

NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR FLURAZEPAM IN PURE AND CAPSULE DOSAGE FORM

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ABSTRACT

We report a new, sensitive, fast, precise, reversed phase-high performance liquid chromatography method for the determination of flurazepam in (capsule) dosage form. The developed method is validated by measuring the linearity, precision, limit of detection (LOD), robustness and ruggedness, drug recovery and the system suitability parameters. The HPLC conditions are; methanol:acetonitrile (80:20 v/v) mobile phase, Chromosil C₁₈ column (250 mm x 4.6 mm x 5 μm) stationary phase, pump pressure (6.5 MPa), flow rate (0.9 ml/min) and the detection wavelength of 230 nm. The measured flurazepam retention time is 5.27 minutes. The limit of detection is 0.05 μg/ml. The linearity range measured is from 20-200 μg/mL with a correlation coefficient (R²=0.9991). The measured parameters indicate the developed method is useful in determination of flurazepam in pure and capsule dosage form.

Keywords: *Flurazepam; RP-HPLC method; Capsule dosage form; UV detection.*

INTRODUCTION

The systematic (IUPAC) name of flurazepam is 7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one. Flurazepam (Fig.1) chemical formula is C₂₁H₂₃ClFN₃O and its molar mass is 387.88 g/mol^{1,2}. It is one of the benzodiazepine derivatives which possess anxiolytic, anticonvulsant, sedative and skeletal muscle relaxant properties. Kinetics, brain uptake and receptor binding characteristics of flurazepam are also studied³.

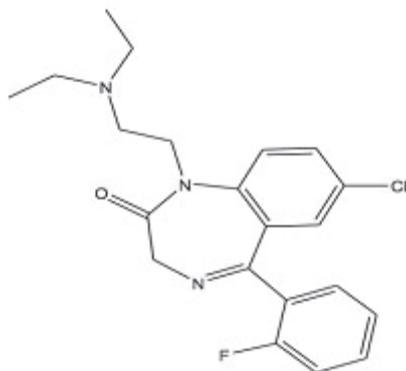


Fig. 1: The chemical structure of flurazepam.

Flurazepam has been investigated as a single drug⁴, with its metabolites⁵⁻⁹ and in combination with other drugs¹⁰⁻¹³. It was determined in plasma⁵, human plasma⁶, human blood plasma⁷, serum⁸ and mouse and rat plasma⁹. Different methods have been employed to determine flurazepam in pure form and in combination by gas liquid chromatography⁵, electron-capture gas chromatography¹², gas chromatographic-mass spectro-

metric method⁹, second derivative spectrophotometry¹¹, high-performance liquid chromatography^{6-8,10,12,13} and PVC membrane sensor method⁴.

In this work, we present development of RP-HPLC method for flurazepam in pharmaceutical dosage form and validate the method by following the ICH guidelines¹⁴ with parameters -linearity, robustness, specificity, ruggedness and precision.

MATERIALS AND METHODS

Materials

All the chemicals used for the reported work like methanol, water and acetonitrile were of HPLC grade. They were procured from Merck Limited, Mumbai, India. Formulations of the drug were purchased from local market.

HPLC instrumentation

For quantitative estimation of flurazepam using RP-HPLC method, an isocratic PEAK HPLC instrument with Chromosil C₁₈ column (250 mm x 4.6 mm, 5 μm) was employed. It was equipped with a LC 20AT pump for solvent delivery and a variable wavelength programmable ultraviolet (UV) - visible detector (SPD-10 AVP). A 20 μL Hamilton syringe was used for injecting the sample solutions. The chromatograms were recorded using the PEAK software and the obtained results were analyzed with Microsoft excel software. The maximum absorption wavelength of flurazepam was determined by an UV-visible spectrophotometer (Techcomp UV 230D) with

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HITACHI software. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Denver SI234 balance was used for weighing the materials.

Preparation of standard solution

The standard (stock) solution for the studies was prepared from 10 mg of flurazepam drug. It was dissolved in 10 ml of methanol in a 10 ml volumetric flask. It was sonicated for 2 minutes to dissolve completely and was filtered through a 0.45 μ m nylon membrane filter paper (Ultipore®, Ultra pore, Mumbai, India). A 1000 μ g/ml standard solution was thus prepared. From this solution required concentrations by proper dilution were prepared.

Method development

Mobile phase volume ratio was developed as a first step of the method development. The choice of the mobile phase depends mainly on the drug under investigation. Standard organic solvents methanol, acetonitrile in pure form were tested separately as mobile phase. These solvents were chosen as they have properties of good compatibility with HPLC systems and their availability. Furthermore, they have lower UV cut off wavelength. From the results of the trial runs, methanol gave better result; hence it is more in volume than acetonitrile. Then different volume ratios of methanol and acetonitrile were tried and finally the 80:20 v/v showed a sharper chromatogram, high theoretical plates and low tailing factor. Hence, we choose this as an optimal mobile phase for the present study.

After optimization of the mobile phase volume ratio, pH of the solution was checked with a pH meter and its value was 4.9. Different concentrations of the flurazepam solution were used at different wavelengths for determining the optimum wavelength.

Optimum flow rate was determined by running the solutions at different flow rates. Keeping in mind the recommended flow rate for the used column with a given internal diameter, the flow rate was also optimized. The active pharmaceutical ingredient (API) concentration was 140 μ g/ml. Thus the finally optimized RP-HPLC conditions used for the present studies were; pH 4.9, detection wavelength 230nm, mobile phase-methanol: acetonitrile (80:20 v/v), pump pressure 6.5 \pm 0.5 MPa, flow rate 0.9 ml/min, run time 10 minutes, peak area was 1149612.8 mAU.

RESULTS AND DISCUSSION

The flurazepam detection wavelength was obtained from spectrophotometric method. The spectra from diluted solutions of flurazepam were recorded separately with an UV spectrophotometer from 200 nm to 400 nm. Fig.2 shows flurazepam absorption spectrum with X-axis as wavelength (nm) and Y-axis as absorbance (%). The maximum absorbance was observed at 230 nm.

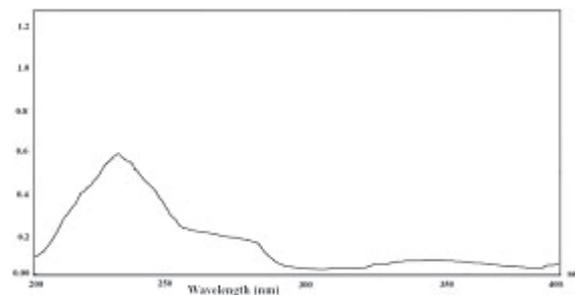


Fig. 2: Absorption spectrum of flurazepam. The maximum absorption was at 230 nm.

The developed method was validated in accordance with ICH guidelines¹⁴. Parameters such as specificity, linearity, precision, accuracy, recovery, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ) were validated. Furthermore the system suitability was also evaluated.

Specificity

Using the standard chromatographic conditions, flurazepam retention time was measured. Peak interference from the excipients was almost non-existent. The mobile phase for flurazepam was methanol and acetonitrile in the ratio (80:20 v/v). Chromatogram of the standard solution of flurazepam is shown in Fig. 3. The measured retention time was 5.27 minutes for a runtime of ten minutes. Furthermore, the system suitability was evaluated with the theoretical plate number and tailing factor. The theoretical plate (TP) numbers in the present study was 5718 and a factor of 2 more than the recommended value and tailing factors have to be less than 2, which was 1.44 for the presented results.

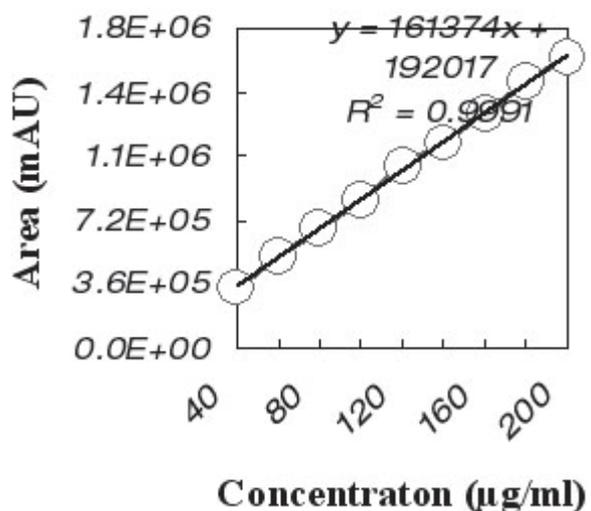


Fig. 3: Chromatogram of the standard solution of flurazepam. The retention time was 5.27 minutes for ten minutes runtime.

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Limit of Detection (LOD) and Limit of Quantification (LOQ)

The parameters, limit of detection and limit of quantification for flurazepam were calculated from the calibration equations. These were determined from the sensitivity during linearity measurements and were calculated from the relation $LOQ=3.3 \times LOD$. The limit of detection was 0.05 $\mu\text{g/ml}$ and the limit of quantification was 0.15 $\mu\text{g/ml}$, respectively.

Linearity

The linearity of the peak areas of flurazepam was determined for ten different concentrations (20-200 $\mu\text{g/ml}$ in steps of 20 $\mu\text{g/ml}$). Calibration graph was constructed by plotting the peak areas as Y-axis and the corresponding ten concentrations ($\mu\text{g/ml}$) as X-axis (Fig.4). A linear function was used to fit the data and the linear regression was tested. It was found to be precise from the value of the correlation coefficient $R^2 = 0.9991$.

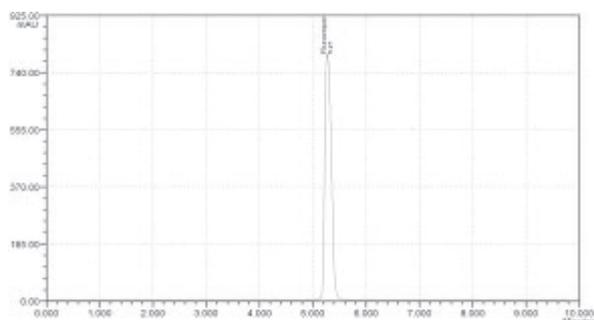


Fig. 4: Linearity results of flurazepam concentration ($\mu\text{g/ml}$) versus peak area (mAU).

Precision

This is one of the most commonly validated parameters for drugs under study through intra-day and inter-day measurements. The repeatability of flurazepam drug was studied with six ($n=6$) samples. This was evaluated by measurement of the peak area for each sample and comparing the relative standard deviation (RSD).

Intra-day precision was studied at 140 $\mu\text{g/ml}$ of flurazepam for all the six samples on the same day while for inter-day precision the same concentration was used for three successive days in a week. The data obtained from intra-day and inter-day measurements for precision checks are presented in Table 1. The peak area given in the table is the normalized mean area of six samples. The normalization was done to one of the peak areas of the six samples. Then the mean was determined. Also given are the standard deviation (SD) of the mean and their RSD. The RSD of all the samples from intra-day measurements is 0.33 and for inter-day measurements 1.45, which is less than 2%, respectively. From the results the method could be said precise.

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Table 1: Flurazepam results for intra-day precision, inter-day precision and ruggedness.

Parameter	Concentration ($\mu\text{g/ml}$) (n=6)	Normalized Area Mean \pm SD	RSD
Intra-day precision	140	1.004 \pm 0.003	0.33
Inter-day precision	140	0.985 \pm 0.014	1.45
Ruggedness	140	1.004 \pm 0.011	1.13

The ruggedness of the method was validated for three samples by two analysts separately. The results are given in third row of Table.1 with peak areas and RSD obtained from the peak areas.

Robustness

The robustness of the method was verified by deliberate changes made to the parameters such as the mobile phase volume ratio, pH of the solution and detection wavelength. They are given in Table 2 along with percentage change in peak areas with respect to the standard solution peak area. Flurazepam of 140 $\mu\text{g/ml}$ concentration was used for these studies. This was checked by consciously changing the mobile phase volume ratio (<2%), pH value (<5%) and wavelength (<1%) from the optimized conditions of the above parameters. The percentage change in peak area was calculated for each parameter and was found to be less than 2% satisfying the ICH guide lines. The values shown in Table 2 indicate robustness of the method.

Table 2: Robustness results of flurazepam for mobile phase, pH and wavelength.

Parameter	Value	area	% of change
Standard	standard	1149613	
Mobile Phase Methanol : Acetonitrile	78 : 22 (v/v)	1132799	1.47
	82 : 18 (v/v)	1167199	1.53
pH	4.7	1153993	0.38
	5.1	1158084	0.74
Wavelength	228 nm	1152060	0.21
	232 nm	1130082	1.7

Recovery

Using the standard addition technique, the accuracy of the method was verified by adding known amount of the standard to the sample of flurazepam. Three different percentage determinations (50%, 100% and 150%) were used to study the recovery of the drug. For each percentage level the analysis was repeated three times ($n = 3$). The recovery percentage of the drug was compared with the actual amount and results are given in Table 3. The mean concentrations and their standard deviation (SD) for each percentage are

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shown. The coefficient of variation (CV) or the relative standard deviation (RSD) in percentage is also given. The recovery mean of the drug and its corresponding SD along with the calculated error percentage (last column in Table 3) is also given. The recovery percentage was good ranging from 98.97% (lower limit) to 100.92% (upper limit) indicating the high accuracy of the method.

Table 3: Recovery results of flurazepam for three percentage concentrations.

Recovery	Target Conc., (µg/ml)	Spiked conc., (µg/ml)	Final Conc., (µg/ml)	Conc., Obtained Mean ± SD	RSD	Recovery (%) Mean ± SD	% Error
50%	40	20	60	59.37 ± 0.04	0.06	98.97 ± 0.06	0.036
100%	40	40	80	79.77 ± 0.89	1.10	99.71 ± 1.12	0.65
150%	40	60	100	100.92 ± 0.97	0.96	100.92 ± 0.97	0.55

^a % Error = RSD/n, no of trials n = 3.

Formulation assay

This analysis was done with commercial formulation Nindral (15mg) capsules. The procedure was repeated two times, by weighing the capsule in powder form. Twenty capsules were weighed; the average weight was 42mg. The powder equal to 10 mg of the drug was dissolved in 10 ml of methanol. From 1000µg/ml solution, 140µg/ml was prepared and this was used for formulation assay studies. The results are given in Table 4.

Table 4: Formulation assay of flurazepam.

Brand name	Available form	Label claim	Concentration	Amount found	% Assay
Nindral	Capsule	15 mg	140 µg/ml	139.01 µg/ml	99.29

CONCLUSION

In conclusion, flurazepam in pure and capsule dosage form was investigated quantitatively using RP-HPLC method. The developed method was simple, precise and accurate for the determination of flurazepam in capsule dosage form. The measured parameters like linearity, precision, ruggedness and recovery validate the developed method. From the statistical analysis, the recovery and formulation assay of the drug was good. This method proves to be suitable for faster analysis of flurazepam in bulk.

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REFERENCES

1. British Pharmacopoeia. Online version, 2012. <http://bp2012.infostar.com.cn/>, 6 April 2014.
2. Anthony C, Osselton MD, Widow B. Clark's Analysis of Drugs and Poisons, Pharmaceutical press, 3rd edition, 2004; pp 943-944.
3. Miller LG, Greenbelt DJ et al. Kinetics, brain uptake and receptor binding characteristics of flurazepam and its metabolites. Psychopharmacology. 1988; 94 (3): 386-391.

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4. Norouzi P, Rashedi H et al. PVC membrane sensor and wire coated electrode for determination of flurazepam. Int J Electrochem Sci. 2011; 6: 2312-2322.
5. Cooper SF, Drolet D, Gas-liquid chromatographic determination of flurazepam and its major metabolites in plasma with electron-capture detection. J of Chromatography B. 1982; 231(2): 321-331.
6. Selinger K, Lessard D and Hill HM. Simultaneous determination of flurazepam and its metabolites in human plasma by high-performance liquid chromatography. J of Chromatography B. 1989; 494: 247-256.
7. Dadgar D, Smyth WF, Hojabri H. High-performance liquid chromatographic determination of flurazepam and its metabolites in human blood plasma. Analytica Chimica Acta. 1983; 147: 381-385.
8. Lau CE, Falk JL, Dolan S, Tang M. Simultaneous determination of flurazepam and five metabolites in serum by high performance liquid chromatography and its application to pharmacokinetic studies in rats. J Chromatography B. 1987; 423: 251-259.
9. Song D, Zhang S, Kohlhof K. Gas chromatographic-mass spectrometric method for the determination of flurazepam and its major metabolites in mouse and rat plasma. J of Chromatography B. 1994; 658 (91): 142.
10. Malfara WR et al. Reliable HPLC method for therapeutic drug monitoring of frequently prescribed tricyclic and non-tricyclic antidepressants. J of Pharm. and Biomed. analysis. 2007; 44(4): 955-962.
11. Omran AA et al. Determination of partition coefficients of diazepam and flurazepam between phosphatidylcholine bilayer vesicles and water by second derivative spectrophotometric method. Journal of Pharm Biomed Anal. 2001; 30: 319-324.
12. Peat MA, Kopyak L. The screening and quantitation of diazepam, flurazepam, chlrodiazepoxide and their metabolites in blood and plasma by electron-capture gas chromatography and high pressure liquid chromatography. J of Forensic sciences. 1979; 24(1): 46-54.
13. Berzas JJ, Casaneda G, Pinilla MJ. Determination of clobazam, clorazepate, flurazepam and flunitrazepam in pharmaceutical preparations. Talanta. 2002; 57(2): 333-341.
14. ICH Harmonized Tripartite Guideline Q2 (R1), Validation of analytical procedures: text and methodology. Current Step 4 Version, 2005.