ANALYTICAL TECHNIQUE FOR THE DETERMINATION OF DIAZEPAM IN SOLID DOSAGE FORMS

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ABSTRACT

Several methods are available in literature for the determination of percentage purity of diazepam (pharmaceutical raw material) either by non aqueous titration or spectrophotometric methods. For the quantitative determination of diazepam from the commercial pharmaceutical preparations, the current official B.P method (nonaqueous titration and spectrophotometric assay) has been utilized and a comparison has been made between results obtained from both methods. The utilization of spectrophotometric method proved to be a better method for determination of the percentage purity of diazepam pharmaceutical raw material and in the solid dosage form. The spectrophotometric method has been found to be a more rapid, accurate and gives reproducible results.

Keywords: Percent purity, Diazepam, Spectrophotometry, non aqueous titration

INTRODUCTION

Diazepam is a benzodiazepine derivative drug occurs as odorless white yellowish solid crystals having slightly bitter taste. It has a melting point of 131.5°C to 134.5°C. Its solubility is slight in water, more in alcohol and freely in chloroform. Its chemical formula is C₁₆H₁₃Cl N₂O, with molar mass of 284.7g, structurally related to quinazolines (Earley et al., 1979).

Diazepam possesses anticonvulsant, sedative, skeletal muscle relaxant properties. It binds to a specific subunit on GABA receptor, where it causes inhibitory effects through hyper polarization of post synaptic membrane. Its site of action includes limbic system, thalamus, hypothalamus and cerebral cortex. The anticonvulsant properties of diazepam are because of binding to voltage-dependent sodium channels while muscle relaxant properties are produced via inhibition of polysynaptic pathways in the spinal cord (Rosman et al., 1993).

Diazepam can be administered orally, intravenously, intramuscularly or as a suppository. When administered orally it is rapidly absorbed with fast onset of action whereas peak plasma levels are achieved in 30 minutes to 2 hours. It is widely distributed throughout the body. It crosses both blood brain barrier and the placenta and is excreted into breast milk. It is metabolized in liver through cytochrome P450 enzyme system, having biphasic half life of 1-2 and 2-5 days producing many pharmacologically active metabolites which are excreted primarily in the urine. (McLean and Macdonald, 1988).

Diazepam is mainly used to treat anxiety, panic attacks, insomnia, symptoms of acute alcohol or opiate withdrawal and status epilepticus, and is an adjunctive treatment of other forms of epilepsy. It is also used as a premedication for including sedation, anxiolysis or amnesia prior to certain medical procedures e.g. endoscopy (Date et al., 1984).

It's most common side effects of the drug are somnolence, impaired motor function, depression, impaired learning anterograde amnesia and cognitive deficits. It has possible pharmacological interactions with barbiturates, phenothiazides, narcotics and antidepressants. Whereas its use should be avoided in conditions of ataxia, severe renal deficiencies, severe

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sleep apnea, depression, myasthenia gravis and acute intoxication. It can also cause physical dependence, addiction known as benzodiazepine withdrawal syndrome. Special caution is needed for its use in pediatric, elderly patients and in pregnant woman and lactating mother (Tesar et al., 1988).

There are many analytical methods to determine the percentage purity of diazepam as the pharmaceutical raw material and in formulation, including titration, colorimetric, gravimetric method, paper, thin layer, column, ion exchange chromatography, UV spectrophotometry, flame photometry, atomic absorptions spectrometry (AAS). Therefore it is important to find out an accurate rapid and reproducible method for the quantitative analysis of diazepam.

The HPLC method for determination of diazepam injection has been carried out by (Maurice et al., 1982). The simple and sensitive liquid chromatography and mass spectrometry method for the determination of diazepam and its major metabolites in rat cerebrospinal fluid has been carried out by (Junying et al., 2003). For the analysis of multicomponent formulations containing phenylpropanolamine hydrochloride, caffeine and diazepam by using LC have been carried out by (Carola et al., 2001).

The simultaneous spectrophotometric determination of phenylpropanolamine HCL, caffeine and diazepam in tablets have been carried out by (Carola et al., 2002). The research on molecularly imprinted solid-phase extraction of diazepam and its metabolites from hair samples was conducted by Marinah et al., 2007). The comparison of capillary electrophoresis and reversed-phase liquid chromatography methodologies for determination of diazepam in pharmaceutical tablets has been carried out by Maria et al., 2005). The High-performance liquid chromatographic method for the determination of benzodiazepines in plasma or serum using the column-switching technique has been carried by Anissa et al. (2000).

MATERIALS AND METHODS

Chemicals and reagents
Perchloric Acid, Glacial Acetic Acid, Acetic Anhydrides, Nile Blue Solution, 1-Naphthol Benzine (0.2 % w/v in glacial acetic acid), Potassium Hydrogen Phthalate, 0.1 N Perchloric acid, 0.1 N H₂SO₄ and diazepam pharmaceutical raw material as well as commercial samples.

Non aqueous titration method
Preparation of 0.1 N perchloric acid solution
Perchloric Acid (72%, 8.5 ml) was added slowly to glacial acetic acid (900ml) with continuous mixing. Similarly the acetic anhydride (30ml) was added, volume was adjusted to one liter with glacial acetic acid allowed the solution to stand for twenty four hours before use. This was approximately 0.1 N perchloric acid.

<p>| Table I: Different commercial brands of diazepam used |
|-----------------------------------------------|-------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Brands</th>
<th>Strength</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>2mg, 5mg</td>
<td>Ethical Laboratories (Pvt.), Ltd.</td>
</tr>
<tr>
<td>Valium</td>
<td>2mg, 5mg</td>
<td>Roche Pakistan Ltd.</td>
</tr>
<tr>
<td>Valipam</td>
<td>2mg, 5mg</td>
<td>Kurative Pak (Pvt.) Ltd.</td>
</tr>
<tr>
<td>Eruopam</td>
<td>2mg, 5mg</td>
<td>Euro Pharma International (Pvt.) Ltd.</td>
</tr>
<tr>
<td>Relaxan</td>
<td>2mg, 5mg</td>
<td>CKD Pharmaceuticals Pak (Pvt.), Ltd.</td>
</tr>
<tr>
<td>Relaxipam</td>
<td>2mg, 5mg</td>
<td>Epla Pharmaceuticals Pak (Pvt.), Ltd.</td>
</tr>
<tr>
<td>Unipam</td>
<td>2mg, 5mg</td>
<td>Unisons Chemical Works (Pvt.), Ltd.</td>
</tr>
<tr>
<td>Neppam</td>
<td>2mg, 5mg</td>
<td>Ferozsons Laboratories (Pvt.), Ltd.</td>
</tr>
</tbody>
</table>

Standardization of 0.1 N perchloric acid

Perchloric acid solution was standardized against potassium hydrogen phthalate. Potassium hydrogen phthalate (0.5 g) was weighed accurately into a 100 ml conical flask and glacial acetic acid (25 ml) was added and then it was warmed until the salt was dissolved. The titration was carried out with 0.1 N perchloric acid using two drops of 1-naphthol benzine indicator till the color changed to green. Each ml of 0.1 N HClO₄ is = 0.02041 g of potassium hydrogen phthalate.

Assay of diazepam raw material
Dissolve 0.500g in 50ml of acetic anhydride R. Using 0.3 ml of Nile blue A solution R as indicator, titrated with 0.1 N perchloric acid until a yellowish green color was obtained. One ml of 0.1 N perchloric acid is equivalent to 28.47 mg of C₁₂H₁₃ClN₂O₄ or each ml of 0.1N HClO₄ is = 0.02847 g of diazepam.

Spectrophotometric method
The percent purity of diazepam was determined by spectrophotometric method. The different concentrations of pure raw material were made in 0.1 N, H₂SO₄ (sulphuric acid) and the absorbance of the solution was taken using Spectrophotometer at 284nm and percentage purity was calculated by using the following formula.

\[
\text{Percent purity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard } E_{1\%}^{\text{cm}}} \times 100
\]
Similarly various samples of diazepam tablets were analyzed by using this method.

**RESULTS AND DISCUSSION**

This study was carried out to compare the percent purity of diazepam as raw materials and active ingredient in the commercial preparations. For this purpose, eight brands of diazepam were purchased from the local market. The drug contents were determined using the non-aqueous titration and spectrophotometric methods. The findings for the percent purity of diazepam are given in Table II. In case of non-aqueous titration, the percent purity ranged from 99.6 to 101.3% as compared to 100 to 102.20% obtained from spectrophotometric method. The comparison of the percent purity values obtained from the both methods has been given in Table III.

<table>
<thead>
<tr>
<th>Sample weight (g)</th>
<th>Volume used (ml)</th>
<th>Percentage Purity %</th>
<th>Concentration (%)</th>
<th>Absorbance</th>
<th>Percentage Purity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>17.6</td>
<td>100.2</td>
<td>0.001</td>
<td>0.450</td>
<td>100.00</td>
</tr>
<tr>
<td>1.0</td>
<td>35.4</td>
<td>101.3</td>
<td>0.002</td>
<td>0.920</td>
<td>102.20</td>
</tr>
<tr>
<td>1.5</td>
<td>52.9</td>
<td>99.6</td>
<td>0.003</td>
<td>1.351</td>
<td>100.07</td>
</tr>
<tr>
<td>2.0</td>
<td>70.5</td>
<td>100.2</td>
<td>0.004</td>
<td>1.801</td>
<td>100.05</td>
</tr>
<tr>
<td>2.5</td>
<td>88.1</td>
<td>100.2</td>
<td>0.005</td>
<td>2.251</td>
<td>100.04</td>
</tr>
</tbody>
</table>

**REFERENCES**


**CONCLUSION**

The best method for diazepam assay in pharmaceutical raw material and in solid pharmaceutical dosage forms was the spectrophotometric method since this method was accurate, sensitive and rapid as compared to other method. Therefore, this method can be used both in raw material as well as commercially available solid dosage form.


