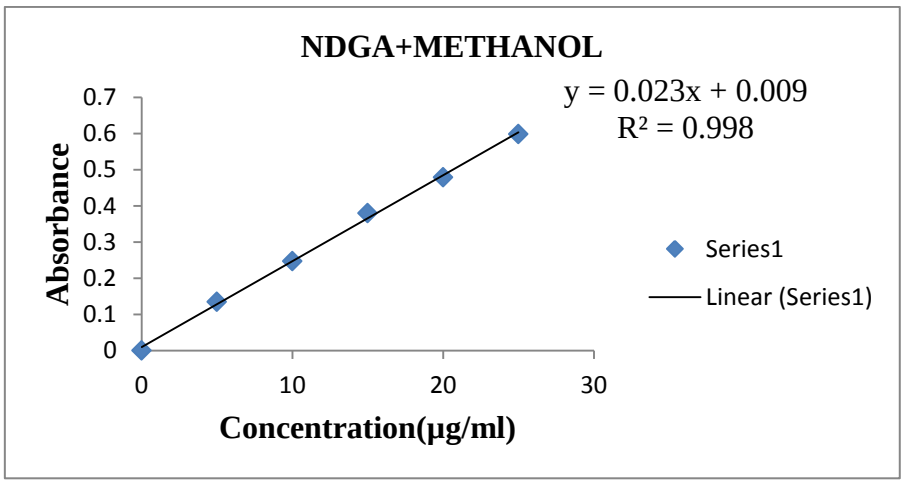

Figure 4: Absorption spectra of Nordihydroguaiaretic acid (Acetonitrile)

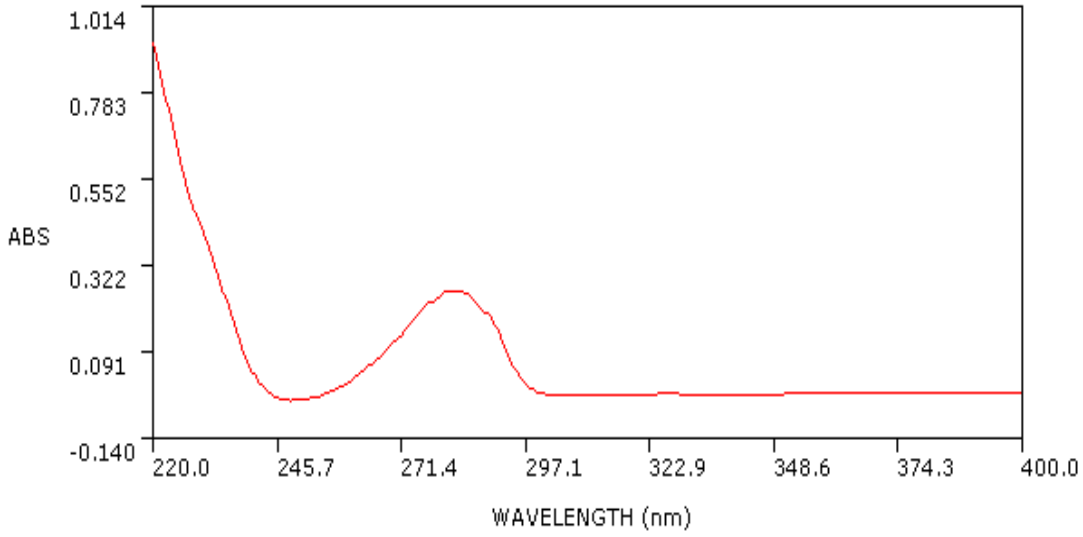

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Figure 5: Calibration curve of Nordihydroguaiaretic acid (Acetonitrile)

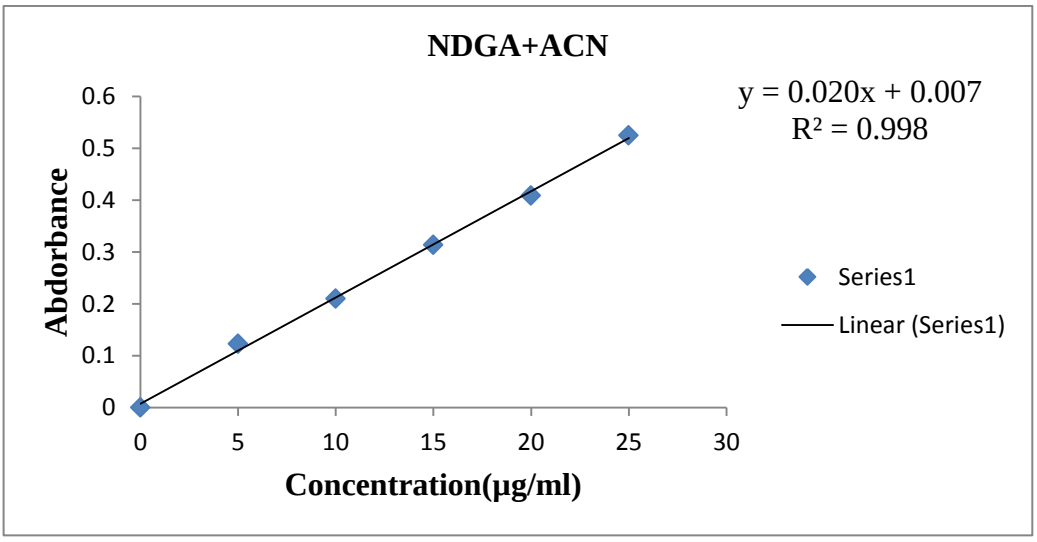

Table 1: Result of calibration of NDGA

S.No	Concentration (µg/ml)	Absorbance at 282 nm in Acetonitrile	Absorbance at 283.6 nm in Methanol
1	5	0.123	0.135
2	10	0.21	0.247
3	15	0.314	0.38
4	20	0.409	0.479
5	25	0.525	0.598

Table 2: Statistical data for NDGA by UV method

Parameter	Nordihydroguaiaretic acid	
Solvent	Acetonitrile	Methanol
Absorption maxima (nm)	282	283.6
Slope	0.020497143	0.023742857
Intercept	0.007285714	0.009714286
Correlation coefficient(R^2)	0.998211275	0.998394613
Range	5-25µg/ml	5-25µg/ml
Regression equation	Y=0.020x+0.007	Y=0.023x+0.009
Limit of detection	1.05µg/ml	1.001µg/ml
Limit of quantitation	3.20µg/ml	3.035µg/ml

*y = mx + c where x is the concentration of NDGA in µg/ml and y is the absorbance at the respective wavelength

Table 3: System Precision data for LH by UV spectrophotometric method

S. No	Interday Precision		Intraday Precision	
	%Amount found ± SD	%RSD	%Amount found ± SD	%RSD
Nordihydroguaiaretic acid in Acetonitrile	99.75 ± 0.000707	0.342425	99.5 ± 0.001155	0.294015
Nordihydroguaiaretic acid in Methanol	99.5 ± 0.000707	0.562354	100.4 ± 0.00577	0.239896

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Table 4: Determination of Accuracy, Recovery studies

Percentage level	In Solvent Acetonitrile		In Solvent Methanol	
	%Recovery ± SD	%RSD	%Recovery ± SD	%RSD
80%	97.3±0.000577	0.221773	92.9 ± 0.001155	0.401402
100%	98.6±0.000577	0.190754	99.3 ± 0.000577	0.164175
120%	99.4±0.000577	0.16751	100.5 ± 0.001	0.247525

Table 5: Result of Assay

Solvent used for the preparation of solution	Amount found (g/ml.)
Methanol	0.014
Acetonitrile	0.01032

Table 6: Ruggedness results for NDGA by UV Method

Standard drug	In Solvent Methanol		In Solvent Acetonitrile	
	Analyst-I	Analyst-II	Analyst-I	Analyst-II
	% Recovary ± SD	% Recovary ± SD	% Recovary ± SD	% Recovary ± SD
15µg/ml	98.55±0.000707	98.36 ±0.001414	100.3±0.0014	100.6±0.0007
20µg/ml	98.26±0.00014	98.5±0.0007	98.75±0.0021	98.25±0.0014

Table 7: Robustness data for NDGA

Standard Drug	In Solvent Acetonitrile		In Solvent Methanol	
	ELICO SL 210	SYSTRONICS 2203	ELICO SL 210	SYSTRONICS 2203
10µg/ml	9.9±0.0014	10±0.0007	9.92±0.002	9.98±0.0014

Table 8: Summary table of validation parameters of Levamisole Hydrochloride by spectrophotometric methods

S.no	Parameter	Results	
		In Acetonitrile	In Methanol
1	Range(µg/ml)	5-25 µg/ml	5-25 µg/ml
2	Correlation coeifficient(r^2)	0.998211275	0.998394613
3	Precision Interday Intraday	 0.342425 0.294015	 0.562354 0.239896
4	Ruggedness Analyst I Analyst II	 98.55±0.000707 98.36 ±0.001414	 98.25±0.0014 98.75±0.0021
5	Accuracy (n=3) Mean % recovery	80% - 97.3±0.000577 100% - 98.6±0.000577 120% - 99.4±0.000577	80% - 92.9 ± 0.001155 100% - 99.3 ± 0.00057 120% -100.5 ± 0.001
6	LOD(µg/ml)	1.05	1.001
7	LOQ(µg/ml)	3.20	3.035

RESULTS AND DISCUSSION

Specific accurate precise and simple UV spectroscopic method was developed for determination of NDGA from its dosage form. The first method was based on Calibration curve method. In the proposed Calibration curve method, the signals were measured at 282nm in solvent Acetonitrile and 283.6nm in solvent Methanol. The Concentration of each drug was obtained by using the Calibration curve.

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. There were no analytical methods for the determination of NDGA in dietary supplements (Chparral tablets). In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, UV Spectrophotometry method for the quantitative estimation of

Nordihydroguaiaretic acid in bulk drug and dietary supplement (Chaparral™) has been developed. The above proposed UV spectroscopic method was simple, easy, does not use polluting reagents and requires relatively inexpensive instruments. The reliability of the UV Method was proven by fulfilling all the validation criteria.

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