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Review Article

A Review on Quantitative and Qualitative Analysis of Diazepam in Pharmaceutical Solid Dosage Form and Biological Samples

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Abstract
Diazepam is a benzodiazepine drug that acts by binding to GABA A receptor facilitating GABA action by increasing the frequency of opening of chloride ion channels hence causing decrease in neuronal activity of the central nervous system. This drug is mostly used as a sedative, hypnotic, sleeping pill, manage panic disorders and an adjunct therapy for epilepsy and alcohol withdrawal. This analysis is key in clinical practice and forensic science hence, we need to know the current trend of analysis required for detection and quantification purposes. Analysis of diazepam is mostly done by chromatographic and spectroscopy methods though other methods like capillary electrophoresis, andimmune-assay have also been implemented for the same use.

Keywords: *Benzodiazepines, biological samples, diazepam, pharmaceutical solid dosage form, qualitative, quantitative.*

INTRODUCTION
Benzodiazepines like Diazepam (DZP) is the drug of choice for many clinicians in the management of psychotic disorders, it has been rampantly dispensed either on prescription or over the counter without a prescription. Diazepam is known for its use in the management of muscle spasms, anxiety, epilepsy, hypnotic, sedative and also as a sleeping pill. Diazepam works by binding to GABA A receptor and facilitating GABA action, increase the opening of chloride ion channels hence influx of chloride ions into the cell causing membrane hyperpolarization the depressing the central nervous system [1].

Qualitative and quantitative analysis of diazepam in solid dosage form and biological samples will enable us to know the therapeutic effectiveness of the drug when taken by patients since chemical equivalence doesn't guarantee biological equivalence, this will also help in ensuring patient safety [2]. So, proper quantitative and qualitative tests need to be done to give better information on the different generic brands and innovator brands so that they can be interchangeably used by patients and also help healthcare providers in getting a brand or generic of choice [3].

Diazepam uses are commonly known and hence it is being highly used for its known effects for other practices which include rape, abduction by criminals making the rate of abuse quite high. Its determination in biological samples can be used in guidance in case of forensic sciences.[4]

Several methods for the qualitative and quantitative methods of analysis of benzodiazepines like diazepam have been cited in many literatures. These include nonaqueous titration, spectrometric methods like Nuclear Magnetic Resonance (NMR), UV visible spectroscopy, Mass Spectroscopy (MS), fluorimetry, Infra-Red spectroscopy (IR). Chromatographic methods like High Performance Liquid Chromatography (HPLC), Ultra-High Performance Liquid Chromatography

(UHPLC), Gas Chromatography (GC), Thin Layer Chromatography (TLC). Chiral separation, immunoassay Potentiometry, polarography and others [5; 6].

Among all these, non-aqueous titration and HPLC are the official methods, but because of the sample preparation step, using appropriate solvents for extraction and also because the sample may degrade during preparation, simple spectroscopic methods have been utilized more. For already commercialized preparations, spectrophotometric methods proved to be more efficient in giving results than the non-aqueous method because they give more rapid accurate and reproducible results. [6]

Qualitative and Quantitative methods done

1. High-Performance Liquid Chromatography (HPLC)

A simultaneous analysis of four (4) frequently prescribed BZP in bulk powders was done using HPLC (Alprazolam, chlordiazepoxide, diazepam, and nitrazepam). Isocratic elution of 1.0 and 1.5ml/min was used on the C18 column with ambient temperature. 4 different mobile phases were studied and they were able to give both quantitative and qualitative information with minimal sample preparation and less amount of sample is needed for analysis. Since less sample used or needed, the eluted solvent can further be used for more analysis when collected. This method is suitable for forensic science and routine control of pharmaceutical dosage forms [7].

The amount of DZP was estimated in tablets using Reversed Phase (RP-HPLC); In the study, the testing was carried out on the mono preparation of DZP tablets and the combination of DZP with other formulations like imipramine. The method was validated for its specificity, linearity, robustness, accuracy, precision, limit of detection and limit of quantification. The method was found to be simple, sensitive, specific, fast and had a high resolution, sharp chromatographic peaks within a run time of 10 minutes[8].

A review was done on papers from 1996-2008 for BZP determined in biological samples and pharmaceutical formulations and it was found that HPLC was the technique for determination of BZP mostly used in biological samples. Liquid-liquid extraction (LLE) was the technique for sample preparation mostly used followed by solid phase extraction (SPE) especially for biological samples. The detection was mostly done using ultraviolet visible (UV) detectors. The use of UV together with HPLC for detection was considered more efficient for the determination or assay of BZP because it was rapid, sensitive, and specific except for ketazolam, temazepam and oxazepam which are not well detected by many methods. Reverse-phase columns are preferred because they require less maintenance and can be used for the separation of drugs with a wide range of polarity [9].

Six BZP were analyzed for their presence in vitreous humor using a combination of HPLC and diode array detector. The analysis was done to validate the method and sample preparation was done by SPE using 10% acetic acid/methanol as elution solvent. Vitreous humor is most effective when the blood and urine samples cannot be got especially for postmortem cases in forensics. It also proved to be a better alternative technique when GC-MS and LC-MS cannot be used or are not available for the analysis to be done. It provided good specificity for the identity of BZP, and the multichannel detector had a maximized sensitivity that proved efficient for the detection of BZP with the concentration found in the vitreous humor, hence this method was a better alternative to be used in forensics[10].

The retention behavior of 6 BZP (alprazolam, bromazepam, diazepam, flunitrazepam, medazepam, nitrazepam) was determined on 4 different stationary phases C18, C8, C6H5 and CN and all maintained at a fixed temperature. The retardation factor (RF)for the 6 BZP was determined and compared when put in 2 mobile phase compositions that had an organic modifier of acetonitrile/methanol and another that had no mobile phase organic modifier. From the analysis, results showed that the RF of BZP put in the mobile phase without an organic modifier was higher for acetonitrile than methanol for all compounds studied on the 4 stationary phases so the organic modifier decreases the retention time for all BZP when added in the mobile phase decreasing analysis time and fast sample identification[11].

DZP content in DZ-coated tablets was analyzed using an eco-friendly RP-HPLC method, the method was developed and validated for precision, accuracy, linearity, range, specificity, selectivity, sensitivity, robustness according to the ICH guidelines. Separation was achieved on LiChrosper 100 RP-18 (250mm x 4mm, 5micrometer particle size). The mobile phase containing a mixture of water and ethanol in a ratio of 40: 60 % v/v and a flow rate of 1.0ml/ min was used under isocratic elution mode. An injection volume of 10microliter and wavelength used for detection was set at 254nm, temperature at 35°C. Greenness assessment and eco scale approach were used to assess the assay and content uniformity of DZP in coated tablets. From the results the green analytical chemistry concept with mobile phase as ethanol and water was an environmentally friendly solvent that can replace organic solvents methanol and acetonitrile because ethanol has a higher elution strength than methanol and acetonitrile. The peak for the retention time of DZP in the eco-friendly method was similar to that obtained from the reference USP method implying that the eco-friendly mobile phase had an elution power comparable to that of the conventional mobile phase. In validation of the ecofriendly method chromatograms of the solvents of the placebos and standards of DZP solutions were tested for assay and content uniformity, and results showed no peak interferences from the solvent or placebo with the elution of DZP. linearity and range were between 0.05 and 0.15mg/ml, triplicate analysis was done and linear regression analysis was evaluated by the least squares regression method. the correlation coefficient was greater than 0.999 showing good linearity. Precision was validated through system and method repeatability which was 0.2% RSD, intermediate precision was not more than 2% making the method to be precise. Accuracy was evaluated using analytical recovery and the percentage of recoveries was in the range of 99.73±0.03% showing good accuracy. The ecofriendly method was simple, fast, and robust so can be used in the routine analysis for assay and content uniformity determination in coated DZP tablets, reliable analytical results can be generated from it hence a better alternative than the reported methods [12].

2. Combination of Spectrophotometric Analysis and Chemical Analysis

In this study, DZP interacted with picric acid and 3,5 di-nitro benzoic acid, and 3,4 di-nitro benzoic acid and it was found that the interaction improved the spectrophotometric determination. The assay of the preparation was done at 475,500, and 500 nm respectively. The results of the assay were compared with official methods and it was found that the method was simple and needed no sample preparation before analysis, samples with very low concentrations can also be determined, and the excipients in the formulation did not interfere with assay results. The accuracy and precision of the method were comparable with official methods, hence the method was recommended for routine analysis and quality control [6].

3. Extractive Spectroscopic Method

Quantitative analysis was conducted on pharmaceutical preparations containing DZP, and it proved to be a successful method for the determination due to its accuracy and precision compared to official methods. The method was based on a 1:1 ion association complex formation that was extractable into chloroform from aqueous phase and measured at 410nm [13].

4. Gas Chromatography (GC)

A review was done to determine the concentration of benzodiazepines (BZP) in bio samples and G.C was found to be the best fit for the determination even in the trace amounts of as low as Nano gram or microgram per milliliter. The bio samples checked were human serum, plasma, blood, rat liver, urine, hair(human/rat), breast milk, and oral fluid. Among the four chromatographic methods done of HPLC, GC,TLC, and MECC (Micellar capillary chromatography), GC-MS proved the best fit to confirm BZP [14].

Quantitative determination of BZP and simultaneous screening was done using dual channel GC having electron capture and nitrogen phosphorus detection. BZP like temazepam midazolam, flunitrazepam, bromazepam, trazolam, nitrazepam, clonazepam, chlordiazepoxide, lorazepam, oxazepam, diazepam, nordiazepam, were able to be quantified, screened in plasma and gave accurate chromatographic information in a single run with in 12minutes. Flurazepam was used as an internal standard, Identical fused silicaSE-54 columns were inserted in a split-split less injector and used[15].

5. Capillary Electrophoresis

This method involves a lot of techniques like capillary zone electrophoresis (CZE), Capillary isoelectric focusing (CIEF), Capillary Gel Electrophoresis (CGE), Capillary Iso Tachophoresis (CITP), Micellar Electro Kinetic Capillary Chromatography (MECC), Capillary Electro Chromatography (CEC). These are the most common methods for analysis of BZP in biological samples[7]

Studies that have mostly been carried out for the separation of 1,4 BZP are by TLC, HPLC, GC and an alternative analytical method of capillary electrophoresis have also been used and it has increasingly gained acceptance as a complementary method to chromatography. The above method is an official method of separation of BZP in European Pharmacopoeia 5th edition and is known for its excellent separation efficiency, high mass sensitivity, use of less amount of solvents and samples and it can do both direct and indirect detection. MECC has proved to be the best method for the separation of BZP using sodium tetra borate buffer with an organic modifier because it is fast, sensitive and reliable, hence the optimized method has proved to be a single analytical method for both monitoring body fluid levels and drug abuse screening in suspects. It has shown use in the identification, separation, and stability determination of BZD[16].

DZP was analyzed by CEC, and samples were put in a magnetic field and flew using electrically charged particles and liquid medium, charged particles moved at various directions and speeds so separation occurred. BZP is hydrophobic in nature so the CEC method is the mostly the method for analysis when hyphenated with MS (TOF). Concentrations of as low as ng/ml can be detected.

The above methods have proved to be complex since the sample preparation step is needed which is complex and also if the sample is not UV detectable then results cannot be obtained [7].

6. Liquid Chromatography Mass Spectroscopy Mass Spectroscopy (LC-MS-MS)

BZP in oral fluids was analyzed and determined using the above method. A quadrupole mass spectrometer in positive electrospray mode was used. Oral fluids were collected usinga Quantisal TM oral fluid collection device that collects a neat oral fluid that can efficiently recover the BZP from the collection pad into the transportation buffer to get high quantitative values and tested. Intraday and interday precision were done to determine their concentration in the oral fluid and the results gave good sensitivity over other published methods. The method qualified ions for identification of BZP at low concentrations in oral fluids hence a currently used method for the determination of BZP in oral fluids[17].

7. Thin Layer Chromatography (TLC)

DZP can be extracted from biological samples and checked for presence using TLC, SPE is the method of choice for sample preparation, and solvents like methanol, chloroform, benzene, ether, acetone and others are used for extraction and transferred to benzophenones which can be easily identified and analyzed. Silica gel plates / RP –C18 plates with florescent agent F254 are used. Single solvent extraction or two-component systems, ternary, and quaternary systems can be used. Detection is done under UV light at 254 nm or IR light with sulfuric acid. Densitometry can also be done and scanned at 380nm, the plate can also be sprayed with Dragendorff's reagent for visualization. TLC can be coupled with liquid secondary ion MS (LSIMS) in case no standard sample is available for comparison. FTIR can also be used for powders which change easily in composition [18]; [19].

8. Fourier Transform Infra-Red (FTIR)

Thus, method is rarely used for the determination of DZP in pharmaceutical solid dosage form. The use of vibrational frequency was seen in this method after sample preparation using chloroform and sonicated. The peaks for identification were seen at 1672 cm^{-1} and 1682 cm^{-1} a baseline correction of 1850 and 1524cm^{-1} was used and provided a relative standard deviation (RSD) of 0.5% for independent measurements that were 5 and the limit of detection was 0.04 mg per tablet in 5 tablets. The method proved to be fast accurate and sensitive since it was in agreement with the already official methods when UV spectroscopy was used as a reference method hence a better alternative.[19]

9. Sonogel Carbon Electrode with Bentonite

Determination of DZP and chlordiazepoxide hydrochloride in tablets and their metabolites in urine (oxazepam) were determined using bentonite at different parameters like pH, buffer type, ionic strength, accumulation potential, scan rate and accumulation time. The quantification and detection limit were 14.0 and 4.0 ng/ml for DZP and 16.0 and 5.0ng/ml for chlordiazepoxide hydrochloride respectively. The analysis had better precision, very low detection limits, and was faster than the voltammetry techniques reported [20].

10. Silver Amalgam Electrode

BZP was determined by voltammetry behavior on a meniscus-modified silver solid amalgam electrode. The pH effect in different media like phosphate, citrate buffer, 0.1$moldm^{-3}$ sodium hydroxide, Britton-Ribinson cyclic voltammetry was used for the study of the electrochemical behavior of BZP and it had a fast, simple and low cost hence favorable for determination of BZP in the sample of urine.[21]

11. ImmunoEnzymatic Assay

This method is used more in forensic science to detect elicit BZP samples. These methods have proven to be inefficient in the sensitivity to detect usually prescribed BZP, e.g. Radio Immuno Assay (RIA), Enzyme ImmunoAssay(EIA), and Immune Fluorescence Assay (IFA). The methods have been improved like the use of Enzyme Multiplied Immuo Technique (EMIT) and Fluorescence Polarization Immuno Assay (FPIA)[7].

A total review of analytical methods for the detection of 1,4 BZP showed that several methods like chromatography (HPLC, UPLC, GC, TLC), chiral separation, capillary electro separation, immunoassay, photometry, electroanalytical methods and many more. Among these HPLC was mostly used for the estimation of BZD like diazepam because it is relatively simple andcan easily be used in combination with other methods like UV and amperometry. The method allows the determination of nonvolatile, polar, high molecular mass and thermally labile BZD which does not apply to other methods like GC. If a non-destructive system is used, the eluted solvent can be collected or recovered and further analysis can be done on it. The concentration of the analyte to be detected can be as low as ng or mg.[5]

CONCLUSION

In the recent qualitative and quantitative tests done on DZP in pharmaceutical dosage forms and biological samples, some methodologies have been phased out because of non-convenient sample preparation techniques and advances in technology that make work easy and fast saving time and resources. The current trends of analysis are more on automated machinery which are easy to operate and less number of processes involved to get results. Hyphenated techniques having both chromatographic and spectroscopic methods are the number one choice for qualitative and quantitative analysis of DZP in both biological samples and pharmaceutical formulations. HPLC has proved to be more advantageous than other methods and when coupled with spectroscopic methods. UV spectroscopy is the most used detection method since it can detect for as low as ng/mg concentrations. However, tandem MS has also proven to be taking over UV because it can be modified further. These quantitative and qualitative techniques are important in clinical practice and forensic science hence better ideas need to be brought in play to improve on the already existing methods and the phased-out ideas can also be improved by incorporating these sophisticated instruments to enhance their activity.

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