CHAPTER IV

EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF HALOPERIDOL IN DOSAGE FORMS
Haloperidol (HAL), chemically, 4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone has tranquilizing and antipsychotic activity. Like other anti-psychotic drugs, HAL produces sedation usually without sleep. It is official in BP1, USP2 and IP3. The IP suggests a spectrophotometric method for its analysis in tablet formulations, while USP and BP methods involve non-aqueous titration and UV-spectrophotometry.

Ramana Rao and Raghuveer4 have reported a spectrophotometric method for the determination of HAL in tablets based on the reaction of alkaline HAL with sodium salt of 1,2-naphtoquinone-4-sulphonic acid in acetate buffer of pH 4.0. The method obeyed Beer's law in the concentration range of 40 - 400 ppm. This method involves heating of the reaction mixture for 30 min on a water bath followed by extraction with 1,2-dichloroethane.

Trivedi et al5 have described simultaneous determination of HAL and propantheline bromide in bulk samples and tablets by UV spectrophotometry by measuring the absorbances of HAL at 244 nm.

Shingbal and Joshi6 have estimated HAL in various pharmaceutical formulations using p-chloranil reagent, in which the reaction mixture was allowed to stand for 15 min for completion of the reaction. The absorbances were measured at 540 nm against the reagent blank. The method obeyed Beer's law over the concentration range of 50–500 ppm. The coloured species was stable for 35 min after which the absorbances decreased with time.
Measurement of absorbances at 243 nm in methanol forms the basis of the UV method for the quantification of HAL reported by Kramarenko and Turkevich.

Formation of red coloured species between HAL and 3,5-dinitrobenzoic acid in alkaline medium forms the basis of the colorimetric method discussed by Haemers and Bossche.

A colour reaction was studied between HAL and bromophenol blue for the assay of HAL by Kramarenko and Turkevich. The method involves extraction of yellow coloured ion-association complex (λ<sub>max</sub> = 400 nm) into chloroform. The calibration graph was linear over the range of 4–36 ppm.

Genomefa has utilised phenylhydrazine as a reagent for the spectrophotometric determination of HAL by measuring the absorbance at 335 nm.

Literature also mentions gas-chromatographic and high performance liquid chromatographic methods for the determination of HAL in dosage forms. As spectrophotometric assays offer significant economical advantages over gas chromatographic and HPLC techniques, the aim of the present investigation was to develop new, sensitive and selective spectrophotometric methods for the determination of HAL in bulk samples and in its pharmaceutical preparations. Despite a few spectrophotometric methods available, the literature on extractive spectrophotometric methods for the assay of HAL is scanty, although these methods are well suited for the determination of HAL in pharmaceutical formulations.
In the present investigation, the author reports the development of three accurate, reproducible and adequately sensitive extractive spectrophotometric methods based on the formation of chloroform-soluble ion-association complexes between HAL and bromocresol purple (BCP), thymol blue (TB) or alizarin red S (ARS) in acidic buffer.

**EXPERIMENTAL**

*Equipments:*

Absorbance measurements were made as described in chapter II. The pH measurements were made on a Schott Gerate pH meter CG 804. Elemental analysis was performed on a Thermoquest elemental analyser EA 1110 CHN.

*Preparation of reagents and chemicals:*

All chemicals were of analytical reagent or pharmaceutical grade. Quartz-processed high-purity water and spectroscopic grade methanol, acetone and chloroform were used throughout.

Aqueous solution of each of BCP, TB and ARS (0.1 % w/v) was prepared separately by dissolving 100 mg of each in a small amount of acetone and then diluting up to 100 ml with distilled water.

The following buffers were prepared following the standard methods:

i. KCl–HCl buffers of pH 1.0-2.2 (by mixing appropriate volumes of 0.2 M each of KCl and HCl).

ii. NaOAc–HCl buffers of pH 0.65-5.2 from 1 M each of NaOAc and HCl.

iii. NaOAc–AcOH buffers of pH 3.72-5.57 (by mixing appropriate volumes of 0.2 M each of NaOAc and AcOH).
iv. Potassium hydrogen phthalate–HCl buffers of pH 2.2–3.6 from 0.1 M each of potassium hydrogen phthalate and HCl

Preparation of standard drug solution:

Stock solution of pure HAL (Torrent Pharmaceuticals Ltd., India) was prepared by dissolving a known amount of HAL in methanol and standardised. Different dosage forms of HAL were obtained commercially from different firms.

Recommended procedures

After a systematic and thorough study of the various parameters involved in the formation of coloured products (as described under results and discussion), the following procedures [Methods: A: BCP, B: TB and C: ARS] were recommended for the assay of HAL in bulk sample and its pharmaceutical formulations.

Analysis of pure drugs:

Method A:

Aliquots of standard drug solutions containing 5–65 µg of HAL were transferred into a series of 125 ml separating funnels. To these were added 3.0 ml of BCP and 2.0 ml of NaOAc–HCl buffer of pH 1.99. A 10 ml of chloroform was added to each of the separating funnels and shaken well. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of the yellow coloured species were measured at 408 nm against the reagent blank. The absorbance values were plotted against the concentrations of the drug (Fig. 4.1).
Method B:

Various amounts of the standard HAL solution containing 10–140 μg of HAL were transferred into a series of 125 ml separating funnels followed by 3.0 ml of TB and 2.0 ml of NaOAc–HCl buffer of pH 3.09. A 10 ml amount of chloroform was added to each of the separating funnels and shaken well. The two phases were allowed to separate and the chloroform layer was run through anhydrous sodium sulphate. The absorbances of the yellow coloured species were measured at 409 nm against the reagent blank. Calibration graph was constructed (Fig. 4.1).

Method C:

Several aliquots of standard solution containing 40–500 μg of HAL were transferred into a series of 125 ml separating funnels followed by 6.0 ml of ARS and 3.0 ml of NaOAc–HCl buffer of pH 1.09. A 10 ml of chloroform was added to each of the separating funnels and shaken well. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of the yellow coloured species were measured at 426 nm against the reagent blank. Calibration graph was plotted (Fig. 4.1).

These calibration graphs were subsequently used for the determination of HAL in pharmaceutical formulations.
Analysis of pharmaceutical formulations:

**Tablets:**

After twenty tablets were finely powdered, an amount of the powder equivalent to 20 mg of the drug was weighed into a 100 ml beaker. Seventy millilitres of methanol were added, the powder was completely disintegrated using a mechanical stirrer and the solution was filtered through a Whatman filter paper No. 40. The filtrate was diluted to the mark with methanol and mixed well. A 50 ml of the solution was further diluted to 100 ml with methanol and an aliquot was analyzed using the procedures given in Methods A, B and C.

**Injections:**

A requisite volume of the injection solution was transferred into a 100 ml calibrated flask and diluted up to the mark with methanol. An aliquot of the solution was analysed as stated earlier in Methods A, B and C.

**RESULTS AND DISCUSSION**

The formation of ion-association complexes due to the interaction between two constituents has been explained in detail in chapter I (under theory of solvent extraction). Ion-pair extraction spectrophotometry has received a considerable attention for quantitative assay of different pharmaceutical drugs\(^{15-20}\). The HAL reacts with BCP or TB or ARS in acidic buffer to form a chloroform-soluble yellow colored 1:1 ion-association complex. The probable structures of the ion-pairs may be represented as shown in Scheme 4.1. The ion-pair complexes exhibit absorption maxima at 408 nm, at 409 nm and at 426 nm for BCP, TB and ARS respectively (Table 4.1). Under the
Experimental conditions the reagent blanks showed negligible absorbance (Fig. 4.2).

**Optimum conditions for the product formation:**

The optimum reaction conditions for quantitative extraction of the ion-pair complexes were established via a number of preliminary experiments. The investigator has performed control experiments by measuring the absorbances at respective $\lambda_{\text{max}}$ values for a series of solutions keeping one parameter constant and varying the others.

**Method A:**

*Effect of buffer/pH:*

The formation of an ion-association complex and quantitative extraction depends on the type of buffer used and its pH. This was studied using various buffers *viz.*, KCl–HCl (pH 1.0–2.2), NaOAc–HCl (pH 0.65–5.2), NaOAc–AcOH (pH 3.72–5.57) and potassium hydrogen phthalate–HCl (pH 2.2–3.6). Less stable or low intense coloured complex formation was noticed with the buffers *viz.* KCl–HCl, NaOAc–AcOH and potassium hydrogen phthalate–HCl. It was also noticed that the optimum color intensity and constant absorbances were obtained with NaOAc–HCl buffer of pH 1.99 (Fig. 4.3).
**Effect of reagent:**

The optimum volume of the reagent required for quantitative formation of an ion-association complex was studied. Figure 4.4 revealed an increase in absorbance readings with the increase in volume of BCP up to 2.0 ml and remained constant in the range of 2.0-4.0 ml (Fig. 4.4). Moreover a significant increase in the absorbance of the blank was noticed at volumes larger than 4.0 ml. Hence a volume of 3.0 ml was used for the study.

**Method B:**

*Effect of buffer/pH:*

The ion-association complex formation between HAL and TB was studied in various buffers as mentioned in Method A. The NaOAc–HCl buffer was found to be more suitable compared to other buffers in terms of sensitivity. In order to establish the optimum pH range, HAL was mixed with TB in selected buffer of pH 0.65–5.2 and the absorbance of the ion-pair complex was measured. It was noticed that the maximum colour of the complex was obtained in the selected buffer of pH 3.09 (Fig. 4.3).

*Effect of reagent:*

The results of the effect of reagent given in Fig. 4.4 indicated that the colour intensity of the complex increased with increase in concentration of the reagent. The large excess of the reagent affected the stability and sensitivity of the complex. The absorbance readings remained constant in the range of 2.0-4.0 ml of 0.1 % TB. Hence, in all subsequent investigations, a 3.0 ml of TB was employed.
Method C:

Effect of buffer/pH:

The formation of the ion-association complex and quantitative extraction was investigated using various buffers as indicated in Method A. It was noticed that the optimum color intensity and constant absorbances were obtained with NaOAc–HCl buffer of pH 1.09 (Fig. 4.3).

Effect of reagent:

It was found that a volume of 4.0 ml of 0.1% ARS was necessary for maximum colour development and longer stability of the complex. The sensitivity and stability of the complex was not affected even if a little excess of the reagent was added (Fig. 4.5). But the absorbances of the blank increased significantly for more than 7.0 ml of the reagent. Hence a volume of 6.0 ml was used for the subsequent work.

Choice of extractant for Methods A, B and C:

Various water immiscible organic solvents viz., carbon tetrachloride, 1,2-dichloromethane, chlorobenzene, toluene, benzyl alcohol, chloroform, ethyl acetate, isoamyl alcohol and diethyl ether were tried for the quantitative extraction of the ion-association complexes of HAL. Chloroform was found to be the most suitable solvent as it was observed that only one extraction was sufficient. Shaking times of 0.5 to 2.0 min produced constant absorbances and hence a shaking time of 1.0 min was followed throughout.
Sequence of addition of reagents for Methods A, B and C:

It was found that no appreciable change was observed in absorbance or colour of the complexes even if the order of addition of reactants was altered.

Precision and accuracy:

The precision and accuracy of the proposed methods were checked using known amounts of HAL (within their Beer's law range). The low percentage relative standard deviation and percentage error values (Table 4.1) calculated from five replicate analyses of HAL indicated good precision and accuracy of the proposed methods.

Optical characteristics of the ion-pair complexes:

The Beer's law limits (Fig. 4.1), optimum photometric range, molar absorptivity and Sandell's sensitivity values, regression equation and correlation coefficient for all the systems are given in Table 4.1. A linear relationship was found between the absorbance at corresponding $\lambda_{\text{max}}$ and the concentration of the colored species. Regression analyses of Beer's law plots at their respective $\lambda_{\text{max}}$ values revealed a good correlation (Table 4.1). Graphs of the absorbance versus the concentration showed zero intercept and are described by a regression equation, $Y = a + bX$ (where $Y$ is the absorbance of a 1.0 cm layer, $b$ is the slope, $a$ is the intercept and $X$ is the concentration of the drug in ppm), obtained by the least-squares method.
Effect of temperature:

The effect of temperature on the stability of the complexes was studied. It was found that the complexes were stable in the temperature range 5 - 35 °C. At higher temperatures, the drug concentration was increased due to volatile nature of chloroform. As a result the absorbance values increased. Hence the studies were carried out at room temperature.

Stability of the complexes:

The ion-association complexes were found to be stable for more than 18 h at room temperature.

Studies on diverse substances/excipients:

The effects of common excipients and other substances were tested in Methods A, B and C for their possible interferences in the assay of HAL. It was observed that the talc, glucose, starch, lactose, sulphate, dextrose, acetate, phosphate and magnesium stearate did not interfere in the determination of HAL at the levels found in dosage forms.

Recovery studies:

Recovery studies were conducted by analysing each pharmaceutical formulation in the first instance for the active ingredient (HAL) by the proposed methods. Three different amounts of pure HAL were added to the previously analysed formulations and the total amount of the drug was once again determined by the proposed methods after adjusting the HAL concentration within Beer's law range. The results were found to be satisfactory.
Stoichiometry of the complexes:

The drug to reagent ratio was found to be 1:1 in all the three methods as evaluated from Job's method of continuous variation and molar ratio methods (Fig. 4.6-4.7). The complexes in solid form were also isolated from the chloroform layer by evaporating the respective chloroform layer on a water bath. The analytical data of the complexes also confirmed the composition to be 1:1. The tentative structures of the ion-pair complexes of HAL are given in Scheme 4.1.

Applicability of the proposed methods:

The reliability and suitability of the developed methods was checked by analysing various dosage forms of HAL marketed under different trade names. The results compare favorably with those of official\textsuperscript{2}/reported\textsuperscript{6} methods (Table 4.2).

Statistical analysis of the results in comparison with the official\textsuperscript{2}/reported\textsuperscript{6} method:

The results obtained by the proposed methods were statistically compared by the Student t-test and by the variance ratio F-test with those of the official/reported method. The Student t-values at 95 % confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed methods and the official/reported method. It was also observed that the variance ratio F-values calculated for \( p = 0.05 \) did not exceed the theoretical value indicating that there was no significant
difference between the precision of the proposed methods and the
official/reported method. The results are tabulated in Table 4.2.

CONCLUSIONS

A significant advantage of the extractive spectrophotometric method is
that it can be applied for the determination of individual compound in a
multicomponent mixture. The developed procedures are simple, sensitive,
rapid and inexpensive. The reagent, BCP was found to be more sensitive
compared to other reagents, TB and ARS used for the assay of HAL. Unlike
the gas chromatographic and HPLC procedures, the instrument is simple and is
not of high cost. The importance lies in the chemical reactions upon which the
procedures are based rather than upon the sophistication of the instrument.
These aspects of spectrophotometric analysis are of major interest in analytical
pharmaceutical chemistry since these offer distinct possibility in the assay of a
particular component in complex dosage formulations. The advantage of using
the proposed methods is that they work with all usual dosage forms of HAL
and the results obtained are reproducible. The procedures do not involve any
critical reaction conditions or tedious sample preparation. The wide
applicability of the new procedures for routine quality control is well
established by the assay of HAL in pure form as well as in pharmaceutical
preparations. With these, the proposed methods could be safely adapted as
additional alternate methods to the existing methods for the assay of HAL in
pure and pharmaceutical formulations especially by small scale industries.
REFERENCES


Scheme 4.1: Reaction mechanism of formation of ion-pair complexes of HAL
Fig. 4.1  Beer's law plots of HAL for ■ BCP, ▲ TB, and • ARS
Fig. 4.2: Absorption spectra of ion-pair complexes of HAL.

1. HAL (12 ppm) + TB + NaOAc–HCl buffer
2. HAL (5 ppm) + BCP + NaOAc–HCl buffer
3. HAL (34 ppm) + ARS + NaOAc–HCl buffer
4. TB + NaOAc–HCl buffer reagent blank
5. BCP + NaOAc–HCl buffer reagent blank
6. ARS + NaOAc–HCl buffer reagent blank
Fig. 4.3 Effect of pH (NaOAc-HCl buffer) on the absorbance of the ion pair complex of ▲ HAL (28 ppm)-ARS, ▼ HAL (4 ppm)-BCP and ■ HAL (7 ppm)-TB.
Fig. 4.4 Effect of volume of BCP and TB (0.1 %) on the absorbances of ion-pair complexes of
- HAL(3.5 ppm)-BCP and
- HAL (7.0 ppm)-TB

Fig. 4.5 Effect of volume of ARS (0.1 %) on the absorbances of ion-pair complex of
HAL (30 ppm)-ARS
Fig. 4.6 Composition of the ion-pair complexes of ▲ HAL-BCP ■ HAL-TB and ○ HAL-ARS by Job's method
[HAL] = [BCP] = [TB] = [ARS] = 1.0 X 10^{-3} M

Fig. 4.7 Composition of the ion-pair complexes of ▲ HAL-BCP ■ HAL-TB and ○ HAL-ARS by Mole ratio method
[HAL] = [BCP] = [TB] = [ARS] = 1.0 X 10^{-3} M
Table 4.1 Optical characteristics, precision and accuracy data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BCP</th>
<th>TB</th>
<th>ARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>408</td>
<td>409</td>
<td>426</td>
</tr>
<tr>
<td>Beer’s law limits (ppm)</td>
<td>0.5–6.5</td>
<td>1–14</td>
<td>4–50</td>
</tr>
<tr>
<td>Optimum photometric range (ppm)</td>
<td>1.7–5.7</td>
<td>2.3–12.8</td>
<td>5.2–47.1</td>
</tr>
<tr>
<td>Molar absorptivity (l mol$^{-1}$ cm$^{-1}$)</td>
<td>$4.20 \times 10^4$</td>
<td>$1.91 \times 10^4$</td>
<td>$5.01 \times 10^3$</td>
</tr>
<tr>
<td>Sandell’s sensitivity (ng cm$^{-2}$)</td>
<td>8.95</td>
<td>19.67</td>
<td>75.00</td>
</tr>
<tr>
<td>Regression equation (Y)$^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope, b</td>
<td>0.1093</td>
<td>0.0509</td>
<td>0.0138</td>
</tr>
<tr>
<td>Intercept, a</td>
<td>0.0128</td>
<td>0.0011</td>
<td>-0.0117</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9996</td>
<td>0.9993</td>
<td>0.9990</td>
</tr>
<tr>
<td>Relative standard deviation (%)$^c$</td>
<td>1.08</td>
<td>1.12</td>
<td>1.08</td>
</tr>
<tr>
<td>% Range of error$^c$ (95 % confidence limit)</td>
<td>0.36</td>
<td>0.42</td>
<td>0.59</td>
</tr>
</tbody>
</table>

$^a$ Y = a + bX where X is the concentration of HAL in ppm.

$^c$ For 5 replicate analyses within Beer’s law limits.
Table 4.2 Analysis of pharmaceutical preparations containing HAL by the proposed methods and their comparison with the official2/reported6 method

<table>
<thead>
<tr>
<th>Drugs*</th>
<th>Label claim (mg/tablet or mg/ml)</th>
<th>Official/reported method</th>
<th>Recovery ** ± SD, % and their comparison with the official/reported method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet 1a</td>
<td>10 99.10 ± 1.10</td>
<td>99.34 ± 0.79</td>
<td>99.18 ± 0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.93</td>
<td>F = 1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.05</td>
<td>t = 1.04</td>
</tr>
<tr>
<td>Tablet 2a</td>
<td>5.0 104.8 ± 0.75</td>
<td>104.09 ± 0.58</td>
<td>104.25 ± 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.67</td>
<td>F = 1.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.83</td>
<td>t = 1.95</td>
</tr>
<tr>
<td>Tablet 3c</td>
<td>5.0 102.0 ± 1.12</td>
<td>101.6 ± 0.79</td>
<td>101.79 ± 1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.88</td>
<td>F = 1.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.43</td>
<td>t = 1.03</td>
</tr>
<tr>
<td>Tablet 4b</td>
<td>1.5 103.0 ± 0.78</td>
<td>102.6 ± 0.86</td>
<td>102.87 ± 0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.21</td>
<td>F = 1.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.67</td>
<td>t = 1.54</td>
</tr>
<tr>
<td>Tablet 5c</td>
<td>1.5 106.9 ± 0.78</td>
<td>104.0 ± 0.94</td>
<td>104.15 ± 0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.45</td>
<td>F = 1.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.65</td>
<td>t = 1.92</td>
</tr>
<tr>
<td>Injection 1a</td>
<td>5.0 103.5 ± 0.93</td>
<td>103.0 ± 0.78</td>
<td>103.22 ± 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.42</td>
<td>F = 1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.71</td>
<td>t = 1.73</td>
</tr>
<tr>
<td>Injection 2b</td>
<td>5.0 101.9 ± 1.11</td>
<td>100.9 ± 1.22</td>
<td>101.29 ± 0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.21</td>
<td>F = 1.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.1</td>
<td>t = 164</td>
</tr>
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</table>

* Manufactured by a Sun pharmaceuticals, b Intas Lab. Pvt. Ltd., c Torrent Labs. Ltd.
** Average recovery from five determinations