Chapter 2

Objectives &
Drug Profiles
(A) OBJECTIVES OF THE PRESENT INVESTIGATIONS

Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods, there can be and there is no 'second' quality in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. In popular practice the quality of medicines or pharmaceutical products is assured through quality control. It is therefore, essential that quality assurance department must adopt 'good laboratory practice' to ensure reliability of pharmaceuticals together with there careful control or our moral obligations arising from the humanism towards the sick human beings with the growth of pharmaceutical analysis involving complex instrumentations, providing simple analytical procedures for complex formulations is a matter of fore most importance.

Drugs and pharmaceuticals play a very significant role in the present days for the prevention, control and curing of different kinds of human diseases. It is a common observation and the practical truth that a single drug of a particular composition is marketed in various brand names by different manufactures. The possibility of minor changes in chemical composition and standard of the drug will have a profound effect on the physiological and biological activities of the patient.

It is very much painful for the present day's scientist in general and to the analytical pharmaceutical chemist in particular to note in the various dailies about entry of the spurious and substandard drugs into market, witch definitely will have an adverse effect on the human beings at large.
It is with this challenge in mind, the author has taken up his thorough investigations to evaluate the purity of the various drugs released into the market. The author has made an extensive survey of the chemical and biochemical literature to know whether the reports involving simple experimental techniques such as the Spectrophotometric techniques are available for ascertaining the assay and purity of the drugs.

Various instrumental techniques (HPLC, GC, Fluorimetry, NMR, IR, UV and Visible Spectrophotometric techniques) are available in the literature for the assay of drugs. These methods are either expensive or do not give reproducible. Usually Spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the Spectrophotometric methods depend only on the nature of chemical reactions involved in color development and not on the sophistication of the experiment.

UV and Visible Spectrophotometric methods are highly versatile, sensitive and reproducible this made the author to develop new Spectrophotometric methods for the estimation of selected drugs having varying used in pharmaceutical preparations.
(B) DRUG PROFILES & REVIEW OF LITERATURE

1) Zolpidem Tartrate:

Zolpidem Tartrate is a prescription medication used for the short-term treatment of insomnia, as well as some brain disorders. It is a short-acting nonbenzodiazepine hypnotic that potentiates gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, by binding to gamma-aminobutyric acid (GABA_A) receptors at the same location as benzodiazepines. It works quickly (usually within 15 minutes) and has a short half-life (2–3 hours).

Its hypnotic effects are similar to those of the benzodiazepine class of drugs, but it is molecularly distinct from the classical benzodiazepine molecule and is classified as an imidazopyridine. Flumazenil, a benzodiazepine receptor antagonist, which is used for benzodiazepine overdose, can also reverse zolpidem's sedative/hypnotic and memory impairing effects. As an anticonvulsant and muscle relaxant, the beneficial effects start to emerge at 10 and 20 times the dose required for sedation, respectively. For that reason, it has never been approved for either muscle relaxation or seizure prevention. Such drastically increased doses are more inclined to induce one or more negative side-effects, including hallucinations and/or amnesia.
Molecular structure:

Nomenclature: \(N,N,6\text{-trimethyl-2-(4-methylphenyl)-imidazo[1,2-\alpha]pyridine-3-acetamide}\)

Molecular formula: \(C_{19}H_{21}N_3O\)

Molecular weight: 307.395 g/mol

Characteristics: Yellowish white colored amorphous solid

Category: Hypnotic

Solubility: Soluble in Methanol, Ethanol, Acetonitrile.

Storage: Stored at below 25°C

Functional groups: Aromatic Tertiary amine, Amide and Carboxylic acid

Trade name: Zolfresh - 5 mg, 10 mg.
1. Possibilities and Problems with identification and determination of “New” Hypnotics (Flunitrazepam, Zolpidem).\textsuperscript{53}

Peter Ondra\textit{ et al.}

Thin Layer Chromatography is suitable for Zolpidem identification in the MB fraction after their L-L extraction. The modified procedure according to Večerkova was employed with Bratton-Marshall sulphuric acid in ethanol as the location reagent for zolpidem (ultraviolet light at 366 nm). Gas Chromatography with Mass Spectrometry was used for zolpidem identification in the MB fraction after its isolation by SPE method (GC TRACE 2000, MD – PolarisQ, Thermo Finnigan), equipped with the capillary column ZB 5 MS (length 15 m × I.D. 0.25 mm × film thickness 0.25 μm), injector temperature 230 °C, column temperature 70 °C, detector temperature 230°C, temperature gradient 70 °C (1 min), programming 15 °C/min to final temperature 260 °C (held for 10 min), TIC mode – m/z range 40-450 AMU, or SIR mode – m/z 235 AMU for trace analysis.

2. Determination of Zolpidem hemitartrate by quantitative HPTLC and LC.\textsuperscript{54}

B.A. El Zeany\textit{ et al.}

Two methods are described for the determination of Zolpidem hemitartrate in presence of its degradation product. The first method was a TLC-UV densitometric one in which the mobile phase methanol: water (20:80) was used for developing the TLC plates. The $R_f$ of Zolpidem hemitartrate was found to be 0.29±0.01 and that of its degradation product was 0.59±0.01. Linearity range was 0.5–4 μg/spot with mean recovery percentage (99.98±0.988)%.

The second method was an HPLC method. HPLC was performed on a Bondapack C\textsubscript{18} column. The mobile phase was composed of a mixture of acetonitrile-0.01 M KH\textsubscript{2}PO\textsubscript{4} (40:60). The pH was adjusted to 3.5±0.1. Flow rate was 1.2 ml/min.
Calibration graphs were linear in the range of 0.5–5 μg/ml with UV detection at 245 nm. Both methods have been successfully applied to pharmaceutical formulations. The results obtained were statistically compared with those obtained by applying the reported methods.


K. S. Patil et al.

A simple, sensitive, rapid, accurate and precise Spectrophotometric method has been developed for the estimation of Zolpidem tartrate in bulk and pharmaceutical dosage forms. Zolpidem tartrate shows maximum absorbance at 238.5 nm with molar absorptivity of $4.4648 \times 10^4$ lit/mol/cm. Beer’s law was obeyed in the concentration range of 2-16 μg/ml. The limit of detection and limit of quantification were found to be 0.038152 μg/ml and 0.114577 μg/ml, respectively. Results of analysis were validated statistically and by recovery studies.

4. Determination and in-process control of Zolpidem synthesis by High-Performance Liquid Chromatography.

L. Laviana et al.

A high-performance liquid chromatographic assay with diode-array detection has been developed for the in-process control of Zolpidem synthesis and for the analysis of the drug and its synthetic intermediates. The separation uses a 4.6 mm i.d. reversed-phase Kromasil C$_{18}$ (150 mm) column, 5 μm particle size with a gradient elution mode of acetonitrile and 0.02 M NH$_4$OAc (adjusted to pH 8.0) as the mobile phase (flow rate 1.0 ml min$^{-1}$). The analysis is performed in 12 min. The method is simple, rapid and highly specific.
2) **Sibutramine HCl:**

Sibutramine HCl, usually available as Sibutramine hydrochloride monohydrate, is an orally administered agent for the treatment of obesity, as an appetite suppressant. It is also under review by the FDA and the European Medicines Agency. It is a centrally-acting serotonin-norepinephrine reuptake inhibitor structurally related to amphetamines, although its mechanism of action is distinct.

Sibutramine is manufactured by Abbott Laboratories, under brand names such as Reductil, Meridia and Sibutrex. It is classified as a Schedule IV controlled substance in the United States, despite having virtual no potential for abuse (due to its lack of appreciable dopaminergic effects). It is likely that the compounds use as an anorectic is the sole reason is it classified as a controlled drug, as “over prescription” of anorectics (as a class) in the mid-20th century resulted in a number of cases of abuse or addiction.

**Molecular structure:**

![Molecular structure of Sibutramine HCl]
Nomenclature : \((\pm)-\text{dimethyl-1-[1-(4-chlorophenyl) cyclobutyl}\)-\(N,N,3\)-trimethylbutan-1-amine\)

Molecular formula : \(C_{17}H_{26}ClN\)

Molecular weight : 279.85 g/mol

Characteristics : White color amorphous solid

Category : Anti-Obesity

Solubility : Soluble in Water, Methanol, Ethanol, Acetonitrile.

Storage : Stored at below 25°C

Functional groups : Tertiary amine

Trade name : Obsesave - 10 mg, 15 mg

1. Spectrophotometric methods for the determination of Sibutramine Hydrochloride from capsules.57

R Valarmathi et al.

A new simple, sensitive Spectrophotometric method in Ultraviolet region has been developed for the determination of Sibutramine hydrochloride in bulk and in capsule dosage form. Sibutramine hydrochloride shows maximum absorbance at 220 nm. Beer's law was obeyed in the concentration range 10-50 micro-g/ml. Result of the analysis were validated statistically and by statistically and by recovery studies.
2. Development and Validation of High Performance Liquid Chromatography method for Analysis of Sibutramine Hydrochloride and its Impurity.\textsuperscript{58}

JG Chandorkar \textit{et al.}

A simple, Precise, Rapid reproducible and selective reverse phase HPLC method has been developed for the estimation of Sibutramine Hydrochloride monohydrate and its Impurity in Bulk as well as Formulation. The analyte was resolved by using Mobile phase (Sodium Dihydrogen phosphate and Acetonitrile) at the flow rate of 1.0 Ml/Min. on Isocratic HPLC system consisting of Jasco Make UV visible Detector of model UV 1575 & Jasco make HPLC pump of model PU 1580. An ODS C-8 RP Column (4.6mm ID, 250mm L, particle size 5 Micron, at wavelength of 230 nm.

3. Simultaneous determination of Sibutramine and N-Di-desmethylsibutramine in dietary supplements for weight control by HPLC-ESI-MS.\textsuperscript{59}

Z Huang \textit{et al.}

A High performance Liquid Chromatographic method, coupled with UV detection and Electrospray Ionization Mass Spectrometry (HPLC-UV-ESI-MS), is developed for the simultaneous determination of the illegal additives Sibutramine and its metabolite N-di-desmethylsibutramine in dietary supplements for weight control. The separation is achieved on a Spherisorb C8 reversed-phase column, employing acetonitrile and an aqueous 0.2% formic acid solution containing 20mM ammonium acetate as mobile phases in a gradient mode. UV detection is used for quantitation at a wavelength of 223 nm. Identification of target compounds is completed by ESI-MS using selected ion recording at m/z 280 for Sibutramine and m/z 252 for N-di-desmethylsibutramine. Calibration curves are linear over the range of 0.025-1.0 mg/mL for Sibutramine and N-
di-desmethylsibutramine. Correlation coefficients are better than 0.9990. The intra- and inter-day precision and accuracy for Sibutramine and N-di-desmethylsibutramine are acceptable. The method is successfully applied to the analysis of natural dietary supplement samples.

4. LC method for the Determination of assay and purity of Sibutramine Hydrochloride and its Enantiomers by Chiral Chromatography.

T. Radhakrishna et al.

Two isocratic Liquid Chromatography (LC) methods have been developed for the purity estimation and quantitative determination of Sibutramine HCl, using 4-chloro aniline and lovastatin as internal standards, respectively. The precision has been checked in terms of F-test variance ratio using latter method as reference. The ratio of variances of the two methods is close to unity, confirming their good precision. The correlation coefficient for linear regression is more than 0.999. The inter and intra-day precision is found to be<1.3% RSD. The accuracy determined as relative mean error (RME) for the intra-day assay is±1.7%. The enantiomeric separation of Sibutramine by Chiral Chromatography method has been described also. This method is capable of separating the two enantiomers with a selectivity of 1.4 and a resolution of 4.0. Both methods are found to be stability indicating and useful in the quality control of the bulk material.
(C) PREPARATION OF REAGENTS

All the chemicals and reagents used are of analytical grade and solutions are prepared in distilled water.

(i) TP OO solution (0.5% w/v):

It is prepared by dissolving 500 mg of tropaeoline - OO (Loba) in 100 ml of distilled water.

(ii) BCG solution (0.5% w/v):

It is prepared by dissolving 500 mg of bromocresol green (Loba) in 100 ml of distilled water.

(iii) WFB solution (0.2% w/v):

It is prepared by dissolving 200 mg of wool fast blue (Fluka) in 100 ml of distilled water.

(iv) EBT solution (0.5% w/v):

It is prepared by dissolving 500 mg of eriochrome black-T (Loba) in 100 ml of distilled water.

(v) HCl solution (0.1N):

It is prepared by diluting 8.5 ml of conc. hydrochloric acid (Merck) to 1000 ml with distilled water.
(vi) **Buffer solution pH 3.5 (potassium acid phthalate – HCl):**

It is obtained by diluting a mixture of 50 ml of 0.2M potassium acid phthalate and 8.4 ml of 0.2M HCl to 200 ml with distilled water and the pH is adjusted to 3.5.

(vii) **Buffer solution (pH 1.5):**

It is prepared by mixing 289 ml of glycine solution (37.52 gm of glycine and 29.24 gm of NaCl are dissolved in 500 ml of distilled water) with 711 ml of 0.1M HCl and pH of the solution is adjusted to 1.5.

(viii) **DDQ (0.1% w/v):**

DDQ (2,3-dichloro 5,6-dicyano-p-benzoquinone) (Loba Chem., India) solution is prepared by dissolving 100 mg in 100 ml of methanol.

(ix) **Preparation of standard Zolpidem Tartrate (ZLPT) drug stock solution:**

The stock solution (1mg ml\(^{-1}\)) of Zolpidem Tartrate (ZLPT) is prepared by dissolving 100 mg of drug in 100 ml of methanol. A portion of this stock solution is diluted stepwise with the methanol to obtain the working standard ZLPT drug solutions of concentrations 50 μg ml\(^{-1}\) and 100 μg ml\(^{-1}\).

(x) **Preparation of standard Sibutramine HCl (SBT) drug stock solution:**

The stock solution (1mg ml\(^{-1}\)) of Sibutramine HCl (SBT) is prepared by dissolving 100 mg of drug in 100 ml of distilled water. A portion of this stock solution is diluted stepwise with the distilled water to obtain the working standard SBT drug solutions of concentrations 50 μg ml\(^{-1}\) and 100 μg ml\(^{-1}\).
(D) INSTRUMENTS EMPLOYED IN PRESENT INVESTIGATIONS

1. UV-Visible Spectrophotometer:

ELICO S.L 164 Double beam U.V-Visible Spectrophotometer manufactured by M/S ELICO Private Limited, Hyderabad, India. is used for all Spectrophotometric studies. The instrument provides a unique monochromatic design and a variety of micro process controlled features to give fast and accurate Spectrophotometric measurements.

Operational principle and constructional features

S.L.164 is a double – beam microprocessor based spectrophotometer designed for the quantitative analysis. Its main features are

1. Wave length scanning system by CPU control with out using sine bar to realize high speed wave length scanning.

2. All in one type of spectrophotometer with CRT and printer incorporated.

3. Back up mode parameters are provided so as to enable single action operation.

4. Easy data processing, since the obtained spectrum is available by the conversation with CRO.
### Specifications of UV-160 A Spectrophotometer

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measuring wavelength</td>
<td>200 – 1100 nm</td>
</tr>
<tr>
<td>Spectral band width</td>
<td>2 nm</td>
</tr>
<tr>
<td>Wave length readability</td>
<td>0.1 nm increment</td>
</tr>
<tr>
<td>Wave length scanning speed</td>
<td>Monochromator setting speed is nearly 3600 nm/min. Fast- nearly 2400 nm/min. Medium-nearly 1500 nm/min. Slow nearly 480 nm/min.</td>
</tr>
<tr>
<td>Wave length accuracy</td>
<td>± 0.5 nm with automatic Wavelength correction.</td>
</tr>
<tr>
<td>Light source switching</td>
<td>Automatic switching according to Wavelength can be selected between 295 nm and 364 nm.</td>
</tr>
<tr>
<td>Photo metric system</td>
<td>Double beam system</td>
</tr>
<tr>
<td>Recording mode</td>
<td>Printout of measured data and calculated results.</td>
</tr>
<tr>
<td>Multicomponent</td>
<td>Mixed samples upto six Components can be determined. Mixed samples can be used as standards. Standards sample data can be stored in the back up memory (up to 16 standards).</td>
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</tbody>
</table>
Light sources: 50 W long life halogen lamp (2000 hrs) and socket type deuterium lamp (500 hrs) with automatic control of maximum sensitivity. Monochromator.

Recorder: Computer controlled thermal graphic printer.

CRT: 9-inch with graphic function 240 X 320 dots.

Sample compartment: Inner size: 1100 nm wide.

Power requirements: With line voltage selector for 100

Weight: 42 Kgs.

2. ELICO digital pH meter:

ELICO digital pH meter manufactured by M/S ELICO Private Limited, Hyderabad, India is used for measuring the pH of buffer solutions. The instrument has a temperature components arrangement. The reproducibility of measurement is within ±0.01 pH.