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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATIION OF DEXTROMETHORPHAN SUSPENSION

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ABSTRACT

One simple, specific, accurate, precise and reproducible method have been developed and validated for the Simultaneous Estimation of Dextromethorphan in suspension. In Reverse Phase High Performance Liquid Chromatography Method the chromatographic system was equipped with Eclipse- XDB C_{18} (150 X 4.6 mm, 5µm) Agilent as stationary phase and UV detector set at 225 nm, in conjunction with a mobile phase of 0.05M Potassium Dihydrogen Phosphate buffer (pH-3.0) and Acetonitrile in the ratio of 80:20% v/v (pH 3.0, adjusted with 1% Orthophosphoric acid) at a flow rate of 1.0 ml/min. The described method was linear over a concentration range of 50-150 µg/ml for Dextromethorphan. The retention time of Dextromethorphan was 3.080 min. The % recoveries of the both the drugs were found to be 100.94-101.91% for Dextromethorphan. Method were statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines and can be used for analysis of combined Tablet. In UV spectrophotometric method was a determination using the First Order Derivative Method at 225 nm over the concentration range 0.5-15 µg/ml for Dextromethorphan. Method were statistically validated for accuracy, precision, LOD and LOQ according to ICH guidelines and can be used for analysis of suspension. The propose method enables rapid quantification and simultaneous analysis of drug from syrup without any interference of excipients. So, the method can be used for routine analysis.

KEYWORDS: Dextromthorphan, RP-HPLC Method, Force Degradation, Method Validation.

INTRODUCTION

Dextromethorphan is an opioid like a drug reflex by a direct action on the cough center in medulla of the brain. Dextromethorphan shows high affinity binding to several regions of the brain, including the medullary cough center. This compound is an NMDA receptor antagonists and act as a non-competitive channel-blocker. It is one of the widely used antitussive and is also used to study the involvements of glutamate receptor in neurotoxicity. Chemically Morphinan, 3-methoxy-17-methyl-,(9α,13α,14α)-3-Methoxy-17-methyl-9α,13α,14α -morphinan (Fig.1). The empirical formula is $C_{18}H_{25}NO$ and its molecular weight 271.4 g/mol.

Literature survey reveals U.V. spectrophotometric, HPLC, methods for estimation of Dextromethorphan Bulk, and Dosage form. Literature survey reveals U.V. spectrophotometric, RP-HPLC, HPLC, methods for estimation of Present work emphasizes on method development for simultaneous estimation of Dextromethorphan in Syrup dosage form by HPLC.

MATERIALS AND METHOD

Instruments

HPLC analysis carried out using RP-HPLC system (Younglin, Korea) equipped with a PDA detector (DAD), C_{18} column (150mm×4.6mm, 5 µm), Chrom software. Digital balance (Libror AEU- 210 (Simadzu), pH meter (Systronics pH-361), Corning volumetric flasks, pipettes of borosilicate glass were used in the study.

Chemicals and reagents

Dextromethorphan was obtained as gift samples from Orbit Pharma. Lastuss-LA syrup was used for in this research was obtained from FDC Pharma ltd, Maharashtra. Water For Injection [HPLC grade] procured from Rankem; Acetonitrile [HPLC grade] procured from SD fine chem., Methanol [HPLC grade] procured from Ranchem. Potassium dihydrogen ortho phosphate [AR grade] & Ortho Phosphoric acid [AR grade] were obtained from Merck Chemicals Ltd, Mumbai. Details are mention in table no.10

Chromatographic Condition

- Stationary phase: XDB C_{18}(150× 4.6mm, 5µm) Agilent
- Mobile phase: 0.05 M Phosphate Buffer (pH 3.0): Acetonitrile (80:20 % v/v) pH adjusted to 3.0 using 1% Ortho- phosphoric acid

- Detection wavelength: 225 nm
- Injection volume: 10 µl
- Flow rate: 1.0 ml/min
- Column Temperature: Room Temperature
- Run time: 03 minutes.

Preparation of Mobile phase

Preparation of Buffer Solution: (0.05M KH_2PO_4)

Accurately weighed 3.4 gm Potassium dihydrogen phosphate (KH_2PO_4**)** and transferred in to 500 ml volumetric flask dissolved and diluted up to mark with HPLC grade water and adjusted pH 3.0 (± 0.05) with 0.1% Orthophosphoric acid solution, filtered and sonicated.

Preparation of Orthophosphoric Acid: 10 ml of Orthophosphoric acid is dissolved in 100ml of HPLC grade water mix well and then it is used for adjustment of pH.

Preparation of Diluent: Mix Water and Methanol in the ratio of 70:30%v/v.

PREPARATION OF STOCK SOLUTIONS

Preparation of Dextromethorphan standard stock solution (500µg/ml)

Weight accurately and transferred 45 mg of Dextromethorphan into 100 ml volumetric flask. Added 50 ml of HPLC grade water and shake to dissolve. Diluted up to the mark with HPLC grade water and mixed thoroughly. The solution was sonicated or 5 minutes. The solution was filtered through Whatman filter paper and first few drops of filtrate was discarded. An aliquot (1 ml) of solution was pipetted out in 10 ml volumetric flask and diluted up to mark with mobile phase to obtain solution (10µg/ml) of Dextromethorphan. Chromatogram of this solution was taken and amount of Dextromethorphan was calculated using regression equation.

Peak ID solution of Dextromethorphan (50 µg/ml)

Pipette out 5.0 ml of Dextromethorphan standard stock solution in to 50 ml volumetric flask and diluted up to the mark with diluent and mix.

Forced Degradation Study

Forced Degradation Studies of the drugs, was performed under different stress conditions as mentioned in ICH guideline Q1A (R2).[14] The standard solution containing 500µg/ml Dextromethorphan was subjected to acidic, alkaline, oxidative, thermal condition. Acidic and

alkaline degradation was performed up to 1N strength of acid/base at different temperature. Oxidative stress studies were carried out for using 3-10% H_2O_2The sample solution containing Dextromethorphan was subjected to degrade in Acid and Alkali condition.

Acid Degradation

1.0ml of Dextromethorphan standard stock solution was pipette out into 50 ml volumetric flask. 1 ml of 0.1N hydrochloric acid heated for 30 minutes at 80ºC temperature and it allowed to cool to room temperature and 1 ml of 0.1 N NaOH was added and diluted up to the mark with diluent and mixed and injected into HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table 2, while Fig: 4 represent the graph.

Alkali Degradation

1.0 ml of Dextromethorphan standard stock solution was pipette out into 50 ml volumetric flask and 1 ml of 0.1 N NaOH heated for 30 minutes at 80ºC temperature and it is allowed to cool to room temperature and 1 ml of 0.1 N HCl was added and diluted up to the mark with diluent and mixed and injected into HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table 2, Fig: 5 represents the graph.

Oxidative Degradation

1.0 ml of Dextromethorphan standard stock solution was pipette out into 50 ml volumetric flask. 1 ml 3 % H_2O_2 was added and then it is allowed to kept at room temperature at 80°C and kept for 30minutes and injected into HPLC system.Similarly solutions were prepared for respective conditions mentioned in Table 2, While Fig: 6 represents the graph.

Thermal Degradation

50mg of Dextromethorphan was weighed and transferred into 100ml of flask and it kept in the oven at 80°C then it was allowed to cool at room temperature and then sample were made. 1.0 ml of Dextromethorphan standard stock solution was pipette out into 50 ml volumetric flask and then it was kept and then diluted up to the mark with diluent and mixed and injected into HPLC system.Similarly solutions was prepared for respective conditions mentioned in Table 2 while fig:7 shows graph.

Preparation of Calibration Curve Solutions

Calibration curves were plotted over a concentration range of 50-150 µg/ml Dextromethorphan. The standard stock solution of Dextromethorphan (50 µg/ml) was further diluted with the mobile phase to obtain the final concentration 50, 80, 100, 120, 150 µg/ml.

For the linearity study 0.5, 0.8, 1.0, 1.2, 1.5 ml of Dextromethorphan stock solution was mixed in five different 10 ml volumetric flasks and volume was made upto the mark using mobile phase to obtain the final concentration 50, 80, 100, 120, 150 µg/ml of Dextromethorphan. The calibration curve was plotted for both the drugs, taking concentration on X-axis and peak area on Y-axis.

Optimization of chromatographic parameter

To optimize the HPLC parameters, several mobile Phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.02 M Potassium Dihydrogen Phosphate + 1% Ortho Phosphoric Acid, pH 3.0 and Acetonitrile (80:20 % v/v)at flow rate of 1 ml/min proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 225 nm at which much better detector responses for drug was obtained. As it was shown in Table 1 the retention times was 2.56 ± 0.04 minutes for Dextromethorphan. The resolution peak 16.243 ± 0.18.

System suitability parameters

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. System suitability tests are an integral part of liquid chromatography. These tests include tests for System suitability test parameters like Resolution, Retention time, Theoretical plate, Tailing factor are shown in table 1.

VALIDATION[26]

Specificity

Upon comparison of the blank, standard & sample chromatogram (Figure 8, Figure 9, and Figure 10), no significant change was observed in the peak area of drug for the standard and sample solution. Also from the comparison of the chromatograms of standard solution & solution prepared using the excipients, it was inferred that the excipients do not exhibit considerable effect on the peak area of Dextromethorphan .Hence, the developed method was found to be specific for estimation of drug.

Linearity

The linearity of the response for Dextromethorphan was determined by preparing standard solutions with concentrations of 50-150µg/ml Dextromethorphan. The calibration curves of Dextromethorphan shown in Figure 12 respectively indicate that the response is linear over the concentration range studied with correlation coefficient (r) value 0.991 Dextromethorphan. Overlain peaks for Dextromethorphan in their linearity range shown in Figure 11.

LIMIT OF DETECTION (LOD)

Limit of Detection was found 0.17 µg/ml for Dextromethorphan

LIMIT OF QUANTIFICATION (LOQ)

Limit of Quantitation was found 0.53 µg/ml for Dextromethorphan

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, three repeated injections of standard solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, three injections of standard solutions were made for three consecutive days and response of drug peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise. (Table 5)

Intraday & Interday

Intraday & Interday Precision data of Dextromethorphan are summarized in Table 5 % RSD for Intraday Precision were found to be 1.31-1.68% for Dextromethorphan. %RSD for Interday Precision were found to be 0.88-1.89% for Dextromethorphan

% **Accuracy**

Accuracy was performed at 50%, 100% and 150% levels by standard addition method. Each concentration was analyzed 3 times and average recoveries were measured as shown in Table 4 Accuracy of the methods was assured, involving analysis of formulation samples to which certain amounts of authentic drug was added. The resulting solution was assayed, and the results obtained for drug was compared to those expected. The good recoveries (Table 7) prove the good accuracy of the proposed methods.

Assay

Applicability of proposed method was tested by analyzing marketed formulation. The Results are shown in table. 8.

RESULTS AND DISCUSSION

Various compositions of mobile phase were used. The best results were obtained with 0.02 M potassium dihydrogen phosphate (pH adjusted to 3.0 using 1% Orthophosphoric acid): Acetonitrile in the proportion of 80: 20 (v/v) at 1.0 ml/min flow rate which gave the retention times were 2.567 min for Dextromethorphan. The optimum wavelength for detection was set at 225 nm at which much better detector responses for drug was obtained. The optical regression characteristics and validation parameters are shown in Table 9.

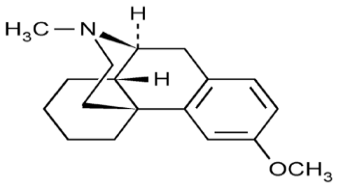

Fig. 1: Chemical Structure of Dextromethorphan

Table 1: System Suitability Parameters.

Peak	Name	Retention Time(n=3)	Area (n=3)	Asymmetry (n=3)	Theoretical Plates(n=3)	Resolution (n=3)
1	Dextromethorphan	2.56 ± 0.04 minutes	1520179±28946.4	1.45	2948± 75.45	16.243 ± 0.18

Table 2: Result of stability study of Dextromethorphan

Degradation method	Optimized Condition	% Degradation Dextromethorphan
Acid	1 ml 1N HCl (30 min 80ºC)	11.59%
Alkali	1 ml 1N NaOH (30 min 80ºC)	3.25%
Peroxide	1 ml 3 % H_2O_2 (30 min 80ºC)	14.3%
Thermal	6 hours at 80ºC	No Degradation

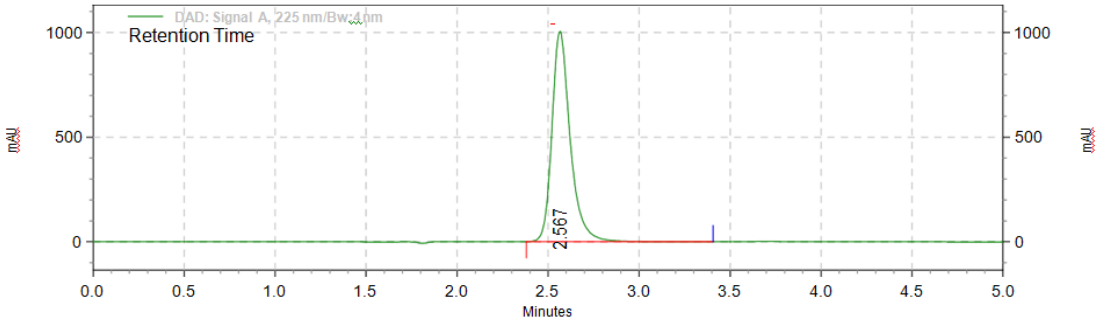

Fig 2: Chromatogram of Dextromethorphan (80:20µg/ml) Retention time 2.56 minutes.

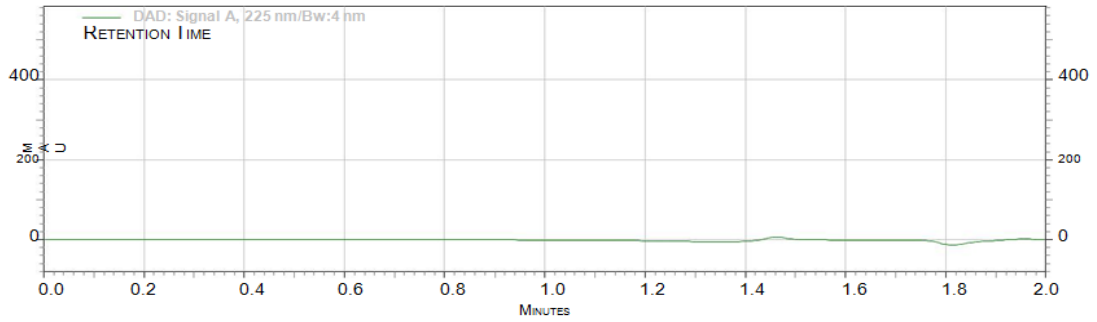

Fig. 3: Chromatogram of Blank Degradation.

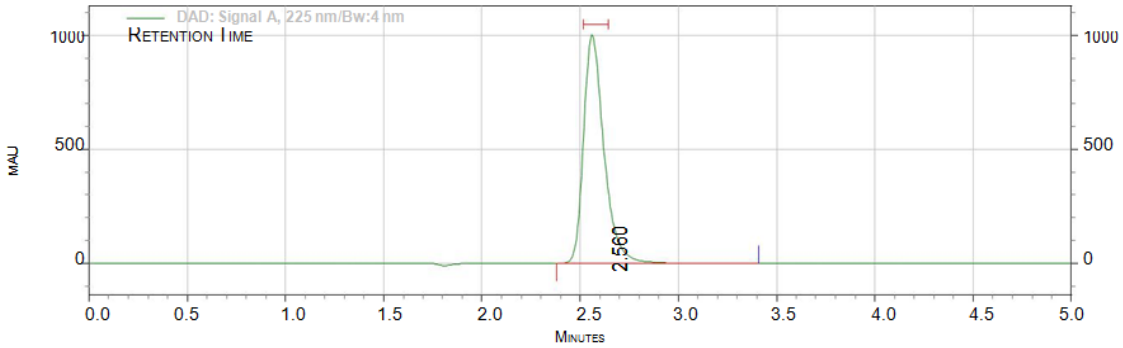

Fig. 4: Chromatogram of optimized condition of Acid Degradation for Dextromethorphan.

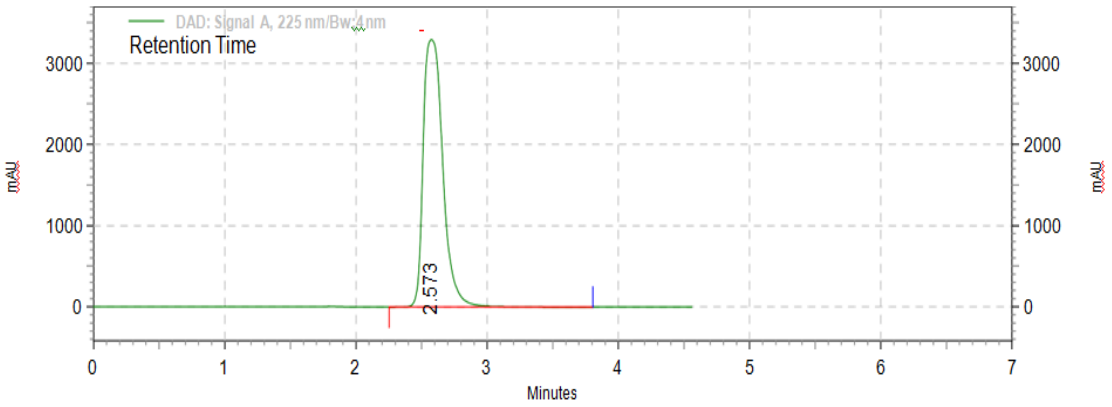

Fig. 5: Chromatogram of optimized condition of alkali Degradation for Dextromethorphan.

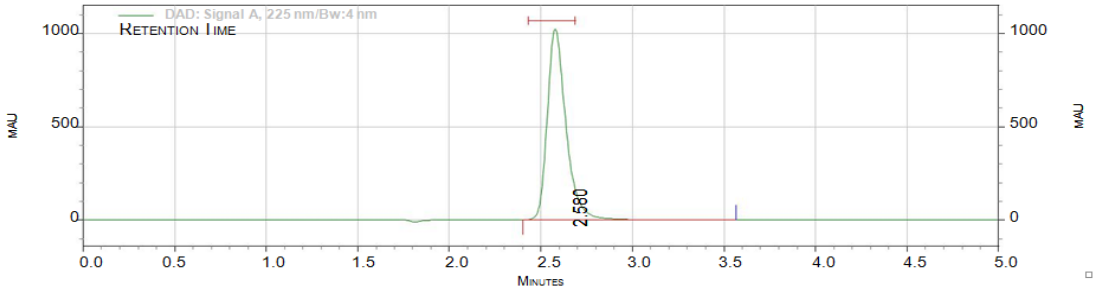

Fig. 6: Chromatogram of optimized condition of Peroxide Degradation for Dextromethorphan.

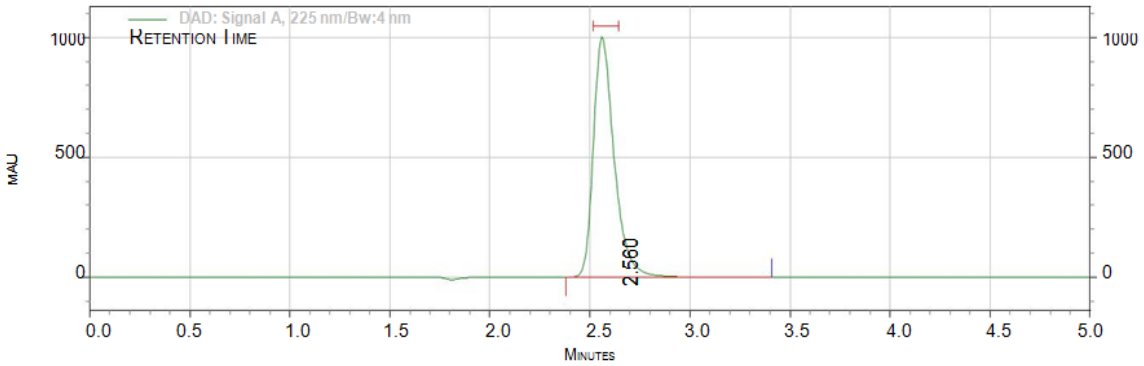

Fig. 7: Chromatogram of optimized condition of Thermal Degradation for Dextromethorphan.

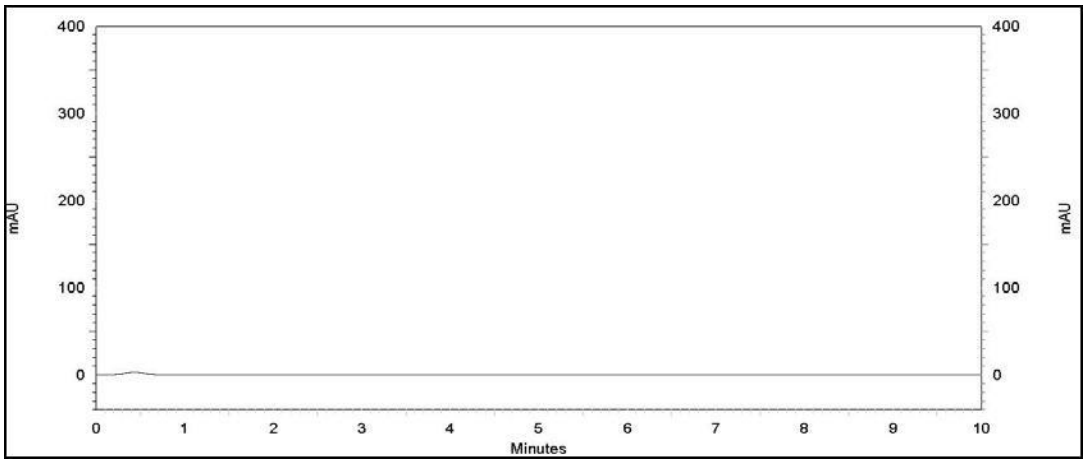

Fig. 8: Specificity chromatogram of diluents.

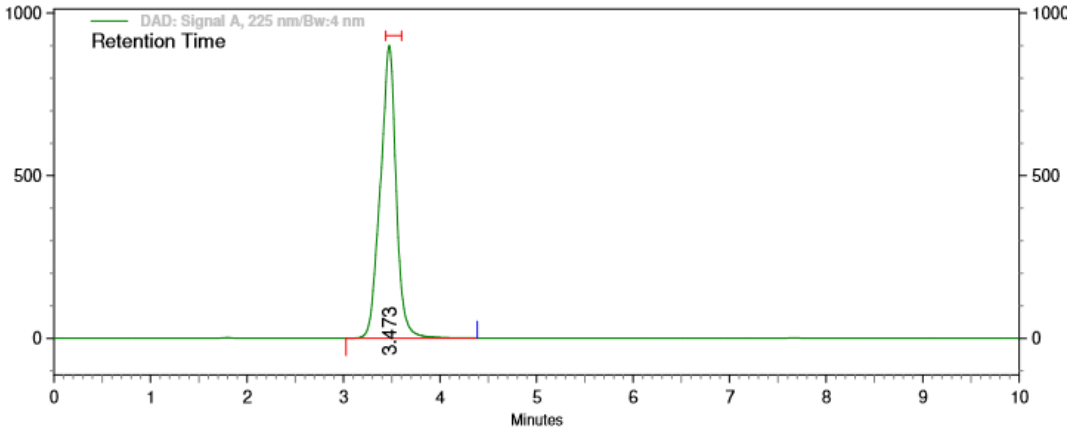

Fig. 9: Specificity chromatogram of standard Dextromthorphan.

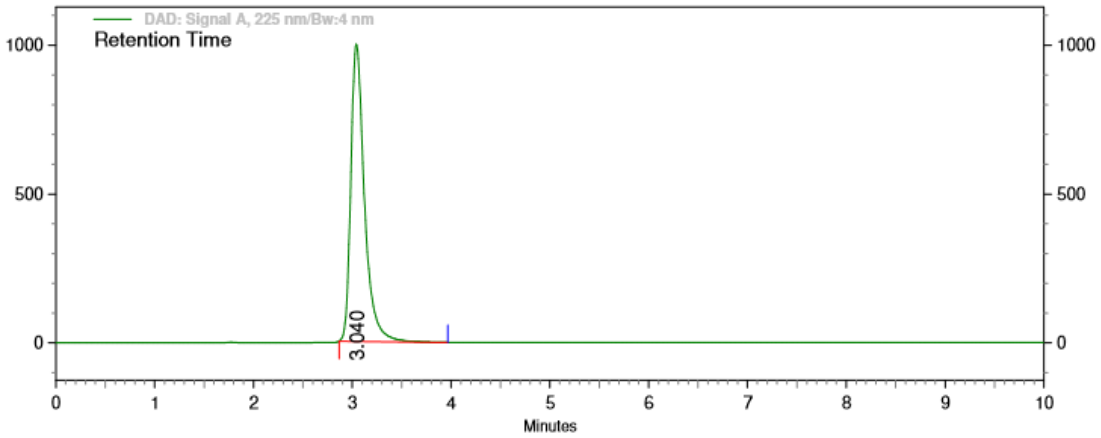

Fig. 10: Specificity chromatogram of sample Dextromethorphan.

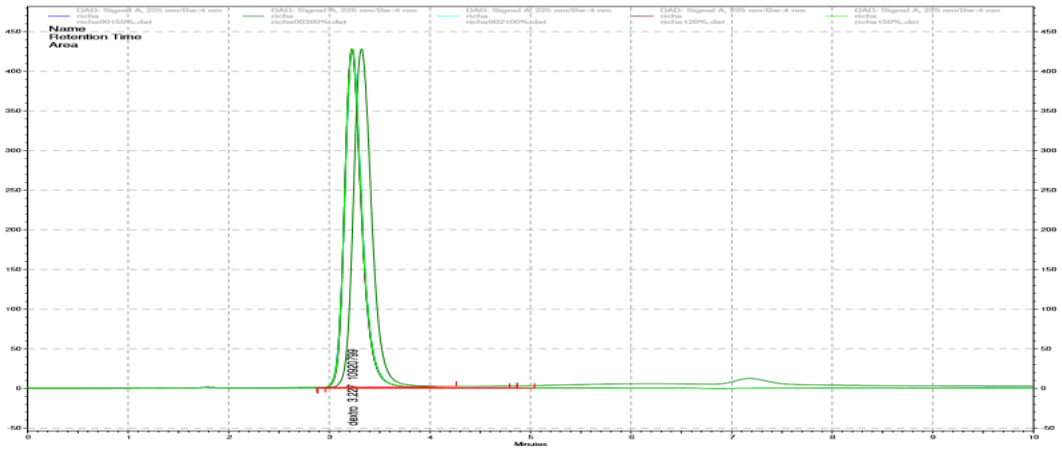

Fig. 11: Overlain linearity chromatogram of Dextromethorphan.

Table 3: Solution preparation for calibration curve.

Linearity solution No.	Dextromethorphan ((µg/ml)	Volume standard stock solution of Dextromethorphan (500 µg/ml)	Diluted up to mark with diluent(ml)
1	50	5	50
2	80	8	50
3	100	10	50
4	120	12	50
5	150	15	50

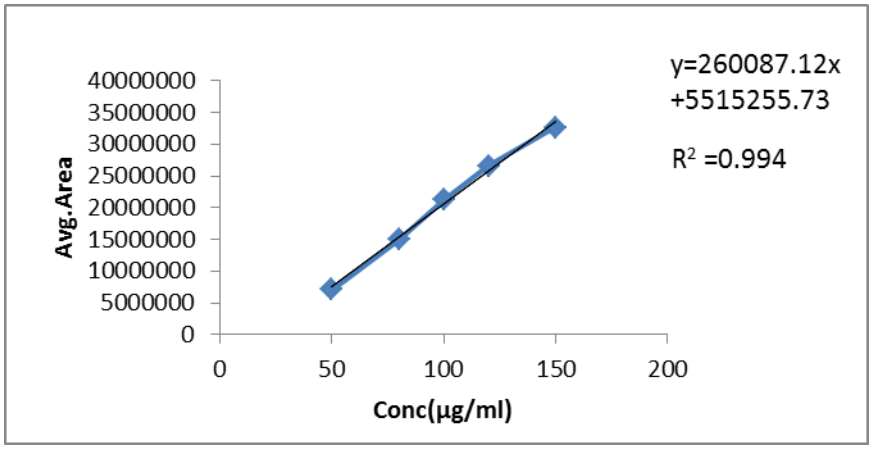

Fig. 12: Calibration curve of Dextromethorphan.

Table 4: Data for linearity and range of Dextromethorphan.

Sr. No.	Conc. (µg/ml)	Average area (n=3)	SD	%RSD
1	50	7081924	54814.8	0.77%
2	80	14920817	65907.14	0.44%
3	100	21261870	309218.22	1.45%
4	120	26592289	378174.3	1.42%
5	150	32577904	575308.8	1.76%

Table 5: Precision Study of Dextromethorphan.

Parameters	Concentration (µg/ml)	%RSD
Repeatability	50	1.27%
Intraday	50	1.31%
	100	1.77%
	150	1.68%
Interday	50	0.88%
	100	1.10%
	150	1.89%

Table 6: LOD and LOQ for Dextromethorphan.

Drug	LOD	LOQ
Dextromethorphan	0.17	0.53

Table 7: Recovery Data for Dextromethorphan.

Drug	Accuracy Level %	Amount of Drug Taken (µg/ml)	Amount Added (µg/ml)	Total amount found (µg/ml)	Amount recovered) ± SD(n=3)	% Recovery ± SD(n=3)
Dextromethorphan	50%	50	25	75	75.32± 0.26	102.33±1.73
	100%	50	50	100	102.67±0.32	103.35±1.6
	150%	50	75	125	125.71± 0.21	102.84±0.84

Table 8: % Analysis of Pharmaceutical Dosage form.

Dextromethorphan Lastuss-LA		
Labelled Amount (mg)	**Amount found (mg)**	**%Assay**
15mg	15.141	100.94%
	15.231	101.54%
	15.287	101.91%
Mean ± SD	15.219± 0.0789	101.46±0.48
%RSD	0.48%	1.15%

Table 9: Optical Regression Characteristics & Validation Parameters.

Sr No.	Parameter		Dextromethorphan
1	Specificity		Specific
2	Linearity/ Range (µg/ml)		50-150µg/ml
3	Regression equation		y = 438785.85x + 523432.13
4	Correlation co-efficient (R^2)		0.991
5	Precision (%RSD)	Repeatability	1.27%
		Intraday	1.31%-1.68%
		Interday	0.88%-1.89%
6	Accuracy(% recovery)	50%	102.33±1.73
		100%	103.35±1.6
		150%	102.84±0.84
7	LOD		0.17
8	LOQ		0.53
9	% Assay		101.46±0.48%w/w

Table 10: Details of Reagents and chemicals.

Material	Company	Grade
Dextromethorphan	FDC	Syrup
Water For Injection	Rankem	HPLC Grade
Acetonitrile	SD fine chem.	HPLC Grade
Methanol	SD fine chem.	HPLC Grade
Potassium dihydrogen phosphate	Merck	AR Grade
Ortho-Phosphoric acid	SD fine chem.	AR Grade
Sodium dihydrogen phosphate	Merck	AR Grade

CONCLUSION

The method was validated for specificity, linearity, precision, accuracy, considering the efficiency of HPLC; attempt has been made to develop simple, accurate, precise, rapid and economic HPLC method for simultaneous estimation of Dextromethorphan in suspension dosage form. This method describe enables the quantification of the API's. The advantages of the method are it is linear, accurate, precise, reproducible and simple. The described method is economic also because of low costs reagents and less run time. The all results were satisfactory and within the range of acceptance criteria and reproducible also. So, above described method can be employed for the quantization of the Dextromethorphan in syrup of

Lastuss-LA for regular and quality control analytical testing. In this proposed method the linearity is observed in the concentration range of 5-15 µg/ml with co-efficient of correlation, (r) = 0.998 Dextromethorphan. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the Dextromethorphan in dosage form without any interference of the excipients.

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