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Development and Validation of A RP-HPLC Method For Estimation of Flunitrazepam In A Tablet Dosage Form

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Abstract: *A simple, sensitive, and precise high performance liquid chromatographic method for the analysis of Flunitrazepamhas been developed and validated for the determination of compound in commercial pharmaceutical products. The compounds were well separated on BDS Hypersil C18 reverse phase column by the use of a mobile phase of mixed phosphate buffer and acetonitrile in a ratio of 40:60 v/v, at a flow rate of 1.0 ml/min with detection wavelength at 248nm. The method was validated in terms of linearity, precision, accuracy, and specificity, robustness and solution stability. The method does require only 10 minutes as runtime for analysis which prove the adoptability of the method for the routine quality control analysis of the drug.*
Key words:*Flunitrazepam, RP-HPLC.*

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I. Introduction

Flunitrazepamis chemically 5-(2-Fluorophenyl)-1-methyl-7-nitro-1,3-dihydro-2H-1,4-benzodiazepin-2-one. A benzodiazepine with pharmacologic actions similar to those of diazepam that can cause anterograde amnesia. Flunitrazepam, also known as Rohypnol among other names, is an intermediate acting benzodiazepine used in some countries to treat severe insomnia and in fewer, early in anaesthesia. Benzodiazepines bind non-specifically to benzodiazepine receptors BNZ1, which mediates sleep, and BNZ2, which affects muscle relaxation, anticonvulsant activity, motor coordination, and memory. It also impairs psychomotor functions similar to other benzodiazepines.

Wavelength selection:
Since the detector selected was UV detector, UV scans Flunitrazepamto determine the detection wavelength.

Procedure:
Weigh accurately 100 mg of standard Flunitrazepamand dissolve it in 100 ml of diluent to get a concentration of 1mg/ml. The prepared solutions was scanned in UV region of 200-400nm. The best possible wavelength were chosen as 248nm Flunitrazepamby using UV spectrophotometer.

Preparation of analytical solutions
Preparation of mixed phosphate buffer solution:
A weighed quantity of 1.1818 g of potassium dihydrogen-o- phosphate (KH_2PO_4) and 0.218 g of dipotassium hydrogen-ortho-phosphate (K_2HPO_4) taken in a 500ml volumetric flask. This is added with 500ml of HPLC water and mixed in an ultra sonicator. The solution pH is adjusted to pH - 3 with orthophosphoric acid.

Preparation of mobile phase:
Mobile phase was prepared by mixing 400ml of mixed buffer solution with 600ml of acetonitrile (40:60 v/v) ratio.

Preparation of a standard Flunitrazepamsolution:
The standard stock solution of Flunitrazepamwas prepared by taking 15 mg of standard drug and transferred in to a 100ml volumetric flask .To which add 10ml of methanol and shake to dissolve. The final volume is made up to 100ml with mobile phase of mixed phosphate buffer pH-3: acetonitrile (40:60) ratio. The concentration of the above solution was found to be 15µg/ml.

Preparation of test solution (marketed formulation):

20tablets were weighed and the average weight is calculated. Take the weight equivalent to and 25mg of Flunitrazepamin to 100ml volumetric flask .The volume is made up to 100 ml with mobile phase. The concentration of the above solution was 25µg of Flunitrazepam HCl. The solution was sonicated for complete solubility.

Assay of marketed formulation:

By using the optimised RP-HPLC method the analysis of the marketed formulation should be done. The procedure is as follows.

Procedure:

The replicate injections of standard were injected in to the system. Then injected test solution in duplicate. The chromatograms were recorded for all injections. From the chromatograms the average area of the peaks was taken for standard and test sample. Then assay percentage can be calculated by using the formula.

Method validation

The method was validated for several parameters like Linearity, Accuracy, Precision, Robustness according to ICH guidelines5,6.

II. Result And Discussion:

Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and / or degradation products and/or impurities.

The prepared mobile phase and sample solutions were injected in to the system and the obtained chromatograms are as follows.

Linearity is the ability to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of regression line.

To study the linearity of the drug the test sample was prepared in different concentrations like 3,6,9,12,15 and 18µg/ml for Flunitrazepam.

Range:

The range of the method is the interval between the upper and lower levels of an analyte that have been determined with acceptable precision, accuracy and linearity. The range for the Flunitrazepamwas found to be 80-120µg/ml.

Accuracy:

The test for accuracy is intended to demonstrate the closeness of agreement between the value found & the value that is accepted as a conventional true value or as an accepted reference value. Accuracy should be assessed using a minimum of nine determinations at a minimum of three concentration levels covering the specified range.

Concentrations containing 80, 100, and 120% solutions were prepared and injected and the chromatograms were recorded. The prepared solutions were injected in to the system and the obtained chromatograms were recorded.

Precision of a method is the extent to which individual test results of multiple injections of standards agree the measured standard deviation. It can be divided into 3 categories.

Method precision: 20tablets were weighed. Take the weight equivalent to 15 mg of Flunitrazepamin to 100ml volumetric flask and diluted to get a concentration of 15µg of Inject the solution in 5 times in to the system.

System precision:

Take the weight equivalent to 15 mg of Flunitrazepamin to 100ml volumetric flask and diluted to get a concentration of 15µg of Flunitrazepam. Inject the solution in 5 times in to the system.

Ruggedness (intermediate precision)

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc.

Limit of Detection

The limit of detection is determined by the analysis with known concentration of analyte and by establishing that minimum level off which the analyte can reliably detected.

Limit of Quantification

Limit of quantification as the lowest concentration of analyte that can be determined with acceptable accuracy and precision by the analytical method.

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. For this study the experimental conditions like flow rate of mobile phase and wavelength were changed and the obtained results were compared

System suitability is defined by ICH as the" checking of a system, before or during analysis of unknowns, to ensure system performance". System suitability criteria may include such factors as plate count, tailing, retention, and/or resolution.

It should also include a determination of reproducibility when a system suitability sample is run. System suitability testing is an integral part of many analytical procedures.

The six replicate injections of standard solution were injected in to the system. The chromatograms were recorded for all injections.

III. Summary and Conclusion

The present study having the description of a new RP-HPLC method development and validation for the estimation of Flunitrazepamin pharmaceutical tablet dosage form. Literature survey revealed that, the RP-HPLC method was not reported so far for the estimation of Flunitrazepamin bulk and its tablet dosage form. Considering the fact, an attempt was made to develop a simple, accurate and precise RP-HPLC method.

A reverse phase assay using a mobile phase of mixed phosphate buffer & acetonitrile in a ratio of (40:60), a BDS Hypersil column (250 x 4.6mm) and ultraviolet detection at 248 nm is employed for the quantization of Flunitrazepamin its tablet formulation. Chromatographic specificity and performance characteristics for procedures are described.

The quantitative results obtained were subjected to statistical analysis to find out the standard deviation values are below 2%, indicating the precision of methodology and low standard error values show the accuracy of the method. The validation of the proposed RP-HPLC method was further confirmed by recovery studies and validated studies.

From the result of the proposed method it is evident that the developed method was simple, specific, selective, accurate, precise, system suitable, economic and can be employed in the routine analytical for the estimation of Flunitrazepam in API and Pharmaceutical tablet dosage form.

Acknowledgement

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Fig 1:UV Spectra of standard Flunitrazepam

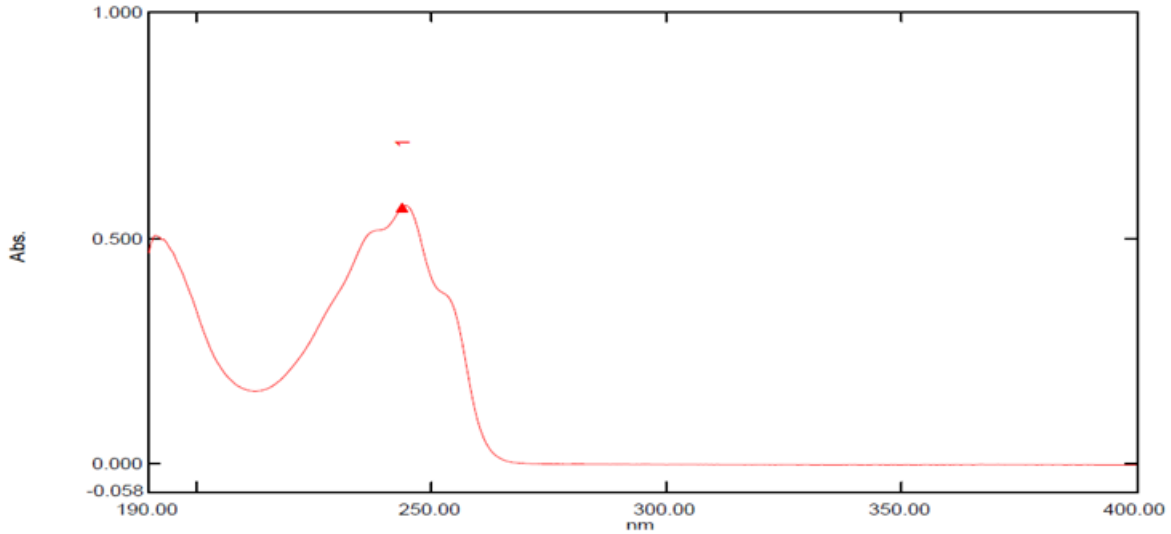
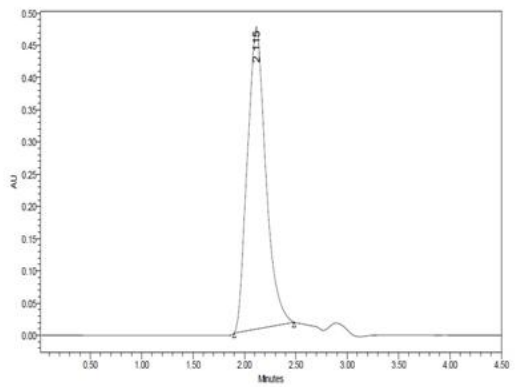


Table 1: Assay of standard Lamotrigine

S. No	Parameters	FLUNITRAZEPAM
1.	Standard area	787.52
2.	Sample area	157.532
3.	Standard wt	15mg
4.	Sample wt	25mg
5.	Label claimed	25mg
6.	Standard purity	99.69%
7.	Avg wt	250mg
8.	Assay	100.46%

F-2Chromatogram for standard FlunitrazepamChromatogram for SampleFlunitrazepam



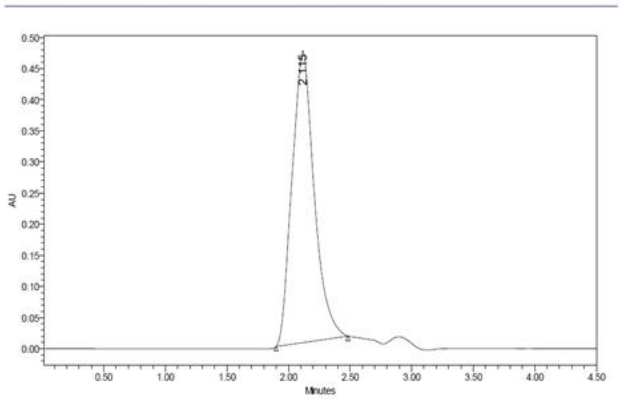


Table 1: Assay of standard Lamotrigine

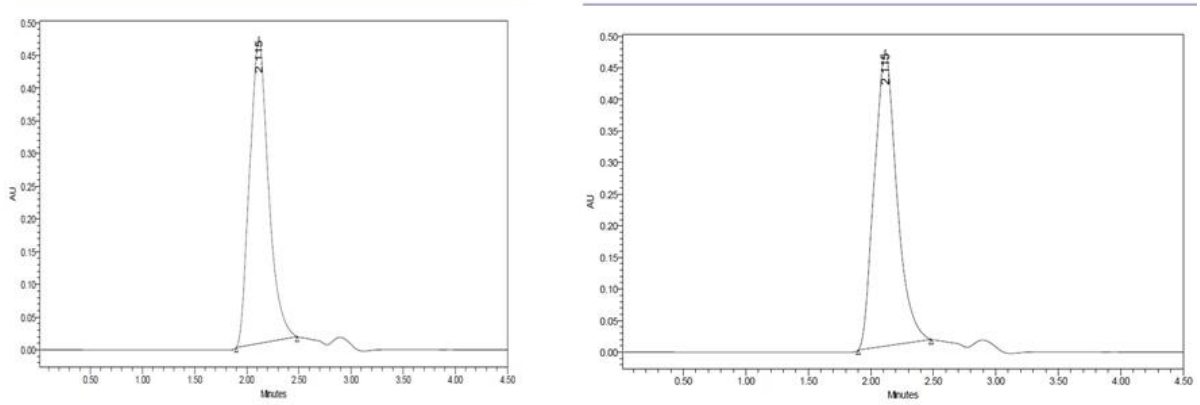


Table 2 : Linearity results ranging from 3-18 microgram/ml

S. No.	Conc. (µg)	Area
1	3	68.577
2	6	136.152
3	9	189.157
4	12	228.469
5	15	284.891
6	18	332.874
Correlation coefficient		**0.997**

Name	Area 1	Area 2	Area3	Avg area	Std V	%RSD
Flunitrazepam15µg	278.6	279.548	279.254	279.134	0.485259	0.173844

Table 3: List of accuracy of standard Flunitrazepam

Name of sample	Accuracy at 80%	Accuracy at 100%	Accuracy at120%
Sample-1	261.957	318.392	368.235
Sample-2	262.788	319.924	369.831
Sample-3	261.659	317.096	366.913
Avg area	262.1347	318.4707	368.3263
Microgram recovery	12.67785	17.11386	19.79298
Percentage recovery	93.90997%	103.7204%	101.5025%
Mean of % recovery	**99.01173%**		

Table 4: List of accuracy resultsFlunitrazepam

S.No	Retention time	Area
1.	2.517	281.641
2.	2.523	281.961
3.	2.517	281.057
4.	2.517	282.752
5.	2.547	281.34
AVG	2.524	281.950
STDEV	0.0052154	7.9134409
%RSD	**0.1599216**	**0.3076973**

Table 4: List of Precision results of Flunitrazepam

S.No	Persons	Retention time	Area
1	**Analyst 1**	**2.115**	**280.452**
2	**Analyst 2**	**2.110**	**283.624**
3	**Average**	**2.52**	**282.638**
4	**STDEV**	**0.0042**	**2.2429**
5	**%RSD**	**0.166**	**0.735**

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