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UV-AUC method development and validation for estimation of Dextromethorphan hydrobromide

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Abstract

A simple, rapid, accurate and economical UV-spectrophotometric method has been developed for estimation of Dextromethorphan hydrobromide from bulk and pharmaceutical formulation. Method applied was area under curve (AUC) in which area under curve was integrated in the wavelength range of 258.4 – 290.8 nm. The λ_{max} of Dextromethorphan hydrobromide in 0.1N HCL was found to be 281 nm. The drug follows linearity in the concentration range 30 - 80µg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 101.42 % was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 %. The % recovery was found to be in the range 99.2%– 101.76%. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of Dextromethorphan hydrobromide in bulk and in pharmaceutical dosage form.

Keywords: Dextromethorphan hydrobromide, UV, validation, quantitative determination

Introduction

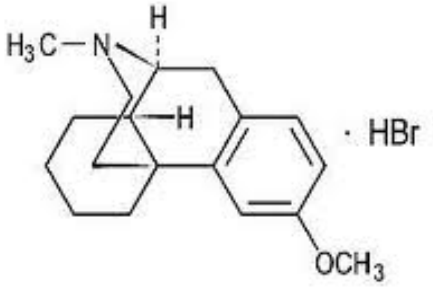

Figure 1: Chemical structure of Dextromethorphan hydrobromide

Dextromethorphan hydrobromide (DEX), [(+)-3-Methoxy-17-methyl-9, 13, 14- morphinan hydrobromide monohydrate] is a cough suppressant, used for the relief of non-productive cough; it has a central action on the cough centre in the medulla. DEX is rapidly adsorbed from the gastro-intestinal tract. It is metabolized in the liver and excreted in the urine as unchanged DEX and demethylated metabolites including DEX, which has some cough suppressant activity [1]. Different methods have been reported for the determination of DEX in the bulk drug, in the dos age forms with other drugs in cough-cold products and in biological samples. HPLC have been reported[2-6] the first and second-derivative technique UV spectrophotometry,[7-10] capillary electrophoresis,[11-13] GC,[14-16] LC[17-20] and TLC.[21,22] Accordingly, the objective of this study was to develop and validate the first order derivative method for the estimation of Dextromethorphan hydrobromide in bulk and pharmaceutical formulation as per ICH guidelines [23].

Experimental

Materials: Dextromethorphan hydrobromide was a gift sample from Torrent Pharmaceutical Limited, Ahmedabad. All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

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Preparation of standard stock solution: Accurately weighed 10 mg of Dextromethorphan hydrobromide was transferred to 100 ml volumetric flask, dissolved in 20 ml 0.1N HCL by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give final strength i.e. 100 µg/ml.

Selection of wavelength for analysis of Dextromethorphan hydrobromide: Appropriate volume 3ml of standard stock solution of Dextromethorphan hydrobromide was transferred into 10 ml volumetric flask, diluted to mark with 0.1N HCL to give concentration of 30µg/ml. The resulting solution was scanned in UV range (200 nm – 400 nm). In spectrum Dextromethorphan hydrobromide showed absorbance maximum at 281 nm (Fig. 2).

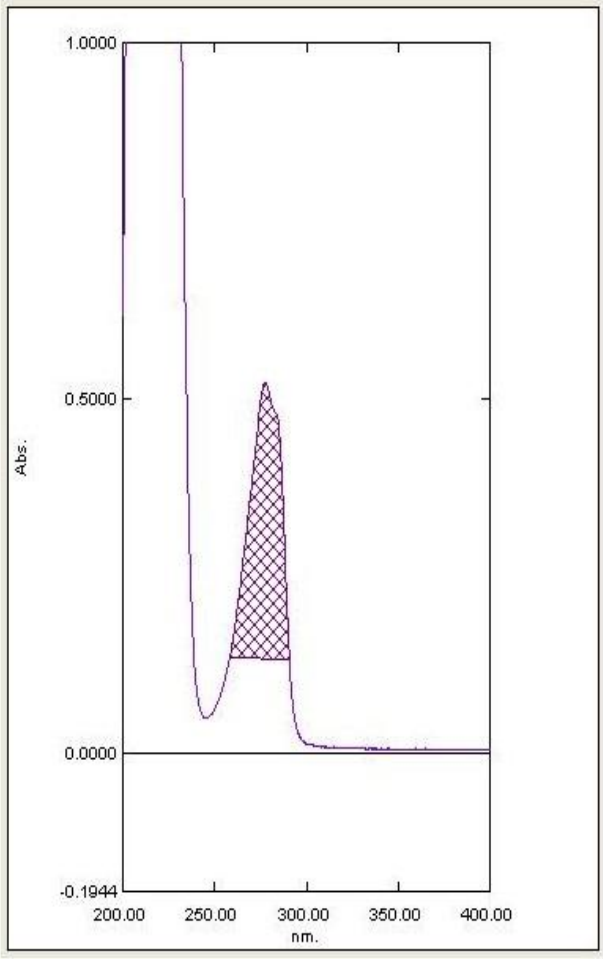

Figure 2: UV Spectrum of Dextromethorphan hydrobromide at 281 nm

Validation of the method: The method was validated in terms of linearity, accuracy, precision, and ruggedness.

Linearity study: Different aliquots of Dextromethorphan hydrobromide in range 3 - 8 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with 0.1N HCL to get concentrations 30, 40, 50, 60, 70 and 80 µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was recorded at 281 nm. The calibration plot was constructed as Absorbance vs concentration (Fig. 3).

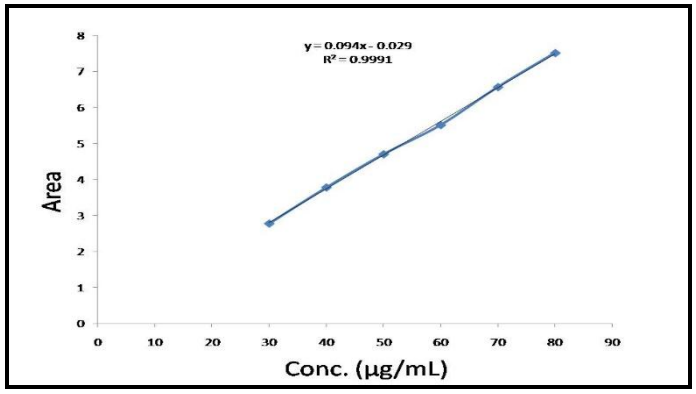

Figure 3: Calibration curve of Dextromethorphan
Y= 0.094X – 0.029, R^2 = 0.9991

Accuracy: To the pre-analysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were re-analyzed by proposed method.

Precision: Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 40, 50 and 60 µg/ml of Dextromethorphan hydrobromide solutions for three times in the same day. Inter-day precision was determined by analyzing the 40, 50 and 60 µg/ml of Dextromethorphan hydrobromide solutions daily for three days over the period of week.

Sensitivity: The sensitivity of measurements of Dextromethorphan hydrobromide lby the use of the proposed method was estimated in terms of the limit of quanfication (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a

measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability: Repeatability was determined by analyzing 50 µg/ml concentration of Dextromethorphan hydrobromide solution for six times.

Ruggedness: Ruggedness of the proposed method is determined for 50 µg/ml concentration of Dextromethorphan hydrobromide by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions.

Determination of Dextromethorphan hydrobromide bulk: Accurately weighed 10 mg of Dextromethorphan hydrobromide was transferred into 100 ml volumetric flask containing 20 ml 0.1N HCL and volume was made up to the mark using same. Appropriate volume 5 ml of this solution was transferred to 10 ml volumetric flask and volume was adjusted to mark using distilled water. The resulting solution was scanned on spectrophotometer in the UV range 200 - 400 nm. The concentrations of the drug were calculated from linear regression equations.

Application of proposed method for pharmaceutical formulation: For analysis of commercial formulation 5 ml of Dextromethorphan hydrobromide eye drop solution was taken in 100 ml volumetric flask and the volume was made up to the mark with 0.1N HCL to give 100µg/ml concentration. From this 5 ml was taken and transferred to 10 ml volumetric flask and volume was made up to the mark with distilled water to give 10 µg/ml concentration. It was scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was recorded at 281 nm. The concentrations of the drug were calculated from linear regression equation.

Results and Discussion

Method Validation: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies: The linear regression data for the calibration curves showed good linear relationship over the concentration range 30 - 80 µg/ml for Dextromethorphan hydrobromide. Linear regression equation was found to be Y = 0.094 X + 0.029 (r^2 = 0.9991). The result is expressed in (Table 1).

Accuracy: The solutions were re-analyzed by proposed method; results of recovery studies are reported in Table 2, which showed that the % amount found was between 99.2% to 101.76% with %R.S.D. >2.

Precision: The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).

Table 1: Linearity study of Dextromethorphan hydrobromide

Conc.	1	2	3	mean ±	SD	RSD
30	2.7834	2.7671	2.78	2.776833	0.007021	0.002528
40	3.7854	3.6992	3.7491	3.744567	0.035337	0.009437
50	4.7067	4.7443	4.6577	4.7029	0.035456	0.007539
60	5.5173	5.6329	5.458	5.536067	0.072625	0.013119
70	6.5712	6.5808	6.5442	6.5654	0.015495	0.00236
80	7.5162	7.5366	7.4624	7.505067	0.031298	0.00417

Table 2: Recovery studies

Drug	Initial amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL, n=3]	% Recovered	% RSD
DEX	30+24	54	52.74	97.11	0.800965
	30+30	60	60.854	101.365	1.862551
	30+36	66	64.975	96.93	0.175081

Sensitivity: The linearity equation was found to be Y = 0.094 X + 0.029. The LOQ and LOD for Dextromethorphan hydrobromide were found to be 1.88 µg and 0.67 µg, respectively.

Table 3: Precision studies

Drug	Concentration [µg/mL]	Intra –day [n=3]	% RSD	Inter day [n=3]	% RSD
DEX	40	100.2767	1.904347	100.34	2.012267
	50	101.6333	0.056807	100.55	0.646445
	60	101.1	0.685282	101.5533	2.611701

Repeatability: Repeatability was determined by analyzing 50 µg/ml concentration of Dextromethorphan hydrobromide solution for six times and the % amount found was between 97.5% to 99.67% with % R.S.D. less than 2 (Table 4).

Ruggedness: Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 5**.** The result showed that the % R.S.D. was less than 2%

Table 4: Repeatability studies

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
	50	49.53	99.07
	50	49.83	99.66
	50	50.3	100.6
	50	49.7	99.4
	50	49.94	99.8
DEX	50	49.84	99.69
	Mean ± SD	49.85667 ± 0.259127	99.70333 ± 0.511573
	% RSD	0.519743	0.513095

Determination of Dextromethorphan hydrobromide in bulk: The concentrations of the drug were calculated from linear regression equations. The % amount found was between 99.07 % to 100.6 % (Table 6).

Table 5: Ruggedness studies

Drug	Analyst - 1		Analyst - 2	
	% Amount found ± SD [n=3]	% RSD	% Amount found ± SD [n=3]	% RSD
DEX	99.65 ± 1.06	1.07	100.20 ± 1.16	1.15

Table 6: Analysis of Dextromethorphan hydrobromide in bulk

Drug	Label-claim [mg]	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
		50	50.88	101.76
		50	49.6	99.2
		50	50.36	100.72
		50	50.82	101.64
DEX	10	50	50.02	100.04
		50	50.22	100.04
		Mean ± SD	50.3033 ± 0.468771	100.6333 ± 0.97305
		% RSD	0.931889	0.966926

Table 7: Analysis of formulation

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	(%) Amount found
	50	49.36	98.73
	50	49.54	99.09
	50	49.83	99.67
DEX	50	48.78	97.5
	50	49.82	99.65
	50	49.22	98.44
	Mean ± SD	49.425 ± 0.39886	98.84667 ±0.821576
	% RSD	0.807053	0.831162

Application of proposed method for pharmaceutical formulation: The spectrum was recorded at 281 nm. The concentrations of the drug were calculated from linear regression equation. The % amount found was between 99.2 % to 101.76 % (Table 7).

Conclusion

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of Dextromethorphan hydrobromide in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

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