Chapter 3

Profile of Drugs

3.1 FLUTAMIDE

3.1.1 Nomenclature

3.1.1.1 Chemical Names

- 2-Methyl-N- (4-nitro-3- (trifluoromethyl) phenyl) propanamide
- α-α-α-trifluoro-2-methyl-4'-nitro-m-propionotoluidide
- 4'-nitro-3'-trifluoromethylisobutyranilide

3.1.1.2 Nonproprietary Names

Flutamide

3.1.1.3 Proprietary Names

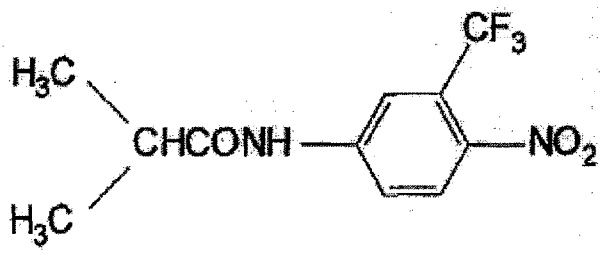
Drogenil, Eulexin, Euflex, Flucinom, Flugeril, Fugerel, Sebatrol

3.1.2 Formulae

3.1.2.1 Empirical

$Cl\ {}_1H_{11}F_3N_2O_3$

3.1.2.2 Structural



3.1.3 Molecular Weight

276.22

3.1.4 CAS Number

13311-84-7

3.1.5 Appearance

Flutamide is a pale yellow crystalline powder (1).

3.1.6 Uses and Applications (2, 3, 4)

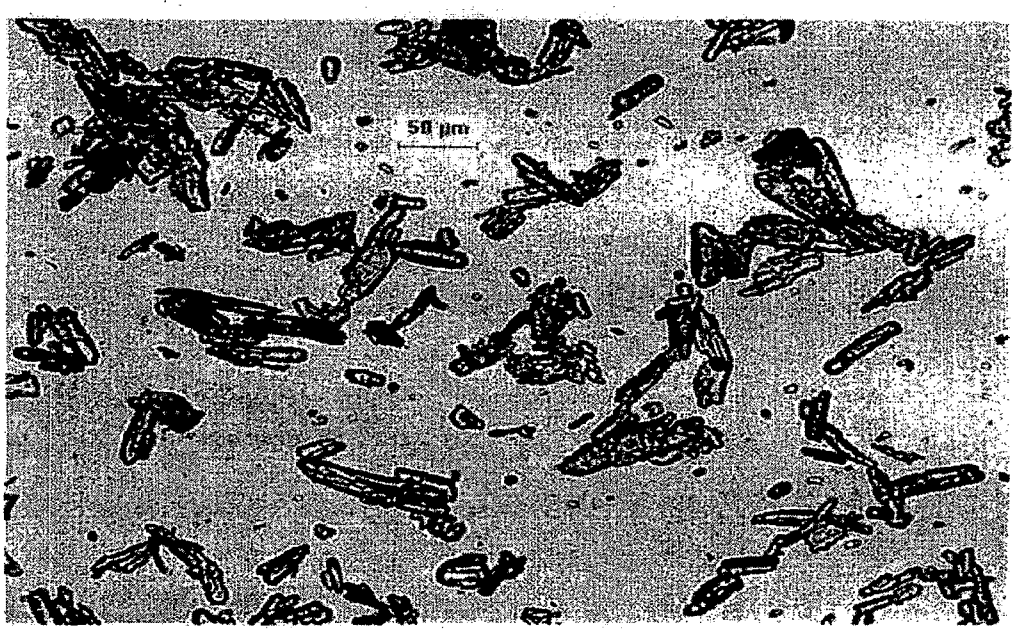
Flutamide is an acetanilide, nonsteroidal, orally active anti-androgen. It exerts its anti-androgenic action by inhibiting androgen uptake and/or by inhibiting nuclear binding of androgen in target tissues. Prostatic carcinoma is known to be androgen-sensitive and responds to treatment that counteracts the effect of androgen and/or removes the source of androgen *(e.g.,* castration). It is indicated for use in combination with LHRH agonists for the management of locally confined Stage B_2-C and Stage D_2 metastatic carcinoma of the prostate.

3.1.7 Physical Properties

3.1.7.1 Particle Morphology

As the photomicrograph in Figure 3.1 indicates, flutamide particles have an elongated (needlelike) shape, and the individual particle size is in the 25 μm range.

Figure 3.1 Photomicrograph of flutamide particles



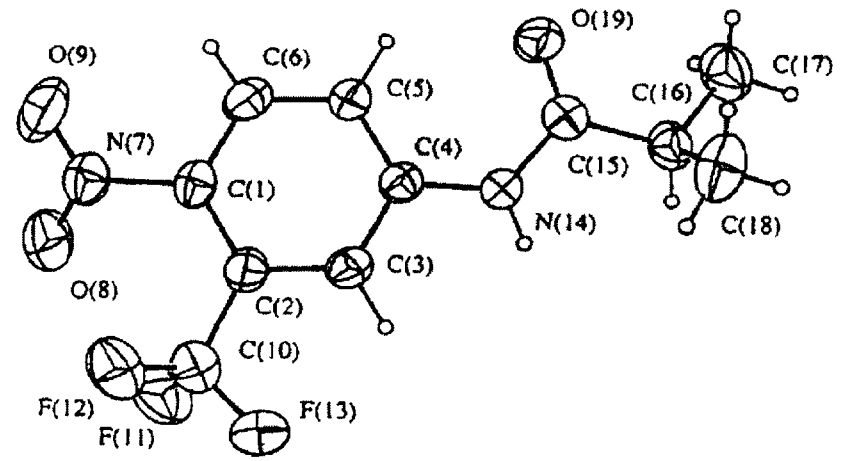
3.1.7.2 Crystallographic Properties

3.1.7.2.1 Single Crystal Structure

The single crystal structure determination of flutamide has been reported (8). The crystal structure reported is shown in Figure 3.2, and the stereo view of the crystal packing is shown in Figure 3.4. The x-ray powder diffraction pattern of flutamide

was obtained using a Philips PW1710 x-ray powder diffraction system as reported (9). The powder pattern obtained is presented in Figure 3.3.

Figure 3.2: Structure of flutamide elucidated from single crystal x-ray data, (8)



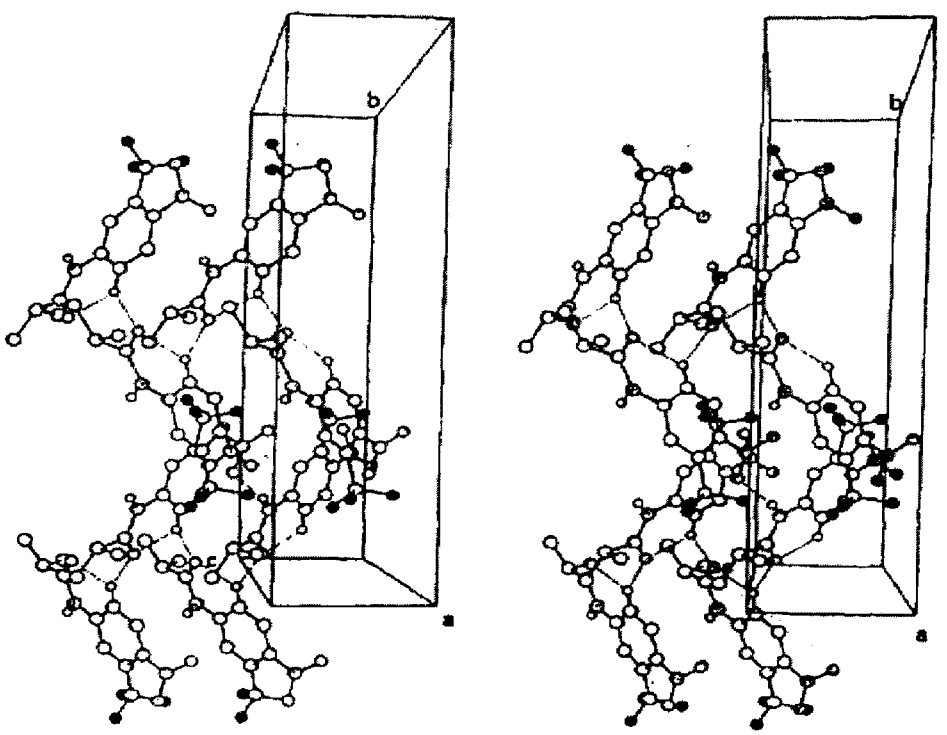


Figure 3.4: Stereo view of flutamide molecules in the unit cell.

Dotted lines represent hydrogen bonds (8)

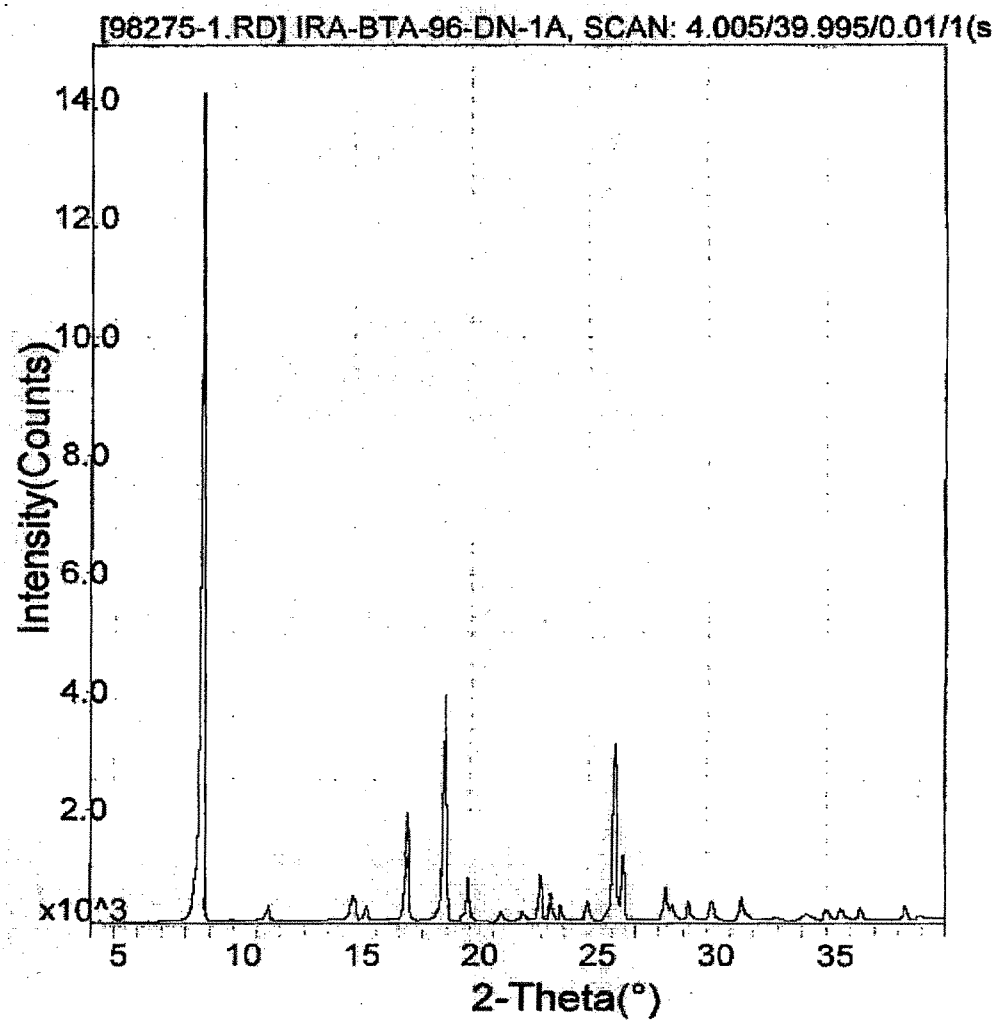


Figure 3.3. X-ray powder diffraction pattern of flutamide.

3.1.7.2.2 Polymorphism

A single polymorph of flutamide is consistently produced. It was reported (9) that the amorphous state (Tg = 272°K) might be obtained by quenching the melt. DSC studies indicate the existence of another polymorph (metastable) form, which is transformed into the stable form at room temperature, but this metastable polymorph was not isolated.

3.1.7.3 Thermal Methods of analysis

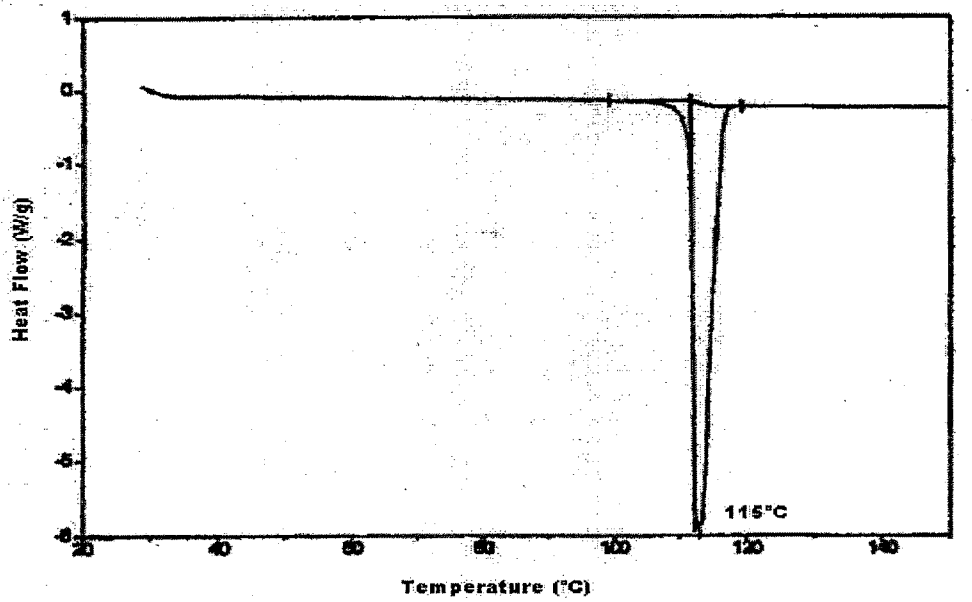
3.1.7.3.1 Melting Behavior

The melting range of flutamide has been reported as 111.5°C to 112.5°C (10).

3.1.7.3.2 Differential Scanning Calorimetry

Differential scanning calorimetry analysis was performed using a TA Instruments model 2920 system as reported (9). The DSC thermogram is shown in Figure 3.5, and exhibits an endothermic transition with an onset temperature of 111.5°C. This feature is assigned to the fusion transition, which is consistent with the above reported value for melting range.

Figure 3.5: Differential scanning calorimetry thermogram of flutamide.



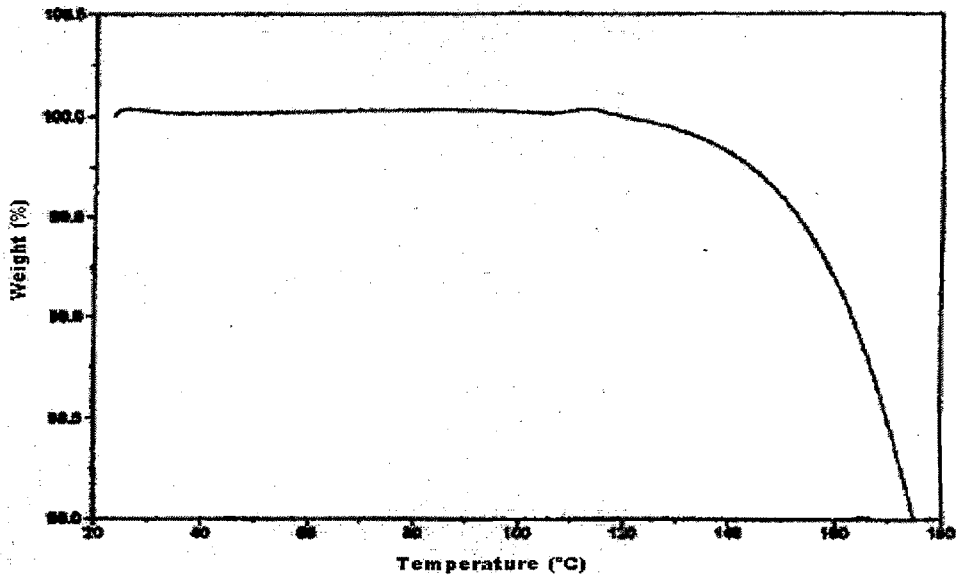
3.1.7.3.3 Thermogravimetric Analysis

A thermogravimetric analysis study of flutamide is shown in Figure 3.6, the TGA thermogram exhibits no weight loss attributable to residual solvents, but does begin to exhibit a significant weight loss starting at approximately 120°C. This is due to the thermal decomposition of the compound, and occurs after the onset of DSC melting at 111.5°C.

3.1.7.4 Hygroscopicity

Moisture uptake for this drug substance is negligible. Weight changes that are less than 0.5% have been reported after exposure over the relative humidity range of 0-95%. Using a moisture balance (VTI) instrument, equilibrium weight gain was recorded over the range 5% to 95% relative humidity at 25°C for two complete cycles and found that the flutamide solid state has a low affinity for water. Based on these observations, flutamide is deduced not to be hygroscopic.

Figure 3.6: Thermogravimetric analysis thermogram of flutamide



3.1.7.5 Solubility Characteristics

Flutamide is insoluble in water, but freely soluble in polar organic solvents such as acetone, alcohol, dimethyl formamide, dimethyl sulphoxide, ethyl acetate, methanol, and polyethylene glycol 400. It is also soluble in chloroform, diethyl ether, and propylene glycol (17).

3.1.7.6 Partition Coefficients

The partition coefficient of flutamide was determined in four different biphasic systems consisting of l-octanol and an aqueous phase, and the results were obtained as mentioned in Table 3.1.

Table 3.1: Partition coefficient of flutamide in different mixtures

Biphasic System	Log P (oil/aqueous)
1-octanol/0.1 N HCl	> 3.4
1-octanol/0.05M phosphate buffer (pH 7)	> 3.4
1-octanol/deionized water	> 3.4
1-octanol/0.1N NaOH	> 2.9

These results show that due to the nonpolar nature and the low solubility of flutamide in aqueous media, it partitions significantly more into the 1-octanol phase than the aqueous phase.

3.1.7.7 Ionization Constants

Using the ACD program (Advanced Chemistry Development, Toronto, Canada), ionization constants (pKa) of 13.1 and -6.4 were calculated for the amide group. No experimental determinations in water have been reported. An apparent pKa in a hydro-alcoholic solution (40 mM Britton-Robinson buffer / ethanol, 80/20) has been determined (11). The pKa for the amide group was determined by a polarographic method to be 4.75, and 4.83 when determined using UV-VIS Spectrophotometry.

3.1.7.8 Spectroscopy

3.1.7.8.1 UV/VIS Spectroscopy

The ultraviolet spectrum of flutamide in a neutral solution (7:3 v/v methanol / water) was reported using a Shimadzu spectrophotometer model 1601. The spectrum is shown in Figure 3.7, and the wavelengths of maximum absorption and the molar absorptivities at each wavelength were determined to be as follows:

Figure 3.7: UV spectrum of flutamide in methanol: water mixture

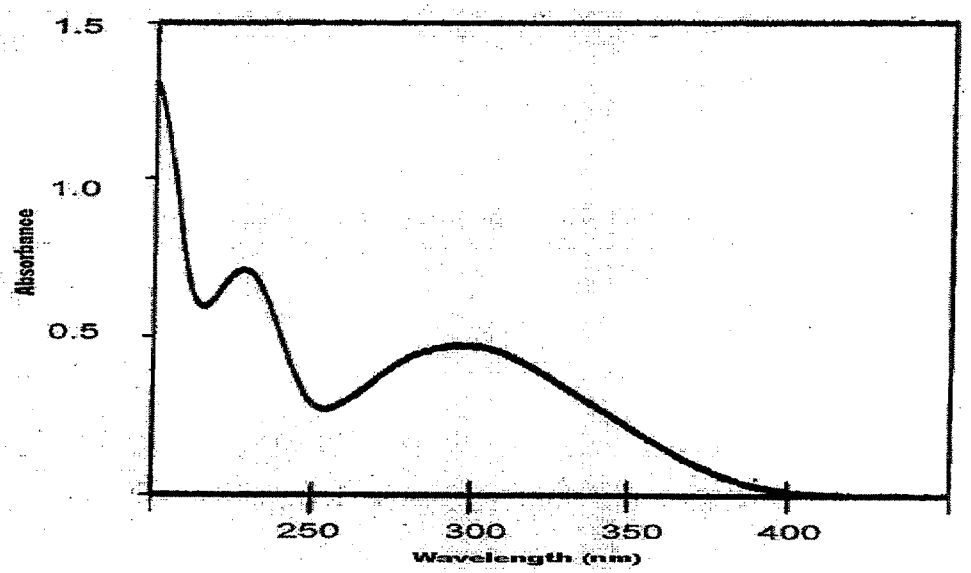


Table 3.2: Molar Absorptivity values of flutamide at various wavelengths

Wavelength Maximum (nm)	Molar Absorptivity (L/mole'cm)
296	1.09×10^4
228	7.24×10^3

3.1.7.8.2 Vibrational Spectroscopy

The infrared spectrum of a mineral oil mull of flutamide was obtained using a Mattson Model 6021 infrared spectrometer. The spectrum shown in Figure 3.8 is consistent with the structure of flutamide, and the infrared assignments are presented in Table 3.3.

Table 3.3: Assignments for the Vibrational Transitions of Flutamide

Energy (cm-1)	Assignment
3356 (s)	N-H stretch
1717 (m)	C--O stretch
1610, 1597 (m)	C=C stretch
1540 (m)	NO_2 stretch
1344 (s)	CF_3
1315 (m)	NO_2
1243 (m)	C-N (amide)
1136 (s)	CF_3
903,862 (m)	1,2,4-trisubstituted benzene
754 (m)	C-N (C-NO_2)

Intensities: m – medium, s = strong

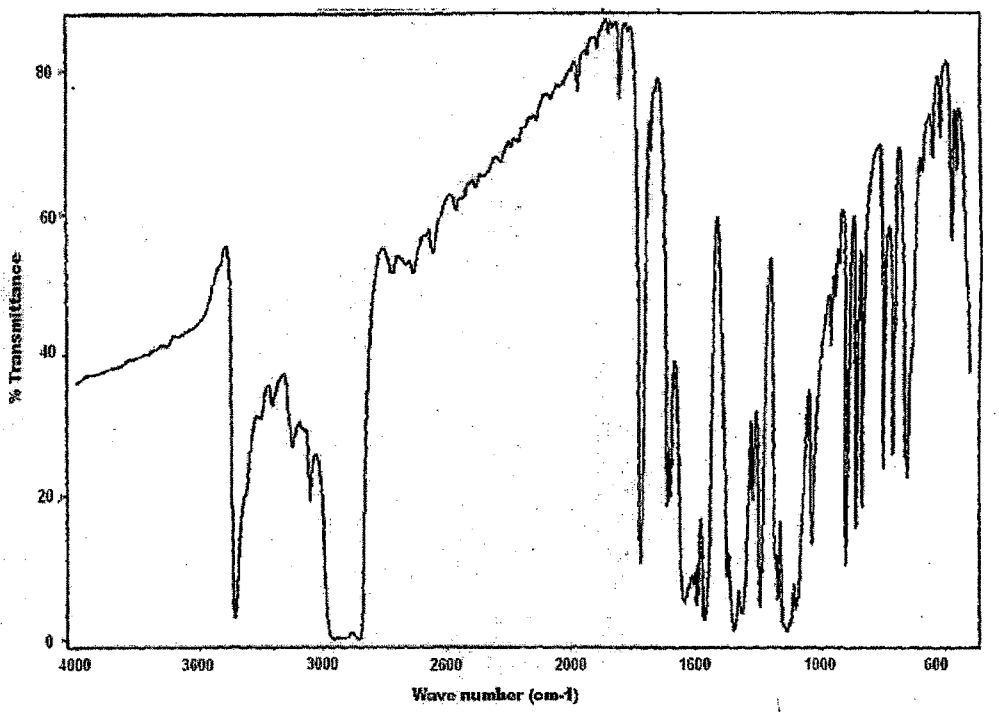


Figure 3.8: IR spectrum of flutamide (mineral oil dispersion).

3.1.8. Methods of Analysis

3.1.8.1 Compendial Tests

The compendial tests for flutamide USP are given in the drug substance monograph (12). The specified testing consists of compound identification, melting range, loss on drying, residue on ignition, heavy metals, chromatographic purity and assay.

3.1.8.1.1 Identification

The identification of flutamide can be made on the basis of the equivalence of its infrared spectrum (obtained in nujol) with that of a standard spectrum. Alternatively the identification can be made on the basis of matching the retention time of the major peak in the HPLC chromatogram of the sample with the major peak in the USP Reference Standard using the USP assay procedure.

3.1.8.1.2 Melting Range

The melting range is determined according to USP general test <741>, and should be between 110 ° and 114°C, with a range of not more than 2°C.

3.1.8.1.3 Loss on Drying

The loss on drying is determined according to USP general test <731 >. The sample is dried at 60°C for 3 hours under vacuum, with a weight loss specification of not more than 1.0%.

3.1.8.1.4 Residue on Ignition

The residue on ignition is determined according to USP general test <281>, and should be not more than 0.1%.

3.1.8.1.5 Heavy Metals

The heavy metals content is determined according to USP general test <231>, Method II, and should be not more than 10 ppm.

3.1.8.1.6 Chromatographic Purity

To perform the Chromatographic purity determination, the following solutions are prepared: Mobile Phase: Prepare a 1:1 v/v filtered and degassed mixture of deionized water and acetonitrile. System Suitability Solution: Prepare a solution containing about 0.004 mg/mL of USP o-flutamide reference standard (RS) and 2 mg/ml of USP flutamide RS in mobile phase. Test Solution: Dissolve 2 mg/ml of flutamide in acetonitrile. Chromatographic system: Use a 4.6 mm x 25-cm column that contains packing L1 (octadecyl silane chemically bonded to porous silica or ceramic microparticles, 3 to 10 pm in diameter). The mobile phase is eluted at a flow rate of 0.5 mL/minute, and detection is made on the basis of the UV absorbance at 240 nm. The injection volume is 20 pl, and the run time continues until the o-flutamide peaks elutes (RRT = 1.4). The System suitability solution must have a resolution of not less than 2.0, and the relative standard deviation for replicate injections of o-flutamide must be not more than 6.0%. The test solution is injected and peak responses are recorded. The specification is not more than 0.5% total impurities.

3.1.8.1.7 Assay

To perform the assay determination, the following solutions are prepared: mobile phase: prepare a 1:1 v/v filtered and degassed mixture of deionized water and acetonitrile. Standard preparation: prepare a 0.5 mg/mL solution of USP flutamide RS in Mobile Phase. System suitability solution: prepare a solution containing 0.4 mg/ml of USP o-flutamide RS and 0.5 mg/ml of USP flutamide RS in mobile phase. Assay preparation: dissolve 50 mg of flutamide, previously dried, in a 100 ml volumetric flask with mobile phase and dilute to volume.

3.1.8.1.8 Chromatographic System

The chromatography system used is the same as for the chromatographic purity procedure. The system suitability solution must have a resolution between o-flutamide and flutamide of not less than 2.0. The standard preparation must have a relative standard deviation for replicate injections of not more than 1.0%. The assay preparation is injected and the peak responses are recorded. The assay value must be between 98.0 to 101.0 percent on a dried basis.

3.1.8.2 Elemental Analysis

Flutamide was analyzed for carbon, hydrogen, nitrogen, and fluorine (17). The carbon, hydrogen, and nitrogen analyses were performed on a Perkin- Elmer Model

240 instrument. Fluorine was determined using an Orion specific ion electrode, and oxygen was calculated by difference. The results obtained are mentioned in Table 2.18.

Table 3.4: % of element present in flutamide

Elements	Theoretical (%)	Found (%)
C	47.83	47.87
H	4.01	3.75
N	10.14	10.06
F	20.63	20.83
O	17.38	17.49

3.1.8.3 Electrochemical Analysis

Differential pulse polarographic assay methods were described (11,13,14) for the quantitative determination of flutamide drug substance and in its formulated products. These methods are based on the reduction of flutamide in aqueous and mixed media to a nitro radical anion, and the corresponding linear relation between the peak current and the flutamide concentration.

3.1.8.4 Spectrophotometric Methods of Analysis

When flutamide is dissolved in hydrochloric acid, color formation occurs with an absorption maximum of 380 nm. This reaction was used to develop a simple and sensitive method for the assay determination of flutamide drug substance, and in formulated product (15). The method was linear over the range 2.5 - 15.0 μg/ml, and the chromophore remained stable for 1 hour in solution. Flutamide tablets may be assayed using UV Spectrophotometry at 304 um (11). Tablets are sonicated and diluted in ethanol. After centrifugation, an aliquot is diluted in 20:80 ethanol / 40 mM pH 8.0 Britton Robinson buffer.

3.1.8.5 Chromatographic Methods of Analysis

3.1.8.5.1 Thin Layer Chromatography

A report (16) summarized the performance characteristics for an over- pressured thin-layer chromatographic analysis (OPTLC) method for flutamide and three related compounds. The report investigated the relationship between various flow rates and the effect on linearity of the calibration graph, the limits of detection and quantitation, and the precision of the data. An additional method for the analysis of

flutamide and its related compounds is known (17). The main characteristics of both methods are mentioned in Table 3.5:

Table 3.5: TLC data of flutamide

Stationary Phase	Eluent	Detection Wavelength (nm)	Sample Concentration (mg/ml)	Reference
Silica gel 60F254	Chloroform	237	Flutamide (1%) and impurities (10^{-4} to 4 x 10^{-2}) in chloroform	16
Silica gel GF	Chloroform / Methylene chloride (90:15)	254	Flutamide (20 mg/mL) in methanol	17

3.1.8.5.2 Gas Chromatography

Table 3.6: GC parameters for flutamide estimation

Instrument	GC with FID and integrator
Column	10% OV-1 on Chromosorb G (AW) treated with DMCS (3 ft x 2 mm id) 80- 100 mesh
Carrier Gas	Nitrogen
Flow Rate	40 cm^3/minute
Temperatures:	
Oven	225°C
Injector	275°C
Detector	280°C
Flutamide Standard Stock	5 mg/cm^3 in chloroform (retention time approximately 2 minutes)
Internal Standard	Chlorphenirarnine maleate (retention time - 3.5 minutes)
Stock solution	10 mg/cm^3 in chloroform
Linearity of Detector Response	1.5-3.0 mg/cm^3

A gas chromatographic method for the assay of flutamide in tablets has been published (19), and the defining characteristics of this method are mentioned in Table 3.6.

3.1.8.5.3 High Performance Liquid Chromatography

In addition to the compendial HPLC methods for purity and assay, an additional method for assay of flutamide drug substance has been developed (17). The critical test parameters can be summarized in Table 3.7.

A recent publication (11) described the following method for assay of flutamide and its impurities in tablets as mentioned in Table 3.8:

Table 3.7: HPLC parameters for flutamide estimation

Column:	300 x 4 mm Bondapak C18 column
Flow rate:	1 mL/minute
Detection:	UV absorbance at 254 um
Mobile phase:	7:4 v/v methanol / 50 mM potassium phosphate
Injection volume:	10 µL
Sample solution:	0.16 mg/mL of flutamide containing 0.09 mg/mL testosterone (internal standard) dissolved in methanol
Flutamide retention time:	12 minutes

Table 3.8: HPLC parameters for flutamide estimation in recent method

Column:	150 x 3.9 mm µBondapak/µPorasil C18 column (operated at 35°C)
Flow rate:	1 mL/minute
Detection:	UV absorbance at 302 nm
Mobile phase:	1:1 v/v methanol / 50 mM phosphate buffer (pH= 3.0)
Injection volume:	20 µL
Sample solution:	1 tablet was sonicated in ethanol and diluted with mobile phase
Retention times:	Flutamide (10.0 minutes), 3-trifluoromethyl-4-nitroaniline (4.0 minutes)

3.1.8.6 Determination in Body Fluids and Tissues

A number of methods have been reported for the analysis of flutamide in body fluids and tissues. One method for the determination of flutamide in dog plasma utilizes mid-bore chromatography (20). Several advantages were outlined; including reduced mobile phase and sample volumes, no formal extraction process, and adequate accuracy, precision and recovery without the use of an internal standard. This method can also be modified for the analysis of human plasma by simply changing the composition of the mobile phase. A reverse phase HPLC method was described to measure levels of flutamide in rats (21). The method used a methyl testosterone internal standard, and was described as being sensitive and precise. Another method reported is an HPLC method with UV detection for the analysis of flutamide, 2 hydroxy-flutamide, and trifluoromethyl- nitroaniline in human plasma (22). An HPLC method with photodiode array detection and gradient elution was developed

for a class-independent drug screen (23), an HPLC method for the plasma analysis of flutamide (24), and two GC methods for the analysis of flutamide and its metabolites in human plasma (25, 26).

3.1.9. Stability

3.1.9.1 Solid-State Stability

Flutamide shows no loss of potency in the solid state when stored in sealed amber glass bottles at room temperature for up to five years (17).

3.1.9.2 Solution-Phase Stability

In aqueous alcoholic solutions, flutamide exhibits maximum stability between pH 3 and 8. Under more acidic or alkaline conditions, the major degradation product observed represents hydrolysis of the amide to form 4-nitro-trifluoromethyl-aniline. Ethanolic solutions (control) showed no degradation for up to 2 weeks when stored at refrigeration and RT, and about 1% degradation at 45°C. Methanolic solutions (0.4 mg./ml) exposed to 500 foot-candle fluorescent light showed no observable degradation over the 8 hour time period studied.

3.1.10 Clinical pharmacology (18)

3.1.10.1 General

In animal studies, flutamide demonstrates potent antiandrogenic effects. It exerts its antiandrogenic action by inhibiting androgen uptake and/or by inhibiting nuclear binding of androgen in target tissues or both. Prostatic carcinoma is known to be androgen-sensitive and responds to treatment that counteracts the effect of androgen and/or removes the source of androgen, e.g. castration. Elevations of plasma testosterone and estradiol levels have been noted following flutamide administration.

3.1.10.2 Dosage and administration

The recommended dosage is 2 capsules 3 times a day at 8-hour intervals for a total daily dose of 750 mg.

3.1.10.2.1 How Supplied

Flutamide, 125 mg, are available as opaque, two- toned brown capsules, imprinted with "Schering 525". They are supplied as follows:

NDC 0085-0525-05 - Bottles of 500

NDC 0085-0525-03 - Unit Dose packages of 100 (10 x 10's)

NDC 0085-0525-06 - Bottles of 180

Store between 2° and 30° C (36° and 86° F). Protect the Unit Dose packages from excessive moisture.

3.1.10.3 Pharmacokinetics

3.1.10.3.1 Absorption

Analysis of plasma, urine, and feces following a single oral 200 mg dose of tritium labeled flutamide to human volunteers showed that the drug is rapidly and completely absorbed. Following a single 250 mg oral dose to normal adult volunteers, the biologically active alpha-hydroxylated metabolite reaches maximum plasma concentrations in about 2 hours, indicating that it is rapidly formed from flutamide.

3.1.10.3.2 Distribution

In male rats neither flutamide nor any of its metabolites is preferentially accumulated in any tissue except the prostate after an oral 5-mg/kg dose of ^{14}C-flutamide. Total drug levels were highest 6 hours after drug administration in all tissues. Levels declined at roughly similar rates to low levels at 18 hours. The major metabolite was present at higher concentrations than flutamide in all tissues studied. Following a single 250 mg oral dose to normal adult volunteers, low plasma levels of flutamide were detected. The plasma half-life for the alpha-hydroxylated metabolite of flutamide is about 6 hours. Flutamide, in vivo, at steady-state plasma concentrations of 24 to 78 ng/mL, is 94% to 96% bound to plasma proteins. The active metabolite of flutamide, in vivo, at steady-state plasma concentrations of 1556 to 2284 ng/mL, is 92% to 94% bound to plasma proteins.

3.1.10.3.3 Metabolism

The composition of plasma radioactivity, following a single 200 mg oral dose of tritium-labeled flutamide to normal adult volunteers, showed that flutamide is rapidly and extensively metabolized, with flutamide comprising only 2.5% of plasma radioactivity 1 hour after administration. At least 6 metabolites have been identified

in plasma. The major plasma metabolite is a biologically active alpha-hydroxylated derivative, which accounts for 23% of the plasma tritium 1 hour after drug administration. The major urinary metabolite is 2-amino-5-nitro-4- (trifluoromethyl) phenol.

3.1.10.3.4 Excretion

Flutamide and its metabolites are excreted mainly in the urine with only 4.2% of the dose excreted in the feces over 72 hours.

3.1.10.4 Indications

Flutamide is indicated for use in combination with LHRH agonists for the management of locally confined Stage B_2-C and Stage D_2 metastatic carcinoma of the prostate.

3.1.10.4.1 Stage B_2-C Prostatic Carcinoma

Treatment with EULEXIN Capsules and the LHRH agonist should start 8 weeks prior to initiating radiation therapy and continue during radiation therapy.

3.1.10.4.2 Stage D_2 Metastatic Carcinoma

To achieve benefit from treatment, EULEXIN Capsules should be initiated with the LHRH agonist and continued until progression.

3.1.10.5 Side effects

3.1.10.5.1 Hepatic Injury

Since transaminase abnormalities, cholestatic jaundice, hepatic necrosis, and hepatic encephalopathy have been reported with the use of flutamide, periodic liver function tests should be considered. Appropriate laboratory testing should be done at the first symptom/ sign of liver dysfunction (eg, pruritus, dark urine, persistent anorexia, jaundice, right upper quadrant tenderness, or unexplained "flu-like" symptoms). If the patient has clinically evident jaundice, in the absence of biopsy-confirmed liver metastases, EULEXIN therapy should be discontinued. In clinically asymptomatic patients, if transaminases increase over 2-3 times the upper limit of normal, treatment should be discontinued. The hepatic injury is usually reversible after discontinuation

of therapy, and in some patients, after dosage reduction. However, there have been reports of death following severe hepatic injury associated with use of flutamide.

3.1.10.5.2 Renal Impairment

Following a single 250 mg dose of flutamide administered to subjects with chronic renal insufficiency, there appeared to be no correlation between creatinine clearance and either C_{max}, or AUC of flutamide. Renal impairment did not have an effect on the C_{max} or AUC of the biologically active alpha-hydroxylated metabolite of flutamide. In subjects with creatinine clearance of <29 mL/min, the half-life of the active metabolite was slightly prolonged. Flutamide and its active metabolite were not well dialyzed. Dose adjustment in patients with chronic renal insufficiency is not warranted.

3.1.10.5.3 Central Nervous System

(drowsiness/confusion/depression/anxiety/nervousness) reactions occurred in 1% of patients.

3.1.10.5.4 Gastrointestinal System

Anorexia 4%, and other GI disorders occurred in 6% of patients.

3.1.10.5.5 Hematopoietic System

Anemia occurred in 6%, leukopenia in 3%, and thrombocytopenia in 1% of patients.

3.1.10.5.6 Skin

Irritation at the injection site and rash occurred in 3% of patients.

3.1.10.5.7 Others:

Edema occurred in 4%, genitourinary and neuromuscular symptoms in 2%, and pulmonary symptoms in less than 1% of patients.

In addition, the following spontaneous adverse experiences have been reported during the marketing of flutamide: hemolytic anemia, macrocytic anemia, methemoglobinemia, photosensitivity reactions (including erythema, ulceration, bullous eruptions, and epidermal necrolysis), and urine discoloration. The urine was noted to change to an amber or yellow-green appearance which can be attributed to the flutamide and/or its metabolites. Also reported were cholestatic jaundice, hepatic encephalopathy, and hepatic necrosis. The hepatic conditions were usually reversible

after discontinuing therapy; however, there have been reports of death following severe hepatic injury associated with use of flutamide.

3.1.10.6 Interactions

Increases in prothrombin time have been noted in patients receiving long- term warfarin therapy after flutamide was initiated. Therefore close monitoring of prothrombin time is recommended and adjustment of the anticoagulant dose may be necessary when EULEXIN Capsules are administered concomitantly with warfarin.

3.1.10.7 Laboratory Tests

Regular assessment of serum Prostate Specific Antigen (PSA) may be helpful in monitoring the patient's response. If PSA levels rise significantly and consistently during flutamide therapy the patient should be evaluated for clinical progression. For patients who have objective progression of disease together with an elevated PSA treatment- free period of antiandrogen while continuing the LHRH analogue may be considered.

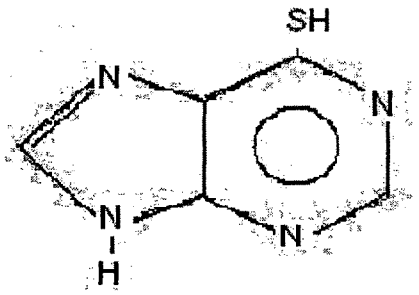
Since transaminase abnormalities, and rarely jaundice, have been reported with the use of flutamide, periodic liver function tests should be considered, eg, when the patient has jaundice or laboratory evidence of liver injury in the absence of liver metastases, flutamide therapy should be discontinued. Abnormalities are usually reversible upon discontinuation.

Laboratory abnormalities including elevated SGOT, SGPT, bilirubin values, SGGT, BUN, and serum creatinine have been reported.

3.2.1 6-MERCAPTOPURINE

3.2.1.1 Name, Formula, Molecular Weight

Mercaptopurine is purine-6-thiol monohydrate (170.19)



3.2.1.2 Appearance, Color, Odor

Mercaptopurine is a yellow, practically odorless, crystalline powder (1)

3.2.2 Physical Properties

3.2.2.1 Infrared Spectrum

Table 3.9 gives the infrared assignments consistent with the structure of mercaptopurine.

Table 3.9: Infrared Spectral Assignments for Mercaptopurine (3)

Band (cm^{-1})	Assignment
3420, 3490	Aromatic NH stretch
3120, 3040, 2780	Aromatic CH stretch
1200	C = S stretch
930	CH band

3.2.2.2 Ultraviolet Spectrum

The UV spectra of Mercaptopurine in 0.1N NaOH, 0.1N HCl, and methanol were taken with a UV/VIS Shimadzu spectrophotometer and are shown in Figure 3.9 to 3.11 (3). Table 3.10 summarizes the UV data.

Table 3.10: UV Spectral Data for Mercaptopurine

Solvent	λ max (nm)	ε
0.1N NaOH	230	14000 (3)
	312	19600 (5)
0.1N HCl	222	9240 (3)
	327	21300 (5)
Methanol	216	8940 (3)
	329	19300 (3)

Figure 3.9: UV spectrum of flutamide in 0.1 N HCl

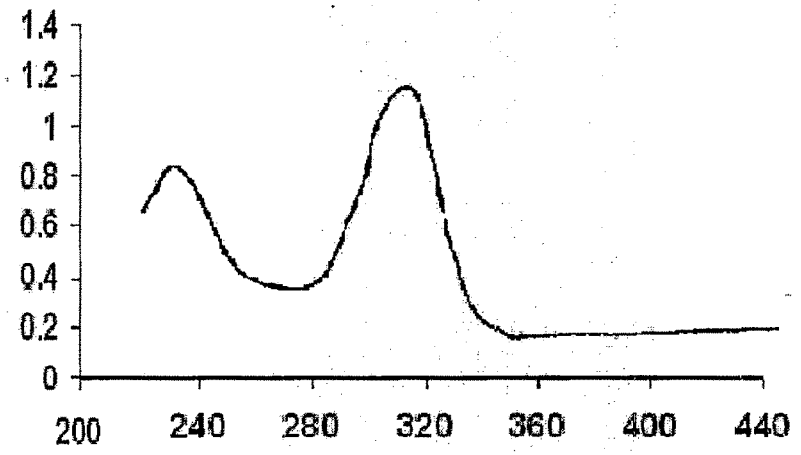


Figure 3.10: UV spectrum of flutamide in 0.1 N NaOH

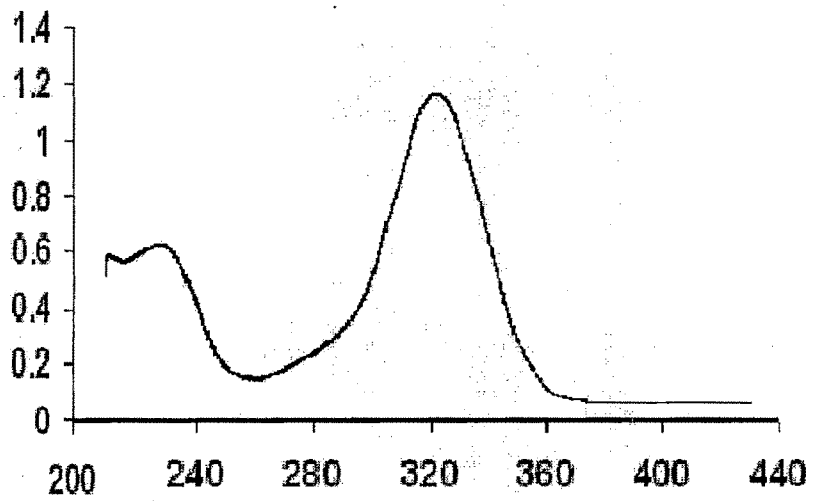
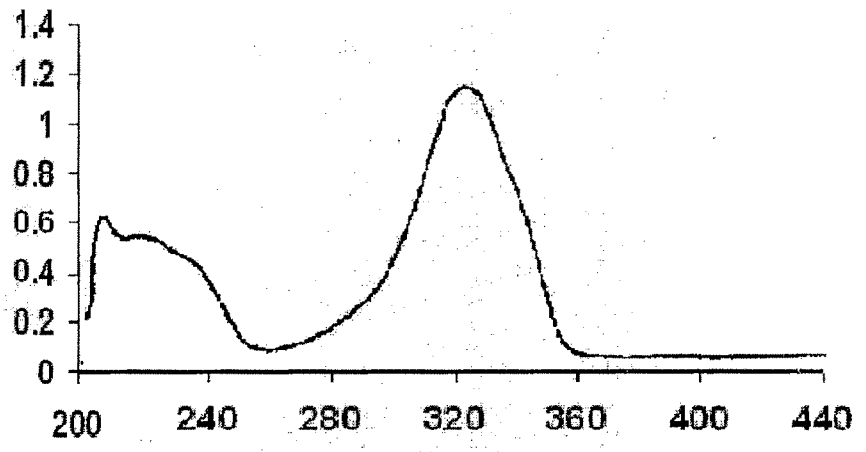


Figure 3.11: UV spectrum of flutamide in Methanol



3.2.2.3 Melting Point

Mercaptopurine decomposes above 308^0C (1).

3.2.2.4 Solubility

Mercaptopurine is insoluble in water, acetone, and ether. It is soluble in hot ethanol and dilute alkali solutions. It is slightly soluble in dilute sulfuric acid (1).

3.2.2.5 Dissociation Constant

The pK_{a1} and pK_{a2} of mercaptopurine determined potentiometrically is 7.77 and 11.17 respectively (7).

3.2.3 Stability

The decomposition of mercaptopurine in 0.1N NaOH, 0.1N HCl, distilled water, and photolytically has been studied (12). When mercaptopurine is refluxed for 6 days in 0.1N NaOH, 4-aminomidazole-5-thiocarboxamide and 4-amino-5-cyanoimidazole are the major products formed. When refluxed for 4 days in 0.1N HCl and 7 days as an aqueous suspension in distilled water, mercaptopurine decomposes primarily to 4-aminomidazole-5-thiocarboxamide. Mercaptopurine in 0.1N NaOH and as an aqueous suspension in distilled water form hypoxanthine, when irradiated for 72 hours with a medium pressure mercury lamp.

3.2.4 Methods of Analysis

3.2.4.1 Elemental Analysis

Table 3.11: Elements present in 6-Mercaptopurine

Element	C	H	N	S
% Calculated	35.28	3.55	32.92	18.83

3.2.4.2 Nonaqueous Titrimetric Analysis

Nonaqueous titration is the official method of analysis in the USP for mercaptopurine. An accurately weighed sample of mercaptopurine (1) is dissolved in dimethylformamide. The solution is titrated with standardized 0.1N sodium methoxide using thymol blue as an indicator. Precautions must be taken against absorption of atmospheric carbon dioxide.

3.2.4.3 Spectrophotometric Analysis

The official USP analysis of mercaptopurine in tablets is a spectrophotometric analysis. A portion of powdered tablets is weighed. Twenty ml distilled water, and 1.5 ml NaOH TS is added to the powder in a 100 ml volumetric flask. The flask is brought to volume with distilled water, filtered, and a portion of the filtrate diluted with dilute hydrochloric acid. The solution is compared against a Reference Standard prepared in a similar manner at 325 nm in 1 cm cells.

3.2.4.4 Polarography

Alternating current polarography has been used to determine decomposition kinetics of mercaptopurine (13). A catalytic wave with Q=0.45 was observed for mercaptopurine in 1 N H_2SO_4.

3.2.4.5 Mass Spectrometry

Quantitative analysis of mercaptopurine in plasma has been accomplished with GC/MS (14). Mercaptopurine was extracted from plasma, derivatized with methyl iodide, and separated by gas chromatography using a 1.83 M, 3% OV-225 column at 200^0C, and detected with a mass spectrometer equipped with a peak monitor. Limit of detection was 20 ng/ml of mercaptopurine in plasma.

3.2.4.6 Chromatography

3.2.4.6.1 High Performance Liquid Chromatography

High performance liquid chromatography was used to determine mercaptopurine and metabolites in cultured cells and animal tissues (15). A 0.18 x 100 cm column packed with Beckman M71 strong cation exchange resin was eluted with 0.4 M ammonium formate (pH 4.6) at ml/hr. the column was kept at 50 C. the retention time of mercaptopurine under these conditions was 39 minutes. Detection was accomplished with a UV detector at 322 nm.

3.2.4.6.2 Column chromatography

Column chromatography has been used to separate mercaptopurine from its metabolites in urine (16). A cation exchange resin, Zeo-carb 225, eluted with 20

%v/v ammonium hydroxide separated mercaptopurine and other 6-thiopurines from a concentrated urine sample.

3.2.4.6.3 Gas Chromatography

A gas chromatographic analysis of mercaptopurine in serum has been described (17). A 1.5M x 6.3mm o.d. column packed with 10% w/w SE-30 maintained at 135^0C was used. Mercaptopurine dramatized with tri methyl anilinium hydroxide had a retention time of 22 minutes.

3.2.4.6.4 Thin Layer Chromatography

The separation of mercaptopurine from mixture of purines and pyrimidines has been accomplished by thin layer chromatography using anion exchange ECTEOLA cellulose plates (18). The plates were developed in acetone: 0.1M H_2SO_4: ethyl acetate (45:10:45). A second development was done in deionized water: acetone (8:2). The R_f value of mercaptopurine was 0.36. Cellulose plates developed in 0.1N HCl, H_2O, and isopropanol: methanol: H_2O: NH_4OH (60:22:20:1) gave R_f values of 0.43, 0.26 and 0.55, respectively (19).

3.2.5 Clinical Pharmacology

3.2.5.1 Mechanism of Action

Mercaptopurine has been in clinical use for over 30 years. It is a 6-thiopurine analogue of the naturally occurring purine bases hypoxanthine and guanine and acts as a cytotoxic antimetabolite. Mercaptopurine requires intracellular anabolism by hypoxanthine guanine phosphoribosyl transferase (HGPTPT) to be cytotoxic. Intracellular activation results in de novo inhibition of purine synthesis and incorporation into DNA. Mercaptopurine is cross resistant with 6-thioguanine. Cytotoxicity is cell cycle phase-specific (S-phase).

3.2.5.2 Pharmacokinetics

3.2.5.2.1 Oral Absorption

Absorption is incomplete and variable (± 50%). Bioavailability is 16-37% largely due to first pass metabolism in the liver (less when given with food).

3.2.5.2.2 Tissue Distribution

In the mouse the concentrate of radioactive mercaptopurine was highest in the gut, almost twice as high as in the blood, and lowest in the brain. Mercaptopurine has some difficulty in passing the blood-brain barrier. The brain concentration is one-tenth the concentration in the blood (20). The presence of a tumor in different sites in rats, as well as in mice, cause lower blood levels of mercaptopurine when compared to non-tumor bearing animals. The apparent volume of distribution is markedly in the presence of a tumor (21).

Distribution	Widely distributed to body tissues, found in breast milk	
	Cross blood brain barrier?	Negligible with conventional doses
	PPB	19% (IV)

3.2.5.2.3 Metabolism

The pathway of metabolism of mercaptopurine is by hydroxylation via the enzymes xanthine oxidase and aldehyde oxidase. Mercaptopurine is transformed into mercaptopurine riboside, 6-thiouric acid, sulfates, and nucleotide metabolites in the liver (20-22).

Metabolism	Rapid intracellular activation	
	Active metabolite(s)	6-thioininosinic acid, 6-thioguanylic acid
	Inactive metabolite(s)	6-thiouric acid, 6-methylthiopurine derivatives

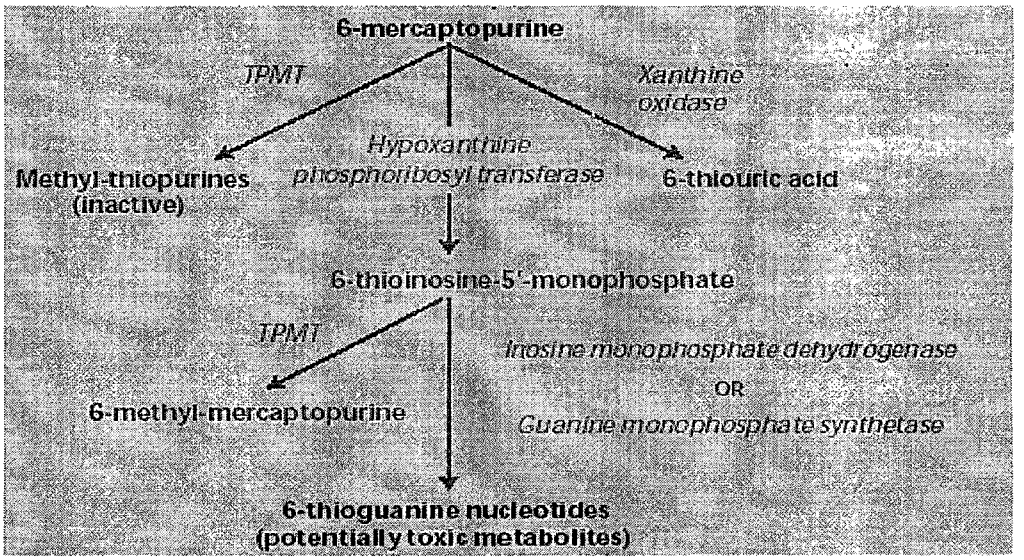


Figure 3.12. Metabolism of 6-mercaptopurine. TPMT, thiopurine methyltransferase

3.2.5.2.4 Excretion

In the rat, by the end of 48 hours after injection, 55% of mercaptopurine was excreted in the urine. The largest proportion was excreted in the first 24 hours (22). For an intraperitoneal injection in the mouse of 1 mg of S^{35} -6-mercaptopurine, 43.5% of the radioactive material was excreted in the urine in the first four hours. At the end of 2 days over 60% of S^{35} was excreted. Approximately the same urinary excretion rate was found with oral doses, but some radioactive CO_2 was detected in the expired air (20).

Excretion	At conventional doses clearance is primarily hepatic and by xanthine oxidase. Renal clearance may become important at high doses	
	Urine	7% of oral dose excreted unchanged in 12 hours
	t ½	90 minutes

3.2.5.3 Indication and Status

Mercaptopurine is chemotherapy that is given as a treatment for some types of cancer. It is most commonly used to treat acute myeloid leukaemia and acute lymphoblastic leukaemia.

1. Acute lymphocytic leukemia
2. Acute myelogenous leukemia
3. Chronic myelogenous leukemia

3.2.5.4 Adverse Effects

The major dose limiting effect of mercaptopurine is **myelosuppression.** Patients with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) prone to develop rapid bone marrow suppression. This problem could be exacerbated by co-administration with drugs that inhibit TPMT, such as olsalazine, mesalazine or sulfasalazine.

Hyperuricemia during periods of active cell lysis, which is caused by cytotoxic chemotherapy of highly proliferative tumors of massive burden (e.g., some leukemias and lymphomas), can be minimized with allopurinol and hydration. In hospitalized patients the urine may be alkalinized, by addition of sodium bicarbonate to the IV fluids, if tumor lysis is expected. However, the dose of mercaptopurine should be reduced to 25%-33% of the usual dose if allopurinol is given.

Table 3.12 Adverse effects of 6-Mercaptopurine

ORGAN SITE	SIDE EFFECT	ONSET			
		I	E	D	L
Dermatologic	Alopecia		E		
	Rash				
	Radiation recall reaction (rare)	I			
	Hyperpigmentation		E		
Extravasation hazard	None				
Gastrointestinal	Nausea and vomiting (mild, rare in children)	I			
	Diarrhea		E		
	Gastrointestinal ulceration				
	Anorexia		E		
	Stomatitis (rare)		E		
	Pancreatitis		E		
Hematologic	Myelosuppression,				
	Nadir 7-14 days, recovery 14-21 days		E		
	Immunosuppression		E		
Hepatic	Elevated liver function tests		E		
	Jaundice (intrahepatic cholestasis (10-40%)		E	D	
	Hepatic necrosis	I	E		
Hypersensitivity	Skin rash		E		
	Drug fever (rare)	I			
Neoplastic	Leukemia				L
Renal/metabolic	Hyperuricemia (during periods of active cell lysis)	I			
	Hematuria and crystalluria (high IV doses)		E		

Dose-limiting side effects are underlined.
I = immediate (onset in hours to days); E = early (days to weeks);
D = delayed (weeks to months); L = late (months to years)

Hepatic toxicity ranges from asymptomatic elevation of liver enzymes with no clinical significance to jaundice (intrahepatic cholestasis) and liver failure. Serious toxicity is manifested by rapid onset of jaundice, ascites, hepatic encephalopathy and/or elevated liver enzymes, usually associated with hepatic necrosis and severe fibrosis. Clinical signs of jaundice are usually evident at 1-2 months but have been reported as early as 1 week and as late as 8 years after initiation of mercaptopurine therapy. Deaths have occurred from hepatic necrosis. The incidence of hepatotoxicity increases when dosage exceeds 2.5mg/kg daily.

Mercaptopurine has the potential to enhance radiation injury to tissues. While often-called **radiation recall reactions**, the timing of the radiation may be before,

concurrent with or even after the administration of the mercaptopurine. Recurrent injury to a previously radiated site may occur weeks to months following radiation.

3.2.5.5 Dosing

Numerous dosing schedules exist and depend on disease, response and concomitant therapy. Guidelines for dosing also include consideration of white blood cell count. Dosage may be reduced and/or delayed in patients with bone marrow depression due to cytotoxic/radiation therapy. Mercaptopurine should be given as a single dose on an empty stomach; doses should be rounded to the nearest 25mg.

3.2.5.5.1 Adults:

Induction: daily: 50-75mg/m^2 then titrates to toxicity/response

Dosage in Myelosuppression: Modify according to protocol by which patient is being treated, if no guidelines available, refer to Appendix 6 "Dosage Modification for Myelosuppression".

Dosage in renal failure: The starting dose or frequency of administration should be modified in the presence of renal dysfunction.

Creatinine clearance (mL/s)	Dosing interval (h)
0.8-1.3	24-36
0.2-0.8	24-48

Dosage in hepatic failure: Adjustment required, no details found.

Dosage in the elderly: Use with caution.

3.2.5.5.2 Children:

Oral: Daily: 2.5mg/kg (Decrease dose by 50% in children less than 3 months of age).

3.2.6 Special Precautions

Mercaptopurine is contraindicated in patients whose disease has demonstrated prior resistance to the drug, or is hypersensitive to any component of the drug. Mercaptopurine is cross-resistant with thioguanine. Patients with hereditary thiopurine methyltransferase deficiency are at a substantially increased risk of toxicity as are patients who are receiving concomitant allopurinol or receiving drugs that inhibit TPMT. Mercaptopurine carcinogenic, mutagenic and teratogenic and

should not be used in pregnancy. Breast-feeding is not recommended due to secretion into breast milk.

3.2.7 Drug-Drug Interaction

Table 3.13: Drug interaction with 6-Mercaptopurine

Agent	Effect	Mechanism	Management
Allopurinol	Increased myelosuppression by mercaptopurine	Inhibits inactivation of mercaptopurine	Decrease dose of mercaptopurine to 25%-33% of usual dose
Anticoagulants	Anticoagulant effect may be decreased	Unknown	Monitor
Doxorubicin and other hepatotoxins	Enhanced hepatotoxicity	Unknown	Avoid/caution
Trimethoprim-sulfamethoxazole	Enhanced myelosuppression	Additive effect	May need to decrease dose of mercaptopurine
Sequential multiple analyzer 12/60	Falsely elevated serum glucose and uric acid values	Unknown	Caution
Aminosalicylates, sulfasalazine	Increased myelosuppression	Inhibition of TPMT	Caution