Simultaneous estimation for Dicyclomine HCl and Simethicone in bulk and oral liquid drop formulation: an RP-HPLC method development and validation

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Abstract

Background: A new, validated, and selective RP-HPLC technique was established for quantitative estimation of Dicyclomine HCl and Simethicone in the pharmaceutical oral liquid drop formulations. The chromatographic conditions were adjusted using Luna C18 (25 cm × 0.46 cm, 5 μm) column, water-methanol mixture (80:20, v/v) as solvent system and pH adjustment up to 7.4 using orthophosphoric acid. The absorbance was observed at 289 nm λmax injecting 20 μl sample solution and maintain the ambient temperature (30 °C) with 1 ml per min flow rate.

Results: The retention times of Dicyclomine HCl and Simethicone were determined 2.962 and 4.091 min, respectively. The developed analytical technique was validated for accuracy, precision, linearity, specificity, sensitivity, ruggedness, and robustness as per ICH guidelines. The LOD was found to be 0.72 and 0.57 μg per ml for Dicyclomine HCl and Simethicone, respectively. But, LOQ was 2.19 and 1.73 μg per ml, respectively.

Conclusions: This validated method showed good precision (RSD% < 1) with acceptable linearity (≥ 0.999). Intra- and inter-day relative RSD of retention times and AUC (area under curve) were found to be less than 2.0%. The method was also significantly useful as economic technique for simultaneous quantitative determination of Dicyclomine HCl and Simethicone in the bulk and pharmaceutical formulation.

Keywords: Dicyclomine HCl, Oral liquid drop formulation, RP-HPLC, Simethicone

Background

Dicyclomine hydrochloride (DIC) is 2-(diethylamino) ethyl-1-cyclohexylcyclohexane-1-carboxylate hydrochloride chemically is used as a parasympatholytic and antispasmodic agent. This drug blocks effect of acetylcholine on parasympathetic receptors in the smooth muscle along with direct relaxing effect on smooth muscle [1–3].

Simethicone (SIM), α-(trimethylsilyl)-ω-methylpolysiloxane(dimethylsilylene), is present with silicon dioxide to enhance defoaming properties of silicon. It is used as an anti-foaming agent to minimize bloating, distress, or pain due to excessive gas [4–8].

The structures of these drugs are given in Figs. 1 and 2. According to the literature, several RP-HPLC methods were availed for quantitative determination of DIC and SIM individually in pharmaceutical dosages forms [9–21]. But, there is no RP-HPLC technique was identified until now for simultaneous estimation of both drug combinations in the bulk and marketed formulation. Our research work focused on developing an economical, validated, and sensitive method for the simultaneous determinations of DIC and SIM in oral liquid drop formulation.
Methods

The analytical samples of DIC and SIM were provided as gift samples by Blue Cross Laboratories Pvt Ltd., Mumbai. They also provided that the gift sample of dicyclomine hydrochloride and simethicone contain 99.87% and 97.38% equivalent quantity of pure drug, respectively. An oral liquid drop formulation (Meftal-Spas Drops manufactured by Blue Cross Laboratories Ltd.) used for the analysis was obtained from market labeled with 10 mg/ml dicyclomine hydrochloride and 40 mg/ml simethicone in the each unit (Fig. 3). Methanol and water of HPLC grades, orthophosphoric acid of analytical grade were purchased from Sigma and Himedia Chemicals supplied by S. K. Traders Indore (M.P.). The samples were observed at 289 nm using the Shimadzu VP Series HPLC system associated with photo diode array detector, LC-10AT pump, and manual injector of 20 μl volumes with rhodyne valve. The samples were analyzed under optimized chromatographic conditions on a Luna C_{18} (5 μm, 25 cm × 0.46 cm i.d.) phenomenex column. An ultrasonic vibrations degasser was used for mobile phase prior to use.

Chromatographic conditions

A methanol and water mixture (20:80) was used as mobile phase. The optimized solvent system was passed through Luna C_{18} column with 1 ml/min flow rate for the interval of 8 min as total analysis time. After injection of 20 μl sample, elution of sample was observed by photo diode array detector at 289 nm.

Preparation of solvent system

A mixture of water 800 ml (80%) and 200 ml HPLC grade methanol (20%) was prepared along with degassing on ultrasonic water bath. The pH of 7.4 for mixture was adjusted using orthophosphoric acid. The final resultant mixture was filtered through 0.45-μm membrane filter.

Preparation of working stock solution

The accurately weighed 10 mg of drug was dissolved using methanol-water mixture in 100-ml volumetric flask. Then, stock solution of 100 μg/ml was obtained through adjust the volume up to the mark using same solvent.

Preparation of sample solution

A volume of 10 ml of oral drop containing amount equivalent to 100 mg Dicyclomine HCl and 400 mg Simethicone was dissolved using about 80 ml of solvent in 100-ml volumetric flask then sonicated for 10 min to obtain clear solution. A volume was adjusted with the same solvent and filtered using 0.45-μ membrane filter. Dilutions prepared to reduce the strength of resultant solution by serial dilution technique and then 20 μl of above solutions were injected into the column under the set chromatographic conditions.
System suitability study
The system suitability was studied with the help of the-oretical plates, tailing factor, and resolution. All the re-sults of above parameters demonstrate the suitability of system for the analysis of this drug combination reported in Table 1.

Purity assessment (percentage purity w/w)
The content of each drug in the marketed formulation was checked and calculated the percentage purity. This study was also confirmed the purity of procured drug gift sample.

Method validation
The developed analytical method was validated according to ICH guidelines (Q2B) [22, 23].

Linearity study
The linearity was observed as linear regression study of absorbance for both drugs with respect to concentration in range 5 to 40 μg/ml. Several aliquots of known strengths were prepared with dilution of working stock solution using mobile phase. The AUC and concentration data for all the aliquots were determined and reported in Table 2. The linearity curves were obtained by plotting a graph between relative AUC and concentration shown in Figs. 4 and 5. Linearity curves were constructed using relative peak area to avoid very high values for intercept.

Accuracy or recovery study
This study was accomplished with standard addition method (n = 3) at 50%, 100%, and 150% analytical concentration. In this method, known quantity of DIC and SIM was mixed to pre-estimated sample solution and further proceeds to the developed HPLC method. Then, experimental and true values were compared. The percentage recovery of Dicyclomine HCl and Simethicone is reported in Table 3.

Precision study
The study was analyzed by taking six independent quanti-tative estimation of standard (10 μg/ml Dicyclomine HCl + 40 μg/ml Simethicone) as well as sample solution (oral drops formulation) on same instrument reported in Tables 4 and 5.

Intra-day and inter-day repeatability study
The drug estimation of standard drug solution was per-formed for thrice on the same day and three consecutive days over duration of a week. The comparison of results was used for the repeatability study of developed analytical method. All the results are shown in Tables 6 and 7.

Sensitivity
The sensitivity of measurement for Dicyclomine HCl and Simethicone by using developed RP-HPLC method was estimated as LOD and LOQ. These indicate the quantitation limit of individual method which is lowest measurable quantity of analyte in the sample. These pa-rameters were determined with the help of equation given as follows:

| Parameter       | Dicyclomine HCl | Simethicone |
|-----------------|-----------------|-------------|
| Tailing factor (T) | 1.04            | 1.36        |
| Theoretical plates (N) | 3882            | 4282        |
| Resolution (Rs) | -               | 5.13        |

Fig. 3 Chromatogram of the mixture of Dicyclomine HCl (10 μg/ml) and Simethicone (40 μg/ml)
LOD = 3.3 × N/B and LOQ = 10 × N/B
where \(N\) is the standard deviation of the peak areas for drug \((n = 5)\) that consider as measure of noise, and \(B\) is the slope of the corresponding calibration curve.

The calculation was completed using the above formulas and found the results given as follows:

Dicyclomine HCl LOQ = 10 × 69.37/316.34 = 2.19 μg/ml
Simethicone LOQ = 10 × 1231.39/7092.7 = 1.73 μg/ml
Dicyclomine HCl LOD = 3.3 × 69.37/316.34 = 0.72 μg/ml
Simethicone LOD = 3.3 × 1231.39/7092.7 = 0.57 μg/ml

Robustness
A solution containing 10 μg/ml Dicyclomine HCl and 40 μg/ml Simethicone was prepared and estimated by two different analysts under the same operational and environmental conditions. A result of reproducibility is compared for robustness study of analytical method reported in Table 8.

Result and discussion
This research work focused on the adequate development of RP-HPLC method with subsequent validations for quantitative analysis of DIC and SIM in oral drop

| S. no. | Dicyclomine HCl | Simethicone |
|-------|----------------|-------------|
|       | Conc. (μg/ml) | AUC | Relative peak area | Conc. (μg/ml) | AUC | Relative peak area |
| 1     | 5              | 3772 | 0.78             | 5              | 46,317 | 3.84 |
| 2     | 10             | 5292 | 1.5              | 10             | 82,562 | 7.63 |
| 3     | 15             | 6855 | 2.24             | 15             | 115,930 | 11.12 |
| 4     | 20             | 8413 | 2.97             | 20             | 149,872 | 14.67 |
| 5     | 25             | 9972 | 3.71             | 25             | 183,292 | 18.17 |
| 6     | 30             | 11,533 | 4.45         | 30             | 218,821 | 21.88 |
| 7     | 35             | 13,091 | 5.18         | 35             | 260,412 | 26.23 |
| 8     | 40             | 14,954 | 6.06         | 40             | 295,971 | 29.95 |

\(r^2\) 0.9995 0.9991
Slope 0.1494x 0.7418
Intercept at \(Y\) axis 0.0007 – 0.0039

Fig. 4 Calibration curve of Dicyclomine HCl for quantitative estimation
formulation using C18 column and PDA detector. The water-methanol mixture (80:20, v/v) was used as mobile phase with adjustment of pH to 7.4 using orthophosphoric acid. The isosbestic point was used as maximum wavelength for the identification of drug samples elution from HPLC column which was found out through scanning of UV spectrum. The RP-HPLC method was performed at various chromatographic conditions with different changes in solvent composition and flow rate, etc. The system suitability parameters like peak resolution, tailing factor, and theoretical plates were used for the optimization of RP-HPLC method which was reported in Table 1.

The resolution was found to be more than 2 that indicate good separation of DIC and SIM with developed RP-HPLC method. The values of asymmetric/tailing factors and theoretical plates for DIC and SIM were also favorable for efficient separation (asymmetric/tailing factors and theoretical plates should be ≤ 2.0 and > 2000, respectively). When a mixture of Dicyclomine HCl (10 μg/ml) and Simethicone (40 μg/ml) was run into HPLC under optimized chromatographic conditions, we were found two symmetric, well-resolved peaks for the retention of DIC and SIM along with retention time at 2.962 and 4.091 min, respectively. A HPLC chromatogram is given in Fig. 3.

The assay was also performed for each drug in marked formulation to check its purity and calculated the percentage purity. The percentage purity of DIC and SIM was found to be 101.06 and 96.98%, respectively.

The validation of developed analytical method was completed with respect to parameters like linearity, specificity, accuracy, precision, repeatability, sensitivity, and ruggedness study.

The different concentrations of working standard solutions of DIC and SIM were given linearity between the ranges of 5 and 40 μg/ml with significant R square value reported in Table 2. The linear line equations for Dicyclomine HCl and Simethicone were \( y = 0.7418x - 0.0039 \) and \( y = 0.1494x + 0.0007 \) and \( y = 0.7418x - 0.0039 \), respectively.

### Table 3 Accuracy or recovery study of Dicyclomine HCl and Simethicone for developed analytical method

| Drug         | Quantity taken | Quantity added | Quantity recovered | % quantity recovered | Mean ± SD | % RSD |
|--------------|----------------|----------------|--------------------|----------------------|-----------|-------|
| Dicyclomine HCl | 100            | 50             | 50                 | 48.7                 | 97.40     | 98.28 ± 0.94 | 0.95 |
|              | 100            | 100            | 100                | 98.2                 | 98.20     | 99.27 |
|              | 100            | 150            | 150                | 148.9                | 99.27     | 99.16 ± 1.28 | 1.30 |
| Simethicone  | 400            | 50             | 200                | 196.2                | 98.10     | 99.16 ± 1.28 | 1.30 |
|              | 400            | 100            | 400                | 102.4                | 100.60    | 100.60 |
|              | 400            | 150            | 600                | 592.8                | 98.80     | 98.80 |
The accuracy of the developed RP-HPLC method was determined by calculating recoveries of Dicyclomine HCl and Simethicone by method of standard addition. Recovery studies were performed in triplicate, and results are reported in Table 3. All the drugs were found to be stable with a recovery of more than 98%. The observed percentage recovery of DIC and SIM was comparable with the corresponding labeled amounts. System and method precision was observed by quantitative analysis of six independent standard as well as oral drop samples. For the repeatability study, the intra-day and inter-day quantitative estimation were carried out for three selected samples concentrations of DIC and SIM. The LOQ for DIC and SIM was found to be 2.19 and 1.73 μg/ml, respectively (Table 3). Robustness of developed method was determined by deliberately changing the analyst at same experimental conditions. All the parameters are summarized in Table 9 were confirmed about the

| Table 4 | System precision study for Dicyclomine HCl and Simethicone standard solutions |
|---------|----------------------------------|
| S. No.  | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | AUC Retention time |
| 1.      | 5345 2.955 | 308,137 4.083 |
| 2.      | 5337 2.959 | 307,545 4.088 |
| 3.      | 5289 2.965 | 309,972 4.095 |
| 4.      | 5298 2.956 | 310,085 4.086 |
| 5.      | 5332 2.963 | 307,951 4.078 |
| 6.      | 5321 2.956 | 306,505 4.094 |
| Mean ± SD | 5320.33 ± 22.37 | 308,365.8 ± 1406.85 |
| % RSD   | 0.42 | 0.14 |

| Table 5 | Method precision study for Dicyclomine HCl and Simethicone sample solutions |
|---------|----------------------------------|
| S. No.  | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | AUC Retention time |
| 1.      | 6044 2.958 | 311,163 4.085 |
| 2.      | 5978 2.965 | 312,897 4.094 |
| 3.      | 6023 2.953 | 309,817 4.087 |
| 4.      | 6021 2.964 | 309,987 4.081 |
| 5.      | 5991 2.961 | 312,926 4.092 |
| 6.      | 5983 2.958 | 309,920 4.095 |
| Mean ± SD | 6006.66 ± 26.43 | 311,118.3 ± 1472.59 |
| % RSD   | 0.44 | 0.15 |

| Table 6 | Repeatability study of intra-day samples for developed analytical method |
|---------|-------------------------------|
| S. No.  | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | AUC Retention time |
| 1.      | 5994 2.968 | 315,826 4.079 |
| 2.      | 6024 2.964 | 313,228 4.089 |
| 3.      | 6052 2.958 | 312,958 4.086 |
| Mean ± SD | 6023.33 ± 29.0 | 314,004.7 ± 1583.66 |
| % RSD   | 0.48 | 0.16 |

| Table 7 | Repeatability study of inter-day samples for developed analytical method |
|---------|----------------------------------|
| S. No.  | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | AUC Retention time |
| 1.      | 6144 2.953 | 317,328 4.093 |
| 2.      | 6112 2.963 | 319,519 4.088 |
| 3.      | 6084 2.96 | 316,491 4.082 |
| Mean ± SD | 6113.33 ± 30.02 | 317,779.3 ± 1563.64 |
| % RSD   | 0.49 | 0.17 |

| Table 8 | Robustness study of developed analytical method |
|---------|----------------------------------|
| Analyst | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | Peak area Retention time |
| I       | 6186 2.955 | 317,382 4.081 |
|         | 6204 2.953 | 319,609 4.088 |
|         | 6257 2.96 | 316,292 4.093 |
|         | 6192 2.959 | 319,508 4.083 |
|         | 6217 2.955 | 319,723 4.086 |
| Mean ± SD | 6211.2 ± 28.22 | 318,502.8 ± 1570.57 |
| % RSD   | 0.45 | 0.10 |

| Table 9 | Analysis of variance study of developed analytical method |
|---------|----------------------------------|
| Analyst | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | Peak area Retention time |
| I       | 6186 2.955 | 317,382 4.081 |
|         | 6204 2.953 | 319,609 4.088 |
|         | 6257 2.96 | 316,292 4.093 |
|         | 6192 2.959 | 319,508 4.083 |
|         | 6217 2.955 | 319,723 4.086 |
| Mean ± SD | 6211.2 ± 28.22 | 318,502.8 ± 1570.57 |
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The accuracy of the developed RP-HPLC method was determined by calculating recoveries of Dicyclomine HCl and Simethicone by method of standard addition. Recovery studies were performed in triplicate, and results are reported in Table 3. All the drugs were found to be stable with a recovery of more than 98%. The observed percentage recovery of DIC and SIM was comparable with the corresponding labeled amounts. System and method precision was observed by quantitative analysis of six independent standard as well as oral drop samples. For the repeatability study, the intra-day and inter-day quantitative estimation were carried out for three selected samples concentrations of DIC and SIM. The LOQ for DIC and SIM was found to be 2.19 and 1.73 μg/ml, respectively. The LOD for DIC and SIM was found to be 0.72 and 0.033 μg/ml, respectively (Table 3). Robustness of developed method was determined by deliberately changing the analyst at same experimental conditions. All the parameters are summarized in Table 9 were confirmed about the

| Table 10 | Analysis of variance study of developed analytical method |
|---------|----------------------------------|
| Analyst | Dicyclomine HCl | Simethicone |
|         | AUC Retention time | Peak area Retention time |
| I       | 6186 2.955 | 317,382 4.081 |
|         | 6204 2.953 | 319,609 4.088 |
|         | 6257 2.96 | 316,292 4.093 |
|         | 6192 2.959 | 319,508 4.083 |
|         | 6217 2.955 | 319,723 4.086 |
| Mean ± SD | 6211.2 ± 28.22 | 318,502.8 ± 1570.57 |
| % RSD   | 0.45 | 0.10 |
good precision (RSD% < 1) with acceptable linearity (≥ 0.999) for validated method. The relative RSD with different studies for this method was also found to be less than 2.0%. The proposed method was successfully applied to the determination of DIC and SIM in their combined dosage form.

### Conclusion

We were found on the basis of reported literature that there was not a significant method for the simultaneous estimation of dicyclomine hydrochloride and simethicone in pharmaceutical formulation. All the methods were reported either only for another combinations or individual drugs. Therefore, it needs to develop new method for their quantitative analysis in the bulk or pharmaceutical formulation. We had developed an analytical RP-HPLC technique successfully for simultaneously determination of these drugs in oral drops formulation. All the validation results for developed method were found to be under the limits as per ICH guidelines. All the results showed that the developed method is new, simple, rapid, and validate. Furthermore, it can be employed for the analysis of Dicyclomine HCl and Simethicone in bulk and oral liquid dosage form. The simplicity, reproducibility, and cost-effectiveness of method complete the objective of research work.

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### Table 9: Summary of validation parameters

| Parameters     | Dicyclomine HCl | Simethicone |
|----------------|-----------------|-------------|
| Linearity range (ng/ml) | 5–40 μg/ml | 5–40 μg/ml |
| Regression equation | $Y = 0.1494X + 0.0007$ | $Y = 0.7418X - 0.0039$ |
| Correlation Coefficient ($r^2$) | 0.9995 | 0.9991 |
| Accuracy (% RSD) | 0.95 | 1.30 |
| Precision (% RSD) | 0.42 | 0.45 |
| Repeatability ($n = 5$) | Intra-day ($n = 3$) | 0.48 | 0.50 |
| | Inter-day ($n = 3$) | 0.49 | 0.49 |
| Robustness | Analyst I ($n = 6$) | 0.45 | 0.49 |
| | Analyst II ($n = 6$) | 0.49 | 0.51 |
| | LOD (μg/ml) | 0.72 | 0.57 |
| | LOQ (μg/ml) | 2.19 | 1.73 |

### Authors’ contributions

We have assured that “all authors have read and approved the manuscript.” All the authors have equal contribution and participation in this research work. PK has analyzed all samples on HPLC instrument and completed the experimental work. He had completed his work under the supervision of SK and MP who help him to elaborate the methodology as well as theoretical approach. AM also helped him in their experimental work and guides to resolve the complications. PW helped him in the data analysis, statistical study, and manuscript editing.

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