ISSN 2515-8260 Volume 9, Issue 7, Summer 2022

An Overview of Different Analytical Methods Reported for the estimation of Flupirtine, Fluindione, Mizolastine, and Dapoxetine Including Impurity Profiling

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Abstract

Quantitative research necessitates an awareness of the actual or average amount of substances present in the sample and makes use of two methods of analysis based on the composition of the material present in the sample. These methods are known as traditional chemical analysis and instrumental analysis, respectively. Quantitative research needs one to be aware of the real or average quantity of chemicals that are present in the sample. In this article, we take a look at the many analytical techniques that have been published for the estimation of flupirtine, fluindione, mizolastine, and dapoxetine in bulk as well as in their particular dosage forms. This study also covers the methods for impurity profiling. It was found that very little work has been done on the chosen medications, and very few techniques have been described for the estimate of pharmaceuticals in bulk and their formulations. This is a problem since there is a great need for this information. There have been no methodologies that have been published on the chosen pharmaceuticals for the purpose of estimating contaminants linked to their synthetic pathway or packaging-related issues. As a result, we have chosen the medications listed above in order to create methodologies for estimating the impurities associated with these drugs.

Keywords: RP-HPLC, Impurity profiling, Method development, Validation, Quantitative analysis

1. Introduction

Instrumental analysis in the field of material chemistry provides evidence on the qualitative and quantitative analysis of chemicals (analytes) that are present in the sample. In order to carry out

this analysis, it is required to have a grasp of both the physical and chemical characteristics of the substances being analyzed[1]. Analytical chemistry, to put it another way, is concerned with the process of separating, identifying, and quantifying the various chemicals present in a sample. In addition to that, it encompasses the magnitude of the chemical consistency and the processing of statistical data[2,3].

Quantitative research requires an awareness of the actual or average amount of substances in the sample and makes use of two methods of analysis based on the composition of the material in the sample, namely traditional chemical analysis and instrumental analysis. Quantitative research also requires an awareness of the actual or average amount of substances in the sample[4–6]. A sample is something that can be recognized as having originated from the atmosphere, water, soil, food, or living organisms. For example, a slice of rock or a piece of meat, some water from the swimming pool of a house, a river, or the ocean, or tissues or blood from humans, livestock, or plants can all be considered samples[7]. The sample is then sent to the laboratory, where it is analyzed for the compounds it contains (the analytes), and the last step consists of determining the proportion of each component that is present in the sample. During analysis, the concentration of the analyte (which is a component of the sample that is being examined) is determined. An overview of different types of analytical system is depicted in Fig. 1[8–12].

In present article, we have reviewed the different analytical methods reported for the estimation of Flupirtine, Fluindione, Mizolastine, and Dapoxetine in bulk and their individual dosage forms including impurity profiling methods. We have selected above stated drugs as we are aiming to develop methods to estimate impurities associated with these drugs.

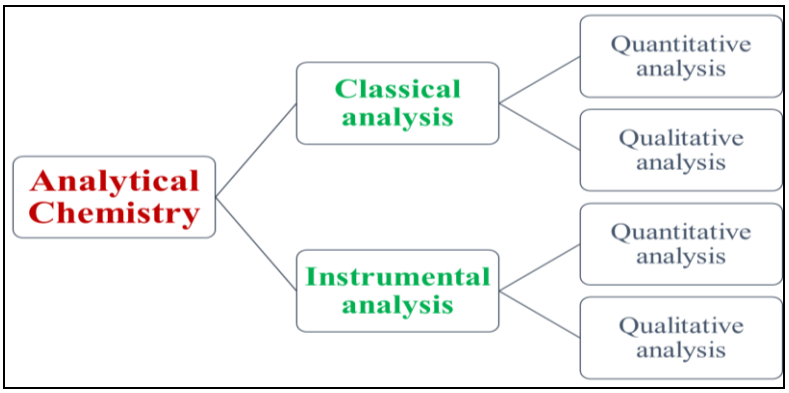

Fig. 1. There are many distinct kinds of analysis systems

2. An overview of analytical methods reported in selected drugs

2.1 Flupirtine

Flupirtine is an aminopyridine that acts as a centrally acting non-opioid analgesic. It was once used as an analgesic for both acute and chronic pain; however, in 2013, the European Medicines Agency restricted its use to only treat acute pain, for a period of no more than two weeks, and only in patients who were unable to take other painkillers[13]. In March of 2018, marketing authorizations for flupirtine were revoked as a result of a recommendation made by the European Medicines Agency. This recommendation was based on the finding that the restrictions that were introduced in 2013 had not been sufficiently followed in clinical practise, and serious liver injuries, including liver failure, continued to occur[14]. Flupirtine is a selective neuronal potassium channel opener (SNEPCO) that also has qualities that make it an NMDA receptor antagonist and a GABAA receptor modulator. In 1984, it was initially made accessible in Europe under the brand name Katadolon, and in the years that followed, various generic brands were produced once the product lost its patent protection[15].

Analgesic treatment for acute pain that ranges from mild to severe may be accomplished with flupirtine. Because of its muscle relaxant effects, it is often used for the treatment of back pain and other orthopaedic conditions. However, it is also used in the treatment of migraines, as well as in cancer, postoperative care, and gynaecology. Because of the potential for liver damage, the European Medicines Agency limited its usage in 2013 to the treatment of acute pain, for a period of no more than two weeks, and only in patients who were unable to take other pain medications. Flupirtine is a triaminopyridine derivative with a chemical structure: ethyl *N*-[2-amino-6-[(4-fluorophenyl)methylamino]pyridin-3-yl]carbamate (Fig. 2.). The 2, 6-dichoro-3-nitropyridine molecule was the fundamental building block in the production of flupirtine. It was initially synthesized in Germany in the 1980s, and Degussa Pharma was the company that brought it to market[16].

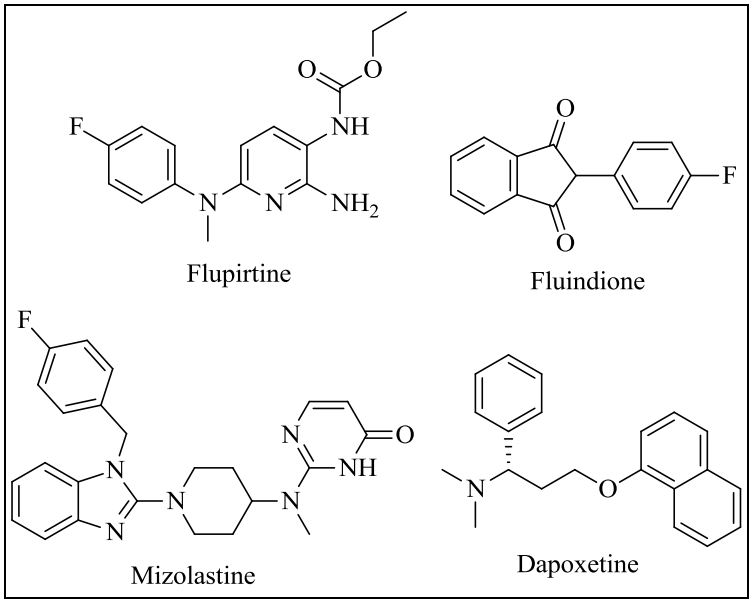

Fig. 2. The structures of selected for the study

Saptarshi Das et al. established a novel reversed-phase high-performance liquid chromatography (HPLC) method for the analysis of flupirtine in raw material and finished products. Orthophosphoric acid, HPLC grade methanol, triethylamine, methanol, and chloroform were used. HPLC Column C18 (150 mm × 25.4 mm) with a mobile phase methanol: water (90:10), flow rate of 1 ml/min, was used injection volume 20 µl in run time 20 min. Then authors performed method development and its subsequent validation, accuracy and analyte of Robust. All the system suitability parameters were within the limit and a sharp peak with better resolution and purity was obtained with the developed method. Recovery studies are between the ranges of 98.0% and 120% with a relative standard deviation at each level of <2.0%, which proves that the method is accurate for the estimation of flupirtine maleate over the range 50%–150% of target concentration[17].

Nagaraju Pappula and Poornima Chintala developed and validated a simple, efficient and reproducible RP-HPLC method for the simultaneous determination of paracetamol and flupirtine maleate in bulk and in pharmaceutical formulations. The chromatographic analysis was performed on a Thermo BDS hypersil C_{18} (250 x 4.6 mm i.d, 5µ) column in isocratic mode using water: methanol (pH was adjusted to 3.35± 0.02 with ortho-phosphoric acid) in the ratio of 50:50 v/v as eluent. The flow rate was 1 ml/min and eluent was detected at 250 nm. The retention time of paracetamol and flupirtine maleate were 3.5 and 5.4 min, respectively. The linear dynamic

range was 650-1950 µg/ml and 200-600 µg/ml for paracetamol and flupirtine maleate, respectively. Percentage recoveries for paracetamol and flupirtine maleate were 100.50 and 99.76 %, respectively[18].

Gayatri Gullipalli et al. developed and validated RP-HPLC for the simultaneous estimation of flupiritine maleate and paracetamol in bulk and pharmaceutical dosage form. The two components were separated using Inertsil ODS, C_{18} (250 mm × 4.6 mm id, 5 µm particle size) column by isocratic elution using mobile phase composition of potassium dihydrogen phosphate: methanol (70: 30) and pH 3.0 was adjusted with orthophosphoric acid. Flow rate used was 1ml/min and detection was carried out at 217nm. Injection volume is 5 µl. The retention time of flupiritine maleate and paracetamol is 2.553 min and 3.620 min respectively. As per ICH guidelines the method has been validated in terms of specificity, linearity, range, accuracy, precision, limit of detection, limit of quantitation, robustness. The method was found to be linear in the range of 50-150µg/ml (R^2=0.999) for flupiritine maleate and 10-30 µg/ml (R^2=0.999) for paracetamol. The limit of detection and limit of quantitation were found to be 2.14 and 7.13 for flupiritine maleate and 2.52 and 8.41 for paracetamol. Flupiritine maleate and paracetamol has the recoveries of 100.2% and 100.8% respectively and their relative standard deviations were less than 2%. All the validation parameters met the acceptance criteria[19].

Golla Murali Mohan J. et al. developed RP-HPLC method for the simultaneous estimation of Paracetamol and Flupiritine Maleate from pharmaceutical formulation. The method was carried out on Agilent C18 (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of Methanol: Water (0.2% TEA, adjusted to pH 3.0 using orthophosphoric acid) in the ratio of 90:10 v/v. The retention time of Paracetamol and Flupiritine Maleate was 3.2 min and 5.1 min respectively with the flow rate of 1mL/ min with VWD detection at 239 nm. The linear regression analysis data for the linearity plot showed good linear relationship with correlation coefficient value for Paracetamol and Flupiritine Maleate were R^2=0.9995 and R^2=0.9996 in the concentration range of 9-63 µg. mL-1 , 3-21 µg. mL^{-1} respectively. The relative standard deviation for intra-day precision has been found to be lower than 2.0 %. The developed method was validated in terms of specificity, selectivity, accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability[20].

Umang Shah et al. reported two methods for simultaneous estimation of Paracetamol and Flupirtine maleate in combined dosage form. The first method is the application of Q-analysis

method (absorbance ratio), which involves the formation of Q-absorbance equation at 302 nm (isobestic point) and at 248 nm, the maximum absorbance of Paracetamol. The linearity ranges for PCM and FLU were 2-35 µg/ml and 0.5- 70µg/ml, respectively. The second method was based on the use of first derivative spectroscopy, in which derivative amplitudes were measured at selected wavelengths (234 nm ZCP of FLU for PCM and 334 nm ZCPof PCM for FLU), without mutual interference. The linearity ranges for PCM and FLU were 2-34 µg/ml and 2- 65 µg/ml, respectively. The accuracy of the methods were assessed by recovery studies and was found to be 99.93% ±0.549 and 100.05% ±0.665 for Q absorbance ratio method and 99.54% ±0.591 and 99.41% ±0.792 for first derivative method, for PCM and FLU, respectively. Authors concluded that these methods are simple, accurate and rapid; those require no preliminary separation and therefore can be used for routine analysis of both drugs in quality control laboratories[21].

Mallikarjunarao Nagasarapu and Gowrisankar Dananna developed a simple, fast, sensitive, and validated HPLC method for the simultaneous estimation of Paracetamol and Flupirtine Maleate in combined dosage form. A Hypersil BDS C18, 150 x 4.6, 5 µ column with mobile phase containing Phosphate buffer (Ph 6.2): Acetonitrile (600:400) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 245 nm. The retention times of Paracetamol and Flupirtine Maleate were 3.1 min and 5.2 min respectively. The correlation co-efficient for Paracetamol and Flupirtine Maleate were found to be 0.99 and 1 respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Paracetamol and Flupirtine Maleate in formulations was found to be 100% and 100% respectively confirms the non-interferences of the excipients in the formulation. Degradation studies reveals that purity threshold is greater than the purity angle hence the peak is said to be pure. Authors concluded that due to its simplicity, rapidness and high precision, this method can successfully applied to the estimation of Paracetamol and Flupirtine Maleate in combined dosage form[22].

P. Giriraj and T. Sivakkumar reported a new, simple, precise, accurate, reproducible, and efficient Vierordt's method or simultaneous equation method was developed and validated for simultaneous estimation of paracetamol and flupirtine maleate in pure and pharmaceutical dosage form. The method was based on the measurement of absorbance at two wavelengths 245

nm and 344.5 nm, λmax of paracetamol and flupiritine maleate in 0.1 N HCl correspondingly. Calibration curves of paracetamol and flupiritine maleate were found to be linear in the concentration ranges of 5–15 μg/mL and 1.53–4.61 μg/mL, respectively, with their correlation coefficient values (R^2) 0.999. LOD and LOQ were 185.90 ng/mL and 563.38 ng/mL for paracetamol and 78.89 ng/mL and 239.06 ng/mL for flupiritine maleate. In the precision study, the % RSD value was found within limits (RSD < 2%). The percentage recovery at various concentration levels varied from 99.18 to 100.02% for paracetamol and 98.47 to 100.09% for flupiritine maleate confirming that the projected method is accurate[23].

Sathish Kumar Konidala et al. developed and validated a simple, validated RP-HPLC method for the simultaneous estimation of Paracetamol and Flupirtine maleate in pharmaceutical dosage. This method was developed by selecting Inertsil, C18, (250 x 4.6mm, 5µ) column as stationary phase and Methanol: O-Phosporic acid (85: 15 v/v) as mobile phase. Flow rate of mobile phase was maintained at 1 ml/min at ambient temperature throughout the experiment. Quantification was achieved with ultraviolet (DAD) detection at 239 nm. The retention times of Paracetamol and Flupirtine maleate were found as 2.78 min and 3.47 min respectively. The detector response was linear in the concentration range of 65-190 µg/ml and 20-60 µg/ml for Paracetamol and Flupirtine maleate respectively, and the regression coefficients found as 0.998 and 0.999 for Paracetamol and Flupirtine maleate respectively. From recovery studies authors concluded that the recovery of Paracetamol and Flupirtine maleate has no interference with any excipients in the formulation. This method has been validated according to ICH guidelines and shown to be Specific, Sensitive, Precise, Accurate, Rugged and Robust. Hence, this method can be applied for routine quality control of Paracetamol and Flupirtine maleate in dosage forms as well as in bulk drug[24].

Eberhard Scheuch et al. reported a quantitative LC–MS/MS determination of flupirtine, its *N*-acetylated and two mercapturic acid derivatives in man. The non-opiate analgesic drug flupirtine was shown in vitro to undergo hydrolysis followed by *N*-acetylation to form D13223, glucuronidation and conjugation with glutathione to form the stable mercapturic acid derivatives M-424 and M-466. To quantify flupirtine and its metabolites in samples obtained in a clinical study in healthy subjects selected on their genotype of NAT2, UGT1A1 and GSTP1, two LC–MS/MS methods were developed. The validation range for flupirtine and D-13223 in serum was

0.5–500 ng/ml. For urine and feces, the validation ranges for flupirtine and D-13223 were 20–5000 ng/ml and 5.0–5000 ng/ml, respectively. M-424 and M-466 could be quantified in urine between 5.0 and 5000 ng/ml. Free flupirtine and D-13223 were separated from serum, urine and feces with liquid–liquid extraction. For flupirtine and D-13223, the chromatography was performed on a XTerra C18 column isocratically with a mobile phase consisting of ammonium formate buffer (pH 3.5 mM) and acetonitrile (50:50; v/v), for M-466 and M-424 a Synergi® Fusion-RP column was used and a linear gradient method with water/HCOOH (pH 3) and acetonitrile. The mass spectrometer operated both with electro spray ionization in positive multiple reaction monitoring mode. The developed methods fulfilled the current FDA criteria on bioanalytical method validation for accuracy (error: −16.9 to 11.2%), precision (1.2–13.4%), recovery, stability and matrix effects over the observed analytical range. Thus, the methods were suitable to quantify flupirtine absorption and metabolic disposition in man after single intravenous and oral dosing (100 mg) and repeated oral administration (400 mg once daily)[25].

P. Haritha et al. reported a simple, accurate and precise RP-HPLC technique for the simultaneous determination of Flupirtine maleate and Paracetamol in pharmaceutical dosage form. The method involves an isocratic elution of drug in a stationary phase of Phenomenex, C18 (150mm × 4.6mm, 5µm) column using a mobile phase composition of methanol and 0.1% (v/v) orthophosphoric acid in the composition ratio of 60:40 v/v with a flow rate of 0.8 mL/min at 270 nm of detection. The injection volume is 20 µL. the method has been validated for specificity, linearity, range, precision, accuracy, limit of detection, limit of quantification, ruggedness and robustness. The retention times for Flupirtine maleate and Paracetmol are about 3.07 and 4.63 minutes respectively. Quantitative linearity was observed over the concentration range of 10.08 to 302.51 µg/mL for Flupirtine maleate and 4.99 to 99.80 for Paracetamol respectively. The regression equations of concentration of Flupirtine maleate and Paracetamol are found to be y = 1774x+4755, y = 39182x + 64154 respectively where y is the peak area and x is the concentration of drug (µg/mL). The % recovery of Flupirtine maleate and Paracetamol are found to be in the range of 97% to103 %. All the validation parameters are within the acceptance range[26].

A rapid, selective and sensitive HPLC–tandem mass spectrometry method was developed and validated by Xiaoyan Chen et al. for simultaneous determination of flupirtine and its active metabolite D-13223 in human plasma. The analytes and internal standard diphenhydramine were

extracted from plasma samples by liquid–liquid extraction, and chromatographed on a C_{18} column. The mobile phase consisted of acetonitrile–water–formic acid (60:40:1, v/v/v), at a flow rate of 0.5 ml/min. Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via atmospheric pressure chemical ionization (APCI). The method has a limit of quantitation of 10 ng/ml for flupirtine and 2 ng/ml for D-13223, using 0.5-ml plasma sample. The linear calibration curves were obtained in the concentration range of 10.0–1500.0 ng/ml for flupirtine and 2.0–300.0 ng/ml for D-13223. The intra- and inter-run precision (RSD), calculated from quality control (QC) samples was less than 7.2% for flupirtine and D-13223. The accuracy as determined from QC samples was less than 5% for the analytes. The overall extraction recoveries of flupirtine and D-13223 were determined to be about 66% and 78% on average, respectively. The method was applied for the evaluation of the pharmacokinetics of flupirtine and active metabolite D-13223 in volunteers following peroral administration[27].

2.2 Fluindione

The administration of vitamin K antagonists is recommended in evidence-based clinical practice recommendations for the prevention of stroke in atrial fibrillation, the treatment of venous thromboembolism, in patients who have mechanical valves, and in the first three months following the installation of bioprotheses[28,29]. Studies have shown that vitamin K antagonists are not used nearly as often as they should be, particularly by older people. This is especially true in the United States. Anti–vitamin K agents, also known as AVKs, are substances that work by inhibiting the reduction reactions that are necessary for recycling vitamin K. This, in turn, leads to a reduction in the production of vitamin K–dependent coagulation factors, including factors II (prothrombin), VII, IX, X, protein C, and protein S. The two primary categories of AVKs are coumarin derivatives, such as warfarin and acenocoumarol, and indanedione derivatives, such as fluindione and phenindione. Other examples of coumarin derivatives include acenocoumarol. Fluindione represents ~80% of AVK prescriptions in France. Chemically Fluindione is 2-(4-fluorophenyl)indene-1,3-dione (Fig. 2)[30–32].

N. Mallikarjuna Rao and D. Gowrisankar developed a stability indicating HPLC and UV Spectrophotometric methods for the determination of fluindione in bulk and its solid dosage forms. HPLC method was developed on a Symmetry (4.6 × 150 mm, 5 µm, Make: ODS) column

with a mobile phase consisting of sodium phosphate buffer pH 3.5: acetonitrile 50:50 v/v, pumped at 1.0 mL/min flow rate. The pH of buffer was adjusted to 3.5 with ortho phosphoric acid. The column was maintained at ambient temperature and 20 µL of solutions were injected. The analyte was quantified spectrophotometrically at 285 nm. Fluindione eluted at 3.5 min. The method was validated reaching satisfactory results for selectivity, precision and accuracy. Forced degradation samples could be simultaneously evaluated, without interferences in the quantitative analysis. For the spectrophotometric analysis, methanol was used as solvent and the wavelength of 285 nm was selected for the detection. Both methods were found to quantify fluindione in bulk and its tablets accurately. Statistical analysis by Student's t-test showed no significant difference between the results obtained by the two methods. Therefore HPLC and UV methods presented the most reliable results for the analysis of fluindione tablets[33].

A simple, sensitive and accurate spectrophotometric method has been developed by E. Brahmani et al. for the determination of Fluindione in bulk form. The λ max of the Fluindione was found to be 278nm in 25%Methanol. The method shows high sensitivity with linearity 2 to 10µg/ml (Regression equation: Y=0.097 + 0.126; r^2 =0.999). The apparent molar absorptivity was found to be 2.63x104mol−1 cm−1 in 25% Methanol. These methods were tested and validated for various parameters according to ICH guidelines and USP. The Detection limit and quantitation limit were found to be 0.2702µg ml–1 and 0.8189µg ml-1 in 25%Methanol respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Fluindione in bulk form[34].

Isabelle Fourel et al. developed and validated a liquid chromatography-tandem mass spectrometry method for the identification and quantification of anticoagulant (anti-vitamin K or AVK) compounds, including rodenticides, drugs, and natural. The proposed method was based on ion-trap technology with electrospray ionization (ESI) and multiple reaction monitoring (MRM) technique. Each AVK is identified by means of its retention time, precursor ion, and two product ions. Plasma samples are extracted by liquid-liquid partition on Toxi-tube B®. The method was validated on dog plasma and gave good results in terms of specificity, linearity, and percent recovery for the 14 AVK tested (warfarin, acenocoumarol, bromadiolone, brodifacoum, chlorophacinone, coumatetralyl, dicoumarol, difenacoum, difethialone, flocoumafen, fluindione, phenindione, and tioclomarol). The limits of detection ranged from 5 to 25 ng/mL. Intraday

repeatability was good, but interday repeatability was more variable though still sufficient for our diagnostic purposes. The technique was successfully applied in a series of clinical investigations to demonstrate its applicability in various animal species and gave very high sensitivity and specificity results[35].

A simple, accurate, precise and rapid validated stability indicating HPTLC method of Fluindione was successfully developed by Mahesh G. Thakare and Mrinalini C. Damle. This method was based on HPTLC separation followed by UV detection at 285 nm. The separation was carried out on merck TLC aluminium sheets precoated with silica gel $60F_{254}$ using chloroform: methanol (9.9: 0.1%v/v) as a mobile phase. Fluindione gave well defined and sharp peak at R_f 0.47 ± 0.02. Calibration curve was linear in range 1000-3000 ng/band. Stress degradation study shows that sample degraded with acid and base hydrolysis, under oxidation, thermal and photolytic stress conditions. The peak purity parameter ensured noninterference by product of degradation. This method can be applied to determination of stability of Fluindione. The suitability of this HPTLC method for quantitative determination of Fluindione was proved by validation in accordance with requirements of ICH guidelines[36].

A simple, accurate, precise, specific and highly sensitive spectrophotometric method developed by Manish Kumar Thimmaraju et al. for the determination of Fluindione in bulk drug .The optimum conditions for the analysis of the drug were established. The λ max of the Fluindione was found to be 230nm in 10% methanol in 0.1N HCl. The method shows high sensitivity with linearity 1 to 5µg/ml. The regression of the curve was Y = 0.097x+0.1277.The apparent molar absorptivity was found to be 4.79x104 mol^{-1} cm^{-1} in 10 % Methanol. The lower limit of detection and the limit of quantitation was found to be 0.09302µg/ml and 0.28186µg/ml respectively. All the calibration curves shows a linear relationship between the absorbance and concentration and correlation coefficient was 0.999.The proposed method will be suitable for the analysis of Fluindione in bulk form[37].

3.3 Mizolastine

Mizolastine is an antihistamine medication that belongs to the second generation and has strong affinity as well as specificity for histamine H1 receptors. Mizolastine been shown to have anti-inflammatory and anti-allergic properties in animal models. It has also been shown to have antiallergic effects in healthy volunteers[38,39]. Mizolastine is an antihistamine medication that is of

the second generation and is used once a day at a dose of 10 milligrammes. Mizolastine, on the other hand, has not been demonstrated to increase the QTC interval in either volunteers or patients, in contrast to terfenadine and astemizole. In the treatment of allergic rhinitis and chronic idiopathic urticaria, mizolastine has the potential to be an effective alternative to existing antihistamine drugs of the second generation. Chemically mizolastine is 2-[[1-[1-[(4-fluorophenyl)methyl]benzimidazol-2-yl]piperidin-4-yl]-methylamino]-1*H*-pyrimidin-6-one (Fig. 2)[40–42].

A sensitive and rapid liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) method has been developed and validated by Li Ding for the determination of mizolastine in human plasma using dipyridamole as the internal standard (I.S.). Plasma samples were simply pretreated with methanol for deproteinization. Chromatographic separation was performed on an Agilent Zorbax C_{18} column with a mobile phase of 10 mM ammonium acetate buffer containing 0.1% formic acid–methanol (20:80, v/v) at a flow rate of 1 mL min^{-1}. The electrospray ionization (ESI) interface was employed in a single quadrupole mass spectrometer. The analytes were protonated in the positive ESI interface and detected in single ion monitoring (SIM) mode. Chromatographic separation was achieved in less than 3.5 min. The linearity was established over the range of 0.5–600 ng mL^{-1}. The lower limited of quantification (LLOQ) of the method was 0.5 ng mL^{-1}. The intra- and inter-run standard deviations were both less than 11.2%. The method was applied to study the pharmacokinetics of the mizolastine sustained-release tablets in healthy volunteers[38].

A fast and selective CZE method was developed by Serena Orlandini for the determination of mizolastine and related impurities. Response surface methodology was applied to study the influence of phosphate/triethanolamine (TEA) buffer concentration, heptakis(2,3,6-tri-O-methyl)-β-CD (TMβCD) concentration, voltage and temperature. The optimum conditions were: 105 mM phosphate/TEA buffer (pH 3.0) containing 10 mM TMβCD, temperature 19°C and voltage 30 kV. Validation of the method was performed in drug substance and drug product. Robustness was evaluated using a Plackett–Burman design, including pH among the considered factors. Applying the optimal conditions, the nine peaks were baseline separated in about 10 min. The method was applied to the quality control of mizolastine in controlled-release tablets[43].

Two simple, sensitive and accurate spectrophotometric methods have been developed by A. Sreelakshmi et al. for the determination of Mizolastine in pure state and in its pharmaceutical

formulations. The developed Method A was based on the formation of picrate salt between picric acid and free base of Mizolastine and it shows maximum absorption at λmax 400 nm and Linearity in the range of 3-15 µg/mL. Method B involves reaction between free base of Mizolastine and chloranilic acid. The developed chromogen in Method B shows maximum absorption at λmax 530 nm and Linearity in the range of 50-250 µg/mL. The results obtained were statistically evaluated and were found to be accurate and reproducible[44].

For the determination of mizolastine in human plasma, three methods were developed based on liquid—liquid extraction, solid-phase extraction and column-switching in combination with high-performance liquid chromatography with ultraviolet detection by V. Ascalone et al. The liquid—liquid extraction method included a back-extraction step that preconcentrates the drug into a small aqueous volume, resulting in very high sensitivity (0.5 ng/ml of plasma); it can be used in conventional bioanalytical laboratories that do not have sophisticated automatic devices. The solid-phase extraction method is performed by using a robotic system (Benchmate). It is completely automated from the initial sampling to the final injection into the chromatograph. It has a good sensitivity (1 ng/ml of plasma), but requires an expensive apparatus and skilled analysts. The column-switching method is based on a solid-phase extraction performed on-line with chromatographic analysis; it is not completely automatic, because some operations are performed manually. The device required for valve switching is not expensive and can be managed by a simple integrator or a personal computer; it is very easy to use and affords a sensitivity (2.5 ng/ml of plasma) that generally satisfies the needs of pharmacokinetic investigations of mizolastine. The conditions were similar for all the three methods: a C_8 type column, an eluent of phosphate buffer and acetonitrile, and a spectrophotometric ultraviolet detector operated at 285 nm[45].

A simple and precise UV spectrophotometric method was developed by C. Narasimharaju B. and G. Devala Rao for the estimation of mizolastine in pharmaceutical dosage forms. The λmax of mizolastine was found to be 289 nm. Linearity for this method lies in the range of 5-15 µg/mL. The proposed method is sensitive, accurate, reproducible and useful for the routine determination of mizolastine in tablet dosage forms. No interference was observed from the excipients[46].

Simple, rapid and accurate high performance liquid chromatographic (HPLC) and spectrophotometric methods are described for determination of antihistaminic acrivastine in

capsules. The first method (method A) is based on accurate, sensitive and stability indicating chromatographic separation method. Chromolith® Performance RP-18e column, a relatively new packing material consisting of monolithic rods of highly porous silica, was used as stationary phase applying isocratic binary mobile phase of ACN and 25 mM NaH_2PO_4 pH 4.0 in the ratio of 22.5:77.5 at flow rate of 5.0 mL/min and 40 °C. A diode array detector was used at 254 nm for detection. The elution time of acrivastine was found to be 2.080 ± 0.032. The second and third methods (methods B and C) are based on the oxidation of acrivastine with excess N-bromosuccinimide (NBS) and determination of the unconsumed NBS with, metol–sulphanilic acid (λmax: 520 nm) or amaranth dye (λmax: 530 nm). The reacted oxidant corresponds to the drug content. Beer's law is obeyed over the concentration range 1.563–50, 2.0–20 and 1.0–10 μg mL^{-1} for methods A, B and C, respectively. The limits of detection and quantitation were 0.40, 0.292 and 0.113 μg mL^{-1} and 0.782, 0.973 and 0.376 μg mL^{-1} for methods A, B and C, respectively. The HPLC method was validated for system suitability, linearity, precision, limits of detection and quantitation, specificity, stability and robustness. Stability tests were done through exposure of the analyte solution for four different stress conditions and the results indicate no interference of degradants with HPLC-method. The proposed methods was favorably applied for determination of acrivastine in capsules formulation. Statistical comparison of the obtained results from the analysis of the studied drug to those of the reported method using t- and F-tests showed no significant difference between them[47].

2.4 Dapoxetine

A medicine known as dapoxetine, which is sold under the brand name Priligy amongst other names, is used to treat premature ejaculation (PE) in males aged 18–64 years old. Dapoxetine's mechanism of action involves blocking the serotonin transporter, which in turn increases serotonin's activity at the postsynaptic cleft and, as a result, promotes ejaculatory latency[48]. Dapoxetine, which is an antidepressant and a part of the family of drugs known as selective serotonin reuptake inhibitors, or SSRIs, was first developed as a treatment for depression. Dapoxetine, on the other hand, is swiftly absorbed and removed from the body, in contrast to the other SSRIs. Because of its rapid onset of action, it is well-suited for the treatment of PE, but it is not effective as an antidepressant[49,50].

Dapoxetine was first developed by the pharmaceutical firm Eli Lilly, but it was later acquired by Johnson & Johnson in 2003. The following year, in 2004, Johnson & Johnson filed an application to the Food and Drug Administration (FDA) for approval of the drug as a New Drug to treat PE. There are a number of nations in Europe and Asia, in addition to Mexico that sell dapoxetine. Dapoxetine has been in the phase III development stage in the United States[51]. In May of 2012, Furiex Pharmaceuticals, which is based in the United States, came to an agreement with ALZA Corp and Janssen Pharmaceutica to market dapoxetine in the United States, Japan, and Canada. At the same time, Furiex Pharmaceuticals sold Menarini the rights to market the drug in Europe, the majority of Asia, Africa, Latin America, and the Middle East. Chemically dapoxetine is (1*S*)-*N*,*N*-dimethyl-3-naphthalen-1-yloxy-1-phenylpropan-1-amine (Fig. 2)[52].

A simple, accurate, sensitive, reproducible and specific spectrophotometric method for the determination of Dapoxetine HCl, in bulk and its pharmaceutical formulations, using methanol as a solvent have been developed and validated. The optimum conditions for the analysis of the drug were established and the developed method was validated with respect to linearity, accuracy (recovery), precision, robustness, ruggedness, LOD, LOQ and specificity. The maximum wavelength (% max) was found to be 291 nm and a good linearity was observed in the concentration range of 5-60 µg/mL having regression equation, y = 0.0164x – 0.0071 with correlation coefficient of 0.9998. The percentage recovery of Dapoxetine HCl was found to be 99.5489 ±0.1599 and % CV (0.16; n=9) indicated a good precision of the analytical method. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.0239 µg/mL and 0.0724 µg/mL, respectively. Robustness and ruggedness of the method was performed by using different % max, instruments, apparatus and analysts. The method was found to be simple, accurate, precise, reproducible, economical and robust. Analytical method validation was found to be within an acceptance criteria according to ICH Q2 R1 guidelines. The proposed method can be applied for routine quality control analysis of Dapoxetine HCl[53].

A rapid and sensitive RP-HPLC method with UV detection (230 nm) for routine analysis of Dapoxetine HCl in a pharmaceutical formulation (Priligy®) was developed by Pratik Mehta. Chromatography was performed with mobile phase containing a mixture of buffer [Tri-ethylamine, pH-4.0 (adjusted with o-phosphoric acid)] and acetonitrile (60:40, v/v) with flow rate was 1.0ml min^{-1}. The procedure was validated for linearity (correlation coefficient=0.9998), accuracy, robustness and intermediated precision. Experimental design was used for validation

of robustness and intermediate results in a decrease of the drug found concentration, while the percentage of organic modifier and pH have no important effect on the response. For intermediate precision measure the variables considered were: analyst, equipment and number of days. The R.S.D. value (0.5%, n=6) indicated a good precision of the analytical method. The proposed method was simple, highly sensitive, precise and accurate and retention time less than 5min indicating that the method is useful for routine quality control[54].

Two simple UV spectrophotometric methods have been developed for simultaneous determination of sildenafil citrate and dapoxetine hydrochloride. For both methods, stock solutions were prepared in methanol followed by the further required dilutions with methanol. Proposed dual-wavelength method and ratio derivative method, the wavelength of maximum absorption for sildenafil citrate and dapoxetine hydrochloride was 292 nm and 231 nm, respectively. In both methods, the linearity range lies between 10 and 60 µg/ml for sildenafil citrate and 2–12 µg/mL for dapoxetine hydrochloride with their respective wavelengths. By dual-wavelength method, the percentage of sildenafil citrate and dapoxetine hydrochloride was found to be 101.3% and 100.3%, respectively. Result obtained in this research work clearly indicated that both these methods were found to be accurate, precise, stable, and robust as indicated by low values of percentage relative standard deviation. Thus, the present study gives an excellent method for the determination of both the drugs in combined tablet formulation[55].

A sensitive and rapid ultra-performance liquid chromatography tandem mass spectroscopy (UPLC-MS/MS) method was developed to determine dapoxetine and its two major metabolites (dapoxetine-N-oxide and desmethyldapoxetine) in human plasma simultaneously. After a simple protein precipitation, the analytes and the combined internal standard (carbamazepine) were separated on an Acquity UPLC BEH C18 column using a mobile phase of acetonitrile and 0.1% formic acid in water with gradient elution. Mass spectrometric analysis was performed using a XEVO TQD triple quadruple mass spectrometer coupled with an electro-spray ionization (ESI) source in the positive ion mode. The MRM transitions are of m/z 306.3 → 261.2, m/z 322.2 → 261.2, m/z 292.2 → 261.2 and m/z 237.1 → 194.2 for dapoxetine, dapoxetine-N-oxide, desmethyldapoxetine and IS, respectively. The linearity of this method was found to be within the concentration range of 1.0–200 ng/mL for dapoxetine; 0.5–100 ng/mL for dapoxetine-N-oxide; and 0.1–5.0 ng/mL for desmethyldapoxetine in human plasma, respectively. Only 4.0 min was needed for an analytical run. Intra-day and inter-day accuracy and precision

were within the acceptable limits of ±15% at all of the concentrations. This assay was successfully used to support a clinical pharmacokinetic study following oral administration of dapoxetine tablets in healthy Chinese subjects[56].

A simple, specific, accurate and precise RP-HPTLC method has been developed and validated by Dhwani A. Shah et al. for simultaneous estimation of Avanafil and Dapoxetine. The chromatographic separation was achieved on Aluminium plates precoated with Silica gel 60 F254 using chloroform: methanol: ethyl acetate: glacial acetic acid (5:2:3:0.2, v/v/v/v) as mobile phase detected at 279 nm. The correlation coefficient for RP-HPLC method was found to be 0.9987 for Avanafil and 0.9991 Dapoxetine and the linearity range was found to be 1040-3640 ng*spot-1 for Avanafil and 80-280 ng*spot-1 for Dapoxetine[57].

A novel, precise, accurate and economic high-performance thin-layer chromatographic (HPTLC) method was developed, optimized and validated by Chetan Prajapati et al. for simultaneous determination of Sildenafil Citrate and Dapoxetine Hydrochloride. The chromatographic separation was performed on precoated silica gel 60 GF_{254} plate with hexane: methanol: diethyl amine 9.2:1.6:1.2 (v/v/v) as mobile phase. The plate was developed to distance of 8.0 cm at ambient temperature. The developed plate was scanned and quantified at their single selected wavelength of 241 nm for Sildenafil Citrate and Dapoxetine Hydrochloride. Experimental conditions such as band size, chamber saturation time, migration time of solvent front, etc. were critically studied and the optimum condition were selected. The drugs were satisfactorily resolved with RF 0.21 ± 0.02 for Sildenafil Citrate and 0.72 ± 0.02 for Dapoxetine Hydrochloride. The method was validated for linearity, accuracy, precision, and specificity. The calibration plot was linear between 2000–12000 ng per spot for Sildenafil Citrate and 1200–7200 ng per spot for Dapoxetine Hydrochloride. The limits of detection for Sildenafil Citrate and Dapoxetine Hydrochloride were 210 and 75ng per spot respectively and limit of quantification for Sildenafil Citrate and Dapoxetine Hydrochloride were 450 and 240ng per spot respectively. It is a user-friendly and important tool for analysis of combined fixed dosage forms. Methods were validated statistically and recovery studies were carried out. The method herein described can be employed for quality control and routine analysis of drugs inpharmaceutical formulations[58].

Three rapid, simple and selective spectrophotometric methods were developed by Maimana A. Magdy for determination of Dapoxetine Hydrochloride (DAP) and Tadalafil (TAD) in bulk and pharmaceutical dosage forms. Method (A) is simultaneous first derivative (1D)

spectrophotometric method in which the peak amplitudes of the first derivative spectra (1D) were measured for both DAP and TAD at 322.4 nm and 230 nm, respectively with no interference from each other. Method (B) is the area under curve (AUC) spectrophotometric method in which the areas under curve in the wavelength ranges 228–240 nm and 242–254 nm are used for determination of DAP and TAD respectively. Method (C) is ratio subtraction combined with extended ratio subtraction spectrophotometry (EXRS) in which TAD was determined by dividing the mixture spectra by the spectrum of 15 μg/mL solution of DAP, while DAP was be determined by dividing the mixture spectra by the spectrum of 30 μg/mL solution of TAD. The developed methods were applied to different laboratory prepared mixtures of DAP and TAD. These methods were validated according to the ICH guidelines with respect to linearity, accuracy, precision, selectivity and specificity, and can be used for routine quality control analysis of DAP and TAD in their dosage forms[59].

A reversed-phase high performance liquid chromatographic method was developed and validated by Gergő Tóth et al. for the simultaneous determination of the related substances of *S*-dapoxetine, including *R*-dapoxetine, (3*S*)-3-(dimethylamino-3-phenyl-1-propanol), *S*-3-amino-3-phenyl-1-propanol, 1-naphtol, 4-phenyl-2H,3H,4H-naphtho[1,2-b]pyran and 1-(2E)-Cinnamyloxynaphthalene. During the screening experiments seven different polysaccharide-type chiral stationary phases (amylose-based Lux-Amylose-1, Lux-i-Amylose-1 and Lux-Amylose-2, as well as cellulose-based Lux-Cellulose-1, Lux-Cellulose-2, Lux-Cellulose-3 and Lux-Cellulose-4) were tested in polar organic mode using a mobile phase consisting of 0.1% diethylamine in methanol, ethanol, 2-propanol and acetonitrile with 0.5 mL min^{-1} flow rate at 20 °C. Best results were obtained on Lux Cellulose-3 column with the ethanol-based mobile phase. To increase the retention factor of two, early-eluting impurities, water was added to the mobile phase. In order to counterbalance the increased total analysis time, higher column temperature (40 °C) and gradient elution, combined with flow-programming` was applied. Using the optimized conditions baseline separations were achieved for all compounds within 30 min. The method was validated according to the International Council on Harmonization guideline Q2(R1) and applied to the analysis of an approved, tablet formulation and dapoxetine-containing products sold on the internet. As expected, in the case of the pharmacy-acquired product, all of the monitored impurities were below 0.1%. However, interesting results were obtained when internet-acquired samples were analyzed. These tablets contained racemic dapoxetine and/or

high concentration of R-dapoxetine impurity. Based on this work polysaccharide-based chiral stationary phases can be successfully applied for the simultaneous determination of achiral and chiral impurities in reversed-phase mode applying gradient elution and flow-rate programs. The study further underlines the importance of not only achiral, but also enantiomeric quality control, whenever counterfeiting of a single enantiomeric agent is suspected[60].

Tae Kon Kim et al. described and validated a rapid and sensitive method for quantitation of dapoxetine in rat plasma by using ultra-performance liquid chromatography–electrospray ionization tandem mass spectrometry (UPLC–ESI/MS/MS). Plasma samples were prepared by protein precipitation with acetonitrile, and sildenafil was used as an internal standard (IS). The mobile phase consisted of 0.5% formic acid/acetonitrile (60:40, v/v); a C18 reversed-phase column (2.0 × 50 mm, 1.7 μm) was used for chromatographic separation. Multiple reaction monitoring (MRM) was used in the positive ion mode for mass spectrometric detection. The calibration curve for dapoxetine was linear (r2 = 0.999) in the concentration range of 1–500 ng/mL. The intra- and inter-day precision was between 3.8% and 8.3%, and the intra- and inter-day accuracy was between 101.1% and 109.0%. Dapoxetine was found to be stable in various conditions with the recoveries >87.0% (RSD <7.2%). The method was found to be specific, precise, and accurate, and no matrix effect was observed. The results suggest that this method can be successfully applied in pharmacokinetic studies of dapoxetine in rat plasma[61].

Dapoxetine hydrochloride (DAP) and Tadalafil (TAD) were separated and determined quantitatively using a validated green high-performance thin layer chromatographic (HPTLC) method in their binary mixtures either as raw materials or in pharmaceutical formulations. The concentration ranges were 0.1–1.6 and 0.2–2.5 μg/band for dapoxetine and tadalafil, respectively, with accuracies of 98.93% ± 0.62 and 99.26% ± 1.39, respectively. Silica gel HPTLC F254 plates were used to carry out the separation. The mobile phase used was a mixture of ethanol–ethyl acetate (1:9 by volume), which is environmentally green and harmless. Densitometric scanning with UV detector was used to detect the separated peaks at 222 nm. ICH guidelines were followed to validate the suggested method, and the results prove that they can be used for regular analysis in quality control laboratories with compatible results[62].

Mehdi Rezaei and Ali Ramazani reported the development and validation of assay test for Dapoxetine Hydrochloride in the pharmaceutical dosage forms by HPLC. The assay method by HPLC was found to be linear in the concentration range of 25 to 150 μg/mL. The mobile

phase was composed of methanol and buffer pH: 7.0 ammonium dihydrogen phosphate (80:20 v/v) at the flow rate of 1 mL/min using UV detection at 232 nm. The analytical results were validated by recovery studies. The percentage recovery method was found to be 99.58-100.75%. The LOD and LOQ were found to 0.008 µg/mL and 0.027 µg/mL. All the parameters of validation were in the acceptance range. This developed method was successfully applied to estimate the amount of Dapoxetine Hydrochloride in the tablets[63].

A new and accurate chiral liquid chromatographic method was developed for the enantiomeric resolution of Dapoxetine hydrochloride, (*S*)-N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylPropan-1-amine, a premature ejaculation in bulk drugs. The enantiomer of Dapoxetine hydrochloride were baseline resolved on a Phenomenex Lux-cellulose-1 (250mm×4.6 mm, 5um) column using a mobile phase system containing hexane: 1-propanol: diethyl amine (97.5:2.5:0.1, v/v/v). The resolution between the enantiomer was not less than 3.5 and interestingly distomer was eluted prior to eutomer in the developed method. The presence of diethyl amine and 1-propanol in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was extensively validated and proved to be robust. The detection limit and quantitation limit of (*R*)-enantiomer were found to be 0.017% and 0.05%, respectively. The recovery of (*R*)-enantiomer was ranged from 90-110% in bulk drug samples. Dapoxetine hydrochloride sample solution and mobile phase were found to be stable for at least 48 h. The proposed method was found to be suitable and accurate for the quantitative determination of (*R*)-enantiomer in bulk drugs[64].

Conclusion

Quantitative research requires an awareness of the actual or average amount of substances in the sample and makes use of two methods of analysis based on the composition of the material in the sample, namely traditional chemical analysis and instrumental analysis. Quantitative research also requires an awareness of the actual or average amount of substances in the sample. In present article, we have reviewed the different analytical methods reported for the estimation of Flupirtine, Fluindione, Mizolastine, and Dapoxetine in bulk and their individual dosage forms including impurity profiling methods. It was observed that very little work has been done on the selected drugs and very less methods have been reported for the estimation of drugs in bulk and their formulations. There were no methods have been reported on the selected drugs for the

estimation of impurities related to their synthetic route or packaging related. Therefore we have selected above stated drugs to develop methods to estimate impurities associated with these drugs.

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