Analytical Methods for Amitriptyline: A Review

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Abstract

Analytical Methods are broadly classified as classical and instrumental methods. The former include: volumetry (titrimetry), gravimetry, gasometry and other old methods. Under the later are embraced spectroscopic methods (UV-Visible, FS, AS, IR, NMR, MS, etc), Chromatographic methods (CC, TLC, HPTLC, HPLC, GC, etc), Electrophoretic methods (CZE) and other instrumental methods. In this paper a brief review of analytical methods applied for analysis of amitriptyline alone and in combination with other drugs in dosage forms and body fluids of human and animal model are presented.

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INTRODUCTION

Tricyclic antidepressants (TCAs) are three ring chemical structures that were widely used in the clinical practice for the treatment of different types of depression like phobias, insomnia, chronic pain syndromes, panic disorder, eating disorders (e.g., bulimia nervosa), premenstrual dysphoric disorderand anxiety disorders[1–4].These disorders affect to the patients both economically and socially which can eventually leads to suicidal behaviour. Antidepressants are usually prescribed in different combinations, causing more probable drug–drug interactions, while dosing is mainly based on trial-and-error [5, 6]. All the tricyclic antidepressants are pharmacologically and structurally similar. Under normal condition these drugs inhibit the reuptake of three important neurotransmitters (Serotonin, norepinephrine and dopamine) in the central

nervous system of brain cells [7]. Amitriptyline hydrochloride is a tricyclic antidepressant and is chemically known as 3-(10, 11-dihydro-5H-dibenzo [a,d] cycloheptene-5-ylidine)-N,N-dimethyl-1-propanamine hydrochloride [8] . It is a white, colourless, crystalline compound which is freely soluble in water. It is used for the treatment of several psychiatric disorders [9 - 11].The usual recommended dose varies between 50 and 200 mg daily. Despite the beneficial effects of amitriptyline hydrochloride the overdoses of the drug had many undesirable side effects and may lead to some disorders like unconsciousness, convulsions, hyperreflexia and cardiac depression [12].

After oral administration, AMI is transformed to its active metabolite (nortriptyline, NOR) by mono-*N* demethylation and by hydroxylation, leading to the formation of E-10-hydroxy (EHAT) and Z-10-hydroxyamitriptyline (ZHAT). Nortriptyline is further demethylated to desmethylnortriptyline (NNT) and hydroxylated to E-10-hydroxy nortriptyline (EHNT) and Z-10-hydroxynortriptyline (ZHNT). The demethylation of amitriptyline and nortriptyline is mainly catalysed by CYP2C19, with the participation of other CYP enzyme forms in higher drug concentrations.

In this review, a wide variety of analytical methods have been reported for the determination of amitriptyline in pharmaceutical preparations and in biological fluids. These methods include spectrophotometry, high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE), and others.

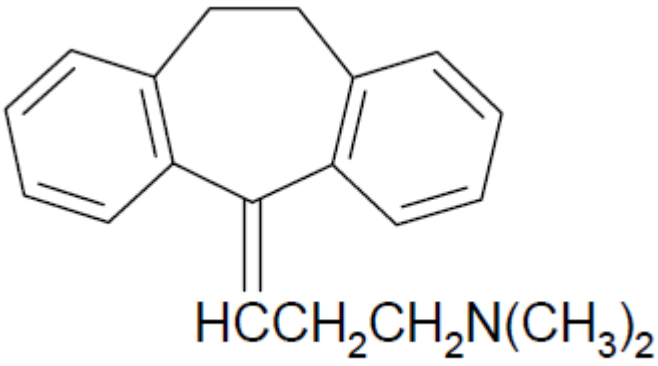

Figure 1; Chemical Structure of Amitriptyline (Adapted from ref. 40)

1. Chromatographic Methods:

High Performance Liquid Chromatography

This research manuscript describes simple yet sensitive, speedy, accurate and precise HPLC method for the analysis of Amitriptyline HCl in tablet form. The sample was analyzed by HPLC instrument using inertsil ODS 3V (150 mm X 4.6 mm, 5 µm Make, GL science) column as stationary phase and Phosphate Buffer: Acetonitrile(55:45 % v/v) as a mobile phase (where PH of the buffer was adjusted to 2.5 by using diluted ortho phosphoric acid) at a flow rate of 1.0 ml/min. UV detector was used for the detection at 254 nm. The retention time for Amitryptiline HCl was found about 4 minute. The linearity for the drug was obtained for the concentration of 45, 80, 100, 120 & 150µg/ml. This method would be successfully applied to pharmaceutical formulations because no significant interferences from tablet excipient were found. The method retained its accuracy and precision when certain variations in method parameters were applied [13].

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Pregabalin (PRE) and Amitriptyline hydrochloride (AMI) in Tablet dosage form. Chromatography was carried on C18 (25cm x 0.46 cm) Hypersil BDS analytical column using mobile phase Buffer (Potassium Dihydrogen Phosphate): Acetonitrile (55:45v/v) pH 4.0 with O-Phosphoric acid at a flow rate of 1.0 ml/min. The detection was carried out at 210 nm. The retention time of PRE and AMI was found to be 5.100 min and 3.227 min respectively. Assay result of marketed formulation of PRE and AMI was found to be 100.84% and 100.46% respectively. The proposed method was validated with respect to linearity, accuracy, precision, selectivity and robustness. Recovery of PRE and AMI was found to be 100.29% and 100.33% respectively. PRE and AMI were scanned in wavelength range of 200-400 nm. The proposed method for estimation of PRE and AMI were found to be simple, precise and accurate is applicable for simultaneous determination of PRE and AMI in marketed tablet formulation [14].

A simple, economic, selective, precise, and accurate Reverse Phase High Performance Liquid Chromatography method for analysis of Amitriptyline Hcl & Chlordiazepoxide in tablet dosage form was developed and validated according to ICH guidelines. The quantification of the drug was carried out by using YMC Colimited C8 (250 X 4.6 mm,5µ) column its equivalent in isocratic mode and maintain column at 400C, using

mobile phase comprising of Ortho phosphoric Acid : Methanol in the ratio of 50:50 v/v (Adjust pH -2 with Orthophosphoric Acid), with a flow rate of 1.0mL/min and the detection wavelength was carried at 253 nm. The retention time for Amitriptyline Hcl & Chlordiazepoxide was found to be 2.502&5.176. The percent assay was found to be 101%&99%. Proposed method was validated for precision, accuracy, linearity & range, specificity and robustness according to ICH guidelines. The method was successfully applied to Amitriptyline Hydrochloride and Chlordiazepoxide combination Tablet dosage form [15].

A new, simple, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of Amitriptyline and Perphenazine in bulk and pharmaceutical formulations was developed. Separation of Amitriptyline and Perphenazine was successfully achieved on Inertsil ODS (250x4.6mm) 5µm column in an isocratic mode utilizing Methanol: ACN: Water (50:30:20) at a flow rate of 1.0 ml/min and eluents were monitored at 253 nm with a retention time of 2.440 and 5.503 minutes for Amitriptyline and Perphenazine respectively. The method was validated and it was found to be linear. The values of the correlation coefficient were found to 0.992 for Amitriptyline and 0.9992 for Perphenazine respectively. The LOD for Perphenazine and Amitriptyline were found to be and 33.8 µg/ml and 4.2 µg/ml. The LOQ for Perphenazine and Amitriptyline were found to be 20.88 µg/ml and 12.12 µg/ml respectively. The percentage recoveries for Amitriptyline and Perphenazine were found to be within the limit indicates that the proposed method is highly accurate. The method was extensively validated according to ICH guidelines [16].

A simple, precise and rapid, reversed phase-high performance liquid chromatography (RP-HPLC) method is developed for simultaneous determination of chlordiazepoxide and amitriptyline hydrochloride in tablet dosage forms. The HPLC conditions used are methanol: acetonitrile (75:25 v/v) mobile phase, Zodiac C18 column (250mm x 4.6mm x 5µm), pump pressure (9.5 MPa) and detection wavelength (221 nm). The measured elution times are 4.87±0.02 minutes for chlordiazepoxide and 7.38±0.02 minutes for amitriptyline hydrochloride, respectively. The method is validated for linearity range, recovery, robustness and sensitivity. Linearity is demonstrated for chlordiazepoxide and amitriptyline hydrochloride in the range 5-30 µg/mL (r2 = 0.9998) and 15-75 µg/ml (r2 =

0.9997). The mean recoveries ranged from 98.81-100.63% and 98.97-101.33% and the limit of detections (LOD) are 0.15 µg/ml and 0.5 µg/ml for chlordiazepoxide and amitriptyline hydrochloride, respectively. The proposed method proved to be specific, robust and accurate and has good signal to noise ratio with well resolved peaks for unambiguous determination of chlordiazepoxide and amitriptyline hydrochloride combination and in tablet dosage form [17].

The precise, accurate, sensitive and very rapid Isocratic Ultra Performance and Liquid Chromatography method has been developed for the estimation of chlordiazepoxide and amitriptyline hydrochloride. The method employs Waters Ecquity UPLC system with UV detector on X Bridge C18 column (4.6 X 50mm, 3.7). The optimum chromatographic separation was attained by usingacetonitrile:0.05M potassium dihydrogen phosphate buffer pH6.8 (70:30v/v) and pH is adjusted to 6.8 using sodium hydroxide(0.1N) as mobile phase at flow rate of 0.3mL /min-1.The UV detection done at 240nm. Chlordiazepoxide and amitriptyline hydrochloride were eluted with the retention times of 0.84 and 1.16 min, respectively. Calibration plots of Chlordiazepoxide and amitriptyline hcl were linear over concentration ranges 10-50 and 25-125 µg/ml respectively. The percentage of relative standard deviation in accuracy and precision studies was found to be less than 1.5.Careful validation proved advantages of high sensitive, selectivity, accuracy, precision, and robust. Both drugs were subjected to stress conditions like acidic, alkaline, oxidative, thermal degradations and both are very sensitive to oxidative degradation. The developed and validated method was successfully used for the quantitative analysis commercially available dosage forms. The total analysis run time 3.0 minutes indicates speed and cost saving intiation of the method developed. No interfering peaks were found in chromatogram indicating that excipients used in the tablet dosage form does not interfered with the estimation of drugs by proposed RP-UPLC method [18].

Stability indicating RP-UPLCmethod was developed for the determination of amitriptyline hydrochloride in bulk and formulations. Chromatographic separation was achieved on a Waters Acquity UPLC system controlled with Empower-2 software.About 5µl of the standard solution was injected into SymmetryC18 (2.1mmx 100mmx 1.7 ìm; BEH) column, the component was separated with a mobile phase of potassium

dihydrogen phosphate buffer (pH3.0±0.05) and acetonitrile in the ratio 35:65 at a flow rate of 0.30ml/min and detected at a wavelength of 239 nm. System suitable parameters such as number of theoretical plates and tailing factor were found to be 12434 and 1.2 respectively. The percent of relative standard deviation in the study of intraday precision and inter day precision were found to be 0.136 and 0.336 respectively. Accuracy of the proposed method was found to be within the limits. Linearity limits, slope, intercept and correlation coefficient were found to be 2.5-20.0, 55605, 3956.2 and 0.9998 respectively.The developed method was found to be robust and rugged. Study of forced degradation indicates that, the percent of degradation was ranged from 8.64% to 18.67% under different degradation conditions. The proposed method can be applied in routine quality control analysis [19].

High performance liquid chromatographic methods are described for the determination of various drugs in biological fluids, using direct injection and a column switching valve. The methods are based on the enrichment of the drug on a reversed-phase concentration column followed by chromatographic analysis using various mobile phases. Members of three major drug groups were examined, Tricyclic Antidepressants, Antihistamines and Benzodiazepines. One of the fundamental requirements of a bioassay is the capability to isolate and detect mixtures of polar and non-polar substances simultaneously, as is often the case with a drug and its metabolites. In the determination of amitriptyline and its metabolites, nortriptyline, 10-hydroxynortriptyline and 10-hydroxyamitriptyline, a direct injection/column switching procedure is described which determines all four analytes simultaneously with excellent recovery. A conventional liquid-liquid extraction procedure is also described which failed to isolate the non-polar metabolites. Both methods are fully validated, compared and applied to samples from patients undergoing treatment with amitriptyline. For the determination of tripelennamine (an antihistamine), in bovine plasma and milk an on-line solid phase extraction technique is described. Bovine plasma proved to be chromatographically cleaner than human plasma but operationally more difficult to handle due to its viscous nature. The extension of the method for clean-up of milk samples was also investigated. With centrifugation prior to injection this proved possible. The method was fully validated. Setistine, a novel antihistamine was also determined using a solid-phase

extraction technique. In the final section a direct injection/column switching procedure for protein bound drugs is described. It is applied to the determination of diazepam and its metabolites desmethyl diazepam, temazepam and oxazepam, in plasma. The method is fully validated and compared to a classical liquid-liquid extraction scheme [20].

This study is aimed at developing a sustainable process for the recovery of valuable drugs from pharmaceutical wastes using ionic liquid (IL)-based aqueous biphasic systems (ABS). Because in pharmaceutical wastes, excipients represent the major contaminants, the search for selective routes for their elimination is of primordial relevance and for that purpose IL-based ABS were evaluated. The effects of different process parameters, namely the IL nature, pH and mixture composition used in the extraction system, were studied and the process was optimized to maximize the extraction of the antidepressant from pharmaceutical wastes. Moreover, the maximum amount of amitriptyline able to be processed using such systems was assessed. The set of ABS investigated herein revealed a high extraction performance, as indicated by the outstanding logarithmic functions of the amitriptilyne partition coefficients ranging from 2.41 ± 0.05 to >2.5 and extraction efficiencies between 66% ± 1% and 100%. The best ABS and conditions were considered in the development of an integrated multi-step purification process. The process here proposed comprises three main stages as follows: the solid–liquid extraction of the antidepressant from ADT 25 pills, its purification using the optimal IL-based ABS and the antidepressant isolation by precipitation with anti-solvent. After the removal of most water insoluble excipients in the first step, with the selected IL-based ABS, it was possible to further eliminate water soluble contaminants. A high capability of extraction and purification, leading to the selective separation of amitriptyline hydrochloride from the main contaminants contained in solid pharmaceutical wastes was achieved. Finally, the isolation of the amitriptilyne in a pure state was successfully accomplished through precipitation with the anti-solvent [21].

Purpose: To assess the doses of activated charcoal currently used in the management of acute amitriptyline-induced drug poisoning and explore the possibility of using lower doses. Methods: Albino male Wistar rats, weighing 200 ± 20 g, were used for the study.

The animals were divided into four groups of eight animals each. The concentration of amitriptyline in rat plasma was measured by high performance liquid chromatography (HPLC) for dose determination of activated charcoal. Chromatograms were established with acetonitrile: 70 mM KH2PO4 buffer (60: 40, v/v) solvent system on an Xterna® ms C18 SUM column (5 µm, 3.9 × 150 mm) and pH was adjusted to 4.5 with ortho-phosphoric acid. Mobile phase flow rate was 1 ml/min and ultraviolet (UV) detection was at 293 nm. Validation of the method was performed to determine its selectivity, linearity, precision, as well as limits of detection (LOD) and of quantification (LOQ). Results: Standard curves were linear, r2 = 0.996, for amitriptyline over the concentration range 10 – 60 ng/ml. Recovery (98.3 to 100.85 %) was in the selected concentration range of 10 - 60 ng/ml. The LOD and LOQ of the method for amitriptyline were 0.109 and 0.332 µg/ml, respectively. The validated method was successfully applied to measure plasma concentrations of amitriptyline and to measure the doses of activated charcoal currently used in the management of acute amitriptyline drug poisoning. Conclusion: The proposed RP-HPLC method enables determination of amitriptyline with good separation and resolution of the chromatographic peaks. Validation revealed that the method is sensitive, accurate and selective. Using half of the standard dose of the activated charcoal gave a comparable effect to the standard dose in reducing drug concentration in the blood. While, using quarter of the standard dose of activated charcoal does not have a cleared effect [22].

Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. Experimental data on the pharmacokinetic interactions between herbal products and drugs are limited. This study attempted to investigate the effect of *Bacopa monnieri* Linn. (Brahmi) formulation on the pharmacokinetics of amitriptyline in rats. In this study, rats were randomly divided into two groups (n = 6 each) which were served as a control (amitriptyline alone) and treatment group (amitriptyline with *B. monnieri*), respectively. Rats in the treatment group received *B. monnieri* (31 mg/kg/day) whereas the control group received normal saline by oral gavage for seven days before a single intragastric administration of 25 mg/kg amitriptyline. Plasma concentrations of amitriptyline were measured up to 24 h after its administration by a developed and validated high-performance liquid

chromatography method. Pretreatment with *B. monnieri* produced a significant increase in the maximum plasma concentration (Cmax), area under the curve (AUC0-t) and elimination half-life (t1/2) of amitriptyline by 16.8%, 26.5%, and 15.5%, respectively, compared to amitriptyline alone. Moreover, oral clearance and volume of distribution (Vss) were decreased by 26.2% and 15.5% respectively. This study concluded that *B.monnieri* significantly enhanced the oral bioavailability of amitriptyline in rats [23].

A new, rapid and sensitive reverse phase HPLC method was developed and validated for the determination of amitriptyline hydrochloride in tablet formulations and urine. The mobile phase used acetonitrile and water, (50 % v/v) adjust pH to 5 using phosphoric acid. The separation was achieved on C18 reversed-phase column (250 mm x 4 mm i.d.). The flow rate was 0.6 ml/min and UV detection at 254nm. The retention time for amitriptyline hydrochloride was 7.3 min. The calibration curve was linear in the range 0.5-3 µg/mL. The mean recovery for amitriptyline hydrochloride is 100.025 %. The assay was precise within day and between days. The method provided excellent sensitivity, recovery, accuracy and reproducibility in therapeutic or toxic concentrations. Common excipients do not interfere [24].

In this work, an efficient sample pretreatment method has been developed by combining salt induced–homogenous liquid–liquid extraction, dispersive solid phase extraction, and dispersive liquid–liquid microextraction based on the solidification of floating organic droplet for the extraction of some widely used tricyclic antidepressant (TCA) drugs (nortriptyline, amitriptyline, desipramine, clomipramine, and imipramine) in human urine samples before their determination by high performance liquid chromatography–ultraviolet detection. In brief, the target analytes are first isolated from urine samples into acetonitrile (ACN) separated by adding a salt. Then the obtained ACN phase is treated with a mixture of appropriate sorbents to remove interferences. Afterward, the purified ACN is mixed with menthol as an extractant and rapidly injected into alkaline HPLC–grade water as a preconcentration step. Next, the obtained solution is placed in an ice bath and menthol collects on top of the solution after solidification. The solidified drop is then withdrawn and injected into separation system after dissolving in 10 µL ACN. Under the optimum experimental conditions, extraction recoveries and enrichment

factors of the selected drugs ranged from 69–84 % and 345–420, respectively. The limits of detection and quantification were obtained at the ranges of 0.22–0.31, and 0.71–1.1 µg L–1, respectively. The relative standard deviations of the proposed method were ≤ 6 % for intra– (n=6) and inter–day (n=4) precisions at a concentration of 10 µg L–1 (each drug). Finally, the suggested approach was applied to determine of TCA drugs in different patients' urine samples. The method could be applied in further TCAs pharmacokinetic and forensic studies [25].

To study correlations between the concentrations, in serum, of amitriptyline and its most important metabolites in clinical response in patients, we developed a “high performance” liquid chromatographic method for the routine determination of amitriptyline, nortriptyline, 10-hydroxyamitriptyline, desmethylnortriptyline, and E(trans)- and Z(cis)-10-hydroxynortriptyline. These compounds are extracted from 1 ml of alkalinized serum in to hexane/isoamyl alcohol (99/1vol). Perazine is the internal standard. To minimize irreversible adsorption of the drugs on to the glassware, 5 µg of maprotiline is added to the organic phase just before evaporation. After a 10-min resolution on silica column eluted with acetonitrile/methanol/NH4OH(1 mol/L), absorbance is measured at 240 nm. Only chlorimipramine, doxepin, procainamide and N-acetylprocainamide may interfere with the assay of the compounds that probably are therapeutically relevant: amitriptyline, nortriptyline, and E-10-hydroxynortriptyline. Uremia, lipemia and icterus also do not affect analysis [26].

2. Gas Chromatography

A gas-chromatographic procedure for the estimation of amitriptyline and its metabolites in serum and urine using a nitrogen-specific detector is described. Specially cleaned glassware and purified solvents are used for the extraction of serum to further minimize extraneous peaks. Trimethylamine is added to serum before extraction to improve the recovery of drugs. Urine is refluxed at pH approximately 1 to hydrolyze the conjugates and to convert hydroxymetabolites to corresponding dehydro compounds. Serum is not hydrolyzed. 2. Two internal standards, one a tertiary amine similar in structure to amitriptyline and the other a secondary amine similar in structure to nortriptyline, are added to the specimen prior

to extraction to obviate the need for accurate measurements of volumes during extraction and analysis. Urine and serum are washed with organic solvents at acidic pH to remove neutral and acidic impurities. Secondary bases are converted to their acetyl derivatives. 3. In the serum of a patient who is on amitriptyline therapy or who has ingested an overdose of amitriptyline, nortriptyline, a pharmacologically active metabolite is also measured. However, detection or estimation of hydroxymetabolites in serum is not clinically relevant. Hydroxylation index of an individual patient is determined by measuring the ratio of nortriptyline to its hydroxymetabolite in urine. 4. Amitriptyline and nortriptyline can be estimated in serum at a lower level of 10 and 20 ng/ml respectively. The procedure is linear over a wide range of amitriptyline and its metabolites. The use of an electronic integrator allows the estimation of different compounds with 100 fold difference in their concentration from the same chromatogram [27]**.**

Gas chromatography was used to determine plasma levels of amitriptyline, nortriptyline and their 10-hydroxy derivatives after conversion to the dehydro compounds by heating with acid. The primary amine 10-hydroxydesmethylnortriptyline is also dehydrated and the dehydro compound coincides on the chromatogram with dehydronortriptyline. Treatment of the extract with salicylaldehyde selectively removed the primary amine, which was determined by difference. Cis- and trans-hydroxydesmethylnortriptyline were isolated from urine by thin-layer chromatography and used to standardize the estimation. The stability of all the metabolites in plasma was investigated. Results are given for hydroxydesmethylnortriptyline levels in the plasma of 41 patients treated with amitriptyline [28]**.**

3. Spectrophotometric Methods:

A simple, accurate and precise absorption correction method has been developed for simultaneous estimation of Amitriptyline hydrochloride and Methylcobalamin in combined tablet dosage form. The method utilizes distilled water as solvent and λmax of Amitriptyline hydrochloride and Methylcobalamin selected for analysis were

found to be 239 nm and 351 nm respectively. The method was validated as International Conference on Harmonization (ICH) guidelines. The linearity rage lies between 20-60 µg/ml (R^2 0.9998) for Amitriptyline hydrochloride and 3-9 µg/ml (R^2 0.9990) for Methylcobalamin. The accuracy and precision were determined and found to comply with ICH guidelines. The method showed good reproducibility and recovery with %RSD in desired range. The proposed method can be applied for routine analysis of both drugs [29].

A new and simple procedure for the spectrophotometric determination of the tricyclic antidepressant drug amitriptyline has been developed. This method is very simple as there is no requirement of prior separation of the complex and reagents are commonly available. The method is based on enhancement of sensitivity of the [AMIYTP]+ β-cyclodextrin and PEG molecules involved in formation of molecules inclusion complex, in presence of polyethylene glycol (PEG) medium. The mole ratio of [AMIYTP]+ β-cyclodextrin and PEG molecules in inclusion complex were determined by the curve fitting method. The value of molar absorptivity of {[AMIYTP: (β CD)] PEG} complex in term of the drug lies in rage of (2.20 - 2.23) × 104 L·mole–1·cm–1 at absorption maximum 242 nm. The slope, intercept and correlation co-efficient were found to be 14.21, 0.0046, and 0.998, respectively. The effect of analytical variables on the determination of the drug and composition of the ion associated complex are discussed in the paper. This method is applicable in the determination of amitriptyline in the pharmaceutical preparations [30].

A new and simple procedure for the spectrophotometric determination of the tricyclic antidepressant drug amitriptyline is proposed. The method is based on enhancement of sensitivity of the [AMIYTP]+ β-cyclodextrin and PEG molecules involved in formation of molecules inclusion complex, in presence of polyethylene glycol (PEG) medium. The mole ratio of [AMIYTP]+ β-cyclodextrin and PEG molecules in inclusion complex were determined by the curve fitting method. The value of molar absorptivity of {[AMIYTP: (β CD)] PEG} complex in term of the drug lies in rage of (2.20 - 2.23) × 104 L·mole–1·cm–1 at absorption maximum 242 nm. The slope, intercept and correlation co-efficient were found to be 14.21, 0.0046, and

0.998, respectively. The effect of analytical variables on the determination of the drug and composition of the ion associated complex are discussed in the paper. This method is applicable in the determina-tion of amitriptyline in the pharmaceutical preparations [31].

A simple, rapid and specific UV spectroscopic method with good sensitivity was developed and validated for simultaneous determination of amitriptyline HCl and chlordiazepoxide in combined pharmaceutical dosage form. The maximum wavelength was found to be 222 nm for amitriptyline HCl and 251 nm for chlordiazepoxide. The linearity range was found to be 2-20 µg/ml for amitriptyline HCl and 1-10 µg/ml for chlordiazepoxide. The value of LOD and LOQ was 0.471 and 1.429 µg/ml for amitriptyline HCl and 0.297 and 0.902 µg/ml for chlordiazepoxide. The low RSD values indicate good precision and high accuracy values indicate accuracy of the proposed method. The method was satisfactorily validated as per ICH guidelines and it can be used for the routine analysis of formulation [32].

The objective of this study was to develop the degradation studies of different brands of amytriptyline hydrochloride in market. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. Amitriptyline (AMT) belongs to tricyclic dibenzocycloheptadiene derivatives. It acts primarily as a serotonin-norepinephrine reuptake inhibitor, used for the treatment of several psychiatric disorders. This drug was subjected to different stress conditions as per International Conference on Harmonization guidelines (ICH). An ultraviolet UV spectroscopic method was developed for analysis of the drug in the presence of the degradation products. Distilled water was used as a solvents. The amount of degraded drugs was calculated by taking the absorbance at 222 nm. According to the assay limit of USP specified that the content should not be less than 95% and not more than 105% of labeled amount. All brands were degraded on basic pH and on acidic pH. In addition to heat exposure all brands were also degraded. It was

concluded that all brands degraded from ranges for all the stresses applied for degradation studies [33].

A simple, rapid, selective and highly sensitive spectrophotometric method is described for the quantitative determination of a tricyclic antidepressant drug, amitriptyline hydrochloride (AMT) in pure and in pharmaceutical preparations. The method is based on the bromination of AMT with known excess of bromine. The unreacted bromine is determined based on its ability to bleach the dye Eiochrome blue black R quantitatively at 530 nm. Beer's law was obeyed over the concentration range 0.0–15 µg/mL. The molar absorptivity value was found to be 1.345×104 L/ moL/cm, with the corresponding Sandell's sensitivity values of 0.0233 µg/cm^2. The limits of detection and (LOD) and quantification (LOQ) are also reported for the developed method. Intra- and inter-assay precision and accuracy of the method was established according to the current ICH guidelines. Applications of the procedure to the analysis of various pharmaceutical preparations gave reproducible and accurate results. Further, No interferences were observed from excipients and the validity of the method was tested against reference method. Percent of relative recoveries values were 98.57% to 100.52% [34].

A binary mixture of amitriptyline HCl and chlordiazepoxide was determined by three different methods. The first method involved determination of amitriptyline HCl and chlordiazepoxide using the first derivative spectrophotometric technique at 219 and 230 nm over the concentration ranges of 1-20 and 2-24 µg/ml with mean accuracies 100.9±0.87 and 99.2±1.0%, respectively. The second method was reversed-phase high performance liquid chromatography using methanol: acetonitrile: 0.065 M ammonium acetate buffer (50:20:30, v/v/v), final pH adjust to 5.5 ± 0.02 with ortho phosphoric acid as the mobile phase and was pumped at a flow rate of 1.0 ml/min. Quantification was achieved with ultraviolet detection at 240 nm over concentration ranges of 0.25-4 and 0.1-1.6 µg/ml; mean accuracies were 100.55±0.62 and 100.71±0.81%, respectively. The third method utilized high performance thin layer chromatography method in tablet dosage form. The method was based on separation of the two drugs followed by densitometric measurements of their spots at 240 nm. The separation was carried out on Merck thin layer chromatographic

aluminium sheets of silica gel 60 F254 using carbon tetrachloride: acetone:triethylamine (6:3:0.2, v/v/v) as mobile phase. The linearity was found to be in the range of 50-600 and 20-240 ng/spot for amitriptyline hydrochloride and chlordiazepoxide, respectively. The methods were successively applied to pharmaceutical formulation because no chromatographic interferences from the tablet excipients were found. The suitability of these methods for the quantitative determination of the compounds was proved by validation [35].

4. Hyphenated Methods:

A. Liquid Chromatography-Tandem Mass Spectrometry

A rapid, specific, and reliable LC-MS/MS-based bioanalytical method was developed and validated in rat plasma for the simultaneous quantitation of amitriptyline and its metabolite nortriptyline. Chromatographic separation of these analytes was achieved on a Gemini C18 column (50 × 4.60 mm, 5 µm) using reversed-phase chromatography. The mobile phase was an isocratic solvent system consisting of 1% formic acid in water and methanol (10:90, v/v), at a flow rate of 0.2 mL/min. The analytical range was set as 0.1-500 ng/mL for amitriptyline and 0.08-500 ng/mL for nortriptyline using a 200 µL plasma sample. The accuracy and precision of the assay were in accordance with FDA regulations for the validation of bioanalytical methods. The validated method was successfully applied to a pharmacokinetic study in six rats after oral administration of amitriptyline (15 mg/kg). This method allows laboratory scientists to rapidly determine amitriptyline and nortriptyline concentrations in plasma [36].

B. Liquid Chromatography-Mass Spectrometry

A Fully automated platform for determination of Tricyclic Antidepressant in serum by was described by Shimadizu [37].

Amitriptyline is a tricyclic antidepressant that is metabolized mainly by CYP2C19 and CYP2D6 enzymes. Higher plasma levels of amitriptyline and its active metabolite, nortriptyline, are associated with an increased risk of adverse events including anticholinergic effects. The aim of this study was to evaluate the effects of CYP2C19

and CYP2D6 genetic polymorphisms on amitriptyline and nortriptyline pharmacokinetics. Twenty-four Korean healthy adult male volunteers were enrolled in the study after stratification by their CYP2C19 and CYP2D6 genotypes. Serial blood draws for pharmacokinetic analysis were made after a single oral 25-mg dose of amitriptyline was administered. Plasma amitriptyline and nortriptyline concentrations were measured by a validated liquid chromatography with tandem mass spectrometry. Population pharmacokinetic modeling analysis was conducted using NONMEM, which evaluated the effects of CYP2C19 and CYP2D6 genotypes on amitriptyline and nortriptyline pharmacokinetics. The biotransformation of amitriptyline into nortriptyline was significantly different between subjects with the CYP2C19*2/*2, *2/*3, and *3/*3 genotypes and those with the other genotypes, with an estimated metabolic clearance of 17 and 61.5 L/h, respectively. Clearance of amitriptyline through pathways other than biotransformation into nortriptyline was estimated as 18.8 and 30.6 L/h for subjects with the CYP2D6*10/*10 and *10/*5 genotypes and those with the other genotypes, respectively. This study demonstrated a quantitative effect of the CYP2C19 and CYP2D6 genotypes on amitriptyline and nortriptyline pharmacokinetics. Production of nortriptyline from amitriptyline was associated with CYP2C19 genotypes, and clearance of amitriptyline through pathways other than biotransformation into nortriptyline was associated with CYP2D6 genotypes. These observations may be useful in developing individualized, optimal therapy with amitriptyline [38].

C. Gas Chromatography-Mass Spectrometry

Antidepressant drugs are widely used for the treatment of depression and other psychiatric disorders and as a result they are involved in numerous clinical and forensic cases. The aim of this study was the development, optimization and validation of a simple, specific and sensitive GC/MS method for the simultaneous determination of amitriptyline and imipramine in human urine samples, clomipramine used as internal standard. The compounds were extracted from urine at basic pH into n-hexane – ethyl acetate (9:1, v/v), back-extracted into acidic aqueous solution. The calibration curves were linear (R2 ≥ 0.990) within the range of 80 –5000 ng/L for

all analytes. Precision expressed as the % RSD was found to be less than 5.5 % for all analytes. The developed method is suitable for routine work. It also used to successfully analyze clinical and forensic samples [39].

Tricyclic antidepressant drugs (TCA) are the second widely used drugs after the analgesics, and involve in acute poisoning cases. In this study we measured unusual high dose of TCA levels in three suicidal poisoning cases. Toxicological screening on patients urine and blood samples were performed with GC/MS, and the quantitation of drugs were done with GC/MS and FPIA methods. Amitriptyline, acetaminophen, and codeine levels were 1135 ng/ml blood, 14.42 µg/ml blood, and 72.9 µg/ml urine respectively in case 1. Amitriptyline levels were found as 505, 429, and 370 ng/ml blood for consequent three days. In Case 2, amitriptyline levels were 883.56 and 137.35ng/ml blood for two days. In Case 3, initial imipramine level was 2560 ng/ml blood. First two patients were fully recovered 4 days after the incident. The third patient died 36 hours after the admission. In acute poisoning cases, early emergency teratment, obtaining rapid and reliable toxicological laboratory results were considered vitally important in poisoning cases. In another study, discontinuous batch liquid-liquid extraction was employed to isolate amitriptyline and nortriptyline from the majority of the foodstuff matrix. And the two compounds were analyzed by GC-NPD [40].

In the present research, a novel microextraction method was used based on dispersive liquid-liquid microextraction using low-density solvent for the determination of trace amounts of amitriptyline and doxepin as model compounds in human plasma and spring water samples before gas chromatography and flame ionization detection. The parameters influencing the extraction efficiencies including type of the extracting solvent, volume of the aqueous sample solution (donor phase), volume of the extraction solvent (acceptor phase), the number of air-injection, and the effects of pH and ionic strength were optimized. Under the optimized conditions, the obtained enrichment factors were >80. By plotting peak areas of the sample solutions versus various concentrations of the analytes, calibration curves were obtained which show the linear ranges of 10-3000 ng/mL with correlation coefficients

of >0.996. The precision of the method was calculated <4.2 and the limit of detection was 1 ng/mL for two analytes. The proposed microextraction method was used for the extraction of the analytes in environmental water and plasma samples and the calculated relative recoveries were all >91% [41].

D. Solid Phase Extraction-Tandem Mass Spectrometry

Tricyclic antidepressant drugs (TCA) are the second widely used drugs after the analgesics, and involve in acute poisoning cases. In this study we measured unusual high dose of TCA levels in three suicidal poisoning cases. Toxicological screening on patients urine and blood samples were performed with GC/MS, and the quantitation of drugs were done with GC/MS and FPIA methods. Amitriptyline, acetaminophen, and codeine levels were 1135 ng/ml blood, 14.42 µg/ml blood, and 72.9 µg/ml urine respectively in case 1. Amitriptyline levels were found as 505, 429, and 370 ng/ml blood for consequent three days. In Case 2, amitriptyline levels were 883.56 and 137.35ng/ml blood for two days. In Case 3, initial imipramine level was 2560 ng/ml blood. First two patients were fully recovered 4 days after the incident. The third patient died 36 hours after the admission. In acute poisoning cases, early emergency teratment, obtaining rapid and reliable toxicological laboratory results were considered vitally important in poisoning cases [42].

Quantitative analysis of tricyclic antidepressant drugs (TCAs) in forensic laboratories traditionally relies on HPLC and immunoassay, however, interfering substances, false positives, and cross reactivity to other compounds may compromise results. An effi cient, fast, accurate, and sensitive SPE/MS/MS method with a wide calibration range was developed for the simultaneous quantitation of eight antidepressant drugs in human serum (Amitriptyline, Nortriptyline, Imipramine, Desipramine, Doxepin, Nordoxepin, Clomipramine, and Norclomipramine). This method employs protein precipitation followed by dilute and shoot on the SPE/MS/MS system, enabling analysis of all eight TCAs at 13 seconds per sample producing > 10x savings in analysis time and solvent consumption compared to typical HPLC methods [43].

Freely dissolved concentrations are considered to be the most relevant concentration in pharmacology and toxicology, as they represent the active concentration available for interaction with its surroundings. Here, a solid-phase microextraction (SPME) coating that combines octadecyl and propylsulfonic acid groups as strong cation exchange sites, known as C18/SCX or "mixed-mode" SPME, is used to measure freely dissolved concentrations of amitriptyline, amphetamine, diazepam and tramadol to different binding matrices, including bovine serum albumin (BSA), human serum albumin (HSA), human plasma and human whole blood. A potential confounding factor in binding studies is that proteins may sorb to the fiber coating leading to incorrect measurement of protein sorption or changes in uptake kinetics to the fiber coating. Sorption of bovine serum albumin (BSA) was observed and quantified using a Lowry assay. BSA binds to the C18/SCX fiber in small amounts, but large changes in uptake kinetics were not observed. All experiments were performed at equilibrium. In addition, however, the effect of depletion and non-equilibrium extraction on the estimation of protein binding affinities was also studied. Binding affinities to BSA and human serum albumin (HSA) were calculated as log KBSA or log KHSA. These values were very similar to reported literature values. Sampling at either equilibrium or non-equilibrium resulted in similar binding affinities. Furthermore, SPME fibers were used to measure freely dissolved concentrations in undiluted human plasma and whole blood. Analysis of SPME extracts could be performed using HPLC-UV or HPLC with fluorescence detection without prior clean-up of the samples. Measured bound fractions in plasma using this SPME approach were comparable to literature reference values. Bound fractions in whole blood were always higher than in plasma, due to red blood cell partitioning. This work shows the potential of SPME as sampling tool for freely dissolved concentrations, especially for highly protein-bound compounds. Conventional SPME coatings such as polyacrylate (PA) or polydimethylsiloxane (PDMS) might be lacking sensitivity when sampling the small neutral fraction of highly protein-bound positively charged compounds, but the C18/SCX fiber is able to sorb the charged species of organic cations, thereby improving sensitivity for these types of compounds [44].

In this study, magnetic framework composites (MFCs) (Fe_3O_4@TMU-10) microspheres were successfully fabricated and applied as an effective sorbent for preconcentration of the two model tricyclic antidepressants (TCAs) amitriptyline and imipramine from biological samples. MFCs were fabricated by a step-by-step assembly, novel, simple and efficient strategy. The shell thickness of the Metal-organic frameworks (MOFs) could also be easily controlled by tuning the number of assembly cycles. By coupling magnetic solid-phase extraction (MSPE) with high-performance liquid chromatography with UV detector (HPLC-UV), a simple, reliable, fast, sensitive and cost-effective method for simultaneous determination of TCAs was developed. Under optimal conditions, the preconcentration factors and relative recoveries of the studied compounds were obtained in the range of 43-50 and 90.5-99.0% respectively. The calibration curves were obtained in the range of 5-800 μg L^{-1} with reasonable linearity ($R^2 > 0.9904$) and the limits of detection (LODs) ranged between 2 and 4 μg L^{-1} (based on S/N = 3). The relative standard deviations of intra- and inter-day tests ranged from 3.1 to 4.6% and from 4.3 to 5.2%, respectively. The results demonstrate that Fe_3O_4@TMU-10 core-shell magnetic microspheres combine advantages of MOFs and magnetic nanoparticles, and are the promising sorbents for rapid and efficient extraction of target analytes from urine and plasma complex biological samples [45].

Rationale: Paper spray (PS) has been developed as a method of choice for point-of-care analysis in many real cases, where its applications can be further expanded with delicate high-throughput design. To achieve this goal, we developed a new PS regime, with the assembly of an induced high voltage into the ion source. Compared to regular DC high voltage, the newly-developed setup is capable of high-throughput, simple configuration and rapid switch between individual papers without complicated electric/mechanic design.Methods: A device of high-throughput induced PS (IPS) was designed by using a two-dimensional (2D) rotating platform equipped with a circular glass plate. The paper substrate was placed on the circular glass plate and separated from the electrode. The method avoids the physical contact between the electrode and the sample. The charged droplets was generated at the

paper tip once an induced high voltage was applied to a wet paper. Results: A relatively rapid analytical speed of 2.6 s per sample was achieved via IPS-MS. Rapid quantification of amitriptyline (AMT) in complicated matrices was obtained within 1 min using isotope internal standard method. Limits of detection for AMT in urine, FBS and blood were calculated to be 0.48, 0.85, 1 ng/mL, respectively. In addition, the high-throughput IPS-MS can be used for chemical reaction monitoring. Conclusions: We have demonstrated the versatility of high-throughput IPS-MS in ambient ionization, which successfully simplified the experimental installation and facilitated the experimental operation. Therefore, we believe that high-throughput IPS-MS analysis will be widely used for discovering drugs and screening reaction, and the present design has a potential for applications in paper chip-MS analysis [46].

Conclusion

A number of analytical methods (HPLC, GC, Spectrophotometric, Hyphenated techniques) were summarized in this paper for analysis of amitriptyline alone and in combination with other drugs in dosage forms and body fluids of human and animal model are presented. The choice of analytical method depends on several reasons like the type and amount of sample, the nature of interferents, the availability of analytical instrument and skilled personnel, presence of further analytical step, data handling, and other reasons. GC-MS is at least expensive and destructive method (the components of the sample cannot be retained if further analysis is needed), HPLC is accompanied by use of large amount of mobile phase which in turn leads to higher cost per analysis, Derivative spectroscopic methods usually require rigorous manipulation of the raw data, Spectrophotometric, Polarographic and Non-aqueous titration methods may have the limitation of low sample throughput.

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