Study of gliquidone degradation behavior by high-performance thin-layer chromatography and ultra-performance liquid chromatography methods

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Abstract
Gliquidone (GQ) is an oral hypoglycemic agent, belonging to second-generation sulfonylurea derivatives. New high-performance thin-layer chromatography (HPTLC) and ultra-performance liquid chromatography (UPLC) methods have been developed and validated and used for complete stability study of GQ following International Conference on Harmonization guidelines. GQ was subjected to stress and forced degradation under hydrolytic, oxidative and photolytic conditions. The drug was found to be unstable under acidic, alkaline and oxidative conditions with the formation of gliquidone sulfonamide (GQS), while a marked stability was confirmed under thermal and photolytic stress conditions. GQS is the British Pharmacopoeial impurity A of GQ and also considered as its synthesis intermediate. The developed chromatographic methods have been utilized for anticipating the degradation behavior of GQ under the studied conditions and then used for quantitation of GQ and GQS either in their pure forms or in laboratory prepared mixtures. The methods were successfully applied to GQ in pharmaceutical formulation. The obtained results were statistically compared with a reported HPLC method showing no significant difference regarding both accuracy and precision.

KEYWORDS
gliquidone, gliquidone sulfonamide, HPTLC, stability, UPLC

1 INTRODUCTION

Gliquidone (GQ) is a second-generation sulfonylurea-type oral hypoglycemic agent, which is widely used in treatment of type II diabetes (Sweetman, 2014; Malaisse, 2006). Chemically, it is 1-cyclohexyl-3-p-[2-[3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo2(1H)-isoquinolyl]ethyl]phenylsulfonyleurea (The British Pharmacopoeia, 2013; Figure 1). GQ is a drug of proven value and listed in British and Chinese pharmacopoeias (Sweetman, 2014). The presence of amide and sulfonylurea moieties in the drug molecule makes it susceptible to hydrolytic degradation (Kurzer, 1952; Sarmah & Sabadie, 2002; Bansal, Singh, Jindal, & Singh, 2008). For GQ, the hydrolytic degradation product was identified as gliquidone sulfonamide (GQS; Figure 1), after its isolation and structural elucidation using IR, MS and NMR analyses. Additionally, GQS is reported to be a GQ impurity (The British Pharmacopoeia, 2013), related substance and its synthetic intermediate (Andrew, 2006). Some analytical methods have been found in the literature for the analysis of GQ either alone by LC-MS/MS (Xiao-lin et al., 2013), in plasma by HPLC (Guo, Li, Chen, Deng, & Tang, 1992; Sridive & Diwan, 2000; Ri-lai et al., 2009; Arayne, Sultana, Mirza, & Siddiqui, 2010b), by spectrophotometry (Arayne, Sultana, Mirza, & Siddiqui, 2010a) or in combination with other drugs by HPLC (Arayne, Sultana, Mirza, & Siddiqui, 2010a; Arayne, Mirza, & Sultana, 2011; Mirza, Arayne, & Sultana, 2013, 2015) and in the presence of its related substance by HPLC (Ling-yun, 2012).

To the best of our knowledge, there has been no report on the complete stability study of GQ under all possible stress conditions. Hence, the present study was designed and aimed to perform and study the complete stability behavior of GQ under the recommended ICH stress conditions (hydrolysis, oxidation, dry heat and photolysis) through development of HPTLC and UPLC methods. In addition, characterization of the hydrolytic degradation product after isolation...
followed by structural elucidation was performed. The present work is a novel study of the degradation behavior of GQ under all possible stress conditions, which is a very important task during drug manufacture, storage and distribution. The proposed method showed high sensitivity and selectivity, short analysis time and consumed small amounts of hazardous chemicals and solvents.

2 | EXPERIMENTAL

2.1 | Instruments

2.1.1 | HPTLC method

In this study, scanning was carried out using CAMAG TLC scanner 3 S/N 130319 with winCATS software (CAMAG, Muttenz, Switzerland). During TLC scanning, the scan mode was absorbance, the source of radiation was a deuterium lamp, the slit dimension was 6.00 × 0.3 mm, scanning speed was 20 mm/s and outputs were chromatogram and integrated peak area. A TLC CAMAG Linomat V autosampler was used, which was equipped with a 100-μL syringe (Switzerland). A UV lamp with a short wavelength of 254 nm (VL-6 LC; Marne la Vallee, France) was used for following up drug degradation during forced degradation. The stationary phase was aluminum sheets (20 × 20 cm) coated with 0.25 mm silica gel 60 F254 (Merck, Germany). A Sonix TV ss-series ultrasonicator (USA) was used for dosage form preparation.

2.1.2 | UPLC method

The UPLC (Dionex, Germany) instrument was equipped with a 3000 model series pump, an autosampler with variable 100-μL loop, a thermostated column compartment and photodiode array detector. Separation and quantitation were performed on a 50 × 2.1 mm (i.d.) Hypersil Gold RP8 column (1.9 μm particle size). Data acquisition was performed on Chromeleon 7.2 software.

2.1.3 | Microanalysis

Degradate structure elucidation was carried out using 1H–NMR spectra which recorded in CDC13 and DMSO-d6 on a Varian Mercury spectrometer (400 MHz; Bruker AG, Switzerland), and IR spectra which were made on a Bruker Vector 22 (Japan) infrared spectrophotometer and expressed in wavenumber (cm⁻¹) using a potassium bromide disk. Mass spectrometry was carried out on a direct probe controller inlet part of a single quadrupole mass analyzer, Thermo Scientific GCMS model ISQ LT using THERMO X-CALIBUR software and mass spectrometry at 70 eV (El).

2.2 | Materials

2.2.1 | Pure samples

Standard GQ was obtained from Sigma-Aldrich (Germany), with certified purity of 98%.

2.2.2 | Pharmaceutical formulation

Glurenor® tablets batch no. EIE2313 were manufactured by Minapharm Pharmaceutical industries (Ramadan City, Egypt). Each tablet was claimed to contain 30 mg of GQ and was obtained from the local market.

2.2.3 | Chemicals and reagents

Methanol and acetonitrile, HPLC grade, were obtained from Tedia, USA. Chloroform, formic acid, glacial acetic acid, hydrochloric acid, hydrogen peroxide and sodium hydroxide were obtained from El-Nasr Pharmaceutical Chemicals Company Abu Zabaal, Cairo, Egypt.

2.3 | Solutions

Stock standard solutions of GQ and GQS were prepared (1 mg/mL) in methanol. A working standard solution was prepared for each component in methanol (100 μg/mL). All solutions were freshly prepared on the day of analysis and stored in the refrigerator to be used within 24 h.

2.4 | Procedures

2.4.1 | Construction of calibration curve

For HPTLC

Aliquots equivalent to 100–800 and 10–200 μg/mL of GQ and GQS, respectively, were separately transferred from their respective stock standard solutions (1 mg/mL) into two series of 10 mL volumetric flasks. A 10 μL aliquot of each sample was applied in triplicate on aluminum plates (20 × 10 cm) coated with silica gel 60 F254 (Merck, Germany). A Sonix TV ss-series ultrasonicator (USA) was used for dosage form preparation.

For UPLC

The UPLC (Dionex, Germany) instrument was equipped with a 3000 model series pump, an autosampler with variable 100-μL loop, a thermostated column compartment and photodiode array detector. Separation and quantitation were performed on a 50 × 2.1 mm (i.d.) Hypersil Gold RP8 column (1.9 μm particle size). Data acquisition was performed on Chromeleon 7.2 software.

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For UPLC
To establish linearity, a series of dilutions in the ranges 1–45 and 0.50–45 μg/mL of GQ and GQS, respectively, were separately prepared in the mobile phase from their respective working standard solutions (100 μg/mL) and then injected in triplicate. Chromatographic separation was carried out on Hypersil Gold RP8 column [50 × 2.1 mm (i.d.), 1.9 μm particle size] using isocratic elution using methanol–water (70: 30, by volume), and pH adjusted to 3 with orthophosphoric acid. The flow rate was 0.6 mL/min. The injection volume was 30 μL with UV scanning at 225 nm at 25°C. The run time was set at 1.5 min and the integrated peak area was used to quantify the studied components.

2.4.2 Application to pharmaceutical formulations
Ten tablets of Glurenor® were accurately weighed, mixed and finely powdered. An accurately weighed portion equivalent to 50 mg of GQ was transferred into a 50 mL volumetric flask and 25 mL methanol was added. The prepared solution was sonicated for 30 min, cooled and completed to volume with methanol. The solution was then filtered and diluted to obtain a solution having a final concentration of 100 μg/mL. Then, 30 μL of the prepared sample was applied to HPTLC plates (3 μg/band). For UPLC method, a solution having the concentration of 20 μg/mL was prepared by appropriate dilution and the chromatographic methods were then continued as mentioned previously. The peak area of each of separated bands/peaks was determined, and the concentrations were calculated from the corresponding regression equations.

2.4.3 Forced degradation study of gliquidone
Different degraded samples were applied in triplicate to obtain concentrations of 2 μg/band for HPTLC and 30 μg/mL for UPLC following the specified chromatographic conditions. GQ percentage degradation was then calculated from the peak area of GQ in each chromatographed sample.

Hydrolytic degradation
Acid and base hydrolysis were performed at 85°C using 0.1 and 1 M of each of HCl and NaOH for 72 h. Four portions of GQ powder equivalent to 25 mg were accurately weighed and separately dissolved in 5 mL acetonitrile in 25 mL volumetric flasks. To each flask, sufficient amounts of 0.1 M HCl, 1 M HCl, 0.1 M NaOH and 1 M NaOH were added and the volumes were completed to the mark. The prepared solutions were refluxed at 85°C for 72 h. The solutions were cooled; volumes were adjusted with acetonitrile to 25 mL. Aliquots of 2.5 mL of each solution was then transferred to 25 mL volumetric flasks, neutralized with either NaOH or HCl and volumes completed with either acetonitrile for HPTLC method or the mobile phase for UPLC method.

Oxidative degradation
For oxidative degradation, two portions of GQ powder equivalent to 25 mg were separately dissolved in 5 mL methanol in two separate 25 mL volumetric flasks, and then the volume was completed with 3 or 30% of H₂O₂. The solutions were refluxed at 85°C for 72 h, cooled and the volume was readjusted to the mark with methanol. Working solutions of 100 μg/mL each were then prepared by transferring 2.5 mL of each sample to 25 mL calibrated flasks; excess H₂O₂ was then removed by heating the solutions at 60°C in water bath until no oxygen bubbles were produced. The volume was then adjusted with suitable solvent and the required final dilutions were then prepared.

Photolytic and thermal degradation
Three thin-layer portions of GQ powder in three Petri dishes were exposed to a UV lamp for 24 h, sunlight for five successive days or 90°C in an oven for 48 h. Then, stock solutions (1 mg/mL) and working solutions (100 μg/mL) were prepared in methanol for each sample.

All forced degradation reactions were followed up every 8 h by TLC in the presence of standard GQ using chloroform–glacial acetic acid–formic acid (10:0.3:0.1, by volume) as a developing system. The degradation product in acidic, alkaline and oxidative stress conditions was separated after complete degradation and disappearance of GQ spot by concentrating the reaction solution to ~30% of its original volume. The produced powder was filtered, washed with water and dried. Furthermore, the structure of the isolated degradation product was elucidated by NMR, IR and MS spectral analyses.

3 RESULTS AND DISCUSSION
Stress stability study of a drug substance is necessary to provide information about potential degradation products, and chemical and physical factors that lead to drug instability (Farid & Abdelwahab, 2015). To our knowledge no stability study has been published for GQ. Hence, our work aimed to develop and validate a full stability study for GQ to anticipate its degradation behavior using HPTLC and UPLC methods. Moreover, the study was further extended for identification of the major degradation product using NMR, IR and MS. The proposed HPTLC method is more advantageous than other reported HPLC methods (Arayne et al., 2011; Ling-yun, 2012; Mirza et al., 2013, 2015), because it offers higher sensitivity and selectivity and can be used in laboratories lacking the facilities to conduct HPLC. The proposed UPLC has the advantages over other reported methods (Guo et al., 1992; Sridevi & Diwan, 2000; Ri-lai et al., 2009; Arayne et al., 2010a, 2010b, 2011; Ling-yun, 2012; Mirza et al., 2013, 2015) of short analysis time, simple sample pretreatment steps and using eco-friendly and simple mobile phase.

3.1 Stability study
Upon subjecting GQ to different stress conditions, it undergoes degradation with different rates as shown from the results in Table 1. GQ was found to be highly sensitive to hydrolytic and oxidative stress conditions while it showed good stability when subjected to photolytic and thermal degradation (Figures 2 and 3). Moreover, it was observed that the degradation rate was increased as the strength of acid, base or hydrogen peroxide increased and the degradation rate in acid was higher than that in base and hydrogen peroxide. Under hydrolytic conditions, only one degradation product was obtained (Figures 2a, b and 3a–d), which was the same
degradation product as obtained upon subjecting the drug to oxidation stress condition (Figures 2c and 3e, f). This could be attributed to the acidic character of hydrogen peroxide solution (Kovaříková, Klimeš, Dohnal, & Tisovská, 2004; Bansal et al., 2008). On the other hand, GQ was stable under stress conditions of dry heat and light as no additional peaks were observed (Figures 2d and 3g–i). The produced degradation product was isolated as a fine, fluffy, off-white powder by concentrating the prepared degradation solutions to about 30% of its original volume. The produced powder was filtered, washed with water, and dried. Then, structure elucidation was carried out using different microanalysis methods.

3.1.1 | ¹H-NMR (DMSO-d₆, 400 MHz)

Spectrum Figure 4(A) shows characteristic chemical shifts, δ 1.46 (s, 6H, 2 CH₃ groups), 2.94 (t, 2H, J = 7.4 Hz, CH₂–10), 3.84 (s, 3H, OCH₃), 4.14 (t, 2H, J = 7.4 Hz, CH₂–9), 7.29 (s, 1H, CH–8 aromatic proton), 7.41 (d, 2H, J = 8 Hz, CH–12,16 aromatic protons), 7.54 (d, 1H, J = 8 Hz, CH–5 aromatic proton), 7.61 (d, 1H, J = 8.8, CH–6 aromatic proton), 7.73 (d, 2H, J = 8.4 Hz, 2 CH–13,15 aromatic proton) and 7.32 (d, 2H, J = 3.2 Hz NH₂, which disappear upon deuteration as shown in Figure 4B).

3.1.2 | IR spectrometry

IR spectrum of degradate (Figure 4c) showed characteristic bands at about 1655 and 1703 cm⁻¹, indicating two carbonyl groups and two additional forked peaks 3325 and 3425 cm⁻¹ indicating an NH₂ group.

3.1.3 | Mass spectrometry

The mass spectrum chart of degradate (Figure 4D) showed a molecular ion peak identified at m/z 402.88 (corresponding to

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Time of degradation (day)</th>
<th>HPTLC percentage degradation</th>
<th>UPLC percentage degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M HCl at 85°C</td>
<td>3</td>
<td>51.20</td>
<td>51.54</td>
</tr>
<tr>
<td>1 M HCl at 85°C</td>
<td>3</td>
<td>67.00</td>
<td>70.00</td>
</tr>
<tr>
<td>0.1 M NaOH at 85°C</td>
<td>3</td>
<td>13.16</td>
<td>15.74</td>
</tr>
<tr>
<td>1 M NaOH at 85°C</td>
<td>3</td>
<td>45.52</td>
<td>46.61</td>
</tr>
<tr>
<td>3% H₂O₂ at 85°C</td>
<td>3</td>
<td>44.36</td>
<td>44.02</td>
</tr>
<tr>
<td>30% H₂O₂ at 85°C</td>
<td>3</td>
<td>55.91</td>
<td>52.44</td>
</tr>
<tr>
<td>Dry heat at 90°C (solid form)</td>
<td>2</td>
<td>0.61</td>
<td>0.41</td>
</tr>
<tr>
<td>Photolysis (solid form)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun</td>
<td>5</td>
<td>0.14</td>
<td>0.80</td>
</tr>
<tr>
<td>UV</td>
<td>1</td>
<td>0.90</td>
<td>0.44</td>
</tr>
</tbody>
</table>

TABLE 1 Summary of forced degradation studies of gliquidone
the molecular weight of GQS). After structure elucidation, GQS was found to be formed as a result of amide group breakage as shown in Figure 1. GQS is reported as IMP-A (The British Pharmacopoeia, 2013) and a synthetic intermediate of GQ (Andrew, 2006).

### 3.2 Method development and optimization

#### 3.2.1 HPTLC method

The main object of the developed stability-indicating HPTLC was to completely separate the peak of the degradation product from that
of the drug. Studying the optimum parameters for maximum separation was carried out by trying different developing systems such as cyclohexane–methanol–glacial acetic acid (10:0.7:0.2, by volume), benzene–methanol–glacial acetic acid (10:1.0:0.5, by volume) and chloroform–methanol (10:1, by volume; 10:0.5, by volume), where GQ and GQS appear as one spot. Chloroform–acetone–glacial acetic acid (10:0.5:0.1, by volume), chloroform–ethyl acetate–glacial acetic acid (10:0.5:0.1, by volume) and chloroform–methanol–glacial acetic acid (10:0.3:0.06, by volume) were then tried, and it was observed that all systems separated GQ from GQS but with bad resolution. Selectivity was improved by removing polar solvents and using chloroform–glacial acetic acid (10:0.3, by volume), but the GQ spot was tailed. Addition of formic acid to the system prevented GQ tailing and resulted in the desired resolution and compact spots with reasonable retardation factor ($R_f$) values. Additionally, removal of glacial acetic acid resulted in the appearance of GQ and GQS as one spot. Finally, chloroform–glacial acetic acid–formic acid (10:0.3:0.1, by volume) is the developing system of choice and the produced $R_f$ values were $0.33 \pm 0.03$ and $0.65 \pm 0.03$ for GQS and GQ, respectively (Figure 2a–d). Scanning was tried at 254 and 225 nm in order to enhance method sensitivity, where the maximum absorbance wavelength was found to be 225 nm (Figure 5), offering highest sensitivity. The band width and interspaces between bands should be carefully selected to avoid the spread of bands outside the tracks and interference between neighboring bands. Different band dimensions were tried; the optimum band width chosen was 5 mm and the interspace between bands was 8.9 mm to obtain sharp and symmetrical separated peaks. The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference from neighboring bands. Different slit dimensions were tried, and $6.00 \times 0.3$ mm proved to be the slit dimension of choice which provided the highest sensitivity (Baghdady, Al-Ghobashy, Abdel-Aleem, & Weshahy, 2013; Naguib, Abdelaleem, Zaazaa, & Hussein, 2014).

Calibration curves were constructed by plotting the integrated peak area vs the corresponding concentrations in the ranges of 1–8 and 0.1–2 μg/band for GQ and GQS, respectively. Regression equation parameters are given in Table 2. The validity of the proposed method was studied by assaying Glurenor ® tablets (Table 3), in addition to applying standard addition technique, which showed that there was no interference from excipients (Table 3).
3.2.2 | UPLC method

UPLC is a promising tool for fast LC method development; its system allows shortening of the analysis time of about 3–9 times compared with conventional HPLC methods (Churchwell, Twaddle, Meeker, & Doerge, 2005; Nováková, Matysová, & Solich, 2006; Tawakkul, Faustino, Sayeed, Khan, & Khan, 2010). Hence, the UPLC instrument offers the advantages of high-resolution with high reproducibility and sensitivity, and decreased time and solvent consumption, which leads little harmful solvent being used and making the method more eco-friendly. Some chromatographic methods were reported for GQ determination, but no UPLC method was reported for its determination. Different factors affecting chromatographic separation have been studied and optimized such as type of the aqueous phase, type and ratio of organic modifier, pH of the mobile phase, scanning wavelength and flow rate. After reviewing of the published chromatographic methods on GQ, C₁₈ was the stationary phase used and methanol was the organic modifier of choice. Hence, method optimization started with using a C₁₈ column (5 cm × 2.1 mm, 2 μm particle size) and an isocratic solvent mixture of methanol–water from 75 to 95% methanol and a flow rate of 1 mL/min, where GQ and its degrade eluted as one peak. By increasing the aqueous phase to 30%, GQ was eluted after 1.7 min while GQS was eluted after 1.2 min, but bad results concerning peak symmetry were observed. Replacing methanol with acetonitrile was tried but with no improvement. Changing pH and flow rate also had no effect. Different concentrations of sodium lauryl sulfate were tried but no enhancement occurred. The stationary phase was then changed to a C₈ column (5 cm × 2.1 mm, 1.9 μm particle size). On using 70% aqueous methanol pumping at a flow rate of 0.6 mL/min, GQ eluted after 0.73 ± 0.03 min while GQS eluted after 0.33 ± 0.03 min but with a tailed peak. Adjusting the pH value to 3 with orthophosphoric acid after trying different pH values, like 3.5, 3.8 and 4, improved the symmetry and peak shape of the studied components.

TABLE 2 Regression and validation parameters of the HPTLC and UPLC methods for determination of gliquidone and gliquidone sulfonamide

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPTLC method</th>
<th>UPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GQ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.00–8.00 μg/band</td>
<td>1.00–45.00 μg/mL</td>
</tr>
<tr>
<td>Slope a</td>
<td>−12.992</td>
<td>0.0018</td>
</tr>
<tr>
<td>Coefficient b</td>
<td>434.88</td>
<td>0.3291</td>
</tr>
<tr>
<td>Intercept C</td>
<td>3499</td>
<td>0.0378</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9991</td>
<td>1.00</td>
</tr>
<tr>
<td>Residual mean</td>
<td>0.000</td>
<td>1.00</td>
</tr>
<tr>
<td>Accuracy (mean R %)</td>
<td>99.61</td>
<td>100.03</td>
</tr>
<tr>
<td><strong>GQS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.10–2.00 μg/band</td>
<td>0.50–45.00 μg/mL</td>
</tr>
<tr>
<td>Slope a</td>
<td>−781.73</td>
<td>0.0073</td>
</tr>
<tr>
<td>Coefficient b</td>
<td>4754</td>
<td>0.19066</td>
</tr>
<tr>
<td>Intercept C</td>
<td>329.89</td>
<td>0.755</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>1.00</td>
<td>0.9991</td>
</tr>
<tr>
<td>Residual mean</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Accuracy (mean R %)</td>
<td>101.02</td>
<td>100.15</td>
</tr>
</tbody>
</table>

**Precision**

| Repeatability (RSD, %) | 0.66 | 0.52 |
| Intermediate precision (RSD, %) | 1.08 | 1.10 |
| LODb                      | 0.28 μg/band | 0.024 μg/band |
| LOQc                      | 0.85 μg/band | 0.072 μg/band |

**Note:**

GQ, gliquidone; GQS, gliquidone sulfonamide.

aFollowing a polynomial regression \( A = aX^2 + bX + C \) where \( A \) is the integrated peak area, \( X \) is the concentration in μg/band, \( a \) and \( b \) are coefficients 1 and 2, respectively, and \( C \) is the intercept.

bLimit of detection, LOD = 3.3 × SD/slope; c limit of quantitation, LOQ = 10 × SD/slope.
To obtain maximum sensitivity different scanning wavelengths were tried, where 225 nm was the wavelength of choice with the best sensitivity and minimum noise (Figure 5). Also, the effect of flow rate was studied; best resolution with minimum analysis time was achieved by delivering mobile phase at 0.6 mL/min. Finally, the optimum chromatographic condition was obtained using C8 column with methanol–water (70:30, by volume), adjusting the pH to 3 using orthophosphoric acid as a mobile phase at flow rate of 0.6 mL/min and with UV detection at 225 nm (Figure 3a–i). After method optimization, linearity was tested where good correlation coefficients were obtained between peak area vs the concentration in the range of 1–45 μg/mL for GQ and 0.5–45 μg/mL for GQS. Regression equations parameters are given in Table 2.

3.3 | Method validation

Method validation was performed according to International Conference on Harmonization guidelines (ICH Guidelines, 2003).

3.3.1 | Linearity
The linearity of GQ and GQS was evaluated by analyzing several concentrations determined in triplicate by the proposed methods. Linearity of the plotted calibration graphs was validated by the high value of the correlation coefficients, residuals means equal zero (Table 2).

3.3.2 | Accuracy
Accuracy of the methods was calculated by applying the developed method for the determination of different concentrations of pure GQ and GQS samples and then applying the corresponding regression equations; the results given in Table 2 confirmed the good accuracy of the reported method. Accuracy was also checked by applying the standard addition technique, involving analysis of marketed samples (Glurenor® tablets) to which certain amounts of pure GQ had been added, and good recoveries were obtained, verifying the accuracy of the proposed methods and revealing no interference from excipients, as shown in (Table 3).

3.3.3 | Precision
Precision was studied with respect to both repeatability and intermediate precision.

Repeatability
Three concentrations (5, 6 and 7 μg/band for GQ and 0.3, 1 and 2 μg/band for GQS for HPTLC, and 10, 20 and 30 μg/mL for UPLC) were determined in triplicate on the same day to estimate intraday variation. Acceptable relative standard deviation (RSD, %) values were obtained confirming the repeatability of the method as given in Table 2.

Intermediate precision
The previous procedure was repeated on the same concentrations on different three days to determine the intermediate precision. Acceptable RSD values were obtained as shown in Table 2.

3.3.4 | Limits of detection and quantitation
On testing sensitivity of the methods, limits of detection and quantitation (LOD and LOQ) were calculated for GQ and GQS according to the ICH Guidelines (2003). Low values were obtained, indicating the high sensitivity of the developed methods (Table 2).

3.3.5 | Robustness
For HPTLC, robustness was evaluated by performing minor changes in the studied chromatographic conditions (e.g. change in glacial acetic acid ±0.02 mL, formic acid ±0.01 mL and saturation time ± 5 min). It was found that these changes did not lead to significant changes in Rf values. While for UPLC, changes have been made in the organic modifier (± 1 mL), flow rate (± 0.05 mL/min) and scanning wavelength (± 2 nm), no dramatic changes in retention time was observed (Table 4).

3.3.6 | System suitability
System suitability parameters were calculated in order to confirm that the overall system was performing well. System suitability testing parameters for the developed methods were calculated and acceptable results were obtained as shown in Table 5.

The results obtained by the proposed methods were statistically compared with the reported HPLC method (Mirza et al., 2013) using
the tabulated ones, confirming that there was no significant difference in determination of gliquidone in its pure form proposed HPTLC and UPLC methods and the reported method for determination of gliquidone and gliquidone sulfonamide.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPTLC method</th>
<th>UPLC method</th>
<th>Reference value (ICH Guidelines, 2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing factor (T)</td>
<td>1.07</td>
<td>1.10</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>1.22</td>
<td></td>
<td>1.22</td>
</tr>
<tr>
<td>Capacity factor (K')</td>
<td>7.5</td>
<td>3.5</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>Resolution (R5)</td>
<td>4.21</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Selectivity(a)</td>
<td>2.14</td>
<td>1.38</td>
<td>1.38</td>
</tr>
</tbody>
</table>

**Table 5** System suitability testing parameters of HPTLC and UPLC methods for determination of gliquidone and gliquidone sulfonamide

Student's t- and F-tests at p < 0.05. The obtained values were less than the tabulated ones, confirming that there was no significant difference between the two methods. This statistical comparison clarified that the proposed method is as precise and accurate as the reported method, Table 6.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC</th>
<th>UPLC</th>
<th>Reported method (Mirza et al., 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>99.61</td>
<td>100.03</td>
<td>99.7</td>
</tr>
<tr>
<td>SD</td>
<td>1.015</td>
<td>1.322</td>
<td>1.634</td>
</tr>
<tr>
<td>Variance</td>
<td>1.031</td>
<td>1.458</td>
<td>2.226</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Student’s t-test* (2.228)</td>
<td>0.984</td>
<td>0.995</td>
<td>—</td>
</tr>
<tr>
<td>F-Test* (4.28)</td>
<td>2.367</td>
<td>1.527</td>
<td>—</td>
</tr>
</tbody>
</table>

*Figures in parentheses are the corresponding tabulated values at p = 0.05.

**Table 6** Statistical comparison of the results obtained by the proposed HPTLC and UPLC methods and the reported method for determination of pure gliquidone in its pure form

**4 | CONCLUSION**

The proposed chromatographic methods succeeded in being a stability-indicating one, where the peaks of degradation product completely separated from the drug with high resolution and selectivity. Furthermore, one of the main advantages of the proposed HPTLC method was its high sensitivity and selectivity; GQ was determined in a range started from 1 μg/mL, unlike other HPLC methods which started from 5 μg/mL, besides using one single scanning wavelength for the two studied compounds, saving time and effort. On the other hand, the main advantage of the proposed UPLC was its short run time compared with that of the published HPLC methods. Also, the suggested UPLC method was found to be greener and more solvent-saving than the reported ones. Finally, the suggested methods are simple, accurate, rapid, highly sensitive and reproducible; these advantages encourage use of the proposed method in routine and quality control analysis of the studied drug.

**ACKNOWLEDGEMENTS**

The authors express their appreciation and thanks to Dr Ahmed Safwat Ahmed for his effort in elucidation of the chemical structure of the degradation product.

**REFERENCES**


How to cite this article: Abdelwahab NS, Elsaady MT, Korany AG, Hegazy MA. Study of gliquidone degradation behavior by high-performance thin-layer chromatography and ultra-performance liquid chromatography methods. Biomedical Chromatography, 2017;31:e4025, https://doi.org/10.1002/bmc.4025