9. PUBLICATIONS

9.1. Publications and patents from thesis work

9.1.1. Publication from thesis work


9.1.2. Patent from thesis work


9.2 Publications and patents from other work

9.2.1. Publications from other work


9.2.2. Patent from other work

ABSTRACT

Objective: The objective of the present study was to develop, and validate a novel reverse phase high performance liquid chromatographic (RP-HPLC) method, for simultaneous determination of saxagliptin (SAXA) and glimepiride (GLIM), in bulk mixtures, and in tablets.

Methods: Determination of the drugs, SAXA and GLIM, was carried out employing ODS C18 column (250 mm X 4.6 mm i. d, 5 µm particle size), with diode array detector at λmax of 230 nm. The mobile phase employed for the current study, composed of two solvents, i.e., A (acetonitrile), and B (0.1 % w/v sodium di-hydrogen orthophosphate buffer, pH 3.8 adjusted with orthophosphoric acid). The mobile phase was pumped at a flow rate of 0.75 ml/min in the gradient mode. The validation study with respect to specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ), was carried out employing the ICH Guidelines.

Results: The developed method was selective and linear for both the drugs, i.e., between 15.63 µg/ml and 250.00 µg/ml for SAXA, and 7.81 µg/ml and 125.00 µg/ml for GLIM, with a correlation coefficient (R²) 0.9977 and 0.9982, for SAXA, and GLIM, respectively. The % recovery obtained was 102.9±0.14% for SAXA, and 101.8±1.96% for GLIM. The LOD and LOQ values for SAXA were obtained to be 1.30 µg/ml, and 3.94 µg/ml, respectively, while for GLIM, LOD was 0.82 µg/ml and LOQ was 2.48 µg/ml. The method also exhibits good robustness for different chromatographic conditions like wavelength, flow rate, mobile phase and injection volume.

Conclusion: The method was successfully employed, for the quantification of SAXA and GLIM, in the quality control of in-house developed tablets, and can be applied for the industrial use.

Keywords: Saxagliptin, Glimepiride, Simultaneous determination, Liquid chromatography, Validation

INTRODUCTION

Saxagliptin (SAXA), (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3-hydroxytricyclo[3.3.1.13, 7]dec-1-yl) acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile, a novel oral hypoglycaemic drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class, has initiated a new therapeutic approach for the treatment of type 2 diabetes (T2D) mellitus. Structurally, SAXA (fig. 1A) is a cyanopyrrolidine derivative, which is designed to provide extended inhibition of DPP-4 enzyme [1]. DPP-4 inhibitors like SAXA, not only inhibit the degradation, but also increase the circulating levels of intact and active glucagon-like peptide-1 (GLP-1), and glucagon-dependent insulinotropic polypeptide (GIP). GLP-1 has multiple important glucoregulatory effects, including promotion of glucose-dependent insulin secretion, and suppression of glucagon secretion [2].

Fig. 1: Structure of, (A): Saxagliptin; (B): Glimepiride

Glimepiride (GLIM) [1-[(p-[2-[3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido] ethyl] phenyl) sulfonyl]-3-[trans-4-methyl-cyclohexyl] urea] (fig. 1B), a first generation sulphonylurea agent, is a commonly accepted drug in the treatment of T2D [3, 4]. It acts by stimulating insulin secretions from the beta cells of the pancreas and is also known to increase peripheral insulin sensitivity, thus decreasing insulin resistance.

Hypoglycaemia is a major concern, when there is a chronic administration of antidiabetic drugs in the management of T2D. However, SAXA exhibited a low risk of hypoglycaemia in comparison to other antidiabetic drugs when used either as a monotherapy or in combination [5-11]. There was no hypoglycemic effect observed when sulphonylureas like GLIM, or Glyburide, was used in combination therapy with SAXA, which could have otherwise appeared if the either drug would have been used alone [12]. Currently, SAXA in combination with sulfonylurea is undergoing clinical trials according to Therapeutic Goods Administration (TGA) guidelines [13]. Both DPP-4 inhibitors and sulphonylureas increase insulin secretion, produce a synergistic effect, and thus suggest their utility in combination therapy [14]. In comparison to the monotherapy of drugs, i.e., either GLIM or SAXA, their simultaneous prescription can lead to same clinical effect at very low doses without eliciting the hypoglycaemia.
MATERIALS AND METHODS

Instrumentation

The chromatographic system used to develop the current technique is an Agilent Technologies 1200 series, Waldbronn, Germany, featuring a binary pump system (G1312A), an automatic injector (G1329A), and a diode array detector (DAD) (G1315D), which was set at 230 nm. Data acquisition was performed using a chromatography software package (EZChrom).

Chemicals and reagents

The drug SAXA and GLIM were procured from Raks Pharma Pvt. Ltd, Vishakhapatnam, A. P., India. All other chemicals and solvents, like acetonitrile, sodium dihydrogen orthophosphate, orthophosphoric acid, and water, employed for the study were HPLC grade, supplied by Loba Chemie Pvt. Ltd, Mumbai, India. Pharmaceutical excipients for preparation were: microcrystalline cellulose pH 101, corn-starch, lactose monohydrate, magnesium stearate and talc obtained from Loba Chemie Pvt. Ltd, Mumbai, India. A placebo for the validation study was prepared with these excipients.

Chromatographic conditions

Chromatographic separation of the active drugs, i.e., SAXA and GLIM was performed using an Innoval C18 column (5 µm, 4.6 mm i. d × 250 mm) made of stainless steel. Two different mobile phases for pumps were: mobile phase A: acetonitrile (ACN) and mobile phase B: buffer. As, isocratic flow was not suitable for simultaneous estimation of SAXA and GLIM, hence gradient flow of mobile phases, at a flow rate of 0.75 ml/min from two different pumps was followed and found to be most suitable. The gradient flow of solvents A and B, comprised of total time period of 30 min, and was in the following order: A: B:30:70 for 0-2 min, A: B:50:50 for 2-25 min, and A: B: 30:70 for 25-30 min. The composition of buffer was 0.1 % sodium di-hydrogen ortho-phosphate, pH 3.8 adjusted with orthophosphoric acid. Both, the ACN and the buffer solution were filtered through a 0.45 µm nylon-membrane filter, and ultra-sonicated for 15-20 min. Samples were filtered through the syringe filter of 0.22 µm pore size, prior to the injection. The injection volume of sample to carry out the chromatography was fixed as 10 µl. The column temperature was maintained at 30 °C.

Stock and working solutions

Separate standard solutions of SAXA and GLIM, were prepared at a concentration of 500 µg/ml, and 250 µg/ml respectively, dissolving the appropriated amount of bulk material in ACN. By adding an equal ratio of SAXA and GLIM solutions, a simultaneous stock solution was prepared, wherein the final concentrations of SAXA and GLIM, were 250 µg/ml, and 125 µg/ml, respectively. Further five levels in the range of 15.63-250 µg/ml for SAXA and 7.81-125 µg/ml for GLIM, were prepared by diluting the stock solutions appropriately with ACN. All drug samples were protected from the light by employing amber coloured glass vials.

Validation study

Specificity

For the specificity study, identification of the active drugs, i.e., SAXA and GLIM was studied individually, and simultaneously, by comparing the raw material (mobile phase=SAXA, mobile phase=GLIM and mobile phase=SAXA+GLIM) with a standard of their respective references (mobile phase=SAXA CRS, mobile phase=GLIM CRS, and mobile phase=SAXA CRS+GLIM CRS).

Another study carried out was to check the absence of any interference, by the excipients that were employed, in the formulation of the tablet form SAXA and GLIM.

Linearity and range

The linearity of the method, used for the analysis of SAXA and GLIM, was evaluated from the standard curve plotted between peak area and analyte concentration. Calibration curves taking five points in each case were generated on the three consecutive days with standard working solutions. The concentration ranges were 15.63-

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

LOD, is the smallest concentration, which the analytical method, on a given instrument and chromatographic conditions, is able to differentiate the compound from the background noise. While, the LOQ, is the smallest concentration which is quantifiable with defined precision and accuracy. To determine LOD, and LOQ separately for SAXA, and GLIM, a sample containing selected minimum concentration for each drug, was injected repeatedly, and analysed to calculate the standard deviation. Finally, LOD and LOQ were calculated as per the equations 1 and 2, according to the ICH guidelines, wherein, “s” is the slope of the curve(s), and standard deviation (σ) of y-intercept of regression line.

\[ \text{LOD} = \frac{3.3 s}{\text{RSD}} \quad (1) \]

\[ \text{LOQ} = \frac{10 s}{\text{RSD}} \quad (2) \]

Preparation of tablets

Wet granulation method was used to prepare the tablet formulation containing the active drugs [34]. The active drugs and excipients were passed through different mesh screens. SAXA, GLIM, microcrystalline cellulose (MCC), lactose monohydrate, and starch, were passed through 30 mesh, while magnesium stearate and talc, were pass through 60 mesh screen. A 10 % w/v aqueous solution of starch was employed as a binder. Drugs (SAXA and GLIM), MCC, and lactose monohydrate were mixed thoroughly in required amount, and sufficient volume of 10 % starch solution was added to obtain a damp mass. Afterwards, the mass was sieved through 20 mesh screen to obtain granules, and were subsequently dried at 60 °C for 1 h. Finally, magnesium stearate and talc were added to the granules, and mixed thoroughly. Lastly, the tablets were compressed by using eight station tablet punching machine.
Dissolution study

The in vitro dissolution study of tablet formulation was determined by using USP Type 2 (paddle type) eight station dissolution apparatus (Eletrolab dissolution apparatus). The dissolution media used was composed of phosphate buffer pH 6.8 USP, and 0.1N HCl USP. The test was performed for 6 tablets in 900 ml of 0.1N HCl, and pH 6.8 buffer, at 50 rpm maintained at 37±0.5 °C. The 5 ml volume of samples was withdrawn at predetermined time intervals, for the period of 2 h (0, 5, 15, 30, 45, 60, 75, 90, 120 min), and replaced with the equal volume of the same dissolution medium. The samples were filtered through 0.2 μm nylon membrane syringe filter. Amount of SAXA and GLIM released from the tablets at each time point, was analyzed by developed RP-HPLC method [35-37].

RESULTS AND DISCUSSION

System suitability

The chromatographic separation of SAXA and GLIM, as explained earlier, was carried out employing an Innoval C18 column (5 µm, 4.6 mm × 250 mm). The chromatographic parameters namely, number of theoretical plates, retention time, an asymmetric factor of the peaks, and HETP, were evaluated and are included in the table 1. The number of theoretical plates for SAXA, was 4659, while for GLIM, it was 21889. The values for asymmetric of peaks were 1.33 and 1.67 for SAXA, and GLIM, respectively. Further, the tailing factor values were obtained to be 1.14 for SAXA, and 1.07 for GLIM.

Fig. 2 revealed that there was no interference in the chromatograms of SAXA and GLIM, with any of the peaks of placebo. None of the peaks of excipients observed at the same retention time of both drugs (fig. 3).

Hence, it can be stated that none of the peaks generated by the excipients treatment interfere with the peaks corresponding to the active ingredients, therefore showing it to be a selective method and suitable for routine analysis.

Validation study

Specificity

The purity of the chromatographic peaks studied for SAXA and GLIM at different time points were well within the established threshold values. It was observed in chromatogram that the excipients interference for pharmaceutical preparation was showing none of the peaks at the same retention time of both drugs (fig. 3).

Excellent linearity was obtained for both the drugs in the range of 15.63-250.00 µg/ml for SAXA and 7.81-125.00 µg/ml for GLIM. The correlation coefficients for both drugs were found to be higher than the critical value of 0.995 (fig. 4 and table 1).

![Fig. 2: Chromatogram of, (A): Placebo chromatogram; (B): Overlay chromatogram of a calibration curve for SAXA and GLIM](image-url)

**Table 1: Chromatographic characteristics of the RP-HPLC method**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAXA</th>
<th>GLIM</th>
<th>Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. Range [µg/ml]</td>
<td>15.63-25.00</td>
<td>7.81-125.00</td>
<td>Non</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>1.30</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>3.94</td>
<td>2.48</td>
<td></td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 19935x+40611</td>
<td>y = 155343x+408956</td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9977</td>
<td>0.9982</td>
<td>0.995 ≥ R²</td>
</tr>
<tr>
<td>Accuracy (%Mean Recovery)</td>
<td>102.98±0.14</td>
<td>101.84±1.96</td>
<td>97-103%</td>
</tr>
<tr>
<td>Precision Interday</td>
<td>Day 1</td>
<td>Day 2</td>
<td>%RSD≤2</td>
</tr>
<tr>
<td>1.225±0.695</td>
<td>0.920±0.815</td>
<td>0.797±0.443</td>
<td></td>
</tr>
<tr>
<td>Precision Intraday</td>
<td>Morning</td>
<td>Evening</td>
<td></td>
</tr>
<tr>
<td>1.225±0.695</td>
<td>1.208±0.680</td>
<td>0.797±0.444</td>
<td></td>
</tr>
<tr>
<td>Retention time</td>
<td>3.28 min</td>
<td>26.12 min</td>
<td>Non</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.17</td>
<td>1.07</td>
<td>%RSD≤2</td>
</tr>
<tr>
<td>Asymmetry of peak</td>
<td>1.33</td>
<td>1.14</td>
<td>%RSD≤2</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>4659</td>
<td>21889</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>HETP</td>
<td>1.50 µm</td>
<td>0.03 µm</td>
<td>≤100 µm</td>
</tr>
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</table>
**Table 2: Linearity range with area**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>SAXA Concentration (µg/ml)</th>
<th>Area (mean±SD*) (mAU)</th>
<th>GLIM Concentration (µg/ml)</th>
<th>Area(mean±SD*) (mAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.63</td>
<td>487570±5157</td>
<td>7.81</td>
<td>2058898±32741</td>
</tr>
<tr>
<td>2</td>
<td>31.25</td>
<td>606590±7596</td>
<td>15.63</td>
<td>2487638±26552</td>
</tr>
<tr>
<td>3</td>
<td>62.50</td>
<td>1239162±6413</td>
<td>31.25</td>
<td>5289563±54861</td>
</tr>
<tr>
<td>4</td>
<td>125.00</td>
<td>2449142±46685</td>
<td>62.50</td>
<td>9870512±28140</td>
</tr>
<tr>
<td>5</td>
<td>250.00</td>
<td>5076379±29842</td>
<td>125.00</td>
<td>19960252±100936</td>
</tr>
</tbody>
</table>

*SD = Standard Deviation, (n=3)

**Fig. 3:** Overlay of SAXA, GLIM, Blank, SAXA+GLIM, and their corresponding CRS

**Fig. 4:** Calibration graph of, (A): GLIM; (B): SAXA (Results are expressed as mean±SD, n=3)

**Precision**

To study the precision for the instrumental system an interday study (n=6 samples for a day) was carried out. The % RSD values were obtained separately for RT and area for both the drugs at different time points (i.e. days). The % RSD values for RT and areas were 0.55 and 0.63, respectively for SAXA however, for GLIM it was 0.93 and 0.73. According to ICH the % RSD values should be less than 2 % for the precision study of the HPLC instrumental system [14], thus it can be concluded that the instrument used for the current study worked correctly for the developed analytical RP-HPLC method, and has been highly repetitive.

For the interday and intraday precision studies, the selected concentration for SAXA were 31.25, 62.50 and 125.00 µg/ml, while for GLIM, it was 15.63, 31.25 and 62.50 µg/ml. All the concentrations for both the drugs, at different time points were analysed in triplicate (n=3).

The % RSD values obtained for the first day were 1.225 for SAXA, and 0.797 for GLIM. Similarly, same set of concentrations were also analyzed in triplicate at different times, within a day, for intraday precision. The % RSD values obtained for SAXA was 1.208, and for GLIM, it was 0.797. For the interday study, the three set of concentrations of same analyte were analyzed at different days, and % RSD values were obtained to be 0.919 and 0.873 for SAXA, and GLIM respectively. As all the values for % RSD is less than 2 %, hence, the developed method is confirmed to be precise according to ICH [14].

**Accuracy (recovery method)**

For the accuracy study from the 9 samples, i.e. n=3 for 50 %, n=3 for 100 % and n=3 for 150 %. The results indicated that the mean of recovery for SAXA was 102.98 %, and % RSD was 0.131, similarly, mean of recovery for GLIM was 101.84 %, and % RSD was 1.924. As
both % RSD values are less than 2 % [14], hence, the reported % recovery by the developed method of the known added amount of analyte in the sample was within the confidence intervals.

Robustness

To evaluate the influence of small variations in the chromatographic conditions, robustness was evaluated, for different chromatographic conditions like wavelength, flow rate, mobile phase and injection volume. The studied wavelength was 230±5 nm, while flow rate was varied from 0.70 ml/min to 0.80 ml/min. The mobile phase ratio was also varied, i.e.,±5 % from the initial values. Further injection volume was kept as 5±1 µl. In all the cases, % RSD values obtained were less than 2 %, indicating that the developed method was consistent with respect to all the studied chromatographic conditions [39-40].

LOD and LOQ

LOD and LOQ were determined for SAXA, and GLIM, as per the ICH guidelines, using the equations 1 and 2, wherein, 'σ' was the standard deviation (SD) of the response, and ‘S’ was the slope of calibration curve. The LOD value obtained for SAXA was 1.30 µg/ml, while for GLIM, it was 0.82 µg/ml. Similarly, LOQ obtained for SAXA, was 3.94 µg/ml, and 2.48 µg/ml for GLIM.

Preparation of tablets

The tablets were prepared by the wet granulation technique as described in the methods. Average weight of tablets was 105±5 mg. Each tablet contained 10 mg of SAXA and 5 mg of GLIM along with the other pharmaceutical ingredients as per the composition is given in table 3.

Table 3: Composition of the tablet

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Per tablet (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SAXA</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>GLIM</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>MCC</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Lactose monohydrate</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Corn-starch</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>Magnesium stearate</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>Talc</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>105</td>
</tr>
</tbody>
</table>

Dissolution study

It is clearly vivid from the graph fig. 5, that there was a continuous increase in the % CDR values of both the drugs, i.e., SAXA and GLIM up to 90 min, in phosphate buffer pH 6.8. The maximum % CDR value obtained for SAXA was 73.94 %, while it was 91.83 % for GLIM.

![Fig. 5: Dissolution profiles of SAXA and GLIM tablet in, (A): pH 6.8; (B): 0.1N HCl (All the values were calculated as mean±SD, n=6)](image)

In 0.1N HCl both the drugs showed up quite a good release up to 90 min. The % CDR was 97 % for SAXA and 96.68 % for GLIM, which is indicating the complete drug release in 0.1N HCl.

CONCLUSION

A simple and new RP-HPLC analytical method has been developed for the simultaneous estimation of SAXA and GLIM for routine analysis in bulk drug mixtures and tablets. The proposed method to determine SAXA and GLIM in tablets has been validated in terms of linearity, precision, accuracy, and selectivity, according to Q2B ICH guidelines and can be applied in routine and in quality control of SAXA and GLIM tablets. It has been proved that the developed method was selective, linear for both the drugs between 50 and 150 % of the work concentration, and between quantification limit, and 150 % for the both of drugs with a correlation coefficient (R²) higher than 0.995. It is also showing good robustness for different chromatographic conditions like wavelength, flow rate, mobile phase and injection volume. The method is convenient because of well-defined chromatographic conditions which can be used for routine analysis in the industrial set-up.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

All authors have none to declare

REFERENCES

CERTIFICATE OF NATIONAL PATENT FILING

Title of the invention: BIOANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF SAXagliptIN AND GLIMEPIRIDE IN BIOLOGICAL FLUIDS

Applicant / Assignee: SHOOLINI UNIVERSITY OF BIOTECHNOLOGY AND MANAGEMENT SCIENCES, Post Office Box No 9, Head Post Office, The Mall, Solan-173212, Himachal Pradesh, INDIA.

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Certified that the above given particulars are correct and duly verified by me.

DATED: 18/06/2018
PLACE: CHANDIGARH

KOMPAL BANSAL  
Patent Agent for the applicant  
(Regn. No.IN/PA/1754)
ANTIOXIDANT, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF BIOACTIVE FRACTION OF LEAVES OF *MYRICA ESCULENTA* BUCH.-HAM ALONG WITH ITS PHARMACOGNOSTIC AND CHROMATOGRAPHIC EVALUATION

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ABSTRACT

Present study is an attempt to investigate the antioxidant, anti-inflammatory and analgesic activity of the standardized ethyl acetate fraction of the leaves of *Myrica esculenta* (ME- EtAC) along with exploration of morphological, microscopical and physico-chemical characters of leaves. Standard WHO procedures were adopted for investigation of microscopical and physico-chemical characters. The antioxidant activity was investigated by using DPPH radical scavenging method, anti-inflammatory activity by Carrageenan induced rat paw edema using Albino wistar rats (150-200g) and analgesic activity by using acetic acid-induced writhing and tail immersion test on Swiss albino mice (18-22g). Pharmacognostical study revealed the presence of various features like covering trichomes, anisocytic type of stomata etc. ME- EtAC expressed potent inhibition of DPPH radical scavenging activity (IC 50 of standard recorded 20.
Characterization of Curcumin di-acetate Aimed at Brain Targeting

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Abstract

In the present study curcumin derivative was synthesized to specifically target to the brain and evaluate the ability of curcumin derivative to cross blood brain barrier compared to the parent compound. The results of the present study showed that curcumin derivative cross the blood brain barrier 10 times more as compared to the parent compound and get converted into the parent compound in brain. Further, the effect of curcumin di-acetate in scopolamine induced experimental dementia of Alzheimer disease in rats was investigated. Rats were treated with scopolamine (1.4 mg/kg, i.p.) alone and with curcumin (25 mg/kg, i.v.), curcumin di-acetate (25 mg/kg, i.v). The changes in behavioral and biochemical parameters were assessed in rats. Scopolamine administered rats showed impaired learning and memory in hippocampus as compared with control. Simultaneous treatment of curcumin and curcumin di-acetate with scopolamine also caused an improvement in the learning and memory activity and (P < 0.05) was found to be significant.

Keywords: Curcumin di-acetate, Brain targeting, Drug delivery, Scopolamine

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Introduction

Curcumin, a major yellow pigment obtained from the rhizomes of turmeric, has a wide array of pharmacological and biological activities including antioxidant, anti-inflammatory, anti-infectious and anti-carcinogenic [1-2]. In addition, curcumin has been shown to have the possibility of slowing the progress of Alzheimer’s disease (AD) by reducing amyloid β, of delaying the onset of kainic acid-induced seizures and of inhibiting the formation of brain tumors. Apart from the anti-amyloid properties [3-4] and anti-tau hyperphosphorylation properties [5] against the two major pathological changes in AD, curcumin is also able to control the resultant changes that occur during the disease like oxidative stress [6], inflammatory stress [7], and cholesterol regulation [4] which are very beneficial while considering alzheimer’s disease therapy.

Alzheimer’s disease is a devastating neurodegenerative brain disorder with progressive loss in memory leads to dementia. It is characterized by the deposition of the senile plaques mainly composed of β- amyloid fragment and neurofibrillary tangles. In the recent past, the plant based molecules attracted attention due to their potential role in dementia. However the efficiency of these herbal compounds also depends on the bioavailability at the right location. Targeting the central nervous system has always been a challenge due to its inaccessibility. Due to the presence of tight junctions within the capillary endothelium severely restricts the delivery of therapeutics to the brain. Enhancing the transport of curcumin has come up as a hopeful alternative to target the brain, by crossing the blood-brain barrier ability. With this background, we have synthesized derivative of curcumin i.e di-acetate to target the brain in the
Stability-indicating HPLC Method for Determination of 7,8,9,10-tetrahydroazepino[2,1b]quinazolin-12(6H)-one, a Potential Anticancer Agent

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7,8,9,10-tetrahydroazepino[2,1b]quinazolin-12(6H)-one (TAZQ) (fig. 1), a synthetic analog of vasicine, has been reported to be an anticancer, bronchodilator, anti-inflammatory, antitussive, antiarthritic and antiasthamatic compound, which is under preclinical development for anticancer activity. The development of a validated reverse phase high performance liquid chromatography method is herein reported for the analysis and stability assessment. The analytical method was optimized using a C18 column and methanol:water (80:20 v/v) as mobile phase at flow rate of 0.9 ml/min. The eluents were monitored at 254 nm. Retention time of 7,8,9,10-tetrahydroazepino[2,1b]quinazolin-12(6H)-one was observed to be 3.9 min. It degraded significantly under alkaline conditions whereas negligible degradation was observed under acidic, oxidative, thermal and photolytic stress conditions. The peak of major degradation product, resulting from alkaline degradation, was well resolved from the peak of 7,8,9,10-tetrahydroazepino[2,1b]quinazolin-12(6H)-one. This method has been found to be linear, accurate, precise, robust, sensitive, specific, suitable and stability indicating.

Key words: Quinazolines, cancer, HPLC, stability-indicating method

7,8,9,10-tetrahydroazepino[2,1b]quinazolin-12(6H)-one (TAZQ) (fig. 1), a synthetic analog of vasicine, is currently under preclinical studies for anticancer activity. Vasicine is a major alkaloid obtained from the leaves of Adhatoda vasica Nees, a well-known medicinal plant used in Ayurvedic and Unani medicine[1]. TAZQ has been extensively studied as bronchodilator[2-7]. It inhibited lung phosphodiesterase activity, lipooxygenase activity, histamine release and antigen-induced mast cell degranulation[8]. It showed synergistic antiasthamatic activity in combination with ambroxol in ovalbumin sensitized guinea pigs[5]. It showed antitussive activity in citric acid cough model in guinea pigs[9]. It’s in vivo metabolic studies were performed in rhesus monkeys and Charles Foster rats.

Various metabolites that were detected did not show any bronchodilatory activity[10,11]. TAZQ showed dose-related reduction in developing adjuvant arthritis in rats. It’s LD50 was lower than 1000 mg/kg p.o, thus was safe at pharmacologically active doses[12]. Structure activity relationship of TAZQ as bronchodilator has been studied extensively[7]. It induced nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB)-mediated apoptosis in human colon carcinoma HCT-116 cell lines[13]. A recent study, showed that its antiproliferative activity is through inhibition of PI3k/Akt/FoxO3a pathway[14].

Although TAZQ has been studied extensively for its biological activities and is currently in pre-clinical studies as anticancer agent (with reference to personal communication with PK-PD Division, IIM-CSIR, Jammu, India), its stability indicating assay method has

Fig. 1: TAZQ (7,8,9,10-tetrahydroazepino[2,1b]quinazolin-12(6H)-one).

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Method Article

Simple, efficient and economical methods for isolation and estimation of novel isoflavone using RP-HPLC

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GRAPHICAL ABSTRACT

The study was undertaken to develop a simplified procedure for the isolation of bioactive isoflavone from \textit{Iris kashmiriana}, using a direct method of isolation, avoiding the use of chromatographic techniques. The compound was isolated by commercially viable procedure. The extraction of powdered drug (500 g) was done with petroleum ether (60–80) using a Soxhlet apparatus (24 h run). The petroleum ether extract (gums and resins 2.13 g) was obtained and the marc (400 g) was subjected to extraction with 95% methanol using a Soxhlet apparatus (24 h run). The methanolic extract (5 g) was subjected to successive fractionation with toluene, chloroform and ethyl acetate and \textit{n}-butanol. On the basis of phytochemical analysis, the glycoside was present in \textit{n}-butanol fraction. The \textit{n}-butanol fraction (1.5 g) was taken in dried methanol, passed through activated animal charcoal and subjected to acid hydrolysis. The isoflavone (250 mg), was obtained after the usual process of separation. The purity of the compound was checked by analyzing TLC (Thin Layer chromatography) and melting

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Development and Validation of a New Chromatographic Method for the Simultaneous Estimation of Serratiopeptidase, Aceclofenac and Paracetamol by RP-HPLC

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Abstract

Background: Method development, validation is an important parameter for the simultaneous estimation of Serratiopeptidase (SERA), Aceclofenac (ACE) and Paracetamol (PCM) by RP-HPLC, is supposed to be a costly and tedious process. The present study revealed using cheap and cost effective solvent system for the simultaneous estimation of Serratiopeptidase (SERA), Aceclofenac (ACE) and Paracetamol (PCM).

Objective: Development and validation of a new chromatographic method for the simultaneous estimation of Serratiopeptidase (SERA), Aceclofenac (ACE) and Paracetamol (PCM).

Methods: Simultaneous determination of SERA, ACE and PCM were carried out by RP-HPLC at the wavelength 327 nm, flow rate 0.4 mL/min, and the mobile phase used was water: methanol in the ratio (50:50 v/v). Further validation parameters such as system suitability, linearity, accuracy, precision, specificity, LOD, LOQ and robustness were taken into account to carry out the validation of the method.

Results: Absorbance maxima for the simultaneous determination were selected by the UV spectrophotometer and that was found to be 327 nm in methanol and water. During the process of RP-HPLC, the linearity was obtained in the concentration range of 2-10 µg/mL for SERA, 100-500 µg/mL for ACE and 20-100 µg/mL for PCM. Correlation coefficient (r) for SERA, ACE and PCM in methanol and water was found to be 0.9817, 0.991 and 0.9949 respectively.

Conclusion: The RP-HPLC method was simple, accurate, precise, and rapid and can be used for the simultaneous determination of SERA, ACE and PCM in bulk and pharmaceutical dosage form. The method is also economical as RP-HPLC grade water methanol in a ratio of (50:50) was used to achieve all the validation parameters.

Keywords: Aceclofenac; Correlation coefficient; Paracetamol; Pharmaceutical dosage; RP-HPLC; Simultaneous estimation; Serratiopeptidase; Validation

Introduction

Paracetamol

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drugs. Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Paracetamol and its combined dosage form with the other drugs have been mentioned in many pharmacopoeias [1,2].

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In-vitro osteoblast proliferation and in-vivo anti-osteoporotic activity of *Bombax ceiba* with quantification of Lupeol, gallic acid and β-sitosterol by HPTLC and HPLC

Shashi Chauhan, Aditi Sharma, Navneet Kumar Upadhyay, Gajender Singh, Uma Ranjan Lal and Rohit Goyal*

**Abstract**

**Background:** *Bombax ceiba* is used traditionally to treat bone disorders, rheumatism, and joint pain. The aim of the study is to carry out osteogenic activity in-vitro and anti-osteoporotic activity in-vivo of stem bark of *B. ceiba* in surgical ovariectomy model in female rats.

**Methods:** Plant drug: *B. ceiba* stem bark was extracted with solvents petroleum ether and methanol using Soxhlet extraction. In-vitro osteoblastic proliferation study was performed using UMR-106 cell lines. Both the extracts were undergone to acute toxicity study as per OECD423 guidelines. Female Wistar albino rats 180-240 g were used (*n*= 6). Surgical ovariectomy was performed under anesthesia to induce bone porosity and loss in all animals except normal control and sham control. Each extract was administered at two dose level: 100 and 200 mg/kg and the standard Raloxifene was given at 1 mg/kg orally for 28 days. The phytochemical study of both the extracts was performed using HPLC and HPTLC.

**Results:** A significant osteoblast cell proliferation and alkaline phosphatase activity were observed with *B. ceiba* extracts in UMR-106 cell lines. Surgical removal of ovaries produced significant (*p* < 0.05) decline in bone mineral density, bone breaking strength, serum ALP, calcium, phosphorus, and estradiol level and marked bone tissue destruction in histology. Administration of petroleum ether and methanolic extract for 28 days significantly (*p* < 0.05) ameliorated the consequences of ovariectomy induced bone porosity and restored the normal architecture of bone, as compared to OVX control. The phytochemical screening of both the extracts were also carried out. The quantification of phytoconstituents showed the presence of β-sitosterol and lupeol in petroleum ether extract, whereas the lupeol is also quantified in the methanolic extract. The presence of gallic acid was quantified in methanolic extract using HPLC.

**Conclusion:** *B. ceiba*: stem bark ameliorated the state of bone fragility and fracture possibly due to estrogenic modulation, as also confirmed by in-vitro osteogenic activity which may be due to the presence of lupeol, gallic acid and β-sitosterol constituents of the plant.

**Keywords:** *Bombax ceiba*, Lupeol, Gallic acid, Ovariectomy, Osteoporosis, Estrogen

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CERTIFICATE OF NATIONAL PATENT FILING

Title of the invention: ANTI-DANDRUFF HAIR STYLING COMPOSITION AND METHOD OF PREPARATION THEREOF

Applicant / Assignee:
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