STABILITY–INDICATING DENSITOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF DONEPEZIL HYDROCHLORIDE AND CURCUMIN IN INSITU NASAL GEL

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Abstract:
A stability indicating high performance thin layer chromatography (HPTLC) method was developed and validated for determination of Donepezil hydrochloride and curcumin in insitu nasal gel. Study was performed on pre-coated silica gel HPTLC plates using toluene: methanol: glacial acetic acid (8: 2: 0.1 v/v/v) as the mobile phase. A TLC scanner set at 254 nm was used for direct evaluation of the chromatograms in the reflectance/absorbance mode. Method was validated according to ICH guidelines. The correlation coefficients of calibration curves were found to be 0.994 and 0.988 in the concentration range of 300–1800 and 120–720 ng band⁻¹ for donepezil hydrochloride and curcumin, respectively. The method had an accuracy of 100.6 % for donepezil hydrochloride and 99.08% for curcumin. The method had the potential to determine these drugs simultaneously from dosage forms without any interference of the excipients. Donepezil hydrochloride and curcumin were also subjected to acid, base, oxidation, heat and photo-degradation studies. The degradation products obtained were well resolved from the pure drugs with significantly different Rf values. As the method could effectively separate the drugs from its degradation products, it can be used for stability-indicating analysis.

Keywords: High-performance thin-layer chromatography, Donepezil hydrochloride and Curcumin, Stability-indicating method

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Please cite this article in press Avinash R. Tekade et al., Stability–Indicating Densitometric Method for Simultaneous Determination of Donepezil Hydrochloride and Curcumin in Insitu Nasal Gel, Indo Am. J. P. Sci, 2018; 05(05).
1. INTRODUCTION:
Donepezil HCl (Fig.1) is chemically 2, 3-Dihydro-5, 6-dimethoxy-2-[(1-(phenylmethyl)-4-piperidinylmethyl]-1H-inden-1-one hydrochloride. It is centrally acting reversible acetyl cholinesterase inhibitor. It is used in the management of Alzheimer’s disease where it is used to increase cortical acetylcholine. & curcumin (Fig 2) is chemically (1E, 6E)-1, 7-bis (4-hydroxy- 3-methoxyphenyl) -1, 6- heptadiene-3, 5-Dione. Curcumin has been shown to have multipotent effects against various symptoms of Alzheimer’s disease. It shows anti-Alzheimer’s activity by exerting neuroprotective, antioxidant, and anti-inflammatory properties.

These two drugs are formulated as an in situ gel in a combined formulation.

![Fig. 1. Structure of Donepezil hydrochloride](image1)

![Fig.2. Structure of Curcumin](image2)

Validated assays have been reported for each drug individually. For analysis of donepezil hydrochloride from human plasma, high performance liquid chromatographic analysis with UV detection [1] LC/MS [2] and HPTLC determination [3] was reported. Spectrophotometric techniques have also been reported for determination of donepezil in bulk and tablet dosage forms [4,5]. High performance liquid chromatography for curcumin individually [6] and in combination with other drugs in plasma and pharmaceutical dosage form [7] LC/MS analysis [8], UV spectrophotometric quantitation methods in bulk and pharmaceutical formulation [9] and HPTLC methods [10] have been reported. There are no reported assay methods that permit the simultaneous quantification of donepezil hydrochloride and curcumin in combined dosage form. The aim of the present work was to develop and validate a new simple, rapid, selective, cost effective and stability indicating HPTLC method for simultaneous determination of donepezil hydrochloride and curcumin in pharmaceutical gel formulation.

2. EXPERIMENTAL

2.1. Materials
Analytical pure samples of donepezil hydrochloride (Dr. Reddy’s Laboratories Ltd, Hyderabad, India) and Curcumin (Rathod Enterprises, Pune, India) were used in the study. The pharmaceutical dosage form used in this study was in house prepared in situ nasal gel containing 4.1 mg of donepezil hydrochloride and 5.9 mg of curcumin. The solvents and chemicals used in the study were of AR grade (Rankem, India).

2.2. Instrumentation
Microsyringe (Linomat syringe 659.004, Hamilton-Bonaduz Schweiz, Camag, Switzerland), pre-coated silica gel 60 F-254 aluminium plates (10 \times 10 cm, 250 μm thickness; Merck, Germany), Linomat 5 applicator (Camag, Muttenz, Switzerland), twin trough chamber (20 \times 10 cm; Camag, Muttenz, Switzerland), saturation pad (Camag, Muttenz, Switzerland), UV chamber (Camag, Muttenz, Switzerland), TLC scanner III (Camag, Muttenz, Switzerland), winCATS version 1.4.0 software (Camag, Muttenz, Switzerland) were used in this study. Microsoft excel was also used to treat data statistically.

2.3. Preparation of standard solutions
Standard stock solutions were prepared by dissolving separately 10 mg of donepezil hydrochloride and curcumin each in 10 mL of methanol to obtain a concentration of 1000 μg mL⁻¹. The standard stock solutions were suitably diluted by methanol to obtain the working standard solutions of both donepezil hydrochloride and curcumin.

2.4. Preparation of sample solutions
An accurately weighed gel equivalent to 41 mg of donepezil hydrochloride was weighed, transferred to a 100 mL volumetric flask and volume made up to about 70 mL with methanol. The solution was sonicated for about 30 min, then diluted to volume with the same solvent and filtered through Whatman filter paper No. 42. Working sample solutions were freshly prepared by diluting suitable volumes of the stock sample solution with methanol.
2.5. Optimized chromatographic conditions
Suitable volumes of standard and sample solutions (μL) were applied to the HPTLC plates, 10 mm from the bottom and 10 mm from the side edges in the form of bands or streaks with band length of 6 mm. The mobile phase consisting of toluene: methanol: glacial acetic acid (8:2:0.1 v/v/v) was used in each chromatographic run. Ascending development technique was carried out in twin trough chambers. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25 ± 2 °C) that was assisted by saturation pads. The distance covered by the solvent front was 8 cm, which took about 15 min. The spots were scanned using the TLC scanner 3 in the reflectance/absorbance mode at 254 nm and all measurements were operated by win CATS software. Concentrations of the separated compounds were determined from the intensity of reflected light and peak areas were used for evaluation.

2.6. Analysis of formulation
The solutions were prepared as discussed above. Suitable working sample solutions (2.0 μL) containing donepezil hydrochloride and curcumin in the concentration 4100 ng: 5900 ng were prepared respectively, applied on HPTLC plates and analyzed under the optimized chromatographic conditions.

2.7. Method validation
The method was validated in compliance with ICH guidelines (ICH, 1994, 1996). The following parameters were used for validation of the developed method.

2.7.1. Linearity
Linear relationship between peak area and concentration of the drugs was evaluated over the concentration range expressed in ng band⁻¹ by making five replicate measurements in the concentrations range of 1000–6000 ng band⁻¹ for both donepezil hydrochloride and curcumin.

2.7.2. Precision
Precision of the developed method was studied by performing repeatability and intermediate precision studies. The sample application and measurement of peak area was determined by performing six replicate measurements of the same band using a sample solution containing 410 ng band⁻¹ of donepezil hydrochloride and 590 ng band⁻¹ of curcumin each.

2.7.3. Limit of detection (LOD) and limit of quantitation (LOQ)
The limits of detection and quantification of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curves of donepezil hydrochloride and curcumin using the formulae as given below.

Limits of Detection=3α/S  Limits of Quantification=10α/S

Where α is the standard deviation of the y-intercepts and S is the slope of the calibration curve.

2.7.4. Robustness
The effect of deliberate variations in method parameters like the composition of the mobile phase, volume of the mobile phase, time from spotting to development and time from development to scanning were evaluated in this study. The effect of these changes on both the Rf values and peak areas was evaluated by calculating the relative standard deviations (RSD) for each parameter.

2.7.5. Specificity
Peak purity of both was assessed to evaluate the specificity of the method. The sample and standard bands were scanned at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions. The specificity of the method was also determined by performing forced degradation studies. Standard stock solutions (1000 μg mL⁻¹) of donepezil hydrochloride and curcumin were employed in the study.

2.8. Stability studies
To evaluate the stability indicating properties of the developed HPTLC method, forced degradation studies were carried out in accordance to the ICH guidelines. The standard drugs were subjected to acid, base, oxidation, wet heat, dry heat and photo-degradation studies.

2.8.1. Acid-induced degradation study
HCl (0. 1 N, 3 mL) was added separately to 5 mL methanolic stock solutions of donepezil hydrochloride and curcumin in 25 mL volumetric flasks. The mixtures were refluxed at 80 °C for 2 hr and the volume was made up with methanol (200 μg mL⁻¹). The forced degradation was performed in the dark to exclude the possible degradation effect of light. The resulting solutions were applied to TLC plates and the chromatograms were run as described above.

2.8.2. Base-induced degradation study
For the base degradation study, NaOH (0. 1 N, 3 mL) was added separately to 5 mL methanolic stock solutions of donepezil hydrochloride and curcumin in
a 25 mL volumetric flask. The mixtures were refluxed at 80 °C for 2 hr and the volume was made up with methanol (200 μg mL⁻¹). The samples were then applied and analysed as described in the acid induced degradation study.

2.8.3. Hydrogen peroxide-induced (oxidation) degradation study

H₂O₂ (3 %, 3 mL) was added separately to 5 mL methanolic stock solutions of in 25 mL volumetric flasks and the volume was made up with methanol (200 μg mL⁻¹). The sample solutions were refluxed at 80 °C for 2 hr. The samples were then applied and analysed as described in acid induced degradation study.

2.8.4. Heat degradation study

For dry heat degradation study, the standard powder drugs were placed in an oven at 80 °C for 24 h. appropriate dilutions were prepared in methanol and then analyzed under the optimized chromatographic conditions.

2.8.5. Photo-degradation study

For the photo-degradation study, the standard powder drugs were exposed to UV light in a photo-stability chamber for 24 h. appropriate dilutions were prepared in methanol and then analysed under the optimized chromatographic conditions.

3. RESULTS AND DISCUSSION:

3.1. HPTLC method optimization

For the selection of appropriate mobile phase for the effective separation of donepezil hydrochloride and curcumin, several runs were made by using mobile phases containing solvents of varying polarity, at different concentration levels. Different mobile phase systems like toluene: methanol, chloroform: methanol, chloroform: diethyl ether: ethyl acetate, chloroform: ethyl acetate: acetic acid at different concentration levels was tried. Among the different mobile phase combinations employed, the mobile phase consisting of toluene : methanol : glacial acetic acid (8:2:0.1 v/v/v) gave the best resolution with sharp well defined peaks with Rf values of 0.23 ± 0.05 and 0.53 ± 0.02 for donepezil hydrochloride and curcumin, respectively. For the selection of analytical wavelength for the quantification of the drugs, the standard spots applied on silica gel were scanned and their overlain spectra were obtained on the HPTLC instrument. From the overlain spectra (Fig. 3), it was observed that both donepezil hydrochloride and curcumin exhibited strong absorbance at about 254 nm which was selected as the analytical wavelength for further analysis.

![Fig. 3: UV spectra comparison of the spots of donepezil hydrochloride and curcumin.](image-url)
3.2. Analysis of marketed formulation
The gel formulation was analysed using the developed method. The chromatogram of gel sample showed only two peaks at $R_f$ value of 0.53 and 0.23 for donepezil hydrochloride and curcumin respectively, indicating that there is no interference of the excipients present in the gel formulation. The content of donepezil hydrochloride and curcumin was calculated by comparing peak areas of sample with that of the standard (Table 1). The densitogram of gel formulation is shown in Fig. 3.

![Typical densitogram of donepezil hydrochloride and curcumin in pharmaceutical dosage form.](image)

Table 1. Assay results of the pharmaceutical gel form.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount present (in mg)</th>
<th>% Amount found$^a$</th>
<th>SD</th>
<th>% RSD$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil hydrochloride</td>
<td>4.1</td>
<td>100.48</td>
<td>0.894</td>
<td>0.894</td>
</tr>
<tr>
<td>Curcumin</td>
<td>5.9</td>
<td>100.10</td>
<td>0.812</td>
<td>0.812</td>
</tr>
</tbody>
</table>

$^a$ denotes average of six estimation.

3.3. Method validation
3.3.1. Linearity
Peak areas were found to have better linear relationship with the concentration than the peak heights. For donepezil hydrochloride, the $r^2$ was found to be 0.994, and for curcumin the $r^2$ was 0.988. Calibration graphs were constructed in the concentration range of concentrations range of 1000–6000 ng band$^{-1}$ for both donepezil hydrochloride and curcumin. The correlation coefficients, y-intercepts and slopes of the regression lines of the two drugs were calculated and are presented in Table 2.

Table 2: Summary of linear regression and validation data.

<table>
<thead>
<tr>
<th>Parameters$^*$</th>
<th>Donepezil hydrochloride</th>
<th>Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>300–1800 ng band$^{-1}$</td>
<td>120–720 ng band$^{-1}$</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>$y = 4821 + 770.81$</td>
<td>$y = 2553.44x + 4540$</td>
</tr>
<tr>
<td>Slope</td>
<td>4821</td>
<td>2553.44</td>
</tr>
<tr>
<td>Intercept</td>
<td>770.81</td>
<td>4540</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.994</td>
<td>0.988</td>
</tr>
<tr>
<td>Limit of detection (LOD)</td>
<td>0.00128</td>
<td>0.0379</td>
</tr>
<tr>
<td>Limit of quantification (LOQ)</td>
<td>0.00157</td>
<td>0.0476</td>
</tr>
<tr>
<td>Repeatability (RSD)</td>
<td>0.00521</td>
<td>0.00565</td>
</tr>
<tr>
<td>Intra-day (RSD)</td>
<td>0.00640</td>
<td>0.005171</td>
</tr>
<tr>
<td>Inter-day (RSD)</td>
<td>0.00685</td>
<td>0.00542</td>
</tr>
</tbody>
</table>

$^*$Denotes average of six estimations.
3.3.2. Precision
Repeatability and intermediate precision of the developed method were expressed in terms of relative standard deviation (RSD) of the peak area. The results showed that the repeatability, intra- and inter-day variation of the results at concentration of were within the acceptable range. The coefficients of variation for both the inter-day and intra-day precision of the method was found to be less than 1% for both drugs (Table 2).

3.3.3. Accuracy/Recovery studies
The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The percentage recovery of donepezil hydrochloride and curcumin at all the three levels was found to be satisfactory (Table 3). For donepezil hydrochloride, the % recovery was found between 99.07% and 100.6% and for curcumin between 97.08% and 101.5%, respectively.

Table 3: Recovery study of the method (using the standard addition method)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Recovery level (%)</th>
<th>Initial amount (ng band⁻¹)</th>
<th>Amount added (ng band⁻¹)</th>
<th>% Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil hydrochloride</td>
<td>80</td>
<td>4.1</td>
<td>3.28</td>
<td>99.07</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.1</td>
<td>4.1</td>
<td>100.6</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>4.1</td>
<td>4.92</td>
<td>100.5</td>
</tr>
<tr>
<td>Curcumin</td>
<td>80</td>
<td>5.9</td>
<td>5.78</td>
<td>97.08</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.9</td>
<td>5.08</td>
<td>99.08</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5.9</td>
<td>5.95</td>
<td>101.5</td>
</tr>
</tbody>
</table>

*Denotes average of three estimations at each level of recovery.

3.3.4. Limit of detection (LOD) and limit of quantitation (LOQ)
The limits of detection and quantitation were found to be 0.00128 and 0.0157 ng band⁻¹ for donepezil hydrochloride and 0.005171 and 0.0542 ng band⁻¹ for curcumin, respectively, indicating the sensitivity of the developed method.

3.3.5. Robustness of the method
The robustness of the method evaluated by assessing the effect of variations in method parameters on peak areas showed low RSD values (less than 1.0%) indicating robustness of the method (Table 4).

Table 4: Robustness study for the developed method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Drug</th>
<th>% SD*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition (±0.1 mL)</td>
<td>Donepezil hydrochloride</td>
<td>0.297</td>
<td>0.296</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>0.543</td>
<td>0.543</td>
</tr>
<tr>
<td>Amount of mobile phase (±5%)</td>
<td>Donepezil hydrochloride</td>
<td>0.984</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>0.982</td>
<td>0.741</td>
</tr>
<tr>
<td>Time from spotting to chromatography (±10 min)</td>
<td>Donepezil hydrochloride</td>
<td>0.539</td>
<td>0.534</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>0.951</td>
<td>0.947</td>
</tr>
<tr>
<td>Time from chromatography to scanning (±10 min)</td>
<td>Donepezil hydrochloride</td>
<td>0.654</td>
<td>0.656</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>0.493</td>
<td>0.490</td>
</tr>
</tbody>
</table>

*Denotes average of three estimations at each level.

3.3.6. Specificity
The peak purity test of donepezil hydrochloride and curcumin spots were assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot and their spectra were overlaid to assess spectral matching.

3.4. Stability studies
The results of the forced degradation study of donepezil hydrochloride and curcumin using toluene: methanol: glacial acetic acid (8:2:0.1 v/v/v) as the mobile phase system are summarized in Table 5.
Table 5: Stability studies for the developed method.

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>Number of degradation products ((R_f) values)</th>
<th>Area of degradation product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>2 (0.23,0.52)</td>
<td>(16.26,14.35)</td>
</tr>
<tr>
<td>Base</td>
<td>2(0.24,0.53)</td>
<td>(15.83,17.86)</td>
</tr>
<tr>
<td>Oxidative</td>
<td>2(0.22,0.58)</td>
<td>(17.17,11.42)</td>
</tr>
<tr>
<td>Heat</td>
<td>2(0.28,0.56)</td>
<td>(12.13,10.11)</td>
</tr>
<tr>
<td>Photo</td>
<td>2(0.22,0.53)</td>
<td>(28.51,17.14)</td>
</tr>
</tbody>
</table>

3.4.1. Acid induced degradation study

Donepezil hydrochloride and curcumin, both were found to undergo acid degradation very rapidly. The reaction in 0.1 N HCl at 80 °C under reflux for 2hr showed extensive degradation for donepezil hydrochloride with additional peaks at \(R_f\) values of 0.23 (about 16.26% degradation). For curcumin, additional peaks were observed with \(R_f\) values 0.52 (about 14.35% degradation) (Fig. 4).

![Figure 4](image_url)

Figure 4. Typical densitogram of donepezil hydrochloride and curcumin and degradation products in the acid degradation study.

3.4.2. Base induced degradation study

In base induced degradation study, donepezil hydrochloride and curcumin showed additional peaks at \(R_f\) values 0.24 (about 15.83 % degradation) and 0.53 (about 17.86 % degradation), respectively (Fig.5).

![Figure 5](image_url)

Fig. 5: Typical densitogram of donepezil hydrochloride and curcumin and degradation products in base degradation study.
3.4.3. Oxidative induced degradation study
In the oxidative degradation study, it was found that both donepezil hydrochloride and curcumin were liable to degradation. Donepezil hydrochloride exhibited degradation peaks at $R_f$ values 0.22 (about 17.17% degradation) and for curcumin at $R_f$ values 0.58 (around 11.42% degradation). The densitogram for the oxidative degradation study is shown in (Fig. 6).

![Typical densitogram of donepezil hydrochloride and curcumin and degradation products in oxidative degradation study.](image)

Fig. 6: Typical densitogram of donepezil hydrochloride and curcumin and degradation products in oxidative degradation study.

3.4.4. Heat degradation study
In the heat degradation study, donepezil hydrochloride and curcumin showed additional peaks at $R_f$ value at 0.28 (about 12.13% degradation) and 0.56 (about 10.11% degradation), respectively (Fig. 7).

![Typical densitogram of donepezil hydrochloride and curcumin and degradation products in heat degradation study.](image)

Fig. 7: Typical densitogram of donepezil hydrochloride and curcumin and degradation products in heat degradation study.
3.4.5. Photo-degradation study
Donepezil hydrochloride and curcumin both showed additional peaks at \( R_f \) value 0.22 (about 28.51 % degradation) and 0.53 (about 17.14 % degradation), respectively, in the photo-degradation study (Fig. 8).

\[ \text{Fig. 8: Typical densitogram of donepezil hydrochloride and curcumin and degradation products in photo-degradation study.} \]

4. CONCLUSION
A combination of donepezil hydrochloride and curcumin is currently available for the treatment of Alzheimer’s disease. As there are no reported methods for their simultaneous estimation, a stability indicating high performance thin layer chromatography (HPTLC) method was developed and validated for the determination of donepezil hydrochloride and curcumin in situ gel formulation on pre-coated silica gel HPTLC plates using toluene: methanol: glacial acetic acid (8:2:0.1 v/v/v) as the mobile phase with densitometric detection at 254 nm. The developed method was found to be simple, rapid, selective, sensitive and suitable for simultaneous determination of donepezil hydrochloride and curcumin. The HPTLC method offers several advantages over liquid chromatographic methods such as the possibility of simultaneous analysis of sample and standard on the same plate, short system equilibrium time, multiple/repeated scanning of chromatograms, higher mobile phase pH, large sample capacity, short run time, minimum solution consumption and no prior treatment for solvents like filtration and degassing. The stability indicating properties established following the recommendations of ICH guidelines also indicated that the drugs could be evaluated in presence of their degradation products and thereby can be employed for the simultaneous estimation of donepezil hydrochloride and curcumin and their degradation products in stability samples in the industry.

ACKNOWLEDGEMENT:
The authors would like to thank Padm. Dr. D.Y. Patil, Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for providing the necessary infrastructural facilities to perform this study.

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