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Quantitative and Qualitative Analytical Techniques for Doxepin Hydrochloride: An Overview

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ABSTRACT

Creation of pharmaceutical analytical techniques has brought revolution in the human health condition and area of medical research and development. Pharmaceutical industries only produce medicines if they have proof that the products produced are free from harmful chemicals, to serve this reason, various analytical techniques were developed on a regular basis by pharmaceutical industries. Doxepin Hydrochloride is tricyclic antidepressant that is used mostly to treat depression and anxiety. Consumption of high concentration of Doxepin hydrochloride can cause serious side effects to the patient. To achieve good quality control and quality assurance, analysis of Doxepin hydrochloride is essential. This paper summarized all the published Doxepin hydrochloride quantitative and qualitative analytical techniques. Analytical techniques reported for determining doxepin hydrochlorides were UV/VIS spectroscopy, high performance liquid chromatography, liquid chromatographic-mass spectroscopy, thin layer chromatography, high performance thin layer chromatography, gas chromatography and radioimmunoassay.

INTRODUCTION

Tricyclic antidepressants have been used for the treatment of depression and other conditions since they were discovered in the 1990s [1]. Tricyclic antidepressants (TCA) are commonly used in the treatment of depression, anti-anxiety and have antihistaminic effects [2]. This displays active core anticholinergic activity and reduces noradrenaline and serotonin reuptake. TCAs are a group of drugs primarily used to treat patients with major depression and other psychiatric disorders including panic disorder, obsessive compulsive disorder, eating disorders, and hyperactivity disorder with attention deficit [3]. These days depressant have become major problem. The analyses of drugs compounds are important for obtaining best therapeutic concentrations and for quality assurance in pharmaceutical preparation. Antidepressants drugs (Tricyclic antidepressants) are commonly used therapeutic management [4]. Researchers have found that some depressed individuals have impaired neurotransmitter rates, chemicals that interact with nerve cells [5]. In many forensic cases such as driving under the influence of drugs, violent crime cases, drug - facilitated sexual assault and other cases of sudden or violent death, these drugs are common. Therapeutic drug monitoring (TDM) is important in circumstances where patients are not responding as planned, either with inhibitors or inducers of CYP450, non-compliance and in patients at risk, such as elderly, poor metabolizes or with liver impairment [6].

Drug profile

Doxepin hydrochloride (DOX) is chemically known as benzoxepin-11-ylidene (3E)-3-(6H-benzo[C][1])-N,dimethylpropan-1-amine. Doxepin is a tricyclic antidepressant and anxiolytic psychotropic agent. It is used to treat depression, anxiety disorders, and chronic idiopathic urticarial as a second line treatment [7]. It has various brand names, including Aponal, Adapine, Doxal (orion), Deptran, Sinquan, Spectra, Doxin, Doxetar and Sinequan. Doxepine is also used for sleep maintenance treatment. For such an indication, Silanor is the trade name. Doxepin has a following structure of the drug which is shown in fig-1[8].

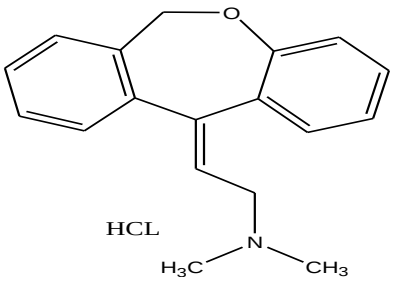

Figure No. 1: Structure of doxepin hydrochloride.

IUPAC Name **:** 3- (dibenzo) [b,e]oxepin -11(6H) -ylidene)-N,N-dimethylpropan-1- amine

Mol Formula **:** $C_{19}H_{21}ClNO$

Mol Weight **:** 279.376 g/ mol

Solubility **:** Water and alcohol

Melting point **:** 192-193ºC

Category **:** Tricyclic antidepressant

Route **:** Oral

Storage **:** 20 to 25ºC

Pka **:** 8.96 [9].

Quantitative techniques

The technique of quantitative analysis helps an analyst to determine the analytes component concentration in the test sample. Analytes separation is mostly done before being analyzed using conventional methods or by instrumental methods during research. Types of chemistry used in quantitative drug testing are titrations, precipitations and extractions. Classical methods use volume or weight as the quantitation parameters [10].

UV/VIS Spectroscopy

The use of UV-VIS spectrophotometry has increased rapidly over the years in the study of the pharmaceutical dosage type. These methods offer low-time and labour advantages. The process is also very accurate. Spectrophotometry is used as a function of wavelength for quantitative measurement of a material's reflection or transmission properties [11].

Rahman N, *et al.* studied commercial dosage spectrofluorimetric determination of Doxepin HCL and developed pharmaceutical preparation based on ion pair-complex. Ion pair complex was extracted by dichromethane and fluorescence intensity is calculated at 560 nm after excitation at 490 nm. Linear range and detection limit were found at 2-14 and 0.55 microgram/ml. This method does not require a clean-up measurement by any laboratory. A successful method of spectrometry was used to analyze the dosage form [12].

Revanasiddapa HD, *et al.* studied the indirect visible spectrophotometric method for determining the individual dosage form of doxepin and dothiepinin pure and the tablet. The lambda max was found to be 590 nm and obeys the law of Beer's in the concentration range of 0-60 µg/ml and the recovery range was 99.26-100.02% for DOX and 99.64-100.36% for DOT. The coefficient of regression was found to be 0.998 and 0.996 for DOX and DOT. LOD and LOQ and all the parameters were validated as per ICH [13].

Sanz R, *et al.* developed three quantitative methods to determine Doxepin for their application to *In Vitro, Ex Vivo* and *In Vivo* studies. For *in vitro* released studies of doxepin quantification simple UV-vis spectrophotometry analysis was chosen with artificial membranes. It was developed to quantitatively detect doxepin which permeated the line reversed phase HPLC method using C18 column with UV-vis detection. HPLC was developed in accordance with the system of tandem mass spectrometer with sample preparation to determine doxepin plasma concentration in pigs. The methods and associated parameters proposed have been validated in accordance with ICH guidelines [14].

Rajendiran N, *et al.* proposed method for determining the interaction between dothiepin and doxepin with bovine serum albumin (BSA) and DNA base (adenine) using UV-Visible fluorescence, ATR-IR, cyclic voltammetry, and molecular docking methods. DOT and DOX interaction with BSA / adenine produced a strong quenching fluorescence and static quenching mechanism. Addition of drugs altered adenine's tautomeric equilibrium structure, oxidation and adenine / BSA interaction peaks moved to high and low potential, respectively.

After binding to DOX and DOT, BSA's secondary structure was observed in ATR-IR. Addition of drugs changed Adenine's tautomeric equilibrium structure, oxidation and reduction peaks of adenine / BSA interaction peaks shifted to high and low potential, respectively. BSA's secondary structure modified in ATR-IR was observed by band shift of amides 1 and 2 when binding to DOX and DOT. Reduction of volumetric current to slow the diffusion was observed in the presence of BSA / adenine binding with BSA molecules [15].

Wu Cheng-ke, *et al.* used a spectrometric approach to analyze the interaction of doxepin hydrochloride, fast green and its application. Standard doxepin hydrochloride solution was used at 1- 4 %. This shows that pH 4.0 doxepin hydrochloride confirms the strong green resonance light resonance scattering signal of fast green. Scattering process maximum at 344 and 486 nm was shown under optimum conditions. RLS intensity is proportional to 8.75x 10^{-3}- 4.88x 10^{-2} mg x mL^{-1} doxepin hydrochloride concentration. Doxepin hydrochloride detection limits are 1.72x 10^{-5} mg x mL^{-1}. Doxepin hydrochloride standard deviation was found to be 0.025 mg x mL^{-1}. The researcher concluded that this procedure was used in pharmaceutical preparations to assess doxepin hydrochloride [16].

Kandagal PB, *et al.* researched and reported spectroscopic (fluorescence, UV-vis absorption, and circular dichroism) techniques to bind doxepin hydrochloride to bovine serum albumin. The method of fluorescence quenching was used to test the binding parameters. Thermodynamic parameter calculations at different temperatures revealed that doxepin hydrochloride's hydrogen bonding interaction with bovine serum albumin. The mean distance between the acceptor (DH) and donor was measured and found at 2.7 nm, according to foster's theory of non-radiation energy transfer. The researcher concluded that DH and ESA were tested for specific ion effects [17].

High performance liquid chromatography

High-performance liquid chromatography or high-pressure liquid chromatography is the most popular analytical technique used for component mixture separation, identification and quantification [18]. High Performance Liquid Chromatography is the fastest chromatographic technique in drug element quality control. It is scalable, robust and suitable for many different applications [19]. The most popular LC technique for pharmaceutical analysis is reversed-phase HPLC (RP-HPLC) [20]. Many chromatographic parameters have been analyzed to optimize the process such as pretreatment of specimens, selection of mobile

stage, row, and selection of detectors. The development of the HPLC method depends on the chemical structure of the molecules [21].

Baezzat MR, *et al,* used high-performance liquid chromatography and cloud-point ultraviolet detection for doxepin hydrochloride determination in human plasma. The process was carried out using the column µBondapakR C18 (4.6 mm id for 300 mm, 3 µm particle size), and the column µBondapakR C18 was also used for isocratic elution of 289 nm wavelength. TritonX-114 non-ionic surfing was used as an extraction solvent. For human plasma, the linear range of doxepin was 0.1-0.9 µg/mL. Maximum detection, pre-concentration factor, 0.08 µg/mL, 50 and 49.0 enrichment factor respectively [22].

Whall TJ. *et al,* established chromatographic high-performance liquid determination of (Z)- and (E) - Doxepin isomers of hydrochloride. The model that has been developed is accurate and precise (the relative standard deviation was 0.3 percent for both isomers). Separation was achieved through the use of HPLC columns loaded with silica microparticles (5-6µm) and acetonitrile-chloroform-diethylamide (750:250:0.2) as a mobile stage, allowing for baseline resolution and simultaneous determination of (Z) and (E)-doxepin isomers [23].

Podili B, *et al.* proposed RP-HPLC stability-indicating method for determining doxepin, benzyl alcohol and capsaicin in forms of bulk and pharmaceutical dosage. Analysis was conducted on column C18 (150mmx4.6 mm, dp=3.5µm) with mobile acetonitrile phase: buffer (40:60v / v, 3.0 gm octane-1-sulphonic acid in 1lt water adjusting pH-2.5 with OPA) at a flow rate of 1.0 ml/min. The detection was carried out by UV detection at 273nm.The calibration curve were linear at range of 6.6-99 µg/ml, 2-30 µg/ml and 0.1-1.5 µg/ml with the limit of detection(s/n=5,4and7) 0.06µg/ml, 0.02µg/ml and 0.001µg/ml for doxepin, benzyl alcohol and capsaicin respectively. The retention time for capsaicin, doxepin and benzyl alcohol were 3.57, 6.10 and 9.10 minutes [24].

Qiong L, *et al*. reported HPLC method determination of doxepin hydrochloride isomers. Method was developed using column Shim pack C (1506mm, 5µ) and mobile phase consisted of (0.2mol. L-1 Potassium di-hydrogen phosphate-methanol (65:35)) and B (0.02 mol. L-1 Potassium di-hydrogen phosphate-methanol (40:60 at flow rate was 1mL / min). The detection happened at 295 nm. The calibration curve for Z-isomer was linear in the range of 221 10µg•mL-1 and 90470µg mL^{-1} for E-isomer and 90470µg mL^{-1} for E-isomer [25].

Badenhorst D, *et al.* published determination of doxepin and Desmethyl doxepin in human plasma using liquid chromatography-tandem mass spectrometry. A Phenomenex Luna C18 5 micron, 150x2.1 mm column with a mobile phase containing methanol-water-formic acid (600 :400:0.5, v/v) was used to separate samples at a flow rate of 0.25 ml/min. Hexane-isoamyl alcohol was used to collect samples. Mobile step consists of methanol-water-formic acid (flow level of 600:400:0.5, v/v. 0.25 ml/min). The author concluded that this method could be used in the bioavailability studies of doxepin drugs [26].

Yan J, *et al.* studied stereoselective and simultaneous measurement by high-performance liquid chromatography of Cis and Trans-isomers of doxepin and N-Desmethyldoxepin in plasma or urine. Chromatography with a silica column and a mobile phase containing hexane-methanol-nonylamine (95:5:0.3, v/v/v) was performed in normal phase system. As an internal standard, UV detector and nortriptyline were used. The mixture of n-pentane-isopropanol (95:5, v/v) was used as liquid-liquid extraction solvents. For each isomer, the LOQ was 1 ng/ml. Calibration curve linearity exceeded the range of 1–200 ng/ml (plasma) and 1–400 ng/ml (urine). All required parameters were performed in accordance with ICH guidance. The researcher concluded that no evidence of deterioration exists [27].

Nyanda AM, *et al.* studied the method of high-performance liquid chromatography to quantify tricyclic antidepressant drugs in human plasma or serum using liquid-liquid extraction methods for testing. Acetonitrile, methanol, and phosphate buffer (0.01 M, pH 7.4) were used for the mobile process at 12:3:5 ratios (v/v/v). Using Supelco Hypersil 5 mm, column 150 3 3.2 mm. Recovery ranged from 89% to 108%. This method was sensitive to 15 ng/mL and linear to 400 ng/mL with an injection of 25 mL [28].

Liquid chromatography-mass spectrometry

LC-MS (Liquid chromatography/mass spectrometry) is developed HPLC method and is considered as important part of liquid chromatograph. Liquid chromatography/Mass Spectrometry made of combination of HPLC and Mass spectroscopy. LC-MS is widely used analytical technique for separation and detection of component. It has very high sensitivity and selectivity and is used in various applications. It is a useful tool for pharmacokinetic drug research is the most commonly used bio-analytical tool [29]. LC tandem spectrometry (LC-MS/MS) is capable of detecting desired components using the multiple reaction monitoring

method, can analyze small volumes of ginsenosides in biological matrix such as urine and plasma, saves time, and is more convenient [30].

For a bioequivalence analysis, Patel NP, *et al.* developed highly sensitive LC-MS / MS method for the measurement of doxepin and its metabolite nordoxepin in human plasma. Analytes were extracted from human plasma by liquid-liquid extraction methods using methyl tert-butyl ether and their international standards (IS). For examination, the column of hypurity C8 (100 mm, 4.6 mm, and 5 mm) was used. Ratio of 93:7 (v/v) of the acetonitrile-methanol mobile mixture (95:5 v/v) and ammonium 2.0 mm. For Dox and NDox with a mean correlation coefficient (r2) of 0.9991 and 0.9993, respectively, a linear dynamic range of 15.03900 pg/mL was developed. The recovery range for Dox and NDox was found to be 86.6% –90.4% and 88.0% –99.1% respectively [31].

Qualitative techniques

Simple chemical test measurement of physical constants may be used to perform qualitative analysis. Melting/boiling point, refractive index and chromatographic technique using standard reference are physical constants for calculating the qualitative properties of product components [32].

Thin layer chromatography

TLC is chromatographic technique used for separate mixtures of component. TLC is low cost, less time consuming and has no complicated technique. Thin layer chromatography is very simple instruments having wide applications in pharmaceutical analysis. It has wide application for identification of compound in given substance. TLC is used for is simple and requires minimal pretreatment [33].

Anna M, *et al.* used TLC with densitometric detector, studied impurity determination in Doxepin and other compounds. Application of densitometric UV detection at 254 nm and peak material absorption showed improved detectability compared to visual inspection under UV lamp. This approach is reliable and highly sensitive as per statistical data analysis. Linear regressions were indicative showing good linear relationships over wide ranges of concentration [34].

High performance thin layer chromatography

High performance thin layer chromatography studies are one of the important identity tests in pharmacopoeial monographs. HPTLC is advanced TLC method. HPTLC increased the resolution of sample to be separated. HPTLC used high quality TLC plate having finer particle sizes in stationary phase [35]. HPTLC is a fast and flexible technique. A wide range of specimens can be analyzed by HPTLC. In many ways, this approach is beneficial as it is easy to handle and takes less time to analyze the complicated material and coarse sample cleaning. HPTLC measures the entire chromatogram without time limits with a number of parameters [36].

Faulkner RD, *et al.* conducted comparative assays using two approaches, HPLC and HPTLC, for doxepin and desmethyldoxepin. For equate the reproducibility and sensitivity of doxepin in plasma with its metabolite desmethyldoxepin, two approaches were used. HPLC produced better reproducibility and showed high sensitivity [37].

Other methods used for determination of Doxepin hydrochloride

Voltammetric technique

The term voltametric is derived from voltamperometry. Jaroslay Heyrovsky was the inventor of a voltammetric technique which he named polarography. A voltammetry technique has less important than chromatographic, electrophoretic and spectroscopic techniques in industry and research. However, it offers great superior solution for specific analytical task. Voltammetric measured current as a function of voltage [38]. Voltammetry provides additional qualitative and selectivity information about the nature of detected analyte on basis of applied current for production. Cyclic voltammetry is the most common format of voltammetry which is extensively used for enzyme based biosensor and their application [39].

Farjami F, *et al*, studied the electrochemical behavior and highly sensitive Volta metric determination of doxepin in pharmaceutical preparation and blood Serum using carbon ionic liquid electrode. Differential pulse voltammetry was applied as an analytical technique for quantification of sub-micromolar concentration of doxepin at optimal conditions, the proposed electrode exhibited great sensitivity toward determination of doxepin compared to other electrodes. Anodic peak current versus doxepin concentration was linear at the range of

0.05-24µM was achieved. The procedure was successfully applied in the drug and blood serum to assess the doxepin content [40].

Radioimmunoassay method

Radioimmunoassay method is a sensitive common bio-analytical methods relying on the reaction of an antigen and an antibody to measure the analyte. In the pharmaceutical analysis area, immunoassays have become one of the most useful methods. Another related and sensitive wide spread bio-analytical methods depending on the reaction of an antigen and an antibody to quantify the analyte. In the pharmaceutical analysis area, immunoassays have become one of the most useful methods. It is now commonly used in specimen identification, diagnosis of disease, control of medicinal medicines, clinical pharmacokinetics, studies of product bioequivalence in drug discovery and in pharmaceutical industry. Immunoassay methods for biological sample processing have inherent specificity and high sensitivity [41].

Rosalyn Sussman Yalow and Solomon Berson created the first immunoassay in 1959. Yellow and Berson used insulin marked with radiation to measure human plasma insulin concentration. For sample analyte concentration cleaning and low assay sensitivity, most RIA formats are recommended. The method of cleaning and concentration typically includes chromatography of ion exchange [42].

Virtanen K, *et al.* reported radioimmunoassay for doxepin and desmethyldoxepin determination in serum. In this method doxepin and desmethyldoxepin recovered were quantitative when drugs were applied to the normal pooled human plasma at different concentrations. By using the extraction technique doxepin and desmethyldoxepin can be measured to 9n mol/from 0.1 ml. Sensitivity may be improved by the extraction of 0.2ml sample [43].

SPE-LC-MS/MS

Liquid chromatography is a technique that is hyphenated. Liquid chromatography (LC-MS) is currently the most widely used mass spectrometry due to its ability to separate and detect wide range of molecules. LC-MS is a flexible method used in the metabolite profiling analysis to solve most of the analytical mission. LC-MS delivers high sensitivity and power of resolution. It also provides excellent reproducibility and better identification. The instrument's strengths are easy to use and low cost. LCMS has issues like tedious sample

preparation, time consuming, and may be prone to error. LCMS is the preferred tool for volatile compound analysis [44].

Gong FJ, *et al.* Used SPE-LC-MS / MS to assess doxepin in the whole blood. Separation was accomplished through solid phase extraction. Ionic electrospray ionization and monitoring of multiple reactions were used. As an internal standard, amitriptyline was used. Doxepin and amitriptyline were retained at 15.15 and 16.94 min. The calibration curve indicated a linear range from 0.005 and 1.00 mg/ml doxepin concentration. Doxepin's detection limit was 0.001 µg/ml and an average recovery rate of 78.0 percent -82.9 percent was observed. Relative average accuracy was less than 2.55 percent and 5.90 percent respectively within the day and between [45].

Gas chromatography

Gas chromatography is a flexible and special analytical method widely used to analyze gaseous materials, liquid solutions volatile solid parts. Mikhail Semenovich Tsvett invented gas chromatography as a separation technique which opened the door to analytical technique which brought revolution in chemical separation and analysis [46].

Gas chromatography is a versatile and unique analytical technique which is commonly used for analysis of gaseous samples, liquid solutions, volatile solid components. Gas chromatography was discovered by Mikhail Semenovich Tsvett as a separation technique which opened the door for analytical technique which brought revolution in chemical separation and analysis [47]. Gas chromatography is widely used in complex sample mixture for product identification, chemical isolation. Gas chromatography is capable of supplying pure components from a mixture. Column is known as the heart of gas chromatography, where different chemical components are isolated on the basis of their affinity to the stationary phase packed in the column [48].

Jack E, *et al*, formulated electron-capture gas chromatography method for testing low nanogram concentrations of doxepin hydrochloride. Upon hexane extraction from the biological matrix with sodium carbonate/sodium bicarbonate dust, biological samples are extracted. In order to form a single product with a high sensitivity to the electron-capture detector, 2,2,2-trichloroethyl chloroformate was used for the derivation of doxepin and desmethyldoxepin. The recovery rate of doxepin and desmethyldoxepin from serum derivatization with polyhalogenated chloroformate reagent is 95 2. + 5% [49].

CONCLUSION

Doxepin hydrochloride is a tricyclic antidepressant that is used p methods used to analyze doxepin hydrochloride was primarily to treat psychotics of anxiety and depression. For sleeping maintenance, doxepin 250 mg capsule is used. In this study, author discussed various analytical methods used for quantitative and qualitative analysis of doxepin hydrochloride. A variety of methods have been used, such as UV/VIS spectrophotometry, HPLC, LC-MS, TLC, and HPTLC. In addition to these important analytical techniques other specific analytical methods such as voltammetric, radioimmunoassay and gas chromatography have been used to estimate doxepin hydrochloride. Highly advance techniques such as solid phase extraction-liquid chromatography-mass spectroscopy and liquid chromatography-mass spectroscopy were also used to analyze doxepin hydrochloride. It can be observed that high performance Liquid chromatography and UV/VIS spectroscopy methods have been widely used for the determination of doxepin hydrochloride.

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