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Review Article

In Depth Investigation of Quantitative Analytical and Bioanalytical Techniques of Paroxetine in Different Matrices: A Review

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Abstract

Analytical method helps in ensuring the safety and efficacy of drugs in different matrices by determining the quantity and quality of Drug. A detailed extensive literature survey is one of the most essential requirements for all focused research activities. Paroxetine is a selective serotonin reuptake inhibitor (SSRI). It is used to treat several diseases including major depressive disorder, obsessive-compulsive disorder, social anxiety disorder, panic disorder, posttraumatic stress disorder, generalized anxiety disorder, and premenstrual dysphoric disorder. Sincere effort has been made in the present review to collate all the relevant literature published in various pharmaceutical journals for determination of Paroxetine in different matrices both individually and in combination with other drugs. In this article main emphasis is given on the various techniques which are used for the estimation of the Paroxetine from various Pharmaceutical dosage forms or in biological matrices. The review highlights the basic as well as advanced techniques performed for estimating Paroxetine. Among different methods, HPLC and UV-Visible spectrophotometry are the most widely used techniques applied by the researchers. Detailed validation parameters are also given for the methods, which helps the researchers to select an analytical technique based on the information sought.

Keywords: *Paroxetine; Analytical Methods; Estimation; Matrices; HPLC*

Abbreviations

SSRI: Selective Serotonin Reuptake Inhibitor; 5-HT: 5-Hydroxy-tryptamine; NE: Nor Epinephrine; TCAs: Tricyclic Antidepressants; ICH: International Conference Of Harmonization; UV-Visible: Ultra Violet Visible; HPLC: High Performance Liquid Chromatography; UPLC: Ultra Performance Liquid Chromatography; HPTLC: High Performance Thin Layer Chromatography; LC-MS: Liquid Chroma-tography-Mass Spectroscopy

Introduction

Paroxetine is a potent and selective serotonin reuptake inhibitor (SSRI) with currently approved indications for the treatment of depression, obsessive-compulsive disorder, panic disorder and social phobia. It is also used in the treatment of generalized anxiety disorder, post-traumatic stress disorder, premenstrual dysphoric disorder and chronic headache. Paroxetine, a phenylpiperidine derivative, is the most potent inhibitor of the reuptake of sero-tonin (5-hydroxytryptamine, 5-HT) of all the currently available antidepressants including the class of SSRIs [1-4]. It is chemically known as (3S, 4R)-3- (2H-1,3benzodioxol -5-yloxy) methyl - 4- (4 fluorophenyl) piperidine (Figure 1). It is a very weak inhibitor of nor epinephrine (NE) uptake but it is still more potent at this site than the other SSRIs. The selectivity of Paroxetine, i.e., the ratio of inhibition of uptake of norepinephrine to serotonin (NE/5-HT) is

amongst the highest of the SSRIs. Paroxetine has little affinity for catecholaminergic, dopaminergic or histaminergic systems and by comparison with tricyclic antidepressants (TCAs) has, therefore, has a reduced propensity to cause central and autonomic side effects. Paroxetine exhibits some affinity for the muscarinic cholinergic receptor but much less than the TCAs [5,6]. It inhibits the serotonin reuptake receptor of the pre-synaptic nerve so that the serotonin amount will get increase in the synaptic cleft and relives various symptoms. It is the strongest candidate of all to inhibit the receptor. It also shows affinity for alpha 1, alpha 2 and beta adrenergic receptors, dopamine D1 and D2 receptors, histamine H1 receptor and sertonergic receptors like 5 HT1A, 5 HT2A and 5 HT2C receptors [7-10]. It also shows some affinity towards muscaranic receptors (Figure 2). Molecular weight of Paroxetine is 329.4 g/mol. Physical properties and taxonomy are mentioned in table 1.

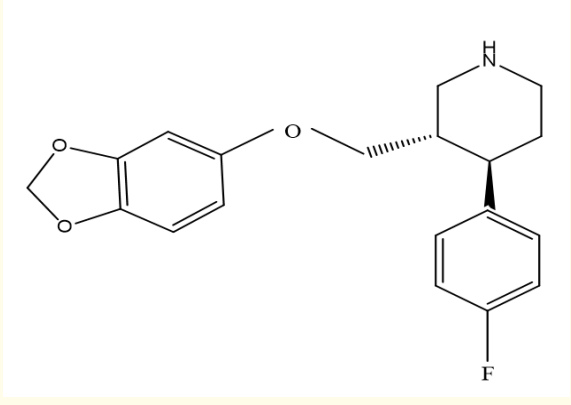

Figure 1: Chemical structure of paroxetine.

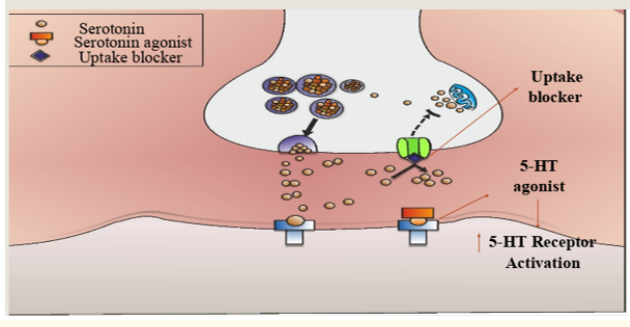

Figure 2: Mechanism of action of paroxetine.

State	White crystalline
Water solubility	5.4 mg/ml
Pka	9.90
Log p	2.53
Melting point	120^0C - 138^0C
Storage	< 30^0C
Kingdom	Organic compounds
Super class	Organoheterocyclic compounds
Class	Piperidines
Subclass	Phenyl piperidines
Direct parent	Phenyl piperidines
Alternative parent	Benzodioxoles/fluro benzenes/alkyl amines/alkyl aryl ethers/aryl fluoride/oxacyclic compounds/dialkyl amines/azacyclic compounds/acetals/organopnictogen compounds/organofluoride/hydrocarbon derivative.
Substituent's	Phenylpiperidine/Benzodioxole/Alkyl aryl ether/Fluorobenzene/Halobenzene/Aralkylamine/Aryl fluoride/Aryl halide/Monocyclic benzene moiety/BenzenoidEther/Secondary aliphatic amine/Acetal/Oxacycle/Secondary amine/Azacycle/Organooxygen compound/Organonitrogen compound/Organofluoride/Organohalogen compound/Hydrocarbon derivative/Organopnictogen compound/Organic oxygen compound/Amine/Organic nitrogen compound/Aromatic heteropolycyclic compound
Molecular framework	Aromatic heteropolycyclic compounds.
External descriptors	piperidines, organofluorine compound, aromatic ether, benzodioxoles.
Direct parent	Phenyl piperidines

Table 1: Physical properties and taxonomy of Paroxetine [10].

Pharmacokinetic of paroxetine

Paroxetine gets metabolized in liver by enzyme cytochrome CYP2D6 and CYP3A4 also contributes in the metabolism. In this change in metabolism can be seen in metabolism pathway due to interference of genetic polymorphs of CYP2D6. Majority of Paroxetine dose undergoes oxidation to form catechol which further get converts to glucuronide and sulfate metabolites with the help of methylation and conjugation reaction (Figure 3 and table 2) [10].

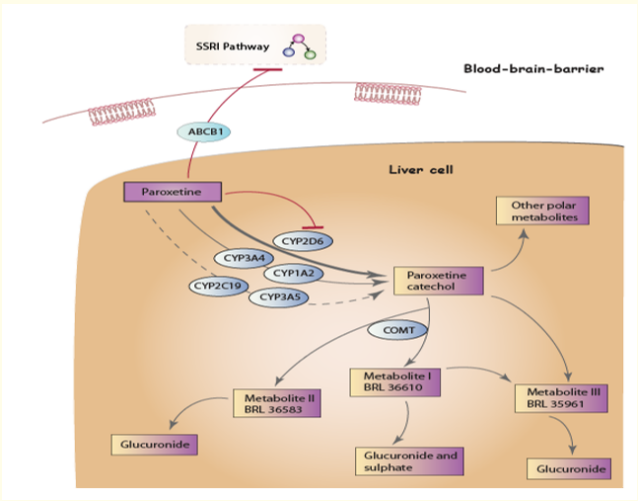

Figure 3: Metabolic pathway of paroxetine.

Bioavailability	30-60%
Cmax	2-8 hours
Plasma protein binding	95%
Metabolism	Liver
Half life	17.3 hours
Excretion	Urine – 64% Feces – 36%

Table 2: Pharmacokinetic parameters of paroxetine.

Clinical trials arena of paroxetine [11]

Paxil (paroxetine hydrochloride) was first developed in 1975 by a Danish company called Ferrosan. During 1980 Ferrosan sold paroxetine to Beecham Pharmaceuticals which later merged with SmithKline and French to become SmithKline Beecham. During 2000 the company merged with Glaxo to form GlaxoSmithKline (GSK) Paxil is indicated for the treatment of a range of conditions which are known as major depressive disorders (MDD). These conditions include post-traumatic stress disorder, panic disorder, social anxiety disorder and obsessive compulsive disorder. In adults the efficacy of paroxetine for depression is comparable to that of older tricyclic antidepressants, with fewer side effects and lower toxicity. The effectiveness of paroxetine in the treatment of MDD was proven by six placebo-controlled clinical trials.

Approvals

Since its launch paroxetine has been approved for a number of conditions.

During 1996 it was approved for treatment of major panic disorder and obsessive compulsive disorder. It is said that major panic disorder will affect three to six million US citizens in their lives. Clinical results showed that in one ten-week-double blind study 76% of subjects treated with 40mg/day of Paxil were completely free of full panic attacks at the end point compared with 44% of subjects who received a placebo.

During 1999 the US Food and Drug Administration (FDA) approved Paxil for the treatment of social anxiety disorder, also known as social phobia, affecting more than 10 million US citizens. Results from three multi-centre, placebo-controlled trials of adults demonstrated that Paxil was significantly more effective than the placebo in treating social anxiety disorder.

During 2000 SmithKline Beecham submitted a Supplemental New Drug Application to the FDA for Paxil, seeking marketing approval of Paxil for the treatment of post-traumatic stress disorder (PSTD). Approval was subsequently granted.

Data from a clinical programme involving nearly 1,200 patients diagnosed with PTSD demonstrated the benefit of Paxil over a placebo in reducing PTSD symptoms as measured by the Clinician Administered PTSD Scale and the Clinical Global Impressions Scale, both established standards.

During 2001 the FDA approved Paxil for the treatment of generalised anxiety disorder. The condition is characterised by excessive anxiety and worry about a number of event or activities. According to GSK, the disorder affects more than 60 million Americans.

Now a days available marketed formulations (In India) are mentioned in given table 3 [12,13].

Generic name	Brand name	Strength	Manufacturer
Paroxetine	Panex Panex-CR	10 /20mg 12.5/25 mg	Micro Labs Ltd. (Synapse)
Paroxetine	Pari-CR Pari	12.5/25 /37.5 mg 10/20/30/40 mg	IPCA Laboratories
Paroxetine	Parotin Parotin-CR	10/20/30/40 mg 12.5/25 mg	Cipla Limited
Paroxetine	Paroxy-CR	12./25/37.5 mg	Bondane Pharma
Paroxetine	Paxidep CR	12./25/37.5 mg	Sun Pharmaceutical Industries Ltd.
Paroxetine	Paxep Paxep-CR	10/20/30/40 mg 12./25/37.5 mg	Intas Pharmaceuticals Ltd
Paroxetine	Paroxy-CR	12./25/37.5 mg	Bondane Pharma
Paroxetine	Paxidep CR	12./25/37.5 mg	Sun Pharmaceutical Industries Ltd.
Paroxetine	Paxep Paxep-CR	10/20/30/40 mg 12./25/37.5 mg	Intas Pharmaceuticals Ltd
Paroxetine	Pxt	10/20 mg	D. R. Johns Lab Pharma Pvt. Ltd
Paroxetine	Raxit	10/20/30 mg	Solus Pharmaceuticals Limited
Paroxetine	Xet	10/20/30/40 mg	Zydus Pharmaceuticals
Paroxetine	Paradise-XR	12./25/37.5 mg	Torrent Pharma
Paroxetine	Paroxet	10/20 mg	Mankind Pharma

Table 3: Marketed formulations of paroxetine in India.

Analytical methods for estimation of paroxetine in bulk drug, pharmaceutical formulation and biological fluids

There are numerous methods have been reported for estimation of Paroxetine in bulk and dosage form as well as in biological fluids.

Spectrophotometric Methods

MC Sharma., *et al.* 2010 developed a UV spectroscopic method which describes the first derivative and absorption maxima method in bulk and tablet dosage form. The method was validated according to the ICH guidelines. Ferric chloride is used as solvent in both the methods. In first derivative and absorption maxima methods 323 nm and 351 nm wavelengths were used respectively. The Result parameters are tabulated in table 4 [14].

Parameters	Results	
	First Derivative	Absorbance Maxima
Linearity range(µg/ml)	10-120	10-120
Correlation range	0.9954	0.9993
%Recovery	98.56	98.56
Precision intraday (%RSD)	0.48	0.26
Precision interday (%RSD)	0.265	0.224

Table 4: Validation parameters reported by M.C. Sharma., *et al*. 2010 [14].

Naik J., *et al.* 2010 reported a validated UV-Spectrophotometric method for the estimation of Paroxetine in tablet. Paroxetine was dissolved in methanol and scanned at 293 nm. From this equation obtained was y = 0.0503x + 0.1139 and it obeys lamberts beer law. The results are tabulated in table 5 [15].

Parameters	Results
Linearity Range (µg/ml)	2-10
Correlation Coefficient	0.9997
% Recovery	100.27

Table 5: Validation parameters reported by Naik J., *et al*. 2010 [15].

Panchumarthy Ravishankar., *et al.* 2016 developed a validated UV-Spectroscopic method for determination of Paroxetine in tablet as well as in bulk. The drug was dissolved in distilled water and scanned at 293 nm. The developed method obeyed beer Lambert law having line equation y = 0.0117x + 0.0014. The results are tabulated in table 6 [16].

B Praveenkumar., *et al.* 2016 developed and validated a UV method to estimate the drug content in bulk as well as tablet. The measurement of the drug was done at 294 nm wavelength using water as solvent. The% assay was to be 99.13%. The other result parameters are tabulated in table 7 [17].

Parameters	Results		
Linearity range (µg/ml)	2-10		
Correlation Coefficient	0.9992		
%Recovery	99.75	99.81	99.85
Precision Interday (%RSD)	0.1944	0.2104	0.2114
Precision Intraday (%RSD)	0.198		
Limit of Detection (µg/ml)	0.33814		
Limit of Quantification (µg/ml)	1.17618		

Table 6: Validation parameters reported by Panchumarthy Ravishankar., *et al*. 2016 [16].

Parameters	Result
Linearity Range(µg/ml)	10-50
Correlation Coefficient	0.999
%Recovery	98.20-100.2
Precision Interday (%RSD)	0.843
Precision Intraday (%RSD)	0.767
%Assay	99.13

Table 7: Validation parameters reported by B Praveenkumar., *et al*. 2016 [17].

L Siva Shankar Reddy., *et al.* 2017 developed a UV method which was validated according to ICH Guidelines. Quantification of drug was done by using methanol as solvent at 538 nm. It was found that the method obeyed the Lambert beer law. The linearity was found in concentration range of 200 - 600 µg/ml. The regression curve was found to be Y = 0.001x + 0.007. The result parameters are tabulated in table 8 [18].

Jalpa U Patel., *et al.* 2018 reported a validated simultaneous Spectrophotometric quantification of Paroxetine hydrochloride and clonazepam in combination. In this method, estimation of both the drugs was done by using ratio derivative method. The overlapping spectra of both the drugs were resolved by using first derivative. The samples were scanned at 312.28 (Clonazepam) and 304.88 (Paroxetine) nm respectively. The concentration ranges of Paroxetine and clonazepam were 10 - 60 µg/ml and 5 - 30 µg/ml respectively while the linear regression plots produced for calibration of Paroxetine and clonazepam are r^2 = 0.9994 and r^2 = 0.9956 respectively. The result parameters are tabulated in table 9 [19].

Parameters	Results
Linearity Range (µg/ml)	200-600
Correlation Coefficient	0.999
%Recovery	106.64
Precision Interday (%RSD)	0.55
Presicion Intraday (%RSD)	0.702
Limit of Detection (µg/ml)	85
Limit of Quantification (µg/ml)	268

Table 8: Validation parameters reported by L Siva Shankar Reddy., *et al*. 2017 [18].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	10-60	5-30
Correlation coefficient	0.9992	0.9958
%Recovery	100.97	100.99
Presicion Interday (%RSD)	1.002	0.528
Precision Intraday (%RSD)	0.535	0.152
Limit of Detection (µg/ml)	1.310	0.087
Limit of Quantification (µg/ml)	0.265	3.971

Table 9: Validation parameters reported by Jalpa U Patel., *et al*. 2018 [19].

M.I. walash., *et al.* 2010 developed a simple and sensitive spectrophotometric method for the determination of each of sertraline (SER) and paroxetine HCl (PXT) in dosage forms. The method was based upon reaction of PXT and SER with 2,4-dinitrofluorobenzene (DNFB) to form colored products. The absorbance of the products were measured at 375 and 390 nm for SER and PXT respectively. The developed method was successfully applied for the determination of SER and PXT in dosage forms. The common excipients and additives did not interfere in their determinations. There was no significant difference between the results obtained by the proposed and the reference methods regarding Student t-test and the variance ratio F-test respectively. The result parameters are tabulated in table 10 [20].

V. Sheeja., *et al.* 2013 developed a colorimetry method using N-naphthyl ethylene diamine (NED) as reagent for the estimation of clonazepam and paroxetine in combined dosage form. The developed method was found to be precise, accurate, rugged and robust

for the simultaneous estimation of Clonazepam and Paroxetine in its combined dosage forms. The result parameters are tabulated in table 11 [21].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	25-225	1-9
Correlation Coefficent	0.981	0.99
%Recovery	99-101.60	99-102
Interday Precision (%RSD)	0.59	0.99
Intraday Precision (%RSD)	0.46	0.97
Limit of Detection (µg/ml)	2	0.2
Limit of Quantification (µg/ml)	16	0.6

Table 11: Validated parameters reported by Sheeja VK., *et al*. 2013 [21].

Spectroflourimetric method

Nawal Alarfaj., *et al.* developed a sensitive spectrofluorimetric method for the determination of Paroxetine–HCl in pharmaceutical formulations and human plasma. The native fluorescence of the drug has been studied under different conditions. Maximum fluorescence intensity was obtained in methanol at 340 nm using 290 nm for excitation. The result parameters are tabulated in table 12 [22].

Parameters	Results
Linearity Range (µg/ml)	0.05-0.40
Correlation Coefficient	0.9999
%Recovery (Formulation) (Plasma)	98.00 ± 0.99 77.70 ± 1.06
Limit of Detection (µg/ml)	0.015

Table 12: Validation parameters reported by Nawal Alarfaj., *et al*. 2010 [22].

Mahmoud A Omar., *et al.* 2013 developed a simple, reliable, sensitive and selective spectrofluorimetric method for determination of certain antidepressant like Sertraline hydrochloride, Fluoxetine hydrochloride, Paroxetine hydrochloride, Thioridazine hydrochloride and Amineptine hydrochloride. The spectrofluorimetric method was based on the charge-transfer reaction of these drugs as n-electron donors with 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π-electron acceptor. The drug-TCNQ complexes showed excitation maxima ranged from 290 - 301 nm and emission maxima ranged from 443 - 460 nm. The proposed method was successfully applied to the analysis of the cited drugs in dosage forms. The high sensitivity of the proposed method allows determination of investigated drugs in spiked and real human plasma. The result parameters are tabulated in table 13 [23].

Ruchita S Das., *et al.* 2012 developed a spectrofluorometric analysis for the quantification of new-generation antidepressant drugs belong to the class of selective serotonin reuptake inhibitors and selective norepinephrine reuptake inhibitors like sertraline, paroxetine, citalopram, venlafaxine, and fluoxetine, in pharmaceutical formulations and urine and plasma samples. Additionally, the results were compared statistically for each analyte in all the three matrices and were found equivalent, which signifies the absence of matrix effect. Thus, the method will be applied successfully to the determination of the cited drugs in pure or dosage forms as well as in biological fluids with good accuracy and precision. The result parameters are tabulated in table 14 [24].

Ibrahim A Darwish., *et al.* 2008 developed a spectrofluorimetric analysis for the quantification of Paroxetine. The method was based on nucleophilic substitution reaction of Paroxetine with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in an alkaline medium (pH 8) to form a highly fluorescent derivative that was measured at 545 nm after excitation at 490 nm. The results obtained by the proposed method were comparable with those obtained by the official method. The proposed method was superior to the previously reported spectrofluorimetric method for determination of Paroxetine in terms of its higher sensitivity and wider linear range. The high sensitivity of the method allowed its successful application to the analysis of Paroxetine in spiked human plasma. The proposed method was practical and valuable for its routine application in quality control and clinical laboratories for analysis of Paroxetine. The result parameters are tabulated in table 15 [25].

Jalpa Patel., *et al.* 2017 developed a synchronous fluorescence spectrofluorometric method for the simultaneous determination of Clonazepam and Paroxetine Hydrochloride in combined pharmaceutical dosage form. This spectrofluorimetry method has been found to have several advantages such as simple spectra, high se-

Investigated drugs	Linear range ng mL $^{-1}$	Intercept (a)	Standard deviation of intercept (Sa)	Slope (b)	Correlation coefficient (r)	LOD ng mL $^{-1}$	LOQ ng mL $^{-1}$
Fluoxetine	50-450	−0.20	2.58	1.45	0.9995	5.35	17.85
Sertraline	50-450	−2.18	2.82	1.36	0.9998	6.23	20.77
Paroxetine	50-550	0.63	2.45	1.10	0.9997	6.68	22.26
Thioridazine	100-650	−1.47	2.68	0.71	0.9992	11.37	37.90
Amineptine	100-750	−0.43	2.37	0.57	0.9990	12.48	41.61

Table 13: Validation parameters reported by Mahmoud A Omar., *et al* 2013 [23].

Parameters	SER	PAR	CIT	VLF	FLX
λe x (nm)	280	244	230	240	220
λe m i (nm)	560	339	300	302	394
Range (ng/μL)	5–500	10–500	5–500	10–500	10–500
Slope (b)	16.04	7.990	14.32	3.960	3.610
Intercept (c)	6.0	1.50	2.60	4.70	3.30
Correlation coefficient (r^2)	0.9995	0.9990	0.9992	0.9986	0.9989
LOD^c (ng/μL)	0.018	0.035	0.016	0.063	0.084
LOQ^d (ng/μL)	0.090	0.108	0. 048	0.186	0.285

Table 14: Validation parameters reported by Ruchita S. Das., et al 2012 [24].

Parameter	Value
λ_{ex} (nm)	490
λ_{em} (nm)	545
Linear range (ng ml^{-1})	80–800
Intercept	5.3550
SD of intercept	1.7646
Slope	0.2304
SD of slope	0.0037
Correlation coefficient (r)	0.9993
LOD (ng ml^{-1})	25
LOQ (ng ml^{-1})	77

Table 15: Validation parameters reported by Ibrahim A. Darwish., *et al* 2008 [25].

lectivity, and low interference. By virtue of its high sensitivity, this method could be applied to the analysis of both the drug in their co-formulated dose forms. The result parameters are tabulated in table 16 [26].

Parameters	Clonazepam	Paroxetine
Linearity range (µg/mL)	1-5	5-25
Correlation coefficient (r^2)	0.9986	0.9982
Slope ± SDb (Sb)	0.470 ± 0.005	0.063 ± 0.009
Confidence limit of slope	0.435 to 0.506	0.058 to 0.068
Intercept ± SDb (Sa)	0.274 ± 0.008	0.087 ± 0.012
Confidence limit of intercept	0.156 to 0.392	0.006 to 0.169
Limit of detection (µg/mL)	0.055	0.033
Limit of quantification (µg/mL)	0.169	0.102
Bartlett's testb (χ^2)	0.0110	0.0054
% Recovery	100.98	100.71
Assay ± SD %	100.451 ± 0.926	99.381 ± 0.300

Table 16: Validation parameters reported by Jalpa Patel., *et al* 2017 [26].

Chromatographic Methods

There are numerous high performance liquid chromatography (HPLC), Ultra Performance liquid chromatography (UPLC), High performance thin layer chromatography (HPTLC) methods have been reported for the analysis of Paroxetine in pharmaceutical formulation, bulk as well as in biological fluids.

M Laksmi Surekha., *et al.* 2012 developed and validated a new RP-HPLC method for the determination of Paroxetine in bulk as well in dosage form. For separation PhenomenaxLunaC18 (ODS) column (150 X 4.6 mm i.d., particle size 5µ) was used. The mobile phase system used for separation purpose was phosphate buffer pH 6: Acetonitrile in ratio of 50:50 and flow rate was 2 ml/min. The elute was scanned at 265 nm and the retention time was 5.563 min. The Validation parameters are tabulated in table 17 [27].

Parameters	Results
Linearity Range (µg/ml)	20-100
Correlation Coefficient	0.999
%Recovery	99.25
Precision Interday (%RSD)	0.0927
Precision Intraday (%RSD)	0.049
Assay (%)	99.8

Table 17: Validation Parameters reported by M Lakshmi Surekha., *et al*. 2012 [27].

Nitasha Agrawal., *et al.* 2013 had developed a validated HPLC method with electrochemical detection to determine the Paroxetine in pharmaceutical preparations. In this method the phosphate buffer 0.01M pH 3 and acetonitrile were runned as mobile phase at flow rate of 1.0 ml/min in ratio of 40:60 respectively. The condition was isocratic throughout the procedure. The electrochemical detection of eluent was done at 0.9 V. The Result Parameters are tabulated in table 18 [28].

Parameters	Results
Linearity Range (ng/ml)	0.5-50
Correlation Coefficient	0.9999
%Recovery	99.7-100.7%
Precision Interday (%RSD)	0.299
Precision Intraday (%RSD)	0.174
Limit of Detection (ng/ml)	0.005
Limit of Quantification (ng/ml)	0.01

Table 18: Validation Parameters reported by Nitasha Agrawal., *et al.* 2013 [28].

Malgorzata lisowska kuzmicz., *et al.* 2013 developed and validated a method for estimation of Paroxetine enantiomers. In this method chiral separation is performed by using two methods (a) ovomucoid immobilized on aminopropyl silane derivatized silicon column was used. The mobile phase was phosphate buffer pH 3.5: Acetonitrile in the ratio of 98: 2 and flow rate 1.5 ml/min. (b) silica based amylase tris carbamate column was used. The mobile phase used was hexane: ethanol: ethanolamine (80: 20: 0.2) with flow rate of 1.0 ml/min. The result parameters are tabulated in table 19 [29].

Parameters	Results	
	Method A	Method B
%Recovery	98.37-101.87	99.53-100.82
Limit of Detection (µg/ml)	0.005	0.006
Limit of Quantification (µg/ml)	0.016	0.020

Table 19: Validation Parameters reported by Malgorzata lisowska Kuzmicz., *et al*. 2013 [29].

Aziz Unissa., *et al.* 2014 had developed and validated RP HPLC method for simultaneous quantification of Paroxetine and Clonazepam in tablet dosage form. In this method Agilent reverse phase column was used for the separation of two drugs by using 15 mM ammonium acetate: methanol was used as mobile phase in ratio of 60: 40. The eluents were monitored at 254 nm. The retention time of Paroxetine and Clonazepam are 5.42 and 4.01 min respectively. The result parameters are tabulated in table 20 [30].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range(µg/ml)	25-125	1-5
Correlation Coefficient	0.999	0.998
%Recovery	100.1	99.47
Method Precision	0.398	0.699
System Precision	0.381	0.695
Limit of Detection(µg/ml)	0.0176	0.892
Limit of Quantification(µg/ml)	0.0532	2.512

Table 20: Validation parameters are reported by Aziz Unissa., *et al*. 2014 [30].

K Haumantha Rao., *et al.* 2014 has developed RP HPLC method for determination of Paroxetine in pharmaceutical dosage form.

The analysis was performed by using Inertial ODS C18 column and mobile phase was phosphate buffer pH 2.6 and acetonitrile in proportion of 70: 30. The flow rate was 1 ml/min and detection wavelength used was 211 nm. The retention time of Paroxetine observed was 3.276 min. The result parameters are tabulated in table 21 [31].

Parameters	Results
Linearity Range (µg/ml)	25-150
Correlation Coefficient	0.999
%Recovery	99.41 - 99.93
Method Precision	0.66
Intermediate Precision	1.3
Limit of Detection (µg/ml)	0.06
Limit of Quantification (µg/ml)	0.18

Table 21: Validation parameters reported by K Hanumantha Rao., *et al*. 2014 [31].

Shrikanth A., *et al.* 2018 developed and validated new RP- HPLC for simultaneous determination of Paroxetine and clonazepam in tablets. The analysis was performed on Inertsil column using phosphate buffer: acetonitrile: methanol (30: 30: 40). The flow rate was 1 ml/min and eluents were detected at 268 nm. The retention time of Paroxetine and clonazepam was found to be 4.867 and 2.367 respectively. The result Parameters are tabulated in table 22 [32].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	60 – 140	2.4 – 5.6
Correlation Coefficient	0.999	0.998
%Recovery	100.08	96.89
Precision (% RSD)	1.21	0.75
Ruggedness (% RSD)	0.036	0.054

Table 22: Validation parameters reported by Shrikanth A., *et al*. 2018 [32].

G Srinivas reddy., *et al.* 2014 developed and validated a stability indicating RP HPLC method for simultaneous estimation of Paroxetine and clonazepam in bulk and its pharmaceutical dosage form. An Agilent Zorbax sb C18 column is used for separation. The mobile phase used was orthophosphoric acid and methanol in ratio of 60: 40. The flow rate was maintained at 0.8 ml/min and effluents were scanned at 270 nm using photo diode array as detector. The retention time of Paroxetine and clonazepam are 3.478 and 3.964 min respectively. The result parameters are tabulated in table 23 [33].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	50 – 150	50 - 150
Correlation Coefficient	0.99	0.99
%Recovery	97 – 103	97 - 103
Precision (% RSD)	0.16	0.14
Limit of Detection (µg/ml)	8.064	2.419
Limit of Quantification (µg/ml)	9.501	2.850

Table 23: Validation Parameters reported by G Srinivas Reddy., *et al*. 2014 [33].

Geetharam Yanamadala., *et al.* 2014 developed and validated the stability indicating HPLC method for simultaneous quantification of Paroxetine and clonazepam in pharmaceutical dosage forms. A reversed phase Kromasil C18 column with mobile phase consisting of Acetonitrile and 0.1% orthophosphoric acid in the ratio of 60: 40. The flow rate was maintained at 1.2 ml/min and effluents are scanned at 260 nm. The retention times of Paroxetine and clonazepam were found to be 3.46 min and 4.55 min, respectively. The result Parameters are tabulated in table 24 [34].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range(µg/ml)	125-750	2.5-15
Correlation Coefficient	0.9999	0.9996
%Recovery	99.4 -100.6	98.1-101.0
Precision (% RSD)	0.38-0.65	0.38-0.65
Limit of Detection (µg/ml)	0.175	0.014
Limit of Quantification (µg/ml)	0.532	0.044

Table 24: Validation parameters reported by Geetharam Yanamadala., *et al*. 2014 [34].

Fawazia Ibrahim., *et al.* 2016 developed and validated a micellar HPLC method for simultaneous estimation of Paroxetine and clonazepam pharmaceutical dosage form. In this monolithic column was used for the separation of these two drugs and mobile

phase used for detection comprises of a mixture solution of 12% n propanol and 0.175 MSDS and pH was adjusted to 6 using ortho-phosphoric acid. The flow rate was kept at 1.0 ml/min and eluent was monitored at 300 nm. The Result parameters are tabulated in table 25 [35].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	4 - 250	1 - 20
Correlation Coefficient	0.9999	0.9998
%Recovery	100.22	100.8
Precision Interday (% RSD)	0.49	0.73
Precision Intraday (%RSD)	0.84	0.64
Limit of Detection (µg/ml)	2.675	0.277
Limit of Quantification (µg/ml)	8.106	0.838

Table 25: Validation Parameters Reported by Fawazia Ibrahim., *et al*. 2016 [35].

N Naidu., *et al.* 2018 developed and validated an assay method by using RP HPLC for simultaneous quantification of both the drugs. The separation of both the drugs were carried out by using Intersil ods 3v column, C18 column. The mobile phase comprises of mixture of buffer (pH7, adjusted with ammonium acetate), methanol, and acetonitrile in the ratio of 3:2:5, at the flow rate of 1.0 ml/min. The detection was carried out 224 nm and the retention time of Paroxetine and clonazepam was found to be 2.367 and 4.867 respectively. The result Parameters are tabulated in table 26 [36].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	60 - 140	2.4 – 5.6
Correlation Coefficient	0.9977	0.9976
%Recovery	100	99.89
Precision (% RSD)	0.56	0.33
Limit of Detection (µg/ml)	2.34	0.03
Limit of Quantification (µg/ml)	7.10	0.09

Table 26: Validation parameters reported by N Naidu., *et al*. 2018 [36].

Niki Vergi-Athanasiou., *et al.* 2007 developed and validate HPLC- fluorescence detection method for estimation of Paroxetine and its metabolite in plasma. The method utilizes a Zorbax Eclipse XDB-C18 5-µm column, a mobile phase composed of acetonitrile-phosphate buffer (KH_2PO_4 0.04 M; pH=3.5) (30:70, v/v) at a flow rate of 1.0 mL/min and protriptyline as internal standard. The total analysis time was 12 min and Paroxetine eluted at 10 min. A fluorescence detector was used with excitation and emission wavelength adjusted at 295 and 350 nm, respectively. The method was applied successfully in the quantitative determination of Paroxetine in plasma samples of patients receiving 20 - 40 mg of Seroxat^R (Paroxetine) and the concentrations were in the therapeutic range. Results of validation parameters are tabulated in table 27 [37].

Parameters	Results		
	Paroxetine	Metabolite A	Metabolite B
Linearity Range (ng/ml)	7-200	12-200	27-200
%Recovery	84%	----------	----------
Precision (% RSD) Intra day Interday	0.26-7.5 4.9	3.4-13 5.5	5.7-15 6.0

Table 27: Validation Parameters Reported by Niki Vergi-Athanasiou., *et al.* 2007 [37].

Bhagyasree T., *et al.* 2014 developed a simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method for simultaneous estimation of Paroxetine and Clonazepam in pharmaceutical dosage form. Chromatographic separation was performed on Agilent Eclipse XDB (C8) (4.6 mm x 150 mm, 5m) column, with mobile phase comprising of mixture of buffer (pH7, adjusted with ammonium acetate), acetonitrile in the ratio of 82:18 v/v, at the flow rate 0.8 ml/min. The detection was carried out at 265 nm. The retention times of paroxetine and clonazepam were found to be 2.36 and 3.14 mins respectively with a run time of 5 mins, theoretical levels for paroxetine and clonazepam were 6753 and 4693 respectively, with a resolution of 5.10. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. The Validation parameters are tabulated in table 28 [38].

B Praveen Kumar., *et al.* 2016 developed HPLC method for estimation of Paroxetine in bulk and pharmaceutical dosage form. The chromatographic method was developed on AGILENT HPLC with

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	100-300	2-6
Correlation Coefficient	0.999	1.0
%Recovery	100	100
Precision (% RSD)	0.13	0.06
Limit of Detection (µg/ml)	2.742	2.40
Limit of Quantification (µg/ml)	9.174	8.0

Table 28: Validation parameters reported by Bhagyasree T., *et al* 2014 [38].

UV detection. The method was optimized by Kromosil-C18, (250 * 4.6 mm, 5µ) column by using Phosphate buffer (pH_ 6): Acetonitrile (60:40) as a mobile phase with 1 ml/min as flow rate. The detection wavelength is 294 nm. The optimized method was validated with good correlation coefficient (0.9999) was found between the concentration range of 20 - 100 µg/ml. The limits of detection and quantitation for the method were 0.7 and 2.4 µg/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. The recovery values were 98.6 - 101.85%. The proposed method was successfully applied for the determination of Paroxetine in bulk and their dosage forms [39].

Erk N., *et al.* 2003 developed Voltammetric and HPLC techniques for the determination of Paroxetine hydrochloride the antidepressant agent Paroxetine hydrochloride was studied by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and osteryoung square wave voltammetry (OSWV). A sensitive method was described for the determination of Paroxetine in its pure form and in human plasma. The linear relationship between concentration and peak current permits the quantification of Paroxetine by CV, DPV and OSWV in the concentration range of 2 x 10(-5) - 8 x 10(-4) M. Applicability to tablets and human plasma analysis has been illustrated. Furthermore, a HPLC method with diode array detection was developed. Linearity was established between 2 x 10(-7) - 6 x 10(-5) M for POT. The described methods were successfully employed with high degrees of precision and accuracy for the estimation of total drug content in human plasma and pharmaceutical dosage forms of Paroxetine [40].

Mireia Segura., *et al.* 2003 developed high-performance liquid chromatography/electrospray ion trap mass spectrometry for Quantitative determination of paroxetine and its 4-hydroxy-3-methoxy metabolite in plasma. Chromatography was performed on a reversed-phase column using acetonitrile/0.02% formic acid (66:34, v/v) as a mobile phase. The mass spectrometer was operated in the multiple reaction monitoring mode. The method was validated over concentration ranges of 0.75 - 100 µg/L and 5 - 100 µg/L for paroxetine and HM paroxetine, respectively. Mean recoveries of 77% for paroxetine and 76% for HM paroxetine were found, with precision always better than 15%. The limits of detection and quantification were 0.20 and 0.70 µg/L for paroxetine, and 0.70 and 2.20 µg/L for its metabolite. The method was applied to the analysis of plasma samples obtained from nine healthy male volunteers administered with a single oral dose of 20 mg paroxetine. After the 20-mg dose, the mean peak plasma concentration was 8.60 µg/L for paroxetine and 92.40 µg/L for HM paroxetine showing a tenfold ratio between the metabolite and the parent drug along the entire time-concentration curve [41].

Massaroti P., *et al.* 2005 reported a bioanalytical method for estimation of Paroxetine from blood plasma. In this the C18 column was used for separation and solution of 0.1% formic acid in acetonitrile: water (6:4) was used as mobile phase. The retention time of Paroxetine and fluoxetine was found to be 1.6 and 1.7 min respectively. Mass spectrophotometric method was performed using Quattro Micro equipment working with an ESI source in the positive ion mode. In this desolvation Nitrogen gas was used and the voltage of ESI source tip was found to be 4.4 kV. The parent ion m/z 330 for Paroxetine and 310 for fluoxetine were selected and analysed by using quadrupole analyser. Further the product ions of m/z 70 for Paroxetine and m/z 44 for fluoxetine were analysed by using third quadrupole analyser [42].

Pattan Shahina Sulthana., *et al.* 2017 developed a simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography for simultaneous estimation of Paroxetine and Clonazepam in pharmaceutical dosage form. Chromatographic separation was performed on INERTSIL ODS 3V column, C18(250x4.6 ID) column, with mobile phase comprising of mixture of buffer (pH7, adjusted with ammonium acetate), methanol, acetonitrile in the ratio of 3:2:5, at the flow rate 1.0 ml/min. The detection was carried out at 224 nm. The retention times of Paroxetine and Clonazepam were found to be 2.367 and 4.867 mins, respec-

tively with a run time of 6 mins, theoretical levels for Paroxetine and clonazepam were 2320 and 3211 respectively, with a resolution of 8.249. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of Paroxetine was found in the range of 60-140µg/mL and that for Clonazepam was found to be 2.4 - 5.6 µg/mL [43].

Schatz DS., *et al.* 2000 developed High-Performance Liquid Chromatography with Coulometric Detection for estimation of Paroxetine, Risperidone and 9-Hydroxyrisperidone in Human Plasma. The drugs were separated on a cyano column followed by coulometric detection. This method described here has sufficient sensitivity to quantitate paroxetine accurately in the range 5 - 500 ng/ml with a lower limit of detection of 1 ng/ml and risperidone and its main metabolite 9-hydroxyrisperidone in the range 2 - 100 ng/ml with a lower limit of detection of 1 ng/ml when 1 ml of plasma was used for the analysis. The precision, accuracy and specificity have been proven, and show that the method is reliable for clinical studies and routine drug monitoring [44].

Umadurai M., *et al.* 2014 reported a validated UPLC method for simultaneous estimation of Paroxetine and clonazepam in tablet dosage form. This method was performed on thermo fischer scientific Hypercel C18 column (50 x 2.1 mm, 1.8 µm) using Acetonitrile: Methanol: Potassium di hydrogen orthophosphate buffer (8:52:40) buffer (pH 3, adjusted with Ortho phosphoric acid) as mobile phase at the flow rate 0.5 ml/min. The samples were scanned at 265 nm while the retention time of Paroxetine and clonazepam was observed as 1.28 and 2.45 min with run time of 4 min. The resolution was found to be 8.09. The Validation parameters are tabulated in table 29 [45].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range (µg/ml)	250-750	10-30
Correlation Coefficient	0.9993	0.9997
%Recovery	100.30	99
Interday Precision (% RSD)	0.55	0.196
Intraday Precision (% RSD)	0.37	0.30
Limit of Detection (ng/ml)	138.36	74.81
Limit of Quantification (ng/ml)	419.29	226.72

Table 29: Validation parameters reported by Umadurai M., *et al*. 2014 [45].

RN Sharma., *et al.* 2007 has developed and validated spectrodensitometric method for estimation of Paroxetine in solid dosage form. The method employed TLC plates pre-coated silica gel 60 F_{254} aluminium sheets as the stationary phase. The mobile phase consisting of ethyl acetate: acetic acid: water in the ratio of 7.5:1.5:1. The chamber saturation time was 15 min and the developing distance was 7 cm. The sample was scanned at 296 nm with scanning speed of 10 mm/sec. The Validation parameters are shown in table 30 [46].

Parameters	Results
Linearity Range(ng/ml)	160-960
Correlation Coefficient	0.995
%Recovery	100.8
Interday Precision (%RSD)	0.89-2.77
Intraday Precision (%RSD)	0.64-2.38
Limit of Detection (ng/spot)	60
Limit of Quantification (ng/spot)	160

Table 30: Validation parameters reported by R.N. Sharma., *et al* 2007 [46].

Purvi shah., *et al.* 2015 developed and validated a HPTLC method for simultaneous estimation of Paroxetine and clonazepam in combined dosage form. The stationary phase used in this method was pre coated silica gel aluminium plate 60 F_{254} while the mobile phase used for separation was n-butanol: glacial acetic acid: water in the ratio of (9:2:0.5). The length of chromatographic run was 8 cm and scanning speed was 20 mm/sec. The data resolution was 100 µm/step. The validation parameters were tabulated in table 31 [47].

Parameters	Results	
	Paroxetine	Clonazepam
Linearity Range(ng/band)	300 - 1800	40-240
Correlation Coefficient	0.9989	0.9958
%Recovery	101.27-101.87	101.46-101.54
Interday Precision (%RSD)	0.520-0.948	1.141-1.546
Intraday Precision (%RSD)	0.753-1.203	1.538-1.788
Limit of Detection (ng/band)	63.416	8.840
Limit of Quantification (ng/band)	195.260	26.789

Table 31: Validation Parameters reported by Purvi shah., *et al*. 2015 [47].

Conclusion

There are numerous reported methods are found for the estimation of Paroxetine in pharmaceutical formulations and biological methods. In nutshell, techniques like RP-HPLC, UV-Visible spectroscopy, Spectro fluorimetry, UPLC, HPTLC and hyphenated technique like LC-MS are the most simple, easy and cost efficient methods for estimation of Paroxetine in various formulations. However, techniques like LC-MS/MS can be used for the determination of Paroxetine in the biological samples like plasma and urine. Therefore, this review helps researchers to widen their ideas on different improved aspects for further studies on the evaluation of the drug.

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Conflict of Interest

Author has no conflicts of interest.

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