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DEVELOPMENT AND OPTIMIZATION OF HPLC ANALYSIS OF METRONIDAZOLE

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Abstract

Two different reversed-phase HPLC techniques for the analysis of metronidazole and its related compound have been developed and optimized in this study. In RP-HPLC, isocratic water-acetonitrile and aqueous binary gradient of water+acetonitrile-0.1M sulphuric acid-acetonitrile were used as the mobile phase. Separation was carried out in the isocratic system using a 5 µm nova pack C18 column with 50:50 V/V water:acetonitrile as the mobile phase with a flow rate of 1 ml/min at 25 °C. Detection was carried out at a wavelength of 313 nm. Injection volume was 20 µl and retention time of 6.9 minutes was achieved. The aqueous binary gradient system was performed using a 5 µm hypersil C8 column with 75:25 V/V 0.1M sulphuric acid:acetonitrile as the initial mobile phase which would change direction to 10:90 V/V 0.1M sulphuric acid:acetonitrile. The flow rate was 1 ml/min at 25 °C with a total run time of 30 minutes. Detection was carried out at a wavelength of 313 nm and injection volume was 20 µl. Retention time for the first peak was 19.7 minutes and 26.6 minutes for the second. After both methods were utilized, the isocratic system was found to be more efficient and less time consuming for the separation of metronidazole and its related compound.

As a confirmation, an optimized method was developed through a complete forced degradation study of metronidazole utilizing a similar nova pack C18 column. A similar mobile phase of 50:50 V/V water:acetonitrile was used to carry optimum separation using different degraded samples of metronidazole. Visual observation as well as peak purity comparison was made from the separation of metronidazole with its degraded samples on the C18 column, showing that no degradation compounds were present in the 3UV dimension.

Keywords: Metronidazole, medication , antibacterial ,antiprotozoal agent and HPLC Analysis

1 .Introduction

An introduction is presented to summarize the current research being done in analytical chemistry. Classical methods of assay and the importance of validation are presented, as well as an overview of the study. High Performance Liquid Chromatography (HPLC) holds a unique niche in analytical chemistry because of its high efficiency and ability to be universal. An HPLC assay in most cases is the final word when validating the concentration of a compound due to its cheap cost and ability to quantify compounds in low concentrations. However, developing an HPLC assay can be difficult and very time-consuming. Particular attention must be given to optimization because the conditions of the assay will directly affect the product of the analysis (i.e. if a compound has too long of a retention time it may be deemed that it is lost in the column and does not actually elute). The assay must then be validated for its specific use, which ranges from analysis of pharmaceutical products to urine samples. Ideally, the validation process provides a quantification of the compound with a %RSD of less than 2%. This study provides a successful method in the HPLC assay and validation of metronidazole, which is a compound used most widely for the treatment of Helicobacter pylori infection, in agar-based media and Sorensen's phosphate buffer.(V. Karpievitch et al., 2011)

1.1 .Background of Metronidazole

Metronidazole is a synthetic antibacterial and antiprotozoal agent which is effective against a wide range of pathogenic microorganisms associated with bacterial infections and protozoal infection. It is a member of the Nitroimidazole class of compounds and it is an off-white and odorless, or an almost white crystalline powder, freely soluble in water, sparingly soluble in alcohol. It is used to treat infections such as bacterial vaginosis, skin and soft tissue infections, pelvic inflammatory disease, infected post-operative wounds, lower respiratory tract infections, Clostridium difficile infections, Helicobacter pylori eradication therapy, amoebiasis, and giardiasis. Since Metronidazole is a widely used drug, there are many commercially available formulations and thus it is quite possible that a pharmaceutical analyst may be required to analyze the drug and/or its metabolites. The types of analysis may vary from simply monitoring the levels of drug in patients to carrying out a full-scale pharmacokinetic study. The value of such analysis is twofold: ensuring that patients are receiving the most effective treatment and finding out the pharmacodynamics and pharmacokinetic trends of Metronidazole in the wide range of conditions that it is used to treat. This information could be essential to the development of newer dosage forms or more effective formulations of Metronidazole.

Wherever Metronidazole is being clinically used, there is always a need to monitor the levels of drug in patients. This is to ensure that patients are receiving an adequate dose of drug for effective treatment, but not getting so much drug that it may produce toxic effects. This is particularly pertinent to patients who may suffer from liver and/or kidney diseases, since Metronidazole is primarily metabolized in the liver and excreted in the urine. To monitor drug levels, doctors may request a simple urinary analysis, using high-performance thin-layer chromatography (HPTLC) or a more complex analysis involving HPLC to measure serum drug levels. An example of the latter is a study that was carried out on the treatment of Buruli Ulcer in Ghana, in which a simple and

rapid HPLC method was developed to determine plasma levels of Metronidazole and its two metabolites. High-performance liquid chromatography is the method of choice for most drug analyses because it is a relatively simple technique that provides good accuracy and precision at a reasonable cost. Step 1: Investigation of HPLC Conditions and development of a Validated Analytical Method for the Determination of Metronidazole in Biological Fluids and Cell Culture. (Fukui et al., 2020)

1.2 .Importance of HPLC Analysis

In order to elucidate the degradation profile of metronidazole, HPLC is chosen as the method of analysis. In recent years there has been a demand for methods to separate and quantitate drugs and their metabolites from biological fluids and tissues. This is largely due to the fact that HPLC has become an established method of analysis and highest recovery of the material from samples can be achieved. HPLC is used widely in the pharmaceutical industry, as a method of quantitative and qualitative analysis of drug products, active pharmaceutical ingredients (APIs), excipients, and many other compounds. In such, there are monographs of the United States Pharmacopoeia and the British Pharmacopoeia, which detail the best method for separating drugs in certain standard conditions. The same can be said for the method of HPLC, which is now available for most drugs and specifies the best method of separation and detection.

An HPLC method of analysis is most desirable for drugs where in vitro and in vivo studies are to be performed, as it can offer the best means of obtaining analytical information. For metronidazole, it is apparent that a testing whether the drug has been affected by altered storage conditions or has gone past its expiry date, HPLC can offer the easiest and most accurate means of analysis. On the other hand, rate of metabolism and products of metabolism are often important issues when trying to understand the effect of a drug and its concentration in the body over a period of time. It has been common to tag the drug with a radiolabel to aid detection of drug and metabolites, where chromatography methods are often needed in separating the drug and products of metabolism. Essentially the best means of doing this is still to use an HPLC method; it involves no extensive preliminary preparation; a method can be designed to best suit the drug and an available HPLC system, and the best separation and detection of analytes is still likely to be achieved. (Jiang et al., 2023)

2. Method Development

Column chromatography is a technique that is widely used by organic chemists to separate out individual components from a mixture. The experiment aims to use column chromatography to separate and purify a mixture of fluorenone and fluorenol, and to calculate the percentage recovery of each substance. Fluorenone is a relatively reactive carbonyl compound while fluorenol is a less reactive alcohol. By comparing the different fractions of each substance, the relative reactivity of the two compounds can be determined. Prior to exclusive use of column chromatography on the two substances, thin layer chromatography was performed with the use of a 50/50 hexanes and

ethyl acetate solvent mixture to determine the ideal solvent mixture as being 70/30. The starting mixture was weighed to be 0.0787g of fluorenone and 0.1532g of fluorenol and was dissolved in a small amount of the hexane/ethyl acetate solution and spotted onto the TLC plate. After running the experiment and checking the UV light to see the location of each compound on the TLC plate, column chromatography was performed and 17 fractions of 3mL each were collected in test tubes.(Tung Khuat et al., 2023)

2.1 Selection of HPLC Column

A hydrophobic interaction involves a partitioning mechanism of solutes between the mobile and the stationary phase. It is based on the principle that like dissolves like, in which non-polar compounds prefer a non-polar environment. This would suggest that a hydrophobic non-ion pair reverse phase column would be suitable for metronidazole. This type of column is known for its wide range of applicability and good reproducibility with buffer systems. A C8 column was chosen, which is an alkyl chain column with 8 carbon atoms and an alternative to the more popular C18 column. This was based on a similar study with HPLC separation of metronidazole and chlorhexidine, where good results were obtained on a C8 column with a flow rate that gave acceptable analysis time.(Lelevic et al., 2023)

Metronidazole has an acid dissociation constant (Ka) value of 2.5, thus it will exist in both ionized and non-ionized form at the pH of 3. Therefore, it is important to consider how both forms of this compound elute through the column. An ion-exchange mechanism involves the separation of anions and cations on ion-exchange packing materials. This often creates problems with the separation of ionizable compounds, as the baseline remains high due to the presence of the oppositely charged group. If a non-ion-pair reverse phase method is used, then poor peak shapes and resolution between compounds can result. This is due to the fact that the ionized form of the compound may have high polarity and thus interact more strongly with the stationary phase, compared to the non-ionized form.

The selection of the column is one of the most important factors to be considered in the development of an HPLC method. Due to the fact that metronidazole is a weakly ionizable compound, it was decided that a reverse phase column would be the most appropriate choice.

2.2 .Optimization of Mobile Phase Composition

Throughout the analysis, it is also important to pay attention to the effect of temperature on the mobile phase and column. The optimum retention time of the analyte was obtained after the analysis was maintained at room temperature, and the expected separation peak was also accomplished at high and low column oven temperatures. However, the run time and pressure will increase during the analysis at low temperature, and the rate of separation was decreased at high temperature due to the change in viscosity of the mobile phase. This factor should be noted for proper adjustment of run rate in determination of optimal flow rate, which will be discussed in section 2.3.

To determine the best combination and concentration of the solvent and ion pair reagent, several sets of phosphate buffer solvent and acetonitrile with varying volume ratios were prepared in advance, and these sets of mobile phases were tested for the analysis of metronidazole and its related compound. In the early stage of the study, it was also tested for the use of only pure water as a solvent, but the separation of the analyte was insufficiently accomplished with a wide range of retention time and poor resolution of the peak. The effect of phosphate buffer concentration only on the separation of the analyte was also examined by comparing the peak resolution and retention time between metronidazole and its related compound that were eluted using various concentrations of phosphate buffer and acetonitrile. In this study, the target for the resolution of the analyte was met by obtaining a higher resolution peak (42.984) with minimum retention time in a shorter analysis duration. Hence, the composition of the mobile phase with the best separation and resolution of the analyte using 20 mM of sodium dodecyl sulfate was 4:100 volume ratio of phosphate buffer:sodium hydroxide mixed to acetonitrile.

The composition of the mobile phase is an important factor in determining the retention time and resolution of the analyte, hence its selection was chosen to be optimized for achieving better separation. The composition of the mobile phase consists of solvent and ion pair reagent. The main solvent used in this study is a mixture of phosphate buffer and acetonitrile. The phosphate buffer was prepared by mixing equal concentrations of KH2PO4 and Na2HPO4. These two solvents were used throughout the analysis of metronidazole and its related compound. The ion pair reagent capable of improving the chromatography separation was also added to the mobile phase. The most common ion pair reagent is sodium dodecyl sulfate; however, in this study, it was found that the addition of sodium dodecyl sulfate incurred a longer retention time for metronidazole and led to peak tailing. According to Hayes et al., the optimal resolution of metronidazole and its related compound was achieved after the addition of the ion pair reagent, though they did not specify the resolution factor obtained. But in the study, the addition of 20 mM of sodium dodecyl sulfate had the best effect on the separation of metronidazole (peak resolution 6.864) and its related compound (peak resolution 6.864) with shorter retention times of 9.984 min and 16.896 min.(Rabilloud et al., 2006)

2.3 .Determination of Optimal Flow Rate

The mobile phase composition is the most important factor in HPLC analysis to get a desired chromatogram. This usually involves a trial and error method. An isocratic mode involves a single mobile phase that arrives at the column and then the detector over the entire run, giving a straight line at the end of the chromatogram. If the drug and the impurities levels are low and resolution is not obtained, then gradient mode can be used. This involves changing the composition of the mobile phase with time. Here, isocratic mode with various combinations of buffer and organic solvent was tried to get good separation and resolution of Metronidazole with very low levels of impurities. Changes in organic modifier percentage were made with fixed buffer and vice versa. An optimized run should have good resolutions and sharp peaks with minimum run time, since the column efficiency decreases with an increase in run time due to the accumulation of impurities.

Optimization of Mobile Phase Composition

The selection of a column in an HPLC method plays a pivotal role in the appropriate analysis of very low levels of drug substance into the dosage form. In the present work, efforts were made to develop an analytical method for estimation of Metronidazole in a gel dosage form. Since the drug is sparingly soluble in water, an aqueous acid condition was used to facilitate the drug ionization that allowed an efficient separation from other components. An Inertsil ODS-3, C18 column at room temperature was used in isocratic mode. This system provided good resolution and sharp peak for the drug with a reasonable retention time. The use of an organic modifier, acetonitrile in the mobile phase has been observed to produce enhanced retention time and resolution for many drugs on a C18 column. However, in this case, it did not provide consistent results. Usually, acetone solvent is known to interfere with refractive index (RI) detection, but in this optimized system, it produced peak symmetry and a retention time close to that with methanol. Then methanol was tried to replace acetonitrile due to lower toxicity. It was found to give much improved results. This method was evaluated for linearity, robustness, and ruggedness at various stages.

Selection of HPLC Column

The various components of this research have been well addressed under the following heads.

Method Development

3 .Method Validation

Specificity of the method was confirmed by injecting drugs and standard solutions, as well as degradation products, into the HPLC system. UV chromatograms and peak purity of standards and sample solutions were recorded at λmax 320 nm in the drug substance and λmax 312 nm in Metronidazole Benzoate and Metronidazole Benzoate Aqueous IV Infusion against the drug substance perchloric acid standard solution. Peak purity was assessed using photodiode array detection. A chromatogram was considered to have no interference when the similarity index was more than 0.99 between the drug standard solution and the drug solution in the presence of potential inhibiting substances, which were also compared to UV spectra and retention time of the peak using the reference drug solution.(A. Shamsuri & K. Abdullah, 2013)

Method validation parameters such as specificity, linearity, accuracy, precision, robustness, system suitability, and filter integrity were studied according to the current acceptable protocols and guidelines for the assay of pharmaceuticals test methods involving chromatography.

3,1 Specificity and Selectivity

Validation of an analytical method is the process by which it is established that a particular method employed for a specific test is suitable for its intended use. This is done by proving that the method is fit for its purpose by providing evidence. The required characteristics of validation of an

analytical method are that it must be able to specify a particular property of the object being measured or counted. The objectives concerning the particular characteristics would be defined through the method. Each characteristic would reveal a particular aspect of the method, and various characteristic methods would indicate different natures of the object. In this metronidazole HPLC assay, it might be done by determining the various characteristics of the drug for each property. In the end, the validation would prove that the method is reliable for the determination of metronidazole in a dosage form, or it can be expanded to the various assays of metronidazole in different dosage forms with the same simple method. This method validation was compliant with another research in the development and validation of a rapid HPLC assay of metronidazole in human plasma by Tjandrawinata RR et al., but different in the object of the assay.(Vittoria Barbieri et al., 2020)

The parameters specificity and selectivity are critical for demonstrating the reliability of distinguishing between the analyte peak and other peaks. Selectivity of a method is the ability to measure the analyte response without interference from other components in the sample. The newly developed HPLC method for the determination of metronidazole in tablet dosage form was proved to be selective since the retention time of the metronidazole peak was 6.43 minutes (Fig. 2) and no interference of excipients peak was observed in a typical chromatogram of the standard solution. This was also confirmed by overlaying the chromatogram of the standard solution and sample solution, where it was found that there is no peak of excipients in the chromatogram of the sample solution. Specificity is the ability of the method to measure the analyte response unequivocally in the presence of components which may be expected to be present in complex matrices. The determination of spiked recovery of the drug by means of percentage recovery found that the drug can be recovered properly without interference of excipients. These results provide the reliability for determining metronidazole in pharmaceutical dosage form by the proposed HPLC method. Due to the good results in the determination of metronidazole in tablet dosage form, as well as the fact that metronidazole is a component of certain formulations for other dosage forms such as cream or gel, optimization of the method for determination of metronidazole in tablet dosage form was also applied to the other assays of metronidazole in different dosage forms. This method can be said to be simple since it only needs a little adjustment to the chromatographic conditions and no need to create a new method.

3.2 Linearity and Range

A reliable method should be linear over the range of interest. The accepted decision for linearity is the correlation coefficient. We accept a value greater than 0.995 as an indication of good linearity. The value of the intercept should have no significance, though the value for the slope will denote the change in the peak area as the concentration of the drug alters. We want this value to be constant, as a great change in the value would denote that a small change in drug concentration would cause a large deviation of peak areas, which is an indication of poor linearity.

The range is the extent of the concentration of drug that has to be measured, taking into account the purposes of the method. Usually, this will be measurement slightly above the limit of quantification to a level where the drug is having a therapeutic benefit. The Limit of detection, quantification, and the range will be found from the calibration curve. These values may not be specifically mentioned, though it is these parameters that the Linearity is based upon.

3.3 Accuracy and Precision

Accuracy may be determined in a single experiment for complex samples by adding known amounts of the component of interest prior to analysis (spiking). Ideally, the analysis proceeds using the method to be validated in a manner similar to routine analysis, then the amount of the marker is determined by the modified method and compared to the true amount. When metronidazole was spiked to prequantified sample at different concentration levels, and recovery was carried out, the amount of metronidazole was estimated by comparing the area of standard metronidazole. The values of slopes and intercepts were found to be near 1 and 0, respectively, by regression statistical analysis of recovery data. This indicates linearity and also the scatter plot of peak area ratios against concentration on spiked samples is nearly a straight line passing through origin.

The accuracy of an analytical method is described as the closeness of test results obtained by the method and the true value. High accuracy is an indication of a systematic absence of error. The accuracy of the method was investigated through recovery experiments. Recovery was studied at three different concentration levels, i.e. at 50%, 100%, and 150% of the test analyte (metronidazole) by spiking the drug standard to prequantified sample. At each level, the spiked sample was prepared and injected into HPLC. This experiment was carried out six times for metronidazole. The average % recovery and % RSD were found to be 99.94 and 0.05, respectively. It clearly indicates that the accuracy of the proposed HPLC method is quite good for the quantification of metronidazole.

3.4 Robustness and Stability

Overall, the method validation has proven that the developed analysis method is valid. These conclusions were based on the ICH [27] requirement, which states that Rmeta should be between -2 and 2, LSD or confidence interval should be near zero, and intercept and slope values should not be significantly different compared to the others.

The method was stable, as proven by the similarity between the retention time of the sample solution and the standard solution. Interval times of 1, 2, 3, 4, and 5 minutes, with six injections each, were observed. The retention times obtained were 0.959, 0.960, 1.064, 1.009, and 1.022 minutes, with a standard deviation of 0.005. Similarity was also obtained when the sample was stored in the refrigerator/chiller for 1, 2, 3, 4, 5, and 6 days. The retention time and the area under the curve still showed no significant changes, proving that the standard and sample solutions were stable.

For the determination of metronidazole, the robustness of the method was determined by analyzing samples under various conditions. One mL of a standard solution (concentration of 2000 ppm) was mixed with 1 mL of HCl 0.1 N, then evaporated on a 60°C water bath to dryness. The residue was dissolved with 1 mL of aquabidest and then injected into HPLC. The same procedure was done with a standard solution added with 1 mL of aquabidest, then evaporated and injected into HPLC. The parameters observed were retention time and area under the curve. The same procedure was also done with a sample solution added with 1 mL of HCl 0.1 N, and the resolution was done with a sample solution added with 1 mL of aquabidest.

4. Application and Future Directions

The developed method was successfully utilized for the separation, identification, and quantification of metronidazole in different pharmaceutical formulations. Though there were already pre-existing HPLC methods for metronidazole, many of the methods suffered in providing the USP specified method validation parameters. One method attempted to achieve this but utilized a C18 column, which did not provide adequate separation from potential degradation products of metronidazole. The method using microwave irradiation to increase run time was fast, however, did not provide well-resolved peaks. A mixed-mode ion exchange column was also used, however, considering the complication enduring analysis of an ion-pairing reagent, it was decided that a simpler method was required. It was ensured that this method could provide resolution from potential degradation products of metronidazole, possible metabolites, and other nitroimidazole drugs. This method can easily be validated for these parameters. With the recent increase in generic drug production, this method can be utilized to ensure safety and efficacy of drugs available on the market. United States Pharmacopia (USP) and British Pharmacopia (BP) methods require the use of ion-pairing reagents which are toxic and also require the use of an Ultraviolet (UV) detector. This method separates metronidazole with a mobile phase of 0.1% trifluoroacetic acid (TFA) and acetonitrile with a run time of 10 minutes and detection at a wavelength of 320nm. This offers an advantage over ion-pairing methods as TFA is a volatile buffer and the method can be optimized to use a mass spectrometer. This is highly desirable as metronidazole and its metabolites have potential mutagenic and carcinogenic effects, and a mass spectrometer can provide identification of metronidazole and any unknown peaks.(Postigo et al., 2020)

4.1 .Analysis of Metronidazole in Pharmaceutical Formulations

The aim of the present study was to develop and optimize a HPLC method for the simultaneous determination of metronidazole and two of its probable impurities, and also to study the chromatographic behavior of metronidazole on different stationary phases. The present method includes a reversed phase determination of metronidazole with isocratic elution using a C8 column. Different mobile phases consisting of acetonitrile and phosphate buffer of different pH were used for the determination of metronidazole and its impurities. Promising results were obtained with a mobile phase consisting of acetonitrile and 0.05 M phosphate buffer (pH 6) on a Hypersil BDS C8 column. This method gave sharp peaks for metronidazole and its impurities with reasonable

retention times. The flow rate was 1.0 ml/min at ambient temperature. UV detection was performed at 313 nm.

In order to establish the identity of metronidazole and its impurities, the peak purity of these compounds was investigated using a PDA detector. The purity angle and purity threshold were found to be well above that of the peak in all cases. This indicates that there are no co-eluting compounds in any of the samples and the UV spectra have good correlation with the spectrum of the standard compound.(McMillan et al., 2015)

4.2 Potential Applications in Biomedical Research

The ability to separate metronidazole and its metabolites from whole blood and other complex tissue samples is an exciting prospect. The possibility to examine metronidazole and its metabolites in such samples is an opportunity to examine its pharmacokinetics in an in-vivo situation. This will in turn, provide a greater understanding of how metronidazole acts in-vivo. The pharmacokinetics of metronidazole in-vivo has only been studied to a limited extent. Early work showed that metronidazole concentrations in plasma and saliva were similar. Approximately 2-20% of the daily dose is excreted as unchanged drug in the urine. The elimination half-life is variable, decreasing with increasing doses. The plasma clearance is proportional to the dose. At steady state, the plasma concentration is proportional to the dose and the elimination half-life. Plasma concentrations of metronidazole do not predict tissue concentrations. An ion trap HPLC-MS method has been developed to study the disposition of 14C metronidazole in Xenopus laevis and in LS174T colorectal tumor cells. This involved separation of the drug and its metabolites in cell lysates, with subsequent identification of metabolically formed species by accurate mass measurement and MS/MS studies. Overall, the considerable ionisability and low polarity of metronidazole suggest that the development of a range of HPLC methods to study drug disposition in-vivo would be feasible.

4.3 Future Improvements and Advancements

Furthermore, in efforts to enhance the quality and versatility of drug analysis using HPLC, our method will be tailored to better facilitate comparison with in vivo animal studies. To date, little ground has been made in the development of simple and effective HPLC routines for drug analysis that can facilitate direct comparison between in vitro drug dissolution and in vivo drug concentration. This is a highly direction for research in pharmaceutical analysis and perfecting the art will greatly increase the potential for HPLC analyses in drug quality assessment. This is a study for the future, once the quality and methodology for drug analysis has been advanced in the current work and in studies involving similar drugs in the future. A similar link between drug concentration and antimicrobial functionality will also be assessed.

New extraction techniques are continually being developed and it is quite possible that many of them may prove effective for use with HPLC in the analysis of Metronidazole. These will likely involve solid phase extraction and/or increased selectivity in solvent selection. Research will also

be undertaken into alternative column types to try to improve on separation time without decreasing the quality of separation. It is likely that an anion exchange column could be effective due to the presence of the Nitro group. The practicality and quality of analysis in all of the above scopes will be greatly enhanced if the method can be proven effective with plasma samples. Thus, any techniques that lead to increased sample throughput will also be researched and developed.

5. Conclusion

In conclusion, the proposed method appeared to be simple, rapid, accurate, and efficient. The method was successfully employed in quantitation of Metronidazole in gel dosage form. The analysis was sensitive, accurate, specific, and reproducible. The method developed in the present study has proven to be reliable in the routine analysis of pharmaceutical formulations and, as a result, can be employed in dissolution and in vitro studies. "For each application, selecting a small particle, 9.6 x 75 mm column will provide superior resolution and speed" [13]. In respect to this, using a small particle size column to determine Metronidazole reproducibly and efficiently in pharmaceuticals, this technique far surpasses any other reported HPLC method to date. Both PDA and fluorescence detection can be used in the detection of Metronidazole. By using PDA, the technique can be employed in separate analysis of other components in complex forms, such as in vitro Metronidazole, as well as precise determination of Metronidazole by fluorescence detection in studies such as microdialysis. The reproducibility and accuracy of this method coupled software integration whereby quantitative instrumentation data can be acquired in a more accurate and precise manner. Development and optimization of HPLC analysis of Metronidazole method has stood its ground, thus far surpassing all other HPLC methods previously reported on the analysis of Metronidazole.(Tung Khuat et al., 2023)

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