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Validated stability-indicating method for estimation of related substances of paroxetine in active pharmaceutical ingredient and its pharmaceutical dosage forms

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ABSTRACT

Validated stability-indicating analytical method was established for the quantitative determination of paroxetine and its related substances in API and it's finished product in the presence of degradation products. To prove the stability-indicating nature of the method, stress studies were carried out. The method was developed by using (Waters, symmetry C18, 250×4.6 mm, 5 μm column) employing water:THF: TFA 90:10:1 (v/v/v) as mobile phase-A and mobile phase-B consist of ACN:THF: TFA the proportion of 90:10:1 (v/v/v) in a gradient mode with a flow rate of 1.5 mL/min was chosen. The column and sample cooler were kept at 45°C and 5°C respectively and 285 nm used as detection wavelength. Significant degradation observed in alkaline conditions, whereas no signification decay in drug stability was observed in other decomposition environments. Method development as well as optimisation studies were done by analysing the samples generated in the stress studies and spiked samples. Mass balance was found to be in the range of 90.3 and 100.1%, signifying the method is stability-indicating. All earlier analysis methods for the analysis of paroxetine have not been entirely validated by considering all the degradation products. The established method validated as per ICH Q2 (R1) and considered as linear, specific, accurate, precise, rugged, robust and found to be suitable for the routine and stability analysis of the product.

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INTRODUCTION

In the United States, antidepressants are one of the most widely used drugs and their usage displays no signs of waning. As per the recent coverage, clinical depression affects about sixteen million people in the USA and is projected to cost the US about $210 billion a year in decline in the productivity and Healthcare requirements. Worldwide revenues of antidepressants likely to rise to about $17 billion by 2020 (Szcześniak *et al.*, 2019).

Paroxetine is considered as a new generation antidepressant medicine and its chemical structure of hydrochloride salt portrayed in Figure 1.

Paroxetine also known as Seroxat or Paxil, is a drug used to treat depression, anxiety, and other mood disorders. The substance acts as a selective serotonin reuptake inhibitor (SSRI). It belongs to a class of drugs, which increases the extracellular level of the neurotransmitter serotonin in the synaptic cleft by limiting of serotonin-reuptake in the presynaptic cell. Paroxetine has in comparison to the conventional tricyclic antidepressants significantly fewer cardiovascular side effects. Paroxetine is a primary compound, which is commonly used as a salt, the most relevant forms being Paroxetine hydrochloride, Paroxetine hydrochloride hemihydrate, and the corresponding maleate, mesylate, and sulfonate salts (Preskorn *et al.*, 2004).

Liquid and Gas chromatographic detection methods are used for the quantitation of paroxetine and its metabolites in biological samples and pharmaceutical preparations. Some methods have been developed for the determination of paroxetine in biological samples like plasma, including HPLC with UV detection (Foglia *et al.*, 1997; Knoeller *et al.*, 1995), fluorescence (without or with derivatisation using dansyl chloride) (Shin *et al.*, 1998; Brett *et al.*, 1987; Lucca *et al.*, 2000), mass spectroscopy (MS) (Juan *et al.*, 2005; Naidong and Eerkes, 2004), diode array detection (Titier *et al.*, 2003; Duverneuil *et al.*, 2003), or gas chromatography combined with Mass spectrometry (Leis *et al.*, 2001; Wille *et al.*, 2005), and applied therapeutic drug level monitoring.

During the literature review, it was identified as there no comprehensive method stated to reveal the quantitative determination of paroxetine and its impurities by HPLC in its drug product as well as in API.

The purpose of current work is to establish a stability-indicating procedure to screen the paroxetine in pharmaceutical preparations by using C18 column and Photodiode array detection. The developed method must be validated as per ICH Q2 (R1) in terms of selectivity, sensitivity, linearity, repeatability, reproducibility, robustness and recovery (ICH Harmonised Tripartite Guideline, 2005).

Experimental

Drug and chemicals

Paroxetine was received as a free sample from MSN Labs (India). The generic formulation was procured from the pharmacists of the local market. Tetrahydrofuran, Trifluoroacetic acid, Hydrochloric acid, Sodium hydroxide and H_2O_2 was obtained from Merck, Darmstadt, Germany. Glacial acetic acid as well as triethylamine brought from Rankem India. Acetonitrile and methanol were procured from JT Baker (Phillipsburg, NJ, USA). Milli-Q water purification system (Millipore, Milford, MA, USA) used for the preparation of water for HPLC studies.

Paroxetine Related Compound E Impurity Standard, Paroxetine Related Compound A (Methoxy Paroxetine)Impurity Standard, Paroxetine Related Compound B (Desfluoro Paroxetine) impurity standard, Paroxetine Related Compound D (Cis-Paroxetine) Impurity standard, Paroxetine Related Compound F (N-Methyl Paroxetine) Impurity standard, Ethoxy Paroxetine Impurity standard and Paroxetine Dimer Impurity Standard Purchased from ClearSynth Labs India.

Apparatus and equipment

HPLC equipped with UV and PDA Detector with data handling system of Waters Empower3 Software used.

Standard preparation

Weigh approximately 30 mg of Paroxetine Hydrochloride working/reference standard into a 100 mL flask, added 60 ml of methanol to dissolve the content sonicated with intermittent shaking, dilute with methanol.

Dilute 1.0 mL of above stock solution to100 mL with methanol and mixed well.

Sample preparation

Calculate the average tablet weight by weighing not less than 20 Tablets.

Transfer the tablets into a mortar and crush them to a fine powder using a pestle. Weigh accurately and transfer the tablet powder, equivalent to 75 mg of paroxetine into a 50 mL flask, added 30 mL of methanol, sonicated for 30 minutes with vigorous shaking, diluted with methanol and centrifuged a portion at $\geq$4000 rpm about 20 minutes and injected into HPLC.

Specificity-Forced degradation studies

Singh and Bakshi's approach was used to carry out the stress investigations on the drug (Singh and Bakshi, 2000).

Paroxetine Hydrochloride sample was forcefully degraded by exposure to degradation conditions of acid, alkaline, peroxide, photostability, moisture and thermal. Control and degradation samples were analysed as per the method. The purity of the peaks for the degradation samples was monitored. Degradation (%) was calculated.

By using 1N HCl and 1N NaOH, acidic and alkaline hydrolysis performed while neutral hydrolysis was done in water and methanol mixture. The

oxidative study was carried out in 30% H2O2 at RT (Room Temperature). The solid drug moulded as a thin layer and also drug solutions were exposed to ~8500 lx fluorescent and ~0.2 W/m2 UV light for various periods to study the effect of photodegradation and for the dark comparison controls were kept concurrently—thermal degradation study performed by at 60 °C for 15 days.

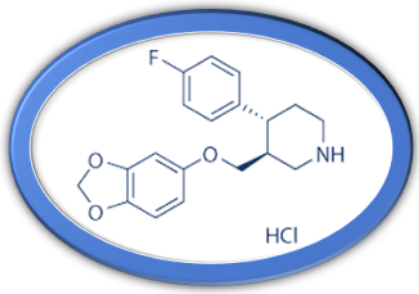


Figure 1: Structure of Paroxetine hydrochloride

Method Validation

Validation of the developed method, following ICH to envisage the performance of a developed method.

Linearity

A sequence of solutions were prepared by using Paroxetine HCl standard and impurities standards from LOQ to 150% of specification level.

Method Precision

It can be determined by injecting six samples prepared by spiking the test preparation with paroxetine impurities at the specification level and calculate the % RSD of Paroxetine and its impurities.

Accuracy

The accuracy can be assessed by using sample solutions, prepared in triplicate by spiking paroxetine impurities into the sample from the LOQ to 150% of specification level and estimated.

Intermediate precision (Ruggedness)

To establish the ruggedness of the test methods, perform the repeatability by preparing six spiked sample solutions separately using the same batch of paroxetine as per the developed LC method. Each solution was injected into the LC using a different column, system and analysed on a different day.

Limits of detection and quantitation

The LOD and LOQ values of paroxetine and its impurities were determined using the values of S/N. Each forecasted LOQ concentration was verified for precision by preparing the solutions containing impurities at about these predicted concentrations and injecting each solution six times into the HPLC by following the test method.

Robustness

Experimental conditions were intentionally altered to verify the robustness of the method, and the relative retention time of the impurities was assessed. Typical variations are flow rate, column oven temperature and organic variation.

RESULTS AND DISCUSSION

Method development and optimisation

Principle objective is to develop the effective HPLC method for the determination of related substances in paroxetine and also to ensure that the established process must be able to determine all the impurities meeting required method validation parameters to utilise for routine as well as QC testing and to verify the quality of marketed formulations. All impurities and paroxetine exhibited satisfactory detection at 285 nm and 45ºC used as temperature of the column during the analysis. The Specimen chromatogram of paroxetine Spiked with Impurities at 0.15% is shown in Figure 3.

Estimation of Forced degradation samples by optimised method

The drug was significantly degraded in the alkaline conditions (1N NaOH, 60 °C and two hours), whereas it remains stable in other forced degradation conditions.

The desirable separation and resolution were obtained using a mobile phase of Water:THF: TFA 90:10:1 (v/v/v)as MP-A and MP-B consist of ACN:THF: TFA the proportion of 90:10:1 (v/v/v) as a gradient mode as T_{min}/A: B; T_{0-30}/90:10; T_{30-60}/10:90; T_{60-75}/90:10. The samples were analysed by the gradient method of 75 min run time, 1.5 ml/min was used as flow rate, column temperature of 45 °C and injection volume used as 20 μl.

Validation of the method

According to the ICH guideline Q2 (R1) (ICH Harmonised Tripartite Guideline, 2005), the established method was validated.

Specificity

Paroxetine was found to be stable under Acid, oxidative, thermal, photolytic and hydrolytic conditions. Only in alkaline conditions significant degradation observed.

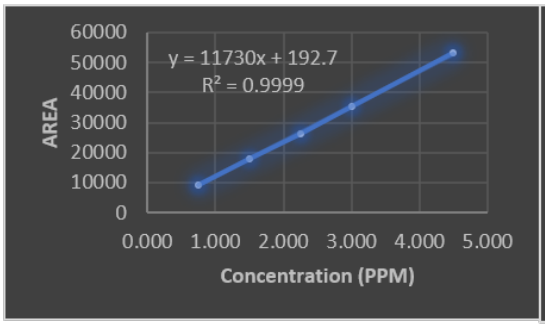
The peak purity values of the paroxetine in all samples of stress conditions confirms that the homogeneity of the peak and there are no co-eluting peaks representing stability-indicating and specific nature. Peak purity passed in each degradation condition; Purity Plots were represented in Figure 4.

Table 1: Results showing Mass balance of developed method

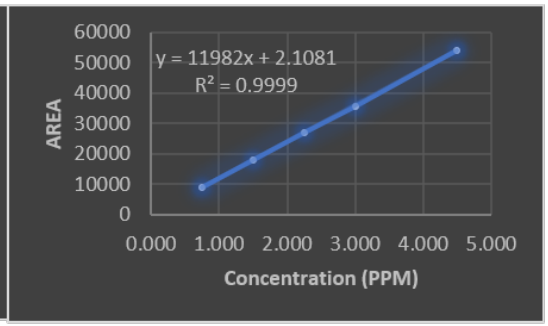
S. No.	Sample name	% (w/w) Total Impurities	% Assay	% Assay + % Total impurities	mass balance
1	Control Sample	0.18	101.0	101.2	–
2	Acid Stress Sample	0.21	100.0	100.21	99.0
3	Base Stress Sample	16.10	83.6	99.7	98.5
4	Peroxide Stress Sample	0.60	99.2	99.8	98.6
5	Thermal Stress sample	0.50	100.6	101.1	100.9
6	Humidity Stress Sample	0.21	100.5	100.7	101.0
7	Photolytic Stress sample	0.10	101.0	101.0	101.2

Table 2: Results are showing the validation parameters of the developed method

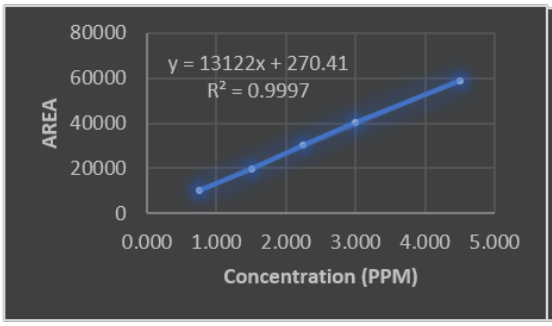
	Method Validation Summary						
Parameter	RC-E	Methoxy Paroxe-tine	Desfluoro Paroxe-tine	Cis-Paroxetine	N-Methyl Parox-etine	Ethoxy Paroxe-tine	Paroxetine dimer
Accuracy(%Recovery)							
LOQ (n=3)	91.0	92.0	94.0	95.8	91.0	97.7	93.6
50% (n=3)	92.6	95.5	96.4	98.1	96.3	98.2	94.5
100% (n=3)	95.9	96.1	95.4	97.7	95.6	99.2	96.1
150% (n=3)	98.3	99.1	100.2	100.3	97.1	99.5	95.9
Precision (%RSD)							
LOQ (n=6)	2.4	2.5	3.5	4.3	3.5	2.8	4.3
100% (n=6)	1.5	2.0	2.2	2.0	1.3	2.3	3.7
150% (n=6)	1.8	0.8	1.4	2.1	0.3	1.6	2.0
Ruggedness: Different day and analyst (%RSD)	2.6	2.1	1.6	2.4	2.8	1.9	1.8
Robustness (RRT)							
Actual flow	0.21	0.70	0.78	0.89	0.95	1.20	2.30
Flow (-0.2 mL)	0.20	0.68	0.77	0.89	0.95	1.20	2.10
Flow (+0.2 mL)	0.19	0.68	0.78	0.88	0.96	1.19	2.40
Temperature (-5°C)	0.20	0.70	0.77	0.89	0.94	1.20	2.09
Temperate (+5°C)	0.21	0.71	0.77	0.89	0.95	1.21	2.48
Organic (-5%) (MP-B)	0.20	0.69	0.78	0.87	0.95	1.19	2.28
Organic (+5%) (MP-B)	0.21	0.70	0.77	0.88	0.95	1.20	2.29
Limit of Detection (mg/mL)	0.019	0.021	0.020	0.015	0.019	0.020	0.024
Limit of Quantitation (mg/mL)	0.040	0.050	0.055	0.041	0.045	0.049	0.050



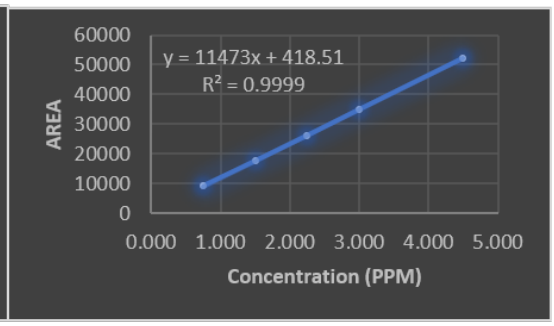
2.a.Linearity of Paroxetine



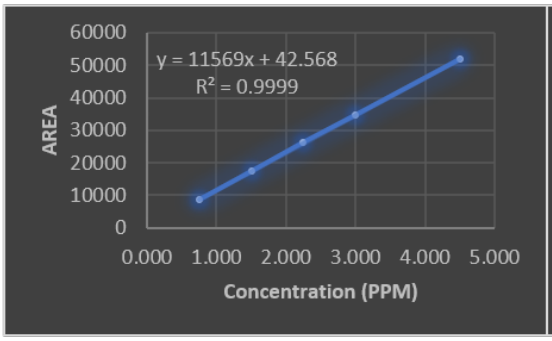
2.b.Linearity of Desfluoro Paroxetine



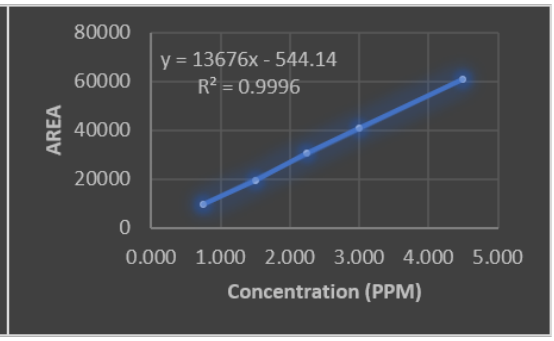
2.c.Linearity of Methoxy Paroxetine



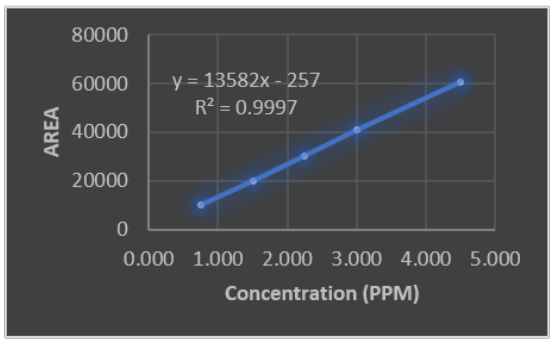
2.d.Linearity of Cis- Paroxetine



2.e.Linearity of N-Methyl Paroxetine



2.f.Linearity of Ethoxy Paroxetine



2.g.Linearity of Paroxetine Related Compound-E

Figure 2: Linearity of paroxetine and its impurities

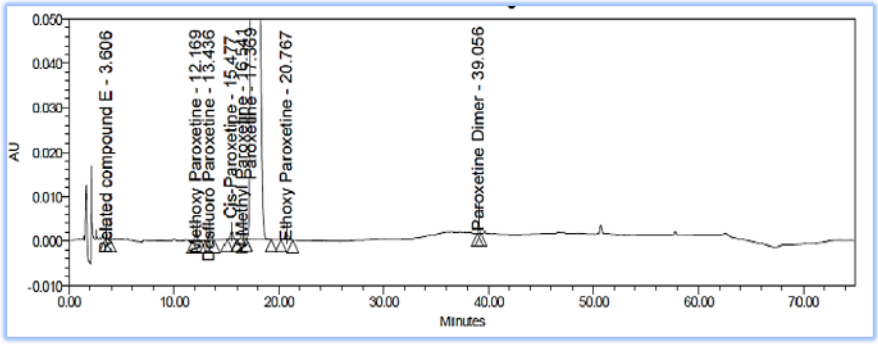
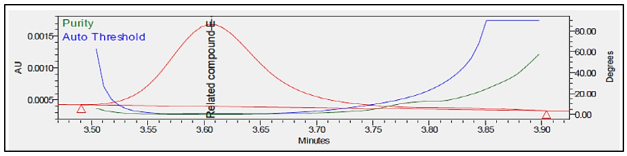
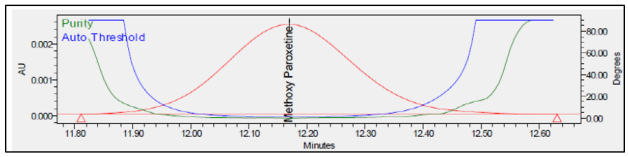


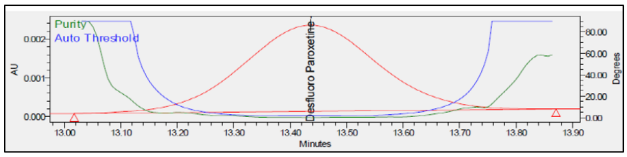
Figure 3: Chromatogram representing paroxetine spiked with Impurities



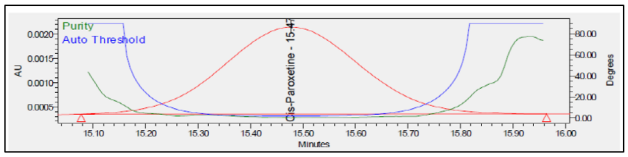
4.a. Purity Plot of Paroxetine Related Compound-E



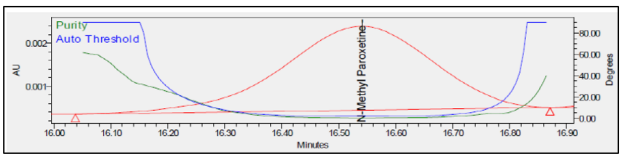
4.b. Purity Plot of Methoxy Paroxetine



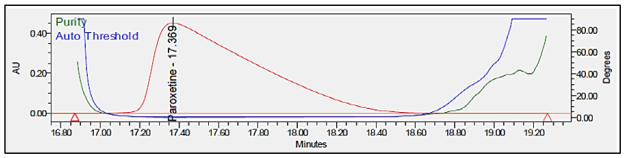
4.c. Purity Plot of Desfluoro Paroxetine



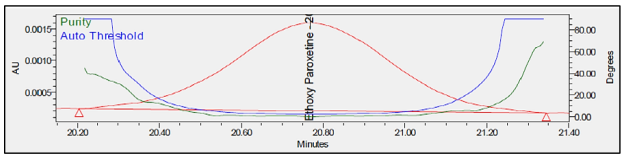
4.d. Purity plot of Cis-Paroxetine



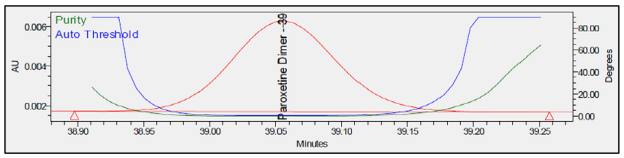
4.e. Purity Plot of N-Methyl Paroxetine



4.f. Purity Plot of Paroxetine



4.g. Purity plot of Ethoxy Paroxetine



4.h. Purity plot of Paroxetine Dimer

Figure 4: Purity Plot of Paroxetine and its impurities

Mass balance was established, and the same was reported in Table 1.

Linearity

From the Linearity graphs Figure 2, the response is linear over the concentration range from LOQ to 150% of specification level for all known impurities for the determination of the related substance of all known impurities in Paroxetine ER Tablets USP.

Accuracy

The results of accuracy indicated that the developed method is satisfactory in terms of efficiency for the estimation of related substances from LOQ to 150% of its specification level; the outcomes are tabulated in Table 2.

Method Precision

The repeatability of impurities % area was found to be less than five, showing the developed method was precise for the estimation of related substances of Paroxetine ER Tablets USP and the results are presented in Table 2.

Intermediate precision (Ruggedness)

Ruggedness data mentioned in Table 2. exemplifies that the developed method is rugged from analyst to analyst, system to system, column to column and day to day variation for the estimation related substance of known impurities in Paroxetine ER Tablets USP.

Limits of detection and quantitation

The reported LOQ data for paroxetine and its impurities suggest that the method has sufficient precision for the quantification of related substances of Paroxetine ER Tablets USP and Table 1 exemplifies the LOD and LOQ values of estimation.

Robustness

The developed method was found to be robust over the intentionally modified chromatographic conditions, and Results are represented in Table 1.

CONCLUSION

Present research on the antidepressant drug is a validated stability-indicating LC method for the quantitative assessment of Paroxetine and its related substances were articulated, and the stability behaviour of drug exposing to different environments of forced degradation was studied. Acceptable separation accomplished from drug and its degradation products formed in stress conditions, representing that the established chromatographic method was accurate, linear, sensitive, specific, reproducible, robust and stability-indicating. This method is suitable for routine analysis and quality monitoring by the assessment of related substance(s) by HPLC in Paroxetine API as well as marketed formulations.

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Conflict of interests

There are no conflicts of interest for this study.

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