Development of reverse phase HPLC method for simultaneous estimation of quetiapine fumarate and curcumin in nano structured lipid carrier formulations

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Abstract—The present work was aimed with the objective of developing simple, precise and sensitive high performance liquid chromatographic method for simultaneous estimation of quetiapine fumarate and curcumin and to execute the developed method in the simultaneous estimation of both the drugs in nanostructured lipid carrier formulations. The method was carried out using various solvents, buffers, at different flow rates for the complete separation and estimation of both the drugs. Using ICH guidelines, the developed method was validated. After various trials the chromatographic conditions were optimized. The complete chromatographic separation and estimation of both drugs was possible using phenomenex kinetex XB-C18 100 A analytical column (3.5 μm, 4.6 mm × 150 mm) and methanol: water 70:30 v/v as a mobile phase, at the flow rate of 1.0 mL/min. Sample of 200 μL was injected and chromatogram was recorded at 290 nm in triplicates. The absorption maximum for quetiapine fumarate was found to be in 290 nm, whereas curcumin possess absorption maxima at 421 nm. The simultaneous estimation of both drugs is possible when estimated at particular single wave length. The peak response for quetiapine fumarate and curcumin was maximum at 290 nm which has been fixed for the analysis. The retention time of quetiapine fumarate and curcumin was 2.10 and 4.60 min respectively.
**Keywords**—curcumin, NLC, RP-HPLC, quetiapine fumarate, simultaneous estimation.

**Introduction**
In present state the recognition of a drug or a combination of drug in various formulations is the crucial need for the upswing of human health. During this journey of development of appropriate formulations from the discovery of drug to marketing, the analytical method development grabs its own pole position. Diversified methods are available for quantification of pharmaceuticals like UV-Visible spectrophotometric, HPTLC, HPLC, UPLC gas-chromatography, and HPLCMS-MS method.[Kiran.B et al]. The firmness of the method made it to capture the unique position in the analysis of formulation consisting either as single drug or its combination. This study sheds its light over the development of analytical method for nano structural lipid carrier formulation comprising of an antipsychotic drug quetiapine fumarate and curcumin.

A dibenzothiazepine derivative, quetiapine fumarate having molecular formula C_{29}H_{33}N_{3}O_{10}S with chemical name of \{2-\{2-\{4-dibenzo[1,4]thiazepine-11-yl-1-piperazinyl\} ethoxyethanol, fumaric acid \{1:2 salts\} is used in this work. The antagonistic action of drug against norepinephrine and serotonin (neuro transmitters). It is also said to have strong affinity and act as antagonist to 5 HT2 receptors and dopaminergic receptors D2. The action of drug may be due to curtailing the action of dopamine and serotonin. It is atypical antipsychotic drug used in the treatment of schizophrenia and bipolar disorders [Parvathi A et al;Rang PH et al;Saller CF et al]. Though the drug has many valuable characteristics it suffers from certain flaws like the oral bioavailability is only 9%, due to extensive hepatic first pass metabolism, having plasma half life of 6 to 7 hours, poor water solubility and also belongs to Pgp substrate. These parameters demand the use of the drug in extensively high dose in order to achieve the desired therapeutic effect which ultimately results in side effects. [Shewta et al 2020;Clarie Davis et al 2007; M.Ribolsi et al 2010;Michael Ridel et al 2007;Arjun Narla et al 2013;Srijit Suttajit et al 2014]

![Figure 1. Chemical structure of Quetiapine fumarate](image)

Curcumin is yellowish–orange color crystalline nature diferuloyl methane isolated from the rhizomes of *Curcuma Longa Linn* belonging to the family of zingiberaceae. Curcumin is commonly known as turmeric. The IUPAC name of curcumin is \{(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, with
chemical formula $\text{C}_2\text{H}_{20}\text{O}_6$, and molecular weight of 368.38 [Sumeet Sood et al 2014; Adhimoolam karthikeyan et al 2020, Kaviyarani Indira Priyadarshini et al 2009]. Curcumin is a lipophilic poly phenolic Phyto compound which has numerous applications in medicinal field as antibacterial, antioxidant, anti-inflammatory, anticancer agents etc apart from facilitating the entry of drug into BBB. [Damodharan Dinakaran et al 2022; Yin –Meng Tsai et al 2011; Hosseininasab et al 2021] The earlier reports and research suggest that curcumin has ability to penetrate BBB when given as adjuvant with anti psychotic drug in management of schizophrenia. [Farooqui et al 2019; Bhat A et al 2019; Soroor Sadegh et al 2014]

![Figure 2. Chemical structure of Curcumin](image)

Hence attempt is made to formulate Quetiapine fumarate and curcumin loaded nano lipid carrier (NLC) [Rohini G Madane et al 2016; Alberto Canfran et al 2015] to treat bipolar disorder schizophrenia. The entry of Quetiapine fumarate to brain is restricted that is facilitated by accompanying the drug along with curcumin, to improve its bioavailability and to target Dopamine receptors at low doses. The nano particulate drug delivery system has proved to be efficient because of its size, tailored surface, solubility improvement and targetability. [Muhammad Raza shah et al 2016] Nanoparticulate drug delivery may be either from polymer based or lipid based. Among them lipid based carriers are biodegradable, less toxic, rapid uptake by brain. Recently the researchers reported that nanostructured lipid carrier is budding area in the field of nanodrug delivery system to treat many diseases. [Sanjay Kumar Singh et al 2016; Sarabjot Kaur et al 2015; Karamsetty V.M et al 2016]

As per literature review there are several methods reported for the analysis of quetiapine fumarate and curcumin individual in bulk, pharmaceutical formulation and biological samples but as of our knowledge the combination not yet reported. [Sethi P et al 2009] But there is a need to develop and validate a method for simultaneous estimation of quetiapine fumarate and curcumin. The method should be simple, cost effective and able to analyze in bulk material, in-vitro release samples and ex-vivo samples. The quetiapine fumarate combine with curcumin was formulated as nano structured lipid carrier system and planned to deliver intranasally to achieve the target site.

### Materials and Methods

### Materials

Dr. Reddys laboratory, Hyderabad India provide gift sample Quetiapine fumarate. Curcumin was obtained as gift sample from natural remedies, Bangalore, India. Potassium dihydrogen phosphate, sodium hydroxide, acetonitrile, methanol, Cholesterol and Vitamin E TPGS were obtained from SD fine chemicals India. Only analytical grade chemicals were used.
**Instrumentation**

A high-performance liquid chromatographic system (Prominence, Shimadzu, Japan) was used. It consists of LC 20 AD liquid pumps with 200 μl sample manual injection loop equipped with photo diode array detector SPD-M20A. Phenomenex kinetex XB-C18 100 A (3.5 μm, 4.6 mm × 150 mm) analytical column with a LC solution v.1.24 Spinchrome-1 software was used to acquire the data.

**Preparation of stock solution**

Standard stock solution of quetiapine fumarate and curcumin (1 mg/mL) were prepared by transferring 10 mg of each quetiapine fumarate and curcumin into a 10 mL volumetric flask containing 4 ml methanol. Sonication was done for 15 minutes and methanol was used for dilution and the solution was filtered through 0.22 μm membrane filter. Aliquot of the filtrate was further diluted with pH 1.2 buffer to get final working concentration of 200, 400, 600, 800 and 1000 ng/mL. Freshly prepared and maintained stock solutions were only used.

**Method Validation**

As per International Conference on Harmonization (ICH guidelines) and US FDA[FDACentre 1994; FDAGuidance 2001] the method was validated. The various parameters including its linearity, recovery, method precision, system precision, system suitability and specificity, intra and inter- day variability and robustness were considered.[ICH guidance for industry 1996; IFPMA 1996]

**Linearity**

Linearity was determined by constructing five point calibration curves for quetiapine fumarate and curcumin using standard solutions at nanogram levels in the range of 200-1000ng/mL. Standard curve was plotted between peak area as function of standard concentration. Linearity of standard curve was evaluated by regression coefficient value.

**Recovery**

The quality and applicability of method was judged by the recovery analysis for quetiapine fumarate and curcumin at three levels like low, middle and high concentrations (80%, 100% and 120%) by standard addition method in triplicates. The pre analyzed samples were spiked followed by known amount of standard solution. The recovery was estimated using developed method. The difference in drug concentration between expected and actual value was estimated. The acceptance criterion for percentage recovery was within 100±2% and % RSD of assay less than 2 %.

**Method Precision and System Precision**

Precision of an analytical method is adopted to check the variability of results between a series of measurements obtained from repeated analysis of same
homogenous sample under mentioned identical experimental conditions. Method precision and system precision was determined for quetiapine fumarate and curcumin by injecting the standard solution / sample solutions performing the analysis for five times.

System Suitability and Specificity

To check the reproducibility and resolution between peaks the system suitability tests were carried out for five replicate of standard solutions and the peaks were analyzed for its tailing factor and theoretical plates. The acceptance limit was RSD # 2% of the peak area and the retention time of two compounds. The chromatographic parameters like capacity factor, (k0), resolution, tailing factor (t) and the theoretical plate number N were also analyzed.[Vitorino.C et al 2013; Nellore Dharani Sai Sreekanth et al 2021] The capacity factor is a measure of where the peak of interest is located with respect to the void volume which is the elution time of non retained components. The degree of separation of two peaks is mentioned as R. The column efficiency is measured by number of theoretical plates.[Asit kumar et al 2021; Ankita Khismatrao et al 2018]. By running chromatogram how many peaks per unit time may be determined.[ Bimlesh Kumar et al 2017; R. D. Jangle et al 2013]

Intra- inter Day and Analyst Variability

The intra-day (repeatability), inter-day (intermediate precision) variability and analyst to analyst variations were determined for quetiapine fumarate and curcumin using standard solutions. The day-to-day variability was checked on the second day by repeating the experiments.

Robustness

In order to demonstrate the robustness of the method, deliberate changes in the chromatographic conditions were made and the robustness study was conducted for quetiapine fumarate and curcumin by purposefully altering the chromatographic conditions. The conditions studied were altered flow rate, altered wavelength and altered solvent ratio.

Method of applicability

Formulation of Nanostructured lipid carrier

Quetiapine fumarate with curcumin loaded NLC were developed by hot emulsification followed by homogenization technique. The solid lipid was fixed minimum of 70% and maximum of 90%, similarly liquid lipids was fixed minimum of 10% and maximum of 30%, were mixed in the ratios chosen, at a temperature 50 °C above the melting point of the solid lipid on a water bath. Cholesterol was taken as solid lipid; oleic acid was taken as liquid lipid. Weighed quantities of the drugs were mixed to it. In a separate beaker, Vitamin E TPGS was added to an affixed volume of HPLC grade water to form the aqueous phase. This was maintained at a temperature equal to the lipid phase and added to the beaker containing the molten lipid phase. Homogenization followed the mixing of
the 2 phases on a homogenizer (Remi, Electronik, Vasai, India, RQT 127/A/D) at 12000 RPM for 10 min resulting in the formation of hot, clear and transparent NLC formulations. [Freitas C et al 1999]

**Determination of drug content**

Drug content of all the formulated quetiapine fumarate and curcumin NLC was evaluated by transferring quetiapine fumarate and curcumin NLC (10 mg) into a 10 mL volumetric flask containing 4 mL of methanol. It was then sonicated for 15 minutes and diluted using methanol and filtered through 0.22 μm membrane filter. Aliquots of the filtrate was further diluted with pH 1.2 buffer and evaluated by RP-HPLC method in triplicates. [Iti Chauhan ID et al 2020; Mohammed Elmowafy et al 2021].

**Entrapment Efficiency (% EE)**

The % entrapment efficiency of QC-NLC was evaluated by direct method. The QC-NLC was centrifugation at 12000 rpm for 30min, the NLC were solubilized in methanol and filtered through 0.22 μm membrane filter. Aliquot of the filtrate was further diluted with pH 1.2 buffer and evaluated by RP-HPLC method. [Koduru Trideva Sastri et al 2020]

\[
\text{% Entrapment efficiency} = \left( \frac{\text{Amount of drug in NLC}}{\text{Initial amount of drug}} \right) \times 100
\]

**Results and Discussions**

**Method development and optimization**

The prime aim of this study was to develop a single isocratic reverse phase HPLC method for the simultaneous estimation of quetiapine fumarate and curcumin. During the optimization of method, the preliminary screening experiments of mobile phase composition at different ratios of acetonitrile: water (50:50, 60: 40, 70: 30 and 80: 20% v/v) were tried. However, all these mobile phase systems gave stable peak for quetiapine at 6.3 min and curcumin at 9 min, but the blank also gave sharp peak at 6.3 min. By considering the interference of blank peak at 6.3 min, the mobile phase has been shifted to combination of methanol: acetonitrile (50:50 - 80:20) and methanol: water (50:50-65:35). After a series of screening experiments mobile phase (methanol: water, 70: 30% v/v) gave better sharp peak for quetiapine fumarate and curcumin with good resolution at a nominal analysis time of 10 minutes.

These results indicate that phenomenex kinetex XB-C18 100 A (3.5 μm, 4.6 mm × 150 mm) analytical column with a mobile phase of methanol: water (70:30% v/v) at a flow rate of 1.0 mL/minute provided good separation and selectivity for the simultaneous estimation of quetiapine fumarate and curcumin even in permeated samples. The absorption maximum for quetiapine fumarate was found to be in 290 nm, whereas curcumin possess an absorption maximum at 421 nm. The peak response for quetiapine fumarate and curcumin was maximum at 290 nm,
hence this wavelength has been fixed for the simultaneous estimation of both drugs. The chromatogram is given in figure no 1.

Figure 1. Chromatogram of quetiapine fumarate and curcumin at 290nm.

**Method Validation**

**Linearity**

The calibration curve obtained for quetiapine fumarate and curcumin were linear over a wide range of concentrations (200-1000 ng/mL) studied. The $R^2$ value of quetiapine fumarate and curcumin at nanogram levels was 0.995 and 0.990 respectively. The regression equation for the calibration curve was found to be $y = 175.7x + 76697$ for quetiapine and $y = 87.94x + 3089$ for curcumin. The result of the linearity study depicts the good linearity for two selected combinatorial drugs. The calibration curves are given in figure 2 and 3 for curcumin and quetiapine fumarate respectively.

**Recovery**

The peak area (% RSD) for the recovery analysis was found to be less than 0.67 and 0.80%, respectively. The % recovery of quetiapine fumarate and curcumin was determined at 80, 100 and 120% and was found to be within 0.241 - 0.261 for curcumin and 0.580 to 0.808 for quetiapine. These results emphasize that the developed methods are precise. A tailing factor of less than 1.179% has been observed for both quetiapine fumarate and curcumin peaks, almost unchanged retention times of both drugs and absence of interference peaks in blank chromatogram indicates the specificity of the developed analytical method. The resolution and capacity factor of the method has been observed that 13.87 and 1.156 respectively. The % RSD of peak area for recovery was found to be less than 1.0%. The results are shown in table no 1.

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Quetiapine</th>
<th>Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSD (%) of peak area</td>
<td>Recovered (%)</td>
</tr>
<tr>
<td>80%</td>
<td>0.261</td>
<td>79.38</td>
</tr>
<tr>
<td>100%</td>
<td>0.676</td>
<td>99.44</td>
</tr>
</tbody>
</table>

Table 1
Recovery analysis of quetiapine fumarate and curcumin
System suitability

The RSD of the peak area for quetiapine fumarate and curcumin were less than 2% which shows that the system is appropriate to analyze two compounds. The peak area (% RSD) for the recovery analysis was found to be less than 0.67 and 0.80%, respectively. A tailing factor of less than 1.179% has been observed for both quetiapine fumarate and curcumin peaks, almost unchanged retention times of both drugs and absence of interference peaks in blank chromatogram indicates the specificity of the developed analytical method. The resolution and capacity factor of the method has been observed that 13.87 and 1.156 respectively. The results are shown in Table no 2.

Table 2
Evaluation of system suitability parameters for the determination of quetiapine fumarate and curcumin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quetiapine</th>
<th>Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.10</td>
<td>4.60</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.944</td>
<td>1.179</td>
</tr>
<tr>
<td>Resolution</td>
<td>-</td>
<td>13.87</td>
</tr>
<tr>
<td>Area</td>
<td>248898</td>
<td>81194</td>
</tr>
<tr>
<td>Capacity factor</td>
<td>0.00</td>
<td>1.156</td>
</tr>
</tbody>
</table>

System precision

The precision of the developed method was evaluated by calculating relative percentage standard deviation for five determinations at high, middle and low concentrations. The precision was also confirmed by inter and intraday variability and analyst-analyst variability under same experimental condition. The % RSD of peak area for system and method precision, inter, intraday and analyst variability was found to be less than 2.0%. The results are shown in table no 3. From the results it is clearly proved that the developed method was precise.

Table 3
Evaluation of the system, method, inter-day and intra-day precision and analyst variation for the determination of quetiapine fumarate and curcumin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quetiapine</th>
<th>Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD (%) of peak area</td>
<td>0.170</td>
<td>0.326</td>
</tr>
<tr>
<td>System precision</td>
<td>0.170</td>
<td>0.326</td>
</tr>
<tr>
<td>Method precision</td>
<td>0.676</td>
<td>0.186</td>
</tr>
<tr>
<td>Analyst-1 variation</td>
<td>0.160</td>
<td>0.301</td>
</tr>
<tr>
<td>Analyst-2 variation</td>
<td>1.916</td>
<td>1.941</td>
</tr>
<tr>
<td>Inter day variation</td>
<td>0.690</td>
<td>0.585</td>
</tr>
<tr>
<td>Intra-day variation</td>
<td>0.294</td>
<td>0.373</td>
</tr>
</tbody>
</table>
Robustness

The robustness of the developed method was studied by altering the flow rate and detection wavelength. The deliberate variations in flow rate and detection wavelength has produced the chromatograms with an acceptable degree of reproducibility (% RSD < 2.5%) and it has shown that the method is robust shown in Table no 4. The wavelength change (290 ± 5 nm) does not produce any significant changes in the chromatogram. These results emphasizes that the developed method was sufficiently robust and unaffected by the changes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test conditions</th>
<th>% RSD of peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Quetiapine</td>
</tr>
<tr>
<td>Altered flow rate (mL/min)</td>
<td>0.9</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>1.75</td>
</tr>
<tr>
<td>Altered wavelength (nm)</td>
<td>285</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>290</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>295</td>
<td>0.92</td>
</tr>
<tr>
<td>Solution stability</td>
<td>Refrigerated temperature (8°C)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Room temperature (25°C)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Inter/Intrady variability

The % RSD of peak area for inter/intra-day variations, system precision and method precision was found to be less than 2.0%. The deliberate variations like altered flow rate and wavelength does not produces any significant changes in the chromatogram, except at the altered wavelength for curcumin, as there is no remarkable changes in the peak areas and retention time that indicates the method to be robust.

Application of Validated Method for the Assay of Nanostructured Lipid Carrier Formulation

The developed method was employed to determine quantity of quetiapine and curcumin in NLC formulations. The drug content of quetiapine fumarate and curcumin was found to be 99.80 and 98.63% respectively. Entrapment efficiency was deducted by direct method. It was found after was centrifugation at 12000 rpm for 30min, the NLC suspension. The amount of drug entrapped in NLC was found after RPHPLC as 98.28% of quetiapine and 97.8% of curcumin. This shows the suitability of the method to determine amount of drug entrapped in NLC formulations.

Conclusion

A novel method was established for simultaneous determination of quetiapine fumarate and curcumin in nano structured lipid formulation. These carrier
systems are designed to deliver the drugs to target the brain to treat schizophrenia, manic and brain related disorders. Being Pgp substrate the drug along with curcumin could achieve the results. NLC formulations of these drugs were formulated using cholesterol as solid lipid, oleic acid as liquid lipid along with Vitamin E TPGS that act as solubilizing agent. The developed method was validated for accuracy, precision, robustness, recovery, system suitability as per ICH guidelines and employed to successfully determine amount of drug in NLC formulations.

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