REVIEW ARTICLE

ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF MELOXICAM IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL SAMPLES

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ABSTRACT
Meloxicam (MX) belongs to the family of oxicams which is the most important group of non steroidal anti-inflammatory drugs (NSAIDs) and is widely used for their analgesics and antipyretic activities. It inhibits both COX-I and COX-II enzymes with less gastric and local tissues irritation. A number of analytical techniques have been used for the determination of MX in pharmaceutical as well as in biological fluids. These techniques include titrimetry, spectrometry, chromatography, flow injection spectrometry, fluorescence spectrometry, capillary zone electrophoresis and electrochemical techniques. Many of these techniques have also been used for the simultaneous determination of MX with other compounds. A comprehensive review of these analytical techniques has been done which could be useful for the analytical chemists and quality control pharmacists.

Keywords: Meloxicam, NSAIDs, analysis, spectrometry, chromatography.

1. INTRODUCTION
Meloxicam (MX), 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carbox-amide-1,1-dioxide (Fig. 1), is a pale yellow powder that is insoluble in water and is slightly soluble in lower aliphatic solvents. It is used for its anti-arthritis and anti-inflammatory action with less gastric and local tissue irritation such as dermal, ocular and rectal tissues. It is a cyclooxygenase-2 (COX-2) potent inhibitor and is comparable to other non steroidal anti-inflammatory drugs (NSAIDs) available in the market. It more effectively inhibits COX-2 than COX-1. It is readily absorbed on oral and rectal administration reaching the peak plasma levels in 6 h. The protein binding is 99% and due to its plasma elimination half-life which is up to 20 h, it is used in single daily doses of 7.5 and 15 mg/day.

Fig. 1. Chemical structure of meloxicam.
2. ANALYTICAL TECHNIQUES FOR THE ASSAY OF MELOXICAM

A number of different analytical techniques have been utilized to study MX and its degradation / metabolic products\(^5\) in order to understand the stability and mode of action of the active drug. The various techniques previously employed for this purpose are presented in the following sections.

2.1. Titrimetric Techniques

2.1.1. Potentiometric titration

A sensitive, precise, economical and quick potentiometric method has been described to determine MX in pharmaceutical preparations. The titration of MX with N-bromosuccinamide (NBS) and N-bromophthalimide (NBP) was carried out in an acidic medium and the end point was determined with the help of a platinum indicator electrode. The concentration range selected was 0.176-1.406 mg of MX with mean percentage accuracy of 100.26% and 100.08% for NBS and NBP, respectively. The method was successfully applied for the analysis of pharmaceutical formulations of MX\(^9\).

Sawant et al.\(^10\) has reported a spectrometric method for the simultaneous determination of MX and paracetamol (PM) in pure form and in formulation at their \(\lambda_{\text{max}}\) at 268.8 and 256 nm, respectively. A second method has been used as the absorbance ratio method in which two wavelengths were selected, one was the isobestic point at 308 nm and other was 256 nm (\(\lambda_{\text{max}}\) of PM). The concentration range of 5-30 \(\mu\)g/ml was taken according to the Beer Lambert's law. Both methods have been validated according to ICH guidelines and can be used for the analysis of MX and PM in pure and tablet dosage form.

2.2. Spectrometric Techniques

2.2.1. Ultraviolet (UV) spectrometry

Sensitive spectrometric and densitometric methods have been described for the determination of MX in the presence of their degradation products (5-methyl-2-aminothiazole and benzothiazine carboxylic acid). These methods involve first derivative spectrometry at 338 nm and TLC densitometry at 365 nm. The mean accuracies of these methods were 99.66\(\pm\)0.91% and 99.99\(\pm\)0.70%, respectively. The concentration ranges used were 5-20 \(\mu\)g and 2-10 \(\mu\)g\(^7\). Mandrescu et al.\(^8\) developed a spectrometric method for the quantitative determination of MX in which a stable chromophore is formed by coupling MX with Al\(^3+\) and measured at 375 nm in the concentration range of 5-30 \(\mu\)g / sample which showed the correlation coefficient \(r^2 = 0.9998\). The limit of detection (LOD) and limit of quantification (LOQ) were 0.68 \(\mu\)g/sample and 2.25 \(\mu\)g/sample, respectively. The system precision with RSD = 1.3794%, the method precision with RSD = 1.6045% and the mean recovery of the method was 100.5%.

A simple, accurate and rapid UV method has been introduced in which MX is determined at 269 nm. In the first step MX was dissolved in 0.1 M NaOH, secondly hydrotrropic agent i.e. 10% trisodium citrate solution was used to dissolve the drug which was water insoluble. The linearity showed the concentration range of 5-30 \(\mu\)g/ml with correlation coefficient of 0.9995 and 0.9987, respectively\(^9\).

For the quantitative estimation of MX in raw material and pharmaceutical dosage forms a simple, precise and economical UV method has been developed and validated. The MX showed \(\lambda_{\text{max}}\) at 360 nm while the first- and second-order derivative spectra indicated sharp peaks at 338 and 281 nm, respectively. According to Beer’s law the concentration range of 5-25 \(\mu\)g/ml was used. The method was validated and found to be precise and accurate\(^11\). In another method Pomykalski and Hopkala\(^12\) made a comparison of classic and derivative UV spectrometric methods for the quantification of MX and mfenamic acid in pharmaceutical preparations. The method described zero-, first- and second-order derivative spectrometry using peak-peak (P-P) and peak-zero (P-O) measurements. The calibration curves were linear in the concentration range of 4.0-14.0 \(\mu\)g/ml for MX and 14.0-24.0 \(\mu\)g/ml for mfenamic acid. Jyothsna\(^13\) proposed a simple and selective method for the quantitative determination of MX by reaction with 2,4-dinitriphenylhydrazine (DNP) in pure form and in pharmaceutical preparations. The method showed...
λ_{max} of DNP at 350 nm with the linearity in the concentration range of 10-50 μg/ml (r²=0.9973). The calculated apparent molar absorbptivity value was 0.4849×10² l/(mol·cm). The method was validated with respect to system suitability, linearity, precision, LOD, LOQ, accuracy (recovery), ruggedness, robustness and stability. According to the ICH guideline Q1A (R2) forced degradation studies were also performed on different brands of MX by using UV spectrometry, e.g. the effect of heat, UV light, acid base etc. The degradation studies of different brands of MX were performed with standard solution of 200 ppm. The working solution was prepared by the addition of 0.1 N HCl, 0.1 N NaOH and deionized water separately. To determine the effect of acid and base, solutions were placed at room temperature, for UV at 320 nm and for heat at 50°C. The behavior of MX showed that maximum degradation takes place with UV light and UV spectrometry is preferred over other methods. Redasani et al.\textsuperscript{15} introduced different UV-spectrometric methods for the quantification of MX in bulk and in tablets. In method I, the zero order spectra were obtained at 269 nm, while in method II wavelength range of 254-279 nm was used to find the area under the curve (AUC), whereas, method III was the first order derivative UV spectrometry in which the zero order spectrum was derivatized into first order (Δλ=2) and 275 nm wavelength was selected for the purpose of determination. In method I and II, MX obeyed linearity in the concentration range of 5-30 μg/ml while in method III, 50-300 μg/ml, respectively with r² as 0.999, 0.998 and 0.999, respectively. Calibration curves were plotted using instrument response between particular wavelengths and concentrations of the analyte in the solution. The proposed methods were successfully applied for the determination of MX in commercial tablets and the amount was found to be 99.81%, 100.15% and 100.07%, respectively. The proposed methods were validated as per ICH guidelines for accuracy, precision, repeatability and ruggedness.

2.2.2. Visible spectrometry

For the quantitative determination of MX in pure form and in pharmaceutical preparations some methods have been introduced in which neutral complex is formed between basic methylene blue and MX in phosphate buffer (pH=8) which was measured at 653.5 nm, the concentration range used was 1-5 μg ml⁻¹ and the mean percentage accuracy was 99.129±1.18%. Another method was reported in which an intense orange red colored product was formed by reacting 2,3-dichloro-5,6-dicyano-p-benzoquinone with MX after heating at 100°C for 5 min. The colored product was then measured at 455 nm, and the percentage accuracy was 100.539±1.04% over the concentration range of 40-160 μg/ml\textsuperscript{16}. In another method visible spectrometry has been used to assay MX after reaction with the dye bromocresol to form an ion-pair of MX in acidic medium, which was extracted into chloroform with a λ_{max} of 415 nm. The Beer's law concentration limit was found to be in the range of 10-50 μg/ml. The molar absorbptivity was found to be 2.17×10³ (mole⁻¹·cm⁻¹), Sandell's sensitivity was 0.1612 (μg/cm²/0.001 absorbance unit), correlation coefficient (r²) was 0.9999 while LOD and LOQ were 0.88 and 2.69 μg/ml, respectively. The result of estimation in marketed formulations was found to be 99.86±0.32% and 97.93±0.32%. The proposed method was applied successfully for the determination of MX in tablets with average recovery of 99.62±0.88% and 100.4±0.768%. The method was then validated statistically as per ICH guidelines, which yielded good results concerning range, linearity, precision and accuracy\textsuperscript{17}.

For the determination of MX in pure form and in pharmaceutical formulations a simple, rapid and sensitive spectrometric method has been developed. The method involved addition of a measured excess of N-bromosuccinimide (NBS) in acid medium followed by determination of unreacted NBS by reacting with indigo carmine (IC) and measuring the absorbance at 610 nm. The optimum reaction conditions and other analytical parameters have also been evaluated. Linearity was observed from 0.2-50 μg/ml and the statistical analysis and comparison of the results by British Pharmacopoeial method was also reported\textsuperscript{18}. Another simple, accurate and sensitive spectrometric method was based on the
formation of the ion-associate complexes between MX and orange G (OG), methylene blue (MB) or copper chloride (CuCl₂). These complexes result in the formation of colored products showing absorption maxima at 358, 652 and 361 nm with the three reagents, respectively. The factors affecting the reactions were also studied. The method was then validated which showed linear relationship with good correlation coefficients (0.9956, 0.9934 and 0.9974) between absorbance and concentrations of the drug in the concentration range 1.0-22.1 μg/ml, under optimum condition. The LOD and LOQ values were in the ranges of 0.40-1.73, 0.66-1.87 and 0.8-1.33 μg/ml, respectively. The recovery was 99.63% with OG, 100.14% with MB and 99.86% with CuCl₂ without the interference of the common excipients. The results of the proposed method were compared with the results of reference methods¹⁹.

2.2.3. Near infrared (NIR) spectrometry
NIR spectrometry is an important component of process analytical technology (PAT) for enabling the rapid analysis of pharmaceutical tablets. NIR-chromometric methods were used to determine the content of MX in tablets. The active content assay was performed on samples corresponding to 80, 90, 100, 110 and 120% of MX content whereas pharmaceutical characterization was performed on samples prepared at seven different compression forces (ranging from 7 to 45 kN) using NIR transmission spectra of intact tablets and PLS as a regression method. The validation of the method showed good accuracy and precision. The method was found appropriate for the direct active content assay in tablets (ranging from 12 to 18 mg/tablet) and also for predicting crushing strength and disintegration time of intact MX tablets. The result showed that the proposed method provides a possibility to predict both chemical property (active content) and physical/pharmaceutical properties (crushing strength and disintegration time) directly, without any sample preparation²⁰.

2.3. Chromatographic Techniques
These techniques have been extensively employed for the separation, identification and determination of drug substances both in the pure form, as mixtures and in the presence of degradation products. Various types of chromatographic techniques include:

2.3.1. Thin layer chromatography (TLC)
A sensitive TLC method has been developed to determine some of the oxicams such as Iornoxicam (LX), tenoxicam (TX) and meloxicam (MX), in the presence of their alkaline degradation products. The drugs spots as LX, TX and MX were measured by densitometric method at 380, 370 and 364 nm, respectively. The solvent systems used for the separation were ethyl acetate:methanol-26% ammonia (17:3:0.35, v/v/v) for LX and TX and chloroform-n-hexane-96.0% acetic acid (18:1:1, v/v/v) for MX. The linear concentration ranges of 0.25-6.0 μg/spot for LX and TX and 0.5-10 μg/spot for MX were obtained. The mean recoveries were found to be 99.80±1.32%, 100.57±1.34%, and 100.71±1.57%, respectively²¹. In another method MX and its degradation products have been separated by TLC with densitometric detection in pharmaceutical preparations. A mixture of ethyl acetate-toluene-butylamine (2:2:1, v/v/v) was used as a solvent system and measurements have been carried out at 297 nm. The method was validated for linearity, precision and accuracy. The LOD and LOQ were 0.96 and 2.90 μg per spot, respectively. The identification of degradation products of MX (i.e. 5-methylthiazol-2-ylamine and 5-(di-iodo-16-sulfanylidine)-6-methylidenecyclohexa-1,3-diene) was done using HPLC-MS/MS method²².

2.3.2. High performance thin layer chromatography (HPTLC)
A simple, selective, precise and stability indicating HPTLC method has been developed to analyze MX as a bulk and in pharmaceutical formulation. A mixture of ethyl acetate:cyclohexane:glacial acetic acid (6.5:3:5:0.02%, v/v/v) was used as mobile phase and densitometric analysis was done at 353 nm. The calibration curve was found linear in the concentration range of 100-500 ng. The ±RSD value of slope was 3183.8±0.358 and regression was 0.9996±0.0321. The RSD values of the precision studies conducted for the system and method were
0.83 and 1.89, respectively. The LOD and LOQ were 30 and 99 ng, respectively, while the value of mean recovery was 100.3%. Hence the method was suggested to be used to analyze MX in the presence of excipients from tablet formulation. Another validated stability indicating HPTLC method was developed for the quantitative determination of MX in the presence of degradation products. It was reported to be rapid, precise, selective and sensitive. The stationary phase was silica gel 60F254 and the solvent system was toluene:ethanol:glacial-acetic acid (4:4:1.6:0.4, v/v/v/v) that gave an Rf value of 0.61±0.02. The densitometric analysis was carried out at 358 nm. The drug response with respect to peak area was linear over the concentration range of 200-700 ng/spot. The mean SD values of the slope, intercept and correlation coefficient were found to be 8.192 (±0.089), 1640.1 (±24.516) and 0.9965 (±0.00083), respectively, whereas the LOD and LOQ values were 23.20 and 70.30 ng/spot, respectively.

2.3.3. High performance liquid chromatography (HPLC)

2.3.3.1. Pharmaceutical preparations
A sensitive and reproducible HPLC method has been described for the quantitative determination of MX in pure form and in pharmaceutical formulations. The drug was determined in the presence of its degradation products over a concentration range of 100-500 μg/ml with mean percentage accuracy of 100.139±0.53%. Another study has also been reported for the separation of 3 drugs from their alkaline degradation products on a RP C18 column. Two different mobile phases were employed such as methanol-acetonitrile-acetate buffer (4.5:0.5:5.0, v/v, pH 4.6) for LX and MX while methanol-acetonitrile-acetate buffer (1.9:0.1:3.0, v/v, pH 4.6) for TX at ambient temperature. The quantification was achieved by UV detection at 280 nm, based on peak area. The linear ranges were 0.5-20 μg/ml for LX and TX and 1.25-50 μg/ml for MX, with mean recoveries of 99.81±1.01%, 98.90±1.61%, and 100.86±1.55%, respectively. The method was validated according to ICH guidelines and was successfully applied to the determination of LX, TX, and MX in bulk powder, laboratory-prepared mixtures containing different percentages of degradation products, and pharmaceutical dosage forms. Similarly, a simple and rapid HPLC method was developed for the quantitative determination of MX using a micro Bondapak 125 A C18 10 μm column. The mobile phase of methanol and water (70:30, v/v, pH 2.6) was set at a flow rate of 2 ml/min and UV detection was performed at 230 nm. The results showed good recovery values of MX in tablets (Melfax 15 mg) from 99.27 to 100.06% using piroxicam (PX) as an internal standard (IS). This method was proved to be useful for the quantitative determination of MX in raw materials, in bulk drugs and other dosage formulations. Another simple, precise, specific, robust and rapid HPLC assay method has been used to measure MX in pharmaceutical formulations. The solutions of MX standard and commercial formulation were prepared in HPLC grade acetonitrile and methanol respectively, in concentration of 5 μg/ml. A mixture of phosphate buffer (0.2 N) and acetonitrile (38:62, v/v) was used as mobile phase and the separation was achieved by using a reversed phase C18 column with a flow rate of 0.5 ml/min. The retention time (tR) was 7.4 min and MX was distinctly and specifically detected at 352 nm. The method was also validated and showed the LOD and LOQ of 0.06 and 6 μg, respectively. A sensitive and selective HPLC method has been used to determine MX in pharmaceutical formulations. A mixture of acetonitrile, water and phosphoric acid was used as solvent system (70:30:0.1, v/v/v/v) on a C18 column and detection at 360 nm. All validation parameters were conducted for intra- and inter-day precision, accuracy, recovery, stability, reproducibility and sensitivity. The method showed simple and fast sample preparation and no pH adjustment to achieve single, sharp and symmetric peak having a tR of 2.1 min at room temperature. The method showed a linear response over a concentration range of 5-200 μg/ml MX with mean concentration accuracy of 99.41%. The inter- and intra-day RSD over the entire concentration range is less than 1.5%.

A validated HPLC analytical method has been
developed for the determination of MX in liposomes. An Ascentis RP amide C_{16} column was used for the separation with a mobile phase composed of acetonitrile and 0.3% formic acid solution (40:60, v/v) adjusted at pH 2.8. The flow rate of the mobile phase was 0.5 ml/min and UV detection was done at 355 nm. PX was used as IS and all the analysis was performed at temperatures of 40.0±0.5°C. The calibration curve was linear over the concentration range of 18-420 ng ml^{-1} and the RSD for precision was <1.03%. The accuracy ranged between 98.53 and 101.41% with a RSD lower than 1.5%. The LOD and LOQ values were found to be 5 ng ml^{-1} and 15 ng ml^{-1}, respectively. A simple and quick reverse phase (RP) HPLC-UV method has been used for the separation and assay of MX in pharmaceutical formulation. An Agilent Zorbax SB C_{18} column was used with methanol and 1% aqueous solution of glacial acetic acid as mobile phase. The elution was performed with composition gradient, MX being detected at 355 nm with a 5 min analysis time. The regression analysis showed good linearity, with correlation coefficient of 0.9997 over the concentration range of 20-2000 ng/ml. The LOD was determined to be 5 ng/ml and LOQ was set at 15 ng/ml. The analysis of the tablets resulted in 85.82% of MX compared to the declared concentration.

2.3.3.2. Biological fluids
A simple HPLC method has been developed to estimate MX in plasma using PX as the IS. A mixture of methanol, acetonitrile and an aqueous solution of (NH_{4})2HPO_{4} (50 mM) (4:1.5, v/v/v) was used as the mobile phase at a flow rate of 1 ml/min. Lichrocart RP-18 (125×4 mm) was used as an analytical column and the analytes were detected at 364 nm. The t_{R} of MX and PX was found to be 2.7 and 1.9 min, respectively. The method showed an accuracy of 102.3% at 0.52 μg/ml and was capable of detecting a minimum concentration of 0.029 μg/ml MX from the biological samples. Similarly the estimation of MX in plasma has also been carried out by simple and rapid HPLC assay method. The separation was done by LiChrosopher C_{18} 511 (125×4.0 mm) analytical column using a mobile phase of sodium acetate buffer (pH 3.3, 170 mmol) and acetonitrile (62:38 v/v). The detection was made at 355 nm and the t_{R} observed for MX and PX (IS) were at 6.0 and 4.0 min, respectively. The response was found linear over a concentration range of 50-1500 ng/ml. The method could also be used for the bioequivalence study of MX formulation. A validated HPLC method for the pharmacokinetic study of MX using whole blood of rats has been developed. The rats received oral doses of 3.2, 5.6 or 10 mg/kg of MX at selected time interval during 24 h after administration. The maximum concentration was achieved in 2 h after administration with a half-life of 9 h. An increase in the value of C_{max} and AUC as a function of dose was observed, and no statistically significant difference was observed in AUC/dose or C_{max}/dose between the doses. However, linearity could not be concluded because of the wide variability observed. A simple and easy column-switching HPLC method has been used to determine MX in rat muscle and plasma. After the clean-up and enrichment, the analyte was transferred to YMC Pack Pro C_{18} column. The elution was monitored with a UV-detector at 360 nm and the coefficients of variation (r^{2}) for muscle and plasma were found to be more than 0.999. The LOQ and LOD of muscle and plasma samples were 50 ng/g and 20 ng/ml, respectively. The accuracy and precision were achieved in the range of 50-2500 ng/g in muscle and 20-2500 ng/ml in plasma.

Jedziniak et al. developed a simple and short HPLC-UV screening method for the determination of residues for seven NSAIDs (carprofen, diclofenac, flunixin, meloxicam, phenylbutazone, tolfenamic acid, vedaprofen) and their three metabolites (4-methylaminoantipyrine, 5-hydroxyflunixin, oxyphenbutazone) in cow’s milk. The sample was prepared by liquid-liquid extraction with acetonitrile in the presence of sodium chloride. The column used was C_{18} with a gradient of acetonitrile and the ammonium acetate buffer pH 5.0. The UV-detector wavelength was programmed in order to improve sensitivity. For most analytes, relatively high recoveries were observed i.e. 76-98% and within-laboratory reproducibility levels were in the range.
of 3.6-17.8%. For phenylbutazone, oxyphenbutazone, and 4-methylaminoantipyrine recoveries were considerably lower (44-68%) and reproducibility was up to 41.9%, which was probably caused by the instability of the analytes. The robustness of the method for different fat contents was successfully investigated. The method was verified by its use in the determination of MX residues in milk samples obtained from MX-treated cows and the obtained results confirmed the usefulness of the developed method for the analysis of NSAIDs residues in milk. The determination of MX and PX in small volume plasma samples has been carried out by a simple, accurate and sensitive HPLC method. The bearded dragon (Pogona vitticeps) was used for MX and crane (Grus rubicunda) for PX. The samples were separated by RP-HPLC using X Bridge C18 column and finally detected at 360 nm. The mobile phase was a mixture of water with glacial acetic acid (pH 3.0) and acetonitrile (50:50, v/v), with a flow rate of 1.0 ml/min. The intra- and inter-day assay variability for MX and PX were less than 10% and the average recovery was greater than 90% for both drugs. This method was found applicable to pharmacokinetics studies requiring multiple sampling from the same animal.

A hand-operated on-line switching HPLC system has been developed for the determination of MX in spiked plasma samples at pH 6.0. The samples were introduced on the ADS precolumn using 0.05 M phosphate buffer. After washing with the buffer the ADS column was back flushed with the mobile phase consisting of 0.05 M phosphate buffer, acetonitrile (30% v/v) and t-butylamine (25 mM) at pH of 7.0. The analyte was then transferred to the analytical column LiChrosart 125-4 LiChrospher RP-8. The eluent was monitored by a UV-detector set at 364 nm and the column-switching method was found fully applicable to plasma injections. The bioequivalence of two formulations of 15 mg tablets of MX and a commercially available preparation as reference in 24 healthy male and female Caucasian volunteers was carried out by a validated non-extractive HPLC-DAD method. The stepwise gradient elution of the method allows injection of a high volume of the sample (500 µl) using Chromolith Performance RP-18e column. The mobile phase consisted of methanol and aqueous 20 mmol/l NaHPO4 buffer solution at pH 6.0. A flow-rate of 2 ml/min achieved a complete chromatographic run (including column equilibration) within 12 min and UV detection at 356 nm with a LOD of 30 ng/ml. The bioequivalence study was based on an open-labeled, randomized, two-period, two-sequence, single dose, crossover design with a 2-week wash-out period between consecutive oral administrations. The main pharmacokinetic parameters such as Cmax, tmax, t1/2, AUD and AUC (0-infinity) were considered as evaluation criteria for the test drug against the reference.

Another simple and sensitive HPLC method using UV detection has been developed and validated to analyze MX in human plasma. The extraction was carried out with diethyl ether and the chromatographic separation was done by using a RP Sunfire C18 column (150 mm×4.6 mm, 5 µm). A mixture of acetonitrile and 20 mM potassium hydrogen phosphate (40:60, v/v, pH 3.5) has been used as mobile phase and UV detection was carried out at a wavelength of 355 nm. The flow rate of mobile phase was 1.2 ml/min and the tR of MX using PX as IS was found to be 11.6 and 6.3 min, respectively. The calibration curve was found linear within the concentration range of 10-2400 ng/ml (r² > 0.9999). The LOQ was 10 ng/ml, mean accuracy was 98-114% and the coefficient of variation (precision) in the intra- and inter-day validation was 1.6-4.3 and 2.4-7.3%, respectively. The pharmacokinetics of MX was evaluated after administering an oral dose of 15 mg to 11 healthy Korean subjects. The AUCinf, Cmax, tmax and t1/2 were 42.4 ± 13.2 µg h/ml, 1445.7 ± 305.5 ng/ml, 4.1 ± 0.3 h and 22.0 ± 4.9 h, respectively. Several other HPLC methods have been developed for the assay of MX in biological fluids.

2.3.4. Reverse phase high performance liquid chromatography (RP-HPLC)
A rapid, simple, economical and stability-indicating
RP-HPLC method was introduced to determine MX in bulk and in pharmaceutical preparations. The C_{18} Hi Q Sil column was used with a mobile phase of acetonitrile-water-glacial acetic acid (55:40:5, % v/v) at a flow rate of 1 ml/min and detection at 355 nm. A single sharp peak with a \( t_r \) of 6.8±0.01 min was observed. The forced degradation of drug was also performed under acidic, basic and oxidative conditions. The method was then validated which showed a regression value of 0.9995 with good linear relationship over a concentration range of 4-20 \( \mu \)g/ml. The accuracy ranged from 99.27 to 100.78% and the % coefficient of variation for both intra- and inter-day precision was found to be less than 2%. MX showed minor degradation peak in acidic conditions at \( t_r \) of 2.24 min and the LOD and LOQ values were found to be 360 and 510 ng/ml, respectively. The proposed method showed good resolution of MX along with its degradants\(^{40}\). Similarly, a RP-HPLC method was developed to analyze MX in the presence of its impurities. The column used was Hypersil Gold C_{18} (250 mm×4.6 mm) as stationary phase. The mobile phase consisted of 0.65% \( \text{KH}_2\text{PO}_4 \) (pH 6) and methanol (45:55 v/v). The method was validated as per ICH guidelines in the concentration range of 5-25 \( \mu \)g/ml at 361 nm\(^{41}\). Similarly, MX has been analyzed through a RP-HPLC method in bulk and in commercial preparations. A C_{18} Hi Q Sil column and a mixture of methanol, water and \( \text{H}_2\text{PO}_4 \) (80:19.9:0.1 %, v/v) was used as the mobile phase. The flow rate was 1 ml/min with detection at 360 nm. The \( t_r \) of MX was 4.58 ± 0.02 min with a sharp single peak. The method was validated for all parameters and the regression data showed linear relationship (\( r^2 = 0.999 \)) over a concentration range of 4-20 \( \mu \)g/ml. The LOD and LOQ were found to be 48 and 680 ng/ml, respectively. The coefficient of variance was less than 2% and accuracy ranged from 99.33 to 100.45% for both the intra-day and inter-day precision\(^{42}\).

A simple, precise, accurate and economical RP-HPLC method has been reported and validated for the assay of MX tablets in USP. The method was developed using pH 7.0 phosphate buffer and acetonitrile (40:60%, v/v) in isocratic mode and Waters C_{18} 5\( \mu \) column. The flow rate and detection wavelengths were fixed at 0.8 ml/min and 344 nm and the \( t_r \) for MX was 2.448 min. Validation of the method was done as per ICH guidelines to test its suitability for intended purpose. The linearity range was set at 10-75 \( \mu \)g/ml with correlation coefficient 0.999 and the accuracy values of 99.53-99.66%. The method was found to be robust against changes in flow rate, organic composition, pH of mobile phase and detection wavelength and was reported suitable for the quality control analysis of MX tablets\(^{43}\). The three oxicams, such as TX, PX and MX were determined through the highly sensitive, accurate and rapid RP-HPLC method in different dosage forms. The C_{18} column was used with the mobile phase of methanol and aqueous buffer solution (\( \text{Na}_2\text{HPO}_4,\text{2H}_2\text{O} \) mixed with citric acid) (60:40, v/v) at pH 5.8. The flow rate was 1.2 ml/min and detection was made at 360 nm. The calibration curve was linear over the concentration range of 1.0-3.0, 1.5-4.5 and 1.8-5.3 mg/l for TX, PX and MX, respectively. The LOD and LOQ for TX were 0.06 and 0.18 mg/l, while they were 0.09 and 0.28 mg/l, for PX, and 0.12 and 0.38 mg/l, respectively for MX\(^{44}\). The C_{18} HI-Q-Sil column has been employed for the quantitative determination of MX in bulk and in pharmaceutical formulation using RP-HPLC with UV detection. The method was simple, economical, sensitive and selective with a mobile phase of acetonitrile, water and 1% aqueous glacial acetic acid (56:34:10 %, v/v/v). The UV detection was carried out at 362 nm with a flow rate of 1 ml/min using PX as IS. The method was validated as per ICH guidelines and demonstrated good resolution between MX and IS. The \( t_r \) for MX and IS were approximately 6.9 and 5.8 min, respectively. The range of reliable quantification was set at 0.3-20 \( \mu \)g/ml while LOD and LOQ were found to be 0.39 and 1.19 \( \mu \)g/ml, respectively. The accuracy ranged from 99.96-103.75% and the % RSD for both intra-day and inter-day precision was less than 2%. The MX showed minor degradation in acidic and basic conditions. There was no degradation of MX in the presence of oxidative, neutral, photolytic, dry and wet heat stress conditions\(^{24}\).
Wali et al. developed a simple, precise and accurate isocratic RP-HPLC method for the determination of MX in bulk drug and tablet dosage form. The separation was achieved on a LiChrospher RP-18 5 μm column using two mobile phases, i.e. 0.1 M K₂HPO₄ (pH 4.0) with H₃PO₄ and methanol. The two mobile phases were mixed in the ratio of 65:35 (v/v) and used with a flow rate of 1.0 ml/min at 35 °C. The tᵣ of MX was about 4.31 min and of its known impurity was about 2.26 min. The photodiode array detector was used to test the purity of the peaks and the chromatograms were detected at 254 nm. The method was validated for linearity, precision, accuracy, robustness, solution stability, and specificity. The method was linear in the concentration range of 150-450 μg/ml with a correlation coefficient of 0.999. The LOD and LOQ were found to be 5 and 50 μg/ml, respectively and the recovery was found to be in the range of 98.57-101.69%. The drug was subjected to the stress conditions such as hydrolysis, oxidation, photolysis, and thermolysis. The degradation products produced as a result of the stress conditions did not interfere with detection of MX and, therefore, the proposed method can be considered stability-indicating. Another RP-HPLC method was developed for the determination of MX in tablets using a C₁₈ Waters Xterra 5 μ column with a mobile phase of phosphate buffer and acetonitrile (40:60, v/v) in isocratic mode. The flow rate of mobile phase was 0.8 ml/min and detection was made at 344 nm with a tᵣ of 2.448 min. The method was validated according to ICH guidelines which showed the linearity range from 10-75 μg/ml with correlation coefficient of 0.999 and accuracy values of 99.53-99.66%. The robustness was done against changes in flow rate, pH of mobile phase, organic composition and detection wavelength. The method was found to be highly useful for the quality control analysis of MX tablets.

The quantitative determination of MX in human plasma has been done by C₁₈ RP-HPLC and tandem mass spectrometry. A single 15 mg oral dose of the drug was given to 26 healthy volunteers. Acetonitrile was used for the precipitation of the protein and PX was used as an IS. The LOQ of MX was found to be 8.96 ng/ml while the mean recovery was noted to be 92%. The assay method was reported to be sensitive, selective and more rapid for the determination of MX in human plasma. A bioequivalence study of 2 MX 15 mg tablets has been carried out. A single dose of each formulation was administered to 24 healthy volunteers (12 males and 12 females) and the plasma samples were obtained over a 96-hour interval. The LC-MS-MS was used to analyze MX using a cross flow counter electrode, an electrospray ionization (ESI) source...
operating in the positive ion mode employed for the multiple reaction monitoring (MRM). A Prevail C8 5 μ column with a mobile phase of acetonitrile-water and 10 mM acetic acid (20:80, v/v) was used. The t½ was found to be 1.8 and 1.4 min for MX and TX (IS), respectively. The LOQ was 0.02 μg/ml and the mean recovery of MX was 95.9%. The geometric mean of MX/movatex 15 mg individual % ratio was 101.3% for AUC (last), 99.9% for AUC (0-infinity) and 107.7% for Cmax. Since the ratios were within 80-125% interval as proposed by the US Food and Drug Administration Agency and accepted by Brazilian ANVISA (Sanitary Surveillance Agency), the results suggested that MX tablets produced by Merck were bioequivalent to movatex for both rate and extent of absorption.

In order to determine the low concentration of MX in human plasma, a highly sensitive and selective LC-MS-MS method has been developed. Both the MX and PX (IS) were chromatographed on a Zorbax SB C18 column using a mobile phase of acetonitrile-water-formic acid (80:20:0.2, v/v/v). A triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via ESI source was used. The LOQ was found to be 0.10 ng/ml and the concentration range of 0.10-50.0 ng/ml showed a linear curve. The method was then also validated. The percentage deviations from nominal concentrations were within ±2.5%, while the intra- and inter-day RSD were <7%. For the evaluation of pharmacokinetics the validated method was also successfully applied to the determination of MX in human plasma collected up to 180 h after a transdermal administration of 30 mg MX.

2.4. Flow Injection (FI) Spectrometry

A flow injection and batch procedure has been used to develop two fast and sensitive spectrometric methods for the determination of MX in pharmaceutical formulations. In methanolic medium a green complex was formed between drug and Fe (III) [2MX/Fe(III)] which is measured at 570 nm. The calibration curve was found to be linear within the concentration ranges 2.0-200 and 5.00-250 mg/l for the batch and flow injection method while the LOD was noted as 0.47 and 0.72 mg/l, respectively. In addition, at 362 nm in a flow injection spectrometric method, the absorbance of the drug was measured in 0.1 M NaOH. The concentration range of 0.5-20 mg/l of the calibration curve was found to be linear and the LOD was 0.04 mg/l. The methods were suggested to be applied to the routine analysis of MX in pharmaceuticals. Baban and Jalla proposed two sensitive and fast spectrometric methods by using batch and flow injection techniques for the determination of MX. There was a formation of primary aromatic amine by azo-coupling reaction of MX with sulphanilic acid in the presence of sodium nitrite in alkaline medium. At 365 nm the calibration curve was linear within the concentration ranges of 1.0-20 and 3.0-30 μg/ml for both the methods. The LOD was 0.5 and 1.0 μg/ml, respectively. The sandell index was 2.539×10⁻² μg/cm² and molar absorptivity was 1.3839×10⁴ l/mole cm. The method was considered to be useful for the routine analysis of MX in its pharmaceutical preparation. Another simple and sensitive indirect method was developed using flow injection analysis (FIA). The method was used to analyze MX, TX and PX as pure material and in pharmaceutical formulations. In acidic medium three drugs were oxidized by a known excess of N-bromosuccinimide (NBS). Then the excess oxidant was treated with chloranilic acid (CAA) to bleach its purple color. As the concentration of the drugs increases the absorbance values increased in a linear pattern. The system obeyed Beer’s law over the concentration ranges of 10-160 μg/ml for MX and PX while 20-200 μg/ml for TX, respectively. For more sensitive and reproducible results, some variables such as the acidity, reagent concentrations, flow rate of reagents and other FI parameters were analyzed. It was also observed that during the analysis, the excipients and additives did not interfere with the drug substances.

In order to determine MX through similar technique, another simple, rapid and sensitive flow injection chemiluminescence (FI-CL) method has been developed. The CL-emitting reaction between MX and KMnO₄ was performed in HCl medium which was enhanced by formaldehyde (H₂CO). The
calibration curve was obtained over the concentration range of 1.0-20.0 µg/ml under optimum conditions. The LOD was found to be 25.6 ng/ml where as RSD was 2.1% for 10.0 µg/ml MX. The proposed method was successfully applied to the determination of MX in capsules without interference from common excipients. Similarly another flow-injection system with chemiluminescent detection was developed for the determination of MX in tablets. During analysis it was found that MX could enhance the CL of tris (2,2’-bipyridine) ruthenium (II)–Ce (IV) system in a medium of sulfuric acid. A good linear relationship with the CL intensity under optimum conditions was shown by MX. The concentration range used was 6.0×10⁻⁴ to 1.0 µg/ml and the LOD was found to be 3.7×10⁻⁴ µg/ml.

2.5. Fluorescence Spectrometry
Four simple and accurate methods were presented for the determination of MX in dosage forms. The methods were based on (A) the direct measurements of the differential spectra at 339.9-384.7 nm, (B) the 1D-values at 322-368 nm and 2D-values at 343.2-385.6 nm, (C) the formation of an ion-association complex between the drug and safranin T with subsequent absorption measurement at 518 nm and (D) fluorescence measurement at 582 nm. All variables were studied to optimize the formation of the ion-association complex. The Beer’s law was found valid over the concentration range of 2-10 µg ml⁻¹ (method A), 1-10 µg ml⁻¹ (method B), 4.0-12 µg ml⁻¹ (method C) and 0.4-1.2 µg ml⁻¹ (method D). The LOD were 0.11, 0.07, 0.10, and 0.33 µg ml⁻¹ for methods A, B, C and D, respectively. The proposed methods were successfully applied for the assay of MX in tablets and suppositories.

2.6. Capillary Zone Electrophoresis (CZE)
For the analysis of MX a CZE assay method has been developed and validated. The influence of buffer concentration, buffer pH, methanol as organic modifier, capillary temperature, applied voltage and injection time was systemically investigated in a fused silica capillary column. For the optimum results, capillary temperature was kept at 25°C, 100 mM borate buffer (pH 8.5) containing 5% methanol was used as mobile phase with applied voltage of 20 kV with a detection wavelength of 205 nm. All the validation parameters were performed which showed good selectivity, accuracy, precision, linearity and sensitivity. In this method six pharmaceutical preparations including two dosage forms were determined. The RSD of 7 replicate analyses for each sample was less than 0.66%. The results were comparable with the spectrometric method.

2.7. Electrochemical Techniques
2.7.1. Voltammetric method
The adsorption behavior of MX has been studied by cyclic, differential-pulse and square-wave voltammetry on a hanging mercury drop electrode (HMDE). A well-defined stripping peak current was obtained at 21.42 V versus Ag/AgCl (saturated KCl) electrode when the drug was accumulated at HMDE in acetate buffer solution (pH 5.0). A voltammetric procedure was developed for the determination of MX using square-wave cathodic adsorptive stripping voltammetry (SW-CASV). The optimum working conditions for the determination of the drug were established. The analysis of MX in human plasma was carried out satisfactorily by this method. The oxidative voltammetric behavior of MX at a carbon paste electrode has been studied in Britton-Robinson (B-R) buffers in the pH range of 2.0-11.5. It gave rise to two voltammetric peaks, corresponding to the oxidation of amide and enol functions, respectively. The oxidation process was found to be independent on the pH of the supporting electrolyte. Using linear-scan voltammetry, the drug yielded well-defined voltammetric response in B-R buffer (pH 3.0). The process could be used to determine MX concentration in the range 5.0×10⁻⁷-5×10⁻⁵ M with a LOD of 1.6×10⁻⁷ M. Application of the method to the determination of the drug in the dosage form, without any interference from the excipients, resulted in acceptable deviation from the stated concentration (RSD = 2.2 %).

The electrochemical reduction of MX has been studied at a mercury electrode using various electrochemical methods in aqueous solutions over a wide pH range. The reduction of the drug produced...
a single reduction step in acidic media, whereas in slightly acidic and neutral media two reduction steps were observed. In alkaline media MX shows a single pH-independent reduction step. The irreversibility of the electrode process was verified by different criteria. At all pH values, reactant adsorption at mercury electrode was observed. Using differential-pulse voltammetry, the drug yielded a well-defined voltammetric response in B-R buffer (pH 4.0) at -1.286 V (vs. Ag/AgCl). This process could be used to determine MX concentration in the range of 1.0×10⁻⁸-5.0×10⁻⁶ M. The method was successfully applied for the analysis of MX in tablet dosage form. The improvement in this method was found by modification of a glassy carbon electrode with an anionic layer of cysteic acid providing electrostatic accumulation of the analyte onto the electrode surface. By cycling potential in cysteine solution modification was found in electrochemical oxidation of L-cysteine. The anodic peak current obtained at +1.088 V (vs. Ag/AgCl) by voltammetry showed linearity in the concentration range of 4.3×10⁻⁸ to 8.5×10⁻⁴ M in the B-R buffer solution (0.04 M, pH 1.86). The LOD (S/N = 3) was found to be 1.5×10⁻⁹ M with a correlation coefficient of 0.999. For the determination of MX this low-cost modified electrode shows good sensitivity, selectivity and stability in pharmaceutical formulation. In another method the adsorptive behaviors of MX was investigated by using glassy carbon electrode that was electrochemically treated by anodic oxidation at +1.8V, following potential cycling in the potential range from -0.8 to 1.0 V versus Ag/AgCl reference electrode. The resulting electrode showed a good activity to improve the electrochemical response of the drug, MX was accumulated at an electrochemically activated glassy carbon electrode (phosphate buffer, pH 6) in a certain time and then determined by linear sweep voltammetry. The oxidative peak currents showed a linear function in the concentration ranges of 0.02-10 mM using a 240 sec pre-concentration time. The pre-concentration medium-exchange approach was utilized for the selective determination of the drug in spiked urine and plasma samples with satisfactory results. The recovery values of the proposed method were 105% (RSD 2.5%) and 100% (RSD 1.8%) for urine and plasma samples, respectively. Also, the proposed method has been successfully used for determination of MX in tablets.

2.7.2. Polarographic methods
Different methods have been used in order to study the voltammetric behavior of MX, using direct current (DC), differential pulse polarography (DPP) and cyclic voltammetry (CV). The influence of several variables including nature of the buffer, pH, concentration, modulation amplitude, scan rate, drop size, etc. have been examined in DPP method. At pH 4.88 acetate buffer gives the best DPP response. Linear calibration curve was obtained with the concentration range of 0.38-15.0 µg/ml. The peak currents were measured with a static mercury drop electrode at -1.49 V versus Ag/AgCl. The method has been validated and applied to the determination of MX in tablets, with a mean recovery of 99.20±0.37%. It was concluded that the developed method was accurate, sensitive, precise, reproducible and useful for the quality control of MX in pharmaceuticals.

CONCLUSION
A number of analytical techniques have been discussed to study MX as pure, in pharmaceutical formulations and in biological samples. Most of the techniques have also been used to study the degradation or metabolic products of MX. Each technique has shown its own advantages over the other but the chromatographic techniques such as HPLC has proved to be the most accurate, precise and accurate method of analysis.

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