CHAPTER 3
 CHAPTER  3  PROFILES OF DRUG AND CO-CRYSTAL FORMERS

3.1  Fluoxetine hydrochloride

Fluoxetine hydrochloride (FH) is a selective serotonin reuptake inhibitor which is clinically effective for the treatment of depression (Stark and Hardison, 1985). Fluoxetine and its major metabolite norfluoxetine act as neuronal inhibitors of serotonin reuptake and result in both increased serotonin concentration at the synaptic cleft and autoreceptor stimulation (Wong et al., 1975; Schmidt et al., 1988). Fluoxetine hydrochloride has been shown to have comparable efficacy to tricyclic antidepressants but with fewer anticholinergic side effects (Benfield et al., 1985; Chouinard, 1985; Stark and Hardison, 1985; Lader, 1988). Fluoxetine hydrochloride has been primarily studied for the treatment of depression, but more recently has been studied for the treatment of bulimia and severe obesity (Ferguson and Feighner, 1987; Freeman and Hampson, 1987; Levine et al., 1987).

3.1.1  General description

Chemical Name:  dl-N-methyl-3-phenyl-3-[(α,α,α-trifluoro-p-tolyl)oxy] propylamine hydrochloride

Empirical Formula:  C_{17}H_{18}F_{3}NO.HCl

Molecular Weight:  345.79

Structure:

Appearance:  White to off-white, crystalline powder

pKa:  8.7
3.1.2 Physical properties (Risley and Bopp, 2005)

3.1.2.1 Melting Range

The melting range of fluoxetine hydrochloride is 158.4-158.9 °C.

3.1.2.2 Solubility Profile

The solubility properties of fluoxetine hydrochloride are listed in Table 3.1. Fluoxetine hydrochloride is freely soluble in methanol and ethanol; soluble in acetonitrile, chloroform, and acetone; slightly soluble in ethyl acetate, dichloromethane, and water (with sonication at pH: 1.2, 4.5, and 7.0). Fluoxetine is essentially insoluble in toluene, cyclohexane, and hexane.

3.1.2.3 Ultraviolet Spectrum

The ultraviolet spectrum of fluoxetine hydrochloride in water is shown in Figure 3.1. The ultraviolet absorbance of fluoxetine hydrochloride is due to individual contributions from CF$_3$-Ph-OR and Ph-R. The maximum absorbance from the fluoronated cresol chromophore occurs at 227 nm with a molecular absorptivity of $\varepsilon = 12,900$. The peak absorbances are listed in Table 3.2.

3.1.2.4 Infrared and Raman Spectra

The major absorption bands for both the infrared and the Raman frequencies (Figure 3.2) and the corresponding assignments are listed in Table 3.3 (Risley and Bopp, 2005).

3.1.2.5 Optical Rotation

The observed rotation for a 10 mg mL$^{-1}$ methanol solution of fluoxetine hydrochloride using the sodium D-line on a Perkin-Elmer 241 MC Polarimeter was determined to be 0.00° indicating a recemic mixture.
Table 3.1. Solubility Properties of Fluoxetine Hydrochloride

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (mg mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ethanol</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Acetone</td>
<td>&gt;33&lt;100</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>&gt;33&lt;100</td>
</tr>
<tr>
<td>Chloroform</td>
<td>&gt;33&lt;100</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>&gt;5&lt;10</td>
</tr>
<tr>
<td>Water (pH 1.2)</td>
<td>&gt;1&lt;2</td>
</tr>
<tr>
<td>Water (pH 4.5)</td>
<td>&gt;1&lt;2</td>
</tr>
<tr>
<td>Water (pH 7.0)</td>
<td>&gt;1&lt;2</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>&gt;2&lt;2.5</td>
</tr>
<tr>
<td>Toluene</td>
<td>&gt;0.5&lt;0.67</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>&gt;0.5&lt;0.67</td>
</tr>
<tr>
<td>Hexane</td>
<td>&gt;0.5&lt;0.67</td>
</tr>
</tbody>
</table>

Figure 3.1. UV spectrum of fluoxetine hydrochloride in water
Table 3.2. Peak Absorbance for Fluoxetine Hydrochloride

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>$\varepsilon_{1%/1}$ (cm)</th>
<th>$\varepsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>227</td>
<td>372.0</td>
<td>12,900</td>
</tr>
<tr>
<td>264</td>
<td>29.2</td>
<td>1,010</td>
</tr>
<tr>
<td>268</td>
<td>29.3</td>
<td>1,010</td>
</tr>
<tr>
<td>275</td>
<td>21.5</td>
<td>745</td>
</tr>
</tbody>
</table>

Figure 3.2. Infrared and Raman spectra of fluoxetine hydrochloride in a KBr pellet and solid form respectively.
### Table 3.3. Infrared and Raman Spectral Assignments for Fluoxetine Hydrochloride

<table>
<thead>
<tr>
<th>Correlation/Assignments</th>
<th>Frequency (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>OH stretch – H(_2)O in KBr</td>
<td>3430 vb</td>
</tr>
<tr>
<td>Aromatic CH stretches</td>
<td>3085, 3060, 3039, 3026, 3010</td>
</tr>
<tr>
<td>Asymmetric CH(_2) and CH(_3) stretches</td>
<td>2986, 2957, 2927, 2916</td>
</tr>
<tr>
<td>Symmetric CH(_2) and CH(_3) stretches</td>
<td>2884, 2961, 2838</td>
</tr>
<tr>
<td>NH(_2^+) - NH stretches and ‘combination bands’</td>
<td>2805, 2790, 2772, 2730, 2638, 2614, 2547, 2490, 2435</td>
</tr>
<tr>
<td>NH(_2^+) deformations</td>
<td>1636</td>
</tr>
<tr>
<td>Phenyl ring vibrations</td>
<td>1614, 1589, 1585, 1516, 1495</td>
</tr>
<tr>
<td>CH(_2) deformations</td>
<td>1474, 1470</td>
</tr>
<tr>
<td>Asymmetric CH(_3) deformations</td>
<td>1455, 1446</td>
</tr>
<tr>
<td>Symmetric CH(_3) deformations</td>
<td>1429</td>
</tr>
<tr>
<td>CF stretches</td>
<td>1329</td>
</tr>
<tr>
<td>Phenyl ring vibrations</td>
<td>1314, 1307</td>
</tr>
<tr>
<td>C-O stretches; aryl/alkyl ether</td>
<td>1257, 1241</td>
</tr>
<tr>
<td>CF stretches; CN stretches secondary amine</td>
<td>1182, 1161</td>
</tr>
<tr>
<td>C-O stretches; aryl/alkyl ether</td>
<td>1122, 1108</td>
</tr>
<tr>
<td>Phenyl ring vibrations</td>
<td>1075, 1069, 1028, 1023</td>
</tr>
<tr>
<td>Ring ‘breathing’ vibrations mono-substituted phenyl</td>
<td>999</td>
</tr>
<tr>
<td>Phenyl CH wags</td>
<td>986, 957, 945, 913, 903, 847, 841, 818</td>
</tr>
<tr>
<td>Phenyl ring vibrations Para dis-substituted</td>
<td></td>
</tr>
<tr>
<td>Phenyl ring vibrations</td>
<td>765, 746, 730</td>
</tr>
<tr>
<td>Phenyl ring vibrations mono-substituted</td>
<td>697</td>
</tr>
<tr>
<td>Phenyl ring vibrations</td>
<td>647, 635, 622</td>
</tr>
</tbody>
</table>
3.1.2.6 Differential Thermal Analysis

The DTA thermogram for fluoxetine hydrochloride, at a heating rate of 5 °C per minute, shows a sharp endotherm at 161 °C indicating a melt. A large exotherm occurs at 241 °C and this is attributed to the decomposition of fluoxetine.

3.1.2.7 Thermogravimetric Analysis

The TGA thermogram for fluoxetine hydrochloride, at a heating rate of 5 °C per minute, shows an initial weight loss at 172 °C followed by continuous weight loss indicating decomposition.

3.1.2.8 X-ray Diffraction Analysis

Figure 3.3 illustrate the X-ray diffraction pattern of fluoxetine hydrochloride.

![X-ray diffraction pattern of fluoxetine hydrochloride](image)

**Figure 3.3.** X-ray diffraction pattern of fluoxetine hydrochloride
3.1.3 Analytical Methods for determination of FH

Several papers are available in the literature on the quality control of pharmaceutical formulations containing fluoxetine by means of different techniques such as spectrophotometry (Naik et al., 1999) or spectrofluorimetry (Prabhakar et al., 1999; Berzas Nevado et al., 2002; Mandrioli et al., 2002), electrochemical techniques (Roque da Silva et al., 1999), gas (Berzas Nevado et al., 2000a; Berzas Nevado et al., 2005) or liquid (El-dawy et al., 2002) chromatography, capillary electrophoresis techniques (Berzas Nevado et al., 2000b; Mandrioli et al., 2002), NMR spectroscopic method (Shamsipur et al., 2007). The British Pharmacopoeia (2007) and the United States Pharmacopeia (2007) report the use of liquid chromatography with UV detection for the determination of fluoxetine in bulk and pharmaceutical preparations.

Analysis of fluoxetine in biological fluids also reported in literature. The determinations are based on different techniques such as gas chromatography (Eap et al., 1996) and HPLC with UV (Alvarez et al., 1998; Rodrigues Chaves et al., 2009), fluorimetric (Raggi et al., 1998) or mass spectrometry (Saber, 2009) detection. Moreover, high-sensitivity capillary electrophoresis has been used for the stereoselective separation of fluoxetine enantiomers in formulation (Piperaki et al., 1995) and in plasma (Rodríguez Flores et al., 2005).

3.1.3.1 Bulk and/or formulation

Naik et al. (1999) reported an extractive spectrophotometric method for estimation of fluoxetine hydrochloride from pharmaceutical formulations. It is based on extraction of colored complex formed between fluoxetine hydrochloride and bromothymol blue in chloroform. This yellow complex has an absorption maximum at 412 nm with molar extinction coefficient $13.6 \times 10^3$ mol$^{-1}$ cm$^{-1}$. The system obeys Beer's law in the range of 1.5-20.0 µg mL$^{-1}$ of fluoxetine hydrochloride.
Prabhakar et al. (1999) reported two spectrofluorimetric method for determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. Both methods are based on the formation of a yellow ion-pair complex due to the action of methyl orange (MO) and thymol blue (TB) on fluoxetine in acidic (pH 4.0) and basic (pH 8.0) medium, respectively. Under optimized conditions they show an absorption maxima at 433 nm (MO) and 410 nm (TB), with molar absorptivities of $2.12 \times 10^{-4}$ and $4.207 \times 10^{-3}$ L mol$^{-1}$ cm$^{-1}$ and Sandell’s Sensitivities of $1.64 \times 10^{-2}$ and $0.082$ µg cm$^{-2}$ per 0.001 absorbance unit for MO and TB, respectively. The colour is stable for 5 min after extraction. In both cases Beer’s Law is obeyed at 1–20 µg mol$^{-1}$ with MO and 4–24 µg mol$^{-1}$ with TB. The proposed method was successfully extended for analysis of pharmaceutical preparations—capsules.

Roque da Silva et al. (1999) reported electrochemical studies and square wave adsorptive stripping voltammetry of the antidepressant fluoxetine. The electrochemical reduction of fluoxetine was investigated by cyclic, linear sweep, differential pulse and square wave voltammetry using a hanging mercury drop electrode in alkaline buffer solution in water and in a water/acetonitrile mixed solvent. Cyclic voltammograms in aqueous solution showed very strong adsorption of fluoxetine on the electrode with formation of a compact film. The effect of addition of different percentages of acetonitrile on the voltammetric response was evaluated. It is shown that acetonitrile protects the electrode surface, thus preventing the adsorption of fluoxetine as a compact film, although reduction occurs at more negative potentials. Adsorption was used to accumulate the drug onto the electrode surface. The adsorbed species were measured voltammetrically by reduction at $-1.3$ V in an aqueous 0.05 M Ringer buffer, pH 12, 20% acetonitrile v/v. Linear calibration graphs were obtained in the range 0.52–5.2 M. The quantification of fluoxetine in
pharmacological formulations existing in the market was performed using adsorptive square wave cathodic stripping voltammetry and compared with data from UV spectrophotometry.

Berzas Nevado et al. (2000a) reported capillary gas chromatographic method with flame ionization detection for the simultaneous determination of fluoxetine, fluvoxamine, and clomipramine without a prederivatization. Optimal conditions for the quantitative separation were investigated: column head pressure (80 kPa), injector and detector temperatures (260 and 250°C), time and temperature for the splitless step (0.75 min and 60°C), size of sample (2 μL), and oven temperature program, providing analysis times shorter than 10 min.

Berzas Nevado et al. (2000b) developed and validated a capillary zone electrophoresis method for determination of fluoxetine and fluvoxamine in pharmaceutical preparations. A background electrolyte solution consisting of 40 mM borate buffer adjusted to pH 9.3, hydrodynamic injection and 8 kV of separation voltage were used, obtaining in these conditions analysis times lower than 2.5 min. Main aspects of the validation method are examined and discussed. Detection limits of 1.0 mg/l for fluoxetine and fluvoxamine were obtained. The proposed method was applied to nine pharmaceutical preparations with recoveries between 97.75 and 102.95 over their nominal contents.

Berzas Nevado et al. (2002) reported cyclodextrin enhanced spectrofluorimetric determination of fluoxetine in pharmaceuticals and biological fluids. The detection limit, according to the error propagation theory, was 9.6 μg L⁻¹ and the detection limit proposed by Clayton was 15.8 μg L⁻¹. Repeatability and relative standard deviation were also determined according to this theory, with satisfactory results.
El-dawy et al. (2002) reported high pressure liquid chromatographic technique for determination of fluoxetine in the capsule dosage form, human plasma and in biological fluid. Analysis is performed with a reversed phase-C18 column with ultraviolet detection at 228 nm. The isocratic mobile phase (1.5 mL min\(^{-1}\)) consists of acetonitrile and triethylamine buffer (48+52, V/V). A linear calibration model (correlation coefficient 0.9986) was developed using pyridoxine as internal standard. The retention times were 2.10 and 3.20 min for pyridoxine and fluoxetine, respectively. The method was applied for the quantitation of fluoxetine in spiked human plasma samples. The detection limit is 5 µg L\(^{-1}\) and the absorbance varies with fluoxetine concentrations in the range (10–300) µg L\(^{-1}\). The mean % recovery±S.D. was found to be 97.99%±2.39. The proposed method was applied successfully for monitoring of fluoxetine in human plasma after single dose administration of one Prozac® capsule.

Mandrioli et al. (2002) reported two different analytical methods for the quality control of fluoxetine in commercial formulations: a spectrofluorimetric method and a capillary zone electrophoretic (CZE) method. The fluorescence emission values were measured at 293 nm when exciting at 230 nm. The CZE method used an uncoated fused-silica capillary and pH 2.5 phosphate buffer as the background electrolyte. The extraction of fluoxetine from the capsules consisted of a simple one step dissolution with methanol/water, filtration and dilution.

Berzas Nevado et al. (2005) described an easy and fast capillary gas chromatographic FID method for the simultaneous determination of three antidepressants, fluoxetine, fluvoxamine and clomipramine without derivatization step in pharmaceutical formulations. The pharmaceutical preparations subject to
validation were: ‘Prozac’ (capsules), ‘Dumirox’ (tablets) and ‘Anafranil’ (tablets)
containing fluoxetine, fluvoxamine and clomipramine, respectively.

Risley and Bopp (2005) discussed a reversed-phase HPLC method for
determination of fluoxetine in capsule and tablet formulations. The mobile phase was
of 50% acetonitrile/49% water/1% triethylamine (water and triethylamine adjusted to
pH 6 with phosphoric acid). A DuPont Zorbax RX column (250 mm × 4.6 mm i.d.) is
used for the separation. The flow rate is 1.0 mL min⁻¹ and UV detection is at 260 nm.

Risley and Bopp (2005) also reported a gas chromatography (GC) method to
determine fluoxetine in dosage forms, due to the reasonable thermal stability and the
volatility of fluoxetine. The chromatographic method was developed on a Hewlett
Packard model 5710 gas chromatograph. A 15 m x 0.53 μm x 1.5 μm DB-1 Megabore
column (J&W Scientific) is used with an oven temperature of 170 °C. The injection
port temperature is 275°C and the FID detector temperature is 250°C. Helium flow
rate is 25.0 mL min⁻¹, oxygen flow rate is 300 mL min⁻¹, and hydrogen flow rate is 40
mL min⁻¹. This procedure involves extraction from basic solution into chloroform.
The chloroform extract is filtered through Whatman filter paper prior to analysis.

Shamsipur et al. (2007) applied ¹⁹F NMR spectroscopy to the quantitative
determination of fluoxetine enantiomers using different chiral recognition agents in
pharmaceutical formulations. Several parameters affecting the enantio-resolution
including the type and concentration of chiral selector, concentration of fluoxetine and
temperature were studied. The chiral selectors investigated are the cyclic
oligosaccharides α-, β- and γ-cyclodextrin and a diamino derivative of methylated α-
cyclodextrin (DAM-α-CD), linear polysaccharides (maltodextrin with dextrose
equivalents of 4.0–7.0, 13.0–17.0 and 16.5–19.5) and the macrocyclic antibiotic
vancomycin. Among the chiral selectors used, DAM-α-CD turned out to give the best
resolution of the $^{19}$F NMR signals of (R)- and (S)-fluoxetine. The calibration curve was linear for (R)- and (S)-fluoxetine over the range 0.10–1.35 mg mL$^{-1}$, the detection limits (S/N = 3) being 5.9 and 7.5 µg mL$^{-1}$ for the pure solutions of (R)- and (S)-fluoxetine, respectively. The recovery studies performed on pharmaceutical samples ranged from about 90 to 110% with relative standard deviations of <8%.

United States Pharmacopoeia (2007) has given high performance liquid chromatography with UV detection for determination of Fluoxetine hydrochloride in bulk, capsules, oral solution and Ion-pair liquid chromatography for fluoxetine in delayed-release capsule and tablets. HPLC method consists of mixture of triethylamine buffer, tetrahydron and methanol (6:3:1 v/v) mixture as mobile phase and C18 stationary phase with detection at 227 nm. Ion-pair chromatographic method consists of ion-pair solution (mixture of glacial acetic acid and sodium pentanesulfonate) and methanol (33:67 v/v) as mobile phase; C18 stationary phase with detection at 227 nm.

British Pharmacopoeia (2007) has given high performance liquid chromatography method for determination of fluoxetine in bulk and ion-pair liquid chromatography for fluoxetine in capsule. The specifications for both the methods are similar to the methods given in United States Pharmacopoeia.

Piperaki et al. (1995) reported a systematic approach to enantiomeric separations in capillary electrophoresis (CE) and liquid chromatography (LC) with chiral mobile phase additives (MPA) or a chiral stationary phase (CSP) in the study of fluoxetine and norfluoxetine with cyclodextrins as chiral selectors. Binding constants and selectivities are determined under the same experimental conditions (mobile phase, buffer composition). The role of the buffer salt was investigated by comparison of binding constants obtained with triethylammonium and sodium acetate buffers.
Profiles of Drug and Co-crystal Formers

Investigation of the effects of derivatisation of the selector in CE and LC with MPA demonstrates the appropriate choice of cyclodextrin type for use in LC. By studying the influence of organic modifier content on separation parameters, CE can predict a useful solvent working range for a CSP.

3.1.3.2 Biological fluid

Eap et al. (1996) reported a gas chromatographic-mass spectrometric method for the simultaneous determination of the plasma concentrations of fluvoxamine and of the Enantiomers of fluoxetine and norfluoxetine after derivatization with the chiral reagent, (S)-(−)-N-trifluoroacetylprolyl chloride.

Alvarez et al. (1998) developed a high performance liquid chromatography (HPLC) method using only 0.1 mL of serum or homogenate from brain areas for determination of fluoxetine and its metabolite, norfluoxetine with ultraviolet detection at 227 nm. The small volume of sample required in this method allows studies in small animals, such as mouse. The method provides recoveries of up to 90% for both compounds. The limit of detection was 5.0 ng mL⁻¹. No interferences were found with tricyclic antidepressant drugs and benzodiazepines, which allows this method to be used in clinical studies.

Raggi et al. (1998) developed an HPLC method with fluorescence detection for the monitoring of fluoxetine plasma levels. For the analytical separation a reversed phase C8 column was used, while the mobile phase was a mixture of acetonitrile and water containing perchloric acid and tetramethyl ammonium perchlorate.

Berzas Nevado et al. (2002) proposed a micellar electrokinetic capillary chromatography (MEKC) for determining fluoxetine and its metabolite (norfloxacin). Optimal conditions for the quantitative separation were investigated. A background
electrolyte solution consisting of 5 mM phosphate buffer adjusted to pH 12.3 and 40 mM of 1-decanesulfonic acid sodium salt (DSS), hydrodynamic injection and 25 kV of separation voltage were used. Detection limits of 0.2 mg L\(^{-1}\) for fluoxetine and norfluoxetine were obtained. The developed method was applied to determine fluoxetine and its metabolite in human serum and urine. The samples were purified and enriched by means of extraction-preconcentration step with a preconditioned C\(_{18}\) cartridge and eluting the compounds with methanol.

Rodríguez Flores et al. (2005) reported nonaqueous capillary electrophoresis (NACE) method to measure fluoxetine and its main metabolite norfluoxetine. Optimum separation of fluoxetine and norfluoxetine, by measuring at 230 nm, was obtained on a 60cm×75μm capillary using a nonaqueous solution system of 7:3 methanol-acetonitrile containing 15mM ammonium acetate, capillary temperature and voltage 25 °C and 25 kV, respectively and hydrodynamic injection. Paroxetine was used as internal standard. Detection limits of 10 μg L\(^{-1}\) were obtained for fluoxetine and its metabolite. This method was used to determine fluoxetine and its main metabolite at clinically relevant levels in human urine. Before NACE determination, the samples were purified and enriched by means of extraction-preconcentration step with a preconditioned C\(_{18}\) cartridge and eluting the compounds with methanol.

Rodrigues Chaves et al. (2009) reported solid phase microextraction and LC-UV method for analysis of new-generation antidepressants (mirtazapine, citalopram, paroxetine, duloxetine, fluoxetine, and sertraline) in plasma sample. Poly(pyrrole) (PPY) coating was prepared on a stainless-steel (SS) wire for solid-phase microextraction (SPME) by electrochemical deposition (cyclic voltammetric). The effect of electrolyte solution (lithium perchlorate or tetrabutylammonium perchlorate) and the number of cycles (50, 100 or 200) applied during the polymerization process
on the SPME performance was evaluated. Important factors in the optimization of SPME efficiency such as extraction time, temperature, pH, influence of plasma proteins on sorption mechanisms, and desorption conditions are discussed. The SPME–PPY/LC method showed to be linear in concentrations ranging from the limit of quantification (LOQ) to 1200 ng mL\(^{-1}\). The LOQ values range from 16 to 25 ng mL\(^{-1}\). The inter-day precision of the SPME–PPY/LC method presented coefficient of variation (CV) lower than 15%.

Saber (2009) reported an instrumental setup including on-line solid phased extraction coupled to capillary liquid chromatography-electrospray ionization-mass spectrometry (SPE-capLC-ESI-MS) to improve the sensitivity for quantification of fluoxetine hydrochloride in human plasma. Prior to injection, 0.5 mL of plasma spiked with metronidazole (internal standard) was mixed with ammonium formate buffer for effective chloroform liquid–liquid extraction. The method was validated in the range 5–60 ng mL\(^{-1}\) fluoxetine, yielding a correlation coefficient of 0.999 (\(r^2\)). The within-assay and between-assay precisions were between (8.5 and 11%) and (6.6 and 7.5%), respectively. The method was used to determine the amount of fluoxetine in a healthy male 14 h after an intake of one capsule of the antidepressant and anorectic Flutin\(^\text{®}\), which contains 20 mg fluoxetine per each capsule. Fluoxetine was detected, and the concentration was calculated to 9.0 ng mL\(^{-1}\) plasma. In the preliminary experiments, conventional LC–UV instrumentation was employed. However, it was found that employing a capillary column with an inner diameter of (0.3 mm I.D. \(\times\) 50 mm, Zorbax C\(_{18}\)) increased the sensitivity by a factor of ~100, when injecting the same mass of analyte. Incorporating an easily automated C\(_{18}\) reversed phase column switching system with SPE (1.0 mm I.D. \(\times\) 5.0 mm, 5 \(\mu\)m)
made it possible to inject up to 100 µL of solution, and the total analysis time was 5.5 min.

3.1.4 Stability (Risley and Bopp, 2005)

3.1.4.1 Stability in Bulk

Floxetine hydrochloride is a very stable molecule under normal storage conditions. The only known degradation products are α-[2(methylamino)ethyl]benzene methanol and p-trifluoromethylphenol. They are formed under acidic stress conditions (3 mg fluoxetine per 1 mL 0.1 N HCl refluxed for 48 hours) or when irradiated for five hours with a mercury arc lamp.

3.1.4.2 Stability in Dosage Form

Fluoxetine hydrochloride is marketed as 20 mg (fluoxetine base) capsules. The active ingredient is stable in the starch formulated dosage form stored at 25°C for five years and 40°C/75% relative humidity for two years. Fluoxetine hydrochloride is stable in a mint syrup formulation (20 mg base/5 mL syrup solution) stored at 25°C for six months. Only a slight increase in related substances was noted at 40°C storage for three months.

3.1.4.3 Stability in Solution

Fluoxetine hydrochloride is stable in water even when stored at 65°C or exposed to ultraviolet light for eight weeks. Fluoxetine hydrochloride is also stable in commercially available beverages (Gatorade®, Spea’s Farm® Apple juice, and Ocean-Spray Cran-Grape® Drink) stored at 25°C for two weeks.

3.1.5 Biopharmaceutical profile

3.1.5.1 Pharmacokinetics

In human volunteers, fluoxetine is readily and completely absorbed from the gastrointestinal tract with peak serum levels occurring 6-8 hours after oral dosing with
capsules (Bergstrom et al., 1984; Lemberger et al., 1985). Fluoxetine and its major metabolite norfluoxetine are distributed in the tissues, predominantly the lung, and gradually released. The elimination half life of fluoxetine is 2-3 days and that of norfluoxetine is 7-9 days (Lemberger et al., 1985). Maximal central nervous system efficacy has been shown to be 8-10 hours post dosing (Saletu and Grunberger, 1985).

3.1.5.2 Drug Metabolism

The main metabolite of fluoxetine is norfluoxetine, an active metabolite with similar physiological activity as its parent compound. Metabolism occurs in the liver by N-demethylation (Lemberger et al., 1985). Studies in animals have shown that fluoxetine is also metabolized into p-trifluoromethylphenol by O-dealkylation. Other known metabolites include glucuronides of both fluoxetine and norfluoxetine.

After oral dosing with C-14 labeled drug, 60% of the activity was recovered in the urine over a 5 week period (2.5-5.0% was recovered as unchanged drug, 10% being norfluoxetine, 5.2% fluoxetine glucuronide and 9.5% norfluoxetine glucuronide). An additional 16% of the radio-labeled material was recovered in facesd (Lemberger et al., 1985).

3.1.5.3 Toxicity

Acute toxicity of fluoxetine was determined in mice, rats, dogs and monkeys. LD₅₀ values are listed below in Table 3.4.

Phospholipids were increased in some tissues of rats and dogs, however, phospholipidosis was found to be reversible after cessation of fluoxetine dosing. In rodents, elevated doses of fluoxetine resulted in the following toxicity signs: increased salivation, tremors, ataxia, leg weakness, and chronic convulsions. In dogs, vomiting, mydriasis, tremors and anorexia were observed. In monkeys, vomiting was the only indication of toxicity. Toxicity studies in animals administered chronic dosing of
fluoxetine indicate that the drug was not a mutagen, carcinogen or teratogen and id not impair reproductive capabilities (Risley and Bopp, 2005).

Table 3.4. $LD_{50}$ Data for Fluoxetine Hydrochloride

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>$LD_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Oral</td>
<td>248</td>
</tr>
<tr>
<td>Mouse</td>
<td>I.V.</td>
<td>45</td>
</tr>
<tr>
<td>Rat</td>
<td>Oral</td>
<td>466</td>
</tr>
<tr>
<td>Rat</td>
<td>I.V.</td>
<td>35</td>
</tr>
<tr>
<td>Dog</td>
<td>Oral</td>
<td>ND*</td>
</tr>
<tr>
<td>Monkey</td>
<td>Oral</td>
<td>ND*</td>
</tr>
</tbody>
</table>

* ND Determined (Dog $LD_0 > 100$; Monkey $LD_0 > 50$)

Side effects noted after dosing with fluoxetine in human volunteers include: nausea, nervousness, insomnia, headache, tremor, anxiety and drowsiness. Uncommon side effects include: psychosis, hallucinations, ataxia, dizziness, sensation disturbances and asthenia.

3.1.6 Modifications of FH molecule

Ah et al. (2005) studied proton magnetic resonance spectra in $D_2O$ of mixtures of fluoxetine hydrochloride (guest) with $\beta$-cyclodextrin (host) and revealed the existence of two different equilibria for 1:1 inclusion complexes in which $-CF3$ substituted ring of the guest is more tightly held by the host cavity. The structures of the two complexes have been proposed which are supported by 2DROESY spectral data. The dissociation constant was also determined.

De Sousa et al. (2008) investigated the inclusion complex formed between $\beta$-cyclodextrin ($\beta$CD) and fluoxetine (FLU) by experimental and computational
methods. From Horizontal Attenuated Total Reflectance (HATR), it was possible to verify a strong modification in the vibrational modes of βCD and FLU, indicating interactions between them. The Nuclear Magnetic Resonance (NMR) experiments confirm these interactions through the change in chemical shifts in $^1$H spectra, reduction in longitudinal relaxation times values, and the Nuclear Overhauser Effect confirm the inclusion of aromatic rings of FLU into the βCD. A high equilibrium constant ($K\approx 6921\pm 316$) and the stoichiometry, 1:1, were obtained by Isothermal Titration Calorimetry (ITC) experiments.

3.2 Maleic acid (MA)

Structure:

![Maleic acid structure](image)

IUPAC name: Maleic acid; (Z)-Butenedioic acid;

Synonym: Toxilic acid; cis-1,2-ethylenedicarboxylic acid.

Molecular formula: $C_4H_4O_4$

Molecular weight: 116.07 g/mol

Appearance: white solid

Density: 1.59 g/cm$^3$, solid

Melting point: 138-139 °C (White crystal from water); 130-131°C (from alcohol and benzene); it is converted in part into the much higher-melting fumaric acid (mp 287°) when heated to a temperature slightly above the melting point.

Solubility: Freely soluble in water (78 g/100 mL (25 °C)) or alcohol; Soluble in acetone, glacial acetic acid; slightly soluble in ether; practically insoluble in benzene.
Acidity ($pK_a$): $pK_{a1} = 1.97, pK_{a2} = 6.07$

Related compounds:

- Related dicarboxylic acids: Fumaric acid, Succinic acid
- Related compounds: Maleic anhydride, Maleimide

Crystal structure: Shahat (1952) reported crystal and molecular structure of maleic acid.

Caution: Strong irritant.

Uses: In manufacturing of artificial resins; to retard rancidity of fats and oils in 1:10,000 (these are said to keep 3 times longer than those without the acid); dyeing and finishing wool, cotton, and silk; preparing the maleate salts of antihistamines and similar drugs.

3.3 Glutaric acid (GA)

Structure:

![Glutaric acid structure](image)

IUPAC name: Pentanedioic acid; 1,3-propanedicarboxylic acid

Molecular formula: $C_5H_8O_4$

Molecular weight: 132.11 g/mol

Appearance: Large monoclinic prism

Density: 1.429 g/cm³, solid

Melting point: 97.5-98 °C

Boiling point: 302-304 °C (with very slight decomposition)
**Solubility:** Solubility in water (g/l): at $0^\circ$: 429; at $20^\circ$: 639; at $50^\circ$: 957; at $65^\circ$: 1118.

Freely soluble in absolute alcohol, ether; soluble in benzene, chloroform; slightly soluble in petroleum ether.

**Acidity ($pK_a$):** $pK_{a1} = 4.34$, $pK_{a2} = 5.22$

**Uses:** Glutaric acid itself has been used in the production of polymers such as polyester polyols, polyamides. The odd number of carbon atoms (i.e. 5) is useful in decreasing polymer elasticity.

### 3.4 L-tartaric acid (LTA)

**Structure:**

![Structure of L-tartaric acid](image)

**IUPAC name:** (2R,3R)-2,3-Dihydroxybutanedioic acid

**Synonym:** Ordinary tartaric acid, natural tartaric acid, d-tartaric acid, (+)-tartaric acid, dextrotartaric acid, L-2,3-dihydroxybutanedioic acid, d-α,β-dihydroxysuccinic acid.

**Molecular formula:** $C_4H_6O_6$

**Molecular weight:** 150.09 g/mol

**Appearance:** Monochinic sphenoidal prisms

**Density:** $1.76$ g/cm$^3$, solid

$[\alpha]_{20}^\circ = +12.0^\circ$

**Melting point:** 168-170 $^\circ$C

**Solubility:** Freely soluble in water; one gram dissolves in 0.75 mL water at room temperature, in 0.5 mL boiling water, 1.7 mL methanol, 3 mL ethanol, 10.5 mL propanol, 250 mL ether. Insoluble in chloroform.
Acidity (pKₐ): pKₐ₁ = 2.98, pKₐ₂ = 4.34

Uses: In the soft drink industry, confectionery products, bakery products, gelatin desserts, as an acidulant. In photography, tanning, ceramics, manufacturing tartrates. The common commercial esters are the diethyl and dibutyl derivatives used for lacquers and in textile printing. As pharmaceutical aid (buffering agent).

3.5 DL-tartaric acid (DLTA)

Structure:

![Structure of DL-tartaric acid]

IUPAC name: 2,3-Dihydroxybutanedioic acid

Synonym: Racemic tartaric acid; racemic acid; dl-tartaric acid; resolvable tartaric acid; uric acid; para tartaric acid

Molecular formula: C₄H₆O₆

Molecular weight: 150.09 g/mol

Appearance: triclinic pmacoidal crystals

Melting point: 206 °C

Solubility: Less soluble in water than L-tartaric acid. pH of 0.1M aq soln: 2.0. Solubility in alcohol (g/100g): 2.006 at 0°; 3.153 at 15°; 5.01 at 25°; 6.299 at 40°. Solubility in ether about 1%.

Acidity (pKₐ): pKₐ₁ = 2.96, pKₐ₂ = 4.24