***Review Paper:***

# Different Analytical Methods for the Determination of Metronidazole

**Abrahem Sarra A.[1], Abdul Kader S.[2] and Ibrahim Suhad A.[1*]**
1. Al-Nahrain University, College of Science, Dept. of Chemistry, IRAQ
2. Ministry of Higher Education and Scientific Research, Minister's office, IRAQ
*su_aziz2015@yahoo.com

## *Abstract*

*Metronidazole is available in most areas of the world and is often used for treating bacterial infections in various parts of human body including the liver, brain, joints, vagina, skin, heart, respiratory tract and stomach or intestines. Therefore, it is essential to develop simple and low-cost analytical methods for such a compound in order to advance quality control.*

*In this study, various analytical papers that identify metronidazole in commercial preparations and biological samples have been analyzed and reviewed. The reviewed literature included spectrophotometric, chromatography and ion selective electrodes.*

**Keywords:** Metronidazole, ISE, HPLC, Spectrophotometric.

## Introduction

Metronidazole is a 5-nitronimidazole derivative and is widely used for treating bacterial infections due to its effectivity towards anaerobic bacteria and anaerobic protozoa[2]. Topical metronidazole (Metrogel) is used to treat a skin condition known as rosacea. Its gel form is often used to treat vaginal bacterial infection[1]. It has the formula $C_6H_9N_3O_3$, shown in figure 1 and a molecular weight of 171.156 g·mol$^{-1}$. Its chemical structure is [2- (2- methyl - 5 -nitroimidazole-1-yl) ethanol]. Metronidazole has a slight odor and is available in the form of crystalline powder of a light yellow to white color[1].

**Table 1**
**Ion Selective Electrodes for Determination of Metronidazole**

| Type of Ion pair for Electrodes | Results |
|---|---|
| Electrochemical ultrasensitive sensor using carbonnano, multi-walled polydopamine/carboxylic - tubes (MWCNTs_COOH) nano composites using improved (GCE) glassy carbon electrode.[5] | Conc. Range: 5 to5000 µ mol /dm$^3$.<br>Detection limit: (S/N = 3)0.25 µmol / dm$^3$.<br>pH: 5.0 to11.0.<br>Reduction peak current: remained 95.2% of its initial value.<br>Recovery: Between 93.4% and 118.3%.<br>Life time:1 month. |
| (MNZ) metronidazole based on 1-butyl-3-methylimidazolium tetrafluoroborate as (IL) ionic liquid and (SWCNT) single walled carbon nanotube. Using IL in the paste, as a binder, increased the electrode response.[6] | Conc. Range: Between $5.00\times10^{-5}$ and $5.00\times10^{-3}$ (mgL$^{-1}$).<br>Detection limit: $1.238\times10^{-5}$ (mgL$^{-1}$).<br>pH: 2.0 to 10.0.<br>Reduction peak current: –0.7 ± 0.05 V.<br>Recovery: 90.33–108.0 %. |
| quantitative and simultaneous (MT) metronidazole detection utilizing (PCHAGCE); (chromotrope 2B) poly, modified, activated glassy carbon electrode.[7] | Conc. Range: $1.0 \times 10^{-5}$- $4 \times 10^{-4}$ mol/L.<br>Detection limit: (S/N = 3) $3.3 \times10^{-7}$ mol/L.<br>pH: 4.0-9.0.<br>Reduction peak current: -0.58 V.<br>Recovery: 99.0-103.0%.<br>Life time: 2 weeks. |
| A sensor of amperometric metronidazole (MTZ) utilizing a recognition element (glycosylated metalloporphyrin) combined with a carbon paste electrode. For the preparation of a MTZ - sensitive active material, 5, 10, 15, 20 - tetrakis [2- (2, 3, 4, 6 - tetraacetyl - β - D -glucopyranosyl) - 1 - O - phenyl] porphyrin (T (*o*glu) PPH2) and its Mn (III) complex, MnT (*o*-glu) PPCl.[8] | Conc. Range: From $2.9 \times 10^{-3}$ to $5.8 \times 10^{-8}$ (M/L).<br>Detection limit: $5.8 \times 10^{-8}$ (M/L).<br>pH: 4.3.<br>Reduction peak current: - 408 mV.<br>Life time: 2 months. |

**Table 2**
**HPLC for determination of Metronidazole**

| Methods | Results |
|---|---|
| HPLC[9] | Column: Eclipse XDB-phenyl column.<br>Mobil Phase: (75:25:1, v/v/v) Sodium acetate (0.05 M): acetonitrile: glacial acetic acid, using phosphoric acid to adjust the pH ed to 4.0.<br>Detector: UV detector.<br>λ: 320nm.<br>Conc. range:0.05 – 30µg/ml<br>LOQ: 0.05µg/ml.<br>$t_R$: 4.06min. |
| RP-HPLC[10] | Column: Column (C18,0.5µm), Shimadzu LC-10ATvp (HPLC), particle size (150×4.6mm).<br>Mobil Phase: de-ionized water and HPLC grade methanol were mixed (ratio of 650:400 ml), using phosphoric acid to adjust the pH to 2.5.<br>Using vacuum filtration unit, a 0.45 µ membrane was utilized to filter the mobile phase. Then, ultrasonic bath was used for degassing for 15 min.<br>Detector: UV detector.<br>λ: 254 nm.<br>Conc range: 10-1000 µg/ml.<br>LOD:0.0158 µg $ml^{-1}$.<br>$t_R$: 7.751±0.00025 min. |
| RP-HPLC[11] | Column: (250 × 4.6mm) column, LiChrosorb® RP-18, packed with (5-µm) octadecylsilyl silica gel.<br>Mobil Phase: A solution of (0.02:20:80 v/v/v) triethylamine, acetonitrile and 0.3% o-phosphoric acid.<br>Detector: ShimadzuSPD-20A ultraviolet-visible (UV/VIS) detector.<br>λ: 290 nm.<br>Conc range: 12.5 to 100.0 µg/ml.<br>LOD: 0.125 µg/ml.<br>$t_R$: 3.42 min. |
| RP-HPLC-DAD[12] | Column: L-2300 column oven, prepared with a 250 × 4.6 mm2 (i.d.), ODS column (5µm).<br>Mobil Phase: Phosphate buffer (50mM) adjusted with 1M HCl to a (4.27 ± 0.01) pH as mobile phase A-methanol.<br>Detector: UV detector.<br>λ: 242 nm.<br>Conc range:1-20 µg/ml.<br>LOD :0.02 µg/ml.<br>$t_R$: 4.5min. |
| RP-HPLC[13] | Column: 250 x 4.6 mm, 5 µ (Phenyl column).<br>Mobil Phase: Instead of (ACN) Acetonitrile, Propylene Carbonate:Methanol 60:40 (Solvent-X) was used.<br>Detector: UV-Vis detector.<br>λ: 310 nm.<br>Conc range: 1.0–2.4µg/ mL.<br>LOD: 15ng/ml.<br>$t_R$:5.2 min. |
| RP-HPLC[14] | Column: (4.6 x150mm), particle size (5µ) (C-18 column), Water's X-bridge.<br>Mobil Phase: a mixture of acetonitrile and phosphate buffer (pH 2.5) in (70:30, %v/v) ratio.<br>Detector: UV detector.<br>λ: 220nm.<br>Conc range: 10-30µg/mL.<br>LOD: 0.042µg/mL.<br>.$t_R$: 3.157 min. |
| RP-HPLC | **Column:** The performance of isocratic elution on a (100mm×4.6 mm) Whatman® Partisil 5 ODS-3 column, (RP) plus Whatman® guard cartridge, particle size (5_m). |

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|---|---|
| | **Mobil Phase:** Degassing and filtering of (0.45_m; Millipore) solution mixture of phosphate buffer. A pH of (4.7; 0.05 M) - methanol (95:5, v/v). The pH was adjusted to (4.0).<br>**Detector:** UV detector.<br>**λ:**254nm.<br>**Conc range:** 0.13 and 300 µg/mL.<br>**LOD:**0.1 µg/mL<br>**$t_R$:**6.8 min. |
| HPLC | **Column:** *C8 ODS (250X4.6mm) column*.<br>**Mobil Phase:** 200ml methanol, 300ml acetonitrile, 100ml THF and (1.56gms/liter K2HPO4) 400 ml water.<br>**Detector:** UV detector.<br>**λ:** *254nm*.<br>**Conc range:** (60-100) ppm.<br>**LOD:** 0.1 ppm.<br>**$t_R$:** 3.14 min. |

**Table 3**
**Spectrophotometric methods for determination of Metronidazole**

| Methods | Results |
|---|---|
| Two Spectrophotometric methods[17] | Sample: metronidazole.<br>Conc range: 5-55 µg/ml and 5-60 µg/ml.<br>Slope: 0.0228 and 0.0174.<br>$r^2$: 0.9996 and 0.9997.<br>%Re: 99.15 and 99.54.<br>$\lambda_{max}$: 510 nm and 480. |
| Spectrophotometric[18] | Sample: metronidazole.<br>Conc range: 2.0-40.0 µg/mL.<br>Slope: $4.38 \times 10^{-2}$.<br>$r^2$:0.9988.<br>LOD: 0.76 µg/mL.<br>$\lambda_{max}$: 326 nm. |
| UV Spectrophotometric[18] | Sample: metronidazole.<br>Conc range: 2-20 µg/ml.<br>$r^2$:0.998.<br>%Re: 98-102%.<br>LOD: 0.763µg/ml.<br>$\lambda_{max}$: 320 nm. |
| UV Spectrophotometric[20] | Sample: metronidazole.<br>Conc range: 2-10µg/ml.<br>$r^2$: 0.9987.<br>%Re: 102.459.<br>$\lambda_{max}$: 277nm. |
| UV Differential Spectrophotometric[21] | Sample: metronidazole.<br>Conc range: 2-14µg/mL.<br>$r^2$:0.999.<br>%Re: 99.93.<br>LOD: 0.15µg/ml.<br>$\lambda_{max}$: 324 nm. |
| Novel UV spectrophotometer[22] | Sample: metronidazole and furazolidone.<br>Conc range: between 10 to 50 µg/mL and between 5 to 25 µg/mL.<br>$r^2$: 0.9992, 0.9996.<br>%Re: 98.16 and 98.44.<br>Slope: 0.0277 and 0.0600.<br>LOD: 0.323 µg/ml, 0.443 µg/ml.<br>$\lambda_{max}$: 319 and 364 nm. |

| | |
|---|---|
| UV-visible Spectrophotometric[23] | Sample: metronidazole.<br>Conc range: 1 to 15µg/ml.<br>r $^2$: 0.9994.<br>%Re: 98.80.<br>LOD: 0.4277 µg/ml.<br>$\lambda_{max}$: 320 nm. |
| Extractional spectrophotometric[24] | Sample: metronidazole. Tinidazole, ornidazole and secnidazole.<br>Conc range: 2.50–22.50, 2.50–30, 7.50–35 and 5–30 µgml$^{-1}$.<br>Slope: $3.15 \times 10^{-2}$ ,$4.56 \times 10^{-2}$, $5.14 \times 10^{-2}$ and $4.66 \times 10^{-2}$.<br>r $^2$: 0.9995, 0.9995, 0.9995 and 0.9996.<br>LOD: $5.33 \times 10^{-2}$, $5.16 \times 10^{-2}$, $5.01 \times 10^{-2}$ and $4.67 \times 10^{-2}$ µg/ml.<br>$\lambda_{max}$: 419, 418, 420 and 416 nm. |

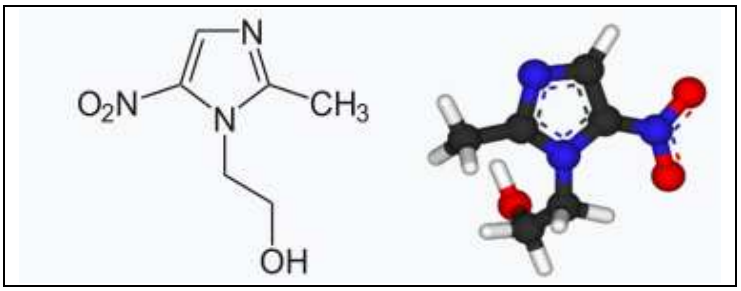


**Figure 1: A (2D) left and B (3D), right, chemical structures of Metronidazole.**

Several analytical methods were conducted by scholars to determine metronidazole. These methods included: concentration range, limit of detection, recovery, life time, type of column, mobile phase, slope, retention time, reduction peak current and the range of pH for metronidazole.

The results of reviewing these methods in this study were listed in tables 1, 2 and 3. In addition, there were other analytical methods for the determination of metronidazole such as: Photo-Fenton Oxidation Technology[3] and Charge-Transfer Complexes Formation.[4]

## Conclusion

In tables 1, 2 and 3, varieties of theoretical studies of three analytical methods for calculating metronidazole were included. It turns out from reviewing these studies that the best way to calculate metronidazole is (HPLC) high performance liquid chromatography. This method gave a wide range of concentrations that were specified in ng/ml and µg/ml, as well as low detection limit plus the most commonly used solvents are acetonitrile and methanol.

As for spectrophotometric and ion selective electrodes, these methods are low cost considering quality control analysis compounds in pharmaceutical preparations. In addition, they are easy to use in terms of their application in calculating metronidazole in pharmaceutical samples.

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