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RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DROTAVERINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, sensitive and precise reverse phase high performance liquid chromatographic method (RP-HPLC) has been developed and validated for the simultaneous estimation of paracetamol and drotaverine hydrochloride in both : bulk drug samples and formulation. The method was carried out on a phenomenex Luna C-18 (250 mm × i.d -4.6 mm, particle size 5 µm) column with a mobile phase consisting of methanol : water (pH adjusted to 3.34 with 0. 1% ortho-phosphoric acid) in the ratio of 52 : 48 (v/v). The flow rate was optimized at 1 mL/min and effluent was monitored at detection wavelength of 246 nm. The total chromatographic run time was 10 minutes and the retention time of paracetamol and drotaverine hydrochloride was 3.18 min and 5.07 min, respectively. The method was proved to be accurate and precise at linearity range of 2-36 µg/mL for paracetamol and 1-60 µg/mL for drotaverine hydrochloride with a correlation coefficient (r^2) 0.999 and 0.9991, respectively. The method is highly reproducible with recoveries ranging from 99.93-100.2% and 99.90-100.16% for paracetamol and drotaverine hydrochloride, respectively. The method was validated for accuracy, precision, robustness, detection and quantification limits as per ICH guidelines. The intra- and inter- day precision and accuracy values were found to be within the limits. Due to its simplicity, accuracy and economical value, the proposed method can be used for routine quality analysis of these drugs in bulk and combined dosage form.

Key words: Paracetamol, Drotaverine hydrochloride, Simultaneous, Linearity.

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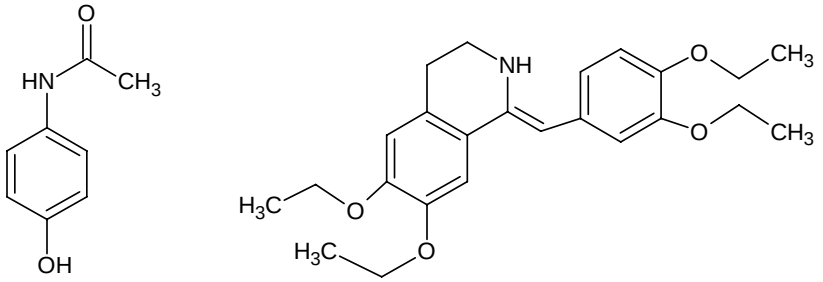
INTRODUCTION

Paracetamol is an analgesic and antipyretic drug. Drotaverine hydrochloride is an antispasmodic drug and it is selective inhibitor of phosphodiesterase-4 and has no cholinergic effects. Chemically, paracetamol is known as N-(4-hydroxyphenyl) acetamide[1] (Fig. 1a). Drotaverine hydrochloride is known as 1Z-1-(3, 4 –diethoxyphenyl) methylene]-6, 7-diethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (Fig. 1b). Literature survey reveals that several analytical methods for determination of paracetamol and drotaverine hydrochloride individually, from plasma and in combination with other drugs[3-26] were reported. So far no RP-HPLC method has been reported for simultaneous estimation of paracetamol and drotaverine hydrochloride from pharmaceutical tablet dosage form. The present study describes simple, sensitive, accurate, rapid and reproducible RP-HPLC method for the simultaneous estimation of paracetamol and drotaverine hydrochloride in bulk and dosage forms with sensitivity and accuracy.



(a) **(b)**

Fig. 1: (a) Paracetamol, (b) Drotaverine hydrochloride

EXPERIMENTAL

Equipment

Shimadzu HPLC system (Shimadzu, Japan) equipped with Spinchrome software consisting of Lc-10AT VP series pump, rheodyne injector with 20 µL fixed volume loop, UV- Visible detector SPD-10AVP was used for analysis. Shimadzu UV-1800 double beam UV-Visible spectrophotometer with 10 mm matched quartz cells was used to record the spectra. Drugs and chemicals were weighed by Metler balance.

Chemicals

Pure drug samples of paracetamol and drotaverine hydrochloride were obtained as gift samples from Trident Pharmaceuticals Ltd., Hyderabad, Andhra Pradesh and Blue Cross

Pharmaceuticals Ltd., Nashik, India. Methanol (HPLC grade) and ortho-phosphoric acid (AR grade) were procured from S.D. Fine chemicals, Ltd., Mumbai. Triple distilled water was used to carry out the analysis. The tablet formulation Drotin ™ plus (manufactured by Martin Harris lab Ltd) with labeled amount of 500 mg of paracetamol and 80 mg of drotaverine hydrochloride was purchased from local drug store.

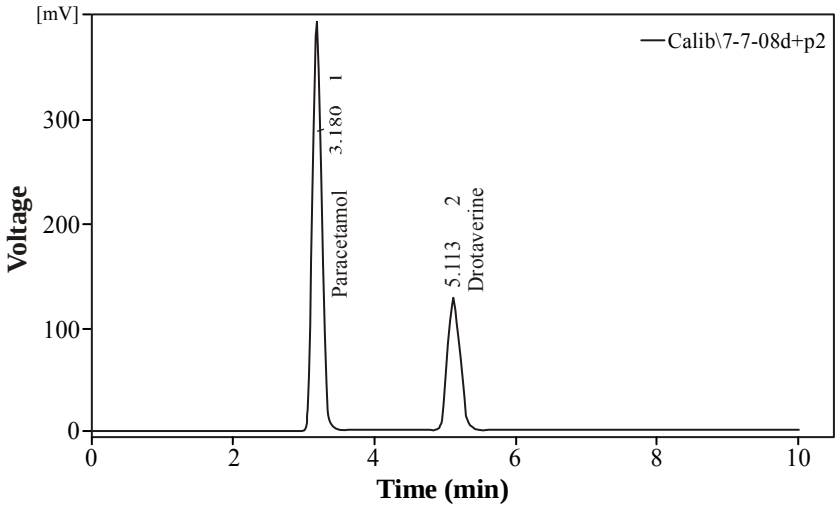


Fig. 2: Typical HPLC chromatogram of paracetamol and drotaverine hydrochloride

Chromatographic conditions

Separation and analysis was carried on LUNA Phenomenex C-18 column with 250 mm × 4.6 mm i.d. having 5-micron particle size. The mobile phase consisting of methanol : water (pH adjusted to 3.34 with 0.1% ortho phosphoric acid) in the ratio of 52 : 48 (v/v) was selected for analysis. The mobile phase was filtered through 0.45 µm membrane filter and sonicated before use. The separation was carried out at room temperature and the mobile phase was pumped at a flow rate of 1 mL/min.The injection volume was 20 µL and run time was kept 10 min. The wavelength was selected by scanning standard solutions of both the drugs over 200 nm to 400 nm and found that both the components show reasonably good response at 246 nm.

Preparation of stock solution of paracetamol and drotaverine hydrochloride

Accurately weighed amounts of paracetamol (50 mg) and drotaverine hydrochloride (50 mg) were transferred to 50 mL volumetric flasks separately and were dissolved in 25 mL of mobile phase. The solutions were sonicated for 5 min and then made up the volume to 50 mL with mobile phase.

Calibration curve

Apropriate aliquots were pipetted out from standard stock solution into a series of 5 mL volumetric flasks. The volume was made up to the mark with mobile phase to get solutions having concenteration range 2-36 µg/mL for paracetamol and 1-60 µg/mL for drotaverine. Triplicate dilutions of each concenteration were injected into RP-HPLC system and chromatographed under conditions the above mentioned conditions and elution was monitered at 246 nm.

Analysis of formulation

Twenty tablets (Drotin TM) were weighed and finely powdered. Accurately weighed quantity of tablet powder equivalent to 25 mg of paracetamol and 4 mg of drotaverine hydrochloride was transferred to a 25 mL volumetric flask. The contents were dissolved by sonicating in mobile phase for 10 min and made up to the mark with mobile phase. This solution was filtered through 0.45 µm membrane filter and filtrate was collected. From the filtrate, different aliquots were taken in separate 5 mL volumetric flask. The contents of the flask were made up to the volume with the mobile phase and mixed well. The solutions were sonicated for 10 min and 20 µL of each sample solution was injected into the column under above mentioned chromatographic conditions. The amount of paracetamol and drotaverine hydrochloride per tablet was calculated by comparing the peak area values of sample and standard solutions.

Validation of method[2]

System suitability tests

System suitability tests were carried out on freshly prepared mixed standard stock solutions of paracetamol and drotaverine hydrochloride for six times. 20 µL of the each solution was injected under optimized chromatographic conditions (Table 1). System suitability parameters for the method are listed in Table 2.

Linearity and range

The linearity of the method is its ability to elicit test results that is directly proportional to the concentration of analyte in sample. The stock solutions were further diluted with mobile phase to get a concentration of 2-36 µg/mL of paracetamol and 1-60 µg/mL of drotaverine hydrochloride. The solutions were filtered through 0.45 µm membrane filter and then 20 µL of filtrate was injected each time into the column. Each concentration was injected six times into the column and the corresponding chromatograms were obtained.

Table 1: Optimised chromatographic conditions

Parameters	Method
Stationary phase (column)	Shimadzu Lc-10AT liquid pump,SPD-10A UV-Visible detector, a Phenomenex C-18 RP-HPLC column (250 × 4.6 mm 5 µm, I.D)
Mobile phase	Methanol : Water (pH adjusted to 3.34 with 0.1% ortho-phosphoric acid) 52 : 48 (v/v)
Flow rate (mL/min)	1
Runtime (min)	10
Column temperature (^oC)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	246

Table 2: System suitability test parameters

Parameters	Paracetamol	Drotaverine hydrochloride
Retention time *(min)	3.184	5.122
Theoretical plates (N)*	3357	4645
Tailing factor (F)*	1.224	1.111
Resolution *	-	7.133

*Average of six observations

Evaluation of the drugs was performed with UV detector at 246 nm and a calibration graph was obtained by plotting peak area vs. the respective concentration of drug. The plots of peak area versus the respective concentration were found to be linear with coefficient of correlation (R^2 = 0.999) for paracetamol and (R^2 = 0. 9991) for drotaverine hydrochloride (Figs. 3 and 4). Linear regression least square fit data obtained from the measurements along with the respective slope (m), intercept (c) and correlation coefficient (r^2) are presented in Table 3.

Specificity

Specificity of the method was established by adding the commonly used excipients to the sample solution. It was observed that there was no interference of the placebo with the principal peaks.

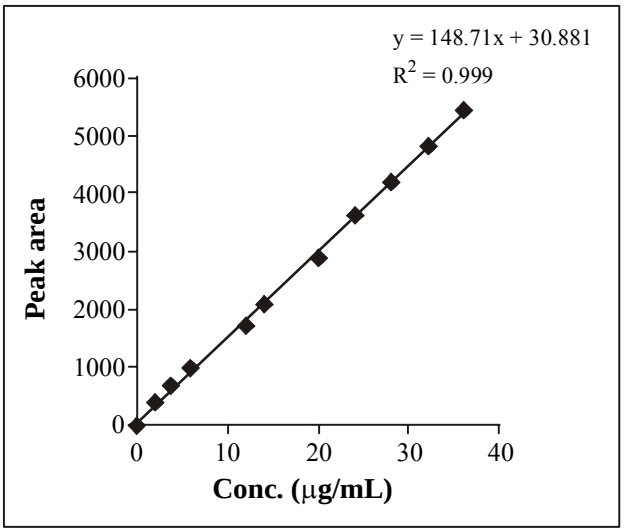


Fig. 3: Calibration curve of paracetamol

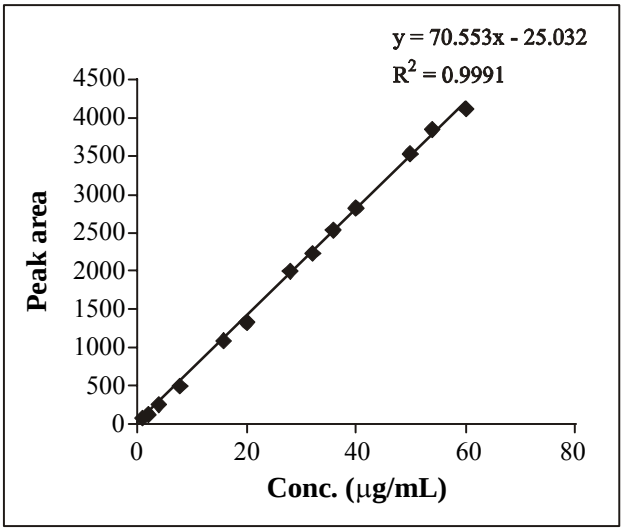


Fig. 4: Calibration curve of drotaverine hydrochloride

Precision

The intra-day and inter-day variations of the method were determined using three replicate injections of three different concentrations and analyzed on the same day and on three different days. The response factor of drug peaks and % RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

Recovery studies

Accuracy of the method was determined by recovery studies of paracetamol and drotaverine hydrochloride. Known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC method. The study was done with three different concentration levels. Each determination was performed in triplicate. The results of recovery analysis are presented in Table 4. The mean recovery is within the acceptance limit and hence, the method was found to be reasonably accurate.

Table 3: Validation parameters

Parameters	Paracetamol	Drotaverine hydrochloride
Linearity and range (µg /mL)	2-36	1-60
Regression equation	y=148.71x+30.881	y= 70.553x-25.032
Slope (m)	148.71x	70.553x
Intercept (c)	30.881	-25.032
Correlation coefficient (R^2)	0.999	0.9991
LOD (µg/mL)	0.4	0.2
LOQ (µg/mL)	1.2	0.6
Intra-day precision (%RSD)*	0.12	0.17
Inter-day precision (%RSD) *	0.13	0.19

* Average of three determinations

Table 4: Results of the recovery study

Amount of drug added (mg)		Amount of drug recovered (mg)		% Recovery± SD *	
Para.	**DH**	**Para.**	**DH**	**Para.**	**DH**
10	10	10.00	1 0.01	100.00 ± 0.021	100.10 ± 0.023
20	20	20.04	19.98	100.20 ± 0.035	99.90 ± 0.020
30	30	29.98	30.05	99.93 ± 0.027	100.16 ± 0.014

*Average of three determinations; Pare. – Paracetamol and DH = Drotaverine hydrochloride

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and limit of quantification of the method were determined by injecting progressively low concentration of the standard solutions of paracetamol and drotaverine hydrochloride solutions with the optimized chromatographic conditions. The limit of detection and limit of quantification was 0.4 µg/mL and 1.2 µg/mL for paracetamol and 0.2 µg /mL and 0.6 µg/mL for drotaverine hydrochloride. The signal to noise ratio is 1 : 3 for LOD and 1 : 10 for LOQ.

Table 5: Results of analysis of formulation by the proposed

Pharmaceutical formulation	Label claim (mg)		Amount found (mg)		% Labeled claim mean ± SD*	
	Para.	DH	Para.	DH	Para	DH
Tablet 1	500	80	500.04	79.93	100.08 ± 0.21	99.91 ± 0.24
Tablet 2	500	80	499.67	80.08	99.93± 0.13	100.1 ± 0.05

*Average of six determinations

Stability of solution

Sample and standard solution were stable for 24 h and do not show any appreciable change in the retention time and peak areas.

Robustness

Robustness of the method was checked by deliberately altering the analytical wavelength ± 2 nm, flow rate ± 0.1 mL/min, slight variation in the pH ± 0.2 and composition of the mobile phase, which showed that retention time values of the peaks of interest remain unaffected by small changes in the operational parameters. No marked changes in the chromatogram demonstrated that the developed HPLC method is robust.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained. Different mobile phases were tried and satisfactory separation with well resolved and good symmetric peaks were obtained with the mobile phase containing methanol : water (pH adjusted to 3.34 with 0.1% ortho-phosphoric acid) in the ratio of 52 : 48 (v/v). Mean retention time for paracetamol and drotaverine hydrochloride were found to

be 3.18 min and 5.12 min, respectively, and resolution between paracetamol and drotaverine hydrochloride was found to be 7.13, which indicates good separation of both the compounds. Calibration curves for paracetamol and drotaverine hydrochloride were obtained by plotting the peak areas and respective concentrations over the range 2-36 µg/mL for paracetamol and 1-60 µg/mL for drotaverine hydrochloride. Regression equation and coefficient of correlation obtained for calibration curves were $y = 148.71x + 30.881$, with $R^2 = 0.999$ for paracetamol, and $y = 70.553x - 25.032$, with $R^2 = 0.9991$ for drotaverine hydrochloride (Figs. 3 and 4). System suitability parameters are presented in Table 2. Intra-day and inter-day studies were carried out by using three different samples on the same day and on three different days. Percent RSD value was below 2%, which indicates that method is precise (Table 3). Percentage recovery was found to be in the range of 99.93 %-100.2% for paracetamol and 99.90%-100.16% for drotaverine hydrochloride (Table 4). The detection limit and quantification limit for paracetamol and drotaverine hydrochloride was found to be 0.4 µg/mL, 0.2 µg/mL and 1.2 µg/mL, 0.6 µg/mL (Table 3). Stability studies were carried out by keeping both the standard and sample solutions of paracetamol and drotaverine hydrochloride for 24 h at room temperature, which showed that retention time and peak areas remained almost unchanged and no degradation products were observed. Robustness of the method was checked after deliberate variation in analytical wavelength, flow rate and pH of mobile phase. The proposed method was used to evaluate commercially available tablets containing paracetamol and drotaverine hydrochloride. Six replicate determinations were performed on the accurately weighed amounts of the contents of the tablets. The percentage assay was found to be 99.93-100.08% for paracetamol and 99.91-100.1% for drotaverine hydrochloride (Table 5).

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, robust, precise, and accurate. The correlation coefficient so obtained for regression analysis showed that good correlation exists between the concentration of analyte taken and peak area ratio. The low % RSD values obtained during precision study confirms acceptable intra and inter-day response variation and percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Thus, this can be used for simultaneous determination of paracetamol and drotaverine hydrochloride in tablet dosage form instead of processing and analyzing each drug separately.

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