A validated RP-HPLC method for the determination of piperidone analogue of curcumin

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Abstract: Curcumin (Diferuloylmethane) is a natural product extracted from the root of Curcuma longa. 5-Bis (4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone, the piperidone analogue of curcumin (PAC), was one of the analogues that, demonstrated potential anticancer effects against breast and colon cancers compared with native curcumin. A simple, accurate, and rapid isocratic reverse phase high performance liquid chromatography (HPLC) analytical method utilizing UV detection was developed and validated for the determination of PAC utilizing C₁₈ column with run time was 7 min. Chromatogram showed a peak of PAC at retention time of 5.8±0.92 min. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. Linear relationship (r > 0.99) was observed between AUP of PAC and the corresponding concentrations over 100-10000µg/mL. The LOQ of this assay was 3.9ng/mL with a corresponding relative standard deviation of 4.8 and 4.0%. The LOD was 13.1ng/mL at a signal-to-noise ratio of >3.

Keywords: Piperidone analogue of curcumin, reverse phase high performance liquid chromatography.

INTRODUCTION

The dried powdered rhizome of Curcuma longa L. is commonly known as turmeric and used worldwide as a food-coloring agent (Martins et al., 2009). Turmeric extract contains curcuminoids, which are phenolic compounds composed mainly of three bioactive substances, curcumin, demethoxy curcumin, and bis-demethoxy curcumin (PAC) (Martins et al., 2009).

Curcuminoids are well-known in traditional medicine because of their ant inflammatory (Jurken, 2009), antibacterial (Martins et al., 2009), antidepressant (Kulkarni et al., 2009), antidiabetic (Wickenberg et al., 2010), antitumor (Wilken et al., 2011), immunomodulatory (Rogers et al., 2010) and gastro protective (Kim et al., 2005) properties. In addition, curcuminoids haves been successfully used in the treatment of Alzheimer’s disease (Ahmed et al., 2010) and cardiac disorders (Morimoto et al., 2010). Turmeric is widely accepted as a spices with the highest antioxidant activity (Wojdylo et al., 2007). Moreover anticancer effects of curcumin have been elucidated in many in vitro and in vivo studies against various cancer types (Dhillon et al., 2008, Sharma et al., 2001, Anand et al., 2008).

Curcumin is characterized by poor aqueous solubility, low gastrointestinal absorption, high rate metabolism, low of bioavailability and low stability. In neutral pH conditions, contributing to their limited clinical uses (Anand et al., 2007a, Nimiyia et al., 2016). In addition, these molecules have poor bioactivity and are unstable in neutral and alkaline aqueous solutions (Yallapu et al., 2012, Tønnesen and Karlsen, 1985).

Khairia M. Youssefa et al. (Youssef et al., 2004) synthesized various curcumin analogues in 2004. 5-Bis (4-hydroxy-3-methoxybenzylidene)-N-methyl-4 piperidone, piperidone analogue of curcumin (PAC) (fig. 1), was one of the curcumin analogues that, showed promising anticancer effects against breast and colon cancers (Al-Hujaily et al., 2011, Al-Qasem et al., 2016). PAC is 27-fold more soluble than curcumin in water(Al-Hujaily et al., 2011).

Various analytical techniques for the quantification of total and isolated curcumin in different matrices have been reported, particularly spectrophotometric methods for the determination of total curcuminoids (Kadam et al., 2013, Silva-Buzanello et al., 2015, Ahmed et al., 2012). However, this approach cannot be used to quantify individual curcuminoids. Methods such as high performance thin layer chromatography (HPTLC) have been used for analysis of curcumin (Rasmussen et al., 2000, Ansari et al., 2005). High-pressure liquid chromatography detection (HPLC) is the most common used method for the determination of curcuminoids and curcumin in turmeric samples, biological samples, or dosage forms (Jadhav et al., 2007, Li et al., 2009, Jangle and Thorat, 2013, do Nascimento et al., 2012, Buadonpri et al., 2009, Jayaprakash et al., 2002, Syed et al., 2015, Koop et al., 2013). Table 1, summarizes the studies in which curcumin was determined using HPLC assay, published in the last two decades.
Although HPLC techniques are characterized by a short run time, to the best of our knowledge, no study has reported a validated assay for PAC with simple and efficient extraction techniques. The retention time for curcumin in literature review varied from 26 to 1.2 min. Our study aimed to develop a rapid, robust, selective, sensitive, and precise HPLC method for the determination of PAC. The assay method was validated for linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ) and used for in determination of drug content of the PAC-liposome formulation (under publication).

**MATERIALS AND METHODS**

**Materials**
PAC was prepared by the Medicinal Chemistry Department, College of Pharmacy, King Saud University, KSA (Youssef et al., 2004). All other reagents and chemicals were of HPLC analytical grade, and were used as received. Water was deionized and purified using a Milli-Q Reagent Grade water system (Millipore Corporation, Bedford, MX 01730, USA).

**Liquid chromatography conditions**
The HPLC system consisted of Waters 1525 binary pump Separation module (Waters, USA) fitted with C\textsubscript{18} column (300 mm × 4.6 mm). The autosampler injection system (Waters 2707) used was a 10µL sample loop. A Millipore Swinnex type filter (pore size =0.45µm) was obtained from Millipore (Bangalore, India). A Waters HPLC system equipped with a Waters 484 variable UV absorbance detector and a Waters 2707 plus auto sampler was used. Waters 515 solvent delivery system was used to operate the gradient flow through a C\textsubscript{18} column (4.6mm × 150 mm, 3µm spherical particles). Acetonitrile: 5% acetic acid (50:50, v/v) was used as the mobile phase at a flow rate of 1 mL/min and the run time was 7.0 min. A Waters 2489 UV/Visible detector used at a wavelength of 392 nm was used for detection. Degassing was achieved via filtration through a 0.45µm Millipore membrane filter and sonication for 10 min. The injection volume was 10µl and detection was at 392 nm. The HPLC system was operated at 25°C. Data were collected with a Breeze Chromatography Manager Data Collection System. A daily standard calibration curve (6 standards ranging from 100 ng/mL to 10000ng/mL) was prepared to determine the unknown PAC concentration.

**Preparation of stock solutions**
The stock standard solution of PAC was prepared in acetonitrile at a concentration of 10µg/mL and stored in 4.0mL glass vials in a refrigerator at 4°C. Different working standard solutions of PAC (100-10000ng/ml) were prepared by diluting of the above mentioned stock solution in pure acetonitrile and were stored at 4°C.

**Validation of PAC HPLC assay**
The RP-HPLC method for PAC assay was validated in term of accuracy, reproducibility, linearity, specificity, LOD, LOQ and robustness according to ICH Harmonized Tripartite Guidelines (Guideline, 2005). Three standard calibration curves were prepared at different times (at least three months) to evaluate the linearity, precision, accuracy and stability.
### Table 1: High performance chromatographic techniques used during last two decades (2000-2017) for quantitative determination of curcumin

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Column temperature °C</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Detector</th>
<th>RT* (min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Curcumin and PAC</td>
<td>25</td>
<td>C18</td>
<td>Acetonitrile: Acetic acid 5% (50:50, v/v)</td>
<td>UV at 392 nm</td>
<td>5.8</td>
<td>Current study</td>
</tr>
<tr>
<td>-Plasma</td>
<td>33</td>
<td>Luna® C18</td>
<td>Acetonitrile: water (50:50, v/v)</td>
<td>UV at 425 nm</td>
<td>13.6</td>
<td>(Fonseca-Santos et al., 2016)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>-</td>
<td>Lichrosorb® CN column</td>
<td>Acetonitrile: citric acid 1.0% (50:50, v/v)</td>
<td>PDA at 425 nm</td>
<td>ND</td>
<td>(Paulucci et al., 2013, Zanarini et al., 2015, Martins et al., 2013)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>40</td>
<td>WondaSil™ C18</td>
<td>Acetonitrile: 10 mM phosphate buffer pH 5 (50:50, v/v)</td>
<td>Electrochemical detector</td>
<td>14</td>
<td>(Long et al., 2014)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>33</td>
<td>ACE C18</td>
<td>Acetic acid 2%: acetonitrile (40:60, v/v)</td>
<td>PDA at 425 nm</td>
<td>7.4</td>
<td>(Morton et al., 2016)</td>
</tr>
<tr>
<td>-Plasma</td>
<td>40</td>
<td>Capcell Pak C8</td>
<td>Acetonitrile: water (40:60, v/v)</td>
<td>VWD at 230 nm</td>
<td>10.56</td>
<td>(Kim et al., 2017)</td>
</tr>
<tr>
<td>-Pharmaceutical dosage form</td>
<td>30</td>
<td>Waters X-bridge® C18</td>
<td>Gradient elution of mobile of 0.05 mM phosphate buffer in Methanol to acetonitrile 1:1 ratio</td>
<td>PDA at 288 nm</td>
<td>36.71</td>
<td>(Korany et al., 2013)</td>
</tr>
<tr>
<td>-Plasma</td>
<td>40</td>
<td>Reprosil-Pur C18</td>
<td>Acetonitrile: water: methanol (34:52:14, v/v/v)</td>
<td>Fluorescence detector at 426 nm</td>
<td>ND</td>
<td>(Rheer et al., 2015)</td>
</tr>
<tr>
<td>-Plasma</td>
<td>-</td>
<td>Symmetry RP C18</td>
<td>Citric acid 1%: tetrahydro-furan (50:50, v/v)</td>
<td>UV/VIS at 430 nm</td>
<td>5.17</td>
<td>(Pak et al., 2003)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>-</td>
<td>C18</td>
<td>Acetonitrile: methanol: water (40:20:40, v/v/v)</td>
<td>UV/VIS at 370 nm</td>
<td>7</td>
<td>(Syed et al., 2015)</td>
</tr>
<tr>
<td>-Pharmaceutical dosage form</td>
<td>35</td>
<td>Luna C18</td>
<td>0.1% ortho phosphoric acid : acetonitrile (45:55, v/v)</td>
<td>PDA at 262 nm</td>
<td>8.6</td>
<td>(Moorthi et al., 2013)</td>
</tr>
<tr>
<td>-Synthetic curcumin</td>
<td>-</td>
<td>C18</td>
<td>Gradient elution of 0.4% acetic acid in acetonitrile</td>
<td>PDA at 422 nm</td>
<td>4.5</td>
<td>(Scotter, 2009)</td>
</tr>
<tr>
<td>-Pharmaceutical dosage form</td>
<td>35</td>
<td>Waters® X Terra MS C18</td>
<td>The gradient program 2% acetic acid in acetonitrile</td>
<td>PDA at 420 nm</td>
<td>8.9</td>
<td>(Lee and Choong, 2011, Ferko et al., 2015)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>55</td>
<td>Phenomenex® fused-core C18</td>
<td>Gradient elution of 0.1% acetic acid in acetonitrile</td>
<td>PDA at 425 nm</td>
<td>1.2</td>
<td>(Oserio-Tobin et al., 2016)</td>
</tr>
<tr>
<td>-Plasma</td>
<td>-</td>
<td>Phenomenex ODS C8</td>
<td>Gradient elution of acetonitrile in 0.1% phosphoric acid, pH 3.5</td>
<td>PDA at 420 nm</td>
<td>44.7</td>
<td>(Xie et al., 2007)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>30</td>
<td>YMC ODS-A C18</td>
<td>Gradient elution of acetonitrile in 0.1% formic acid</td>
<td>UV at 380 nm</td>
<td>26.5</td>
<td>(Li et al., 2011)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>25</td>
<td>Kromasil C18</td>
<td>0.05% acetic acid: methanol (15:85, v/v)</td>
<td>PDA at 420 nm</td>
<td>15.8</td>
<td>(Cheng et al., 2010)</td>
</tr>
<tr>
<td>-Herbal extract</td>
<td>35</td>
<td>Merck Lichsphery C18</td>
<td>Gradient elution of methanol in 0.1% phosphoric acid</td>
<td>PDA at 425 nm</td>
<td>15.2</td>
<td>(Li et al., 2014)</td>
</tr>
<tr>
<td>-Pharmaceutical dosage form</td>
<td>-</td>
<td>Zebarray Eclipse Plus C18</td>
<td>Ethanol: water (90:10, v/v)</td>
<td>UV at 423 nm</td>
<td>2.5</td>
<td>(Yu et al., 2017, Yu et al., 2016, Nguyen et al., 2016, Nguyen et al., 2015)</td>
</tr>
<tr>
<td>-Plasma</td>
<td>-</td>
<td>Macherey Nagel Nucleosil-C18</td>
<td>Water: methanol (80:20,v/v)</td>
<td>UV at 420 nm</td>
<td>ND</td>
<td>(Bahkani et al., 2017)</td>
</tr>
<tr>
<td>-Pharmaceutical dosage form</td>
<td>25</td>
<td>C18</td>
<td>2% glacial acetic acid : acetonitrile (50:50, v/v)</td>
<td>MWD at 425 nm</td>
<td>13.28 ± 0.25</td>
<td>(Stomme et al., 2015)</td>
</tr>
<tr>
<td>22</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>RP-C18</td>
<td>acetonitrile: 0.1% trifluoroacetic acid (50:50, v/v)</td>
<td>UV at 427 nm</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td>Herbal extract</td>
<td>-</td>
<td>Eurospher I 100-5 C18</td>
<td>acetonitrile: water (90:10, v/v)</td>
<td>UV at 420 nm</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>Pharmaceutical dosage</td>
<td>35</td>
<td>Waters spherisorb column C18</td>
<td>acetonitrile: water (60:40, v/v)</td>
<td>UV at 454 nm</td>
<td>11.44</td>
</tr>
<tr>
<td>25</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>Waters uBondapak C18</td>
<td>acetonitrile: citric buffer, pH 3.0 (55:45, v/v)</td>
<td>UV at 428 nm</td>
<td>8.44</td>
</tr>
<tr>
<td>26</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>Thermo BDS C18</td>
<td>Gradient elution of 0.2% formic acid in acetonitrile</td>
<td>PCA at 422 nm</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td>Plasma</td>
<td>-</td>
<td>C18</td>
<td>Gradient elution of acetonitrile in 0.1% trifluoroacetic acid</td>
<td>UV at 570 nm</td>
<td>12.8</td>
</tr>
<tr>
<td>28</td>
<td>Herbal extract</td>
<td>-</td>
<td>Inertsil® ODS-3 C8</td>
<td>Acetonitrile: water, acetic acid (45:55:1), flow rate 1 ml/min</td>
<td>UV- 410 nm</td>
<td>ND</td>
</tr>
<tr>
<td>29</td>
<td>Herbal extract</td>
<td>-</td>
<td>A Tosoh TSK- C18</td>
<td>acetonitrile: methanol: water: acetic acid (41:23:36:1, v/v/v/v)</td>
<td>UV at 422 nm</td>
<td>10.8</td>
</tr>
<tr>
<td>30</td>
<td>Pharmaceutical dosage form</td>
<td>25</td>
<td>Phenomenex C18</td>
<td>Gradient elution of acetonitrile in 50 mM Phosphate buffer pH 5.5</td>
<td>PCA at 420 nm</td>
<td>11.15</td>
</tr>
<tr>
<td>32</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>C18</td>
<td>Acetonitrile: methanol:1.0% acetic acid (30:40:30, v/v/v)</td>
<td>UV at 420 nm</td>
<td>3.3</td>
</tr>
<tr>
<td>33</td>
<td>Pharmaceutical dosage form</td>
<td>40</td>
<td>C18</td>
<td>acetonitrile: water: acetic acid (45:55:1, v/v/v)</td>
<td>LED at 420 nm</td>
<td>20</td>
</tr>
<tr>
<td>34</td>
<td>Plasma</td>
<td>-</td>
<td>C18</td>
<td>methanol: water (80:20, v/v)</td>
<td>UV at 420 nm</td>
<td>2.57</td>
</tr>
<tr>
<td>35</td>
<td>Plasma</td>
<td>-</td>
<td>Merck Lichro-C18</td>
<td>50 mM tetrahydrofuran (THF): citrate buffer (pH 6) (66:34, v/v)</td>
<td>UV at 419 nm</td>
<td>UV at 419 nm</td>
</tr>
<tr>
<td>36</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>C18</td>
<td>Gradient elution of acetonitrile in 5% acetonitrile, 1% TFA</td>
<td>UV at 328 nm</td>
<td>5.3</td>
</tr>
<tr>
<td>37</td>
<td>Plasma</td>
<td>-</td>
<td>Eclipse XDB C18</td>
<td>Acetonitrile : 10 mM monosodium phosphate (pH 3.5) (40:60, v/v)</td>
<td>PCA at 425 nm</td>
<td>22</td>
</tr>
<tr>
<td>38</td>
<td>Plasma</td>
<td>35</td>
<td>C18</td>
<td>Acetonitrile: tetrahydrofuran (4:1) : 1% (w/v) citric acid monohydrate (pH 3.0 ) (65 : 35%)</td>
<td>PCA at 435 nm</td>
<td>3.5</td>
</tr>
<tr>
<td>39</td>
<td>Plasma</td>
<td>40</td>
<td>TSKgel- ODS8 C8</td>
<td>Acetonitrile: 50 mM phosphoric acid (48:52, v/v)</td>
<td>UV at 425 nm</td>
<td>17</td>
</tr>
<tr>
<td>40</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>Spherisorb B3 ODS2-C8</td>
<td>gradient elution of 0.1% TFA in acetonitrile</td>
<td>PCA at 427 nm</td>
<td>14.8</td>
</tr>
<tr>
<td>41</td>
<td>Pharmaceutical dosage form</td>
<td>-</td>
<td>C18</td>
<td>acetonitrile: citric buffer 1% (w/v) (3:2, v/v)</td>
<td>UV at 430 nm</td>
<td>8.8</td>
</tr>
<tr>
<td>42</td>
<td>Plasma</td>
<td>-</td>
<td>Diamonsil C18</td>
<td>Acetonitrile:5% acetic acid (75:25, v/v)</td>
<td>UV at 420 nm</td>
<td>UV at 420 nm</td>
</tr>
<tr>
<td>43</td>
<td>Herbal extract</td>
<td>30</td>
<td>Gemini C18</td>
<td>gradient elution of 3 mM phosphoric acid in acetonitrile</td>
<td>PDA at 425 nm</td>
<td>13.22</td>
</tr>
<tr>
<td>44</td>
<td>Herbal extract</td>
<td>-</td>
<td>Supeleo Exsil- Amino C8</td>
<td>Propanol: water (95:05, v/v)</td>
<td>PDA at 425 nm</td>
<td>11.09</td>
</tr>
</tbody>
</table>
Fig. 3: Mean drug concentration ranging from 1000-10000 ng/ml ± SD. Y denotes dependent variable and X is the independent variable while R is the regression coefficient.

**Accuracy**
The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is obtained by calculating the percent recovery (R%) of the analyte recovered. In this case, to evaluate the accuracy of the developed method, successive analysis (n=3) for three different concentrations (500ng/ml, 250ng/ml and 100ng/ml) of standard PAC solution were performed using the developed method. The data of the experiment were statistically analyzed using the formula [
\[
\% \text{ Recovery} = \frac{\text{Recovered conc.}}{\text{Injected conc.}} \times 100
\]
] to study the recovery and validity of the developed method. The mean recovery should be within 90-110% to be accepted.

**Precision**
Precision of a measurable technique is the degree of agreement among individual tests, when the technique is applied repetitively to analyze multiple replicates in three different occasions. The intraday precision was assessed by analyzing the calibration curves of six replicates of different concentrations of PAC within the same day. The inter-day precision was determined by analyzing of six replicates of different concentrations of PAC on three different days. The total precision of the method was expressed as the relative standard deviation (%RSD). In the current method development and validation protocol, precision was determined by six replicate analyses at a concentration of 5000ng/mL of standard PAC solution using the developed method and % RSD ≤2% was accepted.

**Limit of detection and Limit of quantification**
LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy. These two parameters were calculated using the formula:

\[
\text{LOD} = 3.3 \times \text{SD/S}
\]

\[
\text{LOQ} = 10 \times \text{SD/S}
\]

where SD = standard deviation of response (peak area) and S = slope of the calibration curve.
**Robustness**

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions.

**STATISTICAL ANALYSIS**

In vitro results were expressed as mean ± SD of at least three replicates. The HPLC results of PAC were calculated using linear regression without weighting, according to the equation: $Y = 0.0403X - 0.0119$, where $Y$ is the area under the peak (AUP) ratio of the drug and $X$ is the concentration of PAC. The % RSD was calculated for all values. Student’s t-test was used to inspect the concentration difference at each day and one-way analysis of variance (ANOVA) was used to assess the reproducibility of the assay using IBMSPSS Statistics 21. The level of confidence was 95%.

**RESULTS**

**Development of HPLC method**

In fig. 2, chromatogram A represents the blank mobile phase and chromatogram B represents PAC with an average retention time of 5.8±0.92 min and with no interfering peaks. This is an indication of the specificity of the developed HPLC method. The retention time was comparable with the shorter published data for curcumin (table 1).

**Specificity**

The specificity of the method was monitored by analyzing the placebo and standard solution. No peak was detected close to the retention time of PAC, which proved the high degree of specificity of the method (fig. 2).

**Linearity, limit of quantification, limit of detection**

Linear relationship ($r>0.99$) was observed between AUP of PAC and the corresponding concentrations over 100-10000µg/mL (fig. 3). The mean linear regression equation of the peak area ratios ($Y$) versus drug concentrations ($X$) of PAC was typically of the form $Y = (b ± S.D.)X ± (a ± S.D.)$ and it was $Y=52.42X - 2562.7$ for PAC. The LOQ of this assay was 3.9ng/mL with a corresponding relative standard deviation of 4.8 and 4.0%. The LOD was 13.1ng/mL at a signal-to-noise ratio of >3.

**Recovery, accuracy and precision**

Within-day precision and accuracy of the method were determined from replicate analysis (n=6) of PAC test standards at concentrations within the linear range of the assay for each drug (table 2). The reproducibility of the assay was evaluated by comparing the linear regressions of three standard plots prepared on three different days over a 3-week period. The mean correlation coefficient was >0.999 with % R.S.D. of the slopes of the three lines being 8.4%. ANOVA of the data indicated no significant difference (p>0.05) in the slopes, intra- and inter-day, of the calibration curves. The results confirmed the reproducibility of the assay method. The mean percentage recovery of 100-10000ng/mL PAC was 95.2±4.9%.

**DISCUSSION**

As curcuminoids hydrophobic compounds which is practically insoluble in water (Anand et al., 2007a, Nimiya et al., 2016), it is freely soluble in acetonitrile, so the C18 columns were preferred for HPLC analysis (Jadhav et al., 2007). many studies reviewed the use of C18 for separation of the drug using acetonitrile as the main solvent. These HPLC methods reported in (table 1), particularly older studies, have several disadvantages, including unsatisfactory separation times, poor resolution, complicated solvent mixtures with gradient elution, and long analysis times. The aim of this study was to develop and validate a new simple and rapid analytical method for PAC.

This article describes fast and specific HPLC method for PAC quantification, the drug eluted within 5.8 minutes. A significant reduction in the analysis time is achieved utilizing this method, which also indicate a significant reduction in the solvent consumption.

**CONCLUSIONS**

A simple, rapid and sensitive analytical method was developed and validated for the analysis for piperidone analogue of curcumin (PAC). The chromatographic runtime was also short. The developed analytical method can be reliably used for further in vitro pharmaceutical and pharmacokinetic study of any dosage form containing piperidone analogue of curcumin.

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