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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF DEXTROMETHORPHAN AND QUINIDINE BY RP-HPLC AND UV-SPECTROMETRY

K. Poornima [*1], Y. Madhusudan [2] and K. P. Channabasavaraj [2]

Department of Pharmaceutical Chemistry [1], PES College of Pharmacy, Bangalore, Karnataka, India.
Department of Pharmaceutical Chemistry [2], Government College of Pharmacy, Bangalore, Karnataka, India.

Keywords:

Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), Dextromethorphan, Quinidine, Simultaneous estimation

Correspondence to Author:
Mrs. K. Poornima

Assistant Professor,
Department of Pharmaceutical Chemistry, PES College of Pharmacy
50 Feet Road, Hanumanth nagar,
Bangalore-560050, India.

E-mail: poori.harshit@gmail.com

ABSTRACT: Objectives of study: To develop and validate new, rapid, selective, precise, accurate and economical, isocratic RP-HPLC and UV spectrophotometric method for simultaneous estimation of Dextromethorphan and Quinidine in bulk and in tablet formulations. **Methods:** A RP-HPLC was developed for the simultaneous estimation of Dextromethorphan and Quinidine on Phenomenex C-8 column with the mobile phase of methanol and phosphate buffer (pH 2.5) in the ratio 60:40 v/v at flow rate of 1ml/min and detection at 230nm was used for the study. In UV spectrometry, simultaneous equation method was based on measurement of absorbances at two selected wavelengths 278nm and 331nm for Dextromethorphan hydrobromide and Quinidine Sulfate respectively. Absorbance ratio method based on the measurement of absorbances at isobestic point and wavelength maxima of one drug, selected wavelengths were 278nm (λmax of DMH) and 289nm (isobestic point). The developed methods were validated and recovery studies were carried according to ICH guidelines. **Results:** The peaks of Dextromethorphan and Quinidine were found to be well resolved with retention time of 4.3min and 2.8min respectively, indicating the shorter analysis time. The proposed method was found to be accurate, precise and reproducible. The linearity was established in the concentration range of 1-30µg/ml. Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be within limits for both Dextromethorphan and Quinidine. In UV-spectrometry, both methods obey the Beer Lambert's law in the concentration range of 30-150µg/ml for Dextromethorphan Hydrobromide and 10-70µg/ml for Quinidine Sulfate respectively. **Conclusion:** The methods can be used for routine analysis of formulations containing any of the above drugs or combinations without any alteration in the chromatographic conditions.

INTRODUCTION: Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Drug analysis is the base for the determination of the product. Every year numbers of drugs are introduced into the market.

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Also quality is important in every product or service but it is vital in medicines as it involves life. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance.

Dextromethorphan is an NMDA (N-methyl-D-aspartate) receptor antagonist and acts as a non-competitive channel blocker. It is one of the widely used antitussives, and is also used to study the involvement of glutamate receptors in neurotoxicity [1].

The structure of Dextromethprphan is given in the **Fig. 1**. Quinidine is a class I anti-arrhythmic agent (I a) in the heart. It is a steroisomer of quinine originally derived from cinchona bark. The drug causes increased action potential duration as well as prolonged QT interval. But when given in combination with Dextromethorphan Hydrobromide, it is used for the treatment of Pseudobulbar affect. It is chemically known as (S)-[(2R,4S,5R)-5-ethenyl-1 azabicyclo [2.2.2]octan-2-yl] methoxyquinolin-4-yl)methanol [2]. The structure of Quinidine is given in the **Fig. 2**.

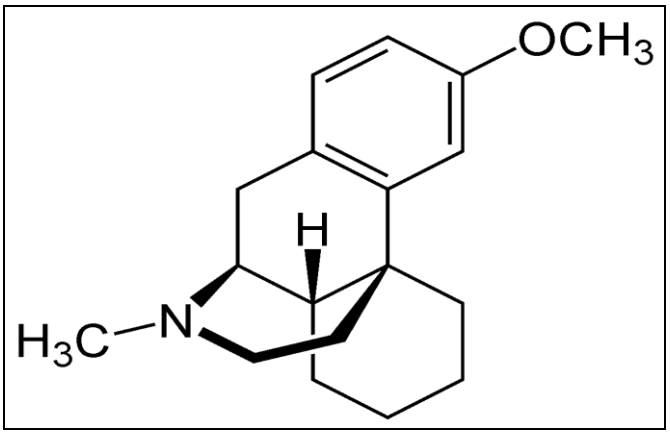


FIG. 1: STRUCTURE OF DEXTROMETHORPHAN

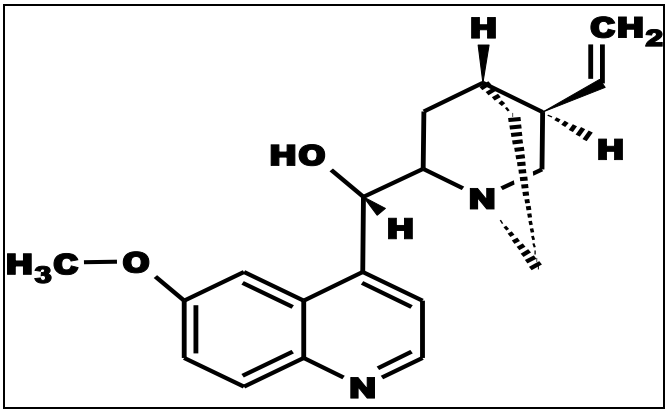


FIG. 2: STRUCTURE OF QUINIDINE

The simultaneous estimation by RP-HPLC methods for Dextromethorphan [3-10] and Quinidine [11-14] with other drugs by RP-HPLC and other spectrometric methods were developed. The Simultaneous determination of Dextromethorphan and Quinidine by Fluorometric [15] method was studied but RP-HPLC and UV spectrometric methods for simultaneous estimation of these drugs were not available. Hence the present study was designed to develop and validate method for simultaneous of Dextromethorphan and Quinidine by RP-HPLC and UV-Spectrometry.

MATERIALS AND METHODS:

Chemicals and reagents: Active pharmaceutical ingredient of Quinidine Sulfate and Dextromethorphan Hydrobromide was obtained as a gift sample from Cadila Pharmaceutical Pvt. Ltd and Ami Life Science Pvt. Ltd, India.

Development of RP-HPLC Method for Simultaneous Estimation:

Instrumental and Chromatographic system: Shimadzu, 20 AT model attached with pump, degasser, auto sampler, Ultra-violet detector.

Chromatographic Column: Phenomenex C-8, (250 x 4.6mm, 5 µm) end capped, Merck made.

Flow rate: 1.0 ml/min.
Acquisition time: 10 min.
Detection wavelength: 250nm
Injection volume: 20µL

Preparation mobile phase: Volume of 700 ml HPLC grade methanol were mixed with 300 ml of 10mM Sodium Dihydrogen Ortho Phosphate buffer, prepared by dissolving 1.56 gm of Sodium Dihydrogen Ortho Phosphate in 1000 ml of Millipore water, filtered with 0.45µ filter paper and sonicated for 10 min.

Determination of wavelength range: Wavelength for detection (UV detector) was selected by injecting the solution consists of Dextromethorphan and Quinidine (10µg/ml) to SHIMADZU- SIL-20A instrument. The overlay spectrum shows isobestic point at 250nm which was selected as wavelength of detection, the overlay spectrum.

Determination of retention time: The retention time for Dextromethorphan and Quinidine were determined individually and in combination by injecting 20µl of working standard solutions at 1ml/min into the chromatograph and UV detected at 245nm. Retention time was observed; chromatogram was recorded.

Preparation of Standard and Sample solutions:

Preparation of working standard solutions of Dextromethorphan and Quinidine: Accurately 10 mg of Dextromethorphan and 10 mg of Quinidine were weighed into clean and dry 10 ml volumetric flasks separately, dissolved with

sufficient volume of diluent. The final volumes were made up to 10 ml with diluent to get the concentration of 1000 µg/ml for Dextromethorphan and Quinidine respectively.

Preparation of working standard solutions of Dextromethorphan and Quinidine: The working standard solutions of Dextromethorphan and Quinidine were prepared in volumetric flasks with diluent to get a different concentration i.e1µg/ml, 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml and 30µg/ml.

Sample preparation: Twenty tablets of Dextromethorphan and twenty tablets of Quinidine were weighed and crushed to a fine, homogenous powder. Quantity equivalent to 10mg is weighed and diluted to 10ml of mobile phase. 10mL is further diluted to 100mlwith mobile phase which is stock B of 100 µg/ ml. Stock B serially diluted to contain 10, 20, 30, 40 and 50 µg/ml of final concentration using mobile phase for both the tablets and are further mixed for the specificity studies.

Optimization of chromatographic conditions: The method was made on trial and error basis and the best resolution was obtained at mobile phase of Methanol: Buffer (10mM Sodium Dihydrogen Ortho Phosphate) in ratio 70:30v/v pH adjusted to 4.0 (with dilute ortho phosphoric acid).

Method Validation: The method was validated as per ICH guidelines. The method was validated in terms of linearity, specificity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) [16, 17].

Linearity and range: 20 µl of each of these working standard solutions of Dextromethorphan and Quinidine ranging from 1 to 50 µg/ml were injected into a chromatograph at flow rate of 1 ml/min. Retention time and peak area obtained were recorded and standard calibration curve was plotted for Dextromethorphan and Quinidine, linearity equations were derived. The Correlation coefficient, % curve fitting were also calculated.

Specificity: 20µl of diluent, working standard of Dextromethorphan and Quinidine were injected separately into the chromatographto examine that the Dextromethorphan and Quinidine peaks are not affected by the mobile phase and diluent and the chromatogram was recorded.

Precision:

System precision: Successive six injections of 20 µl working standard mixture solution (six replicates) were injected into a HPLC chromatograph, the peak area and chromatograms obtained were recorded. The % relative standard deviation was calculated for peak areas of replicates.

Method Precision:

Intra-day Precision: Successive six injections of 20 µl of working standard mixture solutions were injected separately at different intervals in the same day and chromatograms were recorded. The % relative standard deviation was calculated for concentration of drug in replicates.

Inter-day Precision: Successive six injections of 20 µl of working standard mixture solutions were injected separately on different days and chromatograms were recorded. The % RSD was calculated for concentration of drug in replicates

Intermediate Precision: Intermediate precision (Ruggedness) expresses the variations within laboratories variations: (different days, different analysts, different equipment, etc.). The Intermediate precision was performed for Dextromethorphan and Quinidine by different analyst on different instrument using different lot of column on different day.

Accuracy: 20µl solution of the resulting mixture was injected repeatedly into the chromatograph, the peak area and chromatogram obtained were recorded and the % recovery of standard MOXO and were calculated.

Limit of Detection and Limit of Quantification: For estimation of LOD and LOQ, visualization method was followed. In visualization method lower dilutions of working standard solution each of Dextromethorphan and Quinidine of 20µl were injected in to the chromatograph till the drug solution gives response and peak area. The chromatogram and peak area obtained for different concentrations of Dextromethorphan and Quinidine were recorded.

Robustness: For the method developed, flow rate of 1 ml/min was used. The robustness study was carried out with small deliberate change to 0.9 and 1.1ml/min. 20 µl working standard mixture solutions were injected in chromatograph at a flow rate of 0.9 and 1.1 ml/min, the peak area and chromatograms obtained were recorded.

For the method developed, mobile phase comprising of MEOH: Sodium Dihydrogen Ortho Phosphate (70:30v/v) was used. For Robustness study, the ratio of ,MEOH and Sodium Dihydrogen Ortho Phosphate buffer were slightly altered from the ratio of (70:30v/v) to (68:32) and (72:28).20µl of working standard mixture solutions of Dextromethorphan and Quinidine were injected in to the chromatograph with altered mobile phase ratios, the peak areas and chromatograms obtained were recorded, and the % assay was calculated.

System suitability: 20 µl of standard solutions of Dextromethorphan and Quinidine were injected into chromatograph and chromatograms were recorded. From the data obtained system suitability parameters like theoretical plates, tailing factor and resolution were calculated.

Development of UV Spectrometric method for Simultaneous Estimation:

Determination of absorption maxima: Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. Dextromethorphan and Quinidine showed absorbance maxima at 278 nm and at 331 nm respectively. Isoabsorption point was found at 289 nm.

Preparation of standard stock solution Dextromethorphan and Quinidine: The 20 mg of standard Dextromethorphan and 10 mg of standard Quinidine were transferred to 10 ml volumetric flask and dissolved in methanol by sonication. Final volume was made up to the mark, the resulting solution consists of 2000 µg/ml of Dextromrthorphan and 1000 µg/ml Quinidine respectively.

Preparation sample stock solution of Dextromethorphan and Quinidine: The contents of twenty formulated capsules were weighed. A quantity of powder equivalent to 20 mg of Dextromethorphan and 10 mg of Quinidine were transferred to 10 ml volumetric flask and dissolved in methanol by sonication, then filtered through Whatman filter paper no. 41. Final volume was made up to the mark, the resulting solution consists of 2000 µg/ml of Dextromethorphan and 1000 µg/ml Quinidine respectively.

Method A: Simultaneous equation method: Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the UV-1800 Spectrophotometer range from 200 nm to 400 nm. Dextromethorphan and Quinidine showed absorbance maxima at 278 nm and 331 nm respectively. Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. Dextromethorphan and Quinidine showed absorbance maxima at 278 nm and at 331 nm respectively. Isoabsorption point was found at 289 nm and shown in.

Absorbance Ratio Method/ Q-Analysis (Method B): The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. E.g. two dilutions of the same substance give the same absorbance ratio A1 / A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths, one being the λ max of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an iso-absorptive point (Beckett and Stenlake, 2005).

Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400nm to 200nm Dextromethorphan and Quinidine showed absorbance maxima at 278 nm and at 331nm respectively. Iso-absorption point was found at 289nm. The concentration of two drugs in mixture was calculated by using the following equation:

$$C_x = \frac{A_1}{a_{x1}} \left(\frac{Q_M - Q_Y}{Q_X - Q_Y} \right)$$

Assay of capsules by Method A and B: The contents of twenty formulated capsules were weighed. A quantity of powder equivalent to 20 mg of Dextromethorphan and 10 mg Quinidine were transferred to 10 ml volumetric flask and dissolved in methanol by sonication, then filtered through Whatman filter paper no.41. Final volume was made up to the mark, the resulting solution consists of 2000 µg/ml of Dextromethorphan and 1000 µg/ml of Quinidine respectively. Absorbance of each solution against distilled was measured at 278nm, 289nm and 331nm. The absorbance of each solution was substituted in the absorbance ratio method equation to calculate the amount of the drug present.

Linearity: Linearity was studied by preparing standard solutions at different concentration levels. Calibration curves were prepared using the standard solutions of 10µg/ml – 30µg/ml and linear regression analysis was carried out.

Validation of UV spectrophotometric method: The described methods have been validated for the assay of both the major components of bulk drug using following ICH parameters [16, 17].

Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value.

Precision: The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as %RSD.

Intra and inter-day precision: Variations of results within the same day (intra-day) and variation of results between days (inter-day) were analyzed. Intra-day precision was determined by analyzing Dextromethorphan and Quinidine for six times in the same day at 278 nm and 331 nm. Inter-day precision was determined by analyzing both the drugs daily once for three days at 278nm and 331nm.

Reproducibility: The absorbances were measured by another analyst and the values obtained were evaluated by calculating %RSD.

Specificity and selectivity: Specificity is a procedure to detect quantitatively the analyte in presence f components that may be expected to be present in the sample matrix. While selectivity is a procedure to detect the analyte qualitatively in presence of components that may expected to be presented in the sample matrix. Commonly used excipients in capsule formulation were spiked in a reweighed quantity of drugs and the absorbance was measured and calculations were done to determine the quantity of the drugs.

Ruggedness: The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analyst.

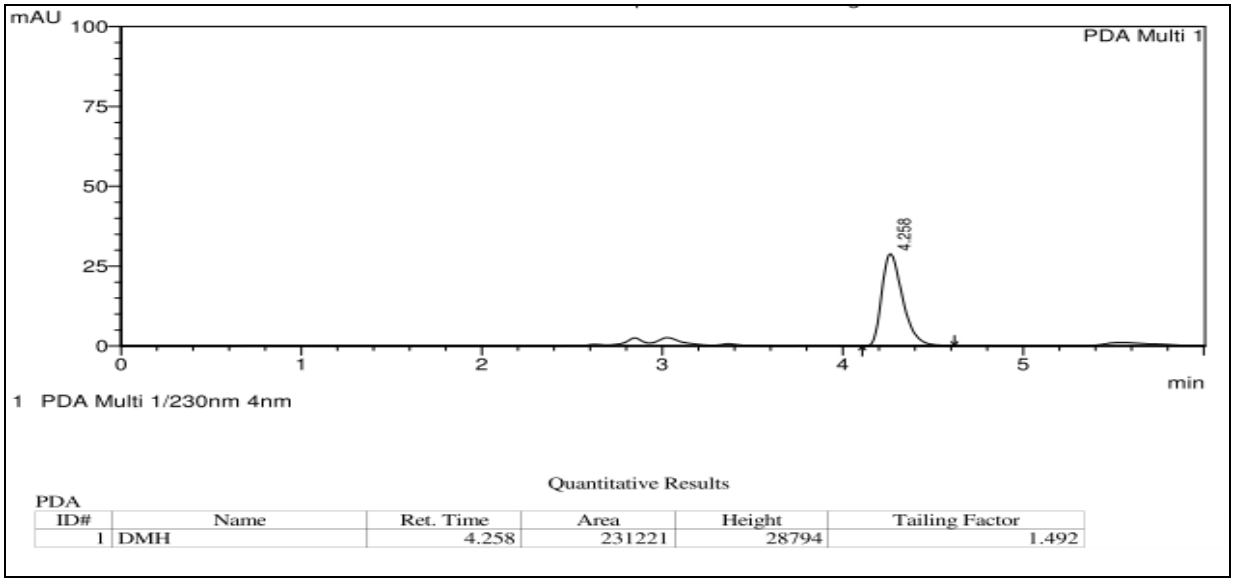
RESULTS AND DISCUSSION:

Developed RP-HPLC Method for Simultaneous Estimation: The RP-HPLC method was developed and validated for simultaneous estimation of Dextromethorphan and Quinidine in pure and combined formulation. The results obtained for the entire project work are presented here.

Column and standardization of the mobile phase: It was found that peaks of Dextromethorphan and Quinidine were well resolved with C 8 column and the solvent system consists of phosphate buffer pH 2.5: methanol (40:60) and this mobile phase was selected for further studies. The 10 µg/ml solutions of Dextromethorphan and Quinidine showed isobestic point at 230nm and it was selected as wavelength maxima for estimation of Dextromethorphan and Quinidine.

Estimation of Retention Time: The Dextromethorphan and Quinidine have shown maximum peak at 30µg/ml concentrations. Retention time of Dextromethorphan and Quinidine was found to be 4.3 min and 2.8 min respectively, when injected as individual components and in combination.

The chromatograms for Dextromethorphan and Quinidine individually and in the combinations are given in the **Fig. 3, Fig. 4,** and **Fig. 5.**

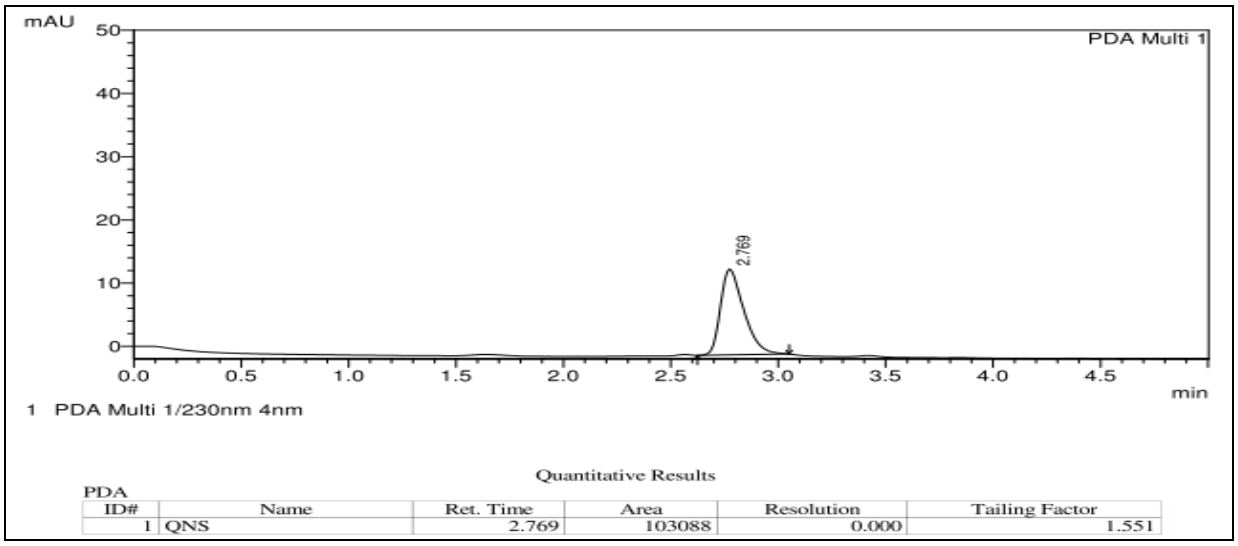


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Height	Tailing Factor
1	DMH	4.258	231221	28794	1.492

FIG. 3: CHROMATOGRAM OF DEXTROMETHORPHAN

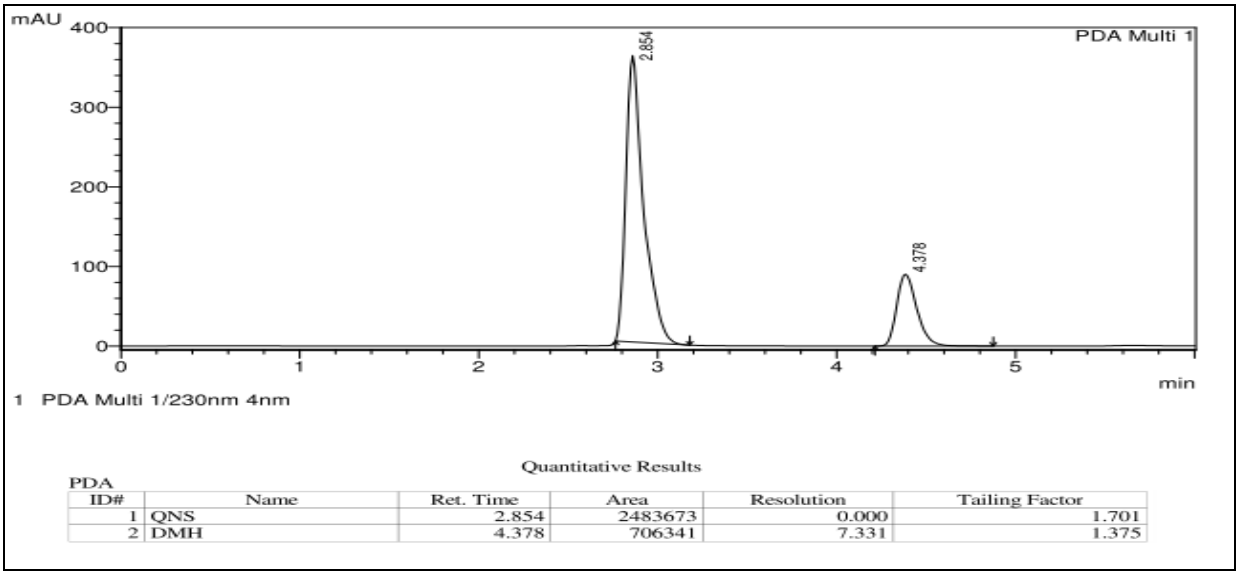


Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Resolution	Tailing Factor
1	QNS	2.769	103088	0.000	1.551

FIG. 4: CHROMATOGRAM OF QUINIDINE



Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Resolution	Tailing Factor
1	QNS	2.854	2483673	0.000	1.701
2	DMH	4.378	706341	7.331	1.375

FIG. 5: CHROMATOGRAM SHOWING DEXTROMETHORPHAN AND QUINIDINE PEAK RESOLUTION AT 30µg/mL

Validation parameters:

Linearity and Range: The linearity response for Dextromethorphan and Quinidine were observed in the concentration range of 1 to 30µg/ml for both the drugs respectively, with Correlation coefficient, percentage curve fittings found to be well within the acceptance criteria limit (See **Fig. 6**).

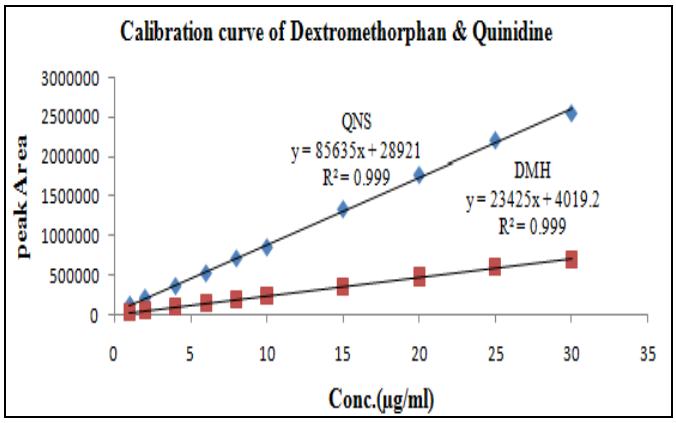


FIG. 6: STANDARD CALIBRATION CURVE FOR DEXTROMETHORPHAN AND QUINIDINE

Specificity: As no peaks were found at retention time of 4.37 min and 2.85 min, the proposed method was specific for the detection of Dextromethorphan and Quinidine (See **Fig. 7**).

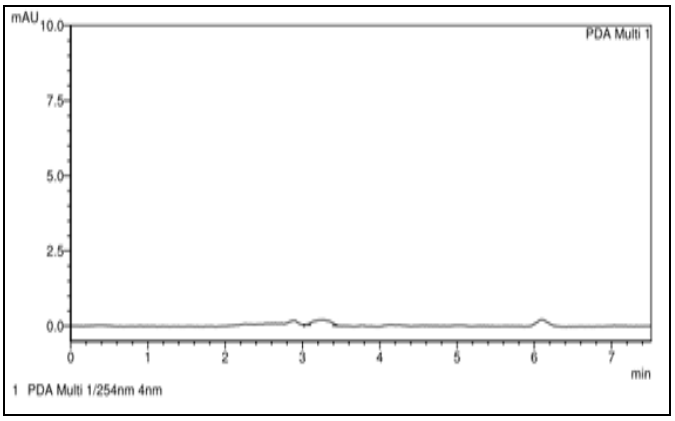


FIG. 7: CHROMATOGRAM SHOWING NO PEAKS IN DETERMINATION OF SPECIFICITY

Accuracy: The mean percentage recovery for Dextromethorphan and Quinidine at three different levels was found to be between 101.7-103.5% and 97.6-98.5% respectively, which was well within the acceptance limit and hence the method was found to be accurate. (See **Table No: 1**, **Table No: 2** and **Table No: 3**)

TABLE 1: REPORT OF RECOVERY STUDIES FOR DEXTROMETHORPHAN AND QUINIDINE

Level	Mean % recovery for Dextromethorphan	Mean% recovery for Quinidine	Acceptance Criteria
80%	101.7	98.5	90-110%
100%	103.5	97.67	90-110%
120%	102.1	98.5	90-110%

TABLE 2: RECOVERY STUDY DATA FOR DEXTROMETHORPHAN

Level	Replicate	Stand Conc. (µg/ml)	Sample Conc. (µg/ml)	Peak Area*	Total Conc. (µg/ml)	Amt of standard recovered (µg/ml)	% Recovery
80%	I	3	5	195060	8	3.05	101.6
	II	3	5	196092	8	3.07	102.3
	III	3	5	194881	8	3.14	104.9
100%	I	5	5	239410	10	5.04	100.8
	II	5	5	238516	10	5.4	100.2
	III	5	5	239445	10	5.05	101
120%	I	7	5	284791	12	7.41	99.97
	II	7	5	285375	12	7.01	100.1
	III	7	5	284443	12	6.9	99.5

*Average of six determinations

TABLE 3: RECOVERY STUDY DATA FOR QUINIDINE

Level	Replicate	Stand Conc. (µg/ml)	Sample Conc. (µg/ml)	Peak Area*	Total Conc.(µg/ml)	Amt of standard recovered (µg/ml)	% Recovery
80%	I	1.5	2.5	369997	4	1.48	98.86
	II	1.5	2.5	368763	4	1.46	97.89
	III	1.5	2.5	369991	4	1.48	98.8
100%	I	2.5	2.5	448774	5	2.40	96.11
	II	2.5	2.5	456653	5	2.49	99.79
	III	2.5	2.5	449792	5	2.41	97.15
120%	I	3.5	2.5	534215	6	3.40	97.15
	II	3.5	2.5	535342	6	3.14	97.53
	III	3.5	2.5	545898	6	3.53	101.05

*Average of six determinations

Precision:

System Precision: The % RSD values of peak area for six replicate injections of Dextromethorphan and Quinidine were found to be 1.57 and 1.77 respectively, which are well within the acceptance criteria limit of not more than 2% (See **Table 4**).

Method Precision: The % RSD was found to be 1.69 and 1.63 for intra-day precision 0.37 and 1.59 for inter day precision of DMH and QNS respectively. In Inter-day precision, the % RSD was found to be 1.5 and 1. For inter day precision; 1.66 and 0.36 for inter day precision of Dextromethorphan and Quinidine respectively. The % RSD values of concentration for intermediate precision for Dextromethorphan and Quinidine were found to be 1.69 and 0.66 respectively. As the results were within the acceptance limits (not more than 2 %) the system provides good method precision (see **Table No: 4**).

TABLE 4: REPORT OF PRECISION FOR DEXTROMETHORPHAN AND QUINIDINE

Precision Parameters	Dextromethorphan % RSD	Quinidine % RSD	Acceptance Criteria
System Precision	1.57	1.77	< 2.0%
Intermediate Precision	1.69	0.66	< 2.0%
Intraday Precision	1.69	1.63	< 2.0%
Interday Precision	0.37	1.59	< 2.0%

Limit of detection (LOD) and Limit of Quantitation (LOQ): From the chromatogram obtained, it was observed that at the concentration of 0.1µg/ml for Dextromethoprphan and 0.06µg/ml for Quinidine peak or response was observed, but no area was found. Hence the LOD for Dextromethoprphan by visualization was found to be 0.1µg/ml and LOQ was found to be 0.2µg/ml respectively and LOD for Quinidine by visualization was found to be 0.1µg/ml and LOQ was found to be 0.2µg/ml respectively (see **Table No: 5**).

System suitability: The system suitability parameters were calculated for Dextromethorphan and Quinidine, results obtained were found to be well within the acceptance criteria (see **Table No: 6**).

TABLE 5: DATA FOR LOD AND LOQ OF DEXTROMETHORPHAN AND QUINIDINE

Volume of stock solution (ml)	Volume made upto (ml)	Concentration (µg/ml)	Peak Area*
0.9	10	0.9	101865
0.8	10	0.8	90487
0.7	10	0.7	81384
0.6	10	0.6	70327
0.5	10	0.5	51388
0.4	10	0.4	44567
0.3	10	0.3	35432
0.2	10	0.2	22757
0.1	10	0.1	11659
0.9 (1µg/ml)	10	0.09	7754
0.8	10	0.08	5879
0.7	10	0.07	2567
0.6	10	0.06	------

*Average of six determinations

TABLE 6: SYSTEM SUITABILITY OF DEXTROMETHORPHAN AND QUINIDINE

System Suitability Factor	Dextromethorphan	Quinidine	Acceptance Criteria
Theoretical plates	4685	2089	>2000
HETP (mm)	32.01	71.780	-
Tailing factor	1.26	1.29	<2
Resolution	5.37		>2

*Average of five determinations

Robustness: It was found that the % assay Dextromethorphan and Quinidine were ranged between 93.74-98.41% and 94.0-101.84% respectively, indicating that the method is robust with respect to slight change change in flow rate. As per our observations, the % assay was 91.59-

98.90% Dextromethorphan and 97.78–107.36 % for Quinidine indicating that the method is robust with respect to slight change in ratio of mobile phase. It was also found that % assay of Dextromethorphan and Quinidine ranges between 95.69-100.70 % and 99.45-105.55% respectively, indicating that the method is robust with respect to slight change in pH of phosphate buffer (see **Table 7, Table 8** and **Table 9**).

TABLE 7: ROBUSTNESS DATA OF DEXTROMETHORPHAN AND QUINIDINE WITH CHANGE IN RATIO OF MOBILE PHASE

Change in flow rate	Dextromethorphan			Quinidine		
	Peak Area*	Amount of drug recovered (µg/ml)	% Assay	Peak Area*	Amount of drug recovered (µg/ml)	% Assay
0.9ml	223616	9.37	93.74	430658	4.70	94.00
1.0ml	234560	9.84	98.41	453410	4.97	99.45
1.1ml	232026	9.73	97.33	463639	5.09	101.84

* Average of five determinations

TABLE 8: ROBUSTNESS DATA OF DEXTROMETHORPHAN AND QUINIDINE WITH CHANGE IN WAVELENGTH

Change in mobile phase ration (MEOH:Buffer)	Dextromethorphan			Quinidine		
	Peak Area*	Amount of drug recovered (µg/ml)	% Assay	Peak Area*	Amount of drug recovered (µg/ml)	% Assay
(60:40)	235726	9.89	98.91	446305	4.88	97.78
(62:38)	219717	9.20	98.24	488507	5.38	107.67
(58:42)	218589	9.15	91.59	487192	5.36	107.36

* Average of five determinations

TABLE 9: ROBUSTNESS DATA OF DEXTROMETHORPHAN AND QUINIDINE WITH CHANGE IN pH

Change in pH	Dextromethorphan			Quinidine		
	Peak Area*	Amount of drug recovered (µg/ml)	% Assay	Peak Area*	Amount of drug recovered (µg/ml)	% Assay
2.4	239940	10.07	100.70	469850	5.16	103.30
2.5	234560	9.84	98.41	453410	4.97	99.45
2.6	228191	9.56	95.69	479398	5.27	105.55

* Average of five determinations

Recovery: The % assay of Dextromethorphan and Quinidine in marketed formulation was found to be 98.59% and 98.75% respectively. The results show that there is no interference from excipients and no impurities were observed in samples for the proposed method (see **Table 10**).

TABLE 10: ASSAY OF DEXTROMETHORPHAN AND QUINIDINE IN MARKETED FORMULATIONS

Peak area*for Dextromethorphan	Peak area*for Quinidine	Amount of drug recovered Dextromethorphan (µg/ml)	Amount of Drug recovered Quinidine (µg/ml)	% Assay for Dextromethorphan	% Assay for Quinidine
235350	451091	9.87	4.92	98.59	98.75

*Average of six determinations

UV Spectrometric method for simultaneous estimation: Simultaneous estimation of Dextromethorphan and Quinidine was carried out by simultaneous equation method by measurement of absorptivities at the λ_{max} of DMH at 278 nm and QNS at 331 nm where as in Second method i.e absorbance ratio method both the drugs were estimated by measuring absorptivities at two different wavelengths. One is at λ_{max}278 nm of DMH and followed by measurement of absorptivities at isobestic point at 289 nm using distilled water was as a blank. Considering above facts, wavelength 278 nm and 331 nm were selected for the estimation of DMH and QNS by spectrophotometry (see **Fig. 8**).

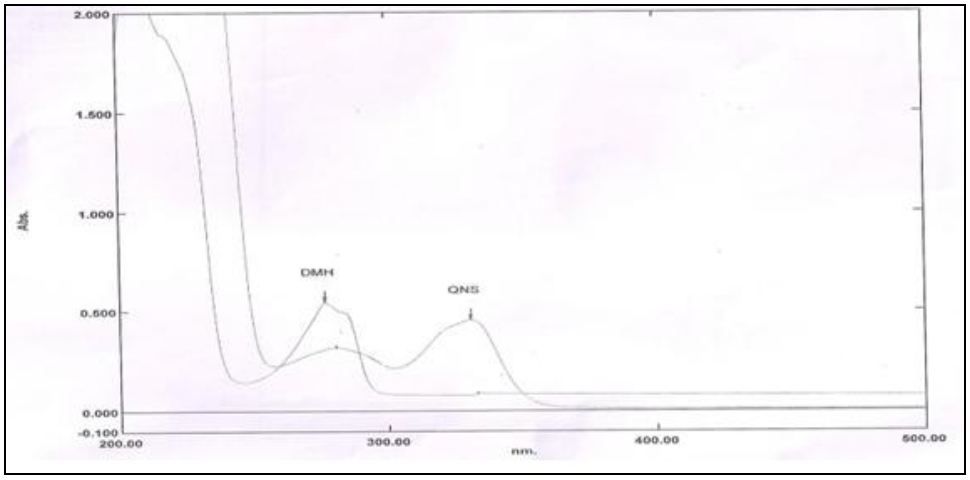


FIG. 8: OVERLAIN SPECTRA OF DMH AND QNS

Linearity: The linearity range was found to be in the concentration range of 30-150µg/ml for DMH and 10-70µg/ml for QNS in both methods. The regression coefficients are reported in **Table 11**.

TABLE 11: STATISTICAL DATA OF DEXTROMETHORPHAN AND QUINIDINE AT 278NM AND 331 NM RESPECTIVELY BY SIMULTANEOUS EQUATION METHOD

Parameters	Dextromethorphan Hydrobromide	Quinidine Sulfate
Detection wavelength	278 nm	331 nm
Linearity range	30-150µg/mL	10-70µg/mL
Slope	0.0157	0.0128
Intercept	0.0052	0.0043
Correlation coefficient	0.9998	0.9998

Method validation:

Accuracy: Results of recovery studies for the determination of accuracy by simultaneous estimation and Q-Analysis method are presented in **Table 12** and **Table 13.**

TABLE 12: DETERMINATION OF ACCURACY OF DEXTROMETHORPHAN AND QUINIDINE BY SIMULTANEOUS EQUATION METHOD

Level %	Vol.tkn into 10ml VF			Conc tkn (µg/ml)		*Absorbance		Conc obt (µg/ml)		% Found	
	Sample soln tkn(ml)	Std soln tkn (ml)									
		DMH	QNS	DMH	QNS	DMH	QNS	DMH	QNS	DMH	QNS
80	0.5	0.8	1.2	64	32	0.683	0.422	63.33	31.9	98.95	99.72
100	0.5	1.3	2.0	80	40	0.765	0.519	78.11	39.2	97.64	98.12
120	0.5	1.8	2.8	96	96	0.922	0.626	94.09	47.35	98.01	98.64

*Average of six determinations

TABLE 13: DETERMINATION OF ACCURACY OF DEXTROMETHORPHAN AND QUINIDINE BY Q ANALYSIS METHOD

Amt. of sample		Amt. of drug added		Amt. Recovered		% recovery	
DMH µg/ml	QNS µg/ml	DMH µg/ml	QNS µg/ml	DMH µg/ml	QNS µg/ml	DMH %	QNS %
40	20	32	16	70.5	34.28	97.91	95.22
40	20	40	20	77.33	39.20	96.66	98.00
40	20	48	24	87.00	43.20	97.96	98.18

Precision: Precision was determined by replicate injection of standard solution. Values of % RSD for precision study obtained was within the acceptance criteria of less than 2%, the proposed method was shown a good degree of precision. The results of Intra and inter-day precision are given the by simultaneous estimation (see **Table 14** and **Table 15**) and Q-Analysis method **Table 16** and **Table17**) are tabulated.

TABLE 14: DETERMINATION OF PRECISION FOR DEXTROMETHORPHAN AND QUINIDINE AT 278 NM BY SIMULTANEOUS ESTIMATION

DMH					QNS				
Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD	Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD
90	0.476± 0.0015	0.31	0.478 ± 0.0005	0.10	40	0.513 ± 0.0020	0.38	0.514 ± 0.0015	0.29

*average of six determinations

TABLE 15: DETERMINATION OF PRECISION FOR DEXTROMETHORPHAN AND QUINIDINE AT 331NM BY SIMULTANEOUS ESTIMATION

DMH					QNS				
Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD	Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD
90	0.476± 0.0015	0.31	0.480±0.0023	0.47	40	0.513± 0.0020	0.38	0.510±0.0018	0.35

*average of six determinations

TABLE 16: DETERMINATION OF PRECISION FOR DMHDEXTROMETHORPHAN AND QUINIDINE AND QNS AT 278NM BY Y Q-ANALYSIS METHOD

DMH					QNS				
Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD	Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD
90	0.476± 0.0015	0.31	0.478 ± 0.0005	0.10	40	0.330 ± 0.0019	0.57	0.328 ± 0.0017	0.51

*average of six determinations

TABLE 17: DETERMINATION OF PRECISION FOR DMH AND QNS AT 289NM BY Y Q-ANALYSIS METHOD

DMH					QNS				
Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD	Conc. µg/ml	Inter-day Absorbance* ± Std. Deviation	% RSD	Intra-day Absorbance *± Std. Deviation (n=6)	% RSD
90	0.233± 0.0014	0.60	0.237 ± 0.0023	0.97	40	0.306 ± 0.0012	0.39	0.310± 0.0018	0.58

*average of six determinations

Reproducibility: Reproducibility was determined by replicate injection of standard solution. Values of % RSD for precision study obtained was within the acceptance criteria of less than 2%, the proposed method was shown a good degree of reproducibility. The reproducibility results for simultaneous estimation (see **Table No. 18** and **Table No. 19**) and Q-Analysis method (see **Table No. 20** and **Table No. 21**) are presented.

TABLE 18: REPRODUCIBITY RESULTS FOR DMH AND QNS AT 278 NM BY SIMULTANEOUS ESTIMATION

DMH					QNS				
Conc. µg/ml	Analyst 1 Absorbance* ± Std. Deviation	%RSD	Analyst 2 Absorbance* ± Std. Deviation	%RSD	Conc. µg/ml	Analyst 1 Absorbance* ± Std. Deviation	%RSD	Analyst 2 Absorbance* ± Std. Deviation	%RSD
90	0.476± 0.0015	0.31	0.480±0.0023	0.47	40	0.513± 0.0020	0.38	0.510±0.0018	0.35

*average of six determinations

TABLE 19: REPRODUCIBITY RESULTS FOR DMH AND QNS AT 331 NM BY SIMULTANEOUS ESTIMATION

DMH					QNS				
Conc. µg/ml	Analyst 1 Absorbance * ± Std. Deviation	%RSD	Analyst 2 Absorbance * ± Std. Deviation	%RSD	Conc. µg/ml	Analyst 1 Absorbance * ± Std. Deviation	%RSD	Analyst 2 Absorbance * ± Std. Deviation	%RSD
90	0.454± 0.001	0.29	0.461±0.003	0.45	40	0.489± 0.0019	0.36	0.504±0.0014	0.33

*average of six determinations

TABLE 20: REPRODUCIBITY RESULTS FOR DMH AND QNS AT 278NM BY Y Q-ANALYSIS METHOD

DMH					QNS				
Conc. µg/ml	Analyst 1 Absorbance * ± Std. Deviation	%RSD	Analyst 2 Absorbance * ± Std. Deviation	%RSD	Conc. µg/ml	Analyst 1 Absorbance * ± Std. Deviation	%RSD	Analyst 2 Absorbance * ± Std. Deviation	%RSD
90	0.476± 0.0015	0.31	0.480±0.0023	0.47	40	0.341± 0.0021	0.61	0.245±0.0028	1.24

*average of six determinations

TABLE 21: REPRODUCIBITY RESULTS FOR DMH AND QNS AT 289NM BY Q-ANALYSIS METHOD

DMH					QNS				
Conc. µg/ml	Analyst 1 Absorbance * ± Std. Deviation	%RSD	Analyst 2 Absorbance * ± Std. Deviation	%RSD	Conc. µg/ml	Analyst 1 Absorbance * ± Std. Deviation	%RSD	Analyst 2 Absorbance * ± Std. Deviation	%RSD
90	0.233± 0.0014	0.10	0.236±0.0026	0.98	40	0.306± 0.0012	0.39	0.309±0.0017	0.55

*average of six determinations

Specificity and selectivity: Simultaneous estimation method and Q-absorbance ratio method were found to be simple, specific, sensitive, precise, accurate and economical for the routine analysis of Dextromethorphan and Quinidine individually or in combined dosage form.

Assay of capsules by Method A and B: The results of determination of percentage purity of capsules containing Dextromethorphan and Quinidineby simultaneous estimation and Q-absorbance method are presented in the **Table 22.** and **Table 23**).

TABLE 22: ASSAY RESULTS OF MARKETED FORMULATION BY SIMULTANEOUS EQUATION METHOD

Formulation	Actual Concentration (mg)		% DMH	% QNS
	DMH	QNS		
Capsule	20	10	96.4	98.0

TABLE 23: ASSAY RESULTS OF MARKETED FORMULATION BY Q-ANALYSIS METHOD

Formulation	Actual Concentration (mg)		% DMH	% QNS
	DMH	QNS		
Capsule	20	10	93.7	94.8

CONCLUSION: The developed RP-HPLC and UV spectrometric methods for the simultaneous estimation of Dextromethorphan and Quinidine were accurate, precise, linear and reproducible. This makes the methods suitable for routine analysis of the combination product in quality control laboratories.

CONFLICT OF INTEREST: All authors are hereby to disclose that there is no conflict of interest including any financial, personal or other relationships with other people or organizations with respect the present work.

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