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RESEARCH ARTICLE

# *Development and Validation of 7-Chloro-1-Methyl-5-Phenyl-3h-1,4 Benzodiazepin-2-One By RP HPLC*

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## ABSTRACT

7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one is the well-known psychoactive drug of class benzodiazepam. A simple, precise, accurate, reproducible RP-HPLC method was developed and validated for the simultaneous estimation of 7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one in bulk and in pharmaceutical dosage forms. Chromatographic separation was carried out on a Thermo-hypersil C8 column (250mm×4.6mm i.d,5µm) utilizing a mobile phase consisting of acetonitrile and 0.01M ammonium phosphate buffer (pH adjusted to 3.0 with ortho phosphoric acid) in the ratio of 55:45 v/v at a flow rate of 1ml/min with UV detection at 242nm. The retention times of 7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one were 2.23 min. The developed method was validated in terms of selectivity, sensitivity, accuracy, precision, linearity, specificity, limit of detection and limit of quantification. The linear range was found to be 20-100 µg/ml.

## KEYWORDS

7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one, selectivity, sensitivity, Reverse Phase HPLC, validation, psychoactive drug

## INTRODUCTION

Benzodiazepines (BZDs) represents one of the important and highly explored class of seven-membered aromatic heterocycles containing two ring nitrogen that are critical for numerous applications in the pharmaceutical industry and organic synthesis of complex molecules [1]. Due to their diverse spectrum of biological activities, they were considered as “privileged structures” in medicinal chemistry.

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Further, they are key synthons for the synthesis of various fused ring compounds [2,3]. As such, further development of BZDs has gained significant attention of organic and medicinal chemists in a quest to discover new and efficacious benzodiazepine based therapeutic agents [4].

7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one have been majorly prescribed in many parts of the world; first as anxiolytics and then as hypnotics [5]. They are extensively indicated for various CNS disorders such as anxiolytics (chlordiazepoxide and diazepam), anticonvulsants (clonazepam, and clobazam), muscle relaxants, anesthesia (midazolam) and insomnia, for some motor disorders and in psychoses (olanzapine and clozapine) [6]. The benzodiazepines were classified as short-acting, intermediate acting and long-acting depending upon their duration

of action [7]. The BDZs exert their effect by binding to the central benzodiazepine receptors which are located at the post and presynaptic membranes. However, certain side effects are associated with the short- and long-term use of benzodiazepines which includes confusion, drowsiness, amnesia, and ataxia [8].

Following the guidance provided by structure-activity relationship studies, compounds with high potency and expanded spectrum of activity, improved absorption, and distribution properties were synthesized and biologically evaluated in various disease areas. Benzodiazepine derivatives were reported to possess various pharmacological activities such as antimicrobial, anticancer, anti-anxiolytic, antidepressant, anticonvulsant, antitubercular, anti-inflammatory, analgesic, antihistaminic and anti-anxiety activities [9-11]. Various benzodiazepine-based compounds have different groups or substituents attached to their core structural motif at positions 1, 2, 5, or 7 respectively [12]. These different side groups affect the binding properties of molecules with the relevant target proteins or receptors (such as BET) and hence modulate their pharmacological properties, the potency of biological response and the pharmacokinetic profile [13].

In presence study, we have reported method for development and validation of 7-Chloro-1-Methyl-5-Phenyl-3h-1,4-Benzodiazepin-2-One by well-known RP-HPLC method.

## 1.2 Experimental Part

### 1.2.1 Apparatus and Software

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD20AV absorbance detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 µl. Data acquisition and integration was performed using Spinchrome software (Spincho biotech, Vadodara). The chromatographic elution of analyte was obtained by using Thermo-hypersil C8 column (250mm×4.6mm i.d,5µm).

### 1.2.2 Reagents and Chemicals

7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one was provided as gift sample from Samir Tech Pvt. Ltd. Vadodara, India. HPLC grade Acetonitrile and Ortho Phosphoric Acid, Dipotassium monohydrogen phosphate AR grade was purchased from Samir Tech Pvt. Ltd. Vadodara, India. The pharmaceutical samples used in the present study include 7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one 5% ointment.

### 1.2.3 Chromatographic Conditions

The mobile phase comprised of Acetonitrile: Dipotassium monohydrogen phosphate buffer pH 3.2 in the proportion of 55:45. Resulting solution was degassed by ultrasonication for 10minutes.

### 1.2.4 Preparation of Standard Solution Of 7-Chloro-1-Methyl-5-Phenyl-3h-1,4-Benzodiazepin-2-One[14]

Stock solution of (1000 µg/ml) was prepared by accurately weighing 10 mg of 7-Chloro-1-Methyl-5-Phenyl-3h-1,4-Benzodiazepin-2-Onein 10 ml volumetric flask. The drug was dissolved in Acetonitrile and the solution was diluted to volume. Further dilutions were made from this stock solution and the injection volume was kept 20 µL. A calibration curve was plotted between concentrations against their respective area for 7-Chloro-1-Methyl-5-Phenyl-3h-1,4-Benzodiazepin-2-One. From the calibration curve, it was found that linearity range is between 20- 100ug/ml.

## 1.3 Analysis of Marketed Formulation Extraction Procedure:

An amount equivalent to 10mg [0.2g for Ointment [15](5%), 0.5g for Gel (2%), and 0.46ml for Injection (2%), 0.1g equivalent to four sprays for Aerosol (10%)] was taken and

dissolved in 10 ml of ACN to get 1000ug/ml of stock concentration. The stock solution was sonicated for 10 minutes and was filtered through Whatman filter paper. From the stock solution 0.6ml was taken in 10 ml volumetric flask. The volume was made up to the mark with ACN to get solution of 60ug/ml. The solution was finally filtered through 0.2um syringe filter was injected into HPLC Transdermal patch (5%). An amount equivalent to 10 mg(0.2g) was taken in 10 ml of Dipotassium monohydrogen phosphate buffer 10 mM of pH3.0 and was magnetically stirred for 2 hours. The solution was then sonicated for 15 minutes and was then filtered through Whatman filter paper. From this stock solution 0.6 ml was taken in 10 ml volumetric flask. The volume was made up to the mark with ACN to get solution of 60ug/ml. The solution was finally filtered through 0.2um syringe filter was injected into HPLC

## 1.4 Result and Discussion

### 1.4.1 Optimization of Chromatographic Conditions

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, ratio of mobile phase and flow rate were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and theoretical plates were calculated. Finally, a simple and inexpensive method was developed by using a combination of Acetonitrile and Dipotassium monohydrogen phosphate buffer in ratio 55:45. Optimized chromatographic conditions are listed in Table 1.

| METHOD PARAMETER | OPTIMIZED VALUE |
|---|---|
| **COLUMN** | Thermo-hypersil C8 column (250mm×4.6mm i.d,5µm) |
| **MOBILE PHASE** | Acetonitrile and 0.01M ammonium phosphate buffer (pH adjusted to 3.0 with ortho phosphoric acid) in the ratio of 55:45 v/v |
| **FLOW RATE** | 1 ml/min |
| **RETENTION TIME tR (MINUTES)** | 2.23 min. |
| **DETECTION WAVELENGTH(nm)** | 242 |
| **TEMPERATURE** | Ambient |
| **INJECTION VOLUME** | 20uL |
| **TAILING FACTOR** | 1.3±0.019 |
| **THEORETICAL PLATES(N)** | 11935±88.33 |

### 1.4.2 Method Validation [16].

#### 1.4.2.1 Linearity

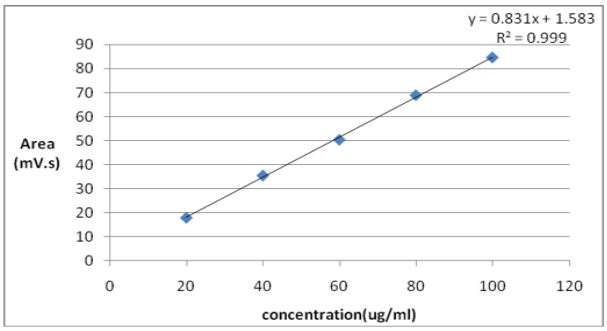


**Figure: 1 Calibration curve of 7-Chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-one**

The calibration curve was constructed by plotting concentrations of 7-Chloro-1-Methyl-5-Phenyl-3h-1,4-Benzodiazepin-2-Oneversus peak areas, and the regression equations were calculated. The linearity of the method was investigated by using concentrations in the range20-100µg/ml.Retention time for 7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-One was found to be2.23 min.. The linear regression equation is Y= 0.831x+1.583(r2=0.999). The plot obtained from linear regressions given in Figure 1.

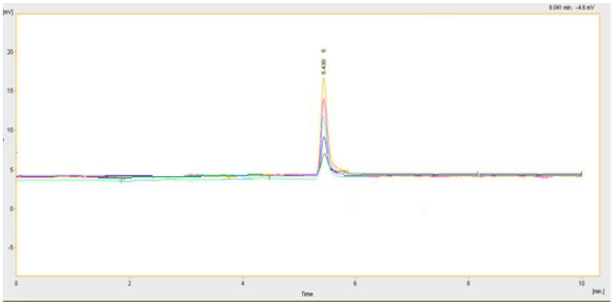

**Figure 2: Chromatogram of 7-chloro-1-methyl-5-phenyl-3H-1,4-Benzodiazepin-2-oneshowing linearity in range 20-100ug/ml at tR 5.43±0.03**

### 1.4.2.2 Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively, where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve[16].The LOD and the LOQ for HPLC were found to be 1.54ug/ml and 4.68ug/ml.

### 1.4.2.3 Precision

The precision of the proposed method was assessed as intraday and inter day precision. Three replicate injections of specific standard at various time intervals on the same day were injected into system for intraday precision and were repeated on three different days for Inter day precision. The % RSD (Relative Standard Deviation) of the results was calculated

**Table 2: Intraday precision of7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-One**

| CONC. (µg/ml) | MEAN AREA (mV.s) | SD | %RSD |
|---|---|---|---|
| 40 | 35.31 | 0.312 | 0.88 |
| 60 | 50.71 | 0.466 | 0.92 |
| 80 | 68.92 | 0.451 | 0.65 |

**Table 3: Inter day precision of7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-One**

| CONC. (µg/ml) | MEAN AREA (mV.s) | SD | %RSD |
|---|---|---|---|
| 40 | 35.23 | 0.351 | 0.99 |
| 60 | 50.63 | 0.611 | 1.20 |
| 80 | 68.63 | 0.650 | 0.94 |

### 1.4.2.4 Accuracy

Accuracy of the method was studied using standard addition method at three different levels (80, 100, and 120%) by recovery experiments. Known amounts of standard solutions containing 7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-One (48, 60,72ug/ml) were added to one of the marketed formulations of concentration 60 ug/ml to reach 80%, 100% and 120% levels. Percentage Recovery was the mean of three determinations at each standard addition level

**Table 4: Accuracy data of 7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-One**

| % SPIKING | CONC TEST (ug/ml) | CONC ADDED (ug/ml) | CONC RECOVERED (ug/ml) | % RECOVERY ± STANDARD DEVIATION |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 80 | 60 | 48 | 48.8 | 101.3±0.55 |
| 100 | 60 | 60 | 59.6 | 99.3±0.42 |
| 120 | 30 | 72 | 72.9 | 101.6±0.42 |

**1.4.2.5 Analysis of Marketed Formulation [6]**

When the 7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-Onemarketed formulation was analyzed by these proposed HPLC method, sharp peaks were obtained at tR 5.43 minutes, when scanned at 263nm. The amount of the label claim measured is given in table 6, all the formulations are within the limits (95%-105%), for patch the limits are (90%-110%)

**Table 6: Assay results of marketed formulation**

| Sr.no | Formulation | % Assay |
|---|---|---|
| 1 | Ointment | 102.1 |
| 2 | Gel | 99.1 |
| 3 | Injection | 100.1 |
| 4 | Aerosol | 99.6 |
| 5 | Patch | 95 |

## CONCLUSION

The proposed reverse phase high performance liquid chromatography method has been developed for the analysis of 7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-Onein their marketed formulation. The method was validated as per ICH guideline. % Assay values of marketed formulation were found to be in the prescribed range. Thus, the proposed HPLC method can be used for routine quality control analysis of 7-Chloro-1-Methyl-5-Phenyl-3H-1,4-Benzodiazepin-2-Onefrom its various Pharmaceutical dosage forms.

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