HIGH THROUGHPUT QUANTIFICATION OF QUINIDINE IN HUMAN PLASMA BY LC/MS/MS FOR THERAPEUTIC DRUG MONITORING

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Abstract

A simple, rapid and sensitive HPLC method for the quantification of quinidine in human plasma has been developed and validated. Quinidine was separated on a C18 column under isocratic conditions using a mobile phase of 85:15 (v/v) 0.2% formic acid and acetonitrile. In these chromatographic conditions, the retention time of quinidine was 1.2 min and the overall time of one analysis was 1.6 min. Plasma sample preparation consisted in protein precipitation with methanol. The detection of quinidine was performed in multiple reaction monitoring (MRM) mode, using an ion trap mass spectrometer with electrospray positive ionization. The linearity domain was established between 0.33 and 13.26 µg/mL. Accuracy (%) and precision (CV%) were less than 7.9% for intra-day assay and 8.9% for inter-day assay, and less than 18.7% and 22.6%, respectively, at the limit of quantification. The recovery ranged between 92.5 and 129.5%. The method is very simple and rapid and can be used for the therapeutic drug monitoring of quinidine.

Keywords: quinidine, liquid chromatography, mass spectrometry

Rezumat

A fost dezvoltată și validată o metodă HPLC simplă și sensibilă de cuantificare a chinidinei din plasma umană. Chinidina a fost separată în condiții izocratice folosind o fază mobilă formată din 85:15 (v/v) acid formic 0,2% și acetonitril. În aceste condiții cromatografice, timpul de rețenție al chinidinei a fost de 1,2 min și timpul de analiză per probă a fost de doar 1,6 min. Prelucrarea probelor a presupus o precipitare a proteinelor din plasma umană cu metanol. Detecepția chinidinei s-a realizat în mod de monitorizare a reacțiilor multiple (MRM), ionizare pozitivă, folosind un spectrometru de masă dotat cu sursă de ionizare tip electrospray și cu analizor trapă ionică. Domeniul de linieritate a fost stabilit între 0,33 și 13,26 µg/mL. Acuratețea și precizia au fost mai mici de 7,9% pentru determinări în aceeași zi și mai mici de 8,9% pentru determinări în zile diferite, și mai mici decât 18,7% respectiv 22,6%, la limita inferioară de cuantificare. Regăsirea se situează între 92,5 și 129,5%. Metoda este foarte simplă și rapidă și poate fi utilizată pentru monitorizarea terapeutică a chinidinei.

Keywords: quinidine, liquid chromatography, mass spectrometry
Introduction

Quinidine, 2-ethenyl-4-azabicyclo[2.2.2]oct-5-yl)-(6-methoxyquinolin-4-yl)-methanol, is a class I antiarrythmic agent that acts by blocking the fast inward sodium current (Fig. 1). After oral administration, its bioavailability is about 50-80%, with a half life of 6 to 8 hours. Quinidine is eliminated by the cytochrome P450 system in the liver and about 20% is excreted unchanged via the kidneys. The currently accepted therapeutic concentration range for quinidine in plasma is 2 to 5 µg/mL [1,2]. Being a drug with large inter-subject variability and narrow therapeutic window, therapeutic drug monitoring is required for quinidine in order to improve its pharmacotherapy and safety. Several methods for the determination of quinidine concentration in human plasma have been reported. Mainly, HPLC-based methods with fluorescence and UV detection were described [1,2,3], but immunochemical methods were reported also [1,4]. LC/MS has been widely accepted as the most used method in the identification and quantitative analysis of drugs and its metabolites due to its superior sensitivity and specificity. There are no papers published to the date regarding the determination of quinidine in plasma by LC/MS/MS.

![Figure 1](image.png)

Chemical structure of quinidine

In the present study we developed a rapid HPLC/MS/MS method for the quantification of quinidine in human plasma in order to be applied in therapeutic drug monitoring or pharmacokinetic studies.

Materials and methods

Reagents

Quinidine sulfate salt dihydrate was reference standard from Sigma-Aldrich. Acetonitrile, formic acid and methanol were Merck products (Merck KgaA, Darmstadt, Germany). Distilled, deionised water was produced by a Direct Q-5 Millipore (Millipore SA, Molsheim, France) water system. The human blank plasma was supplied by the Local Bleeding Centre Cluj-Napoca, Romania.
**Standard solutions**

A stock solution of quinidine with a concentration of 16.5 mg/mL was prepared by dissolving the appropriate quantity of quinidine sulfate reference substance in 10 mL acetonitrile. A working solution was obtained by diluting a specific volume of stock solution with plasma. Then this was used to spike different volumes of blank plasma, providing finally eight plasma standards with the concentrations ranged between 0.33 and 13.26 µg/mL. The accuracy and precision of the method were verified using plasma standards with concentrations of 0.33, 0.83, 5.30 and 10.61 µg/mL quinidine.

**Chromatographic and mass spectrometry systems and conditions**

The HPLC system was an 1100 series model (Agilent Technologies) consisted of a binary pump, an in-line degasser, an autosampler, a column thermostat, and an Ion Trap VL mass spectrometer detector (Brucker Daltonics GmbH, Germany). Chromatograms were processed using QuantAnalysis software. The detection of quinidine was MS/MS using an electrospray positive ionisation (ESI positive). The ion transition was monitored as follows: m/z 325.2 → (m/z 184+253+307). Chromatographic separation was performed at 45 °C on a Zorbax SB-C18 100 mm x 3 mm i.d., 3.5 µm column (Agilent Technologies), protected by an in-line filter.

**Mobile phase**

The mobile phase consisted of a mixture of water containing 0.2% formic acid and acetonitrile (85:15 (v/v), each component being degassed, before elution, for 10 min in an Elma Transsonic 700/H (Singen, Germany) ultrasonic bath. The pump delivered the mobile phase at 1 mL/min.

**Sample preparation**

Standard and test plasma samples were prepared as follows in order to be chromatographically analyzed. In an Eppendorf tube, to 0.2 mL plasma, 0.6 mL methanol is added. The tube is vortexed for 10 s and then centrifuged for 6 min at 5000 rpm. A further 1:5 dilution of the supernatant was prepared because the concentration of analyte was too high for the MS sensitivity. A volume of 0.15 mL of final solution is transferred in an autosampler vial and 2 µl were injected into the HPLC system.

**Validation**

The method validation [5-9] involves verifying specificity, by using six different plasma blanks obtained from healthy human volunteers who did not take quinidine before and any other medication. The linearity of the peak area against standard concentration was verified between 0.33-13.26 µg/mL quinidine by the least squares analysis. The applied calibration model was a quadratic one: \(y = ax^2 + bx + c\), weight 1/y, where \(y\) is the peak area and \(x\), the concentration. Distribution of the residuals (% difference of the back-calculated concentration
from the nominal concentration) was investigated. The calibration model was accepted, if the residuals were within ±20% at the lower limit of quantification and within ±15% at all other calibration levels and at least 2/3 of the standards meet this criterion.

The limit of quantification was established as the lowest calibration standard with an accuracy and precision less than 20%. The intra- and inter-day precision (expressed as coefficient of variation %, CV%) and accuracy (relative difference % between found and theoretical concentration, bias%) of the assay procedure were determined by the analysis in the same day of three samples at each of three levels of concentration in the considered concentration range and one sample of each in three different days, respectively. The recoveries at each of the previous levels of concentration were measured by comparing the response of the treated plasma standards with the response of standards in water with the same concentration of quinidine as the final extract from plasma standards.

**Results and discussion**

The detection of quinidine was carried out by adding multiple fragments from the MS spectrum in order to improve the overall signal. The sum of ions from MS spectrum is (m/z 184+253+307) (Fig. 2). The retention time of quinidine was 1.2 min (Fig. 3) and no significant interference was observed at the retention time in plasma blank samples chromatograms.

![Figure 2](image_url)

**Figure 2**

Ion mass spectra of quinidine (upper spectra- full scan, middle spectra- isolation, lower spectra- fragmentation).
The calibration curves showed a linear response over the range of concentrations used in the assay procedure. The inter- and intra-day precision, accuracy and recovery results are showed in Table I and Table II. The lower limit of quantification (LLOQ) was established at 0.33 µg/mL quinidine. Precision and accuracy at the quantification limit were 11.6% and 3.8% for intra-day determinations and 16.2% and 4.2% for inter-day determinations, being in agreement with the validation guidelines [5-9]. The recovery was consistent and ranged between 92.5 and 109.1% (Tables I and II).

![Chromatogram of the LLOQ plasma standard with 0.33 µg/mL quinidine.](image)

Table I

<table>
<thead>
<tr>
<th>C&lt;sub&gt;theoretical&lt;/sub&gt; µg/mL</th>
<th>Mean c&lt;sub&gt;found&lt;/sub&gt; µg/mL (± S.D.)</th>
<th>Precision (CV %)</th>
<th>Accuracy %</th>
<th>Recovery % (± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>0.34 (± 0.01)</td>
<td>11.6</td>
<td>3.8</td>
<td>101.5 (± 20.1)</td>
</tr>
<tr>
<td>0.83</td>
<td>0.89 (± 0.01)</td>
<td>6.2</td>
<td>7.9</td>
<td>97.7 (± 6.1)</td>
</tr>
<tr>
<td>5.30</td>
<td>5.17 (± 0.07)</td>
<td>6.6</td>
<td>-2.6</td>
<td>100.2 (± 5.9)</td>
</tr>
<tr>
<td>10.61</td>
<td>10.41 (± 0.15)</td>
<td>7.3</td>
<td>-1.9</td>
<td>97.8 (± 5.4)</td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>C&lt;sub&gt;theoretical&lt;/sub&gt; µg/mL</th>
<th>Mean c&lt;sub&gt;found&lt;/sub&gt; µg/mL (± S.D.)</th>
<th>CV %</th>
<th>Accuracy %</th>
<th>Recovery % (± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>0.35 (± 0.02)</td>
<td>16.2</td>
<td>4.2</td>
<td>109.1 (± 12.3)</td>
</tr>
<tr>
<td>0.83</td>
<td>0.87 (± 0.02)</td>
<td>8.9</td>
<td>5.4</td>
<td>99.7 (± 13.0)</td>
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<tr>
<td>5.30</td>
<td>5.04 (± 0.06)</td>
<td>5.8</td>
<td>-5.0</td>
<td>92.5 (± 17.9)</td>
</tr>
<tr>
<td>10.61</td>
<td>9.77 (± 0.10)</td>
<td>5.1</td>
<td>-7.9</td>
<td>96.7 (± 3.7)</td>
</tr>
</tbody>
</table>
Conclusions

The proposed method proved to be rapid, accurate and precise for the quantitative determination of quinidine in human plasma. In comparison with the published articles in literature regarding the quantification of quinidine in human plasma, our method had the advantage of selectivity due to MS detection, high throughput due to both simple plasma preparation and short analysis time. Without using an internal standard and applying a simple sample preparation by protein precipitation, a specific and efficient analysis of plasma samples could be performed. The method can be used for the therapeutic drug monitoring of quinidine.

Acknowledgments

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References

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