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(REVIEW ARTICLE)

Validated analytical methods for estimation of tramadol

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Abstract

Tramadol is an opioid pain medication used to treat moderate to moderately severe pain. When taken by mouth in an immediate-release formulation, the onset of pain relief usually begins within an hour. It is also available by injection. It is available in combination with paracetamol (acetaminophen). There are many reported analytical methods for estimation of Tramadol. The present work list outs the various analytical methods for the estimation of the drug.

Keywords: Tramadol; Painkiller; Estimation methods; Review

1. Introduction

Tramadol is a schedule IV drug in the US is used primarily to treat mild to severe pain, both acute and chronic. Its IUPAC name is 2-[(Dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol. Tramodol hydrochloride is an odorless white to off white crystalline powder. It is readily soluble in water and freely soluble in methanol and ethanol. Its pKa value is 9.41. The water or n-octonol partition coefficient is 1.35 at pH 7. Molecular weight-263.37 g/mole. Structure of compound is given in Fig 1.

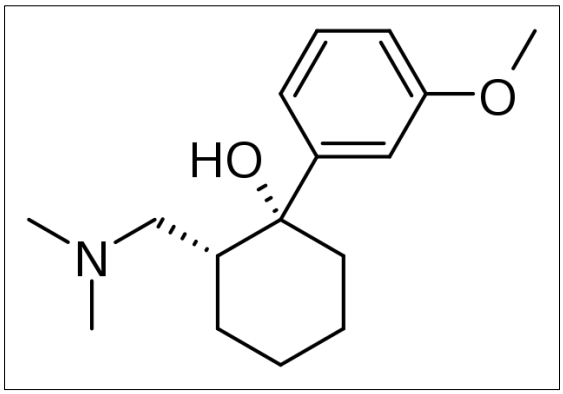

Figure 1 Structure of Tramadol

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2. Various Analytical Estimation methods

2.1. HPLC methods

Tramadol is dosed at 1 mg/kg to treat severe pain in children but the assay for tramadol in plasma samples for pharmacokinetic and toxicology studies does not often consider concurrently administered medications. In this study we developed and validated an HPLC- UV method to quantify tramadol and its main metabolite (O-desmethyl tramadol) in human plasma in the presence of seven potentially interfering drugs. Sample preparation method was developed by combining liquid-liquid extraction and protein precipitation. Chromatographic separation was achieved on a BDS-Hypersil-C18 column (5µm, 250cm, 4.6µm) using a double gradient method. The limit of quantification was 6.7 ng/ml for both tramadol and ODT. The precision and accuracy were in compliance with ICH guidelines. This method was successfully employed to analyse the blood samples of 137 paediatric participants in a tramadol pharmacokinetic trial[1].

Tramadol is a centrally acting analgesic used for prevention and treatment of moderate to severe pain. It is estimated that 0.1% of the administered dose passes into breast milk causing potentially unwanted effects in nursing babies. Pharmacokinetically, breast milk is supposed to be a separate compartment into which the drug is excreted-mainly by passive diffusion. Due to a complex composition of breast milk, a suitable sample preparation procedure is needed with a subsequent chromatographic analysis for drug determination. Among several sample cleanup procedures tested we chose the liquid-liquid extraction procedure using n-hexane as an organic phase with back extraction into aqueous phase since it was considered the most suitable and the most compatible with the subsequent HPLC analysis. The precision and the reproducibility of the method were improved approximately two times by using metoprolol as an internal standard thus making the method also more robust with regard to a variable composition of milk samples. These characteristics, together with low detection limit and short analysis time, proved that the developed method is suitable for monitoring of tramadol in human breast milk[2].

Several clinical studies have shown that the use of a combination of Tramadol and Piroxicam has displayed a better analgesic effect than the individual drug with the occurrence of fewer side effects. As such, there is no analytical method available for Qualitative and Quantitative determination of particular drugs in both combinations. Different analytical techniques can be applied for multicomponent analysis, including; spectrophotometer, chromatography, and electrophoresis. In the present work, the analytical methods of qualitative and quantitative analysis for combinations of Tramadol and Piroxicam have been used employing UV spectrophotometer and HPLC. Two simple, accurate, rapid, and selective analytical procedures were developed and validated for the simultaneous estimation of Tramadol HCl and Piroxicam using UV spectrophotometer and High-performance liquid chromatography with UV detection (HPLC-UV). The absorption Subtraction technique was utilized in the Simultaneous determination of the UV spectrophotometer. The HPLC method employs a C18 Phenomenex column (250cm, 4.6, 5µm), 20µL injection volume, column temperature controlled at 30° C, detection at 282 nm, Trifluoroacetic acid-pure acetonitrile (70: 30 v/v) mobile phase pumped at the rate. Parameters consisting of linearity, precision, accuracy, robustness, ruggedness, detection and quantitation are studied. From the investigations, the Absorptive factor of Tramadol HCl and Piroxicam at both the isosbestic points has been found. The percentage assay of 108.73±1.61 % and 104.54±0.94 % for Tramadol HCl and Piroxicam. The objective of validation of analytical procedures is to demonstrate that it is suitable for its intended purpose[3].

The introduction of sildenafil (SDF) to treat erectile dysfunction has solved a widespread condition with negative on the quality of life. Recently, the co-administration of tramadol (TMD) with SDF to manage premature ejaculation has illegally increased and thus drug-drug interaction studies of these drugs became of great importance. Although certain biological functions have been altered upon co-administration of the two drugs, methods for their determination in vivo to understand their interactions have yet to be published. Herein, therefore, an HPLC method with photometric detection was developed for the determination of a binary mixture of TMD and SDF in rabbit plasma after oral administration. In this study, a reversed-phase chromatography was performed at room temperature on a C18 column with a mobile phase composed of 10 mM Na2HPO4 solution (pH 7.5): acetonitrile (45:55, v/v) at a flow rate of 0.8mL/min using caffeine (CAF) as an internal standard. The detector was set at 220nm. The total analysis time was 6min. Calibration graphs were linear in the concentration ranges of 0.1-10 and 0.05-1 µg/mL with a detection limit of 0.05 and 0.02µg/mL for TMD and SDF, respectively. The method was validated in terms of accuracy, precision, limit of detection and quantitation, recovery, and stability as per US FDA bioanalytical guidelines. In addition, the metabolites N-desmethylsildenafil (UK-103,320) and O- desmethyltramadol were quantified in rabbit plasma after 2h of oral administration using LC-MS/MS. The simultaneous administration of TMD with SDF has affected peak plasma concentration (Cmax), Tmax, area under the concentration-time curve (AUC), and the elimination rate constant (Kel) of SDF. The present study is the first to give valuable insights into the drug- drug interaction and the pharmacokinetic implications associated with the co- administration of SDF and TMD[4].

The optimized method for HPLC determination of tramadol and its metabolite O-desmethyl tramadol in human plasma using sotalol as internal standard has been developed and validated by a new approach. The determination by fluorescence detection was performed on re-eluted solution, obtained after liquidâ€"liquid extraction with ethyl acetate of the three analytes from plasma. The chromatographic separation of tramadol under a gradient elution was achieved at a temperature of 15°C with a RP-18 column, guarded by a C18 precolumn. The mobile phase was a mixed aqueous solution containing ortho-phosphoric acid, triethylamine, acetonitrile and methanol in a complex gradient mode. The quantitative determination of tramadol was performed at different successive pairs of excitation/emission wavelengths (200/300 nm, 200/295 nm, 212/305 nm) with lower limits of quantification: LLOQ=4.078 ng/ml for tramadol, respectively LLOQ=3.271 ng/ml for O-desmethyl tramadol. For the LLOQ limits, were calculated the values of the coefficient of variation and difference between mean and the nominal concentration. For tramadol analyte they were CV%=5.147% and bias%=7.273% in the intra-days and CV%=4.894% and bias%=0.836% in the between-days assay, respectively for the metabolite O-desmethyl tramadol they were CV%=11.517% and bias%=0.337% in the intra-days and CV%=6.41% and bias%=3.259% in the between-days assay. In addition, the stabilities of the analytes were verified in different conditions. Both, tramadol and its metabolite proved to be stable in plasma for four weeks, frozen at 20°C, but also for 48 h at 15°C in the re-eluted solution after liquid-liquid extraction[5].

This paper describes an HPLC method for the determination of tramadol and its major active metabolite, O-desmethyltramadol (ODT), in human plasma. Sample preparation involved liquid-liquid extraction with diethyl ether-dichloro methane- butanol (5:3:2, v/v/v) and back extraction with sulphuric acid. Tramadol, ODT and the internal standard, sotalol, were separated by reversed phase HPLC using 35% acetonitrile and an aqueous solution containing 20mM sodium phosphate buffer, 30mM sodium dodecyl sulphate and 15mM tetraethylammonium bromide pH 3.9. Detection was by fluorescence with excitation and emission wavelengths of 275 and 300nm, respectively. The method was linear for tramadol (3.768ng/ml) and ODT (1.5384ng/ml) with mean recoveries of 87.2% and 89.8%, respectively. Intra- and inter-day precisions were 10.34% and 8.43% for tramadol and 9.43% and 8.75% for ODT at the respective limits of quantitation (3 and 1.5ng/ml). Accuracy for tramadol ranged from 96.2% to 105.3%. The method was applied to a pharmacokinetic study of tramadol in human volunteers[6].

Tramadol, an analgesic agent, and its two main metabolites O- desmethyltramadol (M1) and N-desmethyltramadol (M2) were determined simultaneously in human plasma by a rapid and specific HPLC method. The sample preparation was a simple extraction with ethyl acetate. Chromatographic separation was achieved with a ChromolithTM Performance RP- 18e 50mm—4.6mm column, using a mixture of methanol:water (13:87, v/v) adjusted to pH 2.5 by phosphoric acid, in an isocratic mode at flow rate of 2ml/min. Fluorescence detection (ex=200nm/em=301nm) was used. The calibration curves were linear (r2>0.997) in the concentration range of 2.5-500ng/ml, 1.25-500ng/ml and 5-500ng/ml for tramadol, M1 and M2, respectively. The lower limit of quantification was 2.5ng/ml for tramadol, 1.25ng/ml for M1 and 5ng/ml for M2. The within- and between-day precisions in the measurement of QC samples at four tested concentrations were in the range of 2.5-9.7%, 2.5-9.9% and 5.9-11.3% for tramadol, M1 and M2, respectively. The developed procedure was applied to assess the pharmacokinetics of tramadol and its two main metabolites following administration of 100mg single oral dose of tramadol to healthy volunteers[7].

Tramadol has extracted from the exhaled breath condensate (EBC) samples through the supported liquid membrane consisting of 2-nitrophenyl octyl ether impregnated in the hollow fiber wall, and the lumen of the hollow fiber was filled with 20L of an acceptor phase. Under the optimum conditions of the electromembrane extraction, i.e. the stirring speed of 750 rpm, extraction time of 20min, acceptor pH at 1.0, donor phase pH at 6.0, and an applied voltage of 170 V across the supported liquid membrane, a pre concentration factor of 128-fold with a extraction recovery of 64% was achieved. Acceptable linearity was obtained in the tramadol concentration range of 5-1000 ng/mL (R2 = 0.9999) with a limit of detection of 1.5ng/mL and a limit of quantitation of 5ng/mL. The relative standard deviations for the intra-day and inter-day replications were obtained between 0.4% and 2.5%. The validated technique was successfully used to determine tramadol in real EBC samples[8].

A new method for the determination of 2- [(dimethylamino) methyl]cyclohexanone (DAMC) in Tramadol (as active substance or active ingredient in pharmaceutical formulations) is described. The method is based on the derivatisation of 2-[(dimethylamino) methyl]cyclohexanone with 2,4-dinitrophenylhydrazine (2,4-DNPH) in acidic conditions followed by a reversed-phase liquid chromatographic separation with UV detection. The method is simple, selective, quantitative and allows the determination of 2- [(dimethylamino) methyl]cyclohexanone at the low ppm level. The proposed method was validated with respect to selectivity, precision, linearity, accuracy and robustness[9].

Tramadol (TRA) is a weak opioid analgesic, prescribed to relieve mild to moderately severe pain. However, side effects of TRA overdoses, including vomiting, depression, tachycardia, convulsions, morbidity and mortality are often reported. In this study, an electrochemical sensor based on molecularly imprinted conductive polymer was firstly developed for

the quantitative and non-invasive detection of TRA. Secondly, a voltammetric electronic tongue (VE-Tongue) combined with chemometric methods was used for the qualitative analysis. The MIP sensor was constructed by self-assembling a poly-aniline layer coated with silver nanoparticles (PANI-AgNPs) on a screen- printed gold electrode (Au-SPE). Then, 2-amino-thiophenol was polymerised in the presence of TRA. The electronic device exhibits, under optimal conditions, responses proportional to TRA concentrations (0.01-100µg/mL) with detection and quantification limits of 9.42µg/mL and 28.55µg/mL, respectively. Moreover, its selectivity was proven by insignificant interferences of substances (paracetamol and citric acid). Spiked saliva and urine samples were used for the sensor practical application with a significant recovery above 90% and standard deviations below 4.5%. Besides, urine samples analyses using VE-Tongue and pattern recognition methods show good discrimination, classification, and prediction results with scores above 95%. Correspondingly, both electro-analytical devices could be viable for monitoring drugs in biological matrices[10].

A sensitive and selective electrochemical sensor was fabricated via the drop-casting of carbon nanoparticles (CNPs) suspension onto a glassy carbon electrode (GCE). The application of this sensor was investigated in simultaneous determination of acetaminophen (ACE) and tramadol (TRA) drugs in pharmaceutical dosage form and ACE determination in human plasma. In order to study the electrochemical behaviors of the drugs, cyclic and differential pulse voltammetric studies of ACE and TRA were carried out at the surfaces of the modified GCE (MGCE) and the bare GCE. The dependence of peak currents and potentials on pH, concentration and the potential scan rate were investigated for these compounds at the surface of MGCE. Atomic force microscopy (AFM) was used for the characterization of the film modifier and its morphology on the surface of GCE. The results of the electrochemical investigations showed that CNPs, via a thin layer model based on the diffusion within a porous layer, enhanced the electroactive surface area and caused a remarkable increase in the peak currents. The thin layer of the modifier showed a catalytic effect and accelerated the rate of the electron transfer process. Application of the MGCE resulted in a sensitivity enhancement and a considerable decrease in the anodic overpotential, leading to negative shifts in peak potentials. An optimum electrochemical response was obtained for the sensor in the buffered solution of pH 7.0 and using 2Î¼L CNPs suspension cast on the surface of GCE. Using differential pulse voltammetry, the prepared sensor showed good sensitivity and selectivity for the determination of ACE and TRA in wide linear ranges of 0.1-100 and 10-1000M, respectively. The resulted detection limits for ACE and TRA was 0.05 and 1M, respectively. The CNPs modified GCE was successfully applied for ACE and TRA determinations in pharmaceutical dosage forms and also for the determination of ACE in human plasma[11].

2.2. LCMS Techniques

The purpose of the study was to determine the advantages of different mass spectrometric instruments and commercially available metabolite identification programs for metabolite profiling. Metabolism of tramadol hydrochloride and the excretion of it and its metabolites into human urine were used as a test case because the metabolism of tramadol is extensive and well known. Accurate mass measurements were carried out with a quadrupole time-of-flight mass spectrometer (Q-TOF) equipped with a LockSpray dual-electrospray ionization source. A triple quadrupole mass spectrometer (QqQ) was applied for full scan, product ion scan, precursor ion scan and neutral loss scan measurements and an ion trap instrument for full scan and product ion measurements. The performance of two metabolite identification programs was tested. The results showed that metabolite programs are time-saving tools but not yet capable of fully automated metabolite profiling. Detection of non-expected metabolites, especially at low concentrations in a complex matrix, is still almost impossible. With low-resolution instruments urine samples proved to be challenging even in a search for expected metabolites. Many false-positive hits were obtained with the automated searching and manual evaluation of the resulting data was required. False positives were avoided by using the higher mass accuracy Q-TOF. Automated programs were useful for constructing product ion methods, but the time-consuming interpretation of mass spectra was done manually. High-quality MS/MS spectra acquired on the QqQ instrument were used for confirmation of the tramadol metabolites. Although the ion trap instrument is of undisputable benefit in MS(n), the low mass cutoff of the ion trap made the identification of tramadol metabolites difficult. Some previously unreported metabolites of tramadol were found in the tramadol urine sample, and their identification was based solely on LC/MS and LC/MS/MS measurements[12].

Tramadol is a synthetic opioid drug used in the treatment of chronic and acute pain. An abnormal prevalence of its misuse in elite sport to overcome pain resulting from prolonged physical effort was recently reported. However, besides its antinociceptive effects, tramadol consumption is associated with negative effects such as numbness, confusion, and reduced alertness. This fact prompted the Union Cycliste Internationale to ban the use of tramadol in cycling competitions. Herein, we present the development of a dried blood spot (DBS) sample collection and preparation method followed by a liquid-chromatography mass spectrometry (LC-MS) analysis to rapidly determine the presence of tramadol and its two main metabolites in blood samples. The detection window of each analyte was evaluated and the analysis of performance on various MS platforms (HRMS and MS/MS) was assessed. Tramadol and its two main

metabolites were detected up to 12 h after the intake of a single dose of 50 mg of tramadol in positive controls. In professional cycling competitions, 711 DBS samples collected from 361 different riders were analysed using the developed methodology, but all returned negative results (absence of parent and both metabolite compounds). In the context of professional cycling, we illustrate a valid method bringing together the easiness of collection and minimal sample preparation required by DBS, yet affording the performance standards of MS determination. The proposed method to detect tramadol and its metabolites was successfully implemented in cycling races with a probable strong deterrent effect[13].

This study was designed to assess the fate and the overall potential impacts of the widely prescribed drugs ranitidine and tramadol after their introduction into the aquatic environment. The probability to detect these two drugs in the aquatic environment was studied by analyzing their abiotic and biotic degradation properties. For this purpose, samples were irradiated with different light sources, and three widely used biodegradability tests from the OECD series, the closed bottle test (OECD 301 D), the manometric respirometry test (OECD 301 F) and the Zahn-Wellens test (OECD 302 B), were conducted. The ecotoxicity of the photolytically formed transformation products was assessed by performing the bacterial growth inhibition test (EN ISO 10712). Furthermore, quantitative structure-activity relationship analysis and a risk analysis based on the calculation of the predicted environmental concentrations have also been conducted to assess the environmental risk potential of the transformation products. The possible formation of stable products by microbial or photolytical transformation has been investigated with DOC and LC-MS analytics. In the present study, neither ranitidine, nor tramadol, nor their photoderivatives were found to be readily or inherently biodegradable according to test guidelines. The photolytic transformation was faster under a UV lamp compared to the reaction under an Xe lamp with a spectrum that mimics sunlight. No chronic toxicity against bacteria was found for ranitidine or its photolytic decomposition products, but a low toxicity was detected for the resulting mixture of the photolytic transformation products of tramadol. The study demonstrates that transformation products may have a higher environmental risk potential than the respective parent compounds[14].

A sensitive and selective liquid chromatography-tandem mass spectrometric (LC-MS) method was developed and validated for the determination of Tramadol in human plasma and urine. The analyte was separated on a Diamonsil C18 column with ammonium acetate (5 mmol x L(-1))-methanol (50:50,v:v) adjusted PH by caustic soda at a flow rate of 0.8 ml min(-1), and analyzed by mass spectrometry is in positive ion mode. The ion mass spectrum of m/z were 264.1 for Tramadol and 248.0 for Tinidazole (I.S.), respectively. The weighted (1/x2) calibration curve was linear over plasma concentration range 1.00-400.00 ng/ml and urine concentration range 0.01-16.00 microg/ml, with a correlation coefficient (r) of 0.9995 and 0.9997, respectively. The lower limit of quantification in human plasma was 1.00 ng/ml. The inter-and intra-day precisions (CV%) in both plasma and urine were lower than 10%, the mean method accuracies and recoveries from spiked plasma samples at three concentrations ranged from 98.2 to 100.1% and 61.6 to 62.9%, respectively. The developed method was successfully applied to determine Tramadol in human plasma and urine, and provided suitable profiles for clinical pharmacokinetic study of Tramadol[15].

An ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was developed and validated for the determination of fifteen basic pharmaceuticals, for analysis of post- and ante-mortem whole blood samples. The following compounds were included: amitriptyline and its metabolite nortriptyline, trimipramine, mianserin, mirtazapine, citalopram, paroxetine, sertraline, and venlafaxine (all antidepressants), levomepromazine and quetiapine (antipsychotics), ketobemidone and tramadol (analgesics), alimemazine (sedative antihistamine), and metoprolol (beta-blocker). The sample pretreatment consisted of liquid-liquid extraction (LLE) using ethylacetate:n-heptane (80:20, v/v). Six deuterated analogues were used as internal standards (IS). The compounds were separated using a reversed phase C18-column (2.1mm×100mm, 1.7µm), a flow rate of 0.5mL/min, and gradient elution with 5mM ammonium formate pH 10.2 and acetonitrile. Quantification was done by MS/MS using multiple reaction monitoring (MRM) in positive mode, using two transitions for the compounds and one transition for the IS. The run time of the method was 8min including equilibration time. The calibration curves had R(2) values above 0.995 for all the compounds. The intermediate precision had a relative standard deviation (RSD, %) ranging between 2.0 and 16%. Recoveries of the compounds were ≥81%. The lower limits of quantifications (LLOQs) for the compounds varied from 5.0nmol/L to 0.10µmol/L (1.3-26ng/mL) and the limits of detections (LODs) from 1.0 to 20nmol/L (0.24-5.3ng/mL). LLOQ corresponds to 0.28-5.5pg injected on column. Matrix effects (ME) were between 91 and 113% when calculated against an IS. A comparison with former confirmation LC-MS methods at the Norwegian Institute of Public Health, Division of Forensic Medicine and Drug Abuse Research (NIPH) was performed during method validation. Good correlation was seen for all compounds except sertraline, where the old LC-MS method was showing 33% higher results. The method has been running on a routine basis for more than a year, and has proven to be very robust and reliable with results for external quality samples, including sertaline, corresponding well to consensus mean or median[16].

2.3. GCMS Techniques

To search for pharmaceutical additives in illicit alcoholic beverages referred to the laboratory of Legal Medicine Organization in Iran in 2017. Hundred beverages were sampled. Ethanol content was determined by gas chromatography with flame ionization detection (GC-FID) and then a liquid-liquid extraction combined with reversed-phase high performance liquid chromatography equipped with a photodiode array detector (PAD) was employed for the qualitative analysis. The analysis was confirmed using gas chromatography coupled with mass spectroscopy (GC/MS). In 15% either one or more of the following were detected: tramadol, methadone, diazepam, oxazepam, flurazepam and alprazolam. Tramadol was found with highest frequency. The wide availability of addictive pharmaceutical is leading to fortification of alcoholic beverages on some countries. The addition of such depressant additives should be better known because of the potentially fatal consequences of the combination with ethanol, as well as the potential for adverse effects on behavior[17].

A simple and sensitive GC/MS method for the determination of tramadol and its metabolite (O-desmethyltramadol) in human plasma was developed and validated. Medazepam was used as an internal standard. The calibration curves were linear (r=0.999) over tramadol and O-desmethyltramadol concentrations ranging from 10 to 200 ng/mL and 7.5 to 300 ng/mL, respectively. The method had an accuracy of >95% and intra- and interday precision (RSD%) of ≤4.83% and ≤4.68% for tramadol and O-desmethyltramadol, respectively. The extraction recoveries were 97.6±1.21% and 96.3±1.66% for tramadol and O-desmethyltramadol, respectively. The LOQ using 0.5 mL human plasma was 10 ng/mL for tramadol and 7.5 ng/mL for O-desmethyltramadol. Stability studies showed that tramadol and O-desmethyltramadol were stable in human plasma after 8 h incubation at room temperature or after 1 week storage at -20°C with three freeze-thaw cycles. Also, this method was successfully applied to six patients who had been given an intravenous formulation of 100 mg tramadol with Cmax results of 2018.1±687.8 and 96.1±22.7 ng/mL for tramadol and O-desmethyltramadol, respectively[18].

Synthetic cathinones are an emerging class of designer drugs, frequently with deceptive labels and a multitude of analogs to circumvent drug control regulations. Research regarding the pharmacological effects and toxicity of these amphetamine derivatives is scarce, heightening the risk to the public health and safety. The composition of synthetic cathinone products continually changes and laboratories began to notice ethylone-positive products in late 2011. This report presents nine postmortem cases in whom ethylone was identified. Ethylone was isolated using solid-phase extraction and detected by gas chromatography-mass spectrometry. Seven of the cases had measurable concentrations of ethylone in blood, ranging from 38 to 2,572 ng/mL; ethylone was detected in the blood sample of one case with a concentration below the assay limit of quantification (25 ng/mL), and one case did not have detectable ethylone in blood. Besides ethylone, all but one case were also positive for 11-nor-9-carboxy-Δ9-tetrahydrocannabinol; seven cases had other drugs quantified in blood, including ethanol, alprazolam, benzoylecgonine, diphenhydramine, morphine and tramadol. In five cases where ethylone was present at blood concentrations >400 ng/mL, no other drugs excluding ethanol, cannabis metabolite and doxylamine (one case) were found. The assay also tested for mephedrone, methylone and three dimethoxyamphetamine analogs; no case was positive for these analytes. The present report documents postmortem blood concentrations of ethylone, a novel synthetic cathinone, along with other concurrently identified substances. The findings provide valuable information for developing analytical assays and evaluating a toxic concentration range of ethylone[19].

A sensitive and efficient method was developed for determination of tramadol and its metabolite (O-desmethyltramadol) in human urine by gas chromatography-mass spectrometry. Tramadol, O-desmethyltramadol and medazepam (internal standard) were extracted from human urine with a mixture of ethylacetate and diethylether mixture (1 : 1, v/v) at basic pH with liquid-liquid extraction. The calibration curves were linear (r = 0.99) over tramadol and O-desmethyltramadol concentrations ranging from 10 to 200 ng/mL and 7.5 to 300 ng/mL, respectively. The method had an accuracy of >95% and intra- and interday precision (relative standard deviation %) of ≤4.93 and ≤4.62% for tramadol and O-desmethyltramadol, respectively. The extraction recoveries were found to be 94.1 ± 2.91 and 96.3 ± 3.46% for tramadol and O-desmethyltramadol, respectively. The limit of quantification using 0.5 mL human urine was 10 ng/mL for tramadol and 7.5 ng/mL for O-desmethyltramadol. After oral administration of 100 mg of tramadol hydrochloride to a patient, the urinary excretion was monitored during 24 h. About 15% of the dose was excreted as unchanged tramadol[20].

Over recent years, hair has become the ideal matrix for retrospective investigation of chronic abuse, including for tramadol. However, in order to exclude the possibility of external contamination, it is also important to quantify simultaneously its main metabolite, O-desmethyltramadol (M1), which presence in hair reflects systemic exposure. In the present study a methodology aimed at the simultaneous quantification of tramadol and M1 in human hair was developed and validated for the first time. After decontamination of hair samples (60 mg), tramadol and M1 were

extracted with methanol in an ultrasonic bath (~5 h). Purification was performed by solid-phase extraction using mixed-mode extraction cartridges. Subsequently to derivatization, analysis was performed by gas chromatography-electron impact/mass spectrometry (GC-EI/MS). The method proved to be selective. The regression analysis for both analytes was shown to be linear in the range of 0.1-20.0 ng/mg with correlation coefficients of 0.9995 and 0.9997 for tramadol and M1, respectively. The coefficients of variation oscillated between 3.85 and 13.24%. The limits of detection were 0.03 and 0.02 ng/mg, and the lower limits of quantification were 0.08 and 0.06 ng/mg for tramadol and M1, respectively. The proof of applicability was performed in hair samples from six patients undergoing tramadol therapy. All samples were positive for tramadol and M1[21].

2.4. UV Estimation

The UV spectrophotometric methods for simultaneous quantitative determination of paracetamol and tramadol in paracetamol-tramadol tablets were developed. The spectrophotometric data obtained were processed by means of partial least squares (PLS) and genetic algorithm coupled with PLS (GA- PLS) methods in order to determine the content of active substances in the tablets. The results gained by chemometric processing of the spectroscopic data were statistically compared with those obtained by means of validated ultra-high performance liquid chromatographic method. The accuracy and precision of data obtained by the developed chemometric models were verified by analysing the synthetic mixture of drugs, and by calculating recovery as well as relative standard error (RSE). A statistically good agreement was found between the amounts of paracetamol determined using PLS and GA-PLS algorithms, and that obtained by this analysis, whereas for tramadol GA-PLS results were proven to be more reliable compared to those of PLS. The simplest and the most accurate and precise models were constructed by using the PLS method for paracetamol (mean recovery 99.5%, RSE 0.89%) and the GA-PLS method for tramadol (mean recovery 99.4%, RSE 1.69%)[22].

2.5. HPTLC Techniques

The introduced research presents a novel in vivo quantitative method for assay of mixtures of pregabalin and tramadol as a common combinations approved for treatment of neuropathic pain. Green analytical chemistry is a recently emerging science concerned with control of the use of chemicals harmful to the environment in various analytical methods. Consequently, a green high-performance thin layer chromatography (HPTLC) method was achieved for determination of the mixture in human plasma and urine satisfying both analytical and environmental standards. The separation was achieved on HPTLC sheets using a separating mixture of ethanol-ethyl acetate-acetone-ammonia solution (8:2:1:0.05, by volume) as a mobile phase. The sheets were dried in air then scanned at two wavelengths. For tramadol, 220 nm was chosen; however, pregabalin is an unconjugated drug, so its determination was a challenge. Hence for pregabalin, the plates were sprayed with ethanolic solution of ninhydrin (3%, w/v), to obtain a conjugated complex, which could be assessed at 550 nm. Furthermore, the developed method fulfilled the US Food and Drug Administration validation guidelines, and proved to be useful in therapeutic drug monitoring of this combination. The Eco-scale assessment protocol was implemented to determine the greenness profile of the applied method[23].

Research studies have been carried out to develop a chromatographic and densitometric method suitable for identification and determination of tramadol and impurities. In addition, the stability of tramadol in solutions was investigated, including an effect of solution pH, temperature and incubation time. In the first instance the conditions for identification and quantitative determination of tramadol and impurities in pharmaceutical preparations were established. The separation was performed on silica gel-coated chromatographic plates (HPTLC) using two mobile phases: (I) chloroform-methanol-glacial acetic acid (9:2:0.1, v/v/v); (II) chloroform-toluene-ethanol (9:8:1, v/v/v). The UV densitometry was carried out at lambda = 270 nm. The developed method is of high sensitivity and low detection and determination limits ranging from 0.044 to 0.35 microg. For individual constituents the recovery ranges from 93.23 to 99.66%. The next step was to evaluate the stability of tramadol and determine a method of decomposition under various experimental conditions. It was found that tramadol decomposes in various ways in acidic and basic environments producing (1RS)-[2-(3-methoxyphenyl) cyclohex-2-enyl]-N, N-dimethylmethanamine (imp. B) And (1RS, 2RS)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)cyclohexanol (imp. cis-T) or imp. Cis-T, respectively[24].

First analytical methods were herein developed for determination of pregabalin (PGB) and amitriptyline (AMT) as an active binary mixture used for management of neuropathic pain whether in pure forms or in human biological fluids (plasma/urine). First method is green high-performance liquid chromatography-diode array detector (HPLC-DAD) after derivatization of PGB with ninhydrin (NIN) on a reversed-phase C18 column using a mobile phase consisting of ethanol:water (97:3%, v/v) pumped isocratically at 0.8 mL/min; AMT were scanned at 215 nm, whereas PGB-NIN was scanned at 580 nm. Second method is High-performance thin-layer chromatography (HPTLC), where PGB and AMT were separated on silica gel HPTLC F254 plates, using ethanol:ethyl acetate:acetone:ammonia solution (8:2:1:0.05, by volume) as a developing system. AMT peaks were scanned at 220 nm, whereas PGB peaks were visualized by spraying 3% (w/v) ethanolic NIN solution and scanning at 550 nm. Linear calibration curves were obtained for human plasma

and urine spiked with PGB and AMT over the ranges of 5-100 µg/mL and 0.2-2.5 µg/band for PGB, and 1-100 µg/mL and 0.1-2.0 µg/band for AMT for HPLC-DAD and HPTLC methods, respectively. The suggested methods were validated according to Food and Drug Administration guidelines for bioanalytical methods validation and they can be applied for routine therapeutic drug monitoring for the concerned drugs[25].

Currently, analytical scientists are paying special attention to reducing reliance on hazardous chemicals in various analytical methods. By embracing this concept, we developed an eco-friendly high-performancethin-layer chromatography (HPTLC) method as an alternative for the conventional HPLC method for the determination of an essential human micronutrient, niacin (NIA), which is used improve the lipid profile of patients. Furthermore, the proposed HPTLC method is capable of determining the structurally related impurities of NIA such as pyridine-2,5-dicarboxylic acid, isonicotinic acid, pyridine, and 5-ethyl-2-methylpyridine, which exhibit nephrotoxic and hepatotoxic effects. The separation of this challenging mixture was achieved on HPTLC sheets using a mixture of ethyl acetate/ethanol/ammonia solution (6:4:0.05, v/v/v), and then the dried plates were scanned at 254 nm. The analytical eco-scale assessment protocol was used to assess the greenness profile of the presented method and compare it with the reported HPLC method. The suggested method was found to be greener with regard to the consumption of solvents and the yielding of waste. The results suggest that the described method can be safely implemented for the routine analysis of NIA pharmaceutical dosage without the interference of potential impurities in quality control laboratories[26].

A promising combination of tamsulosin HCl and tadalafil has recently been introduced for treating two prevalent and associated urological disorders: benign prostate hyperplasia and erectile dysfunction. Novel HPTLC methods were designed and validated for assaying the cited drugs in their challenging combined formulation. Separation was achieved using HPTLC silica gel 60 F254 plates as a stationary phase with a densitometric measurement at 280 nm. The proposed methods with two different chromatographic systems were successfully applied: a conventional mixture (method I) of ethyl acetate-toluene-methanol-ammonia (5:3:2:0.5, by volume) and a greener one (method II) with ethyl acetate-ethanol-ammonia (8:2:0.1, by volume). The two methods were evaluated through a comparative study in terms of selectivity, tailing factor, developing time and concentration ranges. The greenness profile for each method was then appraised with several green guides, namely GlaxoSmithKline solvent sustainability guide, Environmental, Health and Safety (EHS) tool, National Environmental Method Index (NEMI) and Eco-scale. Moreover, method specificity and peak homogeneity were evaluated by peak purity assessment using the winCATS® software spectral correlation tool. The methods have potential for being simple, fast, economic and selective, and the greener one could be a good option for sustainable analysis of the drugs[27].

Sofosbuvir (SOF) and ledipasvir (LDS) represent anti-hepatitis C binary mixture. Herein, a fast high-performance thin-layer chromatography (HPTLC) method was developed, validated and applied for simultaneous determination of SOF and LDS in biological matrix. An innovative strategy was designed which based on coupling dual wavelength detection with HPTLC. This strategy enabled sensitive, specific, high sample throughput and cost-effective determination of the SOF-LDS binary mixture. The developed HPTLC procedure is based on a simple liquid-liquid extraction, enrichment of the analytes and subsequent separation with UV detection. Separations were performed on HPTLC silica gel 60 F254 aluminum plates with a mobile phase consisting of ethyl acetate-glacial acetic acid (100:5, v/v). The Rf values for SOF and LDS were 0.62 and 0.30, respectively. Dual wavelength scanning was carried out in the absorbance mode at 265 and 327 nm for SOF and LDS, respectively. The linear ranges were 40-640 and 9-144 ng/band for SOF and LDS, respectively with correlation coefficients of 0.9998. The detection limits were 10.61 and 2.54 ng/band and the quantitation limits were 32.14 and 7.70 ng/band for SOF and LDS, respectively indicating high sensitivity of the proposed method. Consequently, this permits in vitro and in vivo application of the proposed method in rabbit plasma with good percentage recovery (95.68-103.26%). Validation parameters were assessed according to ICH guidelines. The proposed method represents a simple, high sample throughput and economic alternative to the already existing more complicated reported LC-MS/MS techniques. The method would afford an efficient tool for therapeutic drug monitoring and bioavailability studies of SOF and LDS[28].

In recent years, a lot of single-pill combinations (SPC) are manufactured and are used as a promising choice in treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis. However, this trend made a serious challenge to drug analysts because of the difficulty in the analysis of two or more drugs in presence of each other. In this study, two chromatographic methods were developed for the simultaneous analysis of chlorzoxazone (CZ), diclofenac sodium (DIC) and tramadol hydrochloride (TRA) in presence of three of their related substances and potential impurities. Method I involved application of HPLC with diode array detection where Waters Symmetry C8 column was used as stationary phase. Mixture of ortho-phosphoric acid (0.03 M, pH 3) and acetonitrile was used as a mobile system with gradient elution and flow rate 1 mL/min. Peak areas were measured at the wavelengths 218 nm for TRA and 280 nm for CZ and DIC. Peaks eluted at retention times 2.30, 5.75 and 9.74 min for TRA, CZ and DIC respectively. Method II based on HPTLC separation. In this method, HPTLC silica gel 60 F254 plates were used with mobile phase of hexane: ethyl

acetate: methanol: acetic acid (12: 6: 2: 0.1, by volume). Densitometry scanning was performed at 280 nm for all drugs. The retardation factor (Rf) values were 0.14, 0.40 and 0.58 for TRA, DIC and CZ respectively. Both methods were validated according to International Conference on Harmonization (ICH) Guidelines with respect to linearity, ranges, accuracy, precision, specificity, robustness and limits of detection and quantitation. Linearity ranges were 15 - 100µg/mL for the three drugs in method I. In method II, the obtained data were linear in the ranges 300-1800 ng/spot for TRA and 25-150 ng/spot for DIC and CZ. Both methods showed good specificity by resolution of the three drugs from the related substances and potential impurities 2-amino-4-chlorophenol, 2.6- dichloroaniline and impurity A of DIC. Finally, both methods were successfully applied to the analysis of a real sample of combined tablets containing the three drugs. The suggested methods could be applied for the studied drugs in QC-lab[29].

2.6. Voltametry Method

A glassy carbon paste electrode (GCPE) modified with a cation exchanger resin, Dowex50wx2 and gold nanoparticles (D50wx2-GNP-GCPE) has been developed for individual and simultaneous determination of acetaminophen (ACOP) and tramadol (TRA). The electrochemical behavior of both the molecules has been investigated employing cyclic voltammetry (CV), chronocoulometry (CC), electrochemical impedance spectroscopy (EIS) and adsorptive stripping square wave voltammetry (AdSSWV). The studies revealed that the oxidation of ACOP and TRA is facilitated at D50wx2-GNP-GCPE. Using AdSSWV, the method allowed simultaneous determination of ACOP and TRA in the linear working range of 3.34×10(-8) to 4.22×10(-5) M with detection limits of 4.71×10(-9) and 1.12×10(-8) M (S/N=3) for ACOP and TRA respectively. The prepared modified electrode shows several advantages such as simple preparation method, long-time stability, ease of preparation and regeneration of the electrode surface by simple polishing and excellent reproducibility. The high sensitivity and selectivity of D50wx2-GNP-GCPE were demonstrated by its practical application in the determination of both ACOP and TRA in pharmaceutical formulations, urine and blood serum samples[30]

3. Conclusion

The collected methods are various analytical methods for the estimation of Tramodol which is a pain killer. The drug can be estimated by using HPLC method, HPTLC, Voltametric method and UV method, colorimetric method in single as well as in combined dosage form. This collection may be use full for quick glance of various analytical methods for estimation of Tramodol.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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