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AN HPLC METHOD FOR THE INDIRECT QUANTIFICATION OF A QUINONE ADDUCT OF THE DRUG PAROXETINE

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Abstract: An HPLC method has been developed which enables the quantification of low levels of a catechol derivative and a quinone adduct of paroxetine in the presence of excess drug substance. Due to its inherent instability, the paroxetine quinone adduct is not available as a pure compound so that an indirect method was developed for its quantification. This procedure is based on the assumption that one molecule of the catechol (or more precisely the corresponding 1,2-benzoquinone) reacts with one molecule of paroxetine to produce one molecule of paroxetine quinone adduct. In the presence of paroxetine excess, pseudo-first-order kinetics was used to study the formation of the unstable product. A detector response factor for the paroxetine quinone adduct was calculated as a function of the response factor for the paroxetine catechol derivative, after considering a mass balance of the reaction. Using the methodology outlined, quantitative analysis was carried out of the paroxetine catechol derivative and the paroxetine quinone adduct in batches of paroxetine drug substance.

Keywords: HPLC, paroxetine, catechol derivative, quinone adduct, quantitative analysis

Paroxetine (PRX) is a selective serotonin-reuptake inhibitor (SSRI (1) effective for the management of panic disorder as well as obsessive-compulsive disorder (2,3). Both the catechol (PRX-C) and the quinone adduct (PRX-Q) of paroxetine can be found as minor impurities in paroxetine itself due to the reaction shown in the Scheme. The accurate determination of quinone adduct levels is difficult because the instability of this compound does not allow calibration using pre-prepared standard solutions. This paper describes a novel and convenient method for the quantitative analysis of (PRX-Q) in the presence of a large excess of drug substance (PRX) using, in the first instance, pseudo first-order kinetics for the reaction of the oxidized derivative of the catechol (PRX-C) with paroxetine (PRX) to derive a response factor for the quinone adduct (PRX-Q). Quantitative chromatographic analysis of (PRX-C) and (PRX-Q) in the presence of a large excess of drug substance (PRX) was then possible. It can be assumed that one molecule of the catechol derivative (PRX-C), a powerful reducing agent, is oxidized under aerobic conditions to paroxetine 1,2-benzoquinone (PRX-BQ) which then goes on to react with one molecule of paroxetine (PRX) (present in a large excess), thus forming one molecule of quinone adduct (PRX-Q) (see Scheme). Thus one molecule of catechol (PRX-C) is used up in the formation of one molecule of quinone adduct (PRX-Q). Similar reactions of benzoquinones and amines have been previously used for the spectrophotometric determination of either amines (4) or 1,2-benzoquinone (5).

To our knowledge this paper describes the methodology which has not been published before.

EXPERIMENTAL

The chromatographic resolution of (PRX) to (PRX-Q) was performed using Symmetry C18 column, 3.5 μm, 75×4.6 mm (Waters, Watford, UK) thermostated at 35˚C on an Alliance 2690 HPLC system, equipped with a photodiode array detector (Waters, Watford, UK). The mobile phase consisted of acetonitrile (HPLC grade 190 nm, Romil, UK), water (Elga Maxima Ultra Pure Water, Elga Ltd, High Wycome, UK) and ammonium acetate (HiperSolv for HPLC, Merck, Poole, UK) in the following compositions: solvent A, 50 mL acetonitrile/950 mL water/1 g ammonium acetate and solvent B, 800 mL acetonitrile/200 mL water/1 g ammonium acetate. The mobile phase was delivered at 1 mL/min using the following gradient: 100% A to 100% B over 7 min, 100% B held for 1min and then 100% A equilibration for 7 min. Detection was set at a wavelength of 210 nm with a 1.2 nm bandwidth. The injection volume was 10 μL. The sample temperature used was 18˚C for

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the quantitative determination of both (PRX-C) and (PRX-Q), whereas a temperature of 40°C was used for the kinetics experiments.

For a typical kinetics experiment, 200 mg of the hydrochloride of (PRX) and 1.010 mg of catechol (PRX-C) (80% w/w purity) was placed in a 10 mL volumetric flask and dissolved in solvent B. The reaction was followed by HPLC analysis. For the spiking experiment, a stock solution of the hydrochloride of (PRX) (640 mg) was prepared in solvent B (300 mL). Five weights of catechol (PRX-C) standard (0.264 mg, 0.364 mg, 0.772 mg, 1.021 mg, and 1.502 mg) were made up with the stock solution of (PRX) in 50 mL volumetric flasks. Each sample was freshly prepared and analyzed successively. A calibration experiment for catechol (PRX-C) was performed using the following quantities, 0.308 mg, 0.696 mg, 0.992 mg, 1.575 mg, 2.151 mg, dissolved in 50 mL volumetric flasks containing an acidic solvent consisting of acetonitrile, water, acetic acid in the ratio 500: 500:1. The use of acidic solvent assured the stability of catechol (PRX-C).
Four batches of the hydrochloride of paroxetine (PRX) drug substance were analyzed. These batches were experimental batches, which contained comparatively high levels of the catechol (PRX-C) impurity. Samples were freshly prepared in 1.5 mL of acidic acetonitrile/water solvent and kept at 18°C during the period of analysis.

Calculations

To determine the response factor for the quinone adduct (PRX-Q), it was assumed that one molecule of the catechol derivative (PRX-C) is oxidized to paroxetine 1,2-benzoquinone (PRX-BQ) which then goes on to react with one molecule of paroxetine (PRX) (present in a large excess), forming one molecule of quinone adduct (PRX-Q) (see Scheme). As paroxetine (PRX) was in excess in this reaction, pseudo first-order kinetics could be applied. The original concentrations of (PRX-C) and (PRX-Q) in paroxetine samples were then calculated using the formulae derived in the following section.

While the reaction of (PRX) with (PRX-C) is progressing, molar concentration changes for (PRX-C) and the product quinone adduct (PRX-Q) can be expressed as follows:

$$c_c^i - c_c^j = c_q^j - c_q^i \quad (1)$$

where:
c_c^i and c_c^j are the concentrations of catechol (PRX-C) at times i and j;
c_q^i and c_q^j are the concentrations of quinone adduct (PRX-Q) at times i and j.

The molar detector response factor (f) establishes the relationship between the corresponding peak area (A) and the concentration (c):

$$c = fA \quad (2)$$

Equation (1) can be rearranged by incorporating detector response factors and peak areas:

$$(f_cA_c^i) - (f_cA_c^j) = (f_qA_q^j) - (f_qA_q^i) \quad (3)$$

where:
f_c and f_q are the detector response factors for (PRX-C) and (PRX-Q), respectively; A_c^i and A_c^j are the peak areas of (PRX-C) at times i and j; A_q^i and A_q^j are the peak areas of (PRX-Q) at times i and j.
Rearrangement of equation (3) leads to equation (4):

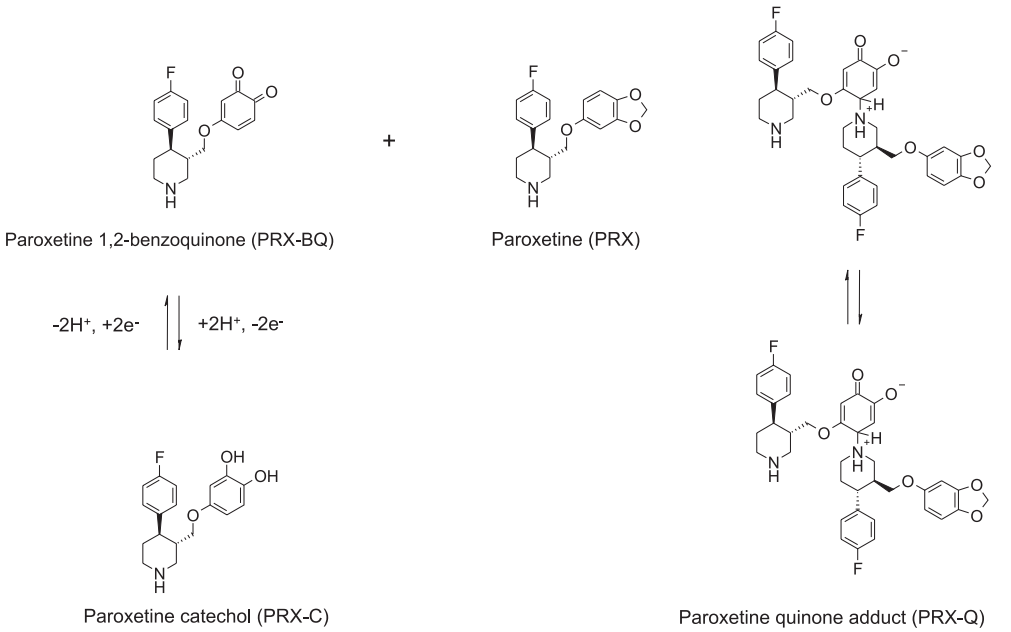
$$(A_c^i - A_c^j) = (f_q/f_c)(A_q^j - A_q^i) \quad (4)$$

From this equation a ratio of the molar detector response factors (f_q/f_c) for the quinone adduct (PRX-Q) and the catechol derivative (PRX-C), can be obtained by plotting ($A_c^i - A_c^j$) versus ($A_q^j - A_q^i$) for several time points. As the reaction of (PRX-C) with (PRX) occurs under pseudo first-order conditions (in a vast excess of (PRX)), it is not necessary to know the starting concentrations of (PRX-C) and (PRX-Q). Changes in the concentrations of these compounds with time can be monitored using exactly the same method we want to use for the quantitative analysis of the quinone adduct (PRX-Q).

The response factor for the catechol (PRX-C), f_c, can be obtained independently by analyzing standards of catechol (PRX-C) and plotting the peak area versus corresponding concentration of the standard (see equation no. 2). Thus knowing the detector response factor for catechol (PRX-C) (f_c) and the ratio of the response factors (f_q/f_c) the detector response factor for quinone adduct (PRX-Q) can be calculated.

As the concentration of (PRX-Q) in typical paroxetine (PRX) drug substance batches is generally very small, a large amount of a sample of (PRX) was necessary for the chromatographic analysis. Preliminary analysis of (PRX) confirmed that a sample of 20 mg/mL has to be used in order to allow the detection of the quinone adduct (PRX-Q) at a wavelength of 210 nm. At such sample concentrations, the paroxetine peak is out of linear range and therefore the content of (PRX-Q) cannot be calculated by using the detector response factor relative to that of paroxetine (PRX).

Content of the quinone adduct (PRX-Q) and the catechol derivative (PRX-C) in a sample of (PRX) is therefore calculated according to the following equation:



Scheme. Formation of paroxetine quinone adduct (PRX-Q) from the oxidised 1,2-benzoquinone derivative of catechol (PRX-BQ) and paroxetine (PRX).

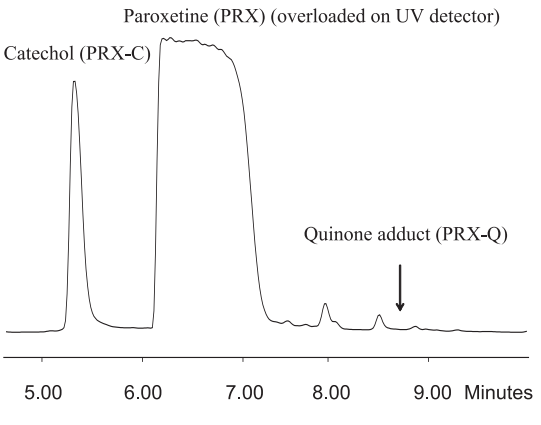


Figure 1. An example of a UV chromatogram obtained at 210 nm for a sample of paroxetine hydrochloride.

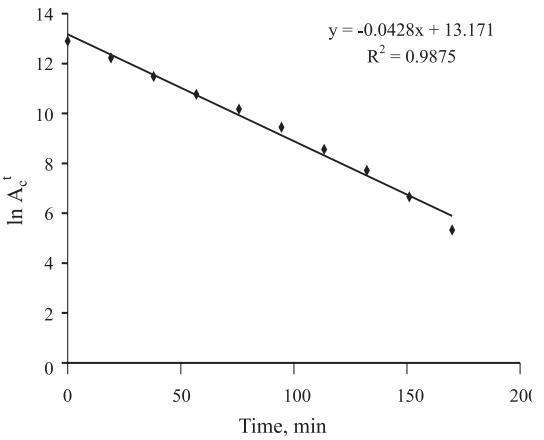


Figure 2. Pseudo first order kinetic plot for the consumption of the catechol (PRX-C) via the 1,2-benzoquinone (PRX-BQ).

$\%m_{c,q} = (f_{c,q}MW_{c,q}A_{c,q} \times V_p \times 100)/m_p$ (5)

where: $\%m_{c,q}$ is the content (w/w) of PRX-C or PRX-Q in a sample of PRX; $f_{c,q}$ is the detector response factor for PRX-C or PRX-Q; $MW_{c,q}$ is the molecular weight for PRX-C or PRX-Q; $A_{c,q}$ is the corresponding peak area; V_p is the volume of the sample solution; and m_p is the mass of the batch sample.

Kinetics

For the reaction described in the Scheme, the concentration of the catechol derivative PRX-C as a function of time can be described by equation (6):

$\ln c_c^t = -kt + \text{constant}$ (6)

where: c_c^t is the concentration of PRX-C at time t, and k is the first-order rate constant of the reaction. In view of the mass balance, the starting concentration of PRX-C, c_c^0, is equal to the actual catechol concentration (c_c^t) plus that of the quinone adduct (PRX-C) c_q^t, that is:

$c_c^t = c_c^0 - c_q^t$ (7)

When all the catechol (PRX-C) is converted to quinone adduct (PRX-Q), its final concentration (c_q^∞) is equal to the starting concentration of PRX-C:

$$c_c^t = c_q^\infty - c_q^t \quad (8)$$

Now equation (6) can be rearranged and the concentration of the quinone adduct (PRX-C) can be expressed as a function of time by equation (9):

$$\ln (c_q^\infty - c_q^t) = -kt + \text{constant} \quad (9)$$

Combining equation (2) with equations (6) and (9), peak areas can be expressed as a function of time by the following two equations:

for catechol (PRX-C):

$$\ln A_c^t = -kt + \text{constant} \quad (10)$$

for quinone adduct (PRX-Q):

$$\ln (A_q^A - A_q^t) = -kt + \text{constant} \quad (11)$$

where, A_c^t and A_q^t are the peak areas for PRX-C and PRX-Q at time t, and A_q^A is the final peak area for PRX-Q.

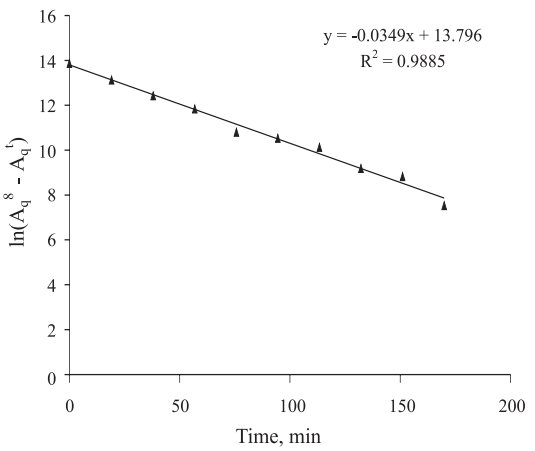


Figure 3. Pseudo first order kinetic plot for the formation of quinone adduct (PRX-Q).

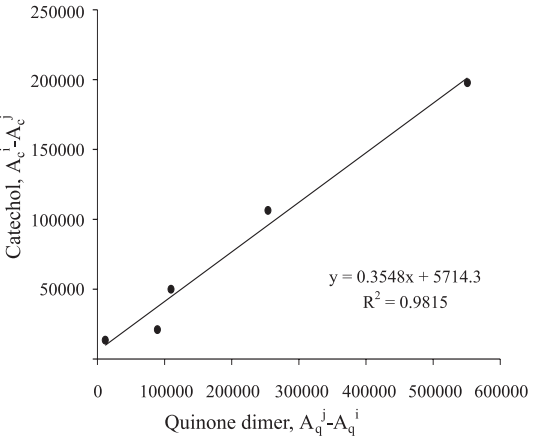


Figure 4. Plot illustrating the relationship for the increase in concentration of quinone adduct (PRX-Q) versus the decrease in concentration of catechol (PRX-C). From this relationship the relative detector response factor (f_q/f_c) at 210 nm was determined as 0.3548.

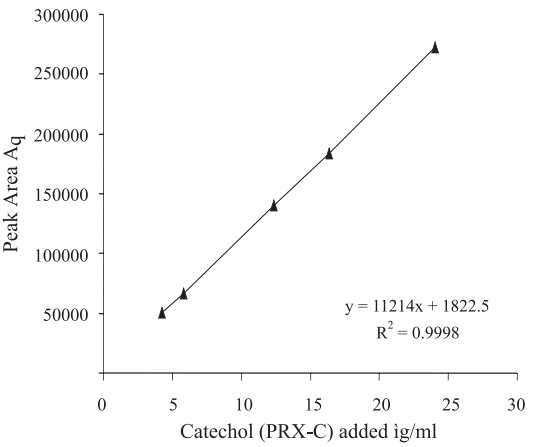


Figure 5. Plot illustrating linear increase of the final peak area of quinone adduct (PRX-Q) on addition of the catechol (PRX-C) to the paroxetine (PRX) stock solution.

Table 1. Factors obtained from experiments and the detector response factor calculated for paroxetine quinone adduct (PRX-Q) used for quantification of both paroxetine catechol (PRX-C) and paroxetine quinone adduct (PRX-Q).

f_q/f_c *	f_cMW_c * g/l	$f_qMW_q = (f_q/f_c)(f_cMW_c)MW_q/MW_c$ g/l
0.3548	1.902×10^{-7}	1.367×10^{-7}

* f_q/f_c and f_cMW_c values are obtained from Figures 4 and 5, respectively.

Table 2. Catechol (PRX-C) and Quinone adduct (PRX-Q) content in batches of Paroxetine (PRX).

Batch Number	Sample Weight mg	Catechol Peak Area	Quinone Adduct Peak Area	Catechol Content % w/w, (%RSD)	Quinone Adduct Content % w/w, (%RSD)
1	30.4	2255683	32064	2.1 (1.9)	0.02 (4.4)
2	30.4	3058023	36080	2.9 (1.2)	0.02 (3.8)
3	30.5	2021025	36953	1.9 (1.1)	0.02 (2.1)
4	29.9	4131728	52865	3.9 (1.4)	0.04 (6.4)

RESULTS AND DISCUSSION

A typical UV chromatogram of paroxetine drug substance showing the presence of catechol (PRX-C) and dimer adduct (PRX-Q) is shown in Figure 1. The 1,2-benzoquinone (PRX-BQ), formed from the oxidation of catechol (PRX-C), reacts rapidly with excess paroxetine (Scheme) and is not normally observed by LC/UV. The linear plots in Figures 2 and 3 confirm that the reaction of the catechol derivative (PRX-C) with excess paroxetine (PRX) to produce quinone adduct (PRX-Q) follows pseudo first-order kinetics. A final peak area for PRX-Q was measured as the observed maximum after approximately 200 minutes of reaction. After this time content of PRX-Q decreased with time.

Good linear correlation was also obtained for the increase in the concentration of the quinone adduct (PRX-Q) versus the decrease in the concentration of the catechol derivative (PRX-C), as shown in Figure 4. From this plot the ratio of the molar detector response factors for PRX-Q relative to that of PRX-C, (f_q/f_c), can be obtained from the slope of the linear regression line. The inverse of the slope obtained from a plot of catechol peak area versus catechol concentration ($y=5256.7x-9167.2$, $R^2=0.9973$) is equal to the product of the molar detector response factor for catechol and molecular weight of catechol, f_cMW_c. The experimentally derived factors and the detector response factor calculated for PRX-Q are given in Table 1.

A spiking experiment provided another confirmation that this approach could be used for the quantitative analysis of quinone adduct (PRX-Q) in paroxetine drug substance. The paroxetine stock solution was spiked with increasing amounts of the catechol derivative PRX-C. Spiked samples were incubated at 40˚C and after all the catechol was consumed, the final peak area for the quinone adduct was determined by HPLC analysis. Figure 5 illustrates the relationship of final peak area of the quinone adduct (PRX-Q) versus catechol (PRX-C) concentration. The good linear correlation obtained was further confirmation of the validity of the present indirect analytical methodology.

Four batches of paroxetine hydrochloride were analyzed for the presence of PRX-C and PRX-Q, and using the approach described weight based concentrations of PRX-C and PRX-Q in these samples were determined (Table 2). For these measurements three repeat injections were performed for each sample. The RSD values shown in Table 2 emphasize the ability of the method to accurately measure low levels of PRX-C and PRX-Q. The mean value of peak areas obtained was then used for the calculation of concentrations of PRX-C and PRX-Q using equation (5) and the factors presented in Table 1. The levels of PRX-C and PRX-Q in four experimental batches of paroxetine drug substance measured by this method are collected in Table 2. These were found to be in the range of 1.9-3.9% w/w and 0.02-0.04% w/w for PRX-C and PRX-Q, respectively.

The methodology described is currently being successfully used to detect levels of catechol (PRX-C) and quinone adduct (PRX-Q) which are substantially lower than those found in the experimental batches used here.

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