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## A stability-indicating LC method for citalopram hydrobromide

K.Rama Seshaiyah<sup>1,2,\*</sup>, Sudip Kr.Samanta<sup>1</sup>, V.Krishna Reddy<sup>1</sup>, VVNKV Prasadaraju<sup>1</sup>, K.Mukkanti<sup>2</sup>,  
V.Ranga Reddy<sup>1</sup>

<sup>1</sup>Dr. Reddy's Laboratories Ltd. Active pharmaceutical ingredients, IPDO, Bachupally, Hyderabad-500072, A.P., (INDIA)

<sup>2</sup>Department of chemistry, J.N.T.University, Kukatpally, Hyderabad-500072, A.P., (INDIA)

E-mail : ramask@drreddys.com; kanuparthi\_ramse@yahoo.co.in

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### ABSTRACT

A simple, inexpensive and rapid LC method has been developed for the quantitative determination of Citalopram, an anti-depressant drug. The method can also be employed for the determination of Citalopram and its impurities in the bulk drug. Degradation studies were performed on the bulk drug by heating to 105 °C, exposure to UV light at an energy of 200 Watt hours/m<sup>2</sup> and to Visible light at an illumination of not less than 1.2 million lux hours, acid (0.5 N hydrochloric acid), base (0.1 N sodium hydroxide) aqueous hydrolysis and oxidation with 3.0% v/v hydrogen peroxide and 0.2% w/w Meta chloro per benzoic acid. Considerable degradation was observed under acid, base and oxidation conditions. Good resolution between the peaks corresponding to impurities produced during synthesis, degradation products and the analyte was achieved on a Symmetry C18 LC column using a mobile phase consisting of a mixture of aqueous potassium dihydrogen phosphate, N,N-Dimethyl octyl amine, methanol and acetonitrile. The degradation samples were assayed against the reference standard of Citalopram and the mass balance in each case was close to 99.9%. Validation of the method was carried out as per ICH requirements.

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### KEYWORDS

Column liquid chromatography;  
Degradation studies;  
Citalopram;  
Method validation.

### INTRODUCTION

Citalopram, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydro bromide (Figure 1), is an selective serotonin reuptake inhibitors developed by Forest Laboratories, Inc.<sup>[1]</sup>. SSRIs increase the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cell, increasing the level of serotonin available to bind to the postsynaptic receptor. Citalopram is used to treat the symptoms of major depression, social anxiety disorder and panic disorder. Citalopram has one

stereocenter, to which a 4-fluorophenyl group and an N,N-dimethyl-3-aminopropyl group bind. Due to this chirality the molecule exists in (two) enantiomeric forms (mirror images). They are termed S-(+)-citalopram and R-(-)-citalopram. (Figure 2)

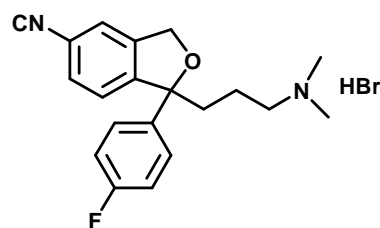
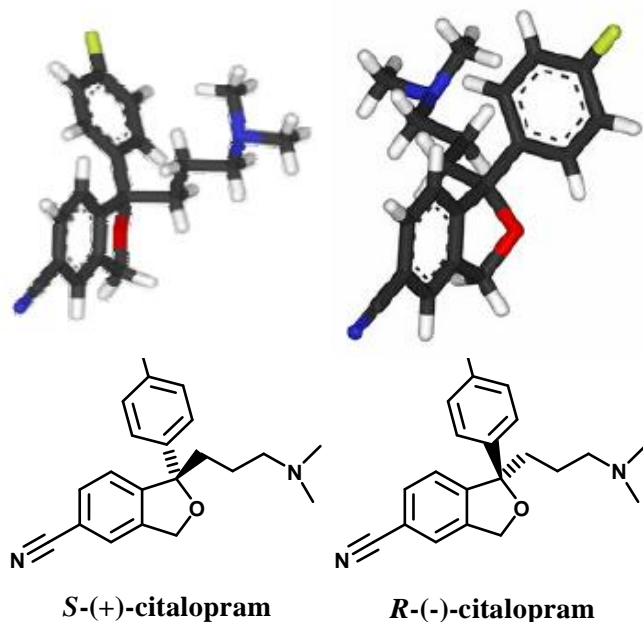


Figure 1 : Citalopram hydro bromide

## EXPERIMENTAL



**Figure 2 : S-(+)-citalopram and R-(-)-citalopram.**

The empirical formula for Citalopram hydrobromide is  $C_{20}H_{21}FN_2O \cdot HBr$ , and the molecular weight is 405.30

The International Conference on Harmonization (ICH) guidelines<sup>[2]</sup> require stress testing of drug substances, which can help identify the likely degradation products, can be useful in establishing degradation pathways and in validating the stability-indicating power of the analytical procedures used. Moreover, a validated analytical method must be applied in stability studies<sup>[3]</sup>.

So far analytical methods for Citalopram was mentioned in United states Pharmacopeia<sup>[4]</sup> and European Pharmacopeia<sup>[5]</sup>. But in both the cases all the 12 impurities are not separated in a single method. Although there are analytical methods for the determination of Citalopram and some impurities by other techniques like LC, LC-MS, LC-MS-MS and Fluorescence detection<sup>[4-10]</sup>. It was, therefore, felt necessary to develop an LC method for the quantitative determination of Citalopram and 12 impurities including process related and degradants. The current work deals with the accelerated degradation of the drug substance under stress conditions like hydrolysis, oxidation, heating and UV light. The work also includes the validation of the stability-indicating method developed. This method can be used for quality control during manufacture and for assessment of the stability of bulk samples of Citalopram.

### Chemicals and reagents

Samples of Citalopram and its twelve impurities namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L (Figure 3 A-L) were received from the Process Research Department of Integrated Product Development Operations of Dr. Reddy's Laboratories, Hyderabad, India. LC grade acetonitrile, methanol potassium dihydrogen orthophosphate and phosphoric acid were purchased from Merck, Schuchardt, Germany. N,N-Dimethyl octyl amine was purchased from Lancaster, Alfa Aesar. High purity water was prepared by using a Millipore Milli Q plus purification system (Bedford, MA, USA).

### Instrumentation

The LC system was a Waters model 2996 equipped with a PDA (Waters Corporation, Milford, USA). The output signal was monitored and processed using Empower software (Waters Corporation, Milford, USA) on a Pentium computer (Digital Equipment Co).

### Chromatographic conditions

Chromatographic separation was achieved on a 5  $\mu$ m Symmetry C18 column, (250 mm  $\times$  4.6 mm), using a mobile phase with a buffer containing a mixture of 0.01 M aqueous potassium dihydrogen orthophosphate and 1.0mL of N,N-Dimethyl octyl amine. The mobile phase A is a mixture of buffer and methanol (95:5, v/v), pH adjusted to 5.8 using dilute phosphoric acid and mobile phase B is a mixture of buffer adjusted the pH to 2.9 and acetonitrile (40:60, v/v). The mobile phase was filtered through a 0.45  $\mu$ m nylon membrane and degassed with helium for 5 min. The mobile phase flow rate was 0.8 mL min<sup>-1</sup>. The LC gradient was time (min)/%B: 0/30, 15/30, 40/80, 45/100, 58/100, 60/30 and 70/30. The column was maintained at 35 °C and the effluent was monitored at 224 nm. The injection volume was 10  $\mu$ L. Mobile phase A and mobile phase B in the ratio of 7:3 v/v was used as diluent during the preparation of the standard and test samples.

### Preparation of standard solutions

A stock solution of Citalopram (1500  $\mu$ g mL<sup>-1</sup>) was prepared by dissolving an appropriate amount in the diluent. Working solutions of 500 and 100  $\mu$ g mL<sup>-1</sup> were

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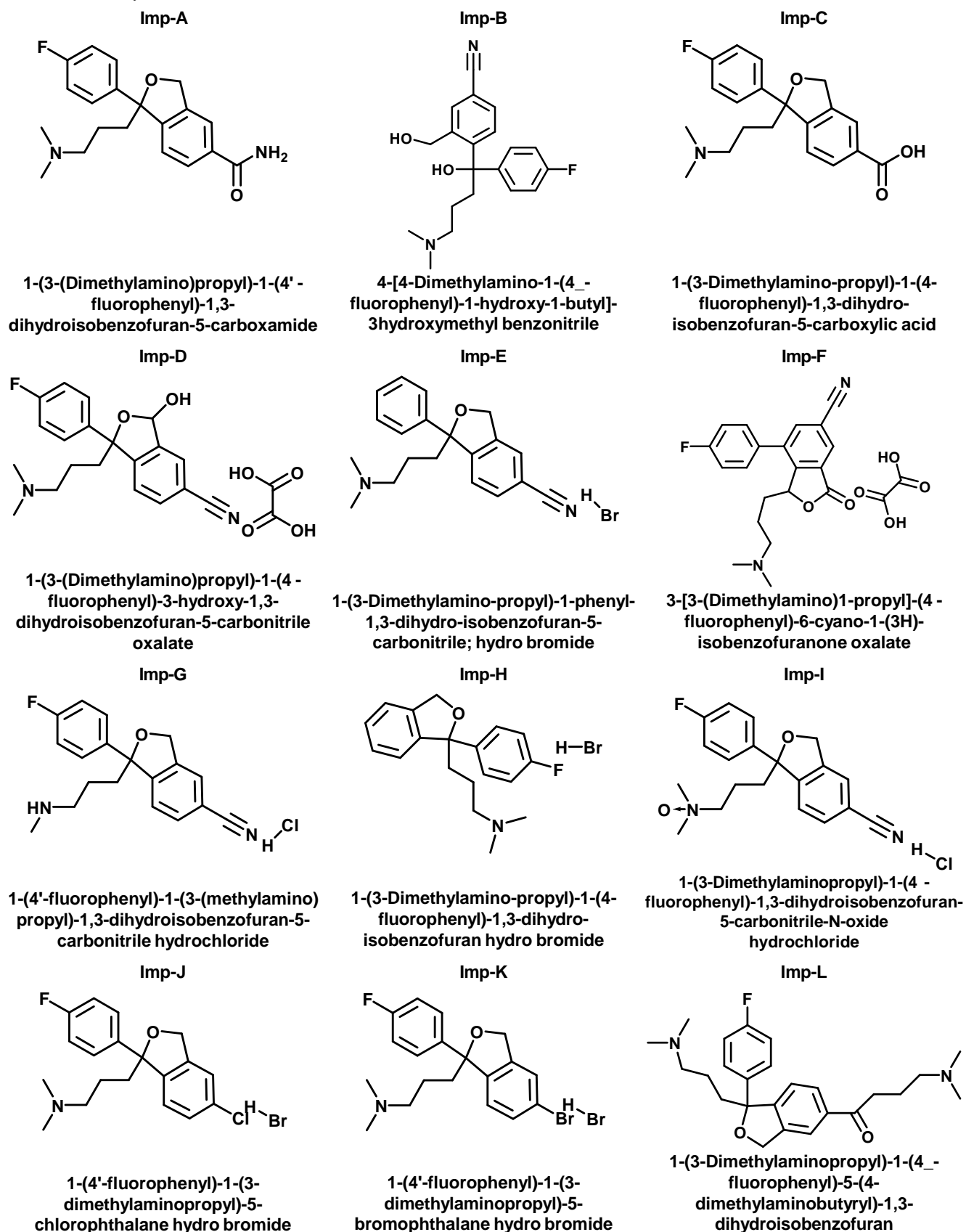


Figure 3 : Structures and chemical names of impurities

prepared from stock solution for impurity determination and assay respectively. A stock solution of impurities (a mixture of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L) at  $500 \mu\text{g mL}^{-1}$  was also prepared in diluent.

### Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21CFR Sect. 211 requires the development and validation of a stability-indicating assay method. In order to determine whether the determination of impurities and the assay method were adequate, a Citalopram API sample was submitted to forced degradation studies. The specificity of the developed LC method for Citalopram was carried out in the presence of its impurities.

The current regulatory guidelines do not indicate detailed degradation conditions in stress testing. However, the conditions used were found to effect a degradation of preferably not less than 5% but not complete degradation. Degradation conditions employed were UV light (200 Watt hours/m<sup>2</sup>), Visible light (1.2 million lux hours), heating to 105 °C, acid hydrolysis with 0.5 N HCl, base hydrolysis with 0.1 N NaOH, water hydrolysis and oxidative degradation using 3% H<sub>2</sub>O<sub>2</sub> and 0.2% meta chloro per benzoic acid. Peak purity testing was carried out on the stressed samples of Citalopram by using the PDA detector. Assay studies were carried out on the stressed samples against a qualified reference standard having a purity of 99.8% and the mass balance (% assay + % degradation) was calculated. Assay was also carried out on a bulk sample by spiking all twelve impurities (Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L) each at the specification level of 0.10%.

### Method validation

#### Precision

Precision was evaluated by carrying out six independent assays of a test sample of Citalopram against a reference standard and calculating the % RSD. The precision of the determination of the impurities was checked by injecting six individual preparations of ( $1500 \mu\text{g mL}^{-1}$ ) Citalopram spiked with 0.10% of Imp-

A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L and calculating the % RSD of area for each compound. The intermediate precision of the method was also evaluated using different analysts and a different instrument in the same laboratory.

#### Limit of detection and limit of quantification

The LOD and LOQ for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were estimated at a S/N of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L and calculated the %RSD for the areas.

#### Linearity

Linearity of test solutions were prepared from stock solution at six concentration levels from 50 to 150% of assay analyte concentration (250, 375, 500, 625 and  $750 \mu\text{g mL}^{-1}$ ). The peak area versus concentration data were subjected to least-squares linear regression analysis. The calibration curve was drawn by plotting Citalopram area injections against the concentration expressed in percentage.

Linearity test solutions for the impurities were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels from LOQ to 150% with respect to the impurities specification level of 0.10% (i.e. LOQ, 0.025, 0.050, 0.075, 0.10, 0.125 and 0.150%). The calibration curves were drawn by plotting the peak areas of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L against the corresponding concentration.

Linearity testing was performed on two consecutive days in the same concentration range for both assay and related impurities. The % RSD value of the slope and y-intercept of the calibration curve was calculated.

#### Accuracy

The accuracy was evaluated at three concentration levels i.e. 250, 500 and  $750 \mu\text{g mL}^{-1}$  of a bulk drug

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sample. The % recoveries were calculated.

Accuracy of the determination of the impurities was carried out in triplicate at 0.05, 0.10 and 0.15% of the Citalopram concentration ( $1500 \mu\text{g mL}^{-1}$ ). The percentages recoveries for the impurities were calculated.

### Robustness

To determine robustness, experimental conditions were purposely altered and the resolution between Imp-H and Citalopram was evaluated.

To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.6 to  $1.0 \text{ mL min}^{-1}$ . The effect of pH on resolution of the impurities was also studied by varying the pH of mobile phase A from 5.6 to 6.0 and mobile phase B from 2.7 to 3.1. The effect of column temperature on resolution was studied at 30 and  $40^\circ\text{C}$ . In all the above conditions, the components of the mobile phase were held constant.

### Solution stability and mobile phase stability

The stability of Citalopram in solution was carried out by leaving both the solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for two days. The solutions were assayed at 24 h intervals up to the end of the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 24 h intervals over 2 days. Mobile phase composition was kept constant during the study period. The % variation in the content of Citalopram was calculated over the period.

The solution stability of Citalopram and its impurities in the related substances method was carried out by a leaving spiked sample solution in a tightly capped volumetric flask at room temperature for 48 h. Content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were determined at 24 h intervals. Mobile phase stability was also carried out for 48 h by injecting freshly prepared sample solutions at 24 h intervals. Content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were checked in test solutions. Mobile phase composition was kept constant during the study period.

## RESULTS AND DISCUSSION

### Optimization of chromatographic conditions

Imp-E, Imp-G and Imp-L was the most likely impurities present in bulk samples produced by Dr. Reddy's laboratories. The main difficulty of the chromatographic method was to get the separation of Imp-D from Imp-E and Imp-G from the Citalopram peak. Attempts were made by using different C18 and C8 stationary phases<sup>[13]</sup>. The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. Effects of pH (2–7) and ionic strength ( $1\text{--}10 \text{ mmol L}^{-1}$ ) were investigated using phosphate and acetate buffers. It was found that the retention time of Citalopram did not significantly alter at pH 2–5 and ionic strength between 1 and  $10 \text{ mmol L}^{-1}$ . But there is a pH effect on the separation of Imp-D from Imp-E and Imp-F and also between Imp-A from Imp-L. The retention time of Imp-I have more effect on the pH of the mobile phase. So the pH of mobile phase A and mobile phase B are the critical on the separation of impurities. The optimum conditions are given in "Experimental". Citalopram, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were well separated with a resolution of greater than 2.0 The tailing factor and the number of theoretical plates for the Citalopram peak were 1.2 and 45,000.

### Results of forced degradation studies

Degradation was not observed in a Citalopram bulk sample during stress conditions like water hydrolysis and heating to  $105^\circ\text{C}$ . Slight degradation was observed in Visible and peroxide conditions. Major degradation was observed in acid, base and oxidation condition with meta chloro per benzoic acid (Figure 4). Imp-D and Imp-F are the major degradants in acid degradation.

Imp-A is the major degradant in base degradation. Imp-D, Imp-G and Imp-I are the major degradants in oxidative degradation. Peak purity test results confirmed that the Citalopram peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.9% (TABLE 1). The assay of Citalopram is unaffected in the presence of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-

G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L, confirming the stability-indicating power of the method.

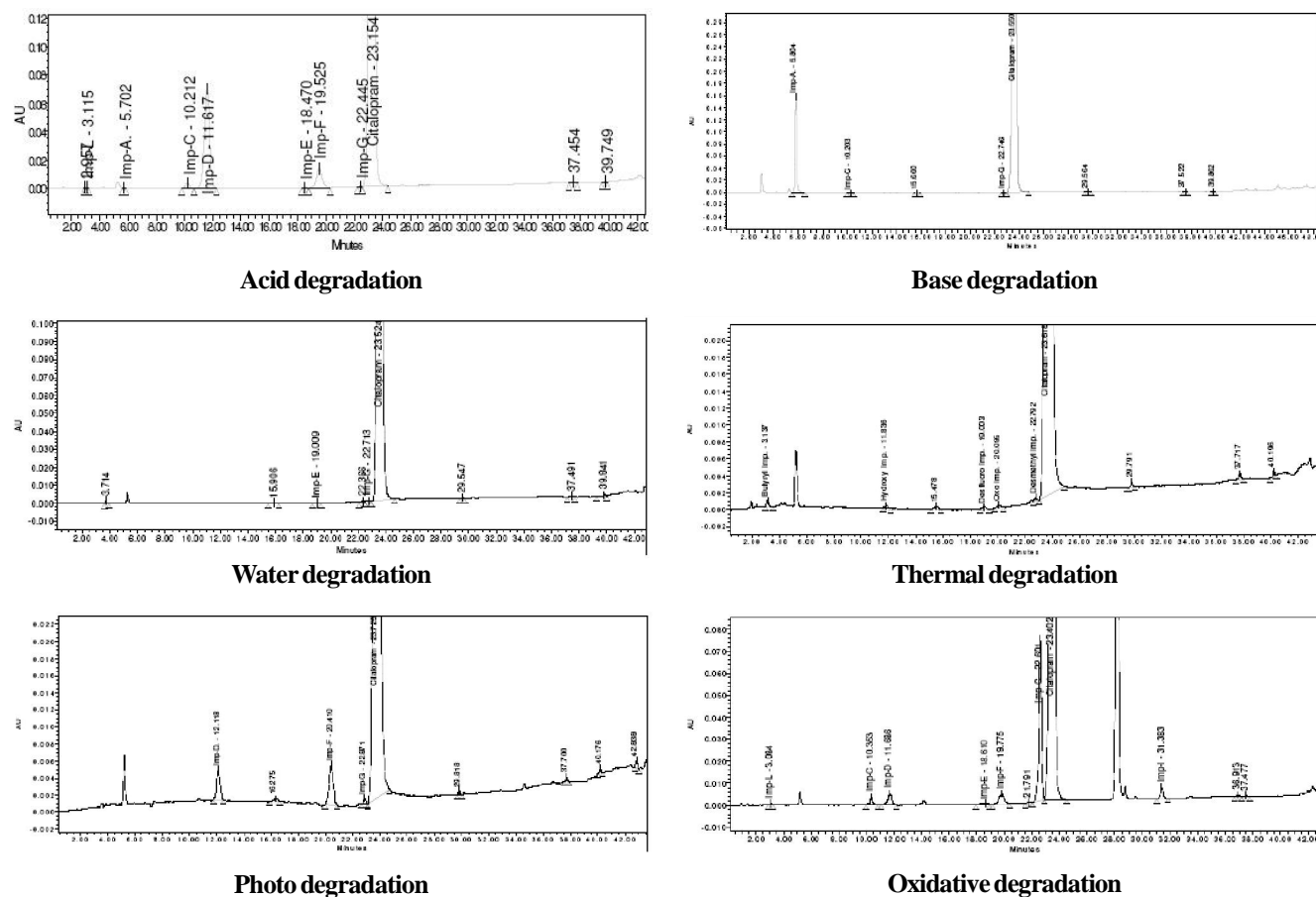


Figure 4 : Typical LC chromatograms of stressed test samples of citalopram

TABLE 1 : Summary of forced degradation results

Degradation Condition	Duration	Assay (%w/w on anhydrous basis)	Mass balance (% assay+ % degradation)	Remarks
Thermal (105°C)	10 Days	99.54	101.0	No significant degradation observed
Acid hydrolysis 0.5N HCl	8 Hours	95.00	100.4	Imp-D and Imp-F are the major degradents
Base hydrolysis 0.1N NaOH	16 Hours	95.40	99.62	Imp-A is the major degradent
Water hydrolysis	48 Hours	99.40	99.38	No significant degradation observed
Oxidative degradation	48 Hours	95.54	99.85	Imp-D, Imp-F, Imp-G and Imp-I are the major degradents
Photo degradation	7 Days	98.70	99.54	Imp-D and Imp-F are the major degradents

Results of method validation studies

Precision

The RSD of the assay of Citalopram was well within 0.3% and the RSD of the area of Imp-A, Imp-B, Imp-

C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L was within 3.6%. The RSD of assay results obtained in intermediate precision studies was within 0.4% and the RSD of area of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-

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I, Imp-J, Imp-K and Imp-L were within 4.0%, confirming the good precision of the method.

### Limit of detection and limit of quantification

The limits of detection (LOD) of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were found to be in the range of 0.002 to 0.005% (of analyte concentration  $1500 \mu\text{g mL}^{-1}$ ) in each case for a  $10 \mu\text{L}$  sample size. The limits of quantification (LOQ) of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were found to be in the range of 0.006 to 0.016%. The precision for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L at LOQ level was below 6.0% RSD.

### Linearity

A linear calibration plot was obtained over the calibration ranges tested, i.e.  $250$  to  $750 \mu\text{g mL}^{-1}$  and the correlation coefficient obtained was greater than 0.9999. Linearity was checked over the same concentration range for two consecutive days. These results show

that an excellent correlation exists between the peak area and concentration of the analyte.

Linear calibration plots for the impurities and analyte were obtained over the calibration ranges tested, i.e. LOQ to 0.15% for Citalopram, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L. The correlation coefficients obtained were greater than 0.999. The results show that an excellent correlation between the peak area and concentration of Citalopram, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L.

### Accuracy

The percentage recovery of Citalopram in bulk drug samples ranged from 99.3 to 99.7%. The percentage recovery of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L in bulk drug samples ranged from 98.2 to 102.2%. Chromatograms of blank and spiked samples at 0.1% level of all three impurities in a Citalopram bulk drug sample are shown in Figure 5.

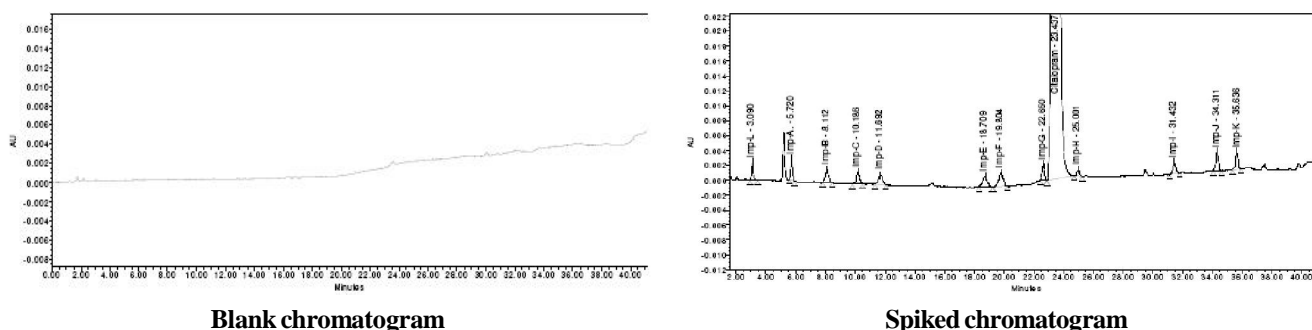


Figure 5 : Typical LC chromatograms of blank and spiked samples (Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L were spiked at 0.1% level in pure Citalopram sample)

### Robustness

In all the deliberately varied chromatographic conditions (flow rate, pH and column temperature), the resolution between Imp-H and Citalopram was greater than 2.0 illustrating adequate robustness of the method.

### Solution stability and mobile phase stability

The RSD of the assay of Citalopram during solution stability and mobile phase stability experiments was within 1.0%. No significant change was observed in the content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F,

Imp-G, Imp-H, Imp-I, Imp-J, Imp-K and Imp-L during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirm that sample solutions and mobile phase used during assays were stable up to 48 h.

## CONCLUSIONS

The simple RP-LC method developed for the quantitative determination of Citalopram and its possible degradation products and impurities is precise, accurate

and specific for the analysis of bulk material. The method was fully validated, showing satisfactory results for all the parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples.

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