Utility of eosin Y as a complexing reagent for the determination of citalopram hydrobromide in commercial dosage forms by fluorescence spectrophotometry

Syed Najmul Hejaz Azmi,* Ahlam Al-Fazari, Munira Al-Badaei and Ruqiya Al-Mahrazi

ABSTRACT: An accurate, selective and sensitive spectrofluorimetric method was developed for the determination of citalopram hydrobromide in commercial dosage forms. The method was based on the formation of a fluorescent ion-pair complex between citalopram hydrobromide and eosin Y in the presence of a disodium hydrogen phosphate/citric acid buffer solution of pH 3.4 that was extractable in dichloromethane. The extracted complex showed fluorescence intensity at \( \lambda_{\text{em}} = 554 \text{ nm} \) after excitation at 259 nm. The calibration curve was linear over a concentration range of 2.0–26.0 \( \mu \text{g/mL} \). Under optimized experimental conditions, the proposed method was validated as per ICH guidelines. The effect of common excipients used as additives was tested and the tolerance limit calculated. The limit of detection for the proposed method was 0.121 \( \mu \text{g/mL} \). The proposed method was successfully applied to the determination of citalopram hydrobromide in commercial dosage forms. The results were compared with the reference RP-HPLC method. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: citalopram hydrobromide; eosin Y; spectrofluorimetry; validation; commercial dosage forms

Introduction

Citalopram hydrobromide is known chemically as (±)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, hydrobromide (CAS: 59729-32-7; \( M_r = 405.35 \)). It is a selective serotonin-reuptake inhibitor, having a broad spectrum of therapeutic activity against depressive disorders. It is approved for the treatment of the symptoms of major depression and is frequently used to treat anxiety, panic disorder and body dysmorphic disorder. It has been found to greatly reduce the symptoms of diabetic neuropathy and premature ejaculation (1).

The assay for citalopram hydrobromide in bulk and in pharmaceutical formulations is cited in The British Pharmacopoeia (2) and is based on liquid chromatography. In view of the great importance of the drug in terms of its optimum oral dose and its wide use in the treatment of depression associated with mood disorders, various analytical methods have been reported including high-performance thin-layer chromatography (3), high-performance liquid chromatography (HPLC) (4–8), cyclic voltammetry (9), capillary zone electrophoresis (10) and spectrophotometry (11). Most of these techniques utilize sophisticated instruments and reagents that are not available in many laboratories or need well-trained personnel to operate them. Two spectrofluorimetry methods are available that are very simple and based directly on determination of the drug in distilled water (12) and sulfuric acid (13).

The aim of this study is to develop an optimized and validated spectrofluorimetric method for the determination of citalopram hydrobromide in pharmaceutical formulations. The proposed method is based on the formation of an ion-pair complex of the drug with eosin Y in the presence of Na₂HPO₄/citric acid buffer (pH 3.4) and is extractable in dichloromethane. The extracted complex showed fluorescence intensity at 552 nm after excitation at 259 nm. The reaction conditions are optimized and validated as per International Conference on Harmonisation (ICH, USA) (14).

Experimental and methods

Apparatus

An Agilent’s Cary Eclipse fluorescence spectrophotometer (Thermo Scientific, Scoresby, Australia) was used for the fluorescence intensity measurements. The instrument is equipped with xenon lamp and 1-cm quartz cells. Excitation and emission wavelengths were set at 259 and 554 nm, with excitation and emission slit widths of 10 nm.

Chromatography was performed with a Dionex-Ultimate 3000 HPLC system (Thermo Scientific) equipped with a 250 mm × 4.6 mm i.d., 5 \( \mu \text{m} \) particle, Acclaim 120 C₁₈ reversed-phase LC column, a variable wavelength program UV/vis detector (WDM-3000), UV/vis photometer detector, pump (HPG-3200 SD), column oven (TCC, 3000 SD) and Chromleon Data System software (v. 5.80 SR11). The column was set at ambient temperature (25 ± 1 °C) and
a flow rate of 1.0 mL/min. The mobile phase was 0.01 M ammonium acetate/methanol (35: 65 v/v, pH 5.0); the wavelength was 239 nm and the injection volume was 20 μL. The mobile phase was cleaned using a Duroapore PVDF membrane filter of 0.45 μm (Merck Millipore Ltd, Tullagreen, Ireland).

A Hanna pH meter (USA) was used for pH measurements and an IR Affinity-1 spectrophotometer (Shimadzu, Kyoto, Japan) was used for IR spectra in wave number region 4000–400 cm⁻¹ using the KBr pellet technique.

Materials

All reagents used were of analytical reagent grade.

A 0.02% eosin Y disodium salt (CAS: 17372-87-1, Mr: 691.85, Fluka Chemie AG, Switzerland) solution was freshly prepared in distilled water.

Na₂HPO₄/citric acid buffer solutions of pH 2.6, 2.8, 3.0, 3.2, 3.4, 3.6 and 3.8 were prepared by mixing 2.18, 3.17, 4.11, 4.94, 5.70, 6.44 and 7.10 mL of 0.2 M Na₂HPO₄ with 17.82, 16.83, 15.89, 15.06, 14.30, 13.56 and 12.90 mL of 0.1 M citric acid in a total volume of 20 mL, respectively (15). The pH of each solution was adjusted with using a pH meter.

The citalopram hydrobromide reference standard drug was a gift of the National Pharmaceutical Industries Company, Oman. The citalopram hydrobromide drug solution was prepared by dissolving 0.01 g of drug in 1 mL of methanol in a 50 mL standard volumetric flask and then made up to the mark with distilled water.

Citolom 20 mg per tablet (National Pharmaceutical Industries Company, Oman) and Citadeep 20 mg per tablet (Cipla, India) are commercial products of citalopram hydrobromide and were purchased from a local market. The excipients used in the commercial tablets are mannitol, silica, magnesium stearate, hypromellose, titanium dioxide, macrogol 6000 and microcrystalline cellulose.

Recommended procedure for the determination of citalopram hydrobromide using proposed method

Aliquots (0.1–1.3 mL) of 0.02% citalopram hydrobromide were mixed with 1 mL of 0.02% eosin Y and 1.2 mL of Na₂HPO₄/citric acid buffer (pH 3.4) in 10 mL standard volumetric flasks and diluted to the mark with distilled water. The contents of the flask were transferred to a separating funnel with 10 mL of dichloromethane and shaken well for 2 min. The lower organic layer was separated and treated with anhydrous sodium sulfate. The fluorescence intensity of the organic layer was recorded at 554 nm after excitation at 259 nm. The amount of the drug was obtained from either the calibration graph or the regression equation.

Procedure for the reference method (7)

A stock solution of citalopram (0.2 mg/mL) was prepared in a mobile phase of 0.01 M ammonium acetate/methanol (35: 65 v/v, pH 5.0). An HPLC column (250 mm x 4.6 mm i.d., 5 μm particle, C₁₈ column) was cleaned using the same mobile phase. A series of standard solutions (5–25 μg/mL) were transferred to 10 mL volumetric flasks and diluted to the mark with same mobile phase. The drug solution was injected (20 μL) into the sample port and eluted using the mobile phase at a flow rate of 1.0 mL/min. The detector wavelength was fixed at 239 nm at ambient temperature and the chromatogram was recorded. The peak area was plotted against the concentration of citalopram in μg/mL to obtain a calibration graph. A linear equation was developed and used to find out the concentration of citalopram in tablets.

Determination of citalopram hydrobromide in pharmaceutical formulations using the proposed and reference methods

Ten commercially available tablets of Citom 20 and Citadeep 20 were weighed and finely powdered. Powder equivalent to 20 mg citalopram hydrobromide was weighed and completely dissolved in 5 mL of methanol (or a mobile phase of 0.01 M ammonium acetate/methanol 35: 65 v/v for the reference method) in a beaker. Sixty millilitres of distilled water (or the said mobile phase for the reference method) was added to the beaker and then filtered through a Whatman No. 42 filter paper (Whatman International Ltd, Maidstone, UK) (or Millipore PVDF membrane filter of 0.45 μm for the reference method) in a 100 mL standard volumetric flask. The residue was washed well with 3 × 10 mL of distilled water (or with the mobile phase for the reference method) for complete recovery of the drug and diluted to the mark with distilled water (or mobile phase for the reference method). The amount of citalopram hydrobromide was determined following the recommended procedures of the proposed and reference methods.

Validation

The proposed method was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, specificity, robustness, accuracy and applicability.

The linearity of the proposed method was evaluated using 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2 and 1.3 mL of 0.02% citalopram hydrobromide corresponding to 2.0, 4.0, 8.0, 10.0, 12.0, 16.0, 20.0, 24.0 and 26.0 μg/mL citalopram hydrobromide. Each concentration was analysed independently five times and the fluorescence intensity was recorded. The fluorescence intensity at each concentration was plotted against the initial concentration of citalopram hydrobromide in μg/mL and the linear regression equation was obtained by least squares treatment of the calibration data. The other statistical parameters of the proposed method were calculated using OriginPro 6.1 software.

The LOD and LOQ for the proposed method were calculated using the following equations:

$$LOD = 3.3 \times \frac{S_0}{b}$$

(1)

$$LOQ = 10 \times \frac{S_0}{b}$$

(2)

where $S_0$ and $b$ are standard deviation and slope of the calibration line, respectively.

The precision of the proposed method was investigated from intraday and interday precisions. Three aliquots of 0.2, 0.6 and 1.2 mL of 0.02% citalopram hydrobromide corresponding to 4.0, 12.0 and 24 μg/mL citalopram hydrobromide were taken and analysed independently five times within a day (intraday precision) and over five consecutive days (interday precision).

The specificity of the proposed method was tested by taking 1 mL of 0.02% citalopram hydrobromide corresponding to 20 μg/mL citalopram hydrobromide in the presence of glucose, fructose, lactose, sodium benzoate, starch, povidone, methyl cellulose and microcrystalline cellulose. The fluorescence intensity was recorded and should not exceed ± 2% at each addition of
foreign species. The maximum tolerance limit of each species at given concentration of drug was calculated using the following formula:

\[
\text{mass/volume (mg/L)} = C \times M_i \times 1000
\]

(3)

where \(C\) and \(M_i\) are the concentration and relative molecular mass of the excipients, respectively.

The robustness of the proposed method was evaluated by pipetting 0.5 mL of 0.02% citalopram hydrobromide solution and observing the influence of small variations in experimental variables such as the concentration of eosin, volume of the buffer solution (pH 3.4), shaking time and solvent.

The accuracy of the proposed method was checked using a standard addition technique. In this technique, 0.5 mL of 0.20 mg/mL of the formulated sample solution was spiked separately with 0, 0.05, 0.1, 0.15 and 0.2 mL of the reference drug sample solution in a 10 mL standard volumetric flask and diluted to the mark with distilled water. Each level was analysed independently five times following the recommended procedure for the determination of citalopram hydrobromide. The nominal concentration of citalopram hydrobromide in the tablet solution was determined by taking the ratio of the intercept and slope.

The accuracy of the proposed method was checked using a direct method. Freshly prepared tablet solutions of Citom for citalopram hydrobromide were analysed independently in five replicates by considering 0.5 mL of 0.02% citalopram hydrobromide and eosin Y at pH 3.4 was studied using the mole ratio method (18). For this purpose, different volumes (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2.2 and 2.4 mL) of 2.5 × \(10^{-4}\) M eosin Y, followed by the addition of 1.2 mL of Na2HPO4/citric acid buffer (pH 3.4) in 10 mL standard volumetric flasks. The contents of the flasks were diluted to the mark with distilled water and were subjected to the recommended procedure for the determination of citalopram hydrobromide. The fluorescence intensity was measured at 554 nm after excitation at 259 nm and plotted against the molar ratio of eosin to the drug. The maximum fluorescence intensity of 310.00 was obtained at 2.0 mL of pure citalopram hydrobromide. It is clear from the result that the combining molar ratio between citalopram hydrobromide and eosin Y is 2:1. The apparent formation constant (\(K_f\)) for the complex between citalopram hydrobromide and eosin Y was calculated using the following expression:

\[
K_f = \frac{(A_{\text{obs}}/A_{\text{exp}})C}{C_D - 2\left(\frac{A_{\text{obs}}}{A_{\text{exp}}}\right)C - (A_{\text{obs}}/A_{\text{exp}})C}
\]

(5)

where \(A_{\text{obs}}\) and \(A_{\text{exp}}\) are the observed and extrapolated fluorescence intensities of citalopram hydrobromide–eosin Y, respectively. \(C_D, C_c\) and \(C\) are the initial concentration of citalopram hydrobromide, eosin Y and limiting concentration (eosin Y) in mol/L, respectively. The \(K_f\) of the complex was found to be 9.61 \(\times 10^{13}\). The apparent Gibbs’ free energy (\(\Delta G^°\)) was calculated using \(\Delta G^° = -2.303 RT \log K_f\) and found to be -74.783 kJ/mol, confirming the feasibility of the reaction.

The Fourier transform infrared (FTIR) spectra of free citalopram HBr, free eosin Y and the citalopram–eosin Y ion-pair complex are shown in Fig. 2(a–c). Citalopram has one potential aliphatic tertiary amine site attached with bicyclic phthalene and undergoes protonation in the presence of Na2HPO4/citric acid buffer (pH 3.4). The FTIR spectra of free citalopram exhibited peaks at 3062 and 3090 cm\(^{-1}\) for C–H stretching (aromatic) vibration, 2935 cm\(^{-1}\) for C–H stretching (aliphatic) vibration, 2229.71 cm\(^{-1}\) for –C≡N aryl group stretching vibration and 1040–1270 cm\(^{-1}\) for (CH)\(_3\)-N-CH\(_2\)- stretching vibration in the range 1554.63–1604.77 cm\(^{-1}\)
Figure 2. Infrared spectra of (a) pure citalopram, (b) free eosin Y and (c) Required correction free citalopram–eosin Y ion-pair complex in KBr (2 mg sample/200 mg KBr).
and a symmetric stretching vibration in the range 1350.17–1458.18 cm\(^{-1}\). The FTIR spectra of the citalopram–eosin Y ion-pair complex exhibited an additional band for a \(-\text{C=}\text{O}\) group due to ionization of the sodium ions in eosin Y for the formation of the ion-pair complex with protonated citalopram. In addition to this, there are some changes and additional bands (1095 cm\(^{-1}\)) appeared in the stretching vibration range 1040–1270 cm\(^{-1}\) for (CH)\(_3\)-N-CH\(_2\)- due to protonation of citalopram at the tertiary amine position. On the basis of our experimental findings and the literature background, the reaction sequence is shown in Fig. 3.

**Optimization of variables**

The variables for the proposed spectrofluorimetric method were optimized by testing several parameters such as reaction time, eosin Y concentration, buffer solutions at different pH, solvents and shaking time for extraction of the complex.

The effect of reaction time on the fluorescence intensity of the ion-pair complex was investigated between 1 and 70 min. It was observed that the ion pair was stable for up to 1 h at 25 ± 1 °C.

The influence of pH on the fluorescence intensity of the ion-pair complex was investigated using 1.2 mL of Na\(_2\)HPO\(_4\)/citric acid buffer solution at different pH values in the range 2.6–4.0 with 1.3 mL of 0.02% citalopram hydrobromide and found to be linear over a concentration range of 2–26 μg/mL. The analytical parameters and the results of statistical analysis of the experimental data are summarized in Table 1. The highest fluorescence intensity of the complex was attained in dichloromethane and there is no extraction of the complex in carbon tetrachloride and ethyl acetate. The fluorescence intensity of the blank solution was also investigated and found to be negligible in the solvent. Therefore, dichloromethane was selected as the best solvent for extraction of the complex.

**Validation**

Under the optimized experimental conditions, the fluorescence intensity was plotted against the initial concentration of citalopram hydrobromide and found to be linear over a concentration range of 2–26 μg/mL. The analytical parameters and the results of statistical analysis of the experimental data are summarized in Table 1. The high value of the correlation coefficient (0.9999) for the proposed method indicated excellent linearity. The experimental intercept of the calibration line was calculated for significance of deviation from the theoretical intercept, i.e. zero using the relation, \(t = a/S_o\) and was found to be 0.768, which is less than the tabulated \(t\)-value (2.365, \(ν = 7\)) at a 95% confidence level indicated an acceptable intercept. This makes the proposed method more

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed method</th>
<th>Reference method</th>
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<tbody>
<tr>
<td>Absorption wavelength (nm)</td>
<td>259</td>
<td>239</td>
</tr>
<tr>
<td>Maximum emission wavelength (nm)</td>
<td>554</td>
<td>–</td>
</tr>
<tr>
<td>Linear dynamic range (μg/mL)</td>
<td>2–26</td>
<td>5–25</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>(F = 0.359 + 17.683 C)</td>
<td>(P A = 0.1711 + 0.7375 C)</td>
</tr>
<tr>
<td>Standard deviation of intercept, (S_o)</td>
<td>0.467</td>
<td>0.136</td>
</tr>
<tr>
<td>Confidence limit of the intercept</td>
<td>1.104</td>
<td>0.348</td>
</tr>
<tr>
<td>Standard deviation of slope, (S_b)</td>
<td>0.030</td>
<td>0.009</td>
</tr>
<tr>
<td>Confidence limit of the slope</td>
<td>0.070</td>
<td>0.023</td>
</tr>
<tr>
<td>Correlation coefficient ((r))</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Standard deviation of calibration line ((S_o))</td>
<td>0.715</td>
<td>0.155</td>
</tr>
<tr>
<td>Variance ((S^2))</td>
<td>0.511</td>
<td>0.024</td>
</tr>
<tr>
<td>Limit of detection, LOD (μg/mL)</td>
<td>0.121</td>
<td>0.632</td>
</tr>
<tr>
<td>Limit of quantification, LOQ (μg/mL)</td>
<td>0.404</td>
<td>2.108</td>
</tr>
</tbody>
</table>

**Figure 3.** Reaction sequence of citalopram–eosin Y ion-pair complex.
appreciable and effective for the determination of drug in pharmaceutical formulations.

Intra- and interday precisions were evaluated by determining the concentration of citalopram hydrobromide at lower, middle and upper concentrations for five repeated measurements within the same day and on five consecutive days, respectively (Table 2). It can be seen from Table 2 that %RSD values were in the range of 0.36–0.75% for intraday and interday precisions. The %RSD values showed that the proposed method is precise and can be used to analyse citalopram hydrobromide in pharmaceutical formulations.

The effect of excipients such as glucose, fructose, lactose, sodium benzoate, starch, povidone, methyl cellulose and microcrystalline cellulose on the determination of 20 μg/mL citalopram hydrobromide was investigated. The fluorescence intensity was recorded at varying concentrations of excipients as additives to known concentrations of citalopram hydrobromide. Table 3 shows the maximum tolerance limit of the studied excipients. Microcrystalline cellulose is practically insoluble in water, whereas methyl cellulose gives a viscous solution. Therefore, they can be removed by filtration only and do not interfere with the determination of citalopram. Larger amounts of tolerated excipients indicated that the proposed method is more specific and selective, and thus can be used to determine citalopram hydrobromide in pharmaceutical formulations in the presence of said additives.

There are six degradation products of citalopram, namely 3-hydroxycitalopram carboxamide, citalopram carboxamide, citalopram cyano N-oxide, citalopram N-oxide, citalopram di-N-oxide and citalopram butanone. At pH 3.4, citalopram cyano N-oxide, citalopram N-oxide and citalopram di-N-oxide did not interfere with formation of the ion-pair complex with eosin Y and hence could not be extracted into dichloromethane. However, 3-hydroxycitalopram carboxamide, citalopram carboxamide and citalopram butanone interfere to some extent in the determination of citalopram, but these products are formed under very stressed conditions (8). Generally, these degradation products are not found in tablet formulations.

The ruggedness of the proposed method was established by deliberately changing the reaction conditions. To prove that the proposed method is rugged, following operational parameters were used: volume of 0.02% eosin Y, 1.2 mL (±0.2 mL); pH 3.4 (±0.2); and shaking time, 2.0 min (±0.5 min). Under these optimal conditions, a solution containing 10.0 μg/mL citalopram hydrobromide (Citom tablet) was analysed using the proposed method. The results showed a mean % recovery ± RSD of 100.21 ± 0.455%. The results indicated the ruggedness of the proposed method.

<table>
<thead>
<tr>
<th>Table 2. Precision of the proposed method</th>
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<tbody>
<tr>
<td>Actual concentration (μg/mL)</td>
</tr>
<tr>
<td>4.0</td>
</tr>
<tr>
<td>12.0</td>
</tr>
<tr>
<td>24.0</td>
</tr>
<tr>
<td>&lt;sup&gt;a&lt;/sup&gt;Mean for five independent analysis</td>
</tr>
</tbody>
</table>

The accuracy of the proposed method was tested by performing recovery experiments using a standard addition technique. The fluorescence intensity of the ion-pair complex for the Citom tablet sample solution spiked with standard drug solution was recorded and plotted, as shown in Fig. 4. It is clear from the graph that the linearity of the regression line for the tablet solution was good (r = 0.999) with an intercept and slope of 176.973 and 17.777, respectively. The concentration of citalopram hydrobromide in the tablet solution was calculated by taking the ratio of the intercept to the slope and was found to be 9.954 μg/mL. The found concentration of drug in tablet solution was subjected to standard deviation, S<sub>xe</sub>, which can be calculated by the following expression:

\[
S_{xe} = \frac{S_{ye}}{b} \sqrt{\frac{1}{n} + \frac{p^2}{b^2 \sum (x_i - \bar{x})^2}}
\]

The value of S<sub>xe</sub> was found to be 0.102 μg/mL. The confidence limit for the concentration of citalopram hydrobromide in tablet was calculated by \( x_t \pm t_{0.025, n-2} S_{xe} \) at n = 2 degrees of freedom and found to be 9.954 ± 0.324 μg/mL. The most attractive feature of the proposed spectrofluorimetric method using standard addition is its relative freedom from pharmaceutical additives and excipients. The pharmaceutical additives and adjuvants are not fluorescent in nature, and therefore did not interfere with the determination process.

![Figure 4. Standard addition plot: 0.5 mL of 0.02% citalopram hydrobromide Citom tablet solution was spiked with 0, 0.05, 0.1, 0.15 and 0.2 mL standard solution of 0.02% citalopram hydrobromide.](image-url)
The applicability of the proposed method for the determination of citalopram hydrobromide in Citom 20 and Citadep 20 tablets has been tested. The results of the proposed method were compared statistically with those of the reference method (7) using point and interval hypothesis tests. Values of $t$ and $F$ at the 95% confidence level were calculated by point hypothesis test for the proposed method with respect to the reference method and found to be less than the tabulated $t$- and $F$-values of 2.036 and 6.39, respectively, thus confirming that there is no significant difference between the performance of the proposed method and the reference method. Bias was also evaluated by interval hypothesis test by means of the recovery results, which are within the acceptable limit of ± 2%. The performance of the proposed method has been compared with other existing methods with regard to linear range, LOD and LOQ values (Table 5). LC-UV (8) and capillary zone electrophoresis (10) methods are expensive and their LOD values are 1 and 0.8 μg/mL, respectively. Moreover, RP-HPLC (5) has a wider linear range, but the lower limit starts at 25 μg/mL, which is not acceptable. In the literature, direct UV spectrophotometric and visible spectrophotometric methods involving chloranil as a reagent have been developed and show linear ranges of 2–12 and 1–25 μg/mL, respectively, but the LOD values are quite high compared with the proposed method. The spectrofluorimetric methods (12,13) have a low linear range, but a high linear range is required for estimation of the drug in commercial dosage forms. Thus, the proposed method is simple, involving a very readily available reagent (eosin Y), has an appreciable linear range, requires less analysis time, and has low LOD and LOQ values compared with the other methods mentioned in Table 5.

### Conclusions

The proposed method was successfully applied for the determination of citalopram hydrobromide in commercial tablets with acceptable recovery results, which are within the acceptable limit of ± 2%.

The proposed method was successfully applied for the determination of citalopram hydrobromide in pharmaceutical formulations in the presence of excipients. Hence the proposed method is more specific and selective. The ease of operation, low-cost reagents, high sensitivity, repeatability and reproducibility of the proposed method make it suitable for routine application in research laboratories and the pharmaceutical industry.

### Table 4. Significance of testing: point and interval hypothesis tests for the determination of citalopram hydrobromide in tablets at 95% confidence level

<table>
<thead>
<tr>
<th>Pharmaceutical formulations</th>
<th>Proposed method</th>
<th>Reference method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD</td>
</tr>
<tr>
<td>Citom 20 mg$^a$</td>
<td>100.21</td>
<td>0.455</td>
</tr>
<tr>
<td>Citadep 20 mg$^b$</td>
<td>100.11</td>
<td>0.447</td>
</tr>
</tbody>
</table>

$^a$Citom 20 and citadep 20 excipients are cellulose microcrystalline, mannitol, magnesium stearate, silica colloidal anhydrous, hypromellose, macrogol and pigment–titanium dioxide.

$^b$Mean for five independent analyses.

$^c$A bias, based on recovery experiments, of ± 2% is acceptable.

### Table 5. Comparison of the proposed spectrofluorimetric method with existing related techniques for the assay of citalopram in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Analytical methods</th>
<th>Linear concentration range (μg/mL)</th>
<th>LOD (μg/mL)</th>
<th>LOQ (μg/mL)</th>
<th>Mobile phase (or solvent)</th>
<th>References</th>
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<tbody>
<tr>
<td>LC-UV method</td>
<td>5–500</td>
<td>1</td>
<td>5</td>
<td>C$_8$ column; acetonitrile and 0.025 mol/L ammonium acetate buffer solution (35: 65 v/v, pH 4.5); retention time 35.78 min; flow rate 0.5 mL/min</td>
<td>8</td>
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<tr>
<td>RP-HPLC</td>
<td>25–150</td>
<td>–</td>
<td>–</td>
<td>C$_{18}$ column; acetate buffer (pH 4.5) and acetonitrile (65: 35 v/v); retention time 3.72 min; flow rate 1.0 mL/min</td>
<td>5</td>
</tr>
<tr>
<td>Capillary zone electrophoresis</td>
<td>5–50</td>
<td>0.8</td>
<td>–</td>
<td>Phosphate buffer, elution time 3.72 min</td>
<td>10</td>
</tr>
<tr>
<td>UV spectrophotometry</td>
<td>2–12</td>
<td>0.5</td>
<td>–</td>
<td>0.1 M HCl</td>
<td>10</td>
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<tr>
<td>Spectrophotometry: chloranil</td>
<td>1–25</td>
<td>1.05</td>
<td>3.46</td>
<td>Methanol, analysis time 5 min</td>
<td>11</td>
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<tr>
<td>Spectrophotometry: chloranil</td>
<td>0.16–0.81</td>
<td>0.003</td>
<td>0.008</td>
<td>Distilled water</td>
<td>12</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>0.1–0.9</td>
<td>–</td>
<td>–</td>
<td>0.05 M sulfuric acid</td>
<td>13</td>
</tr>
<tr>
<td>Spectrophotometry: Eosin Y</td>
<td>2–26</td>
<td>0.12</td>
<td>0.404</td>
<td>–</td>
<td>This study</td>
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</table>
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